Gustavus Adolphus College
Fall Research Symposium Abstract List
Friday, September 17, 2010

Noel Amborn
Karla Marz (Gustavus Adolphus College)

Importance of CRY/PP5 Binding in the Circadian Cycle

Part of the mystery behind the circadian clock is its ability to regulate the rhythms produced by the cycle over 24 hours. The part of the cycle responsible for this regulation are the interlocking loops that interact with the core transcription translation loop. The particular interlocking loop in question is the interlocking loop which results in the phosphorylation and degradation of the PER protein following its dimerization and inhibition of CLOCK and BMAL1. The ability of CRY to bind to another protein, PP5, is suggested to play an important role in delaying the degradation of PER. This is suspected because CRY, when bound to PP5, inhibits PP5's ability to activate CK1. Activation of CK1 is the protein that is responsible for marking PER for degradation and ultimately allowing the core transcription/translation loop to be initiated.

Understanding the underlying importance of CRY/PP5 binding, requires finding the locations responsible for dimerization of CRY and PP5 and disrupting the binding. Eleven separate point mutations previously made to the surface of CRY, were tested using immunocytochemistry. Interpretation of the mutated CRY's ability to bind to PP5 and pull it into the nucleus resulted in the discovery that inaccurate signals were being produced by the antibodies responsible for signifying the location of the PP5. Diagnosis of the source of the inaccuracy was that the primary anti-HA antibody derived from rabbit was non-specifically binding to parts of the mammalian cells as well as the CRY protein produced by the cell. Correction of this problem required creating a new tag for the PP5 protein through subcloning using the Myc vector, and purchasing a new rabbit derived primary antibody.

Justin Anderson
Wei Wu and Patrick Schnable (Iowa State University)

Mapping the Rso Tassel Sterility Gene in Maize

Cytoplasmic male sterility (cms) is a mutation preventing the production of pollen. Maize cms types T, S, and C are an important part of hybrid production unfortunately plagued with disease susceptibility or environmentally induced fertility. In cms-T functional allele (Rf2a) of the *rf2a* gene is linked to fertility restoration. In a normal cytoplasm line, sterility was enhanced in rf2a/rf2a plants combined with some other mutant genes, which we called rf2a significant others (rso). Mapping the rso gene will help us understand the pathway in reproduction system that rf2a is involved. Successful cloning of *rf2a* is complete while the location of rso gene is unknown. This summer a population was

planted controlling for the mutant allele *rf2a*. Study of this population brought about questions of environmental affect on phenotype as well as reiterating the multi-gene hypothesis. This study suggests a two gene linked hypothesis better explains the variety of phenotypes and their ratio than a one gene hypothesis or two non-linked genes.

Hilary Bauer
Seema Kumar, Michael J. Coenen, Susan Demaray, Rebecca S. Bahn (The Mayo Clinic)

Mechanisms of Adipogenesis via PI3-Kinase/Akt pathway in Graves' Ophthalmopathy (GO)

Context: Graves' ophthalmopathy (GO) is an autoimmune disease characterized by increased volume of adipose tissue in the orbit due to enhanced adipogenesis.
Objective: to determine whether TSH or M22 stimulate adipogenesis in orbital preadipocyte fibroblasts from patients with GO and define the roles of Akt and cAMP cell signaling pathways in this process.
Research Design: GO orbital preadipocyte cultures (n=10) were treated for 10 days with bovine TSH (1 or 10.0 U/L) or M22 (1 or 10 ng/ml) in adipocyte differentiation medium. Some cultures were also treated with phosphoinositide 3-kinase (PI3K) inhibitor or an inhibitor of cAMP production. In other experiments, TSH and M22 were added to cultures that were assessed for phosphorylation of Akt (pAkt) or cAMP production.
Results: Increased levels of adiponectin, leptin, and TSHR mRNA were found in GO cultures treated for 10 days with either M22 (mean 2.6 ±0.7 fold; P=0.03) or TSH (13.2 ±5.8 fold, P=0.048). In other studies, both cAMP production and pAkt were stimulated in GO cells treated with M22 or TSH. Inhibition of PI3K activity during 10 days in culture decreased levels of adiponectin mRNA (67 ±12%; P=0.021).
Conclusions: Our results represent a novel finding of autoantibody-mediated adipogenesis via PI3K/Akt signaling.

Bryce Bjork
Greg Ogin

Photothermal Measurements of Optical Coatings for Advanced Interferometric Gravitational Wave Detectors

A possible limiting factor for the sensitivity of future versions of LIGO is thermoelastic damping noise from the thin-film coating of the test masses. This noise is determined by the coefficient of thermal expansion (CTE) and the thermal conductivity (kappa) of the film, and these quantities are often significantly different for materials in thin-film form than for the same material in bulk. We have built an experiment to measure these parameters in coatings using a variant of photothermal displacement spectroscopy, a technique where the sample is heated periodically, and its resulting thermal expansion is measured, as a function of heating frequency, interferometrically. In our experiment, samples are placed in a Fabry-Perot resonator. Pump (heating) and probe (measurement)

beams with orthogonal polarization both resonate in the cavity at the same time. In this talk I will describe how the instrument works and show the data we have obtained.

