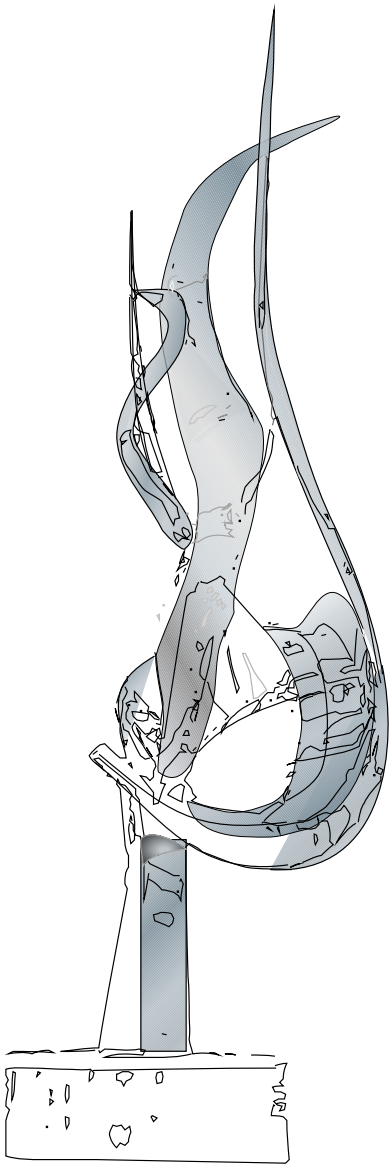
Interdisciplinary Research Collaborative in Biology & Chemistry

IRCBC

Rose-Hulman Institute of Technology



3rd Annual *IRCBC* Undergraduate Research Symposium

Friday
November 3, 2006

9:00 AM to 5:00 PM
Kahn Room

MERCK • AAAS
Undergraduate Science Research
PROGRAM

ROSE-HULMAN
INSTITUTE OF TECHNOLOGY

Welcome to the

3rd Annual IRCBC Undergraduate Research Symposium

Rose-Hulman Institute of Technology

Friday, November 3, 2006

We are honored to welcome you to the 3rd 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 Merck/AAAS Undergraduate Science Research Program, 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 third 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

Ella Ingram
IRCBC Program Coordinator

Symposium Schedule

8:15 AM Registration

9:00 AM *Welcoming Remarks* – Lee Waite, Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology

Scheduled Presentations

Chemical and Biological Functioning of a Constructed Wetland

Ashlee Brewer, **Jill Floyd***, Ella Ingram, Penney Miller, and Michael Robinson*

Simplified Salen Ligand Catalyzed Copolymerization of Cyclohexeneoxide and CO_2

Ross Poland, Jeremy Andreatta, Wonsook Choi, Cass Richers, and Donald Darensbourg*

Efficient Coupling of Aryl Halides and Phenylboronic Acid under Environmentally Friendly Conditions

Amanda Isom and Rebecca DeVasher*

Optimization of the Succinate Dehydrogenase Vital Staining Procedure for Arbuscular Mycorrhizas

Meagan Gallagher and Ella Ingram*

Break

Role of T-bet in the Generation of Pulmonary Atopic Inflammation

A. Mae Huehls, Sarita Sehra, and Mark H. Kaplan*

Genetic and Biochemical Characterization of *Arabidopsis thaliana* Putative Disease Resistance Genes

Emma Hegwood and J. Peter Coppinger*

Anharmonic Vibrations of a 60 Carbon System

Ivan Kornienko and Daniel Jelski*

12:00 PM *Invited Speaker* – Bruce Alberts, Department of Biochemistry & Biophysics, University of California, San Francisco, President of the National Academy of Sciences (1993-2005)

1:00 PM Lunch

2:00 PM Afternoon Session

Purification and Inhibition of Recombinant Vaccinia Virus H1 Protein

Anita Mathur*, Julia Huang and Mark Kaplan*

Determining Cooperativity of the Human Estrogen Receptor Through Tryptophan Fluorescence Quenching

Adam G. Georgas* *and Mark E. Brandt*

The Effect of Alcohols on the Rate of Estrogen Receptor Dimer Exchange

Rebecca J. Waltz*, David M. Knapp, Rachel Krasich, Andrei L. Edwards, and Mark E. Brandt*

The Accuracy of Laser-Induced Breakdown Spectroscopy in Quantifying Carbon in Soils

