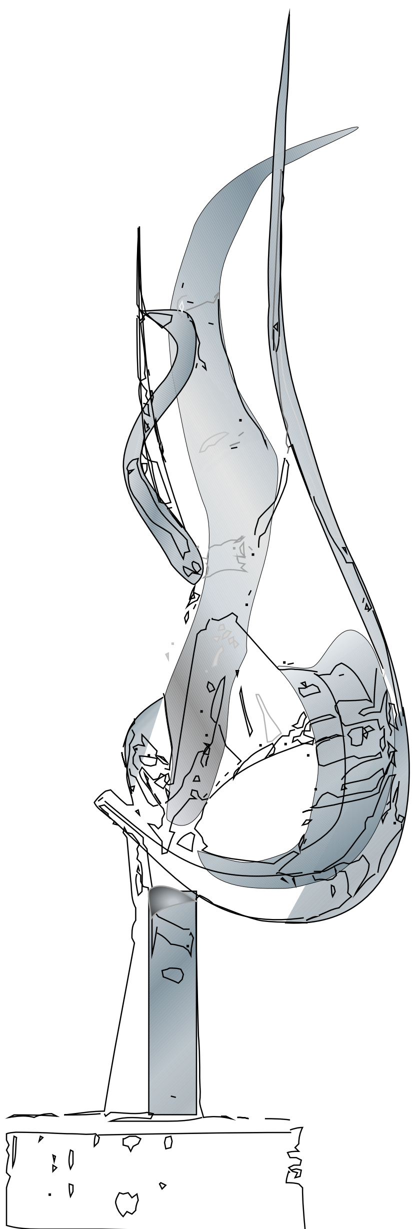
Interdisciplinary Research Collaborative in Biology & Chemistry

IRCBC

Rose-Hulman Institute of Technology



1st Annual *IRCBC* Undergraduate Research Symposium

Friday
October 29, 2004

MERCK • AAAS Undergraduate Science Research PROGRAM

ROSE-HULMAN INSTITUTE OF TECHNOLOGY

Welcome to the

1st Annual IRCBC Undergraduate Research Symposium

Rose-Hulman Institute of Technology

Friday, October 29, 2004

We are honored to welcome you to the 1st 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.

Until relatively recently, the science curricula at Rose-Hulman were supported limited to programs in Chemistry and Physics. However, with the recent formation of the Department of Applied Biology & Biomedical Engineering (ABBE), and the reinvigoration of our biochemistry curriculum, Rose-Hulman students have been provided with new opportunities to explore exciting and burgeoning areas of scientific endeavor. To help students capitalize on these opportunities, we instituted a new interdisciplinary academic major in Biochemistry & Molecular Biology, which is being offered collaboratively by the ABBE and Chemistry Departments. This symposium is intended to further foster and expand on the successes of these new programs.

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.

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.

Richard Anthony
IRCBC Program Coordinator

Mark Brandt
IRCBC Program Coordinator

Symposium Schedule

10:00 AM Registration

10:30 AM Opening Session

Welcoming Remarks **– Arthur Western, Vice-President for Academic Affairs and Dean of the Faculty, Rose-Hulman Institute of Technology**

Scheduled Presentations

Matthew Brittain, "Analysis of Actin in *Stemonitis flavogenita*."

Ian Dailey, "Design of a novel assay for glucose 1,6-bisphosphate production in *E. coli*."

Sara Rohrabaugh, "Characterization of Antibiotic-dependent Yeast Mutants."

Alana Burke, "Functional Substitution of Ribosomal Protein S23 Homologues in Yeast."

12:00 PM Lunch

1:00 PM Afternoon Session

Scheduled Presentations

Chad Zarse, "Advances in Stone Imaging: Improving Shock Wave Lithotripsy Treatment for Patients with Urinary Stone Disease."

Jade Wamsley, "Trace Metal Analysis of Rural Water Samples from the Trafalgar Wisconsian Glacial Aquifers."

