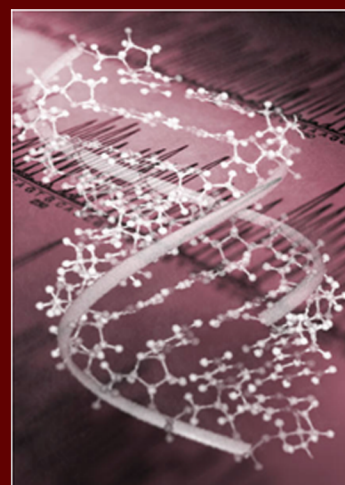
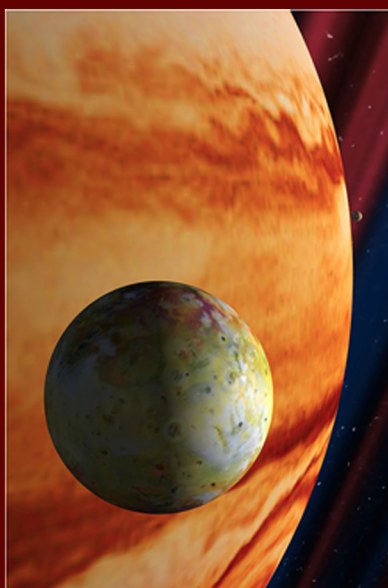
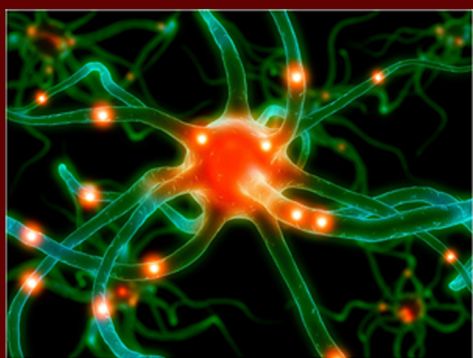




HARVARD COLLEGE

PRISE

PROGRAM FOR RESEARCH IN  
SCIENCE AND ENGINEERING



# ABSTRACTS

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PRISE

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# ABSTRACTS

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## Letter from the Director

I am pleased and privileged to write this introduction to the 2008 Harvard College Program for Research in Science and Engineering (PRISE) Abstract Book. This volume marks the second annual publication of this Fellow-initiated project, which captures the range and variety of scientific exploration among the Fellows that occurs during the ten weeks of PRISE. During the summer, the Fellows have spent countless hours working under the guidance of distinguished Harvard investigators from the Faculty of Arts and Sciences, the Harvard Medical School and affiliate hospitals, the Harvard School of Public Health, and other scientific research enterprises associated with the university. This scientific experience has been augmented by participation in PRISE's interdisciplinary residential community of undergraduate scientists in Leverett House, providing a social environment and the opportunity to interact with peers in a meaningful way.

This third class of 118 Fellows has been irrepressible in their efforts to learn about each others' scientific areas of interest, to connect and engage Harvard-affiliated faculty in both the mission and the operation of PRISE, and to have a fun, productive summer of research, community, and learning. As one of the main goals of PRISE is to encourage and foster the development of young scholars entertaining careers in scientific research, the abstracts herein are a testimony of the experiential strides made in a relatively brief time.

To the PRISE Fellows of 2008: the opportunity to observe and work with you has been a tremendous pleasure. You are an incredibly talented and outgoing group whose creativity and enthusiasm has been nothing short of infectious. As you continue forward, I hope you keep in touch to tell me about your adventures as they continue to unfold.

*Gregory A. Llacer, Director*

*Harvard College Program for Research in Science and Engineering (<http://prise.harvard.edu>)*

## Letter from the Editors

Dear Fellow PRISErs,

Research. All of us have different associations with the word – inquiry, intellectual stimulation, discovery, tedium, trial and error, passion. During the past ten weeks, however, PRISE has allowed us to forge new associations with the word “research” – community, collaboration, and interdisciplinary exchange, among others. Over dinners at Dudley House and lunches in Bauer courtyard, during morning rides on the M2 and spontaneous gatherings in the common room, we've found ourselves unexpectedly drawn into the intricacies of our peers' research. We've been amazed by the wide variety of research questions being tackled, from how fungi use convection to distribute their spores to how magnetic minerals are affected by impact shock waves. At the same time, we've realized just how connected our research can be, and how by sharing ideas, we can improve both our own and others' work. And in the process, we've learned so much and had so much fun – what more could you ask for in a summer? From whale watching to the murder mystery cruise, from Tanglewood to kayaking on the Charles, we have made our PRISE experience as exciting and eventful as possible.

This abstract book is a compilation of 115 productive summers. We hope that it will serve as a unique keepsake and that you will enjoy reading about each other's research. In a matter of weeks, classes will start, and instinctively we will all fall back into our term-time routines, but there is no doubt that the bonds we have formed this summer will continue to bring us together during our remaining time at Harvard and beyond. We hope that you will all continue your PRISE experience by participating in the Harvard Undergraduate Research Symposium (HURS) and the first annual Boston Undergraduate Research Symposium (BURS), both of which are organized by the Harvard College Undergraduate Research Association (HCURA). We look forward to hearing about your success in the months and years to come.