Rachel M. Selby*, L. Edwards, I.B. Gornushkin, B.W. Smith, and J.D. Winefordner*

Break

Method Development and Microfluidic Device Design for Separation and Detection of Modified Nucleosides

Christian Sweeney* *and Daniel L. Morris*

Combustion Toolbox

Mark Vaccari* *and Dan Coronell*

Particle Size Effects in the Pretreatment of Wood Waste Leading to Enhanced Ethanol Production

Amanda Grantz*, David J. Dixon, and Patrick C. Gilcrease*

Prevention of Membrane Pore Formation Via $P2X_7R$-Cx43 Interaction

Christina Chrisman*, M. Cohen, S.O. Suadicani, D.C. Spray, and E. Scemes*

Additional abstract (not scheduled for presentation)

Observation of the Bioluminescent Reaction Between Aequorin and Calcium Ions

Christina. A. Shook* *and Alfred Carlson*

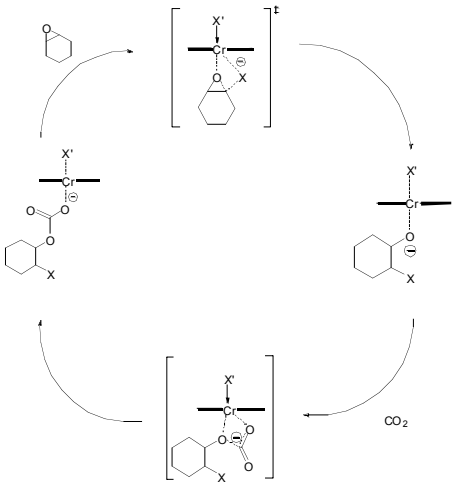
Chemical and Biological Functioning of a Constructed Wetland

Ashlee Brewer*[1], Jill Floyd*[2], Ella Ingram[2], Penney Miller[1], and Michael Robinson[3]
[1]Department of Chemistry, [2]Department of Applied Biology & Biomedical Engineering, and [3]Department of Civil Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Wetlands provide a means of managing water quality because a combination of biological, chemical and physical mechanisms work to improve water quality as water passes through a wetland. Constructed wetlands may function differently than natural wetlands in these respects due to constraints introduced during the construction process, to missing components of the system, or any number of other reasons. Our goal was to characterize the baseline water quality parameters and biological properties of the J.I. Case Wetland and Wildlife Refuge, constructed approximately 30 years ago by the Vigo County Parks Department, to determine if it functions to improve the quality of surface water draining from its agricultural watershed. Environmental monitoring stations at the inlet and outlet monitored dissolved oxygen, pH, conductivity, turbidity, chlorophyll, temperature and depth on a 24-hour basis. We collected water samples from the wetland inlet and outlet three times per week, and performed chemical and algal sampling along transects across the inlet and outlet sites to resolve spatial differences in water quality and biological productivity. We analyzed these samples for dissolved oxygen, pH, dissolved organic carbon (DOC), total nitrogen, and alkalinity. At both the inlet and the outlet, dissolved organic carbon and total nitrogen content of the water were strongly negative correlated. For water flowing into the wetland, we found no relationship between either DOC or total nitrogen and dissolved oxygen. For water exiting the wetland, we found a strong negative relationship between DOC and dissolved oxygen, and a strong positive relationship between total nitrogen and dissolved oxygen. These results suggest that both biotic and abiotic processes to modify water quality were occurring within the wetland to create the observed relationships by the time the water reached the outlet. In the future, we plan to identify these processes and their controls, to eventually compare the functioning of this constructed wetland to the functioning of similar but naturally occurring wetlands.

Simplified Salen Ligand Catalyzed Copolymerization of Cyclohexeneoxide and CO_2

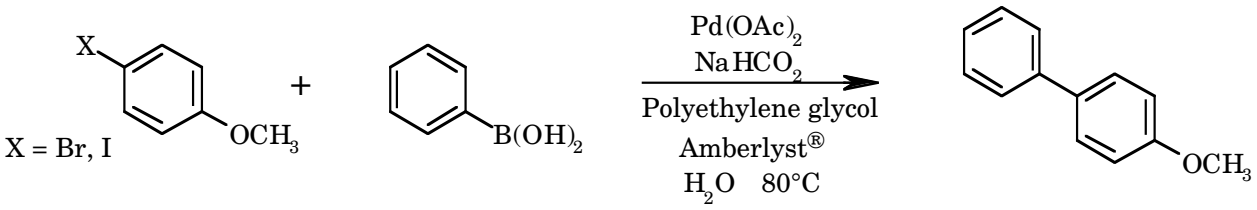
Ross Poland*, Jeremy Andreatta, Wonsook Choi, Cass Richers, and Donald Darensbourg
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803 and Department of Chemistry, Texas A&M University, College Station, TX 77842



