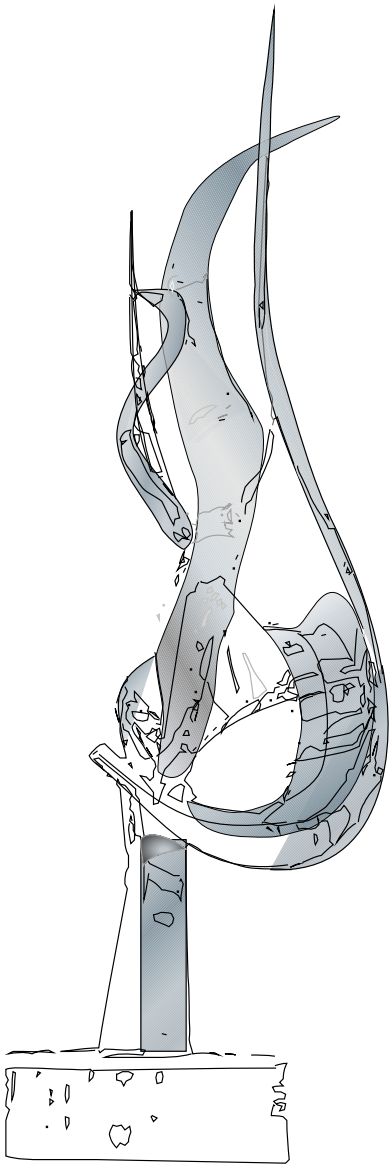
Interdisciplinary Research Collaborative in Biology & Chemistry

IRCBC

Rose-Hulman Institute of Technology



4th Annual *IRCBC* Undergraduate Research Symposium

Friday
November 2, 2007

LILLY ENDOWMENT +INC+

ROSE-HULMAN INSTITUTE OF TECHNOLOGY

Welcome to the

4th Annual IRCBC Undergraduate Research Symposium

Rose-Hulman Institute of Technology

Friday, November 2, 2007

We are honored to welcome you to the 4th Annual IRCBC Undergraduate Research Symposium and we sincerely appreciate your participation. The symposium is coordinated by the Interdisciplinary Research Collaborative in Biology and Chemistry (IRCBC), and is supported by funding from the Lilly Endowment, the Lilly/Guidant Applied Life Sciences Research Center, and Rose-Hulman Institute of Technology.

The IRCBC was created to encourage scientific research by undergraduate students and to help them better understand the exciting educational and research opportunities that lie at the interface of biology and chemistry. An appreciation for laboratory research is central to a working understanding of experimental sciences such as biology and chemistry. By participating in research, students add to current knowledge and, furthermore, they enhance their education and broaden their understanding of the scientific method and its application.

Interdisciplinary research is gaining prominence in both academia and industry, as new techniques from one discipline are applied to problems in other disciplines. By acquiring experience in interdisciplinary research, students become more attractive to potential post-graduate programs and employers. The IRCBC program specifically fosters such interdisciplinary work, and we are pleased to highlight the research of our students, as well as the research of our colleagues in Indiana.

With this fourth annual event, we are delighted to welcome you. Our intention in hosting this event is to offer students an opportunity to share their research interests and progress with their colleagues in a nurturing and supportive environment, and to encourage celebration of the undergraduate research experience. We hope you enjoy the dynamic program of speakers.

Mark Brandt
IRCBC Program Coordinator

J. Peter Coppinger
IRCBC Program Coordinator

Symposium Schedule

8:15 AM Registration

8:30 AM *Welcoming Remarks* – Michael Mueller, Department of Chemistry, Rose-Hulman Institute of Technology

Morning Session I (8:45 – 10:15 AM)

Developing a PCR-Based Assay to Identify Iridoviruses in Amphibian Populations

Meagan Gallagher and Jennifer O'Connor*

Spatial and Temporal Variability of Water Quality in a Constructed Wetland

Whitney Selby, Penney Miller, Ella Ingram, and Michael Robinson*

Analysis of J.I. Case Wetland Bacterial Content Using Both Culture-Independent and Culture-Dependent Methods

Cherie Garvis, J. Peter Coppinger, Ella Ingram, and Penny Miller*

Analysis of the Biological Function of a Constructed Wetland

Britt Hofmann, Ella Ingram, Penny Miller, and Michael Robinson*

Bacterial Diversity of the Oral Cavity in a Small Community

David Bander and J. Peter Coppinger*

Analysis of the White River for Phosphate and Nitrate Levels

Marija Watson and John A. Buben*

A Potential Role for P24 and P41 in Cell Division and Growth Rate in *Mycoplasma pneumonia*

Chandra Lesniak, Jason Cloward, and Duncan Krause*

Morning Session II (10:30 – 12:00 PM)

Proprioceptive Sensitivity of Stroke Subjects with Hemiparesis

Andrew J. Steward, Megan O. Conrad, and Robert A. Scheidt*

The Effect of Ligand and Alcohols on the Rate of Dissociation of the Estrogen Receptor

Michele M. Mumaw and Mark E. Brandt*

Probing the Effect of Alcohols on the Estrogen Receptor Ligand-Binding Domain by Fluorescence Spectroscopy

Evan Breedlove and Mark E. Brandt*

Generation, Display, and Isolation of a Randomly Mutated Polypeptide Library in Yeast

Spencer Perkins and Richard Anthony*

The Application of Microdialysis in the study of Retinal Vascular Physiology: A Feasibility Study

Brent Witten, and Jameel Ahmed*

Estimating Picophytoplankton's Abundance and Contribution to the Global Carbon Cycle

Melissa Chrisman and Adam Martiny*

12:00 PM – 12:45 PM Invited Speaker John Beals, Ph.D.
Research Fellow, Eli Lilly & Company.
"The Role of Protein Engineering in Improving Pharmaceutical Properties of Biotherapeutics."

