

Gustavus 2007 Fall Research Symposium

Friday, September 7

List of Abstracts

Session 1A—Nobel 222

1:30 pm *Conservation genetics investigation of the margined sculpin (*Cottus marginatus*)*

Rochelle Molitor, Joel Carlin

The margined sculpin (*Cottus marginatus*) is an isolated streamfish species found in southeastern Washington and northeastern Oregon. Although it has one of the smallest ranges of any other fish in these states, the population size is presumed large so it has not been granted the status of an endangered or threatened species. However, recent analyses reveal that the habitat in which it lives is rapidly degrading. In this study, DNA from a set of 55 fish was used to assess the species' resulting genetic condition. The entire cytochrome *b* gene from mitochondrial DNA was amplified in 21 samples and sequenced. In addition, 3 nuclear microsatellite loci were amplified in 25 samples. The genetic diversity (heterozygosity and percent sequence divergence) at these loci were analyzed and will be used to provide insight into short term evolution as well as guide future conservation efforts.

1:45 pm *Relating Gene Regions to Phenotype in *Drosophila melanogaster* Female Fertility*

Isaac Weeks, Margaret Bloch Qazi

Females of many animal species perform female sperm storage, a process in which females store viable sperm for extended periods of time. To further elucidate the mechanisms of sperm storage in female fruit flies we performed a deficiency screen of the 2nd chromosome to find genes that have a haploinsufficient effect upon sperm storage. Of the several thousand genes on the 2nd chromosome, we have identified 15 genes with potential sperm storage effects. Through this experiment we may be able to further understand the role of female sperm storage in physiology, development, and sexual selection, as well as learn methods to control animal populations.

2:00 pm *A Computational Model of the Vertebrate Peripheral Auditory System*

Katie Halvorson, Mike Ferragamo, Jan Wotton

The anuran auditory system was utilized to construct a computational model of the auditory periphery that can be applied across multiple vertebrate systems. The model was constructed from anatomical and physiological properties in the literature and electrophysiological data collected in our laboratory. Analysis of the peripheral level demonstrates that the model complements the physiological data in the temporal dimension in areas such as phase-locking and encoding of the signal envelope. In the spectral dimension, the model has also produced similar frequency tuning curve, dynamic range, and adaptation properties to the known physiological data. Continued construction of a multi-layered neural network model centrally (into the dorsolateral nucleus and the torus semicircularis) will allow study of acoustically driven behaviors that could lead to application across homologous mammal auditory systems.

2:15 pm *Desulfation of Cell Surface Glycosaminoglycans by Heparin Degrading Endosulfase, Sulf1*
Vwaire Orhurhu, Lewis Roberts, Jin Ping Lai, Rashid Shire, Vivek Tharayil, Catherine Moser, Kenard Jackson (Mayo Clinic)

Cell surface heparan sulfate glycosaminoglycans (HSGAGs) are complex polysaccharides that have been implicated in several biological functions. Changes in sulfation pattern of this ubiquitous polysaccharide regulate diverse aspects of cancer biology, such as tumorigenesis, tumor progression and metastasis. Studies on the action of SULF1 and SULF2 suggests that they modulate growth factor signaling through specific desulfation of cell surface proteoglycans. However, the two sulfatases appear to have opposing effects on cell proliferation in hepatocellular carcinoma cell (HCC) line, suggesting that they have different effects on cellular proteoglycans. To examine the action of SULF1, we measured the percentage of O - sulfation of HSGAG in three hepatocellular carcinoma cells transfected with an empty vector or SULF1 plasmid DNA. Three hepatocellular carcinoma cells were examined for SULF1 expression using reverse transcriptase polymerase chain reaction (RT-PCR) and immunocytochemistry. Heparan sulfate glycosaminoglycans were purified from hepatocellular carcinoma cell lines. Furthermore, two standard curves were prepared to determine the concentration and percentage O-sulfation of the purified HSGAGs. Hepatocellular carcinoma cell lines (SNU 449, Huh7, and Hep3B) expressing SULF1 showed decrease in O-sulfation compared to HCC cell lines transfected with an empty vector. SNU449 Sulf1-clone1 showed a 28% decrease in O-sulfation, Huh7 Sulf1-clone 4 and clone 5 showed a 12% and 38% decrease in O-sulfation respectively. Hep3B Sulf1-clone 4 also showed a 16% decrease in O-sulfation of HSGAGs. Our results suggests that SULF1 decreases the sulfation of O-Sulfated HSGAGs from HCC cell lines.

