

2006 CBS Summer Undergraduate Research Program Abstracts  
Student Name, Home Institution, Mentor Name/Department

Ranga Atappatu

Swarthmore College

Mentor: Dr. Gwen Jacobs, Dept. Cell Biology and Neuroscience

Abstract:

Collaboration is a vital part of the scientific process. In order for researchers from different labs to reach a common goal and also for the promotion of public scientific education, data sharing is essential. The invention of the Internet has made this task easier. However, for one researcher to understand the work of another the data must be presented in an effective, organized manner.

Neurosys is a database software program created by members of the Center for Computational Biology (CCB) at Montana State University. Within the private laboratory and by outside users, Neurosys is used to arrange information into separate entries that can be then searched across.

However, the work of the CCB concerns the cricket cercal system, a system which is not inherently understandable. The cricket cercal system is a sensory system which is of particular interest to laboratories around the world because of its efficiency and specialization. It is the goal of this lab and others to create a fully functional model of the cricket cerci that could then be adapted for human use such as in cochlear implants and on airplane fuselage.

Our summer goal was to use the powerful Neurosys as a platform to make a large set of morphological data understandable for and available to the public. This was done by making the database concise, clear, and easy to use.

Cole Denton

Drury University (MO)

Mentor: Dr. Edward Dratz, Dept. Chemistry

Abstract:

G protein-coupled receptors (GPCRs) are of high physiological importance and comprise the largest superfamily of transmembrane signaling proteins in the eukaryotic kingdom. Having similar structures, all of these proteins interact with guanine nucleotide binding proteins (G proteins) in essentially the same way.

Rhodopsin, a photoreceptor protein found in the rod cells of the retina, is an excellent model for such GPCRs. A cascade of cellular signals, beginning with the absorption of a photon by 11-cis retinal (found inside the 7 helical structures of rhodopsin), allows for the perception of monochromatic images in dim light. Ultimately, rhodopsin interacts with a G protein in this pathway to transduce the reception of a photon into a nerve impulse that creates vision.

As a result of its physiological significance as well as its classification as a GPCR, rhodopsin has been a relatively well-studied protein model in the recent past. The elucidation of its crystal structure in the past decade has allowed for some valuable insight into the mechanism by which the GPCR interacts with a G protein.

Unfortunately, however, these studies have only been able to determine the structure of the dark, inactive form of the protein. As a result, one of the main goals of this project was to determine the structure of metarhodopsin I, a photoexcited form of the protein. The discovery of this structure would aid in a better understanding of the interaction between GPCRs and G-proteins.

**Kathryn Eilers**

Northern Arizona University

Mentor: Dr. Michael Franklin, Dept. Microbiology

**Abstract:**

Cystic fibrosis is a recessive, life-threatening genetic disorder which occurs in roughly one in every 3,500 American births every year (CFF foundation). The majority of fatalities in cystic fibrosis cases are due to chronic infections of mucoid strains of *Pseudomonas aeruginosa*, a bacterium commonly found in soils. Mucoid *Pseudomonas aeruginosa* are characterized by an overproduction of the polysaccharide, alginate, which acts as a virulence factor by protecting the bacteria from the immune system. All of the genes needed to produce alginate (except AlgC) are located on a single operon; however, the functions of many of these proteins and enzymes are still not understood. The objective of this experiment is to determine the function of AlgX (one of the genes located on the alginate operon) through the generation of unmarked AlgX deletion mutants and site directed mutagenesis.

**Julian Herrera**

Montana State University

Mentor: Dr. Christa Merzdorf, Dept. of Cell Biology and Neuroscience

**Abstract:**

*zic1* is a transcription factor found in the developing nervous system of *Xenopus*. Expression begins in the gastrula stage and continues throughout neural development. The *zic1* protein is found expressed in prospective neurectoderm, in a region that includes the Midbrain/Hindbrain Boundary, MHB. The tissue found at this area is able to organize surrounding tissue to form the midbrain (anteriorly) and the hindbrain (posteriorly). The expression domains of two transcription factors, *otx2* and *gbx2*, form a distinct boundary at the MHB. This *otx2-gbx2* interface initiates the expression of several genes at and around the MHB: *Xwnt1* (MHB and anteriorly), *fgf8* (MHB and posteriorly), *en-2* (MHB only) and *pax2* (MHB only).

The tissue at the MHB experiences a delay in differentiation. While cells around the MHB begin to differentiate into what will become the midbrain and the hindbrain, the cells that make up the MHB remain undifferentiated for a period. The differentiation process can be correlated with the expression timing of several genes in the prospective neural tissue: *Xhr-1* (prior to differentiation), *Xath-1* (in preparation for differentiation), *Xngnr-1* (in preparation for differentiation) and *N-tubulin* (during differentiation).

The goal of this ongoing research is to learn more about *zic1* and its role in the developing neural tissue. We want to understand the regulatory hierarchy and signaling pathways that give rise to the organizing center at the MHB, specifically with relation to *zic1*. We also want to understand the role that *zic1* plays in the delay in differentiation at the MHB.