Production of polycarbonates is a very widely utilized industrial reaction. Polycarbonate plastic is used in many facets of our civilization, whether it be in automobiles, computers, or in medicine. The reaction pathways most used in industry to produce polycarbonates tends to utilize reagents that are hazardous to human health and the environment. A movement known as *green chemistry* attempts to develop more environmentally friendly reaction conditions, reagents, and products. In this example, the polymerization reaction is made more green by negating the need for organic solvents. Green routes to polycarbonate production being studied at Texas A&M University are run in liquid CO_2 with organometallic catalysts. CO_2 serves as both a solvent and a monomer, as does cyclohexeneoxide. In order to accomplish the efficient production of polymer, a salen ligand, which is characterized by an N_2O_2 functionality, was synthesized in an inert atmosphere. The salen synthesized is unique in that it doesn't contain an aromatic ring typical of most salens, and also has a more flexible backbone due to the lack of doubly bound nitrogens. The ring-opening mechanism of the epoxide (2-(trifluoromethyl)oxirane)) coupled with substitution on to a symmetric amine (N1,N2-dimethylethane-1,2-diamine) produced the salen ligand in moderate yield. Chromium(II) was complexed by the ligand after reduction of chromium(III) to chromium(II) with KH as the reducing agent. This yielded the catalyst in a stable, solid state which was then purified using dichloromethane. Once pure, the catalyst and its co-catalyst (tetra-n-butylammonium chloride) were dissolved in cyclohexene oxide. This mixture was then transferred via cannula to a high pressure reactor and liquid CO_2 was added until the pressure in the reactor was approximately 600 psi. This reaction was allowed to run for at least 12 hours and was monitored in situ using a ReactIR spectrometer. The general polymerization mechanism is known, but the distinct properties of this catalyst are still unclear. The results show that there is more to be discovered by functionalizing salen ligands, which will lead to further opportunities for research and hopefully more effective catalysts.

Efficient Coupling of Aryl Halides and Phenylboronic Acid under Environmentally Friendly Conditions

Amanda Isom* and Rebecca DeVasher
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803



Suzuki coupling reactions are ubiquitous in the field of synthetic organic chemistry for the formation of carbon-carbon bonds. More environmentally friendly reaction conditions are desirable for use in the pharmaceutical industry, in particular. A high conversion of 4-iodoanisole to 4-methoxybiphenyl with no production of side products occurs at a 0.75mol% catalyst loading under atmospheric conditions (in air). The identity of the halogen significantly affects the conversion of starting material to product. Attempts to couple 4-bromoanisole and phenylboronic acid yielded considerable formation of phenylboronic acid derivatives. Suzuki coupling was not observed in reactions that did not contain sodium formate. Sodium formate is thought to reduce palladium from the +2 oxidation state to the elemental form of palladium (Pd^0). Cyclic voltammetry (CV) data supported the original hypothesis that the palladium reduction was occurring *in situ* as a result of the sodium formate addition. Amberlyst® A-26 (OH) Ion Exchange Resin (Amberlyst) was employed to increase the pH of the reaction (pH10) and stabilize the Pd^0 catalyst. The catalytic mechanism is thought to be colloidal in nature, owing to the fact that solid palladium exhibits low solubility. Polyethylene glycol Typical M_n4600 (PEG) was also used in the reactions to provide additional stabilization of the Pd^0. Amberlyst and polyethylene glycol loadings also significantly affected the overall yield of the reaction. Catalyst loading was tested in a range from 0.10-2.00 mole percent to determine the optimal catalyst loading; the mole percent is determined in relation to the moles of aryl halide in the reaction. A liquid-liquid biphasic mixture of ethyl acetate-water was developed in order to determine the effectiveness in producing 4-methoxybiphenyl. No change in reaction yield was observed for a 1:1 ratio of water:ethyl acetate solvent system. A liquid-liquid biphasic system is desirable for separation of the organic products from the aqueous stream. This system allows for catalyst recycling at room temperature for up to 3 catalytic reactions with moderate yield. Reactions run under room temperature conditions required longer reaction times than those run at 40°C and 80°C. The reaction reached completion in 4 hours at 80°C without the use of inert atmosphere conditions [$N_2(g)$, $Ar(g)$]. The reaction shown above illustrates a novel, environmentally benign methodology for Suzuki coupling, a reaction employed by the pharmaceutical industry in the synthesis of certain nonsteroidal anti-inflammatory drugs (NSAIDs).

