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01

STUDENT FUNDING
Mohammed I. Almzayyen
MBBC 2009
Vermont Genetics Network-INBRE (NIH) Faculty Project Grant (R. Sandwick)

FACULTY MENTOR
Roger Sandwick
Associate Professor and Chair of Chemistry & Biochemistry

The Role of Ribose-5-phosphate Isomerase in the Formation of 4-Hydroxy-5-methyl-3(2H)-furanone from Ribose 5-Phosphate

Mohammed I. Almzayyen and Roger Sandwick
Department of Chemistry & Biochemistry, Middlebury College, Middlebury VT 05753

The de novo purine synthesis pathway is a series of steps that results in the production of the purines adenosine and guanosine required for the manufacture of cellular RNA and DNA. Critical for generating the purines necessary for cell growth/division, the pathway has received much attention by cancer researchers as an attractive target of chemotherapy. The first two steps in the purine synthetic series are the conversion of phosphoribosyl pyrophosphate (PRPP) to phosphoribosamine (PRA) by the enzyme glutamine-PRPP amidotransferase (GPAT) and then the subsequent chemical transition of PRA into glycinamide ribonucleotide (GAR) by the enzyme GAR synthetase (GARS). The interesting feature of this pair of sequential steps is the relatively high instability of the intermediate metabolite PRA. PRA's lifetime is so short (i.e., $t_{1/2} = 5$ s) that previous research has determined the transfer of PRA between GPAT and GARS must not be by simple diffusion but instead via a direct channeling of the PRA between the enzymes. Failure by other investigators to demonstrate the prerequisite protein-protein interaction has led to a "transient" substrate channeling proposal that lacks further explanation. This investigation attempts to determine the transfer mechanism of PRA between GPAT and GARS. Currently the two enzymes are being cloned and purified either by pMAL® (New England Biolabs) or by pET Directional TOPO® (Invitrogen) expression systems. Enzyme activities are assessed by 31P NMR analysis. Upon obtaining purified GPAT and GARS, further research will look at the kinetics of the paired system to evaluate the potential of substrate channeling.

02

STUDENT FUNDING
Jinna Borgstrom
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Carissa Fritz
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Livia Vastag
Chem 2007
National Science Foundation – Research in Undergraduate Institutions (S. Choi)

FACULTY MENTORS
Sunhee Choi
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Associate Professor of Chemistry, Southwestern University

Synthesis of an Octahedral Nanocontainer

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²Department of Chemistry & Biochemistry, Middlebury College, Middlebury VT 05753

Professor Rawji and her students are visiting researchers in Professor Sunhee Choi’s lab.

Platinum-based drugs such as cisplatin bind to DNA covalently and have been effective against certain types of cancers. However, their side effects, toxicity, specificity for certain cancers only as well as the buildup of resistance against these drugs have led to search for alternatives. Ruthenium containing complexes are potential candidates and have attracted considerable attention. Tris chelate ruthenium(II) complexes are known to be effective in binding to DNA noncovalently. These complexes are shaped like a three blade propeller and have optical isomers which may exhibit different DNA binding modes and/or capacity. In this study, we examine the DNA binding of a tris chelate ruthenium(II) complex with an asymmetric ligand, 2-pyridylbenzoimidizole (pbImH), capable of intercalating with DNA. The nature and extent to which the complex binds has been investigated using circular dichroism (CD) spectroscopy, UV-Visible absorbance titrations and DNA melting. Preliminary data on binding studies as well as the synthesis and characterization of the complex will be presented.
03

STUDENT FUNDING
Seung-An Chyun
CHEM 2007
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FACULTY MENTOR
James Larrabee
William R. Kenan Professor of Chemistry

Magnetic Circular Dichroism of Dicobalt Model Complexes of Metallohydrolases
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Department of Chemistry & Biochemistry, Middlebury College, Middlebury VT 05753

A hydroxide- and carboxylate-bridged dimetal core is a common structural motif in metallohydrolase active sites. One of the key features of this structural unit is the extent of magnetic exchange coupling between the two metal ions. Magnetic circular dichroism is used to measure the extent of coupling between cobalt(II) ions in model complexes that have a hydroxide- and carboxylate-bridged dicobalt(II) core. Two isostructural cobalt complexes were studied. One has two Co(II) ions, the other has a Co(II) ion and a Co(III). The Co(III) ion is diamagnetic and cannot be involved in magnetic exchange coupling, Thus this system allows for exchange coupling to be turned “on” or “off”. The metal ions in the Co(II)-Co(II) complex were found to be ferromagnetically coupled with an exchange coupling constant, $J = 1.7 \text{ cm}^{-1}$. This study is one of the first demonstrations of the ability of MCD to detect and measure weak magnetic exchange coupling.

04

STUDENT FUNDING
Tad Davenport
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Middlebury College Bicentennial Fund for Research Partnerships in the Sciences Fellowship

FACULTY MENTOR
Margaret A. Daugherty
Visiting Assistant Professor in Chemistry & Biochemistry

Investigation of Potential Protein-Protein Interactions in Atherosclerosis
Tad Davenport and Margaret A. Daugherty
Department of Chemistry & Biochemistry, Middlebury College, Middlebury VT 05753

As a group, cardiovascular diseases are the number one killer of men and women in the United States, and atherosclerosis is a major cause of these deaths. Atherosclerosis is often accompanied by arterial calcification. Bone-matrix proteins, such as osteonectin (ON) and osteocalcin (OC), have been found to be intimately associated with calcified arteries, which suggests that calcification is regulated by protein-protein interactions in the extracellular matrix. Previous research has provided an inventory of the proteins involved in the calcification process, but there is still little known about how those proteins interact. The focus of this research is to identify binding interactions between a variety of proteins associated with atherosclerosis, including ON, OC, and plasminogen (Pg). Because ON’s interactions with other proteins (e.g. collagen) have been shown to be influenced by its glycosylation pattern, we plan to study three forms of osteonectin, bone-derived (bON), platelet-derived (pON), and a recombinant, non-glycosylated ON (rON), which possess high-mannose, complex, or no oligosaccharide groups, respectively. Binding interactions will be studied using Analytical Ultracentrifugation (AUC) and Total Internal Reflection Fluorescence Spectrophotometry (TIRFS). Through this research we hope to gain a better understanding of how proteins associate on a microscopic level in the progression of arterial calcification and atherosclerosis.
Graduate Economic Education: A Further Look

Tiziana Dominguez and David Colander
Department of Economics, Middlebury College, Middlebury VT 05753

The objective of this study is to expand our knowledge of US graduate economics education. This is important because an economist’s training is likely to shape their contribution to the field and to society. Previous research has considered students at the top programs in the U.S. This study expands this analysis to lesser-ranked programs. The methodology used was a questionnaire delving into the personal characteristics of the students, their experience in graduate school, and their views of the field of economics. The questionnaire was sent out to all institutions offering Economics PhDs in the U.S. and was completed by 1489 students. The results show that students and the programs are quite different from those at top universities. At lower ranked schools students are more diverse in their views, life experience, and interests. Their choice of graduate school depends less on ranking and more on interest in the program. Students are more likely to be preparing for policy work than for academia. Knowing the differences in graduate economics programs gives us insight into the economic profession and helps future graduate students make more informed decisions about where to go.

