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Abstract Papers

ABSTRACTS of the 2006

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

Winchell Undergraduate

Research SympOSIUM

INTERMOLECULAR INTERACTIONS IN PHE-NYLHYDRAZONE CRYSTALS: LEWIS ACID–LEWIS BASE VS. HYDROGEN BONDING

Trina M. Arola and William H. Ojala (Advisor)

Department of Chemistry

University of St. Thomas, St. Paul, MN

We are working toward preparing new crystalline materials by co-crystallizing molecules we have designated "bridge-flipped isomers." In these isomers, two major parts of the molecule are joined by a bridge of atoms; changing the bridge orientation relates one isomer to the other. We are currently examining phenylhydrazones, in which the bridge-flipped isomerism is Ar–NH–N=CH–Ar' vs. Ar–CH=N-NH–Ar' (where Ar = aryl). Co-crystallization would be facilitated if the bridge-flipped isomers were isostructural (same molecular packing arrangement). We are determining the solid-state structures of phenylhydrazones by single-crystal X-ray diffraction to identify isostructural bridge-flipped isomeric pairs for future co-crystallization experiments. Intermolecular interactions linking molecules into similar chains in the two solid isomers might encourage their isostructuralism, so we have prepared phenylhydrazones substituted with halogen atoms and nitrile groups to encourage intermolecular Lewis acid–Lewis base interactions. We find H-bonding between the bridge N–H and the nitrile group to be a competing and differentiating interaction.

INTERGENERIC HYBRIDIZATION BETWEEN THE SAND DOLLARS *Encope michelini* **AND** *Mellita isometra* **(ECHINODERMATA: ECHINOIDEA: MELLITIDAE)**

Arden Ashley

Department of Biology

Macalester College, St. Paul, MN

Hybridization may be viewed as an obstacle to speciation and evolution or as a source of creative recom-bination leading to positive adaptations. This research presents a case study of a possible intergeneric hybridi-zation event between two groups of echinoids on the Atlantic Coast. In 1974, several unusual sand dollars were collected off of Fort Pierce, Florida. They are unlike any known species and cannot be categorized into any exis-ting genera. They share some characteristics with the genus *Mellita* and some with the genus *Encope,* and it has been theorized that interbreeding between these two gen-era may provide the explanation. If they are not hybrids, they must be considered representatives of a new genus in the Family Mellitidae.

In 2004, research at the National Museum of Natural History was conducted to investigate the origin of these sand dollars. Various morphological characteristics of specimens of the presumed hybrids were compared with those of the presumed parent taxa, *Mellita isometra* and *Encope michelini*. Statistical analysis was not poss-ible due to the small sample sizes; however, this study provides a broad analysis of many characteristics. In some characteristics the hybrids fell directly within the broad limits of variation of the proposed parents. In other cases, the presumed hybrids seemed to be quite distinct, falling outside the range of variation of the presumed parent taxa. Further study is required, including additional breeding experiments, DNA analysis, and additional morphometric data sampling, including examination of the plate structure and analysis of the internal skeleton.

WETLAND POND MACROINVERTEBRATE COMM-UNITIES ON THE ST. OLAF CAMPUS

H. L. A. Austin and M. C. S. Swift (Advisor)

Department of Biology

St. Olaf College, Northfield, MN

Aquatic macroinvertebrates are invertebrates that are visible to the naked eye which live in both pond and stream habitats. The purpose of this study was to learn to identify macroinvertebrates, and to compile a reference macroinvertebrate profile for ten ponds located on St. Olaf College's Natural Lands in Northfield, Minnesota. Fifty-six taxa were identified to genus or species. Samples were provided by Dr. Swift and collected from St. Olaf College. Fifteen taxa were collected and identified from St. Olaf College ponds. The most frequently collected and most abundant macroinvertebrates in St. Olaf ponds were *Hyalella* sp. and *Caecidotea occidentalis,* respectively. There was no correlation between pond age and taxon richness.

EVIDENCE OF DEPOSITION OF COMMERCIAL FERTILIZER IN AN UNPLOWED FLOODPLAIN PRAIRIE REMNANT

Elizabeth Bach and Marty Condon (Advisor)

Department of Biology

Cornell College, Mt. Vernon, IA

Do unplowed areas of remnant tallgrass prairie offer the best examples of unaltered or "natural" prairie systems? Remnants that are on floodplains may show evidence of human alteration even though the area has never been plowed or intentionally altered. My project investigated the soil under Wearin Prairie, a floodplain remnant, for evidence of alteration indirectly caused by human actions. Specifically, my experiments were design-ned to test for the presence of available phosphorus, a component of commercial fertilizer which might be de-posited during flooding events. The results showed a correlation between the concentration of available pho-sphorus and proximity of the sample site to the West Nishnabotna River, implying fertilizer deposition.

MODERATION OF HUMAN RIBONUCLEASE INHIBITOR OXIDATION SENSITIVITY BY SITE-DIRECTED MUTAGENESIS

Johann S. Bergholz, Barbara A Hirschman, Sarah B. Miller, and Kimberly A. Dickson (Advisor)

Department of Biology

Macalester College, St. Paul, MN

Human ribonuclease inhibitor (RI) is a cytosolic protein critical for protecting cells from RNA degradation by ribonucleases. With a femtomolar affinity, the binding of RI with pancreatic-type ribonucleases is one of the strongest protein interactions observed in nature. RI is built from highly conserved, alternating A and B leucine-rich repeats (LRRs), each of which contains an α-helix and β-sheet. Reduced cysteine residues contained in these repeats are critical to protein structure but quickly oxidize under extracellular conditions, complicating efforts to utilize RI as a laboratory reagent. Using site-directed mutagenesis, we will mutate two highly conserved cys-teine residues at positions 10 and 17 of the A-repeats in an effort to increase the stability of RI. In order to main-tain molecular interactions within the tertiary structure of RI position A10 will be replaced with a serine residue, while valine will be substituted for the cysteine residue in position A17. Mutated proteins will be isolated and puri-fied to ensure that structure and function have been main-tained.

PERTURBATION OF AUXIN-MEDIATED TRAN-SCRIPTION VIA SYNTHESIS OF MOLECULAR INHIBITORS

Johann S. Bergholz, Sarah B. Miller, Ronald G. Brisbois (Advisor), and Paul J. Overvoorde (Advisor)

Department of Biology

Macalester College, St. Paul, MN

Auxin, commonly exemplified by indole-3-acetic acid (IAA), is known to play a role in plant growth and development by affecting gene expression. The Aux/IAA family of genes encodes short-lived nuclear proteins that can be induced within five minutes to one hour upon exposure to IAA. The molecular mechanisms of auxin-mediated gene expression, however, are not completely understood. Current research points to the activation of auxin-response genes by directing proteolysis of the Aux/ IAA family of proteins by the ubiquitin protein ligase SCFTIR1, resulting in the activation of auxin response factors (ARFs), a known set of transcription factors that would then arbitrate auxin-mediated gene expression. Compound A, a furylacrylate ester of a thiadiazole hetero-cycle, has been shown to inhibit auxin-mediated tran-scription, but neither the mechanism of action nor the active part of this molecule are known. In this study we synthesized different analogues of compound A to deci-pher the active core moiety of the compound by GUS and qPCR analyses. With these results at hand, we will be able to identify the target for compound A and gain a better understanding of auxin functioning at the molecular level.

CREATION OF AN OXIDATION-RESISTANT RIBONUCLEASE INHIBITOR

Owen Brafford, Catlin Shortridge, and Kimberly A. Dickson (Advisor)

Department of Biology

Macalester College, St. Paul, MN

Human Ribonuclease Inhibitor (RI) is a leucine-rich repeat, horseshoe-shaped protein that binds to pan-creatic-type ribonucleases to inhibit the degradation of RNA. The tertiary structure of RI is characterized by repeating structural elements called A and B units. Each of these units contains an α-helix and a β-sheet. RI contains 32 cysteine residues that create oxidative instability in the protein. Our goal was to replace the cysteine residues with amino acids that would conserve the structure of the protein without compromising its function. We replaced two A21 and five B21 cysteine residues with serine residues. Post-mutagenesis, the mutant RI proteins were characterized for function and binding activity. Possible benefits of an oxidation-insensitive RI would be as a useful laboratory reagent and as a new tool for exploring and modulating interactions with pancreatic-type ribonucleases.

MATE CHOICE MEDIATED BY SIZE AND CHEM-ICAL DEFENSE IN *Nyssodesmus python* (POLYDES-MIDA: PLATYRHACIDAE)

Mercedes Burns and Mark A. Davis (Advisor)
Department of Biology

Macalester College, St. Paul, MN

Large mates are preferred in a variety of species, presumably because larger size typically confers greater fitness. In chemically protected species, mates may prefer more toxic partners for the same reason. The common for-est millipede *Nyssodesmus python* displays sexual dimor-phism in body size and also produces a defensive com-pound containing hydrogen cyanide, making it an organ-ism well suited to an experiment on mate preferences highlighting both size and presence of defense compounds.

In research conducted as part of a study abroad course with the CIEE Monteverde, Costa Rica, program, the influence of size and toxicity on mate preferences in this species was tested. Fifty-eight millipedes were collec-ted and sorted by sex, size, and hydrogen cyanide rank based on a modification of the Grignard Sodium Picrate Test. They were then offered preference between two potential mates of differing size or hydrogen cyanide rank. The 32 mate choice experiments showed a statistic-ally significant preference between both sexes for larger mates; however, preference for mates with differing hydrogen cyanide ranks was not significant. Results suggested that mating with individuals possessing high quantities of defense compounds did not provide a significant fitness advantage. High mortality in this study may result from autotoxicity and exposure to unusually high amounts of ambient hydrogen cyanide during mea-surement of relative hydrogen cyanide levels. A new methodology is recommended in the future to better understand the role of chemically mediated mate choice without the hindrance of high study mortality.

MICROWAVE SYNTHESIS OF IONIC LIQUIDS IN ORGANIC CHEMISTRY LABORATORY

Nathan Burrows and David Blackburn (Advisor)

Department of Natural Sciences

Concordia University, St. Paul, MN

The topics of microwave synthesis, ionic liquids, green chemistry, and neat (solventless) reactions are seldom taught in the undergraduate laboratory setting. This lab provides a method to introduce and experience all of these topics in a simple and easily available method. Students produce butylmethylimidazolium bromide, a hydrophilic low-melting ionic liquid, using a household microwave and a simple equipment setup using common lab glassware. Students can then perform an ion exchange producing butylmethylimidazolium hexafluorophosphate, a hydrophobic room-temperature ionic liquid, as a liquid precipitate. Two of the 12 principles of green chemistry, prevention of waste and atom economy, are exercised in this lab through a neat reaction and purification method.

TURN STRUCTURES OF A MODEL PEPTIDE SYSTEM: INSIGHTS FROM CIRCULAR DICHR-OISM

Sara J. Bush, Kristine L. Carlson, and Kathryn A. Thomasson

Department of Chemistry

University of North Dakota, Grand Forks, ND

Cyclo(Gly-L-Pro-L-Pro)2 (cGPP2) is a peptide model for cis and torsionally strained peptide bonds that exhibits a strong distinctive UV circular dichroic (CD) spectrum. Circular dichroic spectra were computed for the amide pi-pi* transition using the dipole interaction model for various conformations of the peptide. Conformations of cGPP2 were created initially from crystal and NMR data, and followed by energy minimizations via molecular mechanics using three force fields: CVFF, CFF91, and AMBER. A series of dielectric constants, representing various solvent conditions from gas (0) to water (78.5), were used for the minimizations. The minimized struc-tures were examined for structural features such as beta- and gamma-turns. The CD spectra for each conformation were calculated using a variety of parameters, and each result was compared with the published experimental spectrum in acetonitrile.

STRESS FIBER FORMATION IS ESSENTIAL FOR CELLULAR MIGRATION IN CHINESE HAMSTER LUNG FIBROBLASTS

Jenny L. Canine, Joseph J. Provost (Advisor), and Mark A. Wallert (Advisor)

Department of Biosciences

Minnesota State University Moorhead, Moorhead MN

The coordinated reorganization of the actin cytoskeleton is a common cellular event. In a variety of cell types including lymphocytes, the formation of stress fibers is indicative of a stabilized attachment state where the cells no longer migrate. The research presented here demonstrates that stress fiber formation is essential for cellular migration in CCL39 cells, Chinese Hamster Lung fibroblasts. As in virtually all other mammalian cells, the Sodium–Hydrogen Exchanger (NHE) is present and plays a dual role in pH regulation and cytoskeletal attachment to the plasma membrane. In this second role, NHE is also essential for the formation of stress fibers in cells. Previous research from our laboratory has shown that phenylephrine (PE) stimulates NHE and induces stress fiber formation in these cells. To investigate the role of stress fiber formation, CCL39 cells were allowed to grow into a confluent monolayer in a 35 mm culture dish. The cells were then serum deprived 12 to 18 hours. At this point the monolayer was wounded using a standard cell scraper. The cells were then allowed to migrate into the wounded area for 24 hours in one of four conditions: serum-free media, serum-free media with PE, 10% serum media, and 10% serum media with PE. Our data show that in PE-stimulated CCL39 cells, stress fibers are present in the cell immediately adjacent to the wound area and in cells that have migrated into the wound. These studies indicate that stress fiber formation has a direct involvement in cell migration.

STRUCTURAL INSIGHT FROM CIRCULAR DICHROISM

Kristine L. Carlson, Mark R. Hoffmann, and Kathryn A. Thomasson

Department of Chemistry

University of North Dakota, Grand Forks, ND

The tricyclic structure of the substituted diketo-piperazine cyclo(L-Pro-L-Pro) constrains its possible geo-metric conformations to three minimum energy structures. The relationship of these three structures was examined via comparison of UV circular dichroic spectra calculated using the classical dipole interaction model with experi-mental CD (Bowman, R. L.; Kellerman, M.; Johnson, W. C., Jr. Biopolymers **1983,** *22,* 1045). Starting with crystal structure data for the platter conformation, the three conformations were obtained by geometric optimization using MP2, DFT, and three molecular mechanics force fields. The pi-pi* spectrum produced by each conform-ation was distinct but followed a pattern with a negative band at ~ 185 nm and a positive band at ~ 210 nm.