This work was funded in part by the IRCBC under the auspices of the Merck/AAAS Undergraduate Science Research Program.

Optimization of the Succinate Dehydrogenase Vital Staining Procedure for Arbuscular Mycorrhizas

Meagan Gallagher*, and Ella Ingram
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Arbuscular mycorrhizas are extremely common mutualistic associations between plant roots and soil fungi. These symbiotic fungi form structures inside the plant roots for nutrient exchange, with the plant receiving valuable nutrients in exchange for its carbon resources. All strategies to visualize these mycorrhizal associations use techniques to differentiate fungal and root tissues, with the most common of these methods involving the staining of chitin-containing structures. While these methods can distinguish between plant material and fungal material, they do not distinguish between the active and inactive fungal associations. Vital stain techniques, based on the metabolic enzyme succinate dehydrogenase however, do distinguish between active and inactive fungal tissue. In this study the vital staining procedure was applied to the arbuscular mycorrhiza of mayapple *Podophyllum peltatum* L., a common temperate forest herb. The vital staining procedure was tested at various points to obtain a protocol that would yield more desirable results as well as minimize cost and time. Bleaching roots with ammonium hydroxide is recommended, but was found to be unnecessary for visualizing this mycorrhizal association. The amount of time the roots were exposed to KOH for clearing purposes was also tested and a time of 6 hrs was found to be sufficient, although up to 24hrs was also shown to be adequate as well, without significant negative outcomes for the roots. Therefore, depending on time constraints any time within that range would be effective. Soaking the roots in lactoglyerol to destain before mounting was also tested and was found to have no effect on cell clarity. The vital staining procedure was then compared to the non-vital staining procedure using trypan blue to determine differences in the percent of root colonization observed via the two methods, their relative costs and time required. The two procedures resolved a similar amount of fungal tissue in the plant roots, determined using a standard microscopic quantification technique. The final experiment performed showed that the newest roots were colonized by mid-August, earlier than expected based on data obtained via the trypan blue, non-vital staining procedure. Based on the experiments performed and the comparisons made, it is recommended that the vital staining procedure not be used for most experiments where percent colonization or general knowledge is sought, due to the cost, results, and time spent performing procedure. However, it would be useful for studies seeking information regarding changes in the ratio of inactive and active tissue throughout time.

This work was funded in part by the IRCBC under the auspices of the Merck/AAAS Undergraduate Science Research Program.

Role of T-bet in the Generation of Pulmonary Atopic Inflammation

A. Mae Huehls*, Sarita Sehra, and Mark H. Kaplan
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, 47803 and Department of Pediatrics, Herman B. Wells Center for Pediatric Research Indiana University School of Medicine, Indianapolis, IN 46202

The pro-inflammatory cytokine interleukin-17 (IL-17) has been implicated in the pathology of asthma, suggesting a possible role for IL-17-secreting T helper cells (Th17) as effectors in allergic airway inflammation. Development of Th17 cells is negatively regulated by the transcription factor T-box expressed in T cells (T-bet), which also promotes the production of interferon-gamma (IFN-γ). Increased incidence of airway hyperresponsiveness has been reported in T-bet-deficient mice on mixed and BALB/c genetic backgrounds, a phenomenon that could result from the lack of suppression of Th17 development. To test this hypothesis, T-bet-deficient and wild type mice on the C57BL/6 background were sensitized to induce atopic pulmonary reactivity and analyzed for production of IL-17, the Th1 cytokine IFN-γ, and the Th2 cytokines IL-4, IL-5, and IL-13. T-bet-deficient mice displayed increased IL-17 production in comparison to wild type mice but exhibited depressed severity of several inflammatory parameters. The histopathology of the lung tissue and cellular populations of bronchoalveolar lavage fluid were examined and revealed a decreased incidence of eosinophil infiltration in T-bet-deficient mice compared to wild type mice. A concomitant reduction in serum IgE levels in T-bet-deficient mice as compared to wild type mice also was observed. These results suggest that the effects of T-bet deficiency on the development of pulmonary infiltration are dependent on the genetic background of the mice. Furthermore, these results showed that increased development of IL-17-secreting cells is not sufficient to generate airway infiltration.