*Sincerely,*

*The PRISE 2008 Abstract Book Editorial Staff*

Michael Ayoub '10 • Christina Grassi '10 • Dayan “Jack” Li '11 • Charles Liu '11  
Francesca Reindel '11 • Brad Seiler '10 • Lev Shaket '10 • Veronica Shi '11 • Carol Suh '11





# CHEMISTRY AND BIOCHEMISTRY

Leslie Beh  
Pforzheimer 2011

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## Characterization of the SUMO E2 activity of the Polycomb Repressive Complex 2 (PRC2)

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Polycomb Repressive Complex 2 (PRC2) is a highly conserved Polycomb Group (PcG) multiprotein complex that is involved in the faithful maintenance of the repression of Hox genes through repeated cell divisions. Recent data suggests that the fruit fly (*Drosophila melanogaster*) Polycomb Repressive Complex 2 can act as a SUMO (Small Ubiquitin-related MOdifier) conjugating enzyme, being able to catalyze the attachment of a SUMO protein modifier to its target. SUMOylation has important functional consequences, ranging from an alteration of nuclear localization to transcriptional repression. Current experiments are focused at identifying the protein subunit within PRC2 that possesses this SUMO “E2” activity. Firstly, we are attempting to further purify PRC2 subunit protein preps to ascertain that contaminating proteins are not causing the observed SUMO E2 activity. Secondly, we are attempting to express and purify each PRC2 subunit from a bacterial system – free of endogenous SUMO E2 protein – in order to identify the PRC2 subunit that possesses the *bona fide* SUMO E2 activity. The characterization of PRC2 as a novel SUMO E2 conjugating enzyme would expand existing knowledge of SUMOylation mechanisms, and provide new insight into the role of SUMOylation in Polycomb-mediated silencing.

Megan Blewett  
Currier 2011

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## Investigating the role of molecular mimicry in the pathogenesis of multiple sclerosis

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“When all our blandishments would seem defied, we have ideas yet that we haven’t tried.” These words of Robert Frost ring true especially in science. For over fifty years, researchers have thoroughly examined the possibility of a viral cause of multiple sclerosis (MS). Candidate etiological agents have included: measles virus, Epstein-Barr virus, Varicella Zoster virus, and even the polio virus, among many others. While MS may have several causes and a virus may indeed be one of them, the evidence for bacterial involvement in this demyelinating disease has been growing. Broad-spectrum antibiotics like penicillin have been shown to reduce the risk of acquiring MS, the NKT cell populations that appear to be deficient in MS patients recognize LPS-lacking bacteria, and the geographic distribution of MS co-occurs with the distribution of the bacteria *Borrelia burgdorferi*. In addition, *B. burgdorferi* can enter the central nervous system and

remain latent there for over a decade, mirroring the progression of MS. *B. burgdorferi* and *Sphingomonas* are members of a small class of bacteria that do not display LPS, an evolutionary adaptation that allows them to remain relatively undetected by the immune system. In place of LPS, these bacteria express glycolipid antigens, antigens which bear broad structural similarities to the main components of the myelin sheath. A constellation of factors may lead to mimicry-induced demyelination in the CNS; antibodies stimulated by these bacterial glycolipid antigens may cross-react with the myelin sheath. We have investigated the ability of monoclonal antibodies isolated from an MS brain, as well as MS cerebrospinal fluid, to recognize these lipids of the myelin sheath. Ongoing work includes probing for bacterial DNA and determination of antigen-antibody affinities via surface plasmon resonance.

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Cabot 2010

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## Development of a reaction discovery methodology employing mass spectrometry

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Many important chemical transformations employ transition metal complexes as catalysts. Though these catalysts are often designed for specific reactions, they may also have catalytic effects on other chemical substrates. To facilitate efficient evaluation of potential catalysts, this project seeks to develop a reaction discovery methodology using liquid chromatography-mass spectrometry (LCMS). With the help of a data analysis program, we can compare LCMS data for reactions run with and without catalyst to locate signals corresponding to products of a catalyzed reaction, while eliminating signals from compounds that do not react. This should allow evaluation of catalysts on pools containing many chemicals, accelerating the reaction discovery process. We are developing the methodology by applying it to known reactions. Our current goals are twofold: observing the expected peaks and eliminating unexpected ones. Toward the first goal, we have tried incorporating into our substrates functionalities that are more easily ionized, as well as using different ionization techniques that should allow detection of less active molecules. Toward the second, we have written macros that filter out non-product peaks based on various criteria. Once we have developed a procedure that consistently “discovers” known reactions, we will design the reagent pools and begin evaluating potential catalysts.

Abigail Harpstead  
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### Selective inhibition of $\gamma$ -secretase as a drug for Alzheimer's disease

The Laboratory for Experimental Alzheimer Drugs  
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Alzheimer's disease, with age as a major risk factor, is becoming more prevalent in today's society. This disease is a progressive neurodegenerative disorder where patients have a buildup of neuritic plaques made up of  $\beta$ -amyloid (A $\beta$ ) peptides. The A $\beta$  peptide is derived from the Amyloid Precursor Protein (APP) by two enzymes, one of which is  $\gamma$ -secretase. However,  $\gamma$ -secretase is also important in the cleavage of Notch, which has important signaling functions in the body. Therefore, the purpose of the research conducted by LEAD (Laboratory for Experimental Alzheimer's Drugs) is to discover a compound that inhibits  $\gamma$ -secretase from cleaving APP but not Notch. Activity assays followed by either Western blots or ELISA assays are used to test the activity of  $\gamma$ -secretase in the presence of different compounds. These assays are conducted in the presence of the C-100 substrate (to represent APP cleavage) or the N-100 substrate (to represent Notch cleavage). To be a possible drug candidate for Alzheimer's disease, the compound should inhibit  $\gamma$ -secretase from cleaving the C-100 substrate but not the N-100 substrate. LEAD has recently found its first compounds that inhibit  $\gamma$ -secretase 100% from cleaving C-100 substrate at 100 $\mu$ M. The N-100 data is still pending. Future research is going to focus on obtaining N-100 data and trying to find even more potent compounds. Finding a drug that fits these requirements is very important because it would provide the first drug for Alzheimer's disease that actually stops the progression of the disease.