The CFF91 force field was the only classical force field to produce structures whose composite CD resembled experiment, but conformations were not clearly defined by CD due to the high (~ 20 degrees) amide bond torsion angles. Inclusion of water as solvent in the class-ical optimizations through the use of a dielectric constant resulted in very small changes in geometry for each con-formation and little or no shift in weighted CD, although the energies obtained using the CFF91 force field permit-ted real occupation of the chair and boat conformations (18% and 31%, respectively). DFT and MP2 energy calc-ulations indicated that the population of the molecule in the three conformations is roughly equal (MP2: 34% platter, 33% chair, 33% boat). Boltzmann-weighted composite CD supported this conclusion through accurate description of the two pi-pi* peaks with respect to one another.

SOY PROTEIN ISOLATE

Michelle Caron and Gina Mancini-Samuelson (Advisor)

Department of Chemistry

College of St. Catherine, St. Paul, MN

The purpose of this project is to develop a case study for the Advanced Analytical class that focuses on the High Performance Liquid Chromatography instrument. The project focuses on the isolation of Soy Protein Isolate, 11S, and 7S protein from soy flour and other soy products.

MOLECULAR ANALYSIS OF A TRANSCRIP-TIONAL SILENCER IN *Drosophila melanogaster*

Chris Chamberlain and Presley Martin (Advisor)

Hamline University, St. Paul, MN

The MM-50 transgenic line of *Drosophila melanogaster* contains a *D. melanogaster* Alcohol dehydrogenase (Adh) transgene inserted at position 25C of chromosome 2. Previous analysis has shown that expression of this Adh transgene is inhibited by a silencing activity located in an 1180 b.p. region near the 5^1 end of the inserted gene. The sequence of the silencer region revealed the existence of two 20 b.p. homologies, which have been shown to inhibit Adh expression by 50%. The objective of this investigation was to determine what other sequences within the silencing region are required to produce the near 100% inhibition of expression observed when the whole region is present. Seven unique fragments of the silencer sequence were cloned using PCR and purified using standard procedures. Two fragments contain the first homology of the sequence, one fragment contains the second homology, and four fragments contain neither of the homologies. Each of the seven fragments were transformed into plasmids containing a functional Adh gene, thus yielding seven complete plasmids, each containing one of the fragments and one copy of the Adh gene. Each plasmid will be injected into *Drosophila* embryos and the larvae will be assayed for the level of Adh gene expression.

THE EFFECTS OF OPIOID ANTAGONIST NAL-TREXONE IN ANIMALS MOTIVATED TO EAT BY TASTE

Munya Chimukangara and Tim Shaw (Advisor)

Department of Biological Sciences

Bethel University, St. Paul, MN

It is well known that the opioid system plays a significant role in the regulation of palatable food intake. Generally speaking, opioid agonists increase feeding while opioid antagonists decrease feeding in non-food-restricted animals. These drugs, however, have a substan-tially reduced effect in food-deprived animals. These data are based predominantly on studies performed on the peripheral opioid system. Not much is known about the effects of these drugs when administered into the opioid receptors of the central nervous system. However there are data suggesting that food intake regulation is also dependent on brain sites in the hypothalamus. The hypo-thalamus is known to have nuclei associated with energy-and-reward-related feeding. In this study I was therefore trying to find out if the administration of Naltrexone in the hypothalamic paraventricular nucleus (PVN) would decrease food intake more effectively in a food-restricted (energy needs-related) model than in a non-food-restricted (reward-related) model. My results suggest that Naltrex-one more effectively decreases intake of food in the food-restricted model than in the non-food-restricted model. The data also suggest that the PVN is primarily involved in energy-needs-driven-food intake in comparison with reward-driven food intake.

ANALYSIS OF THE EXTRACELLULAR HEMO-GLOBIN FROM *Lumbriculus variegatus*

Jessica Curtis, Rebecca Derby, Melissa Seefeld, Lee Vang, and Kay Tweeten (Advisor)

Department of Biology

College of St. Catherine, St. Paul, MN

The major carrier of oxygen in the California blackworm, *Lumbriculus variegatus*, is an extracellular, high molecular weight molecule. The objective of this project was to characterize this molecule to determine how similar it is to annelid hemoglobins that have already been studied.

The hemoglobin was isolated from homogenates of worm tissue by ultracentrifugation and size exclusion chromatography. The morphology, subunit composition, and size of the hemoglobin were determined by electron microscopy and showed that the structure consisted of two hexagonal-shaped rings that were 265 Å in width and composed of six subunits each. Western blot analysis showed that antibodies against human hemoglobin bound to four of the proteins with molecular weights of 14,900, 15,300,

16,100, and 16,500 daltons, suggesting these are the oxygen-binding proteins. Five to six potential linker proteins with molecular weights ranging from 26,200 to 36,000 daltons were also observed. The glycoprotein composition of *L. variegatus* hemoglobin appeared to be more complex than that of earthworm hemoglobin with all four oxygen-binding proteins and the predominant linker protein being glycosylated. Hemoglobins from *L. variegatus* and *Lumbricus terrestris* were compared by two-dimensional gel electrophoresis to further evaluate the similarities and differences in the proteins composing these assemblages.

THE GENDER GAP IN EARNINGS: DOES URBAN LOCATION MATTER?

Amanda Demeules and Marsha Blumenthal (Advisor)

Department of Economics

University of St. Thomas, St. Paul, MN

Dating back to ancient times one can find references and data pointing to women not earning as much as men. In fact Leviticus (27: 3–4) states that a woman is worth 30 shekels of silver and a man 50 shekels of silver. This paper aims to explore the modern gender gap in earnings in midwestern states, specifically, examining the influence of metropolitan location. Individual data from the *Current Population Survey* will be used to construct an Oaxaca decomposition, controlling for age, educational attainment, race, and marital status.

PROJECTIONS OF RVM NEURONS TO PAIN-RELAY SITES

David H. Do and Martin W. Wessendorf (Advisor)

Department of Neuroscience

University of Minnesota, Minneapolis, MN

Pain, which warns the body of potentially harmful stimuli, could hinder one's ability to act protectively in dangerous situations. It is speculated that, during a "fight or flight" response, neurons in the rostral ventromedial medulla (RVM) of the brainstem send inhibitory signals down the spinal cord to block trans-mission of pain. Activation of μ-opioid receptors in the RVM by drugs such as morphine and heroin utilize these pathways to regulate pain in much the same ways. In contrast, in the case of neuropathic pain, RVM cells appear to work in an excitatory manner to enhance nociception. These phenomena are well documented, but the mechanisms by which they occur are less understood.

The RVM is populated by cholinergic (ChAT) and serotonergic (5-HT) neurons, among others. These neurons, which signal via the neurotransmitters acetyl-choline and serotonin, respectively, may play a key role in pain facilitation by preventing or amplifying signals relayed by neurons in the spinal cord. In this experiment, the potential role of these RVM neurons in pain modula-tion was examined by finding neural connections to the dorsal horn and the spinal trigeminal nucleus, two regions of the nervous system where peripheral sensory information is integrated.

GENETIC SCREEN OF SECRETION MUTANTS IN *Chlamydomonas*

Dina Dobraca, Milkeesso Foge, Srividya Gainedi, Sarah Merkel, Paul Sarjo, and Anton Sanderfoot (Advisor)

Department of Plant Biology

University of Minnesota, Minneapolis, MN

Secretion is an essential activity that plays important roles in numerous cell functions, such as cell-to-cell communication, waste disposal, and cell defense. The purpose of this project is to characterize previously unidentified genes involved in secretion in the model organism *Chlamydomonas reinhardtii,* a unicellular haploid green alga. We expect that *Chlamydomonas* will be a useful model organism for characterizing secretion mutants because only one gene copy of the haploid organism needs to be mutated for the strain to have a mutant phenotype and the unicellular plant may survive with defects that may be lethal in complex, multicellular plants.

Using a forward genetic approach, a collection of mutant strains will be screened for secretion defects using an endogenous secreted enzyme as a primary screen. A secondary screen will use a synthetic, secreted green fluorescent protein (GFP) construct to confirm secretion defects and to determine the location of the blocked secretion product. Briefly, wild-type *Chlamydomonas* will secrete the GFP extracellularly; in a mutant with a secretion blockage, the GFP will accumulate inside the cell at the point of blockage. We are at the stage of creating and confirming the expression of the fluorescent protein constructs. In the future, we hope to use these tools to identify plant-specific secretion genes in *Chlamydomonas* and apply these results to multicellular crop plants.

Readability Levels of High School and College Chemistry Textbooks

Elizabeth A. Drommerhausen and Dr. Jeffrey R. Pribyl (Advisor)

Department of Chemistry and Geology

Minnesota State University Mankato, Mankato, MN

In many science classes the textbook is frequently the determining factor for the content of a course. This is particularly the case where chemistry education is concerned. Just how effective is that heavy, expensive tool? While textbook content is critical, another factor just as critical is often overlooked. That factor is the reading level, also known as the readability level. Chemistry textbooks typically are written at a reading level that is well above secondary students' or college students' abilities. Numerous high school and college chemistry textbooks were analyzed for their readability level using the Fry and Raygor readability graphs. The results of this work confirm that the readability level of chemistry textbooks is often times well above the reading level of the target audience.

THE INVISIBLE HAND OF NATURAL SELEC-TION: SMITH, DARWIN, AND GLOBAL POVERTY

John Dukich and Mark Borrello (Advisor)

Department of Ecology, Evolution and Behavior

University of Minnesota, Minneapolis, MN

Although Adam Smith published *The Wealth of Nations* in 1776 and Charles Darwin published *The Origin of Species* in 1858 their ideas have had a profound impact on modern thought. How have these two ideas, in the context of eighteenth- and nineteenth-century thought, helped to create the global gap between the wealthy and the poor? Smith and Darwin, as well as other thinkers of their time, were influenced by contemporary society and ideas. These ideas, of reductionism, mechanism, and scientific materialism, have been inherited by modern science and modern societies, which in turn have shaped how humanity perceives itself and what's expected of itself. After putting Smith and Darwin in historical con-text, I argue that such a wide gap between the wealthy and the poor has arisen, in part, due to misinterpretations of their respective works as well as due to the inherited ideas of the past 300 to 400 years. I conclude with what is necessary to overcome these obstacles and the implications this would have.

ERK ACTIVATION BY PHOSPHOLIPASE D THROUGH THE α-1 ADRENERGIC RECEPTOR IS Ras DEPENDENT

Matthew P. Duval, Joseph J. Provost (Advisor), and Mark A. Wallert (Advisor)

Biosciences Department

Minnesota State University Moorhead, Moorhead, MN

Phospholipase D (PLD) is considered an important signaling molecule in many growth factor pathways. PLD converts phosphatidylcholine into choline and phosphatidic acid (PA). The PA generated by PLD is thought to recruit Raf to the lipid rafts of cell membranes, leading to stimulation of growth factor signaling com-plexes. Previous experiments in our laboratory have shown the addition of primary butanol inhibits ERK activation by blocking PA production. While this work suggests that PLD is involved in the activation of the ERK signaling pathway, it does not explain its mech-anism. To investigate the role of PLD in ERK activation, two short-chain, cell-permeable phosphatidic acids (1,2-Dihexanoyl-sn-Glycero-3-Phosphate and 1,2-Dilauroyl-sn-Glycero-3-Phosphate) were incubated at several times and concentrations with CCL39 fibroblasts. Both short chain phosphatidic acids (scPA) act as endogenously added PLD product. Both the 6 and 12 acyl scPA stim-ulated ERK activation in a dose- and time-dependent manner with maximum ERK activation observed with the 6 acyl scPA. Ras activation pull down assays conducted with phenylephrine (PE) stimulating cells showed Ras activation. When a primary butanol was added prior to stimulation, PE did not activate Ras. Additional evidence for a Ras-dependent PLD-ERK activation was determined through dominant negative Ras (D/N Ras). Expression of D/N Ras blocked activation of ERK by PE. The ability of D/N Ras to inhibit PA activation of ERK was also invest-igated. This is a novel mechanism for PLD involvement in growth factor pathways. This work was supported by a grant from the NIH, Award number 1 R15 HL074924-01A1.

SEQUENCE AND EXPRESSION OF THE FGF-10 GENE IN *Xenopus laevis* LUNG DEVELOPMENT

Brett D. Einerson and Brian Hyatt (Advisor)

Department of Biological Sciences

Bethel University, St. Paul, MN

During lung bud morphogenesis, reciprocal interactions between the epithelial endoderm and the mesenchyme surrounding it lead

to early branching of the pulmonary system. Members of the fibroblast growth factor (FGF) family, along with their receptors, have been shown to play an integral part in mediating these inter-actions. FGF-10 specifically has been shown to be an essential regulator in lung formation. The FGF-10 gene was isolated from *Xenopus laevis*, and its expression during lung development was examined. *X. laevis* serves as an effective model organism for this study. Gathering information about gene expression in this organism expands our understanding of pulmonary development.

ANALYZE THIS! A CASE STUDY APPROACH IN THE ANALYTICAL CHEMISTRY LABORATORY

Sarah Evans, Gina Mancini-Samuelson (Advisor)

Department of Chemistry

College of St. Catherine, St. Paul, MN

If one contemplates art and science for a minute, artists are really quite similar to chemists. Both are personally engaged in combining, transforming, and experimenting with materials. In my personal opinion, the relation of art to chemistry is, in fact, the most overt among all the scientific disciplines. This research project incorporates art with chemistry creating that unforeseen bond. In this project, a case study was created for the use in Advanced Analytical Chemistry course at the College of Saint Catherine. The case study is about forensic science and art forgery and gives students a scenario discussing the authenticity of a newly discovered piece of artwork and asks the student to determine the authenticity of the painting through laboratory work mainly with the use of Infrared Spectroscopy. To construct the case study, it was my job to gather background information about the artist and specific time period and create standards using pigments from that period and analyzing them with Infrared Spectroscopy

A SPECTRAL ANALYSIS TEST OF THE FEMALE MIMICRY HYPOTHESIS OF DELAYED PLUM-AGE MATURATION

Ben Freeman and Mark A. Davis (Advisor)

Department of Biology

Macalester College, St. Paul, MN

Delayed plumage maturation (DPM) in birds is the retention of subadult plumages by individuals of one sex when sexually mature. The female mimicry hypo-thesis of DPM suggests that subadult males are selected to mimic females in appearance, as has been demonstrated in animals from insects to lizards, and in adult males of one bird species (Langmore et. al. 1999). Additionally, the female mimicry hypothesis has been suggested to apply more widely to birds with DPM and female-like subadult plumages. A full-spectrum analysis of two bird species with the above characteristics, painted buntings (*Passerina ciris*) and American redstarts (*Setophaga ruticilla*), revealed at least one distinguishing plumage patch in *P. ciris* and none in *S. ruticilla*. The distin-guishing plumage patch in *P. ciris* was the lower back, which could be a concealable badge. Thus, the female mimicry hypothesis could, at least from this spectral perspective, apply to both species.