Genetic and Biochemical Characterization of *Arabidopsis thaliana* Putative Disease Resistance Genes

Emma Hegwood*, and J. Peter Coppinger
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

NDR1 (nonrace-specific disease resistance-1) is required for pathogen resistance in *Arabidopsis thaliana*. Studies have shown that null mutations in this gene result in enhanced susceptibility to bacterial pathogens. *NDR1* is flanked by two putative genes, *NDR2* and *NDR3*, which exhibit greater than 80% sequence similarity to *NDR1*. The sequence similarity to *NDR1* suggests that *NDR2* and *NDR3* may also play key roles in pathogen resistance, though it is currently unknown whether *NDR2* or *NDR3* are expressed *in planta*. In an attempt to investigate this hypothesis, we generated epitope-tagged alleles of *NDR2* and *NDR3* in an *Agrobacterium* 35S binary expression vector. These constructs will be transformed into *Arabidopsis* and *Nicotiana benthamiana* to study the post-translational processing and localization of these proteins. Concomitantly, we have initiated a reverse genetics approach to determine the biological function of *NDR2* and *NDR3 in vivo*. Mutant *Arabidopsis* lines containing "knock-out" T-DNA insertions in the *NDR2* and *NDR3* ORFs were identified through published seed stocks. We are currently testing these "knock-out" lines for an altered disease resistance phenotype. The goal of this research is to elucidate the mechanisms of the pathogen resistance pathways in *Arabidopsis*, which may ultimately enrich our understanding of disease resistance in agriculturally significant crops.

This work was funded in part by the IRCBC under the auspices of the Merck/AAAS Undergraduate Science Research Program.

Anharmonic Vibrations of a 60 Carbon System

Ivan Kornienko* and Daniel Jelski
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The soccer ball shaped molecule, known as bucky ball, consists of 60 identical carbon atoms positioned in a sphere. The chemically identical nature of all carbons allows for a relatively simple mathematical model of a large, seemingly complex system. A previously proposed equation for modeling the behavior of atoms in this system was implemented to prove it's validity by using a computational approach. In order for the complex system to be rigorously tested, an efficient language had to be chosen for the implementation. In accordance with this FORTRAN was the choice. These proved to be a challenge because of the complexity of code required and the inability to use modern object oriented techniques. To deal with the problem, modern refactoring and organizational techniques were tailored to FORTRAN to create an efficient and easily modifiable piece of code. Furthermore, to prove the validity of the results provided by the FORTRAN code, a preliminary Java program was written to generate 3d manifestations of the carbon atom positions of the bucky ball.

Purification and Inhibition of Recombinant Vaccinia Virus H1 Protein

Anita Mathur*, Julia Huang and Mark Kaplan

Department of Chemical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803 and Department of Pediatrics, Herman B. Wells Center for Pediatric Research Indiana University School of Medicine, Indianapolis, IN 46202

Variola, a member of the poxvirus family is the causative agent of smallpox and has long been recognized has a potential threat in biowarfare. Vaccinia virus is a highly related poxvirus and is used as the vaccine for smallpox. Since smallpox has been largely eradicated worldwide, infants and adults are no longer routinely immunized. Both Variola and Vaccinia employ a number of immune evasion mechanisms including blocking interferon (IFN) signaling that is required for optimal viral clearance. VH1 is a Vaccinia encoded phosphatase that is important for the viral life cycle and disrupts IFN signaling in part by dephosphorylating tyrosine residues of STAT proteins. Previous attempts to purify recombinant VH1 have yielded inactive enzyme. We have employed an altered protocol in the attempt to obtain purified active enzyme that can be used for in vitro assays. We also tested the efficacy of phosphatase inhibitors in an assay for VH1 activity using recombinant protein bound to the purification resin with the rationale that inhibition of the VH1 phosphatase could be a treatment for poxvirus infection. We tested various inhibitors using the p-Nitrophenyl Phosphate (pNPP) colorimetric assay. Six inhibitors of known phosphatase targets were tested. Significant inhibition was only observed with a broad spectrum inhibitor. An inhibitor of the related mammalian kinase VHR did not have an effect. Purified VH1 will be tested with additional inhibitors and used for additional in vitro assays.

