Student Titles and Abstracts Based on Summer Research 2003

**Bryan Anderson.** IMPLEMENTING A WEB-BASED 24/7 DYNAMIC NMR and JARED IRWIN. A NOVEL BROWSER-BASED NMR DATA DELIVERY AND ANALYSIS TOOL.

In October of 2002, St. Olaf College installed a fully automated Bruker Avance 400 MHz NMR spectrometer. After modifying the original software and writing a custom web-based application, our NMR spectrometer can now be operated remotely 24 hours a day 7 days a week. Using a web-based interface over the college network we can now assign student teams their own slot in our robotic sampler for the entire semester. With a sample in their slot, a student may select any of the 13 allowed experiments, change the parameters for that experiment, check the status of the spectrometer, and analyze the data from their current experiment or any of their past experiments. Students can accomplish all of this without leaving their lab or dorm room. Most importantly, this gives the student more direct contact with the process of running an NMR experiment than just handing the sample to a lab TA and getting back their spectrum during their next lab period. This poster outlines the essential aspects of the software and hardware we developed for this unique NMR installation.

**Laura Berntson.** GENE REGULATION BY mRNA-THIAMINE RECOGNITION.

Specific gene regulation in bacteria occurs by several distinct mechanisms. In certain situations proteins bind to DNA near the transcription start site interfering with the initial formation of messenger RNA. Alternatively, gene regulation can occur after the messenger RNA has been fully transcribed. This post-transcriptional regulation occurs when factors binding to messenger RNA interrupt the initiation of translation.

We first attempted to establish whether the mechanism of *R. etli* thiamin regulation occurs at the transcriptional level. A transcriptional pausing assay was used to distinguish between full length and truncated RNA products depending upon the presence of thiamin. We continued our investigation by testing the translational control model by attempting to resolve discrete structural differences of fully transcribed RNA folded in the presence and absence of thiamin.

Unfortunately, we were not able to detect sensitivity to TPP (thiamin pyrophosphate) at the transcriptional level or determine gross structural differences in the RNA in the presence and absence of TPP.

**Matt Berkseth and Katie Huber.** FISHING FOR ANSWERS: PHYLOGEOGRAPHY OF THREE DARTER SPECIES (*ETHEOSTOMA*)

**Matthew Bills.** DEVELOPMENT OF A HARDWARE AND SOFTWARE RADAR SYSTEM.

The mission of our research group is to study the relationship between ice and climate. For the last four years, a ground based ice-penetrating radar system has been taken to Antarctica to study the internal layers within the ice down to the bedrock, as part of the US-ITASE program. The internal ice stratigraphy helps us understand ice dynamics and the evolution of the ice sheet over time. Over the course of the four field seasons of the
US-ITASE project, we have gathered more than 2 gigabytes of raw data, taken from four major traverses covering more than 4000 kilometers of Antarctica.

For this project we have developed our own radar system using specialized, but primarily off-the-shelf, components, configured to our particular needs. The system requires a very fast digitizing process for the radar signal while being robust enough to survive long traverses in brutal Antarctic weather conditions. This poster describes the requirements for the radar system and how we have tailored both the hardware and software components to address those requirements.

**Michael Bongard, William Kleiber, John Nichol.** FINISHING UNFINISHED BUSINESS HIGH RESOLUTION MOLECULAR BEAM SPECTROSCOPY.

Using St. Olaf’s molecular beam electric resonance spectrometer, we study hyperfine interactions, which are interactions between the nuclei of the atoms in a molecule and the entire molecule itself. Researchers in years past observed RbBr but failed to make significant progress, so we decided to continue gathering RbBr data. We also analyzed past $^6$LiI data to determine whether the electric hexadecapole interaction suggested by prior analyses is real. To this end, we later reloaded the spectrometer with $^6$LiI to gather more data. We also started extensive software development for a new data analysis package.

**Sara Champlin and Yvonne Yang.** STABILITY OF THE DNA HELIX IN COSOLVENT SOLUTIONS: INVESTIGATING DNA HYDRATION, COSOLVENT BINDING, AND ION RELEASE.

In this study, we have investigated the effects of water activity, $a_w$, on the thermal stability of 160 base pair (bp) fragments of calf-thymus (ct) DNA. UV-absorbance spectroscopy was used to measure the melting temperatures ($T_m$) of the DNA helix as a function of $a_w$ determined by vapor pressure osmometry. Ethylene glycol and the dipolar methylglycines cosolvents were used to adjust $a_w$.