THE ROLE OF 5-HYDROXYTRYPTAMINE (5-HT) AND THE 5-HT1A RECEPTOR IN HUMAN T CELL PROLIFERATION

Audrey Geiger, Melinda Hexum, Kate Ruter, and Dr. Jodi Goldberg (Advisor)

Biology Department

Hamline University, St. Paul, MN

Previous research has shown that the neurotransmitter serotonin, or 5HT, is necessary for T cell proliferation. However, these studies have employed heterogeneous culture systems. In order to determine the specific impact of 5-HT on human T cell proliferation, T cells were first purified from whole blood to greater than 98% purity. T cells were then labeled with a fluorescent dye and active-ted in one of two manners. T cells were activated in the absence of other cells by incubating purified T cells with anti-CD3/anti-CD28 coated beads (accessory cell inde-pendent activation). Alternately, T cells were activated in the presence of accessory cells and the T cell mitogen phytohemagglutinin (PHA), a plant lectin (accessory cell dependent activation). The role of 5-HT in this activation was assessed by either adding the 5-HT synthesis inhi-bitor pCPA or by adding 5-HT receptor antagonists to the cultures. Flow cytometry was used to measure T cell proliferation as a decrease in T cell associated fluor-escence. Inhibition of proliferation by pCPA was observ-ed at a lower concentration in accessory cell dependent cultures than in accessory cell independent cultures. Inhibition by Methiothepin, a general 5-HT receptor antagonist, was observed in cells stimulated by both accessory cell dependent and accessory cell independent methods at equal concentration. No inhibition by Nan-190, a specific 5-HT1A receptor antagonist, was observ-ed. Reversal of inhibition of T cell proliferation in these cultures by the introduction of 5HT into the cell cultures has not yet been achieved.

STRUCTURAL STUDY OF ALPHA-AMYLASE: SELECTION OF SCAFFOLDING STRUCTURES AND DOMAIN SWAPPING

Robyn Gilbertson and Paul Tavernier (Advisor)

Department of Chemistry

Bethel University, St. Paul, MN

The structure of *Bacillus licheniformis* alpha-amylase was studied by viewing its pbd file 1BLI with Rasmol. The 11th alpha-helix was selected for use in this project. Similar sequence helices were found using online bioinformatics databases such as Conserved Domain Database and Protein Data Bank. An alpha-helix in hydro-lase from *Pseudomonas stutzeri* was selected for the domain swap. Stratagene's QuikChange II Site-Directed Mutagenesis Kit was used to delete the 30-base-pair DNA sequence coding for the selected alpha-helix from AmyE, the gene for alpha-amylase in *B. licheni-formis*. Plasmid pRL298 containing AmyE was the DNA template used for the mutagenesis. Using the successful deletion plasmid, the DNA sequence for the *P. stutzeri* helix was inserted using the QuikChange Kit. Results were verified by growth on LB agar plates containing kanamycin and starch and by molecular weight deter-mined by gel electrophoresis.

AdS_2 SOLITON DYNAMICS

Kassahun Haileyesus and Tonnis Ter Veldhuis (Advisor)

Department of Physics and Astronomy

Macalester College, St. Paul, MN

The dynamics of a soliton in a two-dimensional curved space-time is worked out. Transformation rules in AdS_2 space are found and Maurer Carton one forms are used to construct a Lagrangian that is invariant under AdS_2 transformations. This Lagrangian is shown to be equivalent to the Lagrangian of a relativistic Harmonic oscillator. Conserved quantities associated with symme-tries are found using Noether's method and they are used to find solutions to the Euler Lagrange equations of mo-tion. From the invariant action, the conjugate momentum is determined and the Hamiltonian is constructed. This Hamiltonian is then shown to be equivalent to that of a conformal mechanics in one dimension.

THE EFFECTS OF LIPOPHOSPHOGLYCAN STIMULATION ON MACROPHAGE GENE EXPRESSION

Cassondra Halsted and Joyce Doan (Advisor)

Department of Biology

Bethel University, St. Paul, MN

Leishmania spp. are protozoan parasites which are transmitted by sandflies or, to a lesser extent, by the sharing of needles. *Leishmania* parasites are found all over the world, although infection with these organisms is much less common in the United States. Different species of the parasite cause cutaneous, subcutaneous, and visceral forms of the disease leishmaniasis. The cutaneous form of the disease is characterized by disfiguring skin lesions. In humans, *Leishmania* parasites cause intra-cellular infection of macrophages. Therefore, the ability of the cell membrane of *Leishmania* spp. to interact with the cell membrane of macrophages is a central event during infection. One of the cell surface molecules present on *Leishmania* spp. that is important for the infection process is lipophosphoglycan (LPG). Exper-iments were designed to figure out the effects of LPG stimulation on macrophages. The expression of specific genes known to be involved in immune responses against protozoan parasites like *Leishmania* spp. was determined using reverse-transcriptase polymerase chain reaction (RT-PCR). It was discovered that LPG alone does not appear to change the expression of many genes that are important to immune responses

EFFECTS OF NEUROTENSIN ON THE SMOOTH MUSCLE TISSUE OF THE MOUSE UTERUS

Nellie Hangge and Teresa DeGolier (Advisor)
Department of Biology

Bethel University, St. Paul, MN

Research has shown neurotensin (NT), a biolog-ically active peptide, to produce various effects in mam-malian smooth muscle tissue. NT inhibits rat intestine yet stimulates guinea pig colon. This study investigated whether NT would have an effect on smooth muscle tissues of the isolated mouse uterus. Oxytocin was used as a positive control for uterine contraction. Uterine horns from mice in the diestrus stage of the cycle that were suspended in the smooth muscle bath and subjected to higher doses of NT (10-7-10-6M) increased contractile frequency. Oxytocin caused an increase in strength of contraction within tissues from mice in estrus at these same doses. These results suggest that NT may play a role in uterine contraction during diestrus and, therefore,

mouse reproduction.

DESIGN AND SYNTHESIS OF TRPV1 ANTAGON-ISTS: PROBING THE D-REGION BINDING SITE USING AMIDOALKYL SUBSTITUENTS

Victoria D. Held and David B. Rusterholz (Advisor)

Department of Chemistry

University of Wisconsin–River Falls, River Falls, WI

TRPV1 (transient receptor potential vanilloid subfamily 1) is a membrane-bound ion channel protein that mediates the pain response elicited by capsaicin, resiniferatoxin, and similar drugs. Structure activity studies of compounds that bind to TRPV1 indicate several binding sites, namely: (1) an aromatic "A" region, (2) a polar "B" region, and (3) a hydrophobic "C" region. Recently a fourth region has been proposed based on the structure of resiniferatoxin. This fourth "D" region is thought to be responsible for the high potency exhibited by resiniferatoxin. A new series of potential TRPV1 antagonists was designed that incorporates molecular features intended to interact with the known A, B, and C binding sites. In order to investigate the interaction of the D binding region, amidoalkyl groups were incorporated into the target structures. The synthesis of several of members of the target series was accomplished using a convergent synthetic strategy. Ultimately these compounds will be tested for their effectiveness as TRPV1 antagonists.

THE EFFECTS OF INHIBITION OF SEROTONIN SYNTHESIS ON T CELL PROLIFERATION

Melinda Hexum, Audrey Geiger, Katelyn Ruter, and Dr. Jodi Goldberg (Advisor)

Department of Biology

Hamline University, St. Paul, MN

Previous research has indicated that serotonin (5-hydroxytryptamine, 5HT) is a signal necessary for human T cell proliferation. The role of 5HT in T cell prolifer-ation was investigated by examining the impact of p-chlorophenylalanine (pCPA), a serotonin synthesis inhib-itor, on the proliferation of purified human peripheral blood T cells activated by accessory cell independent (anti-CD3/CD28 beads) and accessory cell dependent (phytohemagglutinin, PHA) methods. T cell proliferation in response to either method of activation was inhibited by pCPA, though T cells activated by PHA and accessory cells were inhibited at significantly lower doses than pure T cells activated with antibody-coated beads. Inhibition of proliferation with pCPA was most effective when added upon initiation of T cell activation, with inhibitory effects progressively reduced when pCPA was added on day 1 or 2 after activation. Attempts were made to reverse the inhibitory effects of pCPA on T cell proliferation by providing pCPA treated cells with exogenous 5HT or 5-hydroxytryptophan (5HTP), but neither restored prolifer-ation at any concentration tested. These data suggest that T cells are inhibited by pCPA, but that non-T cells may be more sensitive to pCPA's effects. It remains to be determined if the inhibition occurs via a serotonin-dependent pathway.

GAS CHROMATOGRAPHY DETECTION OF TERBUFOS

Cori Hollingsworth, Ian MacRae, and Julie E. Larson (Advisor)

Department of Chemistry

Bemidji State University, Bemidji, MN

Terbufos, an active ingredient in granular pesticide (Counter®-BASF), is applied to cultivated soil in Minnesota's Red River Valley to control the sugar beet root maggot population. The sugar beet root maggot is an insect pest of sugar beets and is capable of inflicting serious economic hardship on sugar producers. For the pesticide to affect insect populations, the active ingredient must remain in the top four to five inches of the cultivated soil, also known as the "kill zone." This study assessed the leachability of Counter® via gas chromatographic detection of terbufos in leachate collected from soil cores subjected to varying amounts of simulated rainfall. This work was conducted under the direction of Dr. Ian MacRae, Northwest Research and Outreach Center, University of Minnesota, Crookston.

SEX SELLS: THE ECONOMICS OF PROSTITU-TION

Anna C. Hovde and Fahima Aziz (Advisor)

Department of Management and Economics

Hamline University, St. Paul, MN

Prostitution is illegal in most countries of the world, but the market for paid sexual services still flour-ishes. The United Nations estimates that prostitution is a $7 billion-a-year industry. Prostitution is openly adver-tised in many local newspapers, and street prostitutes can be seen in major cities even where prostitution is illegal. This study examines why the market for sexual services exists and the costs it imposes on society. The supply and demand sides of the U.S. market are explored in depth with economic models, and the implications of the models are discussed based on available empirical data. New data on the wage differential of prostitutes in the Minneapolis–St. Paul area are also presented. Policy options including prohibition, legalization, and regulation are considered in light of the analysis.

EXPERIMENTAL INVESTIGATION OF AN ELEC-TROPHILIC AROMATIC ADDITION REACTION

Michael Huehn and Jack Waas (Advisor)
Department of Chemistry
Bethel University, St. Paul, MN

In 2005, Chi and coworkers reported an unusual electrophilic aromatic addition reaction (Ad_EAr) (Choi, H. Y.; Srisook, E.; Jang, K. S.; Chi, D. Y. *J. Org. Chem.* **2005,** *70,* 1222-1226). The Ad_EAr and electrophilic aromatic substitution (EAS) pathways share a common initial mechanistic step. After one electrophilic aromatic bromination of the usual variety, the addition of a second bromine is thought to proceed with further addition of methanol rather than deprotonation. This addition of methoxy is observed only under basic methanolic condi-tions, and requires either a pyridine moiety or added pyridine. This is contrary to what is normally observed under acidic or neutral conditions which result in EAS only. Our initial results indicate that the report of the

Ad_EAr occurs in the manner reported by Chi and cowork-ers. These results are based on experiments performed on naphthalene and quinoline derivatives. Chi and coworkers reported their experimental results but did not report any computational results. Our preliminary semi-empirical and DFT calculations compare reaction energies of Ad_EAr with those of EAS. Our research will contribute to the advancement of organic chemistry and have possible applications in pharmaceuticals.

SULFONATION OF COMPLEX BIOMOLECULES BY THE BIOCATALYTIC TRANSFER OF SULFINYL DERIVATIVES

Tuere A. Hughes, (Romas J. Kazlauskas), Christopher K. Savile, and Paul F. Mugford

Department of Biochemistry, Molecular Biology, and Biophysics
University of Minnesota, Saint Paul, MN

Sulfate groups on complex biomolecules in nature, such as hormones, sugars, and aminoglycans, play a key role in their physiological function. These sulfate groups are essential in hormone regulation, cellular degradation, blood coagulation, and cell–cell recognition processes such as cell adhesion, developmental cell signaling, bacterial and viral pathogenesis, and tumor metathesis. A major barrier to determining the structure–function relationships of sulfate groups in biology is the great difficulties in the chemical and biochemical syn-theses of complex sulfonated biomolecules and analogs. Chemical methods cannot select among the many groups with similar reactivity in a complex biomolecule, and biosynthetic methods that mimic natural biosynthesis yield only trace amounts of products. We propose to use unnatural reactions coupled with biocatalytic methods to overcome these difficulties. The use of enzyme catalysis will provide the advantage over chemical methods by showing selectivity for specific groups. The use of unnatural reactions and conditions will allow for the production of large amounts of desired products.

Our aim is to identify proteases and sulfinyl group donors for the biocatalytic synthesis of complex biomolecules containing sulfates. Our working hypothesis is that sulfinyl groups are similar enough in structure and chemical reactivity to acyl groups, that proteases that normally transfer acyl groups will also transfer sulfinyl groups. We propose a two-step method involving an initial protease-catalyzed addition of a sulfinyl group, followed by chemical oxidation to a sulfate. We synthe-sized and tested the enzymatic hydrolysis of dibenzyl sulfite and diphenyl sulfite by reacting with six proteases: subtilisin Carlsberg, subtilisin BL, pronase, pepsin, alpha-chymotrypsin, and penicillin acylase.

ECOLOGICAL GENOMICS: ANALYZING GENE-TIC DATA FROM *Marchantia* USING ISSRs AND THE CEQ 8000

Eun Hyuk Chang, Linda Fuselier (Advisor), and Michelle Malott (Advisor)

Biosciences Department

Minnesota State University– Moorhead, Moorhead, MN

Ecological genomics is an emerging field at the interface of ecology, evolutionary biology, and genomics. The application of genetic information such as genome structure, DNA sequence, DNA variation, and gene function is helping researchers better understand the fundamental mechanisms of evolutionary and developmental biology.