Determining Cooperativity of the Human Estrogen Receptor Through Tryptophan Fluorescence Quenching

Adam G. Georgas* and Mark E. Brandt
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The estrogen receptor protein plays a key role in the development of breast cancer. In order to learn more about the disease and its treatment, the effect of ligands such as the commonly prescribed anti-cancer pharmaceutical, tamoxifen, on the receptor protein must be understood. By measuring the amount of tryptophan fluorescence quenching through fluorescence spectroscopy, the amount of ligand bound to the receptor can be determined. Previous experiments showed that methanol, which is required for Tamoxifen solution preparation, affects the protein's conformation less than other solvents. Under these conditions, the experimental reproducibility increased. Usual methods of curve fitting were not robust, yielding to non-unique fitting parameters. By employing a highly iterative least squares optimization method written in Matlab, unique solutions for the Hill coefficient and $K_{0.5}$ fitting parameters were achieved. Analysis shows that the human estrogen receptor exhibits positive cooperativity with the Hill coefficient being 1.41 ± 0.07 and $K_{0.5}$ being 0.024 ± 0.006 μM. Determining the effects of ligand on the estrogen receptor will give us greater insight in how this protein functions, and may allow researchers to develop improved pharmaceuticals for the treatment of breast cancer.

This work was funded in part by NSF MRI Program Award CHE-0521430.

The Effect of Alcohols on the Rate of Estrogen Receptor Dimer Exchange

Rebecca J. Waltz*, David M. Knapp, Rachel Krasich, Andrei L. Edwards, and Mark E. Brandt
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The ligand-binding domain (LBD) of the estrogen receptor protein can be expressed in *Escherichia coli* as an independently folding peptide covalently attached to maltose binding protein. This 70 kDA fusion protein is cleaved during purification to release the 30 kDa LBD. Both the fusion protein and the isolated LBD form non-covalently associated dimers in solution; the two interacting polypeptides exchange partners with a half-life dependent on the strength of the interaction. Previous studies have suggested that binding of both physiological and non-physiological ligands has a significant effect on the protein dimer exchange half-life. Because estradiol and other ligands have limited solubility in water, these compounds are typically dissolved in organic solvents prior to use. Full characterization of the effects of ligand binding requires an understanding of the effect of organic solvents on the protein. The kinetics of dimer exchange were measured using HPLC gel filtration chromatography following pre-incubation of the homodimeric LBD and fusion protein homodimers in the presence of low concentrations of organic solvent. The half-life of wild-type protein in buffer was about two hours. Low concentrations (0.1 to 0.5% by volume) of small mono-functional alcohols ranging from methanol to 1-butanol reduced the half-life in a concentration-dependant manner. The decrease in half-life was positively correlated with the boiling point of the alcohol tested, suggesting that the effect is mediated by non-polar interactions with the protein. Because changes in the dimer exchange half-life imply perturbations of the protein structure affecting the dimer interface, characterizing these effects may improve understanding of the estrogen receptor protein. The low concentrations of alcohols used further suggest possible physiological significance to these studies.

This work was funded in part by the IRCBC under the auspices of the Merck/AAAS Undergraduate Science Research Program.

The Accuracy of Laser-Induced Breakdown Spectroscopy in Quantifying Carbon in Soils

Rachel M. Selby*, L. Edwards, I.B. Gornushkin, B.W. Smith, and J.D. Winefordner
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803 and Department of Chemistry, University of Florida, Gainesville, Florida 32611

The purpose of this research is to use Laser-Induced Spectroscopy (LIBS) to determine the most accurate method of quantifying carbon in soils by comparing the integrated areas of the carbon (C) and the carbon-nitrogen molecular bands (CN).