Richard Kwant  
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### Determining the role of entropy in the binding of aromatic sulfonamides to carbonic anhydrase

The Whitesides Laboratory  
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Recently the number of drugs developed has decreased while the cost of developing these drugs has increased. This trend results from drug manufacturers' inability to accurately predict binding interactions between proteins and medicines, which are usually small organic molecules. Specifically, we do not understand how the binding of small molecules (ligands) and proteins is governed by entropy, a measure of disorder. Therefore current projects involve using carbonic anhydrase, a protein important in numerous human cellular processes, as a model system to develop methods to predict how molecules will bind to proteins. After synthesizing a number of similar aromatic (cyclic) compounds attached to sulfonamide (SO<sub>2</sub>NH<sub>2</sub>) groups, we use an isothermal calorimeter to measure the energy released when each molecule binds to carbonic anhydrase. This information allows us to determine how entropy affects the affinity

of a ligand for a protein. We also perform x-ray crystallography on samples of protein-bound ligands to determine how the geometry of binding affects entropy and binding affinity. By comparing the geometries, binding affinities, and entropic effects of a number of small molecules, we are currently working on a model to predict how other molecules will bind to carbonic anhydrase.

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Adams 2011

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### The evolutionary fitness of sequence permutations in RNA

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FAS Center for Systems Biology

How did life emerge from inanimate molecules? One hypothesis is that RNA was the driving force behind the evolutionary leap from certain organic and inorganic molecules to life. Therefore, the biochemical properties of RNA played an essential role in shaping the evolution from molecules to the earliest cells on Earth. Our work involves characterizing the evolutionary fitness of RNA according to its sequence permutations. The objective is to determine if certain sequences would make RNA molecules more resistant to degradation and thus better able to survive the harsh conditions of the pre-biotic world. To simulate these harsh conditions, base hydrolysis experiment was carried out on synthesized random sequences. The surviving RNA molecules from the hydrolysis were collected and subsequently purified through gel electrophoresis. This process allowed us to separate fully intact sequences from the ones that were broken down. These nucleotide sequences were then converted to DNA so that they could be amplified using the polymerase chain reaction (PCR) and sent for sequencing. The goal now is to be able to find recurring patterns among the surviving sequences. The biochemical properties of these short sequences and secondary structures could confer an evolutionary advantage whose identification could help characterize the basic genetic features of early life.

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Leverett 2009

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## Investigating the stabilization of Emp1 $\beta$ -turns

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Erythropoietin (EPO) is a glycoprotein hormone that maintains erythropoiesis homeostasis through targeting EPO receptors (EPOR). Recombinant human EPO protein (rHuEPO), a widely used anemia treatment, demands improvement in drug half-life and clearance because its current form is temperature sensitive and requires frequent injections. EPO mimetic peptide 1 (Emp1), a 20-residue EPOR agonist exhibits significant erythropoietic effects in mice, suggesting its potential as a treatment for anemia. EPOR and Emp1 have been shown to interact as dimers, where Emp1  $\beta$ -hairpin turn monomers associate non-covalently with each other as each  $\beta$ -turn is stabilized by an intramolecular disulfide bridge. These interactions suggest critical sites for Emp1 stabilization via incorporations of intramolecular olefin cross-linker through alkene metathesis or triazole ring cross-linker through azide-alkyne cycloaddition. Preliminary studies have screened Emp1 derivatives where the disulfide bridge is replaced by either cross-linker to identify active peptides containing azido-ornithine. Covalent dimerization of promising hits by alkene metathesis has shown comparative responses relative to Emp1 in Ba/F3 proliferation assays. This project aims to optimize the stabilization of Emp1  $\beta$ -turns via synthesis of unnatural amino acid building blocks that form the cross-linkers, and synthesis of peptides containing the olefin and the triazole linkers. Biochemical assays will be performed to determine the metabolic stability and  $K_d$  of stabilized peptides. It is envisioned that the peptides will be used as tools to monitor stages of EPO-derived erythropoiesis in addition to potentially serving as a treatment for anemia.