This, in turn, contributes to our understanding of the ecology of a variety of organisms.

Most genomic applications involve isolation of DNA, polymerase chain reaction (PCR) amplification of specific regions of DNA, and the analysis of the resulting amplification products in a manner relevant to the question being asked. For example, examining the DNA sequence and determining the amount of genetic diversity of a population of organisms can be helpful in addressing

questions involving reproductive behaviors, evolutionary changes, and overall health of the species. Traditionally, analysis of amplification products is done by gel electrophoresis and visual scoring of gels. This can be a time-laborious process when attempting to analyze many samples (*i.e.,* different individuals) from different sample sites or locations in a statistically meaningful manner. This poster describes the use of a new instrument, the CEQ 8000, at MSUM for examining the genetic diversity of *Marchantia* species.

IS THERE A ROLE FOR Rap1 IN THE MAMMAL-IAN CELL CYCLE?

Andrea Johnson and Jennifer L. Cruise (Advisor)

Department of Biology

University of St. Thomas, St. Paul, MN

The small G-protein Rap1 has been linked to a number of signaling pathways involved in the cell cycle, but its exact role is unknown. Prior research demonstrating that endogenous Rap1 expression is down-regulated prior to the onset of DNA synthesis has led to the hypothesis that Rap1 activity regulates the cell cycle. We are testing the predictions that increasing active Rap1 in cells would decrease DNA synthesis and cell proliferation, and decreasing active Rap1 would increase DNA synthesis and cell proliferation, using mammalian MRC-5 (fibroblast) and MDCK (epithelial) cells. These cells were transfected transiently or stably with either an activated Rap1 gene, a control plasmid, or a dominant-negative Rap1 gene. The cell cycle was then monitored through flow cytometry, BrdU labeling index assays, and cell counts using a hemacytometer. Flow cytometry experiments and BrDU labeling studies thus far have suggested that active Rap1 reduces baseline DNA synthesis and response to growth factor stimulation. Dominant-negative Rap1 appears, at least to some extent, to have the opposite effect, increasing baseline DNA synthesis and response to growth factors. In addition, cell counts in MDCK stable cell lines reveal a higher overall proliferation rate in cells expressing dominant-negative Rap1, and a lower rate in cells expressing increased active Rap1, when compared with controls. These preliminary data support our hypothesis that Rap1 has a regulatory effect on the mammalian cell cycle, but further, more specific experimentation is necessary to confirm our results and identify the cell signaling components that may explain this pattern of regulation.

THE ROLE OF OXIDATIVE STRESS IN MATER-NAL mtDNA INHERITANCE

Frank L. Johnson Jr. and Dr. Ellen Brisch (Advisor)

Department of Biosciences

Minnesota State University Moorhead, Moorhead, MN

The purpose of this investigation is to determine if, and to what extent, oxidative stress plays a role in maternal mtDNA inheritance. Upon fertilization, the paternal mitochondria are tagged with ubiquitin and degraded by proteolytic enzymes; therefore the paternal mtDNA is not incorporated into the zygote. Free radical oxygen (O_2^-) is a known byproduct of oxidative phosphorylation and is also known to destroy biological tissues by the mechanisms of oxidation. The methods employed in this investigation are used to determine the extent of oxidative damage to paternal mtDNA. This may offer evidence as to why paternal mtDNA is not inherited.

AN EXAMINATION OF ROCK PHOSPHORY-LATION OF THE NHE1 CARBOXYL TERMINUS

Jessica F. Johnson, Jason T. Grosam, Diane L. Nelson, Mark A. Wallert (Advisor), and Joseph J. Provost (Advisor)

Department of Biosciences

Minnesota State University–Moorhead, Moorhead, MN

The sodium hydrogen exchanger isoform one (NHE1) is key in the regulation of intracellular pH, cell volume, and cell motility in nearly all eukaryotic cells. Regulation of NHE1 activity occurs primarily through post-translational modification of the cytoplasmic carboxyl-terminal tail.

Rho-Associated Kinase (ROCK) is a serine/ threonine kinase involved in upstream signaling pathways that regulate NHE1 activity. We propose that ROCK directly phosphorylates NHE1 in an α1-adrenergic receptor-dependent fashion. This will be demonstrated through the use of an NHE1 carboxyl–terminal tail fusion protein, CTNHE1. Phosphorylation of CTNHE1 via a ROCK dependent pathway will first be examined using cell lysates after stimulation with phenylephrine, a specific α1-adrenergic receptor agonist.

Control and agonist-stimulated lysates will be combined with purified CTNHE1 in an *in vitro* kinase assay. Phosphorylation of CTNHE1 will be assessed by Western blotting using phosphoserine/threonine anti-bodies. To show direct phosphorylation of CTNHE1 by ROCK, purified forms of ROCK will be combined *in vitro* with purified CTNHE1 and analyzed by Western blot. Subsequently, specific ROCK phosphorylation site(s) of NHE1 will be predicted based on known ROCK substrate sequences. Ser/Thr-to-Ala mutations of the CTNHE1 construct will be prepared and analyzed for phosphorylation by ROCK. We expect the net results to show that ROCK directly phosphorylates CTNHE1 in an α1-adrenergic receptor response. Consequently our results could enable greater understanding of disease states in which NHE1 activity is altered, such as tumor formation and migration. This work was supported from a grant from the NIH, award number 1 R15 HL074924-01A1.

SURFACTANT PROTEIN B AND SURFACTANT PROTEIN C CHARACTERIZATION AND EXPRESSION IN *Xenopus laevis*

Natalie S. Johnson and Brian Hyatt (Advisor)

Department of Biological Sciences

Bethel University, St. Paul, MN

Surfactant Protein B (SP-B) and Surfactant Protein C (SP-C) are molecules expressed exclusively in lung tissue. They are expressed early in lung development and continue to be expressed in the adult lung. Previously these two genes had been sequenced only in mammals. The internal gestation of mammals has made it difficult to research the early development of lungs, however. *Xenopus laevis* was therefore examined as a possible model for allowing easier access to, and manipulation of, the embryo during lung development. Sequence analysis of the coding regions of *Xenopus* SP-C and SP-B determined that they have high homology with the human and mouse gene sequences. RT-PCR and *in situ* hybridi-zation techniques showed that the expression of these genes in *Xenopus laevis* was located exclusively in the lung tissue and was also seen in the early stages of lung development. The expression patterns of SP-C and SP-B in *Xenopus laevis* are consistent with those seen in mammalian subjects. In light of these similarities it is believed that *Xenopus laevis* would be a good model for further study of SP-B and SP-C and may also be a good subject for further studies on lung development.

TOXIC AND MUTAGENIC EFFECTS OF ARNICA MONTANA ON BACTERIA, FUNGI & HUMAN LYMPHOCYTE CULTURE

Nate Johnson and Gregg Johnson (Advisor)

Department of Biological Sciences

Bethel University, St. Paul, MN

Arnica (*Arnica Montana)* is an herb of the asteraceae family, that grows in the Rocky Mountains. It has been part of Native American herbal medicine for years as a topical treatment for muscle soreness, as an antibacterial mouthwash and application to wounds. It has also been used as a cardio-stimulant, in homeopathic formulation. It has been reported to have some potential as a cytotoxic chemotherapeutic agent. Its active ingredients are sesquiterpene lactones such as helenalin, which reduce inflammation.

It was the purpose of this investigation to test the toxic effects of Arnica on gram – and + bacteria (Serratia marcescens, and Saracina lutea) and on representative fungi (asperigillus, and penicillium). We also tested the genotoxic effect of Arnica on cultured human lymphocytes using the sister chromatid exchange (SCE) technique as well as counts of micronuclei, and bridges as measures of DNA damage. Our results indicate that Arnica does not possess antibacterial or antifungal properties. It does appear that there is significant increase in SCE, micronuclei, and DNA bridge formation and depressed mitotic index in cultured human lymphocytes treated with Arnica.

EFFECTS OF Rap 1 ON CELLULAR MIGRATION AND ADHESION

Daniel Kolar and Jennifer L. Cruise (Advisor)

Department of Biology

University of St. Thomas, St. Paul, MN

Cellular migration is involved in a number of events including wound healing and development. Rap 1 is a small GTPase that has been implicated in cell migration and adhesion. Rap is known to effect E-cad-herins and integrin-matrix interactions, which influence cell's attachment to each other and to surfaces, respect-ively.

Wound assays can be used to observe the rate of migration in monolayers of cells. In these assays, clearing cells from a region of a culture plate is followed by photomicroscopic monitoring of migration of cells into the wounded area. The rate, density, and pattern of migra-tion can be analyzed in photos over time. We examined the effect of Rap 1 on the migration response in three stably transfected MDCK epithelial cell lines. One expressed an activated Rap1 gene and one a dominant-negative form of Rap1; a control line was stably trans-fected with an empty vector plasmid. Two clones of each cell type were examined. We found migration to be significantly impaired by activated Rap1, compared with controls. This suggests that Rap1 inhibits migration. We also characterized the effect of Rap 1 on the expression of integrin-matrix interactions, using adhesion assays. Ad-hesion to three different coatings: fibronectin, coll-agen, and BSA (control), were tested. Cells expressing active Rap1 adhered more strongly to collagen than did controls.

We have found that active Rap 1 decreases mi-gration and increases adhesion. The relationship between the two processes and the mechanism(s) of Rap1's effect requires further research.

PHOTOLYSIS OF NORFLOXACIN UNDER ENVI-RONMENTALLY RELEVANT CONDITIONS

Andrew Korte and Kristine Wammer (Advisor)

Department of Chemistry

University of St. Thomas, St. Paul, MN

Norfloxacin, a member of the fluoroquinolone class of antibiotics, was examined to predict its photo-chemical fate in natural waters. Contamination poses a threat to humans as low-level environmental presence may give rise to bacterial resistance. Additionally, the products of photolysis may also have adverse effects. Samples of a norfloxacin solution were photolyzed using borosilicate-filtered Hg-vapor lamps and analyzed by HPLC to determine concentrations at set time intervals. Rates for photodegradation in deionized water at a range of pH values were compared with those in 0.2 micron-filtered water obtained from Lake Josephine in St. Paul (which contains dissolved organic matter, or DOM).

Photodegradation rates of norfloxacin were rapid in both deionized water and Lake Josephine water but varied significantly with pH. Rates in the water from Lake Josephine were found to be moderately lower than those found in deionized water. This indicates that degradation rates are not likely to be enhanced by indirect photolysis involving DOM and that laboratory studies using deion-ized water will be useful for predicting environmental half-lives. Future work will focus on photolysis product analysis through mass spectrometry and nuclear magnetic resonance.

ANALYSIS OF VOLATILE COMPOUNDS OF DIAGNOSTIC INTEREST USING A MICRO-DIALYSIS PROBE EXTRACTION TECHNIQUE

Ashley Kramer and Dr. Anthony Borgerding (Advisor)

Department of Chemistry

University of St. Thomas, St. Paul, MN

Microdialysis has become useful in sampling non-volatile compounds in biochemical systems such as different places in the human body. Our lab has had success using microdialysis sampling probes for volatile compound extraction from aqueous solutions into the gas phase. Experiments with breath analysis in the biomed-ical field currently use a breathing apparatus as a diag-nostic tool, to measure volatile compounds in the body such as acetone, formaldehyde and dimethyl sulfide. Our research lab is currently testing our probes on these com-pounds for the long-term goal of using microdialysis as an in-vivo method. We have analyzed these compounds over a variety of concentrations. Acetone, for example, was measured over a concentration range of 0.001-0.5% acetone by weight.

USE OF CORNMEAL FOR ALGAE REMEDIA-TION IN URBAN LAKES

Scott Kronholm and Bryan Anderson (Advisor)

Department of Biological Sciences

Bethel University, St. Paul, MN

In nature, algae levels in lakes and streams are usually found in relatively low numbers due to limiting factors such as nutrient availability. Due to the use of products such as lawn care and industrial fertilizers, which contain high amounts of nitrates and phosphates, there can sometimes be a surplus of the normally limiting nutrients. Algal blooms (large growths of algae) are typically found in metro area lakes and streams due to high nutrient levels. Not only are blooms unpleasant to look at, smell, or swim around, they can be toxic as well. Certain genera of algae produce toxins which, if in sufficient quantity, can make those who drink the water ill and have been known to be fatal in some cases. It has been shown that the use of cornmeal is an effective and relatively inexpensive way to reduce numbers of certain genera of algae. In this research I used four genera: *Anabaena, Oscillatoria, Pediastrum,* and *Spyrogyra.* The process by which cornmeal reduces algal growth is not currently well understood; however, some theories are currently being tested. This research deals with the application of cornmeal rather than the process by which cornmeal effects algae.

COMPUTATIONAL STUDIES OF THE ATMO-SPHERIC IMPACT OF CYCLOALKENE OZO-NOLYSIS

Brianna Kujala and Keith Kuwata (Advisor)

Department of Chemistry

Macalester College, St. Paul, MN 55105

The hydroxyl radical is the most important oxidi-zing agent in the atmosphere. It is responsible for oxidi-zing hydrocarbons, the deposition of acid onto Earth's surface, and the production of peroxyl radicals. In the absence of light, the hydroxyl and peroxyl radicals are produced via non-photolysis methods, most importantly through the ozonolysis of alkenes in the atmosphere. Furthermore, because the time period over which a single hydroxyl radical is present in the atmosphere is so small, computational studies are needed to better determine the amounts of hydroxyl radical being produced in our atmosphere and the rates at which it is being produced. This study looks at the ozonolysis of cyclopropene, a model cycloalkene compound. Through computational studies involving density functional theory and other methods, this work attempts to completely characterize the reaction pathways involved in this mechanism with respect to their rates and overall reaction yields. Reaction energies, and energies of activation, for formation of the exo and endo primary ozonides, for their cycloreversion into anti and syn carbonyl oxides, and for their isomeri-zation into dioxiranes and hydroperoxides have all been determined. Preliminary results, for the exo cylcoaddition transition state as the entrance channel, give fractional yields of 0.6817 for the dioxiranes and 0.3175 for the hydroperoxides. Yields for the endo cycloaddition tran-sition state as the entrance channel are 0.6633 for the dioxiranes and 0.3346 for the hydroperoxides. Yields for formation of the secondary ozonide are negligible.