Prof. Margaret Bloch Qazi
(Gustavus Adolphus College)

Is she wound up? The role of a non-canonical histone on sperm fate in female fruit flies

In most animals with internal fertilization, sperm are retained in the female's reproductive tract between mating and fertilization. Because females can have profound influences on whether or not sperm fertilize their eggs, there is a need to identify female factors influencing sperm fate. The fruit fly, Drosophila melanogaster, is an important model for the study of female influences on sperm fate. A recent screen of female flies for genes implicated in a failure to normally retain sperm in the reproductive tract identified Histone 3.3A (His3.3A). To examine whether or not this gene was necessary for normal sperm retention, His3.3A transcripts were substantially reduced in females using RNA interference. Females with reduced levels of His3.3A show defects in sperm retention relative to controls indicating that this gene is necessary for normal sperm retention by females. The implications for sperm fate and female reproduction will be described. Future experiments and opportunities for student research projects will also be shared.

Spencer Bonnerup
Dwight Stoll (Gustavus Adolphus College)

Development of Rapid LC/MS/MS-based Methods for Confirmatory Analysis of Opiates and Benzodiazepines

Increasing case loads and budget and staffing cuts in forensic laboratories continue to motivate the development of higher throughput methods, particularly for confirmatory analysis of regulated intoxicants. In this work, we have focused on the development of rapid LC/MS/MS methods for the determination of nine opiates including two glucuronide metabolites, and 16 benzodiazepines, including two amino- metabolites. Current approaches in use in forensic laboratories often involve the use of multiple methods because of the large range of hydrophilicity presented by these groups of compounds (e.g., parent drugs and polar metabolites). Here we aim to analyze both the parent compounds and important polar metabolites in a single analysis. To this end we have compared the retention of the target compounds on three different reversed-phase HPLC stationary phases: a conventional C18 type phase, a perfluorinated phenyl (PFP) phase, and a mixed-mode reversed-phase/weak cation-exchange phase. The latter two phases exhibit significant cation-exchange behavior for the compounds studied. Furthermore one of these phases (PFP) is built upon the increasingly popular shell particle morphology which presents significant opportunities to improve the speed of analysis. We see that the two mixed-mode phases not only generally exhibit higher

retention than the C18 type phase, but also exhibit very different selectivity such that the nine opiates can be nearly completely resolved in under four minutes. We find that the mixture of 16 benzodiazepines cannot be completely resolved in a reasonable (i.e., less than 20 min.) time, however we have developed a gradient elution retention model for these compounds that facilitates the development of a separation with no more than three overlapping peaks in an analysis time of five minutes.

Alex Chubick
Karla Marz (Gustavus Adolphus College)

The Differences Between CRY1 and CRY2 and Their Role in Circadian Rhythms

Circadian rhythms are 24-hour rhythms that help regulate biological processes. The core mechanism driving circadian rhythms is a negative feedback system found within the cell. Within this system the protein known as Cryptochrome (CRY) plays an important role, and in mammals there are two types of CRY, CRY1 and CRY2. These two CRYs have opposite effects on the lengths of circadian rhythms. In an effort to understand which structural differences between them are important, CRY 1/2 hybrid mutants were developed in which CRY1 contained different CRY2 residues substituted for its own. It was found that these mutations reduce CRY's activity because the mutations inhibit CRY's ability to localize to the nucleus. To see if the mutations had any other effects, the mutant CRYs were given nuclear localization sequences (NLS) to allow the protein to enter the nucleus. The NLS tag appeared to restore activity to some of the mutants, but the activity of other mutants remained unchanged. These results suggest that the region that was mutated on CRY1 has a role in addition to nuclear localization. Before any conclusions can be made about these results further tests must be performed.

Dawn Comstock
Michael D. Bradley and Roy D. Welch (Syracuse University), Garret Suen (University of Wisconsin-Madison), Kimberly A. Murphy (Gustavus Adolphus College)

An investigation of one-component regulators in *Myxococcus xanthus*

When deprived of nutrients, *Myxococcus xanthus* initiates a complex developmental program that allows large groups of cells to migrate to aggregation centers and begin building multicellular fruiting bodies. Once a fruiting body is molded into its final shape, individual rod-shaped cells within this structure differentiate into dormant, spherical-shaped spores that are resistant to many forms of environmental stress. The multicellular behavior of development remains the most intensely studied area in *M. xanthus* biology, and almost no research investigating the role of one-component regulators has been reported. We interrogated the *M. xanthus* genome to compile a list of open reading frames corresponding to one-component regulators. Plasmid-insertion mutants were made for a subset of these genes, and the mutants were assayed for motility and fruiting body development. Among the mutants, five showed defects in motility or fruiting body

development. We are currently disrupting the remaining one- component regulators with the goal of generating and analyzing plasmid-insertion mutants for all one-component regulators of *M. xanthus*.

Eric Cronin
Bob Douglas (Gustavus Adolphus College)

The Irish in Olmsted County: A Preliminary Study

This study focuses on one of Olmsted County's earliest ethnic groups: the Irish. It involves seeking answers to some basic questions, such as what was the population distribution of early immigrants to the county and where did they settle? Using the 1860 and 1870 Federal Manuscript Censuses and the 1868 County Plat Map, some general conclusions were drawn:
First, townships that had a large number of Irish settlers in 1860, namely, Rochester, High Forest, and Marion continued to expand their numbers in 1870. Whereas townships that began with a small Irish settlement in 1860 continued in 1870 to remain relatively small.