2:30 pm *Melanoides tuberculata and invertebrate diversity of Kelly Warm Springs*
Elias Anoszko, Erin Hotchkiss (University of Wyoming)

Melanoides tuberculata is a small snail native to Asia. It was accidentally introduced into the Kelly Warm Springs drainage of Grand Teton National Park in 2004. Since its introduction, it has become the most abundant invertebrate in the warm reaches of the stream. The dominance of *Melanoides* has been concomitant with decreases in native invertebrate diversity and abundance. While *Melanoides* is limited in Kelly Warm Springs to warmer reaches, of the drainage, the possibility exists for *Melanoides* to be transferred to other warm springs in the Greater Yellowstone Area.

2:45 pm *Distribution and Abundance of Botrychium spp. in Joseph, OR and Bemidji, MN*
Amy Waldner, Whitney Hohman, Stephanie Erlandson, Cindy Johnson-Groh

The genus *Botrychium* includes a number of small, rare ferns known commonly as moonworts. Moonworts depend heavily on their mychorizal (fungal) counterparts, which supply them with most of their needed nutrients. Due to this symbiotic relationship, moonworts remain dormant for many years underground. The purpose of this study was to further analyze moonwort life cycles based on previously studied populations and to discover new populations. The first project was carried out in a forest edge near Bemidji, where populations of moonworts had been relocated due to a pipeline expansion. Previously tagged plants were located and measured, and new plants were tagged. This data will be analyzed to provide information about *Botrychium* life cycles, resilience to relocation, herbivory, and effects of varying weather patterns. The second project was conducted in Eagle Cap Wilderness National Park near Joseph, Oregon. Previously tagged plants were located and measured, and new plants were tagged. In addition, new locations were surveyed to locate potential habitats for *Botrychium spp.*, especially *B. lineare*, a candidate for Federal listing as a sensitive species. In Bemidji, many plots were found to have declining numbers of returning plants with few new plants found. Potential habitats surveyed yielded no new plants. In Oregon, many species were found, mostly in calcium rich, unstable meadows, associated with *Fragaria spp.* and prairie smoke. Declining numbers were also noted, especially in Lostine Canyon, where the plants had previously been abundant. Several surveys conducted in potential habitats throughout valleys in Wollowa-Whitman National Park indicated a variety of species commonly associated with *B. lineare*. Declining populations in both communities could have several causes, including changing weather patterns, herbivory, dormancy, or environmental impacts. For the Bemidji project, declines could be due to relocation following pipeline expansion, and transplant into a new and unfamiliar environment.

Session 1B—Nobel 105

1:30 pm *Are carboxyl groups the most acidic sites in amino acids? From gas phase to solution, considering the thermodynamic properties of variable deprotonation sites*
Maari Hanson, Joe Scanlon, Steve Kass, Chris Cramer, (University of Minnesota)

Energetically relevant conformers of cysteine and tryptophan were generated through a Monte Carlo search utilizing the GMMX force field. Selected conformers were optimized and confirmed as minima via several density functional theory calculations (B3LYP/6-31+G(d,p)). In the gas phase, the conjugate base of cysteine is confirmed to be a thiolate ion however the carboxylate ion is favored under the 1 explicit water molecule model. As for the tryptophan results, the carboxylate ion is predicted to be energetically favored by 1.6 kcal* mol^{-1} over the indole deprotonated counter-ion. This value is supported by experimental results where trimethylsilyl azide reactions showed an approximate ratio of 2:1, carboxylate to indolate ions.