LIBS Advantages
Possibility of remote sensing
In-situ analysis
Little to no sample preparation
Micro-destructive
Applicability to all media (solids, liquids, aerosols)
Simultaneous multi-element detection capabilities

LIBS Disadvantages
Poor precision (typically 5-10%)
Relatively high detection limits
Difficulty in preparing matrix-matched standards
Spectral Interferences

Carbon is a key element for all living organisms, but carbon-containing gases in the atmosphere affect the earth's climate, known as the Greenhouse Effect, which could be detrimental to us if nothing is done. The amount of carbon uptake in soils can represent an important mechanism in the cycling of carbon. By knowing the carbon concentrations in different soils and climates, the effect of global warming may be better understood. In order to complete such research, an easier method and applicable instrument will be needed to gather the immense amounts of measurements required.

Soil is very complex when considering its components; it contains inorganic and organic atoms, ions, and molecules. LIBS is capable of identifying carbon without spectral emission interferences. This, coupled with the above-mentioned advantages explains why LIBS is the forerunner in field analysis, although its precision continues to create problems.

Method Development and Microfluidic Device Design for Separation and Detection of Modified Nucleosides

Christian Sweeney* and Daniel L. Morris
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The DNA base guanine is by far the most vulnerable to oxidative damage by reactive oxygen species (ROS), forming 8-OH-Deoxyguanosine (8-OH-dG), a compound known to be linked to at least 50 diseases and clinical conditions. Therefore, it is of great relevance to develop fast and effective means of separating and detecting of 8-OH-dG from oxidatively damaged DNA.
Capillary Electrophoresis (CE) provides a powerful and fast means of separation on the basis of charge and size by means of an applied electric field, and is readily interfaced with UV/Vis absorption detection. Unfortunately, the sensitivity of UV/Vis detection in CE is limited by the small internal diameters of the capillaries. However, stacking of analytes in the capillary can be achieved when employing electrokinetic injection (EK), thereby decreasing the detection limits. We have employed stacking using a cholate based run buffer in a Micellar Electrokinetic Chromatographic (MEKC) separation. The cholate micelles contribute a partitioning dimension to the separation and allow the separation of neutrals in the sample. We report the results of altering individual parameters in the MEKC separation (concentrations, injection times, injection types, etc.) to achieve an optimal set of separation parameters for unmodified nucleosides and 8-OH-dG. We present the results of reactions involving double stranded calf thymus DNA and ROS separated using MEKC and demonstrate the successful separation and detection of 8-OH-dG in the reaction mixtures.
The results from the MEKC separations were employed to the design of a microfluidic device for performing the separations. The advantages of microfluidic devices for chemical analysis include short analysis times and minimal sample requirements, and we report a design that incorporates a z-shape detection channel allowing for an elongated path length for performing UV/Vis absorption detection. In addition, microfluidic device designs are being evaluated which incorporate a solid phase extraction step prior to separation as a means of preconcentrating the analytes. In addition, a device designed for the reaction of DNA and ROS that integrates the reaction, extraction, and separation steps, preventing sample loss as well as degradation is being developed.

This work was funded in part by the IRCBC under the auspices of the Merck/AAAS Undergraduate Science Research Program.

Combustion Toolbox

Mark Vaccari* and Dan Coronell
Department of Chemical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The CHEMKIN software package was developed by Sandia National Laboratories in the early 1980's to model combustion chemistry at the elementary reaction level. Its format for describing detailed chemical mechanisms has been adopted as an industry standard, resulting in the publication of numerous CHEMKIN combustion mechanisms in scientific journals and on the internet. In 1997, the CHEMKIN software package was commercialized and could no longer be obtained through the public domain. The objective of this work was to develop a combustion chemistry simulator that replicates the primary features of the CHEMKIN package, and which can make use of the large number of CHEMKIN-formatted mechanisms available in the literature. The Combustion Toolbox that resulted from this effort is an Add-In that is seamlessly integrated into the Microsoft Excel environment. The graphical user interface was developed using Visual Basic for Applications, and the computational models were developed using Matlab. The Combustion Toolbox solicits input from the user, translates the selected CHEMKIN mechanism and associated thermophysical property files, dispatches the computational task to Matlab, and performs a variety of graphical post-processing tasks. Models have been developed to simulate detailed combustion chemistry in batch reactors, continuous flow (plug, well-mixed) reactors, well-mixed reactors in series, and homogeneous charge compression ignition (HCCI) engines. The numerical results predicted by the Combustion Toolbox have compared favorably with published experimental data (autoignition delays) and CHEMKIN simulations (composition and thermal profiles).