Rita Strack, "Gel Shift Mutations in His-Patch Thioredoxin Mutants."

2:00 PM Break

2:20 PM Afternoon Session Resumes

Scheduled Presentations

David Knapp, "Expression and Purification of Mutants of the Human Estrogen Receptor Ligand Binding Domain."

Matthew Sung, "Characterization of Human Estrogen Receptor Ligand Binding Domain Mutants."

Amber Brannan, "Cloning and Expression of the L1-CAM Extracellular Fragment."

Analysis of Actin in *Stemonitis flavogenita*

Matthew K. Brittain* and Jeannie T. B. Collins
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Stemonitis flavogenita is a myxomycete which has three stages in its life cycle. These stages begin with spores germinating to an aphanoplasmodium which develops into a coralloid plasmodium. Myxomycetes, slime molds, move throughout their environment via pseudopods during the aphanoplasmodial and coralloid plasmodial stages. This process is under the control of cytoskeletal proteins. Actin is a cytoskeletal protein that polymerizes to form microfilaments. Along with other cytoskeletal proteins, these microfilaments are likely involved in motility of the organism. Actin has been detected in extracts from both the aphanoplasmodial and coralloid plasmodial stages. Based on western blot analysis, *Stemonitis flavogenita* actin is roughly 45 kDa in size. Capillary electrophoresis was used to examine actin from extracts of various stages in the organism's life cycle. Under current conditions, actin interacts with other proteins in the extracts during capillary electrophoresis.

Design of a novel assay for glucose 1,6-bisphosphate production in *E. coli*.

Ian Dailey* and Mark E. Brandt
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

During glycolysis, glucose 1-phosphate is converted to glucose 6-phosphate by phosphoglucomutase. Research suggests that glucose 1,6-bisphosphate is a cofactor for this enzyme, and that some amount of unbound glucose 1,6-P_2 is needed to maintain catalysis by phosphoglucomutase. Glucose 1,6-P_2 may also regulate other metabolic enzymes. It is not known if glucose 1,6-P_2 is present in excess in *E. coli*, though it has been found in considerable excess in several mammalian species. *E. coli* TOPP2 cell lysate was analyzed for the presence of excess glucose 1,6-P_2 by precipitation using ethanol and barium chloride, followed by anion exchange HPLC and direct ultraviolet absorption quantitation. Because of the cost of glucose 1,6-P_2, the precipitation study was first performed using known amounts of glucose 1-P as a test molecule. Detecting glucose 1-P and glucose 1,6-P_2 proved more difficult than anticipated due in part to their low extinction coefficients ($\varepsilon_{210} \approx 1$ (M cm)$^{-1}$). Glucosamine 6-phosphate was cross-linked to 6-aminohexanoic acid Sepharose 4B resin, and this was tested as an affinity resin for the isolation of enzymes that might be responsible for synthesis of glucose 1,6-P_2. A significant enrichment of a sub-population of proteins was observed using this method, though the identity of these proteins was not established. Affinity chromatography should be explored further as a separatory technique for possible synthetic enzymes of glucose 1,6-P_2, and other spectrophotometric methods should be considered for the detection of glucose 1,6-P_2 itself.

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

Characterization of Antibiotic-dependent Yeast Mutants.