1:45 pm *Synthesis and Reactivity of Bio-inspired Copper(I) Complexes*
Dave Pearson, Bill Tolman (University of Minnesota)

A biomimetic study was conducted on the copper-containing enzyme quercetin 2,3-dioxygenase (2,3QD), using two α -diimine ligands (2,3-bis(2,6-diisopropylphenylimino)butane and 2,3-bis(3,5-dimethylphenylimino)butane) that model the enzyme's active site. The ligands were synthesized and characterized by ^1H NMR spectroscopy and various copper(I) complexes were generated. A copper carboxylate-ligand complex was also synthesized to model the enzyme prior to dioxygen attack. The complexes were characterized using ^1H NMR and mass spectroscopy. To determine reactivity, dioxygen was bubbled through at low temperature and analyzed via UV/Vis.

2:00 pm *In Pursuit of Pincer Ligand-Supported Hafnium Alkylidenes*
Emily Pelton, Oleg Ozerov (Brandeis University)

Since their introduction in 1975, early transition metal alkylidene complexes have been the focus of myriad research endeavors, principally due to their utilization in the catalysis of alkene and alkyne metathesis. Several alkylidene complexes have been synthesized and characterized for Group IV metals titanium and zirconium, but the alkylidene chemistry of hafnium remains largely unexplored, as only one such complex has yet been reported. In order to contribute to knowledge and potential applications, we endeavored to synthesize alkylidene complexes via α -hydrogen abstraction, the formation mechanism of other Group IV metal-carbon multiple bonds. The use of a rigid bis(*o*-phosphinoaryl) amine PNP ligand has been shown to support formation of early transition metal alkylidene complexes, and we used this "pincer" ligand to achieve the goal of hafnium alkylidene synthesis.

2:15 pm *Organic Synthesis of Pyrrolizidine Alkaloids*
David Guptill, Nina Serratore, Scott Bur

This project focuses on the synthesis of a bicyclic pyrrolizidine alkaloid derived from an amine and a carboxylic acid. The amine was synthesized from methionine by a series of reactions. The carboxylic acid was synthesized from methyl acrylate and nitromethane. The amine and the carboxylic acid were coupled with DCC and D-MAP to form an intermediary amide. We are currently working on the formation of the bicyclic alkaloid using a Lewis acid as a catalyst.

2:30 pm *Nitrate and Other Anions in the St Peter Drinking Water Sources Protection Area*
Nick Hefty, Jeff Jeremiason

The City of St Peter has been blending shallow nitrate-laden ground water with deeper ground water to meet nitrate drinking water standards. In this project we initiated sampling in four creeks that contribute to the ground water St Peter utilizes for drinking water. We measured nitrates and other anions in four creeks that terminate west of St Peter. An ion chromatograph was used to analyze for numerous anions. Sampling began in early June and continued while stream flow persisted. Elevated nitrate levels exceeding drinking water standards were found in the streams. Methods for treating nitrate-laden water were explored and models of them were tested for efficiency.

2:45 pm *Spectroscopic Characterization of the Active Site of γ -Glutamylcysteine Synthetase*
Colin Boettcher, Kelly Rozenboom, Brenda Kelly

Glutathione plays an important biological role in detoxication in nearly all eukaryotes. Recently, it has been implicated in chemotherapeutic resistance due to its high abundance in many cancer cells and its ability to slow or block chemotherapeutic effects. By inhibiting glutathione synthesis, cancer cells may become more susceptible to some therapeutic techniques. The enzyme, γ -glutamylcysteine synthetase (γ -GCS) catalyzes the first step in the synthesis of glutathione. In the present study, UV/Vis spectroscopy was used to identify substrate analogs that alter the activity of γ -GCS, while fluorescence spectroscopy was used to probe the effect of these analogs on the binding of natural substrates. These studies suggest that the enzyme active site contains two distinct binding sites that may be filled with a cysteine-like molecule. To further investigate the metal-binding sites of γ -GCS, preliminary titration studies with cobalt were performed for future study of the enzyme using NMR.

Poster Session—Nobel Atrium

3:00 pm-3:30 pm

The Attenuation of Pro-inflammatory Proteins and Activated Microglia and Astrocytes in Mice with the use of VIVIT Peptide

Steve Howard, Colin Combs (University of North Dakota)

See abstract in session 2A.