LEWIS ACID/LEWIS BASE INTERACTIONS IN HALOGEN/CYANO-BENZYLIDENEANILINES: A COMPARSON WITH SOME METHYL ANALOGUES

Marika K. Kuspa and William H. Ojala (Advisor)

Department of Chemistry

University of St. Thomas, St. Paul, MN

The object of this study has been to determine whether the two isomeric benzylideneanilines bearing a methyl group in one *para*-position and a nitrile group in the other assume identical molecular packing arrangements in the solid state (are isostructural with each other) and whether this solid-state similarity extends to any of the corresponding halogen/nitrile benzylide-neanilines. A previous study showed that the isomeric *p*-cyano-*p'*-iodobenzylideneanilines pack similarly (but not identically) due to intermolecular Lewis acid–base interactions between the halogen atom and the nitrile group. These interactions are absent from the bromo/ nitrile and chloro/nitrile analogues, which do not form isostructural crystals. We have found that *p*-cyano-*N*-(*p*-

methylbenzylidene)aniline and its "bridge-flipped" isomer *p*-methyl-*N*-(*p*-cyanobenzylidene)aniline do not pack similarly to the halogenated compounds, but they do pack rather similarly (but not identically) to each other. The two crystal structures have similar unit cell volumes and have similar cell axial lengths and interaxial angles, but as in the case of the iodo/nitrile benzylideneanilines, the packing arrangement differs at the three-dimensional level of molecular stacking. The presence of intermolecular Lewis acid–base interactions in the solid state does not guarantee isostructuralism for these isomers; at the same time, the similarity between the methyl/cyano benzylide-neaniline crystal structures at least suggests that the absence of these interactions does not preclude it.

AQUATIC INVERTEBRATE BIOTIC INDEXING OF THE STRAIGHT RIVER

Tyler Kutscheid and Jeff Port (Advisor)

Department of Biology

Bethel University, St. Paul, MN

Aquatic bio-monitoring is becoming an impor-tant tool for both citizens and conservationists alike due to its ability to detect long-term water conditions and its relative low cost. Most biotic indexes tend to make prefer-ential use of the orders Ephemeroptera, Plecoptera, and Trichoptera. However, most Midwestern biotic index values have been developed specifically for Wisconsin streams. In order to develop values specific for Minnesota coldwater streams, better understanding of the inverte-brate fauna native to this region is needed. Over the spring of 2004 a survey of Ephemeroptera, Plecoptera, and Trichoptera was conducted on the Straight River near Osage, Minnesota. Utilizing both qualitative and quantita-tive techniques, 39 genera from 23 families were identi-fied: 13 Ephemeroptera, 20 Trichoptera, and 6 Plecoptera. In addition, several genera rare to Minnesota streams were identified, including *Grammotaulius, Pteronarcys,* and *Ephemerella.* The Straight appears to support large numbers of intolerant taxa including 13 families with a tolerance value of 2 or less. Of particular concern within the Straight is the increase in water temperatures. Many of the thermally intolerant genera such as *Pteronarcys* and *Ephemerella* could and should be used to evaluate the effects of these changes.

MICRODIALYSIS SAMPLING FOR THE ANAL-YSIS OF NITRIC OXIDE

Christine C. L'Abbe and Anthony Borgerding (Advisor)

Department of Chemistry

University of St. Thomas, St. Paul, MN

The purpose of this research project is to design a noninvasive method of rapidly measuring nitric oxide in the brain. This method allows for the study of the nature of nitric oxide and its role in critical physiological func-tions and neurological diseases. Microdialysis probes are used in combination with a chemiluminescence detector to analyze nitric oxide samples at several dilute concen-trations in order to determine the limits and efficiency of the microdialysis measurement system.

CHARACTERIZATION OF ANDROGEN REGULA-TION OF ZEB-1 AND PSA IN 22Rz1 IN PROSTATE CANCER CELLS

Lisa LaGoo and Bynthia Anose (Advisor)

Department of Chemistry

Bethel University, St. Paul, MN

Human prostatic carcinoma accounts for 30% of cancer cases diagnosed in men. According to a February 2006 report of the American Cancer Society, the five-year survival rate for a patient with localized prostate cancer (PCa) was 100%, but once the cancer had metastasized, the survival rate dropped to 34%. This significant decline in prognosis with the onset of metastasis has highlighted the need for identifying metastatic biomarkers in addition to currently utilized cancer transformation biomarkers. The commonly assayed-for prostate specific antigen (PSA), while touted as a transformation biomarker, has recently proven to be problematic in the area of false positive diagnoses. It remains, however, a hallmark gene for studying androgen regulation, as its expression is reliably simulated by androgens such as dihydrotestoster-one (DHT).

The goal of this project is to investigate the effects of flutamide (an anti-androgen) and DHT on the expression of PSA and zinc finger E-box binding protein (ZEB-1). Previous research has identified ZEB-1 as a possible biomarker for the onset of metastasis in PCa. The gene has been shown to be androgen-regulated, and its expression decreases sharply at metastasis. This study will confirm the feasibility of using ZEB-1 as a PCa metastatic biomarker using the highly sensitive technique of real-time polymerase chain reaction. The effects of 1 and 10 nM flutamide, in combination with 1 and 10 nM DHT, on expression of ZEB-1 and PSA will be studied in 22Rv1, an androgen-responsive human prostate carcinoma cell line.

PREPARATION AND CHARACTERIZATION OF SYMMETRICALLY SUBSTITUTED TRIOLS AS NANOSCALE BUILDING BLOCKS IN INORGA-NIC–ORGANIC HYBRID MATERIALS

Brian A. Loy and Wade A. Neiwert (Advisor)

Department of Chemistry

Bethel University, St. Paul, MN

This research project aimed at preparing and characterizing various triol-substituted organic com-pounds of the general form $RC(CH_2OH)_3$ (where R can be various organic groups). These triols are able to covalent-ly bind to redox active vanadium-oxo clusters via self-assembly reactions. If “R” is able to support multiple triols (such as R = benzene), then multiple vanadium complexes can be bridged by these organic linkers, resulting in linear chains (with two triols) or two-dimen-sional arrays (with three triols). Recent efforts have fo-cused on the preparation of a tris-triol species with for-mula $1,3,5–C_6H_3(C{\equiv}CC_6H_4CONH(CH_2OH)_3)_3$. FT-NMR and FT-IR spectroscopy have been used to characterize the compound and the isolated reaction intermediates, with hopes of preparing an X-ray quality crystal of the triol for diffraction studies.

IDENTIFICATION OF WOUND RESPONSIVE GENES IN *Avena sativa* SEEDLINGS USING AFLP FINGERPRINTING

Kathleen Lynch and Debra Martin (Advisor)

Department of Biology

Saint Mary's University of Minnesota, Winona, MN

All plants are faced with the dilemma that they must compete to survive and propagate while being immobile. To protect themselves from competitors on the surface, plants have a coating of cutin and subrin. How-ever, if a wound occurs on the surface, the plant becomes susceptible to injurious invaders. When injured, plants produce special proteins that assist in repair and protect-tion against disease. Wound-induced proteins are believed to be involved in such plant defense mechanisms. The objective of this research project was to identify potential wound responsive genes from oat (*Avena sativa*). To begin, mRNA was isolated from wounded oat leaves. The cDNA was amplified by PCR. Using fluorescently labeled primers, the cDNA was re-amplified to generate AFLP fragments to be analyzed on the LiCor Gene Analyzer.

THE ROLE OF UROKINASE PLASMINOGEN ACTIVATOR IN THE REGULATION OF ERK, STRESS FIBER FORMATION, AND NHE IN CCL39 FIBROBLASTS

Lisa Magstadt, Joe Provost (Advisor), and Mark Wallert (Advisor)

Department of Biosciences

Minnesota State University–Moorhead, Moorhead, MN

Cell migration requires control of several signal-ing mechanisms including reorganization of the actin cytoskeleton and adhesion to the extracellular matrix. Urokinase plasminogen activator (uPA) is a thrombolytic agent that possesses a role both dependent and indepen-dent of binding to its receptor, uPAR. Receptor activation of uPAR localizes proteolytic activity to the leading edge of cellular migration and facilitates cellular penetration of tissue boundaries.

Expression of both uPA and uPAR correlates with invasive cancer cell phenotype; however, the mecha-nism by which uPAR transduces its signals to regulate cell migration remains largely uncharacterized. Our focus is to investigate the signaling of uPA in CCL39 fibro-blasts to determine a role for NHE in cytoskeletal remod-eling and cell migratory events. ERK activation by uPA stimulation has been shown in a few cell lines.

Smooth muscle cell inhibition of the sodium hydrogen exchanger (NHE) reduces cell proliferation and migration caused by uPA. Here we report that both the amino terminal fragment and recombinant uPA stimulate ERK phosphorylation in a bimodal fashion. The early peak of activity was observed within 5 minutes and a later chronic stimulation of ERK was seen after 190 minutes. Both forms of uPA induced the formation of stress fibers in CCL39 fibroblasts and the amino terminal fragment of uPA induced over a two-fold increase in NHE transport. These findings identify a potential new signaling role for uPA and suggest an important role for NHE in cell migra-tion and invasion. This work was supported by a grant from the NIH, Award number 1 R15 HL074924-01A1.

DETERMINING THE ELECTRONIC STRUCTURE OF TaO USING LASER SPECTROSCOPY

Kara Manke, Tyson Vervoort, and Thomas D. Varberg (Advisor)

Department of Chemistry

Macalester College, St. Paul, MN

We have recorded hyperfine resolved laser excitation spectra of the $B^2\Phi_{5/2} - X_1^2\Delta_{3/2}$ and $C^2\Delta_{3/2} - X_1^2\Delta_{3/2}$ electronic transitions of TaO. The electronic spectrum of this molecule was first explored by Cheetam and Barrow using a grating spectrograph. A Ti:sapphire ring laser was used to analyze TaO molecules that were produced with a hollow cathode discharge. We achieved hyperfine resolution using the sub-Doppler technique of inter-modulated florescence spectroscopy. A least-squares fit of the transition frequencies was used to determine improved values for the rotational parameters and values for the magnetic dipole and electric quadrupole para-meters of these states.

CREATION OF A CDC 28 "KNOCKOUT" (BY GENERATING A TEMPERATURE-SENSITIVE LOSS OF FUNCTION) MUTANT TO OBSERVE MITOCHONDRIAL INHERITANCE IN *Saccharomyces cerevisae*

Kyle Marthaller and Ellen Brisch (Advisor)

Department of Biosciences

Minnesota State University–Moorhead, Moorhead, MN

In *Saccharomyces cerevisae,* the inheritance of mitochondria from mother cell to daughter bud during cell division is an essential feature of yeast growth. The anal-ysis of mutants defective in mitochondrial morphology and inheritance has led to the identification of a number of proteins that control mitochondrial inheritance. This experiment focuses on a certain gene, CDC 28, that en-codes a protein that drives the cell through mitosis. Using PCR, a knockout construct was generated. This construct was then isolated via gel electrophoresis and successfully purified. This construct will be transformed into yeast cells and through the use of a copper-induced promoter be selectively activated. Through mitochondrial staining, the role of CDC 28 in mitochondrial inheritance will be observed.

DAY IN THE LIFE OF *Daphnia:* **AN INTENSIVE ACOUSTIC STUDY ASSESSING THE PATCHI-NESS OF ZOOPLANKTON**

Rachel J. McAlpine and Leif Hembre (Advisor)

Department of Biology

Hamline University, St. Paul, MN

Advances in technology using high frequency sonar have allowed researchers to visualize patches of zooplankton in large geographical areas. Sonar may be used to assess the relative importance of abiotic versus biotic factors that influence zooplankton patchiness. In this study sonar was used to determine the spatial distrib-ution of *Daphnia* in Square Lake (Washington Co. MN) over a 24-hr period. Net samples were used to calibrate sonar data and also were used to determine if any taxo-nomic or morphological trends were present in the Square Lake zooplankton community. A strong backscattering layer composed primarily of *Daphnia* existed between 10 and 12 m throughout the daytime sampling periods. Anal-ysis of the habitat requirements of *Daphnia's* two main predators in Square Lake, rainbow trout and *Chaoborus*, along with environmental data about the stratification of the lake, suggests that this thin layer between 10 and 12 m is a refuge for *Daphnia* against predation. However, dur-ing the night time there is strong evidence suggesting *Daphnia* adopt morphological strategies to avoid predation.

PREPARATION AND CHARACTERIZATION OF ZINC(II)-SUBSTITUTED POLYOXOMETALATES AS NANOSCALE BUILDING BLOCKS FOR INORGANIC–ORGANIC HYBIRD MATERIALS

Elisabeth Melander and Wade A. Neiwert (Advisor)

Department of Chemistry

Bethel University, St. Paul, MN

The goal of this project is the synthesis and characterization of zinc(II)-substituted A-type Keggin sandwich polyoxometalate (POM) complexes of the general form $[(Zn(H_2O)_2)_x(WO)_{3-x}(PW_9O_{34})_2]^{-(6+2x)}$ (x = 1, 2, or 3). The trivacant $[PW_9O_{34}]^{9-}$ anion was prepared and characterized according to literature methods. Treatment of an aqueous solution of the POM with Zn^{2+} affords the trisubstituted sandwich $[(Zn(H_2O)_2)_3\ (PW_9O_{34})_2]^{12-}$. Methods to substitute a W(VI) cation in place of the Zn(II) were investigated, and isolated products were characterized by FT-IR and NMR spectro-scopy. In addition to the interesting catalytic properties POMs possess, these sandwich-type complexes have the potential to serve as building blocks for inorganic–organic hybrid materials through coordination of ligands to the sandwiched metal cations. The mono- and di-substituted complexes will limit the polymerization and therefore serve as structural models.