As expected, an increase in cosolvent concentration with a concomitant decrease in water activity lowers $T_m$. Our estimates for the number of released water molecules ($\Delta n_w$) from the DNA duplex upon melting agree with the 3-5 specifically bound water molecules predicated by X-ray crystallography. We also find that the methylglycines bind to single-stranded DNA upon melting, in contrast to previous studies using polyols as cosolvents. Sodium ion release ($\Delta n_{Na^+}$) from the DNA duplex upon melting is dependent on $a_w$ in methylglycine-water solutions, suggesting a possible interaction between methylglycines and the negatively charged DNA phosphate backbone.

**Bunkhuon Chhun.** RAISING *ERISTALIS TENAX* FOR CONDITIONED LEARNING.

*Eristalis tenax* (drone fly) has been known to be an important pollinator and have a direct impact on its environment. This leads to the importance of understanding their learning behavior. To understand their learning behavior, three objectives were established: 1) raising naïve flies for experimentation 2) experimenting with naïve flies by conditioning them to different color flowers from their innate color preference 3) ascertaining an extinction time period in which flies can retain learned behavior. Due to shortage of flies,
it was difficult to accomplish numbers 2 and 3, but we were able to successfully grow naïve flies in captivity from eggs laid by wild flies.

**Mike Erickson.** DEVELOPING ELECTRONICS FOR THE POSITRONIUM THERMALIZATION EXPERIMENT.

With the growing interest in measuring the momentum transfer cross-section of positronium gas scattering we have developed a new apparatus for observing the scattering of positronium in gasses. This new apparatus will allow us to make measurements of the momentum and the lifetime of the positronium. In addition to collecting these two measurements independently, we will be able to collect correlation data between the energy and the lifetime, yielding an increase in the precision of the measurements. The data acquisition and correlation detection hardware has been the focus of my summer's work, as has been developing interface software for both acquisition and data analysis.

**Karen Galles.** SIMULATED ISCHEMIA'S EFFECT ON MITOCHONDRIAL MEMBRANE POTENTIAL IN H9C2 CELLS.

**Leah Gross and Alyssa Anderson.** HOUSE WRENS: THE CONTINUING SAGA.

Understanding how species view their habitat and what features entice them to settle in a particular area is important when determining quality of a certain habitat. While it is commonly known that the house wren (*Troglodytes aedon*) is an edge species commonly found in small forests, few studies have studied habitat preference of wrens on a landscape scale. The purpose of this study was to determine if House Wrens tend to select for or against a particular habitat. Our study area contains four distinct habitat regions with nest boxes, including field edge, meadow, pond, and woods. Factors considered throughout this study included nest box occupancy, mate attraction time, clutch size, parental investment, and overall reproductive success. Between 53-56 nest boxes were monitored daily during the summers of 2003, 2002, and 2001. Observations including nest building activity, male pairing status, dates of first egg and hatching, clutch size, and parental presence and behavior was recorded. Data was analyzed using ANOVA and Chi-squared analysis. Preliminary results of the 2003 data support the null hypotheses that House Wrens do not select for a particular habitat area when choosing nesting sites. If house wrens settle in a certain area in order to try and increase reproductive success, these results suggest that each habitat within the study area provides sufficient resources and protection from predators to minimize nestling loss.

**Kyle Halvorson & Kirsten Rittenbach.** CONTAMINATION ANALYSES OF CAFFEINE TRACERS AND PERSONAL CARE PRODUCTS IN THE CANNON RIVER WATERSHED.

A common fate of anthropogenic (human produced) compounds is to pass unscreened through both digestive systems and wastewater treatment facilities (WWTF), or as agricultural runoff, into natural waters. The United States Geological Survey (USGS) recently conducted a nationwide study in an attempt to analyze the presence of anthropogenic pollutants in natural waters. Such a study has only just begun at the local level. Our research goal was to design and apply analytical methods to determine the
identity and quantity of contaminants in the Cannon River Watershed. River water samples were collected, centrifuged, and filtered using standard procedures. Solid Phase Extraction (SPE) was conducted using 3M Empore C18 and Waters Oasis HLB cartridges. Caffeine and other anthropogenic compounds were analyzed using Gas Chromatography/Mass Spectroscopy detection (GC/MS). Personal care products were analyzed using High Performance Liquid Chromatography (HPLC) under isocratic water/acetonitrile conditions with a Zorbax SB-C18 column. Standard calibration curves were used as a basis for comparison and evaluation. The limit of detection for caffeine was 100 ppt ± 8.42 ppt. Other compounds of interest were not detected in the watershed, and compounds not on our original list were found instead. We want to continue expounding on previous research and use our findings to inform the local community of pollution problems and their possible environmental impacts.