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Proper function of ribosomal proteins is necessary to maintain accurate translation, a necessity for cell viability. Nonsense suppressor mutations allow the ribosome to read through spurious stop codons and often decrease the overall accuracy of translation. In *Saccharomyces cerevisiae*, nonsense suppressors may arise from alterations in several ribosomal proteins. These suppressor mutations often increase sensitivity to the aminoglycoside antibiotic paromomycin. Conversely, antisuppressor mutations may also arise in some of these ribosomal proteins. These mutations are thought to increase the accuracy of translation and may counteract the decreased accuracy and increased paromomycin sensitivity associated with suppressor mutations. Wild-type yeast haploids harbor two genes encoding the key ribosomal protein Rps23. Previous attempts to isolate paromomycin-dependent mutants in wild-type strains proved unsuccessful. However, six novel paromomycin-dependent yeast mutants were recently isolated in our lab. Because these mutants were isolated from a strain bearing a knockout in one of the *RPS23* genes, we believe that the mutation responsible for the antibiotic-dependent phenotype resides in the remaining functional copy of *RPS23*. Here, we describe further characterization of these mutants and our attempts to identify the genetic changes responsible for the mutant phenotype. In one experiment, we investigated the effects of varying paromomycin concentrations on the growth of the mutants. Our results suggest that several different mutations may be responsible for the paromomycin-dependence noted in the different mutant isolates. We also attempted to reverse the paromomycin-dependent phenotype by introducing a wild-type copy of the *RPS23* gene on a plasmid. However, in many cases, the mutants were recalcitrant to transformation or appear to have lost the mutant phenotype during the transformation procedure.

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

Functional Substitution of Ribosomal Protein S23 Homologues in Yeast.

Alana M. Burke*, Sara L. Rohrabaugh and Richard A. Anthony
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Ribosomal protein S23 (Rps23) is an essential component of the yeast ribosome and is well conserved in both prokaryotes and eukaryotes. This protein is known to play an important role in maintaining the accuracy of translation. It has also been shown to be crucial in controlling resistance or sensitivity to certain aminoglycoside antibiotics in prokaryotes and eukaryotes, respectively. The purpose of this research is to determine whether Rps23 homologues from bacteria or higher eukaryotes can function in the yeast ribosome and, if so, the effect that these homologues have on aminoglycoside sensitivity. The *RPS23* gene is essential and is duplicated in haploids. Strains harboring a single disruption of either *RPS23A* or *RPS23B* were constructed for previous work. However, a double knockout strain and a counter-selectable plasmid bearing a functional *RPS23* gene are needed to perform plasmid shuffling experiments. A strategy to construct the required double knockout strain and the appropriate plasmid has been developed. This presentation focuses on current progress and future goals.

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

Advances in Stone Imaging: Improving Shock Wave Lithotripsy Treatment for Patients with Urinary Stone Disease

Chad A. Zarse*, Samuel C. Kim, James A. McAteer, James E. Lingeman, and James C. Williams, Jr.
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Helical CT has become the preferred method to diagnose urinary stones in patients presenting with abdominal pain. Recent studies have shown that CT can display internal structure in stones with remarkable detail as well as predict stone composition. Given that some stones are more susceptible to shock wave lithotripsy than others and that shock wave lithotripsy is known to cause renal damage, ascertaining stone structure and composition at diagnosis via clinical CT could be helpful in choosing among treatment options. Older, low-resolution CT technology proves to be problematic since these studies suffered from an artifact in which stones with low apparent CT attenuation were observed to fragment to a greater extent during lithotripsy than stones with high attenuation, but this was likely due to volume averaging error as a result of interplay between stone size and image resolution. Heterogeneity of stone composition is another complicating factor, which was not foreseeable with older CT technology. Using new quad-slice multi-detector helical CT, we demonstrate that there is sufficient image quality to view the spatial arrangement of heterogeneous components in stones when narrow x-ray slice widths and bone windows are used. Improved resolution and suggested viewing parameters also permits accurate identification of stone composition for four clinically relevant stone types using only attenuation values. We anticipate that continued improvement in image resolution for CT technology will provide more information about stone composition and structure that will ultimately lead to better care for patients with stone disease.