SYNTHESIS AND GENETIC ANALYSIS: SMALL MOLECULE-INDUCED PERTURBATION OF AUXIN SIGNALING IN *Arabidopsis thaliana*

Sarah B. Miller[1], Johann S. Bergholz V.[1, 2], Paul J. Overvoorde[1] (Advisor), and Ronald Brisbois[2] (Advisor)

[1]*Department of Biology*

[2]*Department of Chemistry*

Macalester College, St. Paul, MN

Auxin is a plant hormone that is essential for numerous processes in plant development. However, many of the mechanisms by which this hormone modu-lates such processes remain unclear. Chemical genetics provides a novel means by which to investigate the auxin response pathway in *Arabidopsis thaliana*. A previously conducted high-throughput screen of 10,000 small molecules yielded four compounds that strongly inhibited auxin signaling. Here, we have synthesized various analogs of one of these inhibitory molecules, Compound A. Through quantitative real-time PCR and GUS assays, we aim to assess the inhibitory capabilities of each Compound A analog. This information will provide insight as to the active core moiety of Compound A, which will ultimately aid us in devising a scheme for purification of the protein target of this inhibitor.

SYNTHESIS OF TITANIUM(IV) TRISPHENOLATE CATALYSTS IN RING-OPENING POLYMERIZA-TION

William Mitchell, Sarah Russell, and Gretchen Hofmeister (Advisor)

Department of Chemistry

Carleton College, Northfield, MN

The ring-opening polymerization of lactide is of great interest because a variety of plastics can be made using an annually renewable feedstock. Catalysts with high stereoselectivity are desired to create polymers with commercial application. Although our titanium trisphen-olate catalyst has good stereoselectivity and excellent molecular weight control (PDI < 1.10), the mechanism leading to stereocontrol is unclear. To better understand this system and improve catalyst design, we have been investigating the kinetics of this reaction. Preliminary results are presented, including the dependence of this reaction on lactide. An induction period is observed be-fore polymerization begins, presumably due to the forma-tion of the active catalytic species. Further work will focus around investigating the induction period and determining the dependence of the reaction on titanium.

THE USE OF DNA MICROSATELLITES TO ASS-ESS REPRODUCTIVE BEHAVIOR IN FATHEAD MINNOWS (*Pimephales promelas*)

Jill Moes, Laura Matzke, Rebecca Lande, and Michelle Malott (Advisor)

Biosciences Department

Minnesota State University– Moorhead, Moorhead, MN

The fathead minnow (*Pimephales promelas*) is a freshwater fish with a wide geographic distribution. We are specifically interested in the fish population found in Budd Lake in Itasca State Park, Minnesota. By using a molecular approach to study genetic variation in the population, we are able to further investigate the reproductive behavior seen in fathead minnows. We are using Polymerase Chain Reaction (PCR) to examine specific regions of the DNA called microsatellites. This is allowing us to develop genetic "fingerprints" for these minnows. We are currently researching the levels of genetic diversity with various primers, attempting to further our knowledge of the levels of the genetic variation in this population of fathead minnows. We will also show a comparison of data from gel electrophoresis with the capillary electrophoresis analysis.

THE EFFECTS OF GNC MELATONIN ON THE HUMORAL IMMUNE RESPONSE IN FEMALE CD-1 MICE

Ashley M. Munson and Jeanne Minnerath (Advisor)

Department of Biology

Saint Mary's University of Minnesota, Winona, MN

Melatonin, which is a natural neurotransmitter, is a derivative of serotonin and is released by the pineal gland. Recently, researchers have begun to discover that melatonin has many functions within the body. Among those functions, one is to enhance an immune response. Previous research completed at Saint Mary's University has shown that administration of pure, chemically defined melatonin elicits an increased humoral immune response in mice. However, it is unclear whether or not melatonin capsules

that are commercially available to the consumer have the same effect. This research project examined the effects of General Nutrition Center (GNC) melatonin on antibody production in female CD-1 mice. To complete the study, 30 mice were split into three groups and fed peanut butter with or without melatonin (GNC melatonin or chemically defined melatonin) for nine weeks. Three weeks into the project, the mice were immunized with the foreign proteins, cow cytochrome *c* and ovalbumin, to elicit an immune response. At three different times during the experiment, blood samples were collected and ELISAs were completed to determine whether or not melatonin caused an increase in antibody titers. Results indicate that mice treated with either GNC melatonin or chemically defined melatonin did exhibit an increased antibody titer in comparison with control mice. However, this increase was not statistically significant. These results suggest that commercially available as well as chemically defined melatonin may help enhance the humoral immune response in mice.

NEST PREDATION AND HUMAN INFLUENCE ON THE OLIVE RIDLEY SEA TURTLE (*Lepidochelys olivacea*) **POPULATIONS AT OSTIONAL NATION-AL WILDLIFE REFUGE, GUANACASTE, COSTA RICA**

Marla Nelson and Kathleen L. Shea (Advisor)

Department of Biology

Saint Olaf College, Northfield, MN

Nest predation on the olive ridley sea turtle (*Lepidochelys olivacea*) was investigated at Ostional and Nosara beaches of Ostional National Wildlife Refuge in Costa Rica during January 2006. Predation rates and species composition were compared among 4 regions of beach with different degrees of human disturbance, as well as prior to and following the massive nesting phenomenon ("*arribada*") by female turtles. Dogs, black vultures, and human poachers were the chief predators of nests at Ostional. Nest predation by dogs was significantly higher ($P = .0397$) prior to the *arribada*, while human poaching was significantly higher ($P = .0036$) during the *arribada* of mature female turtles. Mean nest predation by vultures varied significantly between the 4 regions of beach ($P = .0002$) but not in relation to the *arribada* activity. Interviews with various human interest groups invested in the refuge revealed tension and power struggles among the people. In order to ensure the welfare of olive ridley populations at Ostional National Wildlife Refuge, future conservation initiatives must include improved communication and inclusion of community members surrounding Ostional Refuge.

MAP kinase phosphatase-3 Regulation in Colorectal Carcinoma

Ngozika Okoye, Marion Zillhardt, Nicholette Zeliadt, Dr. Elizabeth Wattenberg, and Dr. Laura Mauro (Advisor)

Departments of Animal Science and Environmental Health Sciences

University of Minnesota, Twin Cities, MN

Colon cancer is the third most common type of cancer affecting both men and women in the United States and has claimed the lives of an estimated 56,000 people in 2005 alone. The progression of colorectal cancer is due to accumulation of epigenetic and genetic alterations that often include the mutagenesis of the K-ras oncogene. This mutation causes constitutive activation of K-ras and downstream Ras/Raf/MAPK pathway leading to abnormal cell growth. We are interested in how these cells control this pathway to prevent cancer progression. The goals of out studies are: (1) to determine if a negative regulator of the MAPK pathway known as MAP kinase phosphatase-3 (MKP-3) is expressed in colon adenocarcinoma cells; and (2) if expressed, to determine if the expression of MKP-3 is modulated to control the constitutive activation of this pathway. Colon adenocarcinoma cells were treated with mitogens and analyzed to determine MKP-3 mRNA and protein expression and the phosphorylation (pERK) and activation of ERK kinase. High MKP-3 expression is observed in these cells and is uniquely regulated in relation to activated

pERK levels following mitogenic stimulation of the pathway. To verify whether MKP3 is modulating ERK activation, future studies will be conducted to "silence" MKP3 expression and observe how knocking this gene out will affect regulation of the MAPK pathway.

LONG-TERM OUTCOMES OF PATIENTS WITH MITRAL REGURGITATION UNDERGOING PER-CUTANEOUS CORONARY INTERVENTION

Catherine Pastorius[1], (Advisor Lin Aanonsen[1]), Paul Sanders[2], Timothy Henry[2], Denise Windenberg[2], Jessica Wilson[2], Howard Liang[2], Tom Knickelbine[2], and Kevin M. Harris[2]

[1]*Department of Biology*

Macalester College, St. Paul, MN

[2]*Minneapolis Heart Institute, Minneapolis, MN*

Mitral regurgitation (MR) is abnormal leaking of blood from the left ventricle to the left atrium of the heart through the mitral valve. The most appropriate treatment for patients with MR is often debated. Evidence suggests that MR has prognostic importance in patients undergoing coronary artery bypass surgery. Long-term outcome of those undergoing percutaneous coronary intervention (PCI) is less well defined. We evaluated 711 patients who underwent PCI at our institution in the year 2000 and had qualitative assessment of MR by left ventriculography and/or echocardiography. Perioperative death was recorded and mortality was determined by social security death index. MR severity was divided into three strata: none (n=420, 59%), mild (n=209, 29%), and moderate to severe (n=82, 12%).

Patients with progressively more severe MR were older, more frequently female, with lower left ven-tricular ejection fraction (EF) of 45±14.2, higher inci-dence of previous myocardial infarctions, and higher creatinine (all $p<0.003$). Patients with ischemic MR undergoing PCI have significantly decreased survival rates over 30 days, 1 year, and 5 years, and MR is an important predictor of survival. Given a 5-year survival of only 58%, further study will need to evaluate whether concomitant percutaneous valve repair or coronary artery bypass with repair would improve outcome in patients with moderate to severe MR requiring revascularization.

MEASURING ADVENTITIOUS ROOTING: AN ASSAY TO DETERMINE INDIVIDUAL FUNCTION AMONG THE Aux/TAA GENES

Ben Pederson, Can Sungur, Amelia Mueller, Sam Nalle, and Paul Overvoorde

Department of Biology

Macalester College, St. Paul, MN

The current picture of the spatial and functional relationships between each of the 29 Aux/IAA genes in *Arabidopsis* is incomplete. Together with the ARF gene family, they are involved in auxin-mediated changes in gene expression. Since the Aux/IAA proteins are able to homo- and heterodimerize, the number of combinatorial interactions is large, and offers a possible molecular mechanism to explain developmental events mediated by auxin. One such event is the formation of adventitious roots, which can originate in a variety of tissue locations from clusters of mature cells that renew their cell division activity and develop into a root apical meristem in a sim-ilar fashion as lateral roots. The ability of *Arabidopsis* to form adventitious roots provides it a flexible

way to re-spond to environmental changes or injury and is a quant-ifiable trait that reflects auxin metabolism.

In order to piece together the relationship between the Aux/IAA genes in this auxin-mediated event, and to investigate the potential role of ethylene in the gene-expression pathways governed by auxin, we deve-loped an assay to assess differential capacities of single, double, and triple knockout mutants of the Aux/IAA gene family to form adventitious roots. Furthermore, the application of exogenous auxins allowed us to amplify the formation of adventitious rooting, allowing us to better quantifiably analyze the extent to which each of the Aux/ IAA genes tested was responsible for the formation of adventitious roots. We determined that the triple knockout Aux/IAA (i5i6i19) mutant had an increase in adventitious root formation when compared with single knockout mutants of the same genes. This demonstrates that the IAA proteins translated by i5, i6, and i19 overlap in function, working together to dampen the influence of both endo- and exogenous auxins in adventitious rooting. Moreover, the application of ethylene appears to increase the number of adventitious rooting in most genotypes. These data were further supported by the lack of adven-titious rooting within the ethylene-insensitive mutants etr, ctr and ein.

EXPLORING DINOSAUR PALEOECOLOGY OF A VERTEBRATE MICROFOSSIL ASSEMBLAGE IN THE LATE CRETACEOUS LANCE FORMATION, WYOMING

Walter S. Persons and Raymond R. Rogers (Advisor)

Geology Department

Macalester College, St. Paul, MN

Here I attempted a paleoecological reconstruct-tion using data collected from a vertebrate microfossil assemblage found in the Lance Formation near Glen Rock, Wyoming. Data obtained by counting and meas-uring a large collection (n = 750) of garfish scales from the site suggest that fossils were largely unsorted by hydraulics, which makes it reasonable to assume that paleoecology inferences can be drawn. While hadrosaurs are generally viewed as the most common herbivores of the Late Cretaceous, the herbivorous fauna of the exam-ined site was instead dominated by ceratopsians. This study also yielded teeth from small ceratopsians presumed to be near the age of hatchlings.

THE SYNTHESIS OF META-CHLORO- AND META-BROMO- ANALOGS OF CAPSAICIN: POTENTIAL NEW ANALGESIC AGENTS

Katherine M. Phalen and David B. Rusterholz (Advisor)

Department of Chemistry

University of Wisconsin–River Falls, River Falls, WI

Capsaicin is the substance in hot chili peppers that is responsible for causing the burning pain sensation for which these peppers are so well known. When a capsaicin molecule comes in contact with one of these sensory nerves, it first attaches to a receptor molecule called TRPV1. The goal of this research project was to create several new substances that have affinity for the TRPV1 receptor, but do not activate it. It is hoped that biological testing will reveal that these compounds have the qualities needed in a useful pain-relieving drug. The target structures were based on the capsaicin structure with several modifications. Most important, the target structures contained atoms of bromine or chlorine in the position where capsaicin has a methoxy group. This change was expected to enhance the pain-relieving qualities of the resulting compounds. The synthesis of the target compounds was accomplished by a convergent synthetic strategy. The final step involved the formation of a thiourea linkage between an

isothiocyanate precursor bearing the hydrophobic C region and a benzylamine bearing the halogen-modified A region. Details of the synthesis will be presented and discussed.

A NON-RADIOACTIVE ASSAY TO DETERMINE ISOFORM ACTIVATION OF PLD BY PHENYL-EPHRINE IN CCL39 CELLS

Danielle E. Rastedt, Mark A. Wallert (Advisor), and Joseph J. Provost (Advisor)

Minnesota State University, Moorhead, MN

Phospholipase D (PLD) is an enzyme found in the cells of higher mammals and plants. The process of PLD acting on phosphatidylcholine (PC) to produce phospatidic acid (PA) and choline is important in cell signaling. PA acts as a bioactive lipid activating a number of protein kinases and other effectors and can be further metabolized to diacylglycerol, an activator of protein kinase C (PKC). There are two isoforms of PLD, PLD1 and PLD2. PLD1 activity is activated by the small G proteins RhoA and ARF as well as PKC, while PLD2 is constitutively active and can be stimulated by ARF. There is great interest in understanding which isoform is activated by various hormones. Therefore, several methods have been developed to determine its enzymatic activity. The current method used to determine enzymatic activity is an *in vivo* PLD assay using radioactive lipids. Our plan is to use fluorescent labels to measure PLD activity in a non-radioactive assay. Three types of fluorescent lipids were used in these experiments. Two free fatty acids 4,4-difluoro-5-octyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoic acid (BODIPY C_8, C_5); 4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-s-inda-cene-3-do-decanoic acid (BODIPY C_1, $C1_2$); and 1-myristoyl-2-[, 12-[(7-nitro-2-1, 3-benzoxadiazol-4-yl)amino] dodecan-oyl]-sn-glycero-3-phosphocholine (NBD-PC). We found that both NBD-PC and BODIPY C_1, $C1_2$ but not BODIPY C_8, C_5 were incorporated into the membrane as PC. Furthermore, there is a dose- and time-dependent manner in the labeling of starved CCL39 fibroblasts. We plan to show which PLD isoform(s) is activated by stress hormones in CCL39 cells using this technique.