Afternoon Session I (1:30 – 3:15 PM)

Minimizing $^{18}O/^{16}O$ Back-Exchange in the Relative Quantification of tRNA

Rachel Selby, Colette Castleberry, Mahmud Hossain, and Patrick A. Limbach*

Ethanol Production from Sweet Sorghum

Amanda Grantz and Kimberly L. Ogden*

Factors Influencing Filtration Performance

*Emily Dosmar**

In-line Methods for Pre-concentrating Samples for Micellar Electrokinetic Capillary Chromatography (MEKC) and High-Performance Liquid Chromatography (HPLC) Combined with UV Absorption Detection

Katherine Sorvig and Daniel Morris Jr.*

Probing Metal Ion Binding Sites on DNA using Sparfloxacin

Amanda Gehring and Daniel Morris, Jr.*

Thermodynamic Properties of Heterogeneous Mixtures

Ross Hoehn and J. M. Honig*

Slow Hydrolysis Reactions in Aqueous Iodine Solutions – Spectroscopic and Computational Studies

Sarah E. Waller and Roderick M. Macrae*

Afternoon Session II (3:30 – 5:00 PM)

Enantioselective Synthesis of Aminocyclobutanol

Rachael R. Chase and Richard W. Fitch

Selective Monoalkylation of Tetraethylene Glycol

Yingxiao Liu, Kiersten Yake, and Richard W. Fitch

Comparison of Palladium(0) Catalysts in the Cross-Coupling of Functionalized Aryl Halides

Amanda Isom Eric Smith, and Rebecca B. DeVasher*

Solution Effects of Various Ion Exchange Resins Applied to Palladium-Catalyzed Suzuki-Miyaura Cross-Coupling Reactions

Eric Smith, Amanda Isom, and Rebecca B. DeVasher*

Particle Size Analysis of Catalytically Active Palladium(0) and Various Exchange Resins

Cecilia Latta and Rebecca B. DeVasher*

The Design of Greener Undergraduate Labs: Green Chlorination of Vanillin and a Greener Synthesis of Creatine

Ryan Bernhardt and Carl Lecher*

Developing a PCR-Based Assay to Identify Iridoviruses in Amphibian Populations

Meagan Gallagher* and Jennifer O'Connor
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN

In recent years mass deaths and observed population declines have been occurring at higher rates in amphibian populations. Viruses from the *Iridovirdae* family especially those from the *Ranavirus* genus, have been identified as the major contributor of the increased amphibian mortalities, and are generally referred to as iridoviruses. Iridoviruses are large enveloped virus with icosahedral nucleocapsids and double stranded DNA genomes. They encompass a large host range from invertebrates to ectothermic vertebrates such as fish, reptiles, and amphibians. Due to the effect of iridoviruses on amphibian populations, it is important to be able to detect their presence within populations. Through a PCR-based assay, we can monitor the prevalence and transmission of iridoviruses in wild amphibian populations. Goals included developing an effective protocol, identifying the limit of detection, and testing the protocol on amphibian samples. A PCR-based assay was formulated and tested. The assay showed positive results on multiple samples from nucleic acid extractions from fathead minnow cells infected with FV3 in addition to samples taken straight from *Ranavirus* Frog virus 3 (FV3) stock. Ongoing research includes testing the protocol on amphibian samples and to elucidate the limit of detection for the assay.

This work was funded in part by the Lilly Endowment under the auspices of the IRCBC.

Spatial and Temporal Variability of Water Quality in a Constructed Wetland

Whitney Selby*[1], Penney Miller[2], Ella Ingram[3], and Michael Robinson[4]
[1]Department of Chemical Engineering, [2]Department of Chemistry, [3]Department of Applied Biology & Biomedical Engineering, and [4]Department of Civil Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN

Passing water through wetlands presents a natural way of managing and improving water quality through chemical and biological means. Constructed wetlands may function differently than natural ones due to design constraints. The J.I. Case Wetland and Wildlife Refuge was constructed about 30 years ago by the Vigo County Parks Department. The goal of our project is to monitor the water quality of the J.I. Case Wetland and to determine if it functions to improve the water quality of drainage from its watershed. With the use of environmental monitoring sondes at the inlet and outlet sites, we monitored dissolved oxygen, pH, turbidity, conductivity, temperature, and depth of the water. Water samples were collected twice a week from shoreline sites (1-11) and once a week from a transect within the wetland (A-8) during the 2007 summer. Samples were analyzed for pH, dissolved oxygen, ammonium, nitrate, total organic carbon, total nitrogen, and ultraviolet-visible absorption. The levels of dissolved oxygen and pH indicate the wetland's suitability to support aquatic life. The dissolved oxygen levels show increases during the times of large algal blooms. The pH of the wetland averaged around 7 at the inlet and 8 at the outlet over the course of the summer. A strong positive relationship was shown between dissolved oxygen and pH at the inlet and outlet sites. Total organic carbon levels hovered around 8mg/L but typically were higher at the inlet than the outlet. Total nitrogen levels show the same trend as total organic carbon, however, the levels were low for an agricultural watershed, as confirmed by the low levels of nitrate and ammonium measured via ion selective electrode probes (at the detection limits). Dissolved organic matter (DOM) influences the light regime and nutrient pool in the wetland. Certain UV/Vis indices (spectral slope and SUVA) can distinguish between terrestrial and microbial influences on the character of the DOM and can be used to indicate periods of increased microbial activity. The spectral slope at sites A-8 peaked in mid-July whereas in sites 1-11, no remarkable fluctuation in values was observed. Taken together, this data suggests that microbial processes are exerting control on the character of the DOM at sites A-8 whereas shoreline processes buffer spectral changes in the DOM at sites 1-11. The SUVA values, calculated from the UV absorbance at 254nm divided by the dissolved organic carbon concentration, for sites 1-11 and A-8 are very low, indicative of DOM derived predominantly from microbial sources. The strong correlation between dissolved oxygen and pH, decreases in the carbon and nitrogen concentrations from the inlet to outlet, and changes observed in the character of the DOM suggest that the wetland is functioning to remove nutrients from the water column. In the future we hope to extend our monitoring network to the surrounding watershed north of the wetland to investigate where nitrogen removal occurs.