Please leave a message after the beep: The real language of voicemails

Jessie Evangelista, Rachel Ann Cole, Flannery Murphy and Suzanne Gurland
Department of Psychology, Middlebury College, Middlebury, VT, 05753

People’s reasons for engaging in activities affect how we feel about them. But do we actually treat people differently on the basis of their reasons for their behavior, and if so, how? To answer this question, we asked college students (N = 110) to view one of six video clips in which either a male or female target (“Jamie”) describes himself/herself as engaging in a set of behaviors for self initiated reasons such as enjoyment (autonomous condition), externally determined reasons such as rewards or punishments (control condition), or a mix of both these reasons (ambiguous condition). These conditions were derived from our theoretical model. We then measured participants’ perceptions of “Jamie” through questionnaires and a mock interpersonal interaction that involved leaving a phone message welcoming “Jamie” to an activity that they would be doing together in the future. Voicemails were assessed on several dimensions including Friendliness, the use of inclusive (e.g. “we,” “us”) versus exclusive (e.g. “I,” “you”) pronouns, and the extent to which participants offered help or reassurance to “Jamie.” Preliminary results indicate that while participants were equally friendly and showed similar pronoun usage regardless of “Jamie’s” reasons for engaging in certain behaviors, they tended to offer more help to the “Jamie” who gave externally determined reasons. Based on our theoretical model, we interpret this help-giving as evidence that participants view those with externally-determined reasons as incompetent and in need of help. Thus, participants did treat another person differently on the basis of that person’s stated reasons for engaging in activities. The study is presented with a focus on the process of evaluating the voicemail messages, and will include sample messages and operational definitions for each rated dimension.
Positional Cloning and Characterization of mei2.5, an Ethyl Methane Sulfonate (EMS) Induced Mutation of Akap9 Causing Defective Spermatogenesis in Mice

Sky K. Feuer, Laurie B. Griffin and Jeremy O. Ward
Department of Biology, Middlebury College, Middlebury VT 05753

In order to identify genes involved in mammalian gametogenesis, Ward et al. performed a forward genetic screen for mutations causing infertility in mice (Ward 2003). One mutation identified was the recessive mutation mei2.5. Homozygous mutant males are unable to complete spermatogenesis, arresting at various stages during the process. Homozygous mutant females are fertile. In this study, genetic mapping was used to narrow the region of interest containing mei2.5 to the most proximal 2 million base pairs of chromosome 5. Candidate genes were identified and expression studies were initiated. A complementation assay was performed to determine whether mei2.5 occurred in the A-kinase anchoring protein 9 (Akap9), a gene located in the region of interest and known to regulate the activity of protein kinase A (PKA) during the cAMP signal transduction pathway in both rat brain and heart. The mei2.5 allele failed to complement the mutant Akap9allele thereby identifying mei2.5 as a novel allele of Akap9. Akap9 has been previously shown to be expressed in the testis and microarray data suggests Akap9 expression occurs in Sertoli cells. This study is the first to characterize its role in the testis. Immunoblotting revealed the presence of three Akap9 isoforms expressed in the testis, and identified the 84 kDa isoform as testis-specific. This testis-specific isoform was absent in mei2.5 testis extracts. The expression pattern of Akap9 in a mouse mutant arresting in late prophase I (mei4) was compared to wild type. No difference in Akap9 expression was observed, excluding Akap9 as a sperm specific protein. Genetic mapping and sequencing of the mei2.5 allele identified the mutation as a G>A transversion in the 3′ terminal base of the thirteenth exon. This study represents the positional cloning of the mutation mei2.5 and the initial characterization of Akap9 function in spermatogenesis.

Synthesis, Purification, and Characterization of a Ruthenium (III)-Inosine Complex, and Preliminary Studies of its Autoxidation Reaction

Benjamin Fowler, Justin Bogart and Sunhee Choi
Department of Chemistry & Biochemistry, Middlebury College, Middlebury VT 05753

Ruthenium (III) complexes are promising anticancer drug alternatives to their more toxic platinum analogs. When ruthenium (III) complexes bind with DNA, the resulting adduct can be oxidized in the presence of oxygen. While the involvement of ruthenium (III) in this autoxidation process has been known, the kinetics and mechanism have not been studied in detail. The aim of this study was to synthesize a ruthenium (III)-nucleotide complex and study the kinetics of its autoxidation. Using inosine (Ino) as the reactant nucleotide, a [Ru^III(NH_3)_5(Ino)]^{2+} complex was synthesized, purified using preparative High Performance Liquid Chromatography (HPLC), and characterized with Liquid Chromatography-Mass Spectrometry (LC-MS). Preliminary kinetic studies performed using UV-Visible spectroscopy suggest that the bound inosine is converted to 8-oxoinosine during autoxidation. Additional studies are planned to investigate the detailed mechanism of this autoxidation process and its implication in the anticancer activity of Ruthenium (III) complexes.
Rethinking Homelessness: Understanding Marginalized Groups

Kelly George and David Napier
Department of Sociology & Anthropology, Middlebury College, Middlebury VT 05753

The overarching questions of this inquiry are: Why are certain individuals who may have committed no crime marginalized or excluded from society, or dismissed simply for not adhering to societal norms? Why do certain of those marginalized individuals sometimes choose in turn to lead a way of life that alienates them yet further from those same norms? Though homelessness is often thrust upon many who do not want it, this study examines specifically those who choose homelessness over what they feel to be the bankruptcy of contemporary life as others know it. Our working assumption is that through understanding the lives, ideologies, and values of certain intentionally homeless individuals we can gain insight into processes of marginalization and self-stigmatization. What are the limits of lifestyle abnormality that can be tolerated by a given culture at a given historical moment? Through studying the current ideals and values of modern American society as seen by the homeless, we hope to find a way of understanding how the marginalized offer a unique critique of contemporary life. The purpose of this research, therefore, is to rethink the process of marginalization. By employing anthropological studies of alienation, we will use field data among the homeless to examine how Americans understand and construct marginality. Selections from some 120 hours of video conversations gathered among the homeless by Professor Napier are being transcribed, coded, and analyzed in this project with the view to understanding specifically how alienation is understood and lived by homeless people in America. In addition, we are attempting to understand how social identity gets built around extreme social difference. The sociological concept of “self” and “other” becomes the key trope for articulating among the homeless, constructing “who we are” only by stating “who we are not.” The study, therefore, seeks to rekindle a new interest in genuine difference, by examining in the eyes of the homeless how “self” and “other” are defined and lived. In order to bring these concerns to a more general audience, this study will utilize various artistic media—including fiction, film, and literature, and senior thesis work.