THE EFFECTS OF EXPOSURE TO DIMETHYL-SULFOXIDE ON MALE FERTILITY IN *Mus musculus*

Jennifer Reiman and Randy Krainock (Advisor)

Department of Biology

Saint Mary's University of Minnesota, Winona MN.

Interruptions in the process of spermatogenesis can affect the fertility of the male. Dimethylsulfoxide (DMSO), a common chemical solvent, was previously found to have a negative affect on the male fertility of *Mus musculus:* the data, however, were ambiguous. The objective of this experiment was to test if there was a negative affect on male fertility when DMSO was administered topically or orally. Mice were sorted into three groups: a control group, a topically treated group, and an orally treated group. The topical and oral group mice all received 0.61 µl DMSO/day. After 36 days, the control and treated males were mated with untreated females. No significant differences were found between the groups' offspring litter quantities. Testes were collected and the measured mass and length demonstrated no significant differences among the groups. Sperm was extracted from the epididymis and vas deferens: the total sperm counts and motility were not significantly different. There was a significantly greater number of abnormal sperm morpho-logies with DMSO exposure. Gas chromatography mass spectrometer analysis was conducted on the blood serum of the male mice. There was no detectable DMSO within the serum samples from the mice.

INTERPRETING GENETIC VARIATION IN LO-LLYPOP DARTERS (*Etheostoma neopterum*) AND BLACKFIN DARTERS (*Etheostoma nigripinne*) FROM TENNESSEE

Elizabeth Rivers and Jean Porterfield (Advisor)

Department of Biology

St. Olaf College, Northfield, MN

The lollypop darter (*Etheostoma neopterum*) and blackfin darter (*Etheostoma nigripinne*) are members of a group of freshwater fishes called fantail darters (subgenus *Catonotus).* Recent phylogenetic analysis of cytochrome *b* DNA sequences of the 20 species of *Catonotus* suggested introgression of *E. nigripinne* mitochondrial DNA (mtDNA) into *E. neopterum* populations via hybridization. In order to test the hypothesis that hybridization has occurred between these two species, additional *E. nigripinne* and *E. neopterum* were sampled from populations from 11 sites in and around the Shoal Creek system in Tennessee. Cytochrome *b* sequence data were obtained for 26 fish from these sites and analyzed with a larger *Catonotus* data set. The resulting trees characterize *E. neopterum* as a monophyletic group within paraphyletic *E. nigripinne*. This is consistent with a hypothesis of introgression, although alternate hypotheses must still be considered. These data also suggest that if introgressive hybridization occurred, it was not a recent event.

THE EFFECTS OF ESTROGEN-TREATED *Bonasa umbellus* EGGS IN REGARD TO FERAL PREDA-TION

Anna Robertson and Ray Faber (Advisor)

Department of Biology

Saint Mary's University of Minnesota, Winona, MN

In southeastern Minnesota, the population of the ruffed grouse, *Bonasa umbellus,* has declined, possibly due to egg predation by raccoons or other predators. In order for *B. umbellus* populations to recover, a greater success in the number of eggs hatched is necessary. The purpose of this experiment was to determine whether 17α-ethinyl estradiol could be used to create conditioned taste aversions in egg predators.

Chicken eggs were injected with 10 mg of 17α-ethinyl estradiol mixed in 1 ml of canola oil. The eggs were then rinsed in a weak solution of grouse scent to simulate natural grouse eggs.

Beginning on March 1st, three separate sites, each with 90 eggs, were established and monitored frequently for egg consumption for a period of two weeks. Two sites had treated eggs, the third served as a control. On March 31st, a second two-week period of monitoring egg consumption of untreated eggs began in the same locations. Ultimately, if a correlation can be shown between estrogen-treated eggs and decreased predation rates, the placement of estrogen-treated eggs before the season of *B. umbellus* begins may become an accepted management practice.

CHARACTERIZATION OF INSULIN ADSORP-TION TO GOLD-COATED QUARTZ CRYSTAL SURFACES

Amy Rohly, Megan Schwartzbauer, and Ken Rohly (Advisor)

Department of Chemistry

Bethel University, St. Paul, MN

The purpose of this study is to evaluate the adsorption profile of human recombinant insulin on gold surfaces. Gold-coated quartz crystals were initially sub-jected to a cleaning procedure in order to remove contam-inants from the surface. They were then loaded onto a quartz crystal microbalance (QCM; Q-sense E4), which was the primary method of measuring the adsorption profile. The gold crystals vibrate at approximately 4.95 MHz and adsorbed insulin changes the vibrational fre-quency, which can be converted to mass. The dissipation pattern yields information about the orientation of the insulin on the surface. The gold crystals were also char-acterized by atomic force microscopy (AFM) after the cleaning procedure and after protein adsorption to image the change in surface topography. Since gold has a high affinity for adsorbing impurities, the goal is to obtain cleaning and adsorption procedures that yield repro-ducible data.

1,10-DIOXA-4,7,13,16-TETRAAZACYCLOOCT-ADECANE CAUSES LEAD (II) TO SELF-LUMIN-ESCE WHEN EXCITED BY A LASER

Robert Rounds[†] and Marc Percovik[*] (Advisor)

Departments of Chemistry

[†]*Bethel University, St. Paul, MN*

[*]*Western Michigan University, Kalamazoo, MI*

The 18-crown-6 derivative 1,10-dioxa-4,7,13,16-tetraazacyclooctadecane was synthesized. The compound was mixed with lead(II) nitrate in water, yielding a com-plex. This complex was then characterized using NMR and UV-VIS spectroscopy. Emission of the complex at low temperature was examined with a Nd:YAG laser. It was demonstrated that the binding of the macrocycle lig-and enables the lead(II) ion to emit in the 500-nm range when excited by the laser, and that the empty ligand is incapable of emission in the observed region without the lead(II) ion.

SOLID-STATE STRUCTURES OF SOME *ortho*-SUBSTITUTED BENZYLIDENEANILINES

Benjamin L. Sanders and William H. Ojala (Advisor)

Department of Chemistry

University of St. Thomas, St. Paul, MN

We describe isomeric benzylideneanilines as “bridge-flipped” isomers if they differ only in the orientation of the linkage connecting the two aryl groups (Ar–CH=N–Ar’ vs. Ar–N=CH–Ar’). The existence of crystalline benzylideneanilines in which

disorder exchanges the –CH= and =N– moieties has led us to consider whether bridge-flipped benzylideneanilines could be co-crystallized to produce new solid-state materials. Because co-crystallization requires similarity in molecular conformation, we have determined the X-ray crystal structure of a benzylideneaniline bearing an *ortho*-hydroxyl group to compare the conformation and packing arrangement of this molecule with those of its bridge-flipped isomer. Intramolecular H-bonding produces two different conformations for these isomers, but both are nearly planar. Here we compare the crystal structures of these benzylideneanilines with that of a recently prepared analogue in which the hydroxyl group has been replaced by a fluoro substituent. All three benzylideneanilines assume different packing arrangements and engage in different intermolecular interactions in the crystal.

OPTIMIZATION OF A PROPOSED UNDERGRAD-UATE BIOCHEMISTRY LABORATORY INVOL-VING MOLECULAR BIOLOGICAL TECHNIQUES

Noël Santora and Ashley Mahoney (Advisor)

Department of Chemistry

Bethel University, St. Paul, MN

This project involves the integration of numerous biochemical and molecular biological techniques in order to develop a semester-long undergraduate biochemistry laboratory. This laboratory will include a series of related experiments designed to reinforce the significance and versatility of the employed techniques. The objectives of the proposed laboratory are to subclone the β-galactosi-dase gene from a mammalian vector into an *E. coli* vector using specific restriction endonucleases, to express pos-itive clones and purify β-galactosidase using a nickel resin, to visualize the enzyme of interest using SDS–PAGE and Western blotting, and to determine the activity of β-galactosidase using a specific assay.

CHROMOSOME TRANSGENICS IN OAT–MAIZE ADDITION LINES: IMMUNOCYTOLOGICAL ANALYSIS OF THE ENZYME MALATE DEHY-DROGENASE

Krista Schoeder, Jeanne Minnerath (Advisor), and Richard Kowles (Advisor)

Department of Biology

Saint Mary's University of Minnesota, Winona, MN

Oat-maize chromosome addition lines have been successfully generated at the University of Minnesota. Oat–maize (OM) addition lines are oat plants that have one maize chromosome in their genome. These addition lines have been generated for each of the 10 maize chrom-osome pairs. Studies conducted at Saint Mary's Univer-sity of Minnesota have explored the effects that a chromo-some from a C4 photosynthetic plant, maize, has on a C3 photosynthetic plant, oat. One of the enzymes that play a crucial role in C4 photosynthesis is malate dehydrogenase (MDH). MDH has been found in both the C4 maize plant and the C3 oat plant. In maize, MDH is found in the mesophyll cells where it converts oxalacetate into malate during C4 photosynthesis. Although MDH is present in C3 plants such as oats, the function of the enzyme is anaplerotic; that is, not involved in C3 photosynthesis. The purpose of this research was to confirm the presence and location of MDH in maize, oat, and oat–maize addi-tion line plants using immunocytological techniques. Leaf tissue sections from maize, oat, and oat–maize addition lines 5, 6, and 9 were made and probed with polyclonal antibodies against MDH. Antibody binding was detected using a secondary antibody conjugated to gold particles followed by a silver enhancement. MDH was present in mesophyll cells of maize, oat, and OM addition lines. Maize had the most MDH, and it was located in both the mesophyll and bundle sheath cells. Oat–maize addition lines containing chromosomes 5 or 6 possessed more MDH than oat and other oat–maize addition lines tested. This was expected because multiple

genes for MDH exist in maize, and one is located on chromosome 5 and ano-ther on chromosome 6. The results suggest that the addi-tion of maize chromosomes into the oat genome does cause the oat plant to exhibit a greater presence of MDH enzyme.

AN INCREASE IN THE MITOTIC INDEX OF *Vicia faba* ROOT TIP CELLS WITH MITOGEN COMBI-NATIONS

Beth Schubert and Richard Kowles (Advisor)

Department of Biology

Saint Mary's University of Minnesota, Winona, MN

Many studies depend on obtaining an increase in metaphases and a spread of visible chromosomes in cell division. In order to obtain visible chromosomes such as these in high frequency, the cell cycle must be interrupted in metaphase by disallowing spindle fiber formation. In these experiments, the lateral root tips of *Vicia faba* were treated with colchicine, a chemical that disallows spindle fiber formation, as well as phytohemaglutinin (PHA) and hydroxyurea (HU), which are both mitogens. In all combinations, an increased number of mitoses coupled with the lack of spindle fibers resulted in an overall higher mitotic index in *Vicia faba* root tips. The mitotic index of root tip cells treated in colchicine (10.95%) was higher than that of root tip cells treated in either PHA (6.86%) or HU (4.61%) ($p < .001$ in both instances). However, cells treated with PHA and HU together, but without colchi-cine, did not have a significantly higher mitotic index than the control group treated in water ($p = .45$). The highest mitotic indices were achieved through treatments consis-ting of one mitogen (either PHA or HU) plus colchicine (14.31% and 13.58%, respectively). Using these combina-tions, the mitotic index was increased over colchicine by itself and nearly 6-fold over the control group (mitotic index = 2.75%) ($p < .001$). The reason for the lack of increased mitogenic effects in the presence of both mito-gens may be due to an interaction between PHA and HU. Increasing the mitotic index has useful applications with regard to karyotyping, *in situ* hybridization, and other cytogenetic studies.

INVESTIGATION OF EFFECTS OF TESTOSTER-ONE DERIVATIVES ON HUMAN PROSTATE CANCER

Jamie K. Schwendinger and Bynthia M. Anose (Advisor)

Department of Chemistry

Bethel University, St. Paul, MN

Human prostatic carcinoma is initially an exquisitely androgen-responsive cancer. Clinical admin-istration of anti-androgen steroids promotes a rapid regression of the disease; however, in time, the cancer frequently recurs in a far more aggressive form. The resurgent prostate cancer is typically both metastatic and resistant to androgen ablation. Past research had implicated the testosterone derivative dihydrotestosterone (DHT) as the most potent stimulator of prostate cell growth; this hormone has therefore been the one primarily targeted for androgen ablation. However, many analogs of DHT exist that have not been examined or therapeutically characterized.

In our study, the chemically similar testosterone propionate and dehydroisoandrosterone will be assayed for androgenic capabilities and the potential to stimulate uncontrolled prostate cell growth. 22Rv1 (a human prostatic cancer cell line) will be treated with 10 nM DHT, testosterone propionate, or dehydroisoandrosterone. The effects of these mitogens on the expression of the growth-stimulating gene prostate-specific antigen (PSA) will be tested. PSA mRNA levels will be quantified via the highly sensitive, reverse transcription real-time polymerase chain reaction, technique. Stimulation of PSA expression by DHT is both measurable and clinically significant, and as such provides an excellent control for investigating potentially androgenic agents.

EFFECTS OF PRESCRIBED BURNING ON AVIAN USE OF RESTORED PRAIRIES

Rachel Spike and Dr. Jeff Port (advisor)

Department of Biological Sciences

Bethel University, St. Paul, MN

Periodic fire has long been recognized as a key component of the North American grassland ecosystem. The importance of fire in suppressing woody growth is well documented and its use to control invasive species is a commonly practiced management tool. During the summers of 2003-2005, data was gathered for the purpose of documenting the impacts of a controlled burn on songbird populations utilizing a 21 acre restored prairie. Data collected during 2003 and 2005 comprise non-burn years and provided the opportunity to monitor the avian populations one year pre- and post-burn. The most prominent species were the Clay-colored Sparrow (*Spizella pallida*), Sedge Wren (*Cistothorus plantensis)*, and Song Sparrow (*Melospiza melodia*). Fewer foraging adults birds (= 21.67, SD = 4.76) and only two nests were observed during the burn year, while many more adults (= 52.14, SD =3.17) and 26 and 34 nests were located during the non-burn years of 2003 and 2005, respectively. This study suggests that bird populations can quickly recover from small scale periodic fires and nearby refugia may aid in this recovery.

