CHEMISTRY
GUSTAVUS ADOLPHUS COLLEGE

Chemistry Homecoming Research Symposium, October 1, 2011
Poster Abstracts

Current Gustavus Students

Spencer Bonnerup, 2012: Development of Rapid LC/MS/MS-based Methods for Confirmatory Analysis of Opiates and Benzodiazepines
Spencer Bonnerup, 2012, Tomas Liskutin, 2009, Jonna Berry, 2010, Christopher David Harmes (Gustavus Adolphus College), Kathryn Fuller*, and Dwight Stoll (Gustavus Adolphus College)
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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.

Megan Crow, 2014 and Maja Johnson, 2013: "Photodegradation of the Herbicide Imazethapyr on Corn and Soybean Wax"
Megan Crow, Maja Johnson, and Amanda Nienow (Gustavus Adolphus College)

Imazethapyr is an herbicide commonly used in the Midwestern U.S. on corn and soybean crops. Previous studies have observed photolysis as one of the major pathways of environmental degradation. The goal of this study was to determine the rate of degradation of imazethapyr on plant waxes, considering that the herbicide is applied directly to crops in the field. Imazethapyr solutions were irradiated on glass, aqueous, corn wax, and soybean wax surfaces under simulated UV light and analyzed using HPLC. The imazethapyr degraded the fastest on the aqueous and glass surfaces. The soybean wax was notably slower, and the corn wax showed little degradation. This suggests that the composition of the plant wax has a significant slowing effect on the degradation rate of imazethapyr. Future work will include further characterization of the waxes to understand the reason wax affects degradation, as well as studying degradation of imazethapyr on plant leaves.

Chris Gloede, 2012 Ben Johnson-Tesch, 2012, and Amber Kirk, 2011: "Development of Plasmid Shuffling in *Saccharomyces Cerevisiae* for Mutant Respiratory Enzyme Analysis"
(from Heather Haemig's Spring 2011CHE-360 Proteins course)

Saccharomyces cerevisiae, also known as budding yeast, is a well-known eukaryotic model organism for phenotypic analysis of mutant genes. However, nuclear deletions of respiratory pathway genes in *S. cerevisiae* lead to the shedding of mitochondria, or a Rho- phenotype, which can complicate mutant analysis. We have developed a system for introducing mutant respiratory genes on a plasmid while maintaining the Rho+ phenotype of *S. cerevisae*. Specifically, we created an Ampr/Trp tagged plasmid in which a desired fum1 allele can be easily inserted for phenotypic analysis. This plasmid can be transfected into Rho+ yeast which contain nuclear fum1 deletions and a second plasmid containing the wild type fum1 gene necessary to maintain the Rho+ phenotype. Multiple selection techniques were employed to screen for Rho+ yeast with only the desired version of the fum1 gene. Upon confirmation that we have a successful plasmid shuffle system, we hypothesize that we can introduce fum1 mutants into Rho+ yeast without the difficulties previously encountered in developing the yeast strains needed for phenotypic analysis. Ultimately, implementation of plasmid shuffling will further our ability to study respiratory pathway mutations in a eukaryotic model system.

Carrie Johnson, 2014: "Total Synthesis of Biologically Active Natural Products: Amathaspiramides"
Carrie Johnson, Arash Soheili,* and Uttam Tambar*
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In the field of drug research and development, the study of natural products often leads to the discovery of pharmacophores for new drugs. A pharmacophore is the part of a molecular structure that is responsible for the biological action of a drug. In this work our goal was to develop an enantioselective synthetic strategy to yield the natural product Amathaspiramide A, a polycyclic brominated alkaloid which has been shown to have antimicrobial, antifungal, and cytotoxic activity. The synthetic approach utilized a palladium-catalyzed [2,3]-sigmatropic rearrangement as a key step where a variety of carbonate analogs and proline derivatives were the reactants. The sigmatropic rearrangement was successfully used to generate the core structure of Amathaspiramide A. The proposed completion of the synthesis entails an oxidative cleavage and amide formation.

Ben Johnson-Tesch, 2012: "Insights into Irreversible Binding of Buthionine Sulfoximine and its Derivatives to γ-Glutamylcysteineligase"
Benjamin Johnson-Tesch and Brenda S. Kelly (Gustavus Adolphus College)
Gamma-glutamylcysteine ligase (γ-GCL) is the rate-limiting enzyme in the biosynthesis of glutathione, a biological molecule implicated in chemotherapeutic resistance. Transition-state analogs including buthionine sulfoximine (BSO) and its derivatives (methionine sulfoximine, ethionine sulfoximine, pentathionine sulfoximine, and hexathionine sulfoximine) are hypothesized to be irreversible inhibitors of gamma-GCL, yet little is known about where these compounds bind to gamma-GCL. Using pre-incubation studies, we have confirmed that the sulfoximines are binding irreversibly to E. coli gamma-GCL in the glutamate region of the active site, but not the cysteine site. The rate of the irreversible covalent modification of each sulfoximine is dependent on both the size and stereochemistry of the sulfoximine alkyl tail. Our results indicate the buthionine sulfoximine binds at a rate two-fold faster than the next best sulfoximine inhibitor, and at a rate over four-hundred times faster than the worst of the sulfoximine inhibitors. Current and future work includes detecting conformational changes in the enzyme active site upon sulfoximine binding using fluorescence spectroscopy and electron paramagnetic resonance. This work has been supported by the Howard Hughes Medical Institute and Gustavus Adolphus College.