Radical Addition Reactions of Organometallic Ruthenium Compounds

Matthew Griswold, Jesse J. Keenan and Jeff Byers
Department of Chemistry & Biochemistry, Middlebury College, Middlebury VT 05753

When compared to other chemical reactions, radical reactions are still relatively undeveloped, but are potentially valuable for synthesis based on the ability of radicals to act as either electron rich or electron poor intermediates dependent on their reactive partner. Previous Middlebury researchers have performed the radical addition to Cp-Cr-(n^6-styrene). This complex has proven to be limited in reactivity. Radical reactions with Cp-Ru-(n^6-styrene) and Cp-Ru-1,2-dihydronapthalene have thus far been promising and have indicated a greater degree of reactivity to radical additions with the addition of cyclohexyl iodide and isopropyl bromide to Cp-Ru-(n^6-styrene) and 1-iodooctane to Cp-Ru-1,2-dihydronaphthalene. This process could prove useful for the research and synthesis of pharmaceutical products, however further research into radical additions to ruthenium organometallic compounds is necessary before becoming synthetically useful.
Polyamine Protection of Proteins from Glycation

Andrew Harris and Roger Sandwick
Department of Chemistry & Biochemistry, Middlebury College, Middlebury VT 05753

The Maillard reaction, also known as glycation, is a nonenzymatic reaction between the carbonyl group of a sugar and a free amine group. Glycation is biologically relevant because it occurs between sugars and the free amine groups of proteins. Accumulation of glycated proteins has been associated with the complications of aging, due to long-term addition of sugars to proteins, and diabetes, due to elevated sugar levels. It is hypothesized that glycation can cause a loss of protein function when a sugar is added to a functionally important region of the protein; therefore, preventing accumulation of glycated proteins would be beneficial. Polyamines such as spermidine are ubiquitous throughout the body and have the potential to protect proteins from glycation by being glycated themselves, due to their abundance of free amines. We used UV/Vis spectroscopy, LC/MS and NMR to determine the functional and structural changes of our protein of interest, lysozyme, in the presence of a sugar, ribose-5-phosphate (R5P) or R5P with spermidine. LC/MS and NMR were used to follow glycation of R5P onto lysozyme by measuring changes in mass and presence of phosphate groups on lysozyme, respectively. We used UV/Vis to monitor the rate at which lysozyme degrades the cell wall of Micrococcus lysodeikticus cells. We found that in an incubation with R5P over a period of several days, lysozyme lost its activity more quickly than untreated enzyme, and that this relationship was dependent on the concentration of R5P. As hypothesized, when lysozyme, R5P, and spermidine were incubated together, lysozyme maintained its activity for much longer than lysozyme and R5P only, suggesting spermidine can protect lysozyme from glycation. By exploring means of glycation prevention, we may be able to alleviate the complications involved in aging and diabetes.

Creating Optical Flow Datasets using Hidden Fluorescent Texture

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Optical flow algorithms attempt to calculate how real-life objects move in a video from frame to frame. Tracking dynamic motion through video footage has long been an important area of study in the computer vision research community. Applications include video compression, tracking of people and vehicles, and automatic analysis of security camera footage. Given the large number of optical flow algorithms that have been proposed, it is unfortunate that determining the quality of these algorithms is currently very difficult. To gauge the effectiveness of an optical flow algorithm, it must be tested on an image sequence for which the true optical flow is known in advance. The goal of our work is to generate such ground-truth flow for series of images taken in the laboratory. We build test scenes that can be moved by a computer-controlled motion stage, and apply a fine spatter pattern of fluorescent paint to the objects in the scene. By taking high-resolution images of the slowly-moving scene under UV lighting, we are able to precisely track the texture and extract pixel-accurate ground-truth motion. At the same time, we are able to provide a series of low-resolution images taken under regular lighting with larger motion that will be challenging for contemporary optical flow algorithms to handle. The UV light allows us to cover the scene in an easily trackable texture that is invisible to those who are testing their own algorithms. Our data sets will be made available online so that researchers can evaluate and compare their methods. This provides an important addition to the set of internationally-used computer vision benchmarks hosted at Middlebury College at http://vision.middlebury.edu.
Mutation in Mouse Hei10, an E3 Ubiquitin Ligase, Disrupts Meiotic Crossing-over

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Crossing-over during meiotic prophase I is required for sexual reproduction in mice and contributes to genome-wide genetic diversity. Here we report on the characterization of an N-ethyl-N-nitrosourea (ENU) induced, recessive allele called mei4, which causes sterility in both sexes due to meiotic defects. In mutant spermatocytes, chromosomes fail to congress properly at the metaphase plate, leading to arrest and apoptosis before the first meiotic division. Mutant oocytes have a similar chromosomal phenotype, but in vitro can undergo meiotic divisions and fertilization before arresting. During late meiotic prophase in mei4 mutant males, absence of cyclin dependent kinase 2 (CDK2) and mismatch repair protein association from chromosome cores is correlated with the premature separation of bivalents at diplonema due to lack of chiasmata. We have identified the causative mutation, a transversion in the 5’ splice donor site of exon 1 in the mouse ortholog of Human Enhancer of Invasion 10 (Hei10; also known as Gm288 in mouse and CCNB1IP1 in human), a putative B-type cyclin E3 ubiquitin ligase. Importantly, orthologs of Hei10 are found exclusively in deuterostomes and not in more ancestral protostomes such as yeast, worms, or flies. The cloning and characterization of the mei4 allele of Hei10 demonstrates a novel link between cell cycle regulation and mismatch repair during prophase I.