Mike Helgen and Kieran Cofell Dwyer. DATA ANALYSIS AND 3D MODELING OF 2002 ITASE TRAVERSE ROUTE.

Ice sheets play a major role in the earth's climate, affecting the absorption of solar radiation as well as atmospheric and oceanic heat circulation. The US International Trans Antarctic Scientific Expedition (US-ITASE) has completed four traverses across portions of the West and East Antarctic Ice Sheets to help understand the relationship between Antarctica and the earth's climate and changes over the past two-hundred years. As a member of US-ITASE, the Center for Geophysical Studies of Ice and Climate (CEGSIC) at St. Olaf has been recording deep penetrating ice radar along these traverses. Radar provides information on subglacial bedrock topography and internal ice stratigraphy layers within the ice that depict ice dynamics history. Our work has been focused on analyzing the data gathered in the 2002-03 US-ITASE traverse, particularly the regions surrounding the Byrd Surface Camp and Hercules Dome where we have radar survey grids allowing three dimensional analysis of local ice and bedrock structures. These two areas of Antarctica are of particular interest because they are locations of current (Byrd) and possible future (Hercules Dome) deep ice core climate records.

Lauren Lucas and April Graves. PHYLOGENETIC RELATIONSHIPS AMONG CHAEOBorus SPECIES.

Chaoborus play an important role in the trophic dynamics of lakes and ponds. Unfortunately, the relationships among many Chaoborus species have not been described well. We reexamined morphology-based phylogenies using molecular biology to build a DNA-based phylogenetic map of four species of Chaoborus found in North America. We did this by extracting, amplifying, and sequencing their DNA. We analyzed molecular variation in an 806-827 bp region of the mitochondrial genome (cytochrome oxidase I, Leu-tRNA, and cytochrome oxidase II) using computer programs. We found that our individual samples unambiguously grouped into species, and that there were two clades that were only partially congruent with previous morphology-based phylogenies. We also found individual variation within the four species. Our future projects will expand the Chaoborus phylogenetic tree by including more species and will examine individual variation within a single species widely distributed in areas of North America.
**Graeme McAlister and Jessica Burtness.** IS THE BUNDLING OF ACTIN BY EUKARYOTIC ELONGATION FACTOR 1A ESSENTIAL IN VIVO?

Both eEF1A from yeast and EF1A from *E. coli* function in the same in translation; they bring the aminoacyl-tRNA to the ribosome. In yeast eEF1A also bundles actin. Unlike yeast, *E. coli* lack actin. The ability of EF1A to function in the same role as eEF1A, but in an actin free environment, implies it would make a good model for examining the relationship between actin and eEF1A. To better understand that model, we need to know how similar EF1A and eEF1A are. Specifically, we need to determine whether EF1A can bind and bundle actin like eEF1A. To make this determination a cosedimentation assay was used.

**Alec Otteman.** DIHYDROWYRONE’S EFFECT ON RED BEET TONOPLASTS.

I explored the effect that dihydrowyrone (DHW), a phytoalexin produced by red beans, has on plant membranes. Phytoalexins are a class of secondary compounds plants produce in response to a foreign attack by invading cells. DHW seems to do this by disrupting the H⁺ or proton gradients in these cells. In order to understand DHW’s affects I developed several experiments utilizing red beet tonoplasts as a model membrane. Tonoplasts are the membranes surrounding plant cell’s vacuoles and they maintain the pH of a plant cell by pumping excess H⁺ ions into the vacuole for storage. The vacuole therefore has a fairly low pH in comparison to the rest of the cell. The mechanism tonoplasts use to move H⁺ is a protein pump imbedded in the membrane called the vacuolar H⁺-ATPase. Using a proton transport assay I found that DHW inhibits the efficiency of ATPase and also causes the tonoplast to leak protons back into the cytoplasm at an accelerated rate. The concentration of DHW I used for these experiments fell within the range of concentrations produced by plants naturally. In the future, the techniques and assays that I developed for studying dihydrowyrone could easily be used to characterize other phytoalexins.

**Nguyen-Ngoc T. Pham.** TOWARD UNDERSTANDING THE MEMBRANE SURFACE PROPERTIES DUE TO PHOSPHOLIPASE A2 HYDROLYSIS.

**Matt Nyflot.** DEVELOPMENT OF POSITRON EXPERIMENTS AT ST. OLAF.

The unique characteristics of positrons make them useful for materials science and the testing of quantum theories. Our goals this summer are to found two experiments designed to probe DNA structure using positron detection and to analyze the scattering cross-sections of positronium in gases. My work, specifically, has been to construct the apparatuses necessary for these experiments as well as to design the LabView interface to the temperature controller and assist with data analysis. The completion of these experiments will allow us to perform innovative study of the process by which DNA is denatured in addition to measuring more precise scattering cross-sections essential for testing theoretical models.