Analysis of J.I. Case Wetland Bacterial Content Using Both Culture-Independent and Culture-Dependent Methods

Cherie Garvis*[1], J. Peter Coppinger[1], Ella Ingram[1], and Penny Miller[2]
[1]Department of Applied Biology & Biomedical Engineering and [2]Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN

The J. I. Case Wetland Wildlife Refuge in Vigo County, Indiana was constructed in the 1970s as part of an environmental remediation project. The wetland is monitored for chemical characteristics indicative of biologically adequate water quality and suggestive of functioning in water quality processing. Bacteria contribute to water quality through breakdown of wastes and cycling of nutrients such as carbon and nitrogen in wetlands. Therefore, analyzing the bacterial population of the wetland will provide data to supplement the overall chemical characteristics. I will also examine trends in the spatial and temporal distribution of bacterial populations. The culture-independent bacterial detection technique of genetic sequencing can identify bacterial species, but does not provide quantitative data about the relative size of the bacterial population over time or the relative abundance of individual bacterial species. Thus, procedures for both genetic sequencing and heterotrophic plate counts were developed and tested for suitability. Samples were collected weekly at six sites distributed throughout the wetland, including the inlet and outlet. I preserved part of each sample for heterotrophic plate counts. Preliminary tests of the heterotrophic plate count procedure confirmed the viability of the preserved glycerol samples. After five days of incubation a variety of colony morphologies were observed, including many pigmented colony-forming units. One problem identified with the procedure was that undiluted samples yielded fewer colony-forming units than expected compared to serial dilutions of the same samples. Increased competition for space and nutrients in undiluted samples may decrease the amount of colony-forming units relative to diluted samples. To obtain bacterial DNA for genetic sequencing, wetland samples were filtered and DNA was extracted from the filters using the UltraClean® Water DNA Extraction Kit. I used molecular techniques to obtain purified DNA for genetic sequencing. Genetic sequences were successfully obtained from a wetland water sample during the initial proof of concept experiment. I used the NCBI Blast website to identify the genetic sequences obtained. Initial sequences included a strain of E. coli and chloroplast DNA. One problem with this procedure is that the transformation step of the genetic sequencing procedure has not yielded results since the original proof of concept experiment. Current efforts to verify the repeatability of the genetic sequencing procedure include using newer enzymes and nucleotides during the polymerase chain reaction procedure, and altering the template content of ligations. Once genetic sequences are obtained from the DNA samples, I will analyze the relative community of bacterial species identified at each site over the sampling period. In the future, I will compare the bacterial results to the chemical characteristics of the wetland measured during the sampling timeframe and investigate trends, like whether an increase in bacterial populations is correlated with increased nutrient content in the wetland.
This work was funded in part by the Lilly Endowment under the auspices of the IRCBC.

Analysis of the Biological Function of a Constructed Wetland

Britt Hofmann*[1], Ella Ingram[1], Penny Miller[2], and Michael Robinson[3]
[1]Department of Applied Biology & Biomedical Engineering and [2]Department of Chemistry and [3]Department of Civil Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN

During the past four hundred years, over half of the wetlands in the lower continental United States have been lost. When man-made wetlands are created to replace them, monitoring is necessary. The man-made wetland of interest for this study is the J.I. Case wetland, which was originally created for waterfowl habitat in Vigo County, Indiana. Methods of assessment used at the J.I. Case wetland included amphibian diversity and chemical water analysis. Amphibian diversity is an excellent technique for evaluating wetland health since amphibian habitat includes both the aquatic environment and the surrounding land, which serves as a buffer zone. Therefore, when evaluating a wetland, terrestrial factors should be considered along with aquatic analysis. To determine the diversity, trapping by the pitfall and drift net method occurred. Seven traps consisting of three drift nets and four pitfall traps were set up in seven areas of the wetland to give a sample of different landscapes within the entire system. Trapping occurred five days a week over the period of June through August. Based on both trapping and informal observation, this wetland system had low abundance of amphibians. Only eleven frogs were caught in the traps, and there were very few sightings of amphibians during the entire duration of the research. Further analysis of the area would be done to confirm the low activity. Total nitrogen, total organic carbon, pH, nitrates, and ammonium were tested at the nearest point of water in the wetland from the traps. Water quality at the sites of the amphibian traps confirmed that the wetland had water of adequate quality to promote amphibian life. Terrestrial habitat was then analyzed by vegetation survey to determine if the buffer zone was providing an adequate wetland buffer zone. It was shown that there was a high proportion of UPL and FACU species present at the wetland. The low number of wetland plant species can be explained by the construction of the wetland. A majority of the wetland is contained by a steep border which would provide an ideal environment for non-wetland plants. In an ideal wetland, a high abundance of non-wetland plants would not be found within close proximity to the main water body due to seasonally inundated shores. The biological analysis of this study indicates that the wetland is not fully representative of a natural wetland. Although water quality was adequate, the physical setting of this constructed wetland differentiates it from natural wetlands.