Characterization of the Effects of Ribose-5-Phosphate on Tubulin Assembly

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Protein glycation occurs when a sugar spontaneously attaches to a protein’s amine group. Since sugar enters the bloodstream, glycation occurs at a regular rate in the body, and may leave the involved proteins dysfunctional. Tubulin, a protein necessary for cell division, is found universally in cells, and is likely to glycate quickly since it contains many of the target amines lysine and arginine. Tubulins are 55 kD alpha or beta subunits that bind together to form microtubules with the property of dynamic instability, i.e., growing and shrinking. Tubulin was incubated with ribose-5-phosphate or glucose at 4, 32, or 37 degrees Celsius. 1mM GTP, 5% glycerol, and 1mM Taxol were added to the tubulin to enhance polymerization, and absorbance was measured at 340 nm after 0, 1, 8, and 24 hours of incubation. Rate of increase in absorbance indicated the efficiency of polymerization. The resulting polymers were centrifuged from solution, and treated with reagents that break non-covalent bonds. SDS-PAGE was used to determine the molecular size of the polymers after digestion. Molecules larger than 55kD indicated aggregates covalently bound due to glycation. Within 24 hours, glycation by ribose-5-phosphate lowered the rate of polymerization, although not the final absorbance. The aggregates formed were not broken apart by SDS or 500mM NaCl. Glycation with glucose occurred more slowly, but showed similar results. If glycation causes tubulins to polymerize less efficiently, cells growth is hindered. Moreover, if glycation results in covalently bound aggregates, the property of dynamic instability is lost. Glycation and a resulting decrease in protein functionality are new considerations in managing blood or cellular sugar levels.
Chromium Complexes as Possible Models for Low-Molecular-Weight Chromium-Binding Substance (LMWCr) or Chromodulin

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For three decades, chromium (III) has been known to be an essential element in humans for normal carbohydrate and lipid metabolism. Studies have also shown that many humans suffer from a deficiency of chromium in their daily diets. If chromium is essential to humans, then there should be a biological molecule that is able to bind chromic ions. Several studies have proposed that the binding material is the oligopeptide Low-Molecular-Weight Chromium-Binding Substance (LMWCr), also known as chromodulin. Chromodulin was recently isolated in quantities sufficient for spectroscopic and structural studies. The data suggest that the chromium ions in chromodulin are arranged in an anion-bridged tri- or tetra-nuclear assembly. The goal of this research is to isolate and purify tri- and tetra-chromium complexes that model the proposed chromodulin structure and to take magnetic circular dichroism (MCD) spectra of these complexes. We hypothesize that MCD will be a useful probe for studying the chemistry of Cr(III) in chromodulin. The results on two tri-Cr(III) complexes are very promising.

Ethnic Enclave Formation and Changing Residential Geographies in High Growth Rural Communities

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In recent decades, a new wave of migration into America’s nonmetropolitan regions has tremendously altered the rural landscape. Two distinct groups of migrants comprise a significant portion of this new wave—Hispanics and baby boomers. Between 1990 and 2000, the nonmetropolitan Hispanic population grew over 65 percent (over 1 million people), and nonmetropolitan baby boomers grew by 7 percent (about 900,000 people). The cultural and economic dissimilarities between these two groups of newcomers suggest important differences in their motivations for migrating, as well as significantly different implications for the communities that receive them. Not surprisingly, therefore, a growing body of research has explored these two migration streams individually, often connecting Hispanic migration to the relocation of manufacturing plants into rural areas and baby boomer migration to places with an abundance of natural amenities. However, very little has addressed the possibility that the two migration streams are linked. This project is thus part of a larger study exploring the connection between immigration and domestic migration streams into nonmetropolitan America. In particular, this project examines changes in residential patterns in counties and census tracts within the Tetons, Ozarks and South Atlantic, three regions that have experienced high levels of both Hispanic and baby boomer migration. The results identify a tendency for the two groups of newcomers to cluster in geographically disparate places, especially in regions where Hispanics are employed in traditional manufacturing industries, such as meatpacking, rather than in the growing services sector. This clustering indicates a growth in residential segregation, in some ways resembling the formation of ethnic enclaves, which are generally thought to be an urban phenomenon, thereby introducing unfamiliar and challenging issues into America’s rural landscape.
Physical Characterization of Purβ Using Differential Scanning Calorimetry

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Purβ is a gene regulatory factor implicated in the transcriptional and translational repression of genes encoding contractile proteins found in the heart and vasculature. This protein is unusual in that is preferentially targets single-stranded DNA and RNA molecules. Moreover, it has an unusually high glycine content of 22% which has complicated attempts to obtain structural information on this protein. As yet no crystal structure has been obtained for either the protein as a whole or any of its domains. In order to further our understanding of the physical properties of Purβ, our research is pursuing indirect studies on its structure using Differential Scanning Calorimetry (DSC). The recombinant protein was expressed in bacteria and subsequently successfully purified to homogeneity using a variety of chromatographic techniques. Through the use of DSC we hope to determine the range of reversibility in the thermal melting transition of Purβ from the native to unfolded state in order to understand the reversibility of the folding transition and we are attempting to determine the specific heat of the protein as a function of temperature in order to better understand molecular contributions to the stability of Purβ.

A Novel Pathway for the Formation of HMF from Ribose-5-Phosphate

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The Maillard reaction is an interaction between the carbonyl groups of sugars and deprotonated nitrogen atoms in proteins that is responsible for the brown color of cooked foods. It has far-reaching implications in both food chemistry and health issues such as diabetes mellitus. Specifically, some of the products of the Maillard reaction, known as advanced glycation end-products (AGEs), have been shown to cause damaging protein cross-links and to initiate inappropriate inflammatory responses. The focus of this project is a characterization of the Maillard reaction between the oxygen storage protein myoglobin and the important physiological sugar ribose-5-phosphate (R5P). The reactants (1 mg/mL myoglobin and 50 mM R5P) were incubated at physiological conditions (37ºC and pH 7.4) for a number of days. The products were then analyzed using a liquid chromatographer-mass spectrometer and a UV-visible spectroscopy. Myoglobin and R5P were found to produce hydroxymethyl furanone (HMF), a common Maillard product but one which had not previously been observed in an R5P reaction. HMF is known to be a downstream product of the sugar ribulose-5-phosphate, an isomer of R5P that requires the enzyme R5P isomerase for conversion. Since our reactions do not contain this enzyme we have been trying to determine a mechanism for the conversion. We have found that myoglobin and R5P produce significant amounts of HMF, whereas R5P and apomyoglobin (myoglobin without its iron-containing heme group) do not. Additionally, neither a solution of iron ions nor pure heme were found to change R5P to HMF, leading us to believe that both the protein and the heme portions of myoglobin are required for the conversion. It is speculated that the reaction proceeds through one of the dicarbonyl intermediates typical of AGE formation.
Final Processing of Side-scan Sonar and Sub-bottom Profiling (CHIRP) Data for Use in the Lake Champlain Basin