**Michael Olson.** CURRENT COMPARATIVE TABLE: A BIOINFORMATICS TOOL FOR DATA MANAGEMENT AND HOMOLOG IDENTIFICATION.
Elizabeth Ross. THE EFFECT OF APHIDICOLIN ON CONJUGATION IN TETRAHYMENA THERMOPHILA.

Two wrongs can make a right in Tetrahymena thermophila, a model eukaryotic cell. Tetrahymena undergoes a complex sexual reproductive cycle called conjugation. There are cell lines called star cells that abort conjugation when mated to a diploid or another star cell, probably due to severe aneuploidy. In the presence of a DNA synthesis inhibitor such as Aphidicolin (APD), conjugation between to diploid cells is also aborted. However, abortive conjugation between [star x diploid] mating can be rescued by applying APD. We demonstrate that APD must be present during Pachytene of Prophase I in order to rescue a [star x diploid] mating. To explain the effect produced by APD on these cells we’ve proposed a model that involves a bypass of the Pachytene checkpoint arrest in the star cell, and a bypass of a separate global developmental checkpoint in the diploid cell. The regulation of cell cycle events is important in maintaining the integrity of the genome. Failure of regulation and checkpoints in the cell cycle can lead to various disorders and diseases. The regulation of mitosis has been well described, but until recently very little research had been done in understanding regulation of meiosis. Our model brings us closer to understanding conjugation in Tetrahymena thermophila as well as the regulation of meiosis in eukaryotic cells.

Erica Savage. USING GENOMICS TO DEVELOP A FISH EXPERIMENTAL MODEL OF TUBERCULOSIS.

Tuberculosis differs from most bacterial diseases in that, rather than being completely eliminated, bacteria reach an equilibrium with the host’s immune system, resulting in clinical latency. Since 1/3 of the world’s population currently has latent TB which may reactivate as they age or contract HIV, this period is of extreme importance to epidemiologists. Microarray work by our collaborators has identified the “Dormancy Regulon”, a group of 50 bacterial genes upregulated in response to low oxygen conditions suspected to play a role in latency. Here we describe our work establishing an in vivo experimental model of tuberculosis using zebrafish and the mycobacterium pathogen known as Mycobacterium marinum. As an initial step, we have established an infection protocol, and visualized M. marinum infection of zebrafish in real time using the florescent G-13 bacterial strain. To test the role of the Dormancy Regulon in our fish model, we datamined the incomplete M. marinum genome project and identified homologs for most (38 of 50) of the genes in the Dormancy Regulon. We then isolated promoters for the homologs the dormancy genes Rv2031, Rv2626, Rv2627, and Rv3130 using PCR. Cloning of these fragments into an GFP (green fluorescent protein) expression construct is currently underway.

Johanna Savage. THE NUCLEAR EXCHANGE JUNCTION OF TETRAHYMENA THERMOPHILA: 1) THE DEVELOPMENT OF AN ISOLATION PROTOCOL FOR FUTURE MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION 2) FENESTRIN CHARACTERIZATION AND THE EXCHANGE JUNCTION OF BCD MATING CELL LINES.

The understanding of membrane fusion represents one of the great biological enigmas of our time. Membranes are defined by their ability to physically isolate one structure or organism from another. Hence, the occurrence of membrane fusion presents an
interesting paradox. The study presented here focuses on the membrane fusion event of conjugation (sexual reproduction) in *Tetrahymena thermophila*. Two separate, but related, studies focused on the isolation of the conjugation junction (nuclear exchange junction) and its subsequent characterization in bcd mutant cells. With regard to the first study, isolation of nuclear exchange junctions was successful using a 0.12% Triton X-100/50% Ethanol fixation protocol followed by ultrasonication. Secondly, using an antibody to the protein “fenestrin,” we learned that there is no significant developmental differences between the exchange junctions of wild type and bcd mating cells. These studies provide a solid foundation for the development of future studies.