Bacterial Diversity of the Oral Cavity in a Small Community

David Bander* and J. Peter Coppinger
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN

The human dental cavity has over 500 naturally occurring bacterial species ranging from beneficial microbes to potential pathogens. Previous small studies in unrelated individuals have identified oral bacteria common within the general population. These studies also suggest that some bacterial species may be individual-specific due to genotype, lifestyle, and diet. Rose-Hulman is an ideal location to investigate the influence of diet and living environments on human oral diversity in a small community; the on-campus residents of Rose-Hulman share close living quarters, common dining and housekeeping services, and participate in many similar campus activities. Using universal PCR primers, we are randomly amplifying bacterial 16SrRNA DNA isolated from mouth swabs of students. 16SrRNA PCR products will be sequenced and compared to microbial genomic databases to identify bacterial species. Given the daily interactions among Rose-Hulman students living in residence halls, we hypothesize that students living on-campus will tend to have more similar mouth flora than those students living off-campus.

This work was funded in part by the Lilly Endowment under the auspices of the IRCBC.

Analysis of the White River for Phosphate and Nitrate Levels

Marija Watson* and John A. Buben
School of Mathematics and Sciences, Marian College, Indianapolis, IN

During the past spring and this current fall season, water samples were collected from various locations on the White River for analysis of phosphate and nitrate. Phosphate and nitrate are beneficial as aquatic nutrients, but in excess, can lead to eutrophication and reduced water quality. Previous periodic sampling of the river in Indianapolis had revealed a relatively constant concentration of phosphate of about 0.2 ppm. The purpose of our study was to attempt to determine the extent and origin of phosphate and nitrate in the river. During the spring, a set of water samples was collected from about 10 locations, beginning east of Muncie, and extending through Anderson and Noblesville, and down into Indianapolis. Sample sites were selected so that results might allow for a determination of whether the nutrient pollutants originated largely from point or non-point sources. Results from the spring samples revealed that phosphate levels ranged from 0.02 ppm near Muncie to 0.11 ppm on the north side of Indianapolis, and both decreases and increases in phosphate concentrations were observed over various segments of the river. Nitrate levels ranged from a low of 0.4 ppm near Muncie to a high of 1.7 ppm at Noblesville, and then decreased further down river. The set of water samples for the fall are scheduled for collection near the end of September. Results from the analysis of those samples will be compared with the results from the spring, and general conclusions regarding the gradation of pollutants in the river, their origin, and an estimation of bulk magnitude will be presented.

Proprioceptive Sensitivity of Stroke Subjects with Hemiparesis

Andrew J. Steward*[1], Megan O. Conrad[2], and Robert A. Scheidt[2]
[1]Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN and [2]Department of Biomedical Engineering, Marquette University, Milwaukee, Wisconsin

Approximately 50% of stroke subjects exhibit residual motor deficits, including hemiparesis, 6 months post stroke (Rosamond, 2006). The contribution of impaired sensory function to residual motor deficits remains unclear. The first aim of this study is to compare the ability of hemiparetic stroke subjects and non-stroke subjects to detect spatial displacements (i.e. fixed magnitude hand perturbations) at various locations in their workspace. Detectability (d') values were estimated using standard psychophysical techniques. Second, it aims to identify the perturbation magnitude for which subjects can indicate the presence of perturbation 50% of the time. Stroke subjects were at least 6 months post-stroke with their dominant hand affected. The first experiment used a planar, two degree-of-freedom five-bar linkage robotic arm to position the subject's hand at 21 different locations, two times each, once with a perturbation of fixed (1.4 cm) magnitude and once without. Subjects indicated after each trial whether the perturbation was present. This identified any spatial bias in the sensitivity of detecting mechanical perturbations across subject groups. In a second experiment the subjects experienced perturbations of varying magnitudes at a single spatial location and after each trial stated whether or not they sensed the perturbation. Detectability (d') values for stroke subjects were significantly lower than that of control subjects. Stroke subjects had significantly higher threshold values than control subjects. It is unclear whether the results are due to deficiencies in descending modulations of spinal networks influencing muscle spindle receptor sensitivities, or to impaired central processing of sensory information. This project was supported by the National Science Foundation REU Program under grant #0452503 and NSF BED 0238442.

The Effect of Ligand and Alcohols on the Rate of Dissociation of the Estrogen Receptor

Michele M. Mumaw* and Mark E. Brandt
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN

The development of breast cancer is affected by the estrogen receptor. In order to better understand the effects, the interaction of the estrogen receptor is being studied with different small molecules in solution. Estradiol, the primary human estrogen, behaves as a ligand that binds to the estrogen receptor. The binding location of estradiol is the ligand-binding domain (LBD), the primary focus of this study. The LBD is expressed in E. coli and purified from the fusion protein which is composed of ligand-binding domain and maltose binding protein. When separate, the fusion protein and the ligand-binding domain are each homodimers, but when in solution together the homodimers slowly dissociate and a heterodimer forms. The rate of dissociation of the estrogen receptor was measured using HPLC gel filtration chromatography. Conditions of the experiment such as temperature, alcohols present in different concentrations, and the presence of estradiol were varied to see how the rate would be affected. Since estradiol is only slightly soluble in water, it was first dissolved in methanol because it was found to affect the protein's structure less than other solvents. It was found that the presence of alcohols increased the rate of dimer exchange in a concentration-dependent manner, with large alcohols being more effective than smaller ones. Increasing the temperature also resulted in a higher rate of dimer exchange. In contrast, when estradiol was added to the solution, the rate of dimer exchange was decreased. In conclusion, it was determined that the alcohols and estradiol had a measurable effect even though minimal concentrations, 0.1% to 0.5% and 10 μM respectively, were used. The results of these studies will help better understand the response of the estrogen receptor to different life styles.