INTERMOLECULAR INTERACTIONS AND MO-LECULAR PACKING IN ISOMERIC BENZYLID-ENEANILINES

Jill M. Spude and William H. Ojala (Advisor)

Department of Chemistry

University of St. Thomas, St. Paul, MN

Pairs of benzylideneanilines we have designated “bridge-flipped isomers” differ only in the orientation of the chain or bridge of atoms connecting the two aryl groups; the isomerism is Ar-CH=N-Ar’ vs. Ar-N=CH-Ar’. Our goal is to prepare new solid materials having properties that can be controlled or modified by co-crystallizing various proportions of bridge-flipped benzylideneaniline isomers. Mutual solid-state solubility is most extensive for components that have the same molecular packing arrangement in their respective crystals, so we are preparing benzylideneaniline bridge-flipped isomeric pairs and determining their crystal structures to identify isostructural pairs that would be especially suitable for co-crystallization. Among these structures we have recently discovered our first isostructural pair: 2-trifluoromethyl-*N*-(2-methylbenzyl-idene)aniline and 2-methyl-*N*-(2-trifluoromethylbenzyl-idene)aniline. Unlike the only other isostructural bridge-flipped benzylideneanilines of which we are currently aware, 4-chloro-*N*-(4-methylbenzylidene)aniline and 4-methyl-*N*-(4-chlorobenzylidene)aniline, our compounds assume an ordered molecular packing arrangement in the solid state.

CELL CYCLE ANALYSIS OF MURINE B-LYM-PHOCYTES GROWN IN THE PRESENCE OF MELALEUCA OIL

Tom Stewart and Debra Martin (Advisor)

Department of Biology

Saint Mary’s University of Minnesota, Winona, MN

Melaleuca oil is a natural health product that is manufactured in a natural process, and therefore the FDA does not regulate it. Melaleuca oil is isolated from the Australian tea tree and is said to have healing affects on a multitude of different ailments including both topical and internal medical issues.

Previous research has shown that Melaleuca oil had an inhibitory effect of on mammalian cell growth and viability. This experiment was intended to determine if the cell cycle of the B-lymphocytes is what the Melaleuca oil is affecting. The cells used were murine Z70, a pre B-lymphocyte. Suspensions of these cells were treated with a concentration of Melaleuca oil that was known to have inhibitory affects on cell division (0.01%). Control sus-pensions of the same cells were treated with glycerol in the same concentration. The suspensions were then anal-yzed using flow cytometry to determine the possible cell cycle effects. The cell cycle results from the flow cyto-meter will be presented and discussed.

PHENYLEPHRINE STIMULATES CELL MIGRA-TION THROUGH PHOSPHOLIPASE D ISOFORM 1 AND NOT ISOFORM 2

Lisa Streitz, Blake Heinz, Joe Provost (Advisor), and Mark Wallert (Advisor)

Department of Biosciences

Minnesota State University–Moorhead, Moorhead, MN

Phospholipase D (PLD) is involved in tumor-genesis in several cell lines through the formation of phosphatidic acid (PA) and its downstream metabolites. PLD regulates proliferation and cytoskeletal rearrange-ments. Two mammalian PLD isoforms are known: PLD1 and 2. Small G proteins, protein interactions, and protein kinases regulate both. PLD2 is thought to be the predom-inant isoform involved in signaling. Our experiments sought to determine the PLD isoform responsible for mitogenic events. Phenylephrine (PE), an α1-adrenergic receptor agonist, leads to the activation of ERK, stress fibers, and cell migration in Chinese hamster lung fibro-blasts. Using dominant–negative (DN) PLD isoforms, we determined that PLD1 is responsible for these actions.

In earlier studies, 50 μM PE stimulated ERK 3–5 fold in a PLD-dependent fashion. Expression of DN PLD 1 but not 2 decreased PE induced ERK activation. Addi-tionally, in stress fiber experiments, transfection with DN PLD2 had little effect while DN PLD1 abrogated PE-induced stress fibers. In wounding assays, PE enhanced cell migration. DN PLD1 specifically blocked this increase. Preliminary data suggest that cells transfected with DN PLD2 migrate into the wound whereas cells with DN PLD1 do not. Our data show that PLD1, not PLD2, mediates α1-adrenergic regulation of migratory events and define a unique role for PLD1 in signaling. This work was supported by a grant from the NIH, Award number 1 R15 HL074924-01A1.

INFLUENCE OF PHOTOPERIOD ON MAMMAL-IAN CIRCADIAN FUNCTION: CAN MY MOUSE TELL SUMMER FROM WINTER?

Jeremy J. Stubblefield, Leo S. McNamara, Kumiko Ijichi, and Dwight E. Nelson (Advisor)

College of St. Thomas, St. Paul, MN

In mammals, circadian rhythms in behavior and physiology are driven by endogenous circadian oscillators. Environmental light cycles entrain these central pacemakers to synchronize an organism's internal physiology and behavior with daily changes in the

environment. Seasonal modulations of behavior and physiology are also thought to involve the circadian system through the effects of daylength on circadian release of pineal melatonin. We are examining photoperiod-induced changes in the circadian system in C57BL/6 mice—a strain commonly regarded as insensitive to photoperiod due to a lack of melatonin. Mice (n = 12/group) were entrained to long (16L:8D) or short days (8L:16D) and then released into constant darkness (DD). Light pulses were delivered to each mouse in DD to assess the photic responsiveness of the circadian system using phase shifts. Free-running period and duration of activity were also measured. To test whether the full duration of light was necessary for photoperiodic changes, we also tested mice (n = 12/group) pre-entrained to "skeleton" LD cycles that matched the short day (1h L:6 h D:1h L:16h D) and long day photoperiod (1h L: 14 h D: 1h L:8h D). Mice entrained to long days displayed significantly smaller shifts (-82+/-11min) than mice entrained to short days (-167+/-11min; ANOVA; Tukey, $F=19.15$, $P<0.0001$). Shifts following full photoperiods were not significantly different from those measured following skeleton photoperiods (ANOVA; Tukey, $P>0.94$). We obtained similar results for circadian period and duration of activity. Surprisingly, there is a very large influence of photoperiod on circadian functions in "non-photoperiodic" C57BL/6 mice.

OXIDATION-RESISTANT RIBONUCLEASE INHIBITOR

Can Sungur, Anna Krawisz, and Kimberly A. Dickson (Advisor)

Department of Biology

Macalester College, St. Paul, MN

Ribonuclease inhibitor (RI) is a cytosolic protein that inhibits the activity of ribonuclease A and angiogenin by binding to their active sites with fentomolar affinity. Its previously described anti-angiogenic properties can currently only be of use intracellularly due to the oxid-ation sensitivity of RI. This research attempts to create a ribonuclease inhibitor that is oxidation-resistant by exchanging 11 cysteines for other amino acids. The amino acid substitutions were chosen to preserve the polarity and bulk of the cysteines; this avoids interrupting the second-dary structures of RI. Mutations were verified by sequen-cing. Binding assays were conducted to characterize the affinity of mutagenized RI for RNase A. This oxidation-resistant RI may have important implications for the extracellular inhibition of angiogenin, and thus, for the prevention of angiogenesis and tumor metastasis. Also, it may prove to be a valuable laboratory reagent for explor-ing or modulating interactions with ribonucleases.

ACTIVATION OF MATRIX METALLOPROTEIN-ASE 9 BY PHENYLEPHRINE REQUIRES SODIUM HYDROGEN EXCHANGER 1

Jennifer M. Taves, Joseph J. Provost (Advisor), and Mark A. Wallert (Advisor)

Biosciences Department

Minnesota State University, Moorhead, Moorhead, MN

Matrix metalloproteinases (MMP) are a group of enzymes that play a critical role in digesting the extracellular matrix. Degradation of the extracellular matrix by MMP in migrating cells provides a vital function for tumor metastasis and angiogenesis. The link between extracellular MMP activity and the sodium hydrogen exchanger (NHE) has been suggested, yet not identified. We studied the relationship between NHE and MMP activity in CCL39 fibroblasts containing NHE1, PS120 cells (NHE1 null derived from CCL39 cells), and PS127 cells (PS120 cells expressing NHE1). Initial studies with CCL39 cells found resting cells had moderate MMP9 activity. This activity increased 2.5 fold after 12-hour phenylephrine (PE) stimulation. Western blot analysis of culture media identified MMP9. We found MMP9 activity was dependent upon expression and activation of NHE1. In both CCL39 and PS127 cells, MMP9 was activated in the presence of 100 μM PE. In PS120 cells no MMP was activated in the presence of PE. Incubation of cells with amiloride before PE addition resulted in a notable decrease in MMP9 activation compared with control. Incubation of cells with 0.5% butanol prior to PE stimulation decreased MMP9 activity similar to the control level, while expression of either dominant–negative phospholipase D1 or 2 caused a decrease in MMP9 activity less than untransfected cells.

This work, for the first time, describes an agonist-induced relationship between NHE1 and MMP and a new potential role for NHE1 in tumor formation. This work was supported by a grant from the NIH, Award number 1 R15 HL074924-01A1.

SMALL MAMMAL VEGETATION PREFERENCE IN RESTORED PRAIRIE

Jennifer Thorson and Teresa DeGolier (Advisor)
Department of Biology

Bethel University, St. Paul, MN

One of the difficulties in restoring damaged habitats is knowing what made up the original habitat and what components would most benefit the organisms in the habitat. In prairie restoration it is important to determine carefully what vegetation types would be optimal for the land, the vegetation, and the animals in the habitat. This study examines (1) four different types of vegetation on old farmland that has been converted to restored prairie, (2) the small mammals that utilize these restorations, and (3) effects of restoration burns on the small mammal populations. The four types of vegetations were old fields composed of cool-season grasses, low-diversity-warm season grasses, high-diversity-sixth-year plantings, and high-diversity-seventh-year plantings. Small mammals including the meadow vole *(Microtus pennsylvanicus)*, meadow jumping mouse (*Zapus hudsonius*), and white-footed mouse (*Peromyscus leucopus*) were trapped, marked, and released. *Zapus hudsonius* showed strong preference for low-diversity-warm-season grasses following a burn, while *Microtus pennsylvanicus* showed a strong preference for cool-season grasses following a non-burn year. These results reject the null hypothesis that small mammal populations are independent of vegetation type. These results also support the require-ment of a diversity of reconstruction types to support a diversity of mammal types.

HOW DOES HOSTING THE OLYMPIC GAMES IMPACT EMPLOYMENT IN THE HOST CITY?

Lee Tucker and Dr. Mark Kanazawa (Advisor)

Department of Economics

Carleton College, Northfield, MN

Despite the size and prestige of the Olympic Games, few studies exist to determine whether or not the Games benefit host cities. Existing studies suggest that the Olympics may lead to increased employment, but they reach little consensus on the size or length of that impact. Controlling for the effects of GDP and price levels, I measure the size and shape of the "Olympic effect" with a series of time-period dummies and a fixed-effects model. My study examines all Summer Games from 1984 to 2004 in the first panel study of employment surrounding the Olympics. Using a Prais–Winsten method to correct for heteroscedasticity and AR(1) autocorrelation, I find evidence of a significant employment increase lasting in general from 6 years before to 1 year after the Olympic Games, with a marginally significant boost lasting up to 8 years afterward. I also find that higher Olympic expenditures are negatively correlated with the size of the Olympic effect, and that the employment impact of the Olympics may be larger in wealthier countries.

CHROMOSOME TRANSGENICS IN OAT–MAIZE ADDITION LINES: IMMUNOCYTOLOGICAL ANALYSIS OF

THE ORGANELLE ENZYME MAL-ATE DEHYDROGENASE

Autumn Valk, Jeanne Minnerath (Advisor), and Richard Kowles (Advisor)

Department of Biology

Saint Mary's University of Minnesota, Winona, MN

Oat–maize addition lines result from the cross between oat plants (C3 photosynthesis) and maize (C4 photosynthesis). One complete maize chromosome is successfully incorporated into the oat genome. The lines are generated at the University of Minnesota and are available for maize chromosomes 1 through 10. Oat–maize addition lines have been used to determine to what extent maize chromosomes contribute to C4 photosyn-thesis. Both C3 and C4 plants contain the protein malate dehydrogenase (MDH). Unlike in C3 plants, MDH plays a crucial role in C4 photosynthesis by catalyzing the mutual conversion of malate and oxaloacetate. The object-tive of this research was to validate the presence and location of MDH in oat, maize, and oat–maize addition lines 3, 5, 6, and 9. Leaf tissue sections from each plant were probed with polyclonal antibodies against MDH. A secondary antibody conjugated to gold particles and enhanced with silver reagent was added to determine the concentration of MDH present. The presence of the protein MDH was clearly shown in mostly the mesophyll cells of all plants tested. Maize was found to have a greater quantity of MDH than oat and the oat–maize addition lines, which verifies the importance of MDH in C4 photosynthesis. Furthermore, these results demon-strate that C3 oat plants can exhibit C4 characteristics following incorporation of one entire maize chromosome into the oat genome.

SYNTHESIS AND CHARACTERIZATION OF *N*-PHENETHYLPYRIDINECARBOXAMIDES AS POTENTIAL INDUCERS OF APOPTOSIS

Brianna Zemke and Karl P. Peterson (Advisor)

Department of Chemistry

University of Wisconsin–River Falls, River Falls, WI

Apoptosis is a biological process in which cells "switch-on" a series of pathways that lead to programmed cell death. This process operates to control cell growth and tissue organization throughout the life of an organism. Faulty regulation of apoptosis has been implicated in such common disease states as Alzheimer's disease, Parkinson's disease, and cancer. Small molecules that have the ability to induce or inhibit apoptosis are of tremendous interest as potential pharmaceutical agents and as tools to study the molecular biology of apoptosis. Studying these types of molecules provides insight into the mechanism of how thet interact in the apoptotic pathways. Understanding these interactions could allow for new small molecules to be designed that have an enhanced ability either to induce or inhibit apoptosis. The synthesis and characterization of a series of *N*-phenethylpyridinecarboxamides that bear structural similarity to compounds recently reported to selectively induce apoptosis in cancer cells will be presented.