Second, there was a geographically un-even distribution of Irish settlement. That is, some townships had many more Irish than others. The greatest concentration of Irish, or the "Core Area" was immediately south and east of the city of Rochester in the townships of Rochester, High Forest, and Marion.

Michael Davidson, Christian DeMarais, Samuel Grace, and Henry MacCarthy
(Gustavus Adolphus College)

Thrill Me: The Story of Leopold and Loeb, a musical directed by Gustavus Adolphus College Assistant Professor of Theatre and Dance Henry MacCarthy will be performed at the Lowry Lab Theater in St. Paul beginning Thursday, Sept. 16. Performances are scheduled for 8 p.m. Sept. 16, 17, and 18 as well as a 3 p.m. matinee performance on Sept. 19. The production of this performance was supported by a Gustavus Adolphus College Presidential Faculty/Student Collaboration Grant in summer 2010. The cast and directors will present a talk about this project.

Quentin Ebner
Rajan Murgan (Gustavus Adolphus College)

A Numerical Approach to Heisenberg's Anisotropic Spin Chain

An important quantum model in physics that is exactly solvable, known as Heisenberg's anisotropic spin-1/2 chain is reviewed, in particular its construction. Its spectrum (energy levels) is investigated using the well known Baxter $T - Q$ equation of the model. This is carried out by exploiting a numerical method known as McCoy's method. Two-fold

degeneracies of the energy levels are explained using the results obtained from numerical studies.

Ian Gibbs-Hall
Dwight Stoll (Gustavus Adolphus College)

Carbon Based Stationary Phase Characterizations: For Applications in Extreme Separations

Despite a long history (ca. 30 years) of use in gas and liquid chromatography, carbon-based stationary phases are poorly understood and continue to suffer from several fundamental shortcomings. A relatively new member of this family of materials, Carbon-Clad Zirconia (CCZ), differs from the more well known Porous Graphitic Carbon (PGC) in that the underlying zirconia plays a significant role in the retention of certain classes of compounds. The goal of this work is to quantify the role of the zirconia surface chemistry in the retention of cationic compounds in particular. Plots of the retention factor (k?) of protonated amines against counterion concentrations are a useful tool for quantifying the ion exchange and reversed phase contributions to retention in a given column. This method was used on varying CCZ columns with amine-containing analytes and the results show a great amount of ion exchange interactions occur on CCZ materials having a low coverage of the zirconia surface This work shows that both reversed-phase and ion exchange contributions to retention are very important when using the CCZ material, particularly for the separation of hydrophobic cations, and that the effects are so strong that the material is not practically useful for the separation of highly hydrophobic amines.

Steve R. Groskreutz
Dwight R. Stoll (Gustavus Adolphus College)

Development of Hybrid Multi-Dimensional High Performance Liquid Chromatography for Fast, Targeted Trace Analysis in Complex Matrices

In this work we describe the development of hybrid multi-dimensional HPLC (HMDLC) for targeted analysis of low-level constituents in complex matrices. Currently MDLC is separated into two fundamentally different separation modes: comprehensive, which focuses on 10s to 100s of compounds, and heartcutting , which is a targeted approach. Comprehensive separations take each portion of first dimension effluent and send it for further separation on the second dimension column in multiple 20-30 second slices. Heartcutting methods take the small region of first dimension chromatogram containing the compound of interest and send one similar sized portion to successive columns for further separation.

The hybrid method discussed here is a combination of both methodologies by taking six 1 to 5 second slices of the target analyte peak and performing a 15-30 second separation on each slice in nearly real time in the next dimension. The principle aim of

this work is to show by comprehensively separating the heartcut portion of the first dimension chromatogram multiple advantages arise in terms of analyses of multiple compounds, data analysis, and ease of use. We will present initial results of the application HMDLC to the analysis of phenytoin and triclosan in wastewater treatment plant effluent.

Mandy Halfen
Brandy Russell (Gustavus Adolphus College)

Cloning of Myohemerythrin and Metalloprotein II from *Nereis diversicolor* and Metal Binding in *Phascolopsis gouldii*

Found in several sipunculid and annelid worms is an iron binding protein called myohemerythrin (myoHr). One species of annelid, *Nereis diversicolor*, contains another very similar protein, Metalloprotein II (MPII). MPII is different from MyoHr in that it binds one cadmium atom instead of two iron atoms. To determine the specificity of metal bind in these proteins, the iron atoms in MyoHr from *Phascolopsis gouldii* were removed and then iron or cadmium were incorporated back into the protein. ICP-MS and Uv-vis spectroscopy were used to determine the ratio of the reconstituted metal to the protein. Our data suggest that both metals reincorporate at a lower ratio than found in the native protein. Progress was made on obtaining MyoHr and MPII from *Nereis diverisicolor* for use in this type of study. The genes that code for these proteins were cloned and the process of purification was started, but further identification of the purified protein is necessary before we can begin to use them in future reconstitution studies.