Anne McCabe  
Currier 2011

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## Studying NRAMP protein structure via x-ray crystallography

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The NRAMP family of transporters (Natural Resistance Associated Macrophage Proteins) pumps divalent metal ions ( $Mn^{2+}$ ,  $Fe^{2+}$  or  $Co^{2+}$ ) across biological membranes. In humans, NRAMPs are located in macrophages, where they help to defend against bacterial infections by robbing invading bacteria of essential metal ions. A second NRAMP isoform, expressed at the epithelial tissue of the intestine, mediates uptake of dietary iron. To discover how NRAMPs work, we sought to determine the 3-dimensional structure at near atomic resolution. Protein crystallography requires the protein in question to stack as a crystal, which diffracts X-rays and the resulting pattern is analyzed to deduce the protein structure. We began by cloning various bacterial NRAMP genes into so-called vectors for expression in *E. coli*. To pull the NRAMP protein out of the crude mixture of bacterial proteins, we used a histidine tag before the se-

quence that binds to  $Ni^{2+}$  coated beads. The purified protein is used to grow crystals in various solutions of salts and buffers. In addition to the crystal studies, we set up an assay to determine residues that are essential for protein activity and possible ion specificity. We identified conserved residues reported to line the active site and mediate ion transport (deduced from other studies) and mutated those to non-conserved residues. The mutated NRAMP genes were expressed in *E. coli* and used in cobalt uptake assays. The amount of cobalt in the cells corresponds to the level of function of the mutated transporter.

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## Discovery of new antibiotics in *Photobacterium luminescens*

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In recent years, the discovery of new antibiotic compounds has tapered, despite advances in combinatorial chemistry and high-throughput screening. At the same time, antibiotic resistance has increased, thereby making the discovery of new antibiotic compounds all the more crucial. Genomic sequencing of bacteria and fungi—traditional sources of new small molecules—has revealed the presence of many putative natural product gene clusters whose products have not yet been discovered, indicating an untapped source of potential antibiotics even in well-studied microorganisms. *Photobacterium luminescens*, a worm endosymbiont and insect parasite, is one such bacterium and the focus of our studies. A two-pronged approach was taken to uncover novel antibiotics produced by this organism. The first involves creating a *P. luminescens* gene library in *Escherichia coli* and screening those clones for antibiotic activity. The second involves culturing *P. luminescens* under a variety of conditions and analyzing UV spectra of the extracts on a Liquid Chromatography-Mass Spectrometer. The genomic approach yielded one potential active clone out of 4200. However, the methodology will be altered to increase the sensitivity of the antibacterial screen, hopefully exposing more active clones. The second approach yielded three potentially novel compounds produced under iron-rich or iron-limiting conditions. Further structural determination remains to be performed on these compounds before any definitive conclusions may be drawn.

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Nwamaka Uzoh  
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### **Investigating CD39: Using detergents to determine kinetics and structure**

The Guidotti Laboratory  
Dept. of Molecular and Cellular Biology, FAS

CD39 is a member of the ecto-nucleoside triphosphate dephosphohydrolase (e-NTPDase) family of proteins, which is a group of enzymes responsible for nucleotide hydrolysis at the surface of the cell. CD39 normally hydrolyzes ATP to AMP. CD39 is anchored in the membrane by two transmembrane domains, which regulate the active site in the extracellular region. Removal of either alpha-helical transmembrane domain results in a loss of about 90% of total activity. Furthermore, the transmembrane domains show signs of rotational mobility, determined by crosslinking cysteine residues with disulfide bonds. It is believed that the elasticity of the membrane may regulate the mobility of the alpha helices and in turn the overall activity of the protein. We chose to examine the effects of three different detergents on the activity of the protein: Octyl Glucoside (OG), lysophosphatidylcholine (LPC), and 1,2-diheptanoyl-sn-glycero-phosphocholine (DHPC). Of the three, OG reduces the activity of CD39 the most, followed by DHPC, then LPC. A sucrose density gradient was used to spin detergent-solubilized protein in either a gradient with detergent or without detergent. High activity at both the top and the bottom of the gradient suggests both that CD39 may exist in lipid rafts and that it may oligomerize. We plan to examine this with two mutated forms of CD39:  $\Delta$ NT (lacking the amino terminal) and with a C13S mutation (which is necessary for palmitoylation), which, we hypothesize, might be integral to dimerization.

Peyton Shieh  
Adams 2010

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### **The total synthesis of phorbol**

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Phorbol is a tetracyclic diterpene first isolated from the oil of the croton plant. Phorbol esters are potent tumor-promoters, acting through the competitive binding of the regulatory domain of protein kinase C. While the compound can be obtained from biological sources, its isolation is particularly difficult. In our proposed synthesis of phorbol, we plan to create three of the rings from a macrocyclic intermediate using two transannular aldol reactions. The favored conformation of this macrocyclic intermediate should yield the correct stereochemistry at the ring junctions. This summer, we have created an efficient synthesis to a five-carbon aldehyde precursor to the macrocycle, allowing us easier access to large amounts of the macrocyclic intermediate. Currently, the transannular aldol reactions can correctly make three of the four ring junction stereocenters. Future work is aimed towards fixing the incorrect stereocenter and completing the synthesis of phorbol.