Dan Marino, 2014 and Emily Seelen, 2013: "Metals and Organic Carbon Cycling in an Ombrotrophic Peatland"
Dan Marino, Emily Seelen, Ben Carlson, Jeff Jeremiason (Gustavus Adolphus College), Stephen Sebestyn,* Martin Tsui,** and Meghan Jacobson**
*USDA Forest Service, Northern Research Station, **University of Minnesota

Relationships between dissolved organic matter (DOM), trace metals including mercury were examined in the S2 wetland of the Marcell Experimental Forest. DOM is known to bind trace metals and is a key component controlling trace metal transport from wetlands. However, the complex and largely unknown character of DOM prevents the construction of reliable models to predict delivery of toxic metals from wetlands. As several studies have reported increased DOM transport from wetlands, a better understanding of complex DOM-metal relationships is critical. In this study, we utilize the heavily instrumented and studied S2 wetland and an array of sampling locations within S2 to further understand the complex relationship between DOM and trace metals. Samples were collected in 2010 and 2011 from the outflow weir, lagg and bog porewaters, and in subsurface flow from the uplands. DOM was characterized by measuring total and dissolved organic carbon and by UV spectroscopy. Distinct differences were found between Hg and many other metals, helping us to better understand Hg dynamics in the S2 system. Hg and several metals were found in significant quantities in the subsurface runoff. Metals that bind to soil organic carbon, such as Pb and As, and redox sensitive metals such as Mn and Fe, were low in the subsurface runoff, relative to the weir and lagg porewaters. Hg, which also has high affinity for soil carbon, had higher or similar concentrations in the subsurface runoff as the weir and lagg porewaters, demonstrating different binding affinities for soil and/or dissolved organic carbon.

Carl Schiltz, 2014: "Developing a Method to Regulate Production of Authentic mRNAs in Yeast"
Carl Schiltz and Jeff Dahlseid (Gustavus Adolphus College)

In the yeast *Saccharomyces cerevisise* the regulation of galactose-metabolizing enzymes is carried out by a genetic switch consisting of a regulatory protein and a DNA sequence called an upstream activating sequence (UAS). The switch promotes transcription in the presence of galactose and suppresses transcription in the presence of dextrose. The regulatory function of the switch has been applied to other genes through fusion of the UAS from the *GAL1* gene to the gene of interest. The goal of this research is to develop a systematic method for fusing the *GAL1* UAS to a gene of interest, such that authentic mRNA is produced from the fusion. We have previously succeeded in using this approach with the *CTF13* gene. To test if this approach can be applied to other genes, we used the method on the *PPR1* gene. A plasmid DNA containing the fusion was transformed into yeast and the *PPR1* mRNA produced from the fusion was then found to be the correct size. Yeast transformants grown in galactose-containing media showed an increase in *PPR1* RNA abundance of more than 10-fold compared to those grown in dextrose-containing media, confirming the expected function of the *GAL1* UAS. Our next step is to determine the transcription start site for the *GAL1* UAS-*PPR1* fusion, which will address the authenticity of the mRNA. Confirming the effectiveness of a systematic approach for making *GAL1* UAS gene fusions will offer a reliable tool for further research, especially that on RNA decay.

Mike Sterling, 2014: "Toward developing intramolecular Diels-Alder reaction involving 2-trialkylsilyloxyfurans"
Mike Sterling, and Scott Bur (Gustavus Adolphus)

Initial attempts at developing an intramolecular Diels-Alder reaction involving 2-trialkylsiloxyfurans were unsuccessful. NMR and computational studies provided insight into the reaction dynamics of intermolecular reactions. Based upon these insights, new substrates for the intramolecular reactions were designed. Progress has been made on synthesizing the new substrates.

Spring 2011 Graduates

Garrett Clause, 2011, Jiawen Li, 2011, and Travis Anderson, 2011: "Cost-Effective Purification of Fumarase from Porcine Myocardial Tissue"
(from Heather Haemig's Spring 2011CHE-360 Proteins course)

Fumarase is one of two enzymes extensively studied in the Gustavus Chemistry 255 laboratory. Currently this fumarase is purchased from a supplier for about $65 per 500 units. Kanarek et al (1964) reported a method of purifying fumarase from porcine tissue using ammonium sulfate fractionation and subsequent re-crystallization. The aim of this study is to cost-effectively purify fumarase from porcine myocardial tissue by adapting and optimizing these published protocols to the materials and equipment available at Gustavus. We hypothesize that we will be able to purify fumarase in high yield and purity comparable to that reported by Kanarek et al. It will be very beneficial from an economical standpoint for Gustavus to be able to purify its own fumarase with purity and activity comparable to that obtained from a supplier but at a much lower cost.