Josh Hammer and Carl Stenoien
Joel Carlin and Eric Elias (Gustavus Adolphus College)

Rupert Anderson Award in Systematics: Enhancement of Gustavus Adolphus Vertebrate Museum's Freshwater and Marine Fish Collection

Steps were taken to improve and preserve the Gustavus Freshwater and Marine Fish Collection using funds provided by the Rupert Anderson Award in Systematics. At the onset of the project, the collection included mislabeled jars, minimal taxonomic organization, and outdated nomenclature, deeming the collection less accessible to faculty and students. The Rupert A Anderson Award, which funds collection improvement efforts, supported two Gustavus students in their efforts to enhance the collection to a working level. We gained experience in curation, fish identification, and technical skills related to web-based databases through our work with this collection and a visit to the University of Minnesota's Bell Museum. Accomplishments include the creation of a master list and map of collection specimens, the creation of a web-based digital archive of some specimens, the reorganization of specimen shelves, standardized jar labeling, and other efforts to better preserve existing specimens. These improvements

made this collection more useful for current and future classroom use, as well as potential research endeavors.

Kristen Jahr
Scott Bur (Gustavus Adolphus College)

New approach to discovering drug to protein interactions for pharmaceuticals

Drug research typically requires synthesizing a whole library of compounds to screen against one known defective receptor or protein in the human body; however, by instead allowing the cells to make a library of proteins to screen against one known biologically active compound, we can eliminate time and resource consuming organic synthesis and therefore speed up the process of drug discovery. This specific project focuses on the interactions between proteins and the drug nitrofurazone, an anti-bacterial. By synthesizing a derivative of this drug, attaching it to an affinity chromatography column, and running proteins from lysed cells through, we can discover how this drug interacts with proteins and find other possible uses for this drug. Preliminary chromatography runs have yielded inconclusive results; therefore, further work is required on this project.

Zainab .O. Jaji
Brandy Russell (Gustavus Adolphus College)

Unfolding studies of Myohemerythrin (Myohr) from *Phascolopsis gouldii*

During the summer, I studied the folding process of Myohemerythrin (Myohr) through its unfolding. The main purpose of studying the unfolding process of this protein it to hone our understanding on the relationship presence of certain metals in a protein and the stability it confers to the protein structure. An unfolding experiment was designed to observe this process.

In the experiment, various concentrations of guanidine hydrochloride (GdHCl) were added to a fixed concentration of Myohr. The samples were incubated and the fluorescence spectra were taken. An unfolding curve of the plot of intensity against concentration of GdHCl was generated. This was gotten based on the analysis of the emission intensity of each spectrum at 350nm. The curve showed two sigmoidal transitions that represent the stages in protein unfolding.

Based on previous studies from circular dichroism, UV/Vis and present findings, the 1^{st} transition is suggested to be due to smaller conformational changes while the 2^{nd} transition is due to the disruption of the metal sites and the unfolding of the alpha-helices in Myohr.

Ben Johnson-Tesch
Brenda Kelly (Gustavus Adolphus College)

Insights into Irreversible Binding of Buthionine Sulfoximine and its Derivatives to γ-Glutamylcysteineligase

γ-Glutamylcysteineligase (γ-GCL) is an important rate-limiting enzyme in the biosynthesis of glutathione, a biological molecule implicated in chemotherapeutic resistance. Transition-state analogs like Buthionine sulfoximine (BSO) and its derivatives (Methionine sulfoximine, Ethionine sulfoximine, Pentathionine sulfoximine, and Hexathionine sulfoximine.) are hypothesized to be irreversible inhibitors of γ-GCL, yet little is known about the how or where these compounds bind to γ-GCL. Using two-system pre-incubation assays, we have determined that these sulfoximine inhibitors are binding irreversibly to γ-GCL in the glutamate region of the active site. We have also determined the rate constant for the irreversible covalent modification of each sulfoximine and found the rate to be dependent on both the size and stereochemistry of the sulfoximines. Future plans include detecting conformational changes in the enzyme active site and metal binding site upon sulfoximine binding using fluorescence spectroscopy.

Courtney Kerestes
Scott Bur (Gustavus Adolphus College)

Intramolecular Diels-Alder Reactions of Silyloxyfurans

The synthesis of a fused aromatic and lactone ring by way of a cascade Diels-Alder reaction was explored in order to replicate precursors of biologically active molecules. The methodology of intramolecular Diels-Alder reactions using varied silyloxyfurans and dienophiles was studied, with emphasis on the deprotonation of the furan. After deprotonation, benzaldehyde was added, followed by a fumarate derivative that would give the Diels-Alder precursor, which then was expected to spontaneously undergo the Diels-Alder reaction. A monomethyl fumaric chloride was synthesized to make one end accessible for the addition to the furan and the other end activating for the Diels-Alder reaction. After this methodology is solidified, different furans, aldehydes, and fumarates will be used to expand this scheme and make use of its ability to create highly substituted aromatic rings.