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Trace Metal Analysis of Rural Water Samples from the Trafalgar Wisconsian Glacial Aquifers

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Past studies have shown that the ingestion of specific metals has been found to cause severe health problems. Some of the metals of highest concern can be found in natural deposits in hazardous areas, such as water tables, while others are waste of industrial processes which run into streams causing contamination of drinking water sources. The Environmental Protection Agency (EPA) has published regulations for the amount of hazardous metals that are allowed in drinking water. In this experiment, four water samples were taken from rural wells supplied by the Trafalgar Wisconsian glacial aquifer. The samples were analyzed for hazardous metal concentrations to determine if EPA regulations were exceeded. Ultra-trace metal cation analysis employed the use of UV/VIS Spectroscopy, Flame Atomic Absorption Spectroscopy (FAAS), Flame Atomic Emission Spectroscopy (FAES), ICP Emission Spectroscopy (ICP-ES), and ICP Mass Spectroscopy (ICP-MS). Samples from the same well exhibited ranges of values: FAAS analysis indicated concentrations from 4 to 50 ppb Cr and 10 to 200 ppb Pb, while the ICP-MS analysis indicated concentrations from 8 to 40 ppb Cr and 1 to 10 ppb Pb. The maximum contaminant level established by the EPA is 50 ppb Cr and 15 ppb Pb for these two metals. From the current data, no discernable trends were observed. In conclusion, metal concentrations were found to be 300 ppb Pb, 280 ppm Cd, and 5000 ppm Fe for water used as the standard in several of the analytical methods. The systematic error yielded inconclusive results for UV/VIS Spectroscopy, FAAS, FAES, and ICP-ES. To improve the quality of experimentation, a pure standard should be used in these methods to produce more accurate results.

This work was funded by the NSF and the RSEC.

Gel Shift Mutations in His-Patch Thioredoxin Mutants

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Determining the oligomerization states of many different proteins can be a time-consuming and difficult endeavor. However, we have begun to develop a novel, high-throughput method of determining the oligomerization states of proteins based on how varying the charge causes proteins to run differently on native gels. To create proteins with different charges, we are taking three different approaches. First, we are introducing single amino acid changes via site-directed mutagenesis into a fusion protein consisting of His-Patch thioredoxin fused to the dimeric GCN4 leucine zipper. To affect the overall charge, seven different amino acids within the His-Patch thioredoxin with a positive or neutral charge were changed to glutamic acid or aspartic acid individually in an attempt to create a more overall negative charge. Second, mutations to lysine residues were made in the region between Trx and GCN4 consisting of four consecutive glutamic acid residues to make the overall charge of the protein more positive. These mutants are currently being screened for sequence verification. Finally, we are introducing cassettes encoding charged amino acids into the unique cloning site of *Rsr*II. I tested one verified mutant (K57E) for expression and purification; however, the mutant protein was not detected on an SDS-polyacrylamide gel after purification. More work must be done to verify mutant proteins, create noticeable gel shifts, and to further develop this method of determining oligomerization states.

This work was funded by the NSF.

Expression and Purification of Mutants of the Human Estrogen Receptor Ligand Binding Domain

David Knapp*, Matt Sung, and Mark Brandt.
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The human estrogen receptor ligand binding domain (ER LBD), the independently folding ligand-binding region of the larger Human Estrogen Receptor, is known to undergo a conformational change in the presence of a ligand. The exact nature of this change is not well understood. Previous studies have indicated that the α-helix at the C-terminus of the LBD may undergo significant changes in conformation when the ligand is bound. Unfortunately, as there is no X-ray crystal structure for the unbound protein, other methods analysis must be considered. Two new mutants of the ER LBD were therefore synthesized with substitutions at position 531, at the base of this α-helix, with the intention of observing the difference in behavior between the mutants and the wild type protein with respect to ligand-binding and dimerization. The wild-type and mutant forms were expressed in *E. coli* TOPP2 cells as a fusion protein with maltose binding protein (MBP). The initial fusion protein was expressed in initial yields of over 120 mg per Liter of culture solution. Recovery, as expected, dropped considerably with cleavage of the fusion protein. The proteins were loaded onto an anion exchange column, washed, and eluted with a linear salt gradient, thus separating the LBD and MBP. The fractions containing LBD were pooled and run over an amylose affinity column to remove any remaining traces of MBP. With this new method of purification, enough of the relatively unstable mutant was purified for study. As dimer interaction affinity is difficult to study, kinetic experiments were run on the purified protein to determine a related property, their rate of dissociation. Because the fusion protein and the LBD are known to dimerize, by combining purified solutions of LBD and fusion protein the concentration of these homodimers and the formation of a new heterodimer could be observed as a function of time. These experiments, performed both in the presence and absence of various ligands, will ultimately provide insight into how small molecules can affect the form and function of both the human ER LBD and other proteins important to human metabolism.