#### *zic1*

Transcription factors bind to regions of DNA and activate/inhibit the transcription of target genes. *zic1* uses a zinc finger binding domain as its DNA attachment mechanism. *zic1* is of the structure such that the zinc fingers lie between a non-functional peptide chain (C terminus) and a functional peptide chain (N terminus).

The lab has synthesized two variations on the *zic1* theme: *zic1*▲C and *zic1* D. *zic1*▲C is a more reactive *zic1* version since it does not carry the unnecessary C terminus end. *zic1* D is a less reactive *zic1* version since it will still bind to DNA where normal *zic1* would bind, but it has none of the functionality of normal *zic1*.

**Mark Kaeppler**

University of Wisconsin, Madison

Mentor: Dr. Michael Babcock, Dept. Psychology

#### Abstract:

The hippocampus is a structure located in the temporal lobe forming part of the limbic system; it is critical for learning and spatial memory (Fig. 1). When regions of the hippocampus are properly stimulated, a cascade of molecular events leads to processes of neuronal plasticity (i.e., LTP) thought to underlie learning and spatial memory. An important chemical messenger along this chain of events is calcium-calmodulin kinase II (CaM-KII), an extremely abundant protein in the hippocampus, constituting 2% of the total protein in this region. CaM-KII is an enzyme composed of 8-12 subunits, consisting primarily of  $\alpha$  and  $\beta$  subunits in a 3:1 ratio, respectively (Fig. 2). Upon activation, CaM-KII translocates from the cytosol to the postsynaptic densities and participates integrally in synapse potentiation. The objective of the present study was to use a viral vector to up-regulate the expression of  $\alpha$ CaM-KII protein to observe how behavior reflective of spatial memory might change. The project used a recombinant adeno-associated virus (rAAV) to transfect cells of the rat hippocampus, thereby forcing the cells to produce sense mRNA for the production of the  $\alpha$ CaM-KII enzyme. The vector was microinjected bilaterally into rat hippocampi using stereotaxic surgery. After three weeks recovery, rats were tested in paradigms sensitive to changes in hippocampal  $\alpha$ CaM-KII, including a Morris water maze and an open field task. We hypothesized that rAAV vector transfection of sense mRNA for  $\alpha$ CaM-KII would result in significant changes in rat behavior as a result of physiological and anatomical alterations within the hippocampus.

**Abby Kelly**

Montana State University

Mentor: Dr. Ben Lei, Dept. Veterinary Molecular Biology

#### Abstract:

Human pathogen *Group A Streptococcus* (GAS) commonly causes strep throat and pharyngitis, which may lead to rheumatic fever. The organism also causes severe lethal invasive infections such as fasciitis and streptococcal toxic shock syndrome.

The ability of GAS to cause severe lethal invasive infections is believed to partially depend on its ability to grow rapidly in non-immune human blood. Identifying and characterizing genes, which are critical for GAS growth in human blood, are thus important in identification of new therapeutic targets and vaccine candidates for treating and preventing GAS infections.

Of particular interest are extracellular proteins, which are important in the life cycle, virulence and pathogenesis of bacterial pathogens. A GAS mutant defective in a gene encoding for a putative secreted protein was found to lack the ability to survive in human blood, lose resistance to phagocytosis, and be attenuated in virulence. These phenotypes are caused by the absence of the major cell surface protein M protein, which is a virulence factor and is required for the organism to forgo attack by phagocytes.

Transcription profiling by cDNA microarray analysis shows that *mrr* is down regulated in this mutant and, therefore, hypothesized to be involved in regulation of M protein. To test the hypothesis a *mrr* mutant (*mrr:aad*) was created. The *mrr* mutant has decreased levels of *emm* transcript, lacks M protein, is susceptible to phagocytes, and is unable to survive in human blood. The data suggests that Mrr is necessary for the expression of M protein.

Adam Larson

Millikin University

Mentor: Dr. Lynn George, Dept. of Cell Biology and Neuroscience

Abstract:

Neural development is regulated by a highly conserved and explicit system of gene regulation mediating differential cell fate and survival. The Trk family receptor tyrosine kinase is largely expressed in neural crest derived sensory neurons within the peripheral and sympathetic systems. Using a 560 bp minimal enhancer sequence driving eGFP expression we have created a reporter construct for the nociceptive TrkA. The construct expression reveals novel data on TrkA expressing neurons and their spatiotemporal regulation during the second wave of neurogenesis. The results provide initial data on putative transcriptional/translational regulation present in TrkA expressing neurons.

Guthrie Lewis

University of Montana

Mentor: Dr. Valerie Copie, Dept. of Chemistry

Abstract:

Understanding the structure of proteins can allow for helpful insight into how they can adapt to their environment. Organisms can evolve to live in extreme environments throughout the world. We prepared viral proteins for NMR through various steps of protein purification. Our preliminary results indicate we have prepared a pure protein sample of E73 for NMR analysis. These results indicate that we should be able to determine the 3-D structure of E73. Once the structure of E73 is found then we will gain valuable information to understand how the protein can adapt to an environment with such high temperatures, toxic metal concentrations, and low pH.