Rima Kharbush
William Walters, Christian Lauber, and Rob Knight (University of Colorado at Boulder)

Development of the Microbiota in Newborn Mammalian Species

The microbes associated with the mammalian body play a critical role in a host organism?s health. Recent advances in sequencing and computer technology have

facilitated the analysis of these microbial communities at an unprecedented level. In human adults, microbes associated with different body parts (i.e. the gut, mouth, skin) are distinctly different from one another; however, in newborns microbial communities appear undifferentiated. These discoveries leave us with an incomplete picture of how the microbial developmental process occurs following birth and how various factors affecting this process may later affect the host organism's health. This study was designed to track the development of microbes associated with various mammalian species over time starting at birth. Samples were collected from different body sites on newborn mammals as well as from their mothers and unrelated individuals. Microbial DNA was extracted, amplified with tagged primers, and sent for sequencing. Data will be available in the near future, hopefully providing preliminary information regarding patterns in the development of the microbiota over the first two weeks of life. Further work on this study is ongoing in the hopes of increasing our knowledge base of the role microbes play in human health and disease patterns.

Amber Kirk
Chanakha Navaratnarajah and Roberto Cattaneo (The Mayo Clinic)

Mapping the dimer-dimer interface of the measles virus hemagglutinin tetramer

The measles virus hemagglutinin (H) protein binds to specific cellular surface proteins thereby triggering fusion of the virus with the cell membrane via the fusion protein. The structure of the H-protein, determined by X-ray crystallography, exhibits a dimer arrangement. However, using Blue Native polyacrylamide gel electrophoresis (BN-PAGE), we have shown that the H-protein exists as a tetramer. Subsequently, we used molecular modeling to generate six possible tetramer structures. The aim of this study was to determine if one of these generated poses is a good model for the native H-protein tetramer structure. To discriminate between the models, we mutated key amino acids involved in the different proposed interfaces using site directed to disrupt the dimer-dimer interaction. We developed a protocol to use BN-PAGE to rapidly determine the oligomerization state of each mutated H-protein. Fusion assays were used to determine any functional effects caused by the mutations. 14 different single amino acid substitutions were investigated and showed no significant effect on H tetramer formation or fusion function. It was hypothesized that disrupting a single amino acid involved in the dimer-dimer interface may not have a significant impact on the stability of the tetramer. Therefore 11 double amino acid substitutions were created to target two amino acids involved in the same interface, and we are currently determining effects on tetramer formation and function. While we have developed a robust assay to determine the oligomerization state of the H-protein, our mutagenesis has not yet identified the best model of the dimer-dimer interface.

Michelle Kirkvold
Scott Bur (Gustavus Adolphus College)

Intramolecular Diels-Alder Reaction in Silyloxyfurans

Our goal this summer was to understand the behavior of silyloxyfurans while they undergo the intramolecular Diels-Alder reaction and to use this information to create a pathway to synthesize biologically active molecules. The Diels-Alder reaction is a pericyclic mechanism that creates a six-membered ring from a conjugated diene and a dienophile. The key structure we were working with includes a core furan, a protecting silane group, and a variable carbon chain. The retrosynthetic reaction scheme includes the Diels-Alder mechanism to form the three ring system containing nitrogen, which would hopefully have biological activity.

Matthew Klun and Garrett Stoddard
Steve Miller (Gustavus Adolphus College)

Spectroscopic and Computational Characterization of Transition Metal-Pyrazole Complexes

A series of aqueous first row transition metal-pyrazole complexes were made and characterized. UV-Vis spectra were collected for each complex at varying metal:ligand ratios to determine the ratio at which the ligand sphere of the metal is saturated. Fluorescence and concentration dependent UV-Vis and spectra were then collected for the coordinatively saturated aqueous complexes. Infrared spectra were collected for the complex-chloride salts for comparison to the bare ligand. Finally, computational methods were used to examine the metal-ligand bond for the copper (II) complex. Our results indicate that ligand binds to the metal in a monodentate, s fashion through the non-hydrogenated N atom of the ligand.

Laura Leland
Jeff Dahlseid (Gustavus Adolphus College)

Investigation of mRNA Degradation by the Exosome in S. cerevisiae

Post-transcriptional regulatory mechanisms of gene expression are an interesting and important field of study. By better understanding how these pathways function in baker's yeast we can better identify when errors occur, which would be significant for future cancer research. One example of a post-transcriptional regulatory mechanism is to regulate mRNA levels, which in some cases is mediated by a protein complex called the exosome that degrades targeted mRNA. Previous studies in the Dahlseid lab have found that the exosome indirectly affects the expression of a specific gene called *CTF13*, which has an important role in mitosis. Our goal is to determine the steps in the *CTF13* regulatory mechanism by identifying a possible intermediate, presumably a transcription

factor, which is targeted by the exosome. This can be done by engineering yeast to display an identifiable phenotype when *CTF13* mRNA production is promoted. We can then introduce possible transcription factors and assess the produced phenotype to determine whether they promote *CTF13* mRNA production. This summer we created an insertable reporter gene to provide for the desired phenotype and current work is aimed at introducing it in yeast to create a reporter strain for use in identifying intermediate(s).

Jiawen Li
Brenda Kelly (Gustavus Adolphus College)

Identification and Monitoring of γ-Glutamylcysteineligase Inhibition in Cells

Glutathione (GSH), an intracellular reductant, acts as a detoxicant in cells in both mammalian and bacterial systems. Previous research has shown that GSH is overexpressed in some human tumor cells, thus it is implicated in chemotherapeutic resistance. Therefore, studies related to GSH production may be informative for therapeutics. One approach to alter glutathione production is through inhibition of γ-Glutamylcysteineligase (γ-GCL), the enzyme that catalyzes the rate-limiting step of GSH biosynthesis. Within a purified *E.Coli* γ-GCL enzyme system, four molecules (D-Ethionine, D-Cysteine, L-2,4-diaminobutyric acid and L-2,3-diaminopropionic acid) have been found to significantly inhibit γ-GCL activity. Future plans include application of these inhibitors to Mouse macrophage cells to determine the resulting GSH levels in *vivo*. GSH levels in cells will be detected using Liquid chromatography-mass spectrometry (LC/MS).