Multi-Domain Peptide Hydrogels: Synthesis, Purification, and Analysis of Nanofiber Self-Assembly

Molly Beernink, Jeffrey Hartgerink (Rice University)

Particular multi-domain peptides were chosen for their tendency to combine into stable gels in cell culture media at neutral pH and relatively low peptide weight percentages. The solid phase synthesis of these peptides was optimized as, due to their tendency to form intermolecular bonds, stronger than normal conditions were needed for both deprotection and amino acid coupling during synthesis. Purification of the charged peptides via HPLC was investigated using many solvent systems. Characterization via CD temperature and concentration studies showed β -sheet conformation of the peptide in most cases. The mechanical strength and desired chemical conditions of the peptide hydrogels were tested and found to successfully form gels able to be used for cell proliferation matrixes in possible tissue engineering applications.

PNP-Supported Hafnium Complexes
Emily Pelton, Oleg Ozerov (Brandeis University)

See abstract in Session 1B.

Characterization of Non-Zeolite Pores in SAPO-34 Membranes
Jeremy Bedard, John L. Falconer (University of Colorado—Boulder)

Several experiments were performed on a SAPO-34 zeolite membrane in order to study the effect methanol has on its structural framework. Previous studies have shown expansion interaction between foreign compounds and zeolite membranes. It has also been documented that methanol has a strong adsorption interaction with SAPO-34. None of these studies have been absolutely adequate to truly characterize the interaction between SAPO-34 and methanol. The permeation methods of transient gas, pervaporation, and vapor permeation were utilized in an effort to characterize this interaction. Large molecule, r134a and ethanol, flux was studied with and without the presence of methanol. No clear and consistent result was achieved. It is not certain at this point if methanol expands, shrinks, or has no effect on the SAPO-34 membrane. More experiments must be run at different conditions to further the understanding of methanol's effect on SAPO-34 zeolite.

The Photolysis of Sulfonyl Group Herbicides: Nicosulfuron and Flumetsulam.
Mallory Richards, Amanda Nienow

The herbicides Flumetsulam from the Triazolopyrimidine group and Nicosulfuron from the Sulfonylurea group are relatively new classes of herbicides that function by inhibiting a plant's enzymes, eventually killing the plant.¹ These herbicides have been detected in surface water samples from the Midwestern United States and have structures that may be susceptible to photochemical degradation.¹ In this work, solutions of Flumetsulam and Nicosulfuron prepared in either Millipore water or filtered Mississippi River water were irradiated in a Rayonet photoreactor. The Rayonet can be equipped with lamps with maximum output at 254, 300 or 350 nm. Evidence of photochemical degradation is present in the exposure of Flumetsulam to 254 nm UV light but not to 350 nm UV light. Nicosulfuron undergoes photolysis at 254nm and 300nm but not at 350nm wavelength. The degradation of Nicosulfuron at 300 nm yields a rate constant of $k=0.0404 \text{ min}^{-1}$, and as it undergoes photolysis by-product formation is observed. In the experiments to date, no difference has been observed in the photochemical degradation rate constants of the samples in Millipore water or Mississippi River water.

Channel Morphology of No Thoroughfare Canyon, CO
Kathryn Ladig, Richard Gigi (Mesa State College)

No Thoroughfare Wash is an un-gauged intermittent stream that drains No Thoroughfare Canyon, the second largest drainage in Colorado National Monument. Despite remaining dry most of the year, late summer and early fall bring intense monsoonal storm systems that often cause flash floods. The goal of this study was to characterize the channel morphology of No Thoroughfare wash in order to better understand the channel-forming discharge regime of the current channel configuration and determine rates of incision into valley fill in the upstream region.

Session 2A—Nobel 222

3:30 pm *The Circadian Clock, cAMP Levels, and Light Influence the distribution of Myosin III and Actin in Limulus Photoreceptors*

Rachel Dorr, Barbara-Anne Battelle (University of Florida)

The American horseshoe crab (*Limulus polyphemus*) is a useful model to gain insight on changes in visual sensitivity caused by signals from circadian clocks. In *Limulus*, a clock-driven efferent neural input to the eyes at night changes the structure and function of photoreceptors increasing their sensitivity to light. This clock-driven efferent input together with cyclic light act in conjunction to produce the full range of diurnal changes observed in photoreceptors. We examined the effects of clock input and light on the distributions of two important proteins in photoreceptors, myosin III (LpMYO3) and actin. Both *in vitro* and *in vivo* experiments were conducted to examine the conditions that lead to the return of LpMYO3 and actin to their day-time distributions, and whether the nighttime distribution could be caused by the activation of the cyclic AMP cascade. Our findings show nighttime distribution of LpMYO3 and actin can be reversed by light at night, or darkness during the day.