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

Characterization of Human Estrogen Receptor Ligand Binding Domain Mutants

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The biological effects of estrogen are regulated by the Human Estrogen Receptor (ER). Hence, understanding the interaction of estrogen and other ligands with the ER Ligand Binding Domain (LBD) can prove quite useful. LBD was expressed in high yield as a fusion protein with Maltose Binding Protein (MBP). The MBP-LBD fusion protein was cleaved with hydroxylamine to release the LBD peptide.

Two mutant LBD peptides, in which Lysine-531 was mutated to glutamine (K531Q) and to proline (K531P), and the wild-type LBD were expressed in TOPP-2 strain *E. coli* cell cultures, and then both fusion proteins and isolated LBDs were purified. Dimer exchange experiments were done to measure the rate at which the MBP-LBD and LBD homodimers disassociate and the resulting rate at which the MBP-LBD/LBD heterodimer forms. The amount of each dimer form was quantitated by performing gel filtration chromatography with an absorbance detector interfaced to a computer using Logger Pro. The reaction was allowed to run for about 26 hours for complete equilibration. The actual concentration of the three dimers at each time-point was measured by finding the peak areas of the respective peaks. The homodimer concentrations were shown to decrease in an exponential fashion, allowing the rate constant for dissociation to be determined. Typically, half-lives for the homodimer disassociation process were about 1.9 hours.

The experiment is not without its problems, however. One problem that plagues this experiment is protein aggregation during purification and the dimer exchange experiment itself. Protein aggregation is evidenced by the 8-9 minute peak in the chromatograms; this peak represents the void volume as well as any aggregated protein in the sample. The wild-type MBP-LBD and LBD proteins are relatively stable and soluble when compared to the mutant types of these proteins. The mutant proteins readily aggregate making data analysis more complex. Another problem was that the heterodimer and MBP-LBD peaks were incompletely resolved in the chromatograms. To determine the area of these two peaks non-linear regression analysis was used to fit log-normal curves to both of the peaks. The log-normal curve is a good approximation for most protein absorbance peaks, however, it is not perfect and thus error is introduced in the calculations.

Despite these problems, successful dimer exchange experiments were completed for the wild-type and mutant proteins. The data collected will eventually allow us to more fully understand the mechanism of ligand binding and thus the integral proper folding properties of the ER protein.

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

Cloning and Expression of the L1-CAM Extracellular Fragment

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The final objective of this project is the immobilization of two neuronal growth stimulating proteins on implantable biomaterial bridges to stimulate and direct axonal regeneration following spinal injury. Currently the focus is on the neural cell adhesion molecule (L1-CAM), a membrane glycoprotein required for proper neuronal cell migration in the central nervous system during development. As neurite outgrowth is thought to be controlled by the L1 extracellular immunoglobulin domains, detailed primer design along with PCR techniques were used to selectively clone the extracellular region of L1 for transfer into a baculovirus expression vector. Transfection of Sf9 insect cells with the baculovirus results in protein expression accompanied by the incorporation of a poly-histidine tag that aids in purification and oriented immobilization on a biomaterial bridge for spinal repair. Future work will deal with neurotrophic factor-3 (NT-3) which is also involved in controlling the development, survival, and phenotypic differentiation of nerve cells. After achieving successful expression of L1-CAM and NT-3, directed immobilization of different combinations of the two proteins will be used to investigate how they interact when stimulating neuronal cell outgrowth.

Funding for the program was provided by NSF REU grant EEC 0139624. Thanks also to Clemson University for Ken Webb's start-up funding through NIH grant P20 RR-016461.