Steve Lundberg and Keisha Bates
Colleen Jacks (Gustavus Adolphus College)

Tying the Knot: Providing Connections Between General Biology and Chemistry

In 2008, Gustavus received a grant from Howard Hughes Medical Institute with an overarching theme of improving the connections between biology and chemistry. In order to do this, new laboratory and classroom curriculum needed to be developed. One new chemistry and four new biology labs were designed and created. The labs for biology included a microscopy lab involving algae lab, a cell organelle lab, an ecology lab involving a field trip to the arboretum, and a respiration lab using Vernier probes to quantify. The lab for chemistry was a spectrophotometric determination of phosphorus concentration. These labs were written up and included in the 2010 lab manual. Additional laboratory ideas were written up to further the laboratory development in future years. Classroom unity between the two courses was expanded through supplementation of the existing model. A repository of case studies was created and made available to all the BIO-101 and CHE-107 professors. These case studies were enhanced with primary literature, videos, websites, and other additional resources that help establish a connection between biology and chemistry. Through the creation of new

labs and supplemental classroom material, it is the hope that students will experience a clear tie between biology and chemistry.

Daniel McDougall
Jessie Petricka (Gustavus Adolphus College)

Designing and Building an Ion Trap

My presentation will address the work done over the summer on building and designing an ion trap lab with Professor Jessie Petricka. The theory behind ion trapping and mass spectrometry will be given as background, and then the designing and building process will be explained. The experimental setup will be described, and finally, some results from the end product will be shown. The apparatus is used to trap ions of a particular mass, which will be used in the future for dealing with cooling of molecules down to supercool temperatures. This new lab will be used in the future for students at Gustavus to work further with Jessie on these new projects.

Daniel Mellema
James Greenleaf (Mayo Graduate School)

Modal Analysis of Shearwave Propagation in Arteries and Tube-like Structures

It has previously been shown that the elasticity of kidney, liver, heart and skeletal muscles can be determined through Shearwave Dispersion Ultrasound Vibrometry (SDUV). This method determines the elasticity of the material by utilizing the variation of the shear wave velocity with frequency, or dispersion, and approximating the material as a finite plate. Adapting this technique to arteries, the propagation speed of shear waves through cylindrical tubes was analyzed using two theoretical models for different cases of fluid coupling.

Zhang modeled tube-like structures as an isotropic medium with internal fluid coupling using first-order deformation theory, which approximates the tube as a finite plate. The Berliner model takes into account fluid coupling for internal and external fluid approaches the cylindrical model from the constitutive equations.
The theoretical results were compared to experimental data previously collected using SDUV as well as Finite Element Method (FEM) models for both latex tubes and excised pig carotid arteries. The theoretical model validated the experimental results for the elasticity of tubes but had some discrepancies in the cases for arteries. The effects of internal fluid pressure were examined by observing the effect on the elasticity. Future work will involve adapting the current models for pressure loading.

Audrey Messelt
Scott Bur (Gustavus Adolphus College)

Synthesis of Biologically Active Molecules via 1,5-electrocyclization

Heterocyclic compounds make up 67% of the compounds listed in the Comprehensive Medicinal Chemistry (CMC) database, nitrogen-containing rings composing a large number of these. New, more efficient ways of creating these compounds opens up a wide range of possibilities for synthesis of natural and medicinal products. A 1,5-electrocyclization reaction may have the potential to create nitrogen-containing heterocycles. Electrocyclization, a type of pericyclic reaction, converts pi to a sigma bonds and forms a ring. By using an amine, in the case of this synthesis a phthalimide derivative, as starting material, a five-membered nitrogen-containing ring is formed by electrocyclization—the end product being a polycyclic heterocycle. Proof of viability of this synthesis opens up new possible pathways for the making of biologically active products with a similar core structure.

Linnea Schmidt
Mi-Jeong Woo and Jonathan Wendel (Iowa State University)

Expression patterns of *METACASPASE 9* in Wild and Domesticated Cotton

Domesticated cotton fibers share little resemblance with wild cotton fibers as the former are long and white and the latter are short and brown. The genomic sequences of the wild and domesticated cottons are quite similar; therefore, the differences in fiber morphology are likely due to the variable levels of expression of different genes. We chose to examine the expression levels of *METACASPASE 9 (MC9)* in the wild and domesticated forms of *Gossypium hirsutum* as this gene is known to be highly up-regulated in all domesticated cottons relative to their wild counterparts. This is indicative of parallel domestication yielding a similar pathway to enhance fiber quantity and quality. In addition, *MC9* is involved in oxidative response, which has been shown to increase fiber elongation. Cloning of *MC9* was successful and primers sets for the three putative *MC9* genes successfully amplified the wild and domesticated *G. hirsutum* DNA and cDNA. Future work would include quantifying *MC9* in *G. hirsutum* and examining the expression levels of *MC9* in other domesticated cottons, *G. barbadense* and *G. arboreum*.