3:45 pm *Neurotrophin Regulation of Synaptic Vesicles and Transmission*

Brandon Baartman, Carlos Mantilla, Gary Sieck (Mayo Clinic)

Inactivity of the right hemidiaphragm in the adult rat has been seen to induce remodeling of the neuromuscular synapse. Our lab has created multiple models of diaphragm inactivity and has shown that neuromuscular transmission is enhanced in one model, and worsened in another. Examination of the pre-synaptic terminal of these models has indicated that the enhancement in neuromuscular transmission corresponds with an increase in synaptic vesicle pool size, and the worsening in neuromuscular transmission corresponds with a decrease in synaptic vesicle pool size. Additionally, regulatory molecules in the nervous system, neurotrophins, have been shown to enhance neuromuscular transmission when applied via pre-incubation at the neuromuscular synapse. In the current study, we examine the effect of one of these neurotrophins, brain-derived neurotrophic factor (BDNF), when applied at the motoneuron level on both transmission and synaptic vesicle regulation. Preliminary data suggests that BDNF does indeed enhance neuromuscular transmission in both control and inactivity models, possibly through regulation of synaptic vesicle cycling. Further tests are underway examining expression levels of certain synaptic vesicle-associated proteins.

4:00 pm *Type Specific Diagnosis of Herpes Simplex Virus (HSV) Infections Using Chemiluminescence Immunoassays*

Sarah Duncan, Beth Frenzel (DiaSorin--Stillwater, MN)

HSV-1 infection primarily causes painful blisters near the mouth called cold sores. However, an increasing percentage of genital herpes, 10-15% is caused by HSV-1. HSV-2 infection results in genital herpes with 20% of Americans over the age of 12 infected. Rapid and specific testing for HSV is necessary for patient to begin treatment to diminish viral shedding. A patient's viral concentration of HSV-1 or HSV-2 antibodies can be determined using the fully automated Liaison[®] instrument for chemiluminescence immunoassays. Method comparison with the Focus Diagnostics HerpeSelect[®] Immunoblot kits showed an overall agreement of 96.8% (201/208) with the two HSV-1 assay methods. Overall agreement of 96.8% (201/208) was obtained between the two HSV-2 assay methods. In addition to accurate and reliable determination of HSV-1 and HSV-2 infection, the LIAISON[®] also offers time and cost savings.

4:15 pm *Metal binding specificity in myohemerythrin*
Veronica Taylor, Brandy Russell

Myohemerythrin (myoHr) is an oxygen transport protein containing a di-iron active site found in *Phascolopsis gouldii*. Metalloprotein II is a di-cadmium transport protein found in *Nereis diversicolor* with 80.8% identity with myoHr. This makes MP II an unusual protein for cadmium binding, a metal considered as waste in most biological systems. MyoHr is not found in nature with bound cadmium. Separate iron and cadmium incorporations of myoHr were performed on unfolded apoprotein to assess the ability of myoHr to bind Cd²⁺. Ultraviolet-visible spectroscopy detected successful incorporations of both Fe²⁺ and Cd²⁺. MyoHr is not found with cadmium bound in nature presumably because it has a higher affinity for iron than cadmium.

4:30 pm *The Attenuation of Pro-inflammatory Proteins and Activated Microglia and Astrocytes in Mice with the use of VIVIT Peptide*
Steve Howard, Colin Combs (University of North Dakota)

Brain inflammation is noticeable in neurodegenerative diseases like Multiple Sclerosis, Alzheimer's and Parkinson's and current treatments to suppress inflammation have a multitude of side effects due to the non-specific nature of the drugs. The VIVIT peptide shows potential as a neural inflammation suppressant through the specific inhibition of activation of the transcription factor NFAT. In vitro studies of THP-1 monocytes with inflammatory stimulus and VIVIT treatment showed a transduction of the peptide into the cells with a decrease in pro-inflammatory proteins CCR-5 and Cox-2. In vivo studies on C57BL/6J mice showed that the peptide was able to pass the blood-brain barrier as well as attenuate activated microglia and astrocyte levels in the hippocampus and the substantia nigral regions of the brain.