This work was funded in part by the Lilly Endowment under the auspices of the IRCBC.

Probing the Effect of Alcohols on the Estrogen Receptor Ligand-Binding Domain by Fluorescence Spectroscopy

Evan Breedlove* and Mark E. Brandt
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN

The human estrogen receptor, a member of the family of nuclear receptors, is an intracellular receptor protein which exists as a dimer and cooperatively binds estrogen. Recently, it was discovered that the rate of dimer dissociation is significantly altered by the presence of short-chain alcohols. We therefore decided to investigate whether alcohols alter the fluorescence spectrum of the estrogen receptor ligand-binding domain (LBD). A change in the fluorescence spectrum could be the result of a change in the environment around the LBD fluorophores or of a direct interaction between the fluorophore and the alcohol. The tryptophan analog n-acetyl tryptophanamide showed no statistically significant change in fluorescence in the presence of short-chain alcohols. In contrast, the fluorescence intensity of the LBD decreased by as much as 20% in the presence of 0.13 M 1-propanol. This suggests that alcohols do not have a quenching effect on the LBD tryptophan residues. Instead, 1-propanol appears to be interacting with the LBD, eliciting a conformational change in the protein that alters the tryptophan fluorescence. Thus, fluorescence spectroscopy appears to offer an approach to characterizing the effect of alcohols on the estrogen receptor LBD.

Generation, Display, and Isolation of a Randomly Mutated Polypeptide Library in Yeast

Spencer Perkins* and Richard Anthony
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN

Yeast surface displays from a library of randomly mutated polypeptides were quantitatively screened for potential novel variants using receptor bound magnetic bead selection. Site directed mutagenesis of the proteins binding region will create fragments that will populate a randomly generated mutant library. Using gap repair in yeast, the fragments will be reinserted into the gene. In order to insure a maximal protein display, optimization of surface proteins must be achieved. Fluorescent intensities will be monitored to determine the appropriate time length to induce protein expression in the cells. Magnetic beads coated with appropriate receptors will be used to isolate novel mutants. Competitive binding assays can be used to determine the changed kinetic efficiencies of the novel proteins verses the native one. Using directed evolution, a known mutant whose binding efficiencies has been increased can be randomly mutated and populate a new library. Upon screening of the new library, even more efficient mutants are expected to be found.

This work was funded in part by the Lilly Endowment under the auspices of the IRCBC.

The Application of Microdialysis in the study of Retinal Vascular Physiology: A Feasibility Study

Brent Witten*, and Jameel Ahmed
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN

The focus of this project was to examine the control mechanism that links activity in retinal neurons with the blood supply to the retina. It has been documented that excited retinal neurons have increased oxygen consumptions, which is offset by increased blood flow. It has also been shown that increased retinal blood flow is accompanied by an increase in nitric oxide levels. NO is a known vasodilator, however little is known about the local control signals that induce the release of NO. There are many candidate molecules that could serve as part of the cascade for retinal arteriole dilation, one being adenosine which was the molecule of focus for this project. A goal of this research project was to gain a working knowledge about microdialysis and how it can be applied for sampling the signal molecules around the retinal neurons in the viscous humor. Microdialysis probes were inserted into solutions of known concentrations of adenosine to look at probe recovery rates. The samples were analyzed by HPLC to measure the absorbance of adenosine in the solution. Absorbance is directly related to the concentration of the substrate. It was found microdialysis probes with a 1mm semi-permeable membrane had a recovery rate of 3.80% and the 2mm probe had a recovery rate of 6.01%. 7 rat experiments were preformed during the course of the project. In the last two experiments a microdialysis probe was inserted into the rat's retina. In these studies additional difficulties involving the insertion of the probe and positioning of the probe were observed. These preliminary trials did not yield any readings of adenosine, but it is not known if this was the caused by a problem with the microdialysis setup or if the adenosine levels were too low to be detected.

The main result from the lab was a working knowledge of microdialysis and how it can be applied to the rat retina. Microdialysis is a feasible and repeatable method that allows access to the retina without large trauma. The next step will be to submit an eye with a microdialysis inserted to flickering light and look changes in the absorbances of the samples signaling a change of concentration of a molecule. Once that molecule can be identified, further studies can be performed to observe its role in the retinal vascular dilation cascade.

This work was funded in part by the Lilly Endowment under the auspices of the IRCBC.

Estimating Picophytoplankton's Abundance and Contribution to the Global Carbon Cycle

Melissa Chrisman* and Adam Martiny
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN

Prochlorococcus and *Synechococcus* are thought to be important contributors to the net primary production in the world's oceans. It has also been thought that these organisms are influential to the global carbon cycle and climate control. The main purpose of this experiment is to determine exactly how much *Prochlorococcus* and *Synechococcus* cells contribute to the global carbon cycle. In order to determine to what degree these cells contribute to the global carbon cycle, it is pertinent to determine how much carbon a single cell takes up and fixes. Once it is determined how much carbon is taken up by a known number of cells in a colony in a certain amount of time, one can establish a relationship between cell abundance and carbon uptake.