Laura Secor
Dwight Stoll (Gustavus Adolphus College)

Towards the development of a three-dimensional HPLC method for the quantitation of estrogen hormones and derivatives

Natural and synthetic estrogens, a class of endocrine-disrupting chemicals, have been shown to have potent effects on human and animal reproductive systems at

concentrations of a few ng/L. The study of these effects requires reliable quantitation of the relevant compounds, which is especially difficult in a matrix as complex as seawater. In this work we describe progress toward the development of a three-dimensional (3D) liquid chromatography tandem mass spectrometry method for the rapid, selective, and sensitive quantitation of common natural and synthetic estrogens, as well as their sulfate and glucuronide conjugates. The 3D separation technique requires the use of three columns exhibiting different interactions with the target analytes. Thus far we have evaluated the retention properties of three different reversed-phase materials for a group of four neutral steroids and two sulfate conjugates. We find that the three columns – a pentafluorophenyl phase, a carbon-based phase, and a conventional C18 phase – exhibit separation selectivities that are sufficiently different to be useful in a 3DLC separation of steroids and steroid conjugates from complex matrices. These initial data will be used in ongoing work to optimize the separation of the target steroids from real samples.

Noah Setterholm
Scott Bur (Gustavus Adolphus College)

Factors affecting the rate of Diels-Alder reactions involving 2-trialklysilyloxyfurans.

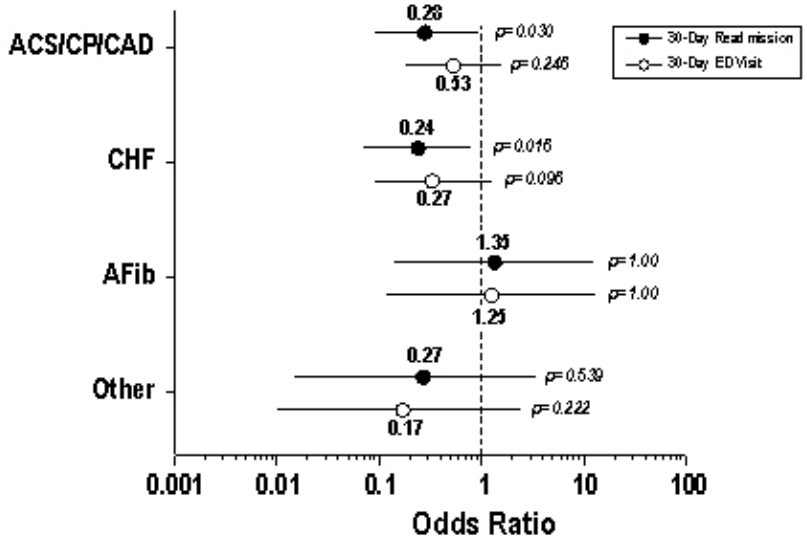
Increasing the size of the silyl groups on 2-trialkylsilyloxy furans and the ester groups on maleate dienophiles reduces the rate of Diels-Alder reactions. While the exo-adduct resulted from the reaction between 2-silylxyfurans and maleic anhydride, endo-adducts resulted from the reactions with maleate esters. Analysis of transition state structures for the cycloaddition, calculated at B3LYP/6-31G*, revealed significant asynchronicity in the forming bonds, with selectivity arising from steric interactions that effect torsional strain about the shorter bond.

Cydni Smith
Sherry Bumpus, Eva Kline-Rogers, Adam Kosteva, Cydni Smith, Daniel Montgomery, Kim A. Eagle, James Froehlich, Melvyn Rubenfire (University of Michigan)

A Nurse Based BRIDGE Transitional Care Model Reduces Readmissions and ED Visits poster

Introduction: Readmissions and emergency department (ED) visits are common and costly. A pilot study of a nurse practitioner based intervention, participation in the BRIDGE clinic reduced 30-day readmissions and ED visits.
Methods: A retrospective study was conducted in all patients referred to BRIDGE, from June 2008 to February 2009. We compared patients who attended BRIDGE and those who did not, in terms of age, diagnoses, comorbidities, time to follow-up visit with a cardiologist, unplanned readmissions, and ED visits.
Results: Of 342 patients referred to the BRIDGE clinic, 230 (67%) attended. Patients who attended the BRIDGE clinic were less likely to be readmitted or seen in the ED than those who did not (readmit odds ratio 0.34, 95% CI 0.18-0.68; ED 0.42, 95% CI 0.21-

0.82). With the exception of AFib, attending the BRIDGE clinic was associated with a significant decrease in rehospitalizations and ED visits (see figure).



Conclusion: The BRIDGE clinic is an effective model for improving care across the hospital-to-home transition for a variety of cardiac conditions. Patients with acute cardiac diagnoses who attended the BRIDGE clinic were less likely to be readmitted in 30 days or be seen in the ED.