Session 2B—Nobel 105

3:30 pm *Controlled Release Polymer/Drug Conjugates with Anti-Cancer Applications*
Morgan Wells, Thomas Hoyer and Chris Macosko (University of Minnesota)

A cancer drug taken intravenously typically diffuses homogeneously throughout the body. However, using polymer nanoparticles, drugs may be localized in tumor tissue - leading to a variety of benefits including lower side effects and smaller effective doses. Impingement mixing was used to improve drug loading efficiency to a maximum of 80%. Most importantly, the synthesis of silyl prodrugs allowed for controllable drug release rates via hydrolysis. This drug delivery system may be applied to a variety of diseases.

3:45 pm *Synthesis and Characterization of Crystalline Ruthenium Complexes for Use as Semiconductors*

Chris Leonard, Kent R. Mann (University of Minnesota)

Presently, a bevy of new materials are being investigated as viable alternatives to silicon-based semiconductors. Previous research has shown that crystalline ruthenium complexes with a diimine moiety have tunable electrical properties arising from their unique crystal structures, which may be applicable as a semiconducting material. This project specifically pertains to the synthesis and characterization of bis(2,2,6,6-tetramethyl 3-heptene-3,5-dionate) (1,10-phenanthroline) Ru(II). Theoretically, the crystal packing of this complex should improve upon its previous analogues, optimizing its electrical properties. Preliminary electron mobility tests showed that the thin-film testing device failed to initiate a current, though alternate methods of material application are to be tested in order to conclusively determine the complex's semiconductive efficacy.

4:00 pm *The Effect of Sulfate Addition on Methylmercury Concentrations in a Marcell Experimental Forest Wetland*

Eliza Swedenborg, Jeff Jeremiason

My research was part of an ongoing study in a Marcell Experimental Forest wetland, which lies around 20 miles north of Grand Rapids, Minnesota. A sulfate solution is added to the wetland via a sprinkler system designed to simulate acidic rainfall once in the spring, summer and fall. Water samples are taken before and after the addition. The samples are analyzed for concentrations of methylmercury. Data shows an increase in methylmercury concentrations after the spring and fall additions. Sulfate-reducing bacteria are suspected to be key players in this change.

4:15 pm *The Changing Faces and Spaces of Lutheranism in the Minneapolis Synod*

Brad Utecht, Mark Bjelland

Our analysis of selected ELCA congregations in the Minneapolis Synod displayed the diversity of theology and congregational makeup within the synod. Our research included charismatic, multi-racial, and reconciling in Christ churches which differ from the stereotypical Minnesotan Lutheran Church image. Spatially, the old parish model for church members has been superseded by longer commutes as a result of suburbanization and neighborhood change. Church members tended to commute according to the sectoral model of urban migration, driving from suburbs back into the city along sectoral lines. Not all churches were spatially similar however. Significant differences were found between niche churches and more typical Lutheran Churches. Similarly, demographics within each church's trade area and core varied according to distance from downtown Minneapolis and sectoral differences.

4:30 pm *Preparation and Reactivity of [1.1.1] Propellane*
Joel Rindelaub, Alex Burum, Brian O'Brien

Cubane and similar highly strained hydrocarbons have garnered a great deal of interest in recent years. They have been nitrated to form explosives 30% more powerful than HMX, and added to pharmaceutical compounds, increasing the lipophilicity and degradation time of drugs. We report our initial efforts in the preparation of [1.1.1]propellane, a grotesquely strained, yet kinetically stable molecule. The general synthesis involves a carbene addition to 3-chloro-2-(chloromethyl)-1-propene, followed by a metal-halogen exchange with methylolithium, with characterization by reaction with thiophenol, followed by ¹H NMR and ¹³C NMR. Additional reactions aimed at polymerization or attachment to drugs may lead to advances in materials science and drug design.