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Vast amounts of side scan and sub-bottom (CHIRP) profiling data on Lake Champlain over the last 12 years. The side-scan sonar survey represents a nearly complete view of the bottom of Lake Champlain which reveals the present day processes occurring in the lake, such as how the water moves throughout the lake by identifying high erosion and depositional zones. Side-scan sonar also detects objects lying on the bottom, such as historical shipwrecks. I am currently finalizing the Side-Scan database that contains all the sonar images taken from 1997 until present day, which will be used to create detailed maps and sub-maps available in both public and private arenas. These maps will be highly advantageous to anyone conducting research on the lake as well as for recreational purposes such as scuba diving and fishing. The CHIRP sonar surveys, which identify the sub-bottom sedimentary layers by sending out a spectrum of acoustic signals (3-12 kHz) that are able to penetrate to depths as great as 100m. The CHIRP Sonar not only provides a general idea of the different sedimentary layers occurring through out the lake, but the changing water and land levels of the previous historical periods of Lake Champlain, including Lake Vermont and the Champlain Sea. Additionally, CHIRP also provides necessary data on the best possible locations for coring. Coring is a method of extracting a column of sedimentary layers from the bottom of the lake which can then be dated and analyzed for changing climatology of the region over the past 12,000 years. As a research assistant, I have operated the CHIRP Sonar on board Middlebury College’s vessel R/V Baldwin as well as UVM’s research vessel Melosira for hydrographic and sedimentological surveys.

INTRODUCTION:   Post-traumatic stress disorder (PTSD) is a psychiatric disorder that results from exposure to a traumatic experience and is characterized by three re-experiencing symptoms, avoidance/numbing symptoms, and hyperarousal symptoms. Individuals with PTSD have been shown to demonstrate cognitive biases toward the processing of trauma-relevant information. The present study used the N400 component of the event-related potential (ERP) to analyze trauma-relevant biases in sentence processing. ERPs are averaged waveforms of brain activity, calculated using information from the electroencephalogram (EEG). The N400 is a component of the overall waveform that is associated with language and varies as a measure of semantic expectancy. The less expected a word is, the larger an individual’s N400. This study reanalyzed ERP data of 18 trauma survivors (13 of whom were war veterans), 7 with PTSD and 11 without PTSD, in order to look at the relationship between N400 variance and PTSD status. METHOD: Sixty-three sentences were presented to each participant; 21 sentences with expected endings, 21 with unexpected endings, and 21 with war-related trauma endings. Throughout the sentence presentations, the EEGs of participants were recorded. EEG data was then processed and analyzed. RESULTS: Results indicate that there was an N400 effect as indexed by larger N400s to incongruent (relative to congruent) final words in the non-PTSD sample. At the Cz electrode, a significant "Group" (PTSD vs. no PTSD) by "Condition" (expected, unexpected, trauma-relevant) interaction was found. Participants diagnosed with PTSD showed smaller N400s for both unexpected and trauma-relevant sentence endings compared with non-PTSD participants. Although waveforms at Pz indicated general N400 activity, when the Pz electrode was included in the analysis, results were not significant. DISCUSSION: This preliminary study suggests that the N400 may be a valuable component to study in PTSD and that basic attentional abnormalities may interfere with their ability to create normal semantic expectancies.
Investigating the “middle men” in *S. mutans*-induced cariogenesis.

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*Streptococcus mutans*, one of many bacteria that reside in the oral cavity, plays a central role in dental caries formation. This process is highly dependent on the development of a plaque biofilm and subsequent bacterial fermentation of dietary carbohydrate. To survive the accumulation of metabolic acid byproducts in plaque, *S. mutans* mounts an acid tolerance response [ATR], which allows further demineralization of the tooth enamel. Clearly, an improved understanding of the mechanisms by which *S. mutans* persists in the plaque biofilm can lead to novel treatment and prevention strategies aimed at combating *S. mutans*-induced caries. My work as a 2007 Summer Roddy Fellow centers on the *S. mutans* gene products that mediate biofilm formation and acid tolerance. Specifically, we hypothesize that the GcrR and TnrA transcriptional regulators serve as intermediary modulators of these and other virulence attributes. Previous work in the Spatafora laboratory supports control of *gcrR* and *tnrA* transcription by a SloR metalloregulator through demonstrations of direct SloR binding to the *gcrR* and *tnrA* promoter regions and differential expression in real time qRT-PCR experiments. To address GcrR and TnrA as putative “middle men” of “cross-talk” between SloR and the players of *S. mutans* aciduricity and plaque formation, I performed qRT-PCR experiments that reveal increased expression of the SloR metalloregulator at acid pH 5.0 relative to physiological pH 7.5, consistent with a role for SloR in modulating the *S. mutans* acid tolerance response via GcrR. Cloning of the *tnrA* gene into a pMal vector system is underway [in parallel with Cheryl McClurg’s *gcrR* cloning experiments] so that the resulting fusion proteins may be applied to column chromatography assays to elucidate the GcrR and TnrA binding partners. Taken together, these experiments can reveal the *S. mutans* GcrR and TnrA regulons that promote biofilm formation and acid tolerance in this pathogen.

Listening in on SloR-GcrR crosstalk in the oral pathogen *Streptococcus mutans*

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*Streptococcus mutans* is the principal causative agent of human dental caries, a disease that derives from interactions involving the host, the microbe and dietary factors. The cariogen is able to form a complex biofilm on the tooth surface, where it metabolizes dietary carbohydrates and generates acid by-products, leading to tooth enamel demineralization. Such acid production lowers the pH of the plaque and stimulates an acid tolerance response [ATR] that allows *S. mutans* to persist at low pH. Indeed, continued investigations of the *S. mutans* ATR could promote the development of novel therapeutics aimed at alleviating the formation of active carious lesions. The results of gel mobility shift assays previously conducted in the Spatafora laboratory support direct binding of the *S. mutans* SloR metalloregulatory protein to the *gcrR* promoter region, and accumulating evidence implicates GcrR as a major mediator of the *S. mutans* ATR. This study focuses on the cross-talk between *S. mutans* GcrR and other constituents of the ATR at the level of the proteome. As a 2007 Curry Research Fellow, I cloned the *S. mutans gcrR* gene into a pMal expression vector to foster the isolation and purification of a GcrR-MBP fusion protein in *E. coli*. The fusion protein will be cross-linked to an amylose resin and used as bait in column chromatography experiments to capture *S. mutans* proteins that interact with GcrR. We hypothesize that SloR will be among the proteins we identify, and that other GcrR binding partners will include both known and novel contributors to the *S. mutans* ATR. Subsequent to their identification, functional analysis of the GcrR target proteins will begin using a reverse genetics approach. Taken collectively, this research will expand on our currently limited understanding of GcrR and its involvement in the ATR that promotes *S. mutans* persistence in the human oral cavity.
Analysis of Devonian Granite Plutons in the Vicinity of Hardwick and Waterbury, Vermont

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The famous granites of Vermont’s Northeast Kingdom intruded roughly 355 million years ago during the creation of the Appalachian Mountains. Though the granite plutons have been mapped and quarried extensively, little is known about the conditions of their intrusion or how individual plutons are related. Our current project involves collecting samples from a number of small plutons south of Hardwick, Vermont and analyzing them using an Inductively Coupled Argon Plasma Spectrometer (ICAP) to determine their elemental composition. By examining amounts of both major and trace elements, it is possible to discover how the granites intruded and what rocks melted to form them. Preliminary results suggest that the plutons have very similar compositions, indicating a possibility that they may be part of a large pluton similar to those mapped further to the northeast or that they possess a shared magma source. Further work will determine the true extent of their similarities and their relationship to other granite bodies in the Northeast Kingdom and elsewhere in New England.