Kathryn Odell

Berry College

Mentor: Dr. Ben Lei, Dept. Veterinary Molecular Biology

Abstract:

Group A *Streptococcus* (GAS) causes several common infections, such as strep throat and impetigo, as well as the rarer necrotizing fasciitis. GAS is a major human pathogen, and the M proteins on its cell wall are largely associated with its virulence. The M proteins were shown to promote the bacterium's ability to survive in human blood by inhibiting phagocytosis.

A mutant strain of *Streptococcus pyogenes* was found unable to survive in human blood; the gene inactivated in the mutant encodes an esterase. The M protein expression in the mutant is down-regulated compared to wild type GAS. *Because the mutant cannot survive in human blood and its M protein expression is down-regulated, we hypothesize that the activity of the esterase is required for the expression of the M protein.* To begin testing this hypothesis, an inactive esterase is needed. To prepare an inactive esterase, the putative catalytic residue 180Serine was replaced with an alanine. The mutant protein was purified and found to be inactive in this study.

Microarray analysis revealed that the transporters for non-preferred carbohydrate sources, such as maltose and mannose, are up-regulated in the mutant strain at the mid-exponential growth phase. This up-regulation suggests that the mutant consumed glucose earlier than the wild type strain. *Thus, the mutant strain was predicted to consume glucose at a faster rate than the wild type bacteria.* This hypothesis can be tested directly by growing both strains (wild type and esterase mutant) and comparing the glucose consumption of each as the bacteria grow. Surprisingly, we found no difference in the glucose consumption of wild type and the Se180Ala mutant.

Justin Schwartzenberger

Montana State University

Mentor: Dr. Martin Teintze, Dept. of Chemistry

Abstract:

CCR5, a G-protein coupled receptor, is a co-receptor for HIV. Researchers hypothesize that blocking the site on CCR5 which binds to HIV's glycoprotein gp120 should stop HIV infection. Several antagonists which bind CCR5 have already been developed and some are entering clinical trials. The purpose of this research project is to crosslink photoactivatable analogs of the CCR5 antagonists and use mass spectrometry to determine the locations on CCR5 to which the antagonists bind. Understanding how they bind will aid in designing drugs that are more specific for CCR5. Rhodopsin, another GPCR, has been used as a model protein for developing purification and mass spectrometry techniques. The protocol developed for, the GPCR model protein, rhodopsin allows for about 50% sequence coverage using the LCMS. Similar results were achieved using the same protocol with CCR5. However, these results are not optimal for crosslinking studies, so we are in the process of trying new mass spectrometry methods. After running a CNBr digested sample of CCR5 on the LCMS Micro TOF we are missing only the ends of the protein and a large fragment in the middle. New protein cleavage options such as BNPS - Skatole must be utilized to fragment the large peptide further.

Cinnamon Spear

Dartmouth College

Mentor: Dr. Mark Burr, Center for Biofilm Engineering

Abstract:

Constructed wetlands have been widely researched and are now being applied in Europe. They employ natural biological interactions to remediate different types of wastewaters. There is little evidence of this new natural means for wastewater treatment in the United States aside from a few instances in Florida and the southeast. Moreover, the research that has been done with these constructed wetlands has not yet thoroughly investigated the microbial communities or processes occurring within it. If developed and implemented, constructed wetlands would be able to clean up wastewater in a more inexpensive, less invasive way, while using natural environmental organisms and processes to degrade the waste. As a result, there would be fewer synthetic chemicals introduced into the water and soil for degradation. Ultimately, the runoff from these wetlands should be less destructive to the surrounding environment since the effluent released is the metabolic by-product of native plants and organisms.

The microbial communities present in constructed wetland environments (such as the plant rhizosphere) are poorly understood. Without an understanding of the microbial interactions occurring within, these wetlands might not be most effectively used on a large scale. There seems to be an abundance of research done on constructed wetland performance but nothing that specifically addresses the composition or function of the microbial community. Some of the most pressing questions that need to be answered are with regard to community structure and function in relation to plant species, season, and wastewater type. One functional group of microorganisms of particular interest is the sulfate reducing bacteria (SRB).

This project was done in hopes of characterizing and identifying the sulfate reducing bacteria present in the MSU constructed wetlands. There were two plants species being investigated for sulfate removal: Cattails- *Typha* and Bulrush- *Schoenoplectus*. The samples used for our analysis were taken from an unplanted control, as it maintained the most anoxic conditions throughout the year.

**Sulfate Reducing Bacteria:**

SRBs are among the most ubiquitous organisms on the planet. These strictly anaerobic organisms utilize sulfate as a terminal electron acceptor in the anaerobic oxidation of organic substrates. As a result, sulfate ( $SO_4$ ) is reduced to the gaseous compound, hydrogen sulfide ( $H_2S$ ) and released into the external environment and decomposed (Figure 2). SRBs are critically important as they are the only known organisms to perform this function. Their role in the geochemical cycling of sulfur is vital to many biological processes, particularly those relating to animals and humans. Almost every possible anaerobic environment contains sulfate reducing bacteria, however they're mainly found below the soil surface where anoxic environments are ideal for sulfate reduction.