Jared Walker Smith. EFFECTS OF PRAIRIE BURNING AND HABITAT FRAGMENTATION ON SMALL MAMMALS.
Two different yet connected studies were conducted in two prairies burned a year apart. Small mammal abundance, specifically prairie deer mice (*Peromyscus maniculatus bairdii*) and meadow voles (*Microtus pennsylvanicus*), was compared between the two prairies using live trapping methods. Meadow voles were found to be most abundant in the earlier burned prairie, and deer mice and meadow voles were found to be equally abundant in the later burned prairie. An enclosure was created to study how path width affects movements of meadow voles. They were found to be hesitant to cross areas cleared of vegetation with widths greater than ten feet. Both studies show the importance of cover to meadow voles and how even a small loss of cover can disrupt meadow vole populations.

Sarah Solarz. SCREENING ANTI-SENSE MUTATIONS FOR CELL DIVISION PHENOTYPES IN *TETRAHYMENA*.
By screening a *Tetrahymena thermophila* antisense library, 48 putative mutations were found. These mutated cells were harvested and cloned in tube cultures to grow. DNA from the individual mutant isolates were screened to distinguish the desired single gene recombinants from the multi-gene recombinants. One of the 48 mutants—D3—was chosen for in-depth molecular follow up. We explored whether re-transformation with the antisense DNA would consistently produce the mutant phenotype. When the antisense rDNA was reintroduced to wild type cells, the phenotype was not re-created. This suggests that the mutation was not a genetic one, but rather, a “cortical phenotype” known as a doublet. This can occur when cells undergo a mechanically induced fission arrest, and the new daughter cell remains attached to the parental cell. Eventually the daughter cell will move from an posterior position to a parallel alignment.

Additionally, experiments were performed to optimize transformation parameters. The number of pulses was tested for one, two, and three pulses. Two and three pulses did not destroy the *T. thermophila*, however, they did not improve the number of successful transformants either. Also, the time at which electroporation is carried out was tested. Normally, this time is set at ten hours after mating begins. Time trials spanning from nine and a half, ten, and ten and a half hours were performed. The best time from these
trials suggests that nine and a half hours is the optimal time to conduct transformation by electroporation.

**Laura Taylor and Katherine Van Heuvelen.** MODELING ENZYME ACTIVE SITES: GROUP VI METALS

Molybdenum, a Group VI metal, is found in the active sites of enzymes in a wide range of organisms. These active sites share a common structure, with molybdenum bonded to the dithiolene ligand molybdopterin. We are attempting to synthesize model compounds of the chemically active portions of these enzymes. Previously, our lab synthesized a model compound containing two molybdenum atoms joined by a single bond and bridged by a simple dithiolene ligand, tfd (S2C2(CF3)2). A few organisms can substitute tungsten, also a Group VI metal, for molybdenum in enzyme active sites; is our model equally versatile? Can a heterobimetallic compound, containing two different Group VI metals, be synthesized without compromising the dithiolene ligand bridging?

NMR, mass spectrometry, and crystallography data show that both compounds containing molybdenum-chromium and molybdenum-tungsten bonds can be successfully synthesized. Further work is being conducted to isolate intermediates and to form a bimetallicheterodimer that mirrors the structure of the original model compound.

**Erin Telste.** DESIGN AND SYNTHESIS OF CONFORMATIONALLY CONSTRAINED RNA OLIGONUCLEOTIDES.

RNA is known to adopt unique structures such as a U-turn, tetra loop, bulged G-motif, or a kink turn. These motifs have been characterized, but mechanisms of RNA folding and details of the flexibility of individual internucleotide linkages has yet to be investigated. We wish to examine these mechanisms of RNA folding by introducing conformationally constrained ribose analogs thus being able to regulate the degree of bend of the RNA strand. Our immediate goal was to synthesize a series of ribose analogs with varying degrees of bend and then use these to create pre-organized RNA structures, which could be compared to naturally occurring RNA structures and used as future reference for investigation of unknown RNA motifs.

**AmberBeth VanNingen and Leigh Cooper.** EARLY GROWTH PATTERNS OF CONIFERS.

As part of the St. Olaf College Natural Habitat Restoration program two plots of conifers were planted in 1993 and 1999 to establish areas similar in species composition to coniferous forests found in Northern Minnesota. The purpose of this project was to continue monitoring studies of conifer growth in these plots. For each tree we measured height, diameter, and male and female cone production. We also took GPS positions of all the trees to produce maps for future research. Results showed significant differences in height among species Jack pines were the tallest and balsam firs were the shortest. The height of trees initially grown in plastic tree shelters or with fabric mats was not significantly different from trees with no growth treatment. Soil tests for nutrients and moisture showed few differences among conifer plots a prairie, and an agricultural field. Future studies will continue to monitor tree growth and gain additional information about growth patterns through tree cores and measurement of reproductive output.