Particle Size Effects in the Pretreatment of Wood Waste Leading to Enhanced Ethanol Production

Amanda Grantz*, David J. Dixon, and Patrick C. Gilcrease
Department of Chemical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803 and Department of Chemical and Biological Engineering South Dakota School of Mines and Technology Rapid City, SD

A twin screw extruder and a ball mill were explored as means of physical pretreatment in the conversion of wood waste to ethanol. Sieve analyses were performed to compare the particle size distributions of wood subjected to different pretreatment methods. Enzymatic hydrolysis was employed to quantify glucose recoveries for particular pretreatment schemes. While particle size distribution analyses show milling to be a more effective particle size reduction method than extrusion, extrusion proved more successful than simple grinding in enhancing cellulase enzyme recovery of glucose for fermentation to ethanol. Extruded wood produced glucose recoveries 10 times greater than those obtained from raw feed material and 25 percent greater than those obtained from ball-milled wood. Scanning electron microscopy revealed significant structural differences among untreated, extruded, and ball-milled wood. The twin screw extruder shears wood to mechanically breaking apart fiber structure. Compared to traditional particle size reduction methods, the effects of using a twin screw extruder in the pretreatment of biomass leading to enhanced ethanol production are unique.

Prevention of Membrane Pore Formation Via $P2X_7R$-Cx43 Interaction

Christina Chrisman*, M. Cohen, S.O. Suadicani, D.C. Spray, and E. Scemes
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$P2X_7Rs$ are ATP-gated ion channels expressed in brain, colon, heart, prostate, and skeletal muscles. Activation of these receptors leads to the opening of large pores, permeable to molecules of up to 900 Da. This membrane pore may be responsible for exacerbating cell damage during pathological conditions such as inflammation, ischemia, spreading depression, and trauma. Previous studies indicate that interactions between P2 receptors and gap junctions may occur. Gap junctions are clusters of intercellular channels that connect the intracellular milieu of adjacent cells and allow the passage of molecules of up to 1 kDa in size. Each cell of an adjacent pair contributes six connexins (Cxs) to form a hemi-channel (connexon), and the pairing of two connexons forms the gap junction channel.

The aims of this study were to determine the proteins of the Cx43-$P2X_7R$ complex using antibody arrays; to determine if there was an interaction between $P2X_7R$ and Cx43 in the brain, and, if so, to determine the role played by Cx43 in the $P2X_7R$-induced pore formation. Using antibody array technique we found that in the brain Cx43 and $P2X_7R$ interact, result confirmed by immunoprecipitation assays. Dye uptake experiments performed on Cx43-null neuroblastoma (N2A) cells and two subclones of a macrophage cell line, J774 (*G8* – a subclone where Cx43 binds to $P2X_7R$, and *A1* – a subclone where Cx43 does not bind to $P2X_7R$) showed that Cx43-null N2A cells over-expressing $P2X_7R$ and J774-A1 cells took up 30% more dye than J774-G8. These results suggest that Cx43-$P2X_7R$ interaction prevents membrane pore formation.

Observation of the Bioluminescent Reaction Between Aequorin and Calcium Ions

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The bioluminescent jellyfish, *Aequoria victoria*, is known to emit a bluish light due to the presence of a Ca^{2+}-binding photoprotein called aequorin. Aequorin is composed of a non-covalent complex of apoaequorin, coelenterazine and molecular oxygen. The protein has been shown to contain three allosteric Ca^{2+}-binding sites. On binding Ca^{2+} to all three sites, the coelenterazine is converted to coelenteramide, and light is released (λ_{max} = 470 nm). Because of these reaction characteristics, the rate of ES to EP conversion can be measured by measuring the rate of photon release.

Using the stop-flow apparatus of the Fluorolog-Tau-3 spectrofluorometer, the rate of photon release was detected under conditions of varied temperature, Ca^{2+} ion concentration, and pH level of the buffer. It was hypothesized that the conversion of ES to EP would be a first order reaction. Higher concentration of Ca^{2+} ions and pH levels closest to pH 7.5 were predicted to cause an increase in the reaction rate.

The data collected indicated a strong first order relationship between the temperature of the reaction and the reaction rate constant, as described by the Arrhenius equation. As the calcium concentration was increased (from 0.0005M to 0.1M Ca^{2+}), the rate of reaction increased up to a point and then began to drop off. Between the buffer pH levels and the log of the reaction rate constant, a somewhat logarithmic relationship was observed.

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