In this summer research project, a map of *Prochlorococcus* and *Synechococcus* distribution and abundance was created for the first time since these organisms have been discovered about 21 years ago. Results of carbon uptake/cell that will be determined in this research project will be used to then create a map of the world showing which areas of the world these cells contribute the most to the global carbon cycle.

It has been determined by climate researchers that the ocean's temperatures will increase by about 2 degrees in the next century. The next step in this research lab will be to see how these cells respond to different changes in temperature and ocean composition. It is hypothesized that by an increased ocean temperature, less nutrients will be available in the oceans overall. Because *Prochlorococcus* cells prefer warmer waters that are lacking nutrients, it is thought that in the next 100 years, more *Prochlorococcus* cells will inhabit the oceans than the amount that currently exists. More *Prochlorococcus* cells in the ocean means more ocean-air exchange of carbon. This means that more carbon will be put into the atmosphere by the increased number of *Prochlorococcus* cells, which in turn will further increase the world's temperature, resembling a positive feedback system. Data of distribution and carbon uptake from this summer laboratory experiment will be used to get precise data in future experiments to test this hypothesis.

Minimizing $^{18}O/^{16}O$ Back-Exchange in the Relative Quantification of tRNA

Rachel Selby*, Colette Castleberry, Mahmud Hossain, and Patrick A. Limbach
Rieveschl Laboratories for Mass Spectrometry, Department of Chemistry, University of Cincinnati, Cincinnati, OH

Posttranscriptional modifications are measured using a tool that utilizes the ribonuclease T1 (RNase T1) digestion of transfer ribonucleic acids (tRNAs) in order to isotopically label tRNA fragments with ^{18}O. These labeled digestion products are quantified using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS). While conducting standard control experiments, Meng and Limbach reported potential problems of the back-exchange of ^{18}O to ^{16}O which consequently decreases the accuracy and precision of this tool [2]. Here, the causes of the back-exchange were studied including the effect of time and temperature. The forward ^{18}O to ^{16}O reaction as well as the reverse ^{16}O to ^{18}O reactions were studied. In both directions back-exchange increased as the digestion products remained in 37°C warm water bath for 8 hours. No relatively significant back-exchange occurred while the digestion products remained in 20°C or 4°C conditions for the same amount of time.

The back-exchange of ^{16}O to ^{18}O was a first order reaction in the warm water bath with a rate of $2.85 \pm 0.11 \times 10^{-5}$ s^{-1}, until 26 hours when it appears to level off.

Ethanol Production from Sweet Sorghum

Amanda Grantz* and Kimberly L. Ogden
Department of Chemical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN and Department of Chemical and Environmental Engineering, University of Arizona, Tucson, AZ.

Ethanol yield was used to evaluate the effect of various sugar preservation schemes on the fermentation of sweet sorghum juice. Juice samples were preserved by refrigerating at 7°C, autoclaving at 121°C for 15 minutes, or adding citric acid (40 mg/100 ml) or lime (4 g/150 ml). The fermentation of refrigerated sweet sorghum juice containing no preservative yielded 9% (v/v) ethanol. Experiments with other preservation schemes yielded at most 4% (v/v) ethanol. No method considered here was found satisfactory for the preservation of sweet sorghum juice for ethanol production on the laboratory or industrial scale. Fermentation was optimized at the neutral juice pH 5.

Factors Influencing Filtration Performance

Emily Dosmar*
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN

The purpose of this study was to understand the principles governing filtration processes and to determine methods for improving overall filtration throughputs by exploring the effect of mixing and shear on filtration.
Methods for improving overall filtration throughputs by exploring the effect of mixing and shear on filtration. Static or dead ended filtration and tangential flow filtration modes were evaluated. Filtrate flow rates and throughputs were measured as a function of pressure, pore size, and filter thickness. Homogenous and heterogeneous (courser to finer) filter combinations were used to determine if resistance to flow would be influenced by filter thickness alone. Solute-containing solutions (Sanilac and skim milk) were used as model filter-plugging solutions. Throughput studies were conducted using a range of filter combinations in a dead ended mode in order to determine the effectiveness of serial filtration and shear and mixing via a tangential flow mode.
Filtration flow rates were shown to be directly proportional to pore size and pressure, and indirectly proportional to filter thickness. Resistance to flow was shown to be sensitive to the tightest filter in a filter train and insensitive to the thickness contributed by courser pre-filters. Filtration throughputs were seen to increase with the recirculation of solute-containing fluids. Flux showed a dependence on the rate of crossflow shear forces on the membrane's surface.

In-line Methods for Pre-concentrating Samples for Micellar Electrokinetic Capillary Chromatography (MEKC) and High-Performance Liquid Chromatography (HPLC) Combined with UV Absorption Detection

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The compound 8-hydroxy-2´-deoxyguanosine (8-OH-dG) is a widely accepted marker indicative of oxidative DNA damage and is associated with over 50 different diseases and clinical conditions. Pre-concentration of samples is often required to aid in the detection of the low concentrations of 8-OH-dG in samples of biological significance. We report a method for in-line pre-concentration in which multiple sample volumes are injected onto an HPLC column followed by UV absorption detection. Multiple samples (up to a total volume of 300 μL) could be loaded using a standard 20 μL injection loop with little or no loss of column efficiency. We also investigated a similar method for pre-concentration of samples separated by micellar electrokinetic capillary chromatography (MEKC) with UV absorption detection. An in-line, solid-phase extraction based method for pre-concentrating samples was employed using a previously reported photoinduced polymerization technique for trapping octadecylsilane (ODS) spheres in capillaries. The method allows for entrapment of ODS spheres to produce varying lengths of stationary phase without the use of retaining frits and their associated problems, such as bubble formation and void spaces. A 1-2 cm length of 4 μm ODS spheres was formed at the end of a capillary using this method to demonstrate its ability to pre-concentrate samples containing 8-OH-dG.