### **The use of lipid lowering agents (statins) in children: patterns and associated adverse events**

The Shannon Laboratory  
Clinical Pharmacology Unit and Preventive Cardiology Clinic,  
Children's Hospital Boston

Increased pediatric obesity rates have led to earlier onset of hyperlipidemias which have an established association with heart disease. Though obesity is the primary and most modifiable risk-factor, familial hypercholesterolemia, a genetic defect, also results in failure to remove excess harmful low-density lipoproteins (LDL) from the blood. When diet modifications and increased exercise fail to adequately lower lipid levels, the class of drugs called "statins" lower LDLs by inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A reductase which determines the rate of hepatic cholesterol synthesis. Our study examines the clinical course of children prescribed statins, identifying adverse drug events (ADE) associated with long-term use and potential risk factors for development. Statins pose a theoretical risk of significantly altering crucial homeostasis in children because cholesterol is critical for liver and neuron development, anchoring transcellular neurotransmitters, and steroid hormone production. We have conducted a retrospective cohort study of children, ages 8-18, followed in Children's Hospital Boston's Preventive Cardiology Clinic (PCC) who were prescribed statins like Zocor® (simvastatin), Lipitor® (atorvastatin) and took them for at least 2 years since July 2004. Medical records were reviewed with clinical and laboratory variables of interest extracted and analyzed statistically, by subgroup and regression modeling. Though still in progress, the investigation is essential as pharmaceutical companies and health care providers continuously approach children as "miniature adults" who can be given fractionated medication portions. The biochemistries of children and adults differ drastically, yet safety profiles of only 25% of drugs prescribed to children have been characterized for use in pediatric population use (AAP).



# COMPUTER SCIENCE

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Pablo Azar  
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## Learning conjunctions through evolution

Supervised by Leslie Valiant  
Dept. of Computer Science, FAS

Computational Learning Theory is a field that studies artificial intelligence algorithms from a theoretical perspective. These algorithms usually take data about the world as input and return predictions or other useful information as output. Recently, Leslie Valiant of Harvard developed a framework for analyzing algorithms that behave like primitive reproducing organisms. These algorithms start with a random output. They learn by producing copies of this output with random mutations, and keeping only the mutations that increase a given fitness function. This aims to capture the behavior of a primitive organism that becomes better at a task through generations of random mutation and natural selection. In early 2008, Feldman established important positive and negative results about what can and cannot be weakly learned under evolution. He left open the problem of whether conjunctions and disjunctions can be strongly learned. In this paper, we will show that conjunctions and disjunctions can be strongly learned when we assume that the attributes over which we are learning follow a product distribution. It is still an open question whether conjunctions and disjunctions can be learned under general distributions, or whether wider classes of functions can be learned under product distributions.

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## Simulation environment and locomotion algorithms for soft robotics

Supervised by Robert Wood  
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Due to the potential to produce robots capable of accomplishing a wide range of new tasks including deformation to a fraction of the robots original size allowing for movement in confined areas, soft robotics has emerged as a newly developing field of intense micro-robotics research. Traditional robotic development is characterized by mostly rigid components, actuators in defined locations, and pre-defined methods of locomotion. Using soft actuators, soft robotics aims to produce robots that can change their shape to increase range of motion and environmental flexibility. Designing and testing potential robot designs by computer software simulation rather than real-world robotics minimizes design costs as revisions are easily made to a robot design without waste of expensive fabrication materials and time. In this research project, a software simulation environment was designed for soft robotics simulation. A soft robot in simulation is represented as a graph in which vertices represent soft universal joints and edges represent soft linear actuators. A simula-

ed demonstration of a soft robot consisting of four hexagons capable of folding and treadmill locomotion was implemented in simulation. Since simulation is limited by physical accuracy, implementation of dynamics is currently under consideration. Future revisions could include support for tensegrity based robotics, robot skins, or alternative methods of robot locomotion.

Prabhas Pokharel  
Adams 2009

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## Building a verified compiler for the lambda calculus

Supervised by Greg Morrisett  
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School of Engineering and Applied Sciences

Having mechanically verifiable proofs about computer programs seems deeply desirable — imagine the benefits of a downloadable software that comes with a machine-verifiable guarantee assuring it is not malicious. To do this, one would need to write a program and then a proof of its “correctness” in a high-level (ie. human-understandable) programming language. A compiler would then translate such a program to low-level assembly language, which is a computer-interpretable code of 1s and 0s. To trust the assembly code however, we would like to mechanically verify that the compiler itself does these translations correctly. During the summer, I have been working with Joe Zimmerman on building such a verified compiler for a simple but powerful programming language called the lambda calculus. In the programming language Coq, we have written various languages (which serve as phases of translation) and translations between them, along with proofs of the fact their independently expressed meanings are preserved through the translations. We have completed a verified compiler to an intermediate language known as A-normal form, and will continue working on translations to assembly code. Future work will involve finishing the compiler, as well as writing a compiler from a higher level language to the lambda calculus that we are translating.

Brad Seiler  
Lowell 2010

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### Photography with all the colors of the rainbow: Building a database of natural hyperspectral images

The Zickler Lab,  
Electrical Engineering and Computer Science, School of Engineering and Applied Sciences

Despite how breathtaking things may appear, human color vision is limited to only three distinct types of color receptors. An infinite number of distinct spectral radiance functions appear identically to human eyes. Film and digital photography, because they are designed for human consumption, have the same limitation. Using new liquid crystal filter technology, we developed a system for capturing hyperspectral images of the natural world, where the spectral radiance function of each pixel in a 1.2 mega-pixel image can be captured in under one minute with great of precision. These images produced are independent of the light source, spectral sensitivity of the camera, and other sources of irregularity, so that they represent the true reflective properties of the objects in the image. Software controls the system to determine the number and duration of exposures necessary to capture the whole image, and also integrates the hundreds of images captured together into a single manageable format for research. We developed this system to test algorithms for color constancy, which work by identifying a transformation from a given trichromatic image to the same image under canonical lighting. Using hyperspectral imaging, we can test these algorithms on images with varying light sources against ground truth. Opportunity also exists to use these images in psychological studies or the development of texture or object recognition algorithms.