Epistemic Development during Adolescence

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With funding for a four-year research project from the NSF, this summer our lab has been preparing for a multi-method study on the epistemic development of adolescents. Epistemology, or the study of beliefs about knowledge and knowing, is concerned with the process of how people know what to believe and why. The goal of this project is to advance the understanding of how individuals progress in their ability to assess and evaluate sources of information, how they justify what they know, and in turn, how this progression is related to other learning variables, such as cognitive development and academic achievement. Students from central Vermont public schools (initially in 6th, 8th, 10th, and 12th grades) will be assessed twice, roughly one year apart, in the following three ways: (1) an applied, think-aloud online search task as if researching for a paper for a science class; (2) a retrospective interview of that process, and an interview about epistemic understanding; and (3) a web-based survey to assess epistemic and cognitive development. This summer, we have conducted a systematic review of the literature on epistemological development and instrumentation to choose an adequate measure of epistemic beliefs, an interview protocol, and a measure for assessing cognitive development in adolescents. We also reviewed a preliminary study that used the online searching task to refine the think-aloud protocol for the first phase of our study beginning in the fall of 2007. This study will expand the knowledge of the developmental path of personal epistemologies during adolescence, an area which has previously been under-explored. The results will help teachers recognize a critical variable in the learning process and address how people seek and evaluate evidence, resolve competing truth claims, evaluate sources of authority, and reconcile scientific data and personal experience. This has broad implications for both school tasks and informal learning throughout life.
Superoxide Dismutase Antioxidant Activity in Developing Bovine Follicular Fluid

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Oxidative stress is fundamental to female reproduction. Naturally present oxidants, such as the common superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), have been shown to interfere with embryo development as well as pre-fertilization, during follicle maturation. Oxidative stress (OS) compromises the developmental competence, or the ability for successful oocyte fertilization and later embryo development. To combat OS, a multifaceted and complexly regulated system of antioxidants exists within developing follicles. Superoxide dismutase (SOD) is the enzyme that catalyzes the breakdown of the superoxide anion into hydrogen peroxide and has shown to have developmental significance. Furthermore, follicles of higher levels of maturity, indicated by follicle size, have exhibited increased developmental competence in relation to smaller follicles. Follicular fluid, the protective liquid surrounding the cumulus-protected oocyte in the follicle, has received significant attention in recent years and presents a relatively unexplored component in follicle maturation. Follicular fluid could provide an evaluation method of specific follicles for their developmental competence permitting improved selection of quality oocytes for use in in vitro fertilization therapies and ultimately allowing sterile couples to conceive a biological child. This research investigated the SOD activity of bovine follicular fluid and any correlation to follicle maturity. Bovine follicles were dissected from ovaries collected from a local slaughterhouse and categorized according to maturation stage. Follicular fluid was aspirated, purified by centrifugation, and frozen. SOD activity was quantified by formazan salt-based spectrophotometry. Total follicular fluid protein was quantified by a bicinchoninic acid assay. SOD activity was expressed in units of activity per milligram total protein, and evaluated in terms of follicle maturation stage and estrous cycle of the dissected ovary. Results show increased SOD activity in small follicles as compared to medium and large. Further research will focus on compiling additional experimental replicates.

Organochlorine and Pyrethroid Pesticide Profiles in an Otter Creek Delta Sediment Core

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Persistent, bioaccumulative, and toxic (PBT) organochlorine (OC) pesticides were used heavily in agriculture between 1945-1965. Because agriculture accounts for nearly one third of the land use in the Otter Creek Watershed, it is important to investigate the long-term persistence of OC pesticides in freshwater ecosystem sediments. Pyrethroids, a newer group of synthetic pesticides are increasingly being used. Pyrethroids are less bioaccumulative, less toxic to mammals, and more biodegradable than OCs; however, the high toxicity of pyrethroids to aquatic organisms renders them an important class of compounds to study. OC and pyrethroid pesticide concentration profiles in VT sediment cores have not been previously reported. In this study, OC and pyrethroid pesticide concentration profiles were measured in a sediment core obtained from the Otter Creek Delta in Lake Champlain. Sediment samples (22) were collected at 2-5 cm intervals along the core, corresponding approximately to years 1910-2006. Sonication extraction and Florisil cleanup was used; pesticides were quantified using gas chromatography (GC) with electron capture detection, and compound identification was confirmed by GC-mass spectrometry. The method was verified by spiking a pristine soil, obtained from Church Woods in Shelburne, VT with known quantities of pesticides (~70% recovery for all pesticides). Preliminary results show increased concentrations of DDT and its degradation products (DDD and DDE) at depths of 30-75 cm, corresponding approximately to the years 1930-1960. The appearance of DDT in the profile prior to its widespread agricultural use is attributed to uncertainty in the sediment dates. Further analysis is underway.
Use of Autonomous Underwater Vehicles (AUV) and Their Effectiveness in Mapping Hydrodynamic Variability in Lake Champlain