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Probing Metal Ion Binding Sites on DNA using Sparfloxacin

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Sparfloxacin is a quinolone drug employed in treating certain types of pneumonia and chronic bronchitis that exhibits its health benefits by binding to DNA. Metal ions and DNA bases have different binding affinities with sparfloxacin. We investigated the relative binding affinities ofFe(II), Cu(II) and Cr(III) with calf thymus DNA, the nucleosides 2´-deoxyguanosine and 2'-deoxycytosine, the nucleotide 2´-deoxyguanosine-5´-monophosphate and sparfloxacin using UV-Vis absorption and fluorescence spectroscopy to probe the affinities of the different metal ions for DNA bases and/or phosphate groups. Metal ions that bind to a DNA base block sparfloxacin from binding to the base. However, metal ions bound to the phosphate group tend to enhance binding possibly by a "bridging" effect. Binary systems composed of sparfloxacin in the presence of a given metal ion, DNA, or mononucleoside/mononucleotide and ternary systems containing sparfloxacin, a specific metal ion, and either DNA or mononucleoside/mononucleotide were examined. Preliminary data on the influence of Fe(II), Cu(II) and Cr(III) on the binding of sparfloxacin to DNA or mononucleosides/mononucleotides will be reported along with the relative affinities of these metal ions for binding sites on DNA bases and/or phosphate groups.

Thermodynamic Properties of Heterogeneous Mixtures

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We analyzed the excess heat capacity, C_p, involved while changing the temperature of a binary heterogeneous mixture consisting of two components A and B with different molar volumes V_a and V_b. This represents a generalization of a well established similar analysis of a binary mixture involving components of equal molar volume. We study the effects of varying the ratio V_b/V_a on C_p when the temperature is raised from low values to beyond the critical point, above which a uniform solution is established. We present numerical calculations based on a lengthy thermodynamic analysis of the free energy ascribed to this binary system. The calculations show significant deviation from the published results in literature for components with equal molar volume.

Slow Hydrolysis Reactions in Aqueous Iodine Solutions – Spectroscopic and Computational Studies

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In aqueous solution elemental iodine, I_2, undergoes a complex series of pH-dependent hydrolysis reactions. Fast processes lead to a pre-equilibrium state which then evolves slowly over time. In this paper iodine hydrolysis is studied spectrophotometrically in the low-pH regime where the slow kinetics occurs over days or weeks. The results obtained are compared to a stochastic model based on parameters from the literature. Additionally, time-dependent density functional theory calculations are carried out on several solvated intermediates in order to assist in characterization of the spectra.

Enantioselective Synthesis of Aminocyclobutanol

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Our laboratory is interested in the enantioselective synthesis of amino-alcohols as useful synthons for the synthesis of natural products and other bioactive molecules. We have particular interest in cyclic amino-alcohols as precursors to choline derivatives which may have interesting biological activity and selectivity at acetylcholine receptors (AChR), an important class of neurotransmitter receptors in the central nervous system (CNS). Cyclic derivatives are conformationally restricted and may serve useful roles in the assessment of active conformations for both nicotinic and muscarinic AChR subtypes. Our synthetic efforts have focused particularly in the synthesis of 2-aminocyclobutanols. These are available from 1,3-butadiene via cyclobutene and enantioselective ring opening of the corresponding epoxide. Our synthetic efforts to date will be described.

Selective Monoalkylation of Tetraethylene Glycol

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Our laboratory has been interested in the selective functionalizaiton of polyethylene glycols (PEG) as useful synthons for the preparation of PEG oligomers and conjugation with drugs to useful pharmacological effect. Such selectivity is not easily obtained however, given that one has two identical functions at each end of the molecule. Moreover, the presence of multiple oxygens as ligands to metals employed as bases presents a dilemma as to elucidating the active species to be alkylated (aggregates versus chelated monomer). We examined several alkalai metal hydrides as bases for the alkylation of tetraethylene glycol with methyl iodide, varying equivalents of base and MeI. Reactions were monitored by TLC, NMR and GC-FID, the latter being most useful. Optimal conditions were found to be a modest excess of LiH and MeI. Details of the optimization and implications for other selective functionalization of diols will be discussed.