Chelsea Zhang  
Kirkland 2010

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### A lens into CitySense: visualizing an urban-scale wireless network

Supervised by Matt Welsh, Sensor Networks Lab  
Electrical Engineering and Computer Science, School of Engineering and Applied Sciences

Research in wireless networking has advanced rapidly, ranging from systems that gather sensor data from remote wilderness sites to globally distributed systems that provide Internet services. Most research-focused wireless networks are small; the larger ones are deployed in enclosed environments like office buildings and laboratories. To study the feasibility and applicability of an urban-scale wireless network, we deploy a 100-node sensor network, called CitySense, in Cambridge, MA. The nodes, mounted on rooftops and streetlights, will contain sensors that collect weather, pollution and noise data. CitySense will serve the main purpose of providing an urban testing environment for researchers' network programs. Besides its applications to urban pollution monitoring, location-customized WiFi access and others, CitySense addresses questions such as: (i) how to distribute processing tasks across nodes with limited

computing power and (ii) how to maintain a stable network across environmentally exposed, unstable nodes. To monitor the health of the CitySense network, we have created software that visualizes the state of wireless connections between nodes. My application analyzes variables like data transmission rate (TCP throughput) and loss rate of transmitted packets temporally, spatially and summarily. Preliminary analysis has shown that TCP throughput across outdoor nodes varies by time of day and correlates negatively with distance between nodes. The high noise levels of measurements and apparent leakages in TCP throughput over time remain unexplained; further development and use of my visualization tool should help answer such questions.

Joe Zimmerman  
Quincy 2010

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### Towards effective certified software

Supervised by Greg Morrisett  
Electrical Engineering and Computer Science, School of Engineering and Applied Sciences

While not in widespread use at present, the prospect of computer-assisted and -verified proofs of software correctness holds great potential. In mission-critical systems, for instance, such proofs would be invaluable: one could prove with absolute certainty that a programmer's error could not cause a spacecraft to veer off-course, a hospital's life-support system to malfunction, and the like. The applications are similarly compelling in computer security and software engineering in general, as source code libraries could be developed alongside guarantees that they perform their function without risk of bugs or exploits. Towards this goal, we have worked this summer to develop software to compile programs from the untyped lambda calculus (a model of computation originally described by Church) to x86 assembly code (which can be assembled and run on most modern processors), and for each step of the compilation process a corresponding mathematical proof of correctness (formalized and mechanically verified by the Coq proof assistant). Upon completion, this software would function as the last phase of a certified compiler for Ynot, a new programming language with many novel features for writing provably correct programs.

# EARTH AND PLANETARY SCIENCES

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*Peter Hedman  
Kirkland 2010*

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## **Shock waves in magnetic minerals**

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Mars exhibits no planetary magnetic field today, but regions of remnant surface magnetism record the presence of a planetary field until 3.5 billion years ago. Martian meteorites found on Earth also exhibit remnant magnetism and may be useful in studying the nature and disappearance of Mars' planetary field. However, the magnetic signatures in these rocks have likely been altered by exposure to impacts on the surface of Mars. In order to interpret these signatures, we must understand how magnetic minerals are affected by the shock wave conditions of impact. Samples of schist bearing the magnetic mineral pyrrhotite are prepared by sectioning into discs and are characterized for density, porosity, and sound speed. A 40mm single-stage gas gun is used to launch a metal, disc-shaped projectile at the samples, which are embedded within an aluminum capsule and recovered after the shot. The remnant magnetism of the rock samples is mapped with a SQUID magnetic microscope before and after shocking. Samples are now being prepared and characterized using the magnetic microscope but have not yet been shocked. Future work includes the construction of an electromagnet to study the effect of exposing rock samples to external magnetic fields during impact.

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## **Shocking samples of icy sand**

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The analysis of crater formation on planets requires studies of high-velocity impact events; shock wave experiments on the materials that make up these craters give data similar to such events. By hitting a sample at a known speed (2.1 km/s), we can find the resulting temperatures and peak after-shock pressure, parameters required for crater modeling. The current sample under analysis is a mixture of ice and quartz. The radiance of the sample during and after the shock is recorded for four infrared and one visible wavelength; voltage measurements are converted into radiance temperatures by Planck's law for blackbody radiation. By fitting plots of the change in temperature over time to a model of theoretical radiance, we can find the absorption coefficients of the shocked and unshocked sample; we can then examine the post-shock radiance to find the relative proportion of extra hot spots to shocked samples and their respective temperatures. Our data showed us the progression of the

shock through the sample, but our final temperature conclusions are uncertain due to the presence of air. Next we plan to shoot a sample of porous ice in order to fully examine the effects of air, and we have also been designing a way to create an ice-quartz sample that avoids air bubbles. We hope to then understand the effects of shocking icy ground.