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The evolving technology of autonomous underwater vehicles (AUVs) is an exciting new advancement in the field of marine science. Old methods to study the water column can be labor, equipment and time intensive in order to gain accurate data. Old methods included the use of ROVs (unmanned underwater vehicles controlled from the surface), manned underwater vehicles and shipboard hydrographic surveys typically taken with CTDs (conductivity/temperature/depth) sensors. AUVs, on the other hand, can provide massive amounts of data with a minimum of user intervention while at the same time surveying spatial and temporal domains that would be dangerous or impossible. For example, dynamic changes in the water column occur during extreme wind events. Fortunately, the size and cost of these instruments are continually being reduced. Additionally, the software that controls the AUVs, as well as the sensors installed on them, are presently being tested and improved to better map ocean and lacustrine environments. As part of a pilot program with the U.S. Navy, Tom Manley (geology department) will be using two new AUVs (model Iver2 from OceanServer Technology, Inc.) to test the accuracy of these devices in Lake Champlain. As a basis to compare the AUV results to, a two-ship (UVM Melosira and Middlebury College’s R/V Baldwin), 106-station CTD survey will be taken each day for 4 days (starting July 29th) in the Thompsons Pt – Split Rock Gap region (~4 km²). Concurrently, the AUVs will be deployed while each CTD survey is underway. AUV and CTD daily data sets will be three-dimensionally modeled and characterized using earthVisons4® (Dynamic Graphics, Inc) software. Statistical comparison of the AUV and CTD final 3-D grids of the various parameters (temp, fluorescence, and turbidity) will provide information as to the reliability and accuracy of the AUV data in a complex hydrodynamic regime. Additionally, representatives from the U.S. Navy, YSI (providing the sensors), DGI (earthVisons4®), and OceanServor will be on site for validation purposes as well as looking at the ability to acquire, model, characterize, and display information in a real-time environment.

Kinetics and Mechanism of the Bonding of Pt Anticancer Drugs to DNA nucleotides and Oligomers

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Platinum anticancer drugs have been instrumental in the treatment of many types of cancers. The anticancer activity of such platinum complexes is thought to derive from the formation of Pt-DNA adducts. Originally, only platinum (II) complexes were utilized therapeutically; however, recent studies have shown that platinum (IV) complexes (e.g., tetraplatin) exhibit similar anticancer activity. These platinum (IV) complexes are thought to bind to DNA nitrogenous bases via a different mechanism than their platinum (II) counterparts. The mechanism by which platinum (IV) compounds interact with DNA has been previously investigated by the Choi lab; however, it has not been proven that platinum (IV) compounds bind ligands in the axial position. In order to confirm the proposed mechanism, tetraplatin (PtIV(dach)Cl4) that was synthesized with chlorine isotopes (37Cl axially and 35Cl equatorially) was reacted separately with 9-ethylguanosine and 5’-dGMP in the presence of a Pt (II) catalyst (PtII(en)35Cl2). Using Liquid Chromatography-Mass Spectrometry (LC-MS) to identify the products of this reaction, the proposed axial binding mechanism was strongly supported. Additionally, it is of interest to study the rate at which tetraplatin binds to oligomers because DNA polymers are a primary substrate in vivo. The rate of reaction of DNA oligomers (three nucleotides in length) were observed by High Pressure Liquid Chromatography (HPLC). Tetraplatin was found to bind to TGT at the slowest rate. On the other hand, TTG and GTT were found to be bound at similar rates with TTG being slightly faster. Together, these results should further our knowledge of platinum drug anticancer activity.
Characterization of iron-dependent biofilm formation in *Streptococcus mutans*

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The ability to form complex, multi-species biofilms, or plaque, is a key virulence attribute for the caries-causing bacterium *Streptococcus mutans*. The bacterial composition of the plaque biofilm is well characterized however, genetic modulators of biofilm formation remain relatively unexplored. As part of this research effort, the Spatafora laboratory studies iron and/or manganese dependent regulation of *S. mutans* genes that contribute to virulence. Although iron is an essential micronutrient, intracellular concentrations must be tightly regulated at the level of transport to avoid generating lethal reactive oxygen species. We hypothesize that multiple regulators have evolved in *S. mutans* to facilitate essential metal ion homeostasis. Interestingly, *in silico* analysis of the *S. mutans* UA159 genome revealed a homolog of the ferric uptake regulator (*furR*) known to mediate iron homeostasis in other gram-positive and -negative bacteria. A UA159-derived *furR*-null mutant, called GMS802, demonstrated an enhanced ability to form biofilms in an iron-replete medium, and when grown as planktonic cells, GMS802 doubling times were significantly increased relative to wild-type. These findings are consistent with repression of biofilm formation by FurR and implicate FurR involvement in iron transport.

Work currently in progress includes construction of *furR* fusions to *gfp* and *gusA* reporter genes so that fur expression may be monitored in UA159 and GMS802 backgrounds under iron-replete and -limiting conditions. Future plans include modifying an inverted microscope to generate 3D reconstructions of *S. mutans* biofilms for statistical analysis. Taken collectively, these experiments will bring the study of *S. mutans* genes and their metalloregulation into the context of a biofilm that more closely approximates plaque in the human oral cavity.

Does inhibiting glutamate alter the development of sensitization to alcohol in mice?

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Previous work has shown that alcohol pre-exposure increases levels of self administration in a drinking paradigm and may do so via either decreasing the aversive effects of alcohol (tolerance) or increasing the rewarding effects (sensitization) or both. Evidence suggests that the development of tolerance may contribute to increased levels of consumption. Thus, in order to assess the impact of tolerance on alcohol consumption, the current study attempted to block sensitization to the locomotor stimulating effects of alcohol using a known NMDA antagonist Dizocilpine (MK-801). Male Swiss mice were administered two injections per day for 5 days of either 0.1 mg/kg of MK-801 or 2.0 g/kg saline followed thirty minutes later by either 2.0 g/kg dose of alcohol or 2.0 g/kg of saline. These injections yielded 4 groups: MK-801/Saline (MS), MK-801/alcohol (MA), Saline/Saline (SS), and Saline/alcohol (SA). On day six mice were given a challenge injection of 2.0 g/kg alcohol. A subset of mice from the SS group was administered saline on this day to serve as a control for the acute stimulatory effects of alcohol in the other groups. The mice were then placed in an open field maze and stimulation was measured by the number of line crossings during a five minute period. We predicted that chronic alcohol exposure would result in a sensitized locomotor response in our SA group as compared to the SS group given alcohol. We also predicted that MK-801 would block the sensitizing effect of chronic alcohol administrations in the MA group. In response to the challenge alcohol injection, all mice showed a locomotor stimulatory effect as compared to the SS group given saline only. However, sensitization of that response was not observed in the SA group nor was it seen in the MA group. In fact, mice in the MA group tended to show depressed locomotor stimulation relative to the other the alcohol groups. Thus, in this paradigm, it is unclear if MK-801 impedes sensitization but, it may blunt the acute stimulatory effect of alcohol. If MK-801 is shown to block the development of sensitization in this paradigm, it will allow for future research to focus specifically on the observation of tolerance and the role it plays in the consumption and alcohol dependence process.
Effects of Testosterone on Long-term and Short-term Spatial Memory of Adult Male Rats