Zach Eastling, 2011, Kristen Jahr, 2011, and Courtney Kerestes, 2011: "Kinetic Analysis of *Saccharomyces cerevisiae* Fumarase Mutants"
(from Heather Haemig's Spring 2011CHE-360 Proteins course)

Fumarase plays a role in respiratory metabolism in the citric acid cycle where it catalyzes the hydration of fumarate to S-malate. Various missense mutations of the human fumarase gene have been found to be associated with multiple cutaneous and uterine leiomyomata (MCL) as well as type II renal cell cancer. Four different human missence mutations, H135R, S158I, M454I, and V394L have been indirectly analyzed through analogous *S. cerevisiae* mutations, H114R, S137I, M432I, and I372L, respectively. The four mutations were constructed and cloned in the *S. cerevisiae* FUM1 gene (fumarase) containing a terminal histidine tag. Utilizing the histidine tag's affinity for a Ni2+ column, all four mutant fumarase proteins, as well as a wild-type fumarase protein, were successfully purified from *S. cerevisiae*. Kinetic parameters of each mutant were determined and compared to the wild type fumarase to determine what affect each mutation has on fumarase. The information gained from analysis of these clinical mutations in *S. cerevisiae* could eventually lead to a better understanding of why humans suffer from fumarase mutations.

Emily Mueller, 2011, Joe Finocchiaro, 2011, and Laura Secor, 2011: "Development of a Kinetics Laboratory Study of Tyrosinase Inhibition for Gustavus Biochemistry Students"
(from Heather Haemig's Spring 2011CHE-360 Proteins course)

The focal enzyme for the biochemistry laboratory curriculum at Gustavus alternates between fumarase and tyrosinase. The experiments throughout the course are essentially equivalent for each, apart from inhibition studies; no experiment has been designed for the study of inhibition of the catalysis of L-DOPA to dopachrome by tyrosinase. In order to develop a cost-effective experiment for the inhibition of the enzyme's activity, kinetic assays were performed at 475 nm using a Cary50 spectrophotometer for the following cheap, known inhibitors from literature: quercitin, benzoic acid, p-coumaric acid, and kojic acid. Quercitin was insoluble in the water solvent, and thus the data collected was unusable. Benzoic acid and p-coumaric acid needed to be dissolved in methanol, but both were seen as effective inhibitors at 1 mM concentrations. Kojic acid, a water-soluble inhibitor, was highly effective at a lower concentration of 0.01mM. Secondary testing was performed with p-coumaric acid and kojic acid to find optimal inhibitor concentrations. Kojic acid was chosen for the design of the procedure for the tyrosinase inhibition experiment in the Gustavus Laboratory manual, as it was water soluble and has long been established as an inhibitor of tyrosinase in the literature and in industry. The instructions include a division between the class in doing the experiment at two optimal pH values, 6.5 and 8.0, mirroring the fumarase procedure where the students are elected to study inhibition of either the forward or reverse reaction.

Gustavus Faculty

"UV-Visible determination of aqueous Cu(II)-pyrazole species"
Steve Miller (Gustavus Adolphus College) and Matthew Klun, 2011

Chemists often treat aqueous mixtures of transition metals and ligands as if they form a single solvated complex. However, ligand addition is an equilibrium process, so a number of species (e.g. ML_2, ML_3, etc.) must actually exist. If each complex absorbs visible light, they all contribute to the overall color of the solution; the color of these solutions are therefore a function of all species present, their concentrations (according to the Beer-Lambert law), and their molar absorptivity profiles. We have studied solutions of Cu^{2+} and pyrazole ($C_3H_4N_2$) using UV-visible spectrophotometry. The results indicate that $Cu(C_3H_4N_2)_x^{2+}$, x = 0 – 4 all exist in aqueous solution; we also report the molar absorptivities of each species (400-800 nm) and the values of $\Delta_f G°$ for each sequential ligand addition. Fluorescence spectra and computational analyses will provide a basis for further characterization of the aqueous Cu^{2+}/pyrazole system.

Characterization of novel carbon-based stationary phases for analytical and preparative HPLC
Dwight Stoll (Gustavus Adolphus College), Jon Thompson*, Steve Groskreutz, 2012, Ian Gibbs-Hall, 2013, Laura Secor, 2011, and Doug Fryer*
***United Science, LLC**

We have recently developed a series of novel carbon-based materials for use in analytical and preparative separations, and as solid-phase extraction media. The analytical materials show unique characteristics compared to other commercially available carbon phases as they are substantially more stable at high pressures and exhibit acceptable mass transfer characteristics. Users of carbon-based phases are aware that some compounds are very difficult to elute from existing carbon-based materials. The new materials described here significantly address this problem through both the ability to adjust the carbon loading on the underlying substrate, and the use of a relatively inert substrate. We will show how these materials can be used for highly relevant separations that could not be accomplished with traditional C18 bonding chemistries.