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## **Modeling China's wind power potential**

The McElroy Group  
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China's current energy consumption and torrid pace of economic growth demands us to develop new renewable energy opportunities such as wind. In order to develop an appropriate wind power modeling approach, we have researched energy models produced by both private and public organizations that aim to forecast the composition of the energy economy under different economic and resource constraints. Although these examples model countries with very different energy and electricity economies than China, they serve as technical examples for constructing an appropriate and useful Chinese model. Due to present data limitations, a complex model such as the United States National Renewable Energy Laboratory's (NREL) Wind Deployment System Model (WinDS) is not currently feasible for China. Thus, the redefined objective for this project is to model the cost of building a new, dedicated transmission system from wind sources to load centers in China. A more technical analysis of the methods and data used to produce the previously studied complex models will be essential to constructing the desired Chinese model. The completion of a successful model will be useful for both Chinese energy policy makers and renewable energy developers. A similar model could be built for China's solar power potential as well.

# ENGINEERING AND BIOENGINEERING

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## Magnetically-induced collagen alignment

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Collagen is the most abundant structural protein in multicellular animals, structurally involved in dense regular connective tissue, bone, and cornea as a cellular scaffold and functionally involved in the maintenance of cell morphology, orientation, and migration as a main component of the extracellular matrix (ECM). An aligned (anisotropic) collagen matrix is useful for recreating the environment seen *in vivo* in dense regular connective tissue, bone, and cornea. However, currently only one method has been reported of creating these anisotropic gels without specialized equipment. Attempts were made to optimize this method by creating collagen gels of various concentrations and supplements, and applying a magnetic field during and/or after gelation. Attempts at alignment have been predominantly unsuccessful, although experiments continue to affect alignment, encapsulate and maintain cells inside the anisotropic matrix. Eventually we hope to manipulate the scaffolding property of collagen to study the effects of spacial control and mechanical stress on cellular gene expression.

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## *In silico* model of acetate production in *S. cerevisiae*

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A main goal of systems biology is to reduce complex metabolisms present in living organisms to simpler mathematical models and use these models to predict how changes to the organism will affect its chemical processes. Currently, one method of modeling these systems involves storing information about an organism's component reactions into a "stoichiometric matrix", which then can be used to predict potential compound yields resulting from deleting reactions from the genome. The goal of this project was to enable the existing modeling program to simulate adding one or more reactions from the Kyoto Encyclopedia of Genes and Genomes to the organism in addition to gene knockouts; this aim was achieved using the MATLAB programming language. The resulting matrix can then be solved using linear programming to find the hypothetical increase or decrease of a specified compound resulting from the modified metabolism. As a test case, this model predicted several foreign reactions that, when introduced into the *Saccharomyces cerevisiae* (yeast) genome, could significantly increase the organism's acetate production. Future directions for the project include growing these mutant strains of yeast and using their increased levels of acetate production to support *Clostridium kluyveri*, an anaerobe that naturally produces the potential biofuel materials hydrogen and butyrate.

## Supercontinuum generation in tapered optical fibres

The Loncar Laboratory  
School of Engineering and Applied Sciences

The goal of this project is to create a supercontinuum—high intensity white light. This light is meant to help improve the resolution of microscopy and spectroscopy instruments by allowing more signals to be emitted from a sample. Today, the most common man-made white light sources are tungsten filaments. However, oftentimes too much heat is generated in these tungsten filaments, melting these light sources. This research intends to create a supercontinuum by shooting high-intensity infrared pulses into a tapered glass optical fibre. Then, the intrinsic nonlinear properties of the glass convert the infrared light into white light. The nonlinear properties of glass are directly proportional to the intensity of the pulses within the glass. White light is only generated when a certain threshold intensity is reached. Thus the tapers serve to intensify the amount of light passing through an amount of glass. The tapered glass used in this process needs to be a thousandth of a millimeter in diameter. To create such delicate tapers, the glass fibres must be pulled over a hydrogen torch. The thickness of the tapers is then controlled by varying the parameters of the pull. Because this process is so intensive, no definitive results have been recorded. However, future plans include optimizing the pulses of the infrared laser and increasing the efficiency of the transfer of light from the laser to fibre.

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## Exploring the mechanism of anaerobic electron transfer in microbial fuel cells by bacteria of the genus *Shewanella*

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Microbial Fuel Cells (MFC's) are devices that harness the energy of microbial metabolisms to produce electrical currents. They have the potential to provide cheap, clean, renewable energy to power electronic devices and could therefore improve the quality of life in places that have limited access to electricity. However, current MFC's are not commercially viable due to their low power outputs and gaining a better understanding of the biological processes that drive and facilitate power production in these systems would be an important step toward improving the technology. The reason MFC's work is that many bacteria are able to survive in low oxygen environments by utilizing solid substrates as external electron acceptors. This project explores the theory that these bacteria use electrically conductive pili, known as nanowires, to directly deposit their electrons onto substrates. We cultured strains of the facultative anaer-



obes *S. oneidensis* and *S. putrefaciens* in aerobic media and then transferred them to MFC's where they grew and produced currents under anaerobic conditions on slides made of different conductive materials. Using scanning electron and fluorescence microscopy we have been able to image the bacteria on these slides and expect to see nanowire formation in response to low oxygen levels coupled with the availability of external electron acceptors. If this is indeed the mechanism by which many microbes are able to produce electrical currents, then future studies could focus on enhancing the expression of these nanowires through genetic engineering, thereby significantly improving the power output of MFC's.