Carl Stenoien
Patrick Crumrine (Rowan University)

Larval Odonate Predation on Free Swimming Cercariae: Effects of Predator Density, Predator Diversity, and Prey Type

Recent studies show that parasites represent large amounts of biomass in some ecosystems and may play significant roles in food webs, ultimately affecting the dynamics of communities. Non-host predation of parasites may indirectly benefit hosts by reducing the abundance of parasites in the environment. This study used three species of larval odonate predators and two species of trematode cercariae prey as a model system for investigating non-host predation. Predator type, predator density, predator diversity and prey type were manipulated in short term microcosm trials. Most predator treatments resulted in significantly greater cercariae mortality than controls. High predator density treatments resulted in less mortality than predicted by a multiplicative risk model, especially with small, less active prey items. There was also a significant difference in mortality between the two cercariae species with larval odonates consuming more large cercariae prey. In the high predator density treatments, it is likely that interactions between odonate predators, such as interference competition, reduced per capita cercariae consumption. However, there was an interaction between predator density and prey type suggesting that competitive interference was lower with larger cercariae prey. This research suggests that odonate predator density may impact

transmission of trematode parasite from snails to amphibians and may have important applications for amphibian conservation.

Kimberley Sukhum
Peter Hoch (Washington University)

The effects of stress conditions on floral and vegetative growth in endemic glade species

Glades are unique habitats in the Ozarks in Missouri. Certain species of flora are found only on these glades. Because of habitat loss, it is important to understand why these species are restricted to glades in order to help preserve them. Glade habitats have more exposed rocks, higher temperature, thinner soils, and faster runoff than their neighboring forest area. This makes them more stressful habitats. We studied how the endemic species (i.e., *Delphinium treleasei, Echinacea paradoxa, and Scutellaria bushii*) are better adapted to the abiotic factors of the glades than a more widespread closely-related taxon (i.e., *Delphinium carolinianum, Echinacea pallid*, and *Scutellaria ovata,* respectively), and whether a trade-off between vegetative and floral traits existed in the stressed environment. This was done through greenhouse and growth chamber experiments, in which each species was grown in control and high-stress conditions, specifically low precipitation and higher temperature. Vegetative and floral traits were compared within and across genera. For both traits, the endemic species were not less affected by the stressful conditions in comparison to their widespread sister-taxa. These results lead to more questions about the mechanism that may affect the range restriction of these endemic glade species.

Jamison Utzig
Jeff Jeremiason (Gustavus Adolphus College)

Mercury in Itasca County Lakes

Total mercury and methylmercury in water were analyzed in selected lakes from Itasca County. The goals of this project were to assess variability of mercury levels in these lakes and to develop a method to analyze methylmercury with the Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). Samples from the lakes were generally collected 3-4 times in 2008 and 2009. Mercury levels tended to by highly variable within lakes, but generally the larger lakes exhibited lower total mercury concentrations than the smaller lakes. Methylmercury trends within and between lakes could not be assessed as only a few samples were analyzed. Most of our time was spent developing a method for the ICP-MS. In the new method, methylmercury and other forms of mercury are ethylated creating volatile mercury species that are trapped on a polymer. The ethylated mercury species are released from the polymer, separated via gas chromatography, and introduced directly to the ICP-MS. We found that analysis time could be reduced with this method and in the future the method will be further refined by using isotopically-labelled methylmercury as an internal standard.

Brian Welch
Bill Soeffing (University of Sioux Falls)

Examination of denitrification and biofilm development by *Pseudomonas aeruginosa* (ATCC? 27853) incubated in agitated and static conditions

Denitrification is an energy-efficient metabolic pathway for *Pseudomonas aeruginosa* population growth and maintenance. Biofilm formation in high population densities allows for an anaerobic microenvironment in which metabolic denitrification is favored. The reduction of nitrate (NO_3-) to nitrite (NO2-) in *P. aeruginosa* cells subjected to static and agitated incubation conditions, with regard to biofilm development, was examined in this study. *P. aeruginosa* (ATCC? 27853) was incubated at 37^{o}C and tested on an hourly basis for the presence of nitrites. Rates of denitrification, as well as nitrite concentrations, were shown to be greater in cells subjected to static conditions with biofilm development. Furthermore, cellular aggregates forming under agitated incubation conditions may be responsible for nitrate reduction in the absence of biofilm formation.

Xiu Xiao
Jeff Dahlseid (Gustavus Adolphus College)

Post Transcriptional Regulation of Metalloprotein II

Nereis diversicolor (hagworms) has been shown to live in sediments contaminated with toxic metals at levels that are usually inhospitable to biological organisms. *Nereis diversicolor* might be able to survive under these conditions, at least in part, because of the function of Metalloprotein II (MPII) in the worm's gut. Demuynck *et al.* (2004) observed that MPII binds the heavy metal cadmium and that in the presence of cadmium, MPII protein levels increased while MPII RNA levels stayed the same. Metal binding may confer resistance to cadmium in the environment. Furthermore, these observations suggest that when the worms are exposed to cadmium, the translation or stability of MPII increased. If translation is increased, it might suggest that MPII is a regulator of itself. We are engaged in studies to verify the functions of MPII using *Saccharomyces cerevisiae* (Baker's yeast) as a model system. We aim to test MPII's resistance to cadmium by measuring growth of yeast with MPII gene on different concentrations of cadmium. In the future we will explore if and how MPII is regulated by translation or protein stability when it's exposed to cadmium, and measure RNA and protein levels.