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Testosterone can improve some forms of spatial memory and testosterone administration to castrated male rats enhances cell survival in the dentate gyrus region of the hippocampus. Blocking hippocampal neurogenesis impairs long-term but not short-term spatial memory. Hence, testosterone may improve long-term spatial memory by enhancing hippocampal cell survival. Adult male rats were bilaterally castrated. Hippocampal dependent spatial memory was tested using a water maze. During six training days, the castrated rats were injected with either 0.1 ml of sesame oil (N=16) or 0.1 ml of testosterone propionate (0.5 mg/rat; N=16). The rats were placed in a circular pool containing a submerged platform, and the water was made opaque with white paint. The room had various visual cues so that the rats could learn the location of the hidden platform. The rats were allowed to remain on the platform for 15 s once they found it or, if after 90 s, a rat failed to find the platform it was placed on the platform by the experimenter. A computer assisted tracking system was used for data collection. The rats were trained for six days with four trials each day. One week after completion of the acquisition trials (short-term memory), half of the rats were returned to the water maze for a probe trial and the other half were tested with a probe trial two weeks after the acquisition trials (long-term memory). During the probe trial, the platform was removed and the rats were allowed to swim freely for 90 s. The amount of time spent in the quadrant where the platform was previously located is used as an index of the rat’s spatial memory. Surprisingly, our preliminary results indicate that rats injected with oil performed better during the acquisition trials compared to the ones injected with testosterone. Probe trials are currently in progress.

Magnetic Circular Dichroism of a Methionine Aminopeptidase-Fumagillin Complex

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Methionine aminopeptidase (MetAP) is a dimetallic enzyme that is important in humans for angiogenesis. As such, it has been suggested as a possible target for anti-cancer treatment. In the dicobalt enzyme, a water molecule bridges the two Co(II) ions. A known inhibitor of MetAP, fumagillin, has been isolated from the fungus Aspergillus fumigatus. Recently, a crystal structure was published showing fumagillin in the active site of human MetAP. The structure shows a hydrogen bond interaction between fumagillin and the bridging water ligand in the active site. Since the bridging water ligand is important for magnetic coupling between the metals in the active site, we hypothesize that the magnetic coupling in the MetAP-fumagillin complex will be different that in resting MetAP. We have studied both the resting MetAP and the MetAP-fumagillin complex by magnetic circular dichroism spectroscopy. We have detected magnetic coupling between the Co(II) ions in the MetAP-fumagillin complex where there is no detectable coupling in the resting enzyme.
Measuring the Expansion of the Supernova Remnant Puppis A

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Every day the sun fuses hydrogen into helium, which produces energy for life on earth. Eventually the sun will run out of hydrogen, and then it will fuse helium into carbon and oxygen before quietly dying as a white dwarf. By contrast, for stars larger than ten times the mass of the sun, the temperature may get high enough for additional, potentially explosive, nuclear reactions to occur. Unlike the sun, these stars undergo a violent and dramatic end known as a supernova in which the collapse of the stellar core produces a shockwave that blasts the star apart. In a few seconds a supernova releases as much energy as the sun will in its entire ten billion year lifetime. Not only are supernovae among the most violent events in the universe, they are also the ultimate source of heavy element production — virtually all the atoms in our bodies came from ancient supernovae. Puppis A is one of only three known oxygen-rich supernova remnants in the Milky Way Galaxy. These types of supernovae are particularly interesting because they are young, and their filaments are mostly pure heavy elements that originated in the core of the star before it exploded, uncontaminated by interaction with the interstellar medium. In images of Puppis A taken at the Cerro Tololo Inter-American Observatory in Chile from 1989 to 2006, we can identify several dozen of these filaments. I am working to measure their velocities and can then extrapolate back to find a point of origin and an age for Puppis A.

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Observational Learning in Octopus bimaculoides

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The octopus (Octopus bimaculoides) is currently a model to investigate observational learning. Observational learning, the ability of an observing subject to learn a task from a previously trained subject, is specific to very intelligent organisms, and may be a precursor to cognition and conceptual thought. Organizational similarities between the central nervous system of octopod and vertebrate brains suggest that learning studies in O. bimaculoides may parallel learning and conceptual thought in humans, and other vertebrates. To study O. bimaculoides, a 300-gallon salt water aquarium had to be constructed. Two 125-gallon tanks filter through a 50-gallon sump with a protein skimmer, 2000-gph flow rate, and fine-micron filtration to provide 42.5 gallon chambers for six individually housed animals. Salt water quality is maintained at a specific gravity of 1.026, with ammonia and nitrite levels of ~0.00 ppm, 10 ppm nitrates, and a pH of 8.3. Early trials indicate that O. bimaculoides are capable of solving simple tasks, such as removing pieces of squid from wooden probes, and have easily learned to associate food with a human. Future studies address how subjects can be trained to distinguish between a “food” and “neutral” stimulus, and that an untrained animal can discriminate through observation of a trained subject alone. After establishing this baseline for observational learning, we will examine whether NMDA and 5-HT antagonists are capable of inhibiting such learning. Studies of such advanced forms of learning in octopods and other cephalopod mollusks suggest potential abilities and mechanisms for complex learning in all animals.
Examining the expression of the antioxidant enzyme catalase in bovine granulosa cells and follicular fluid

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A developing trend has been the delay of childbearing to later in life, when odds of pregnancy are reduced due to decreases in oocyte quantity and quality. As a result, an increasing number of couples experience difficulty conceiving. To optimize the treatment methods available to subfertile and infertile patients, it is necessary to examine the factors that contribute to oocyte quality. Oocyte quality refers to an egg's ability to mature, be fertilized, and support the development of an embryo. One factor of known importance to fertility and reproduction is oxidative stress, which arises from the imbalance of pro-oxidants (reactive oxygen species; ROS) and antioxidants. Although oxidative stress has been linked to several key ovarian processes, little is known about its effect on antral follicle development, a process during which the oocyte and its supporting granulosa cells mature and gain competencies in preparation for ovulation. In the present study, we examine the expression of the antioxidant enzyme catalase (responsible for reducing hydrogen peroxide, a toxic ROS, to water) in granulosa cells and follicular fluid from bovine follicles of three maturation stages. Bovine ovaries were obtained from a local slaughterhouse. Follicles were dissected out and categorized as small (diameter of 2-5mm), medium (6-8mm), or large (>8mm) to account for different maturation stages. Granulosa cells or follicular fluid was obtained and their respective protein was pooled by follicle category. Western blots were performed to examine catalase expression. Preliminary data show that catalase is expressed in granulosa cells and follicular fluid of all three follicle categories, though more data is needed to determine whether its expression is developmentally regulated. Further work will also focus on characterizing the expression of another important antioxidant enzyme, glutathione peroxidase. Altogether, these experiments may provide insight into the mechanisms responsible for combating oxidative stress in the oocyte's microenvironment.