Comparison of Palladium(0) Catalysts in the Cross-Coupling of Functionalized Aryl Halides

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An environmentally friendly methodology was developed for the catalytically-mediated, aqueous-phase cross-coupling of 4-iodoanisole and phenyl boronic acid at elevated temperatures in an oxygen atmosphere. The scope of this methodology has been further developed to include various functionalized aryl halides and phenylboronic acids. In this study, we focus on the use of a previously developed methodology prepared for Suzuki coupling adapted for the Heck coupling reaction, and the comparison of targeted ligands for use as catalysts in cross-coupling reactions. It appears that the palladium(0) catalyst is of greater general utility in the cross-coupling of aryl halides and phenyl boronic acids than in the cross-coupling of aryl halides and vinyl compounds. Due to the lack of activity and selectivity of palladium(0) particles as catalysts in the cross-coupling of aryl halides and vinylic groups, a sterically-demanding, water-soluble phosphine ligand, tri(4,6-dimethyl-3-sulfonatophenyl) phosphine trisodium salt (TXPTS), was added in the attempt to increase the activity and selectivity of the catalyst. TXPTS was chosen based on its aqueous solubility properties and reported activity as a ligand for palladium(0) catalysts in other cross-coupling systems. In addition to the previously reported activity, the ligand was chosen based on its relative tolerance to an oxygen atmosphere and the potential for favorable coulombic attraction to the positively charged anion exchange resin, Amberlyst® A-26(OH). The conditions favorable for the Suzuki-Miyaura coupling reaction do not appear to be general for the Heck coupling reaction in the formation of carbon-carbon double bonds. However, the heterogeneous catalyst system provides a robust system for carbon-carbon bond formation upon the reaction of various functionalized aryl halides and phenylboronic acids.

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Solution Effects of Various Ion Exchange Resins Applied to Palladium-Catalyzed Suzuki-Miyaura Cross-Coupling Reactions

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Green chemistry focuses on the utilization of cheaper, more environmentally friendly and efficient use of substrates and reaction conditions in the synthesis of products. The Suzuki-Miyaura coupling reaction provides a means to reduce the amount of organic solvents in the production of pharmaceuticals. The Suzuki coupling reaction uses a palladium catalyst to produce biphenyls by combining an aryl or vinyl boronic acids with aryl or vinyl halides. Catalytically-mediated trials in organic media have historically produced products in high yield. In our focus on reactions in an aqueous-polyethylene glycol [M_n4600] (PEG) environment, anionic resins such as Amberlyst® A-26(OH) have been implemented to increase yield of cross-coupling product. Yield further increased with the addition of PEG, which implies a relationship between the resin, PEG, and the substrates. In this account, we focus on optimizing and characterizing this relationship by the identity and pH of the exchange resin in addition to PEG substitution. A study was performed on neutral and basic anionic, cationic, and cellulose-based resins at nominal pH and at pH 12 *via* addition of dilute, aqueous sodium hydroxide. It was found that product yield increased for all resins at elevated pH levels, and that product yield and selectivity were greatest in solutions containing Amberlyst® A-26(OH). A PEG study compared PEG with varying molecular weights alongside cellulose and starch materials employed as potential phase transfer additives. It appears that the reaction conditions consisting of Amberlyst® A-26(OH), Carboxymethyl Cellulose, palladium(II) acetate (0.75 mol%), sodium formate, and water at 80°C provided the most favorable reaction conditions in our study of the cross-coupling of phenylboronic acids and aryl halides.

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Particle Size Analysis of Catalytically Active Palladium(0) and Various Exchange Resins

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Methods of improving experiments in a way that is friendly to the environment continue to be an issue among scientists that work with potentially environmentally unfavorable products. Heterogeneous reaction mixtures provide one method of separating the organic product stream from the heavy metal catalysts employed to increase the efficiency of certain organic reactions. In an aqueous-organic system that involves a palladium(0) catalyst, specific resins were chosen based on their ability to separate out materials for a cleaner product. In order to determine if the palladium particles would be able to adsorb onto the surface of the resins, the size of the particles making up the resin and the size of the palladium particles were measured. Palladium(II) acetate was reduced using sodium formate as a reducing agent, and the resulting particle sizes were measured as the reduction proceeded for a solution containing approximately 5% palladium. The palladium(0) particles were found to have significant populations around 1 μm and 100 μm in at least one dimension. Six resins were tested based on their performance in a standardized aqueous-organic reaction, and the particle sizes were investigated against that of palladium(0) particle size. It appears that the palladium(0) particles formed after treatment with sodium formate are smaller than the palladium(II) acetate aggregates.

The Design of Greener Undergraduate Labs: Green Chlorination of Vanillin and a Greener Synthesis of Creatine

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Green chemistry is a useful tool to increase awareness and teach sophisticated problem solving skills in a chemistry context, and provides unique opportunities for innovation in the organic chemistry curriculum. However, there is an ongoing need for educational laboratory materials that teach the tools and strategies of green chemistry in parallel with other fundamental chemical concepts and techniques. To meet this need, Marian College is developing green experiments for the organic chemistry laboratory curriculum. This presentation will highlight research leading to the implementation of two experiments, a green chlorination of vanillin and a greener synthesis of creatine. These labs emphasize lower waste production and the use of alternative, more environmentally benign reagents which can be safely utilized by sophomore organic chemistry students on open benches.

A Potential Role for P24 and P41 in Cell Division and Growth Rate in *Mycoplasma pneumonia*

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With a minimal genome size of 816 kbp, the causative agent of atypical pneumonia, *Mycoplasma pneumoniae*, is a cell wall-less obligate parasitic bacterium that is one of the smallest and least complex identified free living organisms. Despite *M. pneumoniae's* apparent simplicity, it uses a complex membrane bound terminal organelle to facilitate attachment to host cells. This organelle is also the leading end in cell movement and functions in cell division. Cytoskelatal proteins P41 and P24 are localized in this tip, and it has been shown that a mutation in the P41 gene results in the detachment of the terminal organelle and the loss of function for both P41 and P24. This study focused on the effect that the loss of either P41 or P24 has on the growth rate of the organism. *M. pneumoniae* with the loss of P41 was shown to have a faster growth rate than bacteria with the loss of P24 and both mutants appeared to have a slower growth rate than wild-type.