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## Controlling current production in

### *Shewanella oneidensis*

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The metabolically versatile bacterium *Shewanella oneidensis* adapts to anaerobic environments by transporting electrons to its exterior. Produced during metabolism, these electrons are normally accepted by intracellular oxygen. In oxygen poor environments, they are instead accepted by diverse external environmental substrates. When grown in anaerobic media supplemented with lactate as a carbon source, *S. oneidensis* readily transports electrons to an electrode leading to an external oxygen source - the final electron acceptor. Using the detectable current as readout, we seek to engineer *S. oneidensis* to report the cells' internal states. A previous study demonstrated that *S. oneidensis* mutants deficient in the *mtrB* gene produce a smaller current than wildtype cells, and the introduction of exogenous *mtrB* into these mutants returns current production to normal levels. We aim to control the expression of *mtrB*, and thus current production, with synthetic genetic circuits introduced as plasmids containing the *lacI* and TetR chemical sensing systems for lactose and tetracycline, respectively, and the Cph1-EnvZ light sensing system. Such a biosensor allows direct computer interfaces, and may facilitate the development of broadly applicable electrical reporter systems. The standardized assembly of these circuits allows their inclusion in an open registry of synthetic genetic parts.

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## Liposome drug delivery

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Interfering RNA (RNAi) has potential therapeutic applications in regulating gene expression; however, its use is limited by inefficient delivery. We can enhance the transfection of RNAi using pH dependent liposomes coated with poly(ethylene glycol) (PEG)-b-polycation polymers. The PEG coating enables protection from immune recognition during circulation (pH 7.4) and triggering the release of the PEG layer within the cellular endosome (pH ~5.5) allows subsequent intracellular delivery of RNAi. Polycationic blocks, poly[2-(dimethylamino) ethyl methacrylate] (DMA) (31 or 62 monomers) or polylysine (K) (21 monomers), act as anchors for the PEG (113 monomer) protective block on the liposomes. Incorporation of 1,2-dioleoyl-3-dimethylammonium-propane (DAP), a titratable lipid (pKa ~ 6.7), allows the liposome's net charge to increase as the pH shifts from 7.4 in the bloodstream to 5.5 within the endosome. The net increase in liposome cationicity repels the polycationic blocks, which decreases the PEG polymer adsorption, and allows the liposome to fuse with the endosomal membrane; thus, releasing the RNAi into the cytoplasm. Our pH dependent (PD) liposomes encapsulate RNAi that demonstrate GAPD knockdown in human umbilical vein endothelial cells (HUVEC), and our *in vitro* data indicates that polymer desorption from PD liposomes may enhance RNAi-mediated gene knockdown. Future research includes encapsulating drugs that combat hemorrhagic shock and site specific targeting with antibodies.

# MATHEMATICS

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## Zeta functions with almost periodic coefficients

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We refine an implicit function theorem of Neuberger and explore applications to problems in complex dynamics. This theorem allows us, for example, to express particle trajectories in a magnetic field as continuous functions of parameters describing the field. In particular, we attempt to show that trajectories of points under the Chirikov (standard) map, which arises in the study of particle confinement, are stable under small perturbations of a parameter. In the course of examining these stability results, we are led to consider a class of almost periodic Dirichlet series which generalize Riemann's zeta function: letting  $f$  be a periodic function and  $\alpha$  be a Diophantine irrational, we consider a Dirichlet series with coefficients  $f(n\alpha)$ . We investigate the analytic continuation of these functions under various assumptions on the Diophantine type of  $\alpha$  and the function  $f$  and give conditions under which continuation to the set of complex numbers with positive real part is possible. In the future, we hope to find conditions under which such almost periodic Dirichlet series admit meromorphic continuations to the entire complex plane.

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## Vertex algebras and D-modules

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Vertex algebras are a mathematical structure originally introduced by physicists. In the 1980s, mathematicians applied these structures to prove a conjecture known as Monstrous Moonshine, a set of phenomena linking finite group theory to areas such as number theory and complex analysis. Vertex algebras have since found applications throughout mathematics. The goal of this project is to understand vertex algebras from two perspectives. As originally conceived, a vertex algebra consists of a vector space together with a collection of formal power series whose coefficients are linear operators on the vector space. (This collection of data is subject to a number of axioms.) A first objective was to understand the basic definition and properties of vertex algebras, as well as some concrete examples of such objects arising from the representation theory of infinite-dimensional Lie algebras. This having been accomplished, in the next phase of the project the aim is to find out how vertex algebras can be understood in the context of geometry. In particular, a vertex algebra can be understood as associating certain geometric information to a small disc in the complex plane. The appropriate context in which to develop this viewpoint is the language of D-modules, which are a way to study systems of partial differential equations using algebra. To date, the emphasis of this phase of the project has been on developing the necessary technical facility with D-module theory to apply it to vertex algebras.

## Improving convergence of metropolis

algorithms on graphs  
Supervised by Joseph Blitzstein  
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The goal of Markov chain Monte Carlo (MCMC) algorithms is to simulate a probability distribution on a space when computing constraints or the complexity of the state space prevent direct sampling. MCMC explores the space via a Markov chain which is designed to converge to the target distribution in total variation distance. We make the additional assumption that the state space has a graph structure which the algorithm is required to obey. In this context, a particular case where MCMC converges very slowly is graphs with structural or distributional "bottlenecks," areas which the chain has difficulty traversing. One measure of bottlenecking is given by the Poincaré coefficient; using this coefficient to add a small number of edges to bypass bottlenecks was found to be highly effective. A less computationally intensive method is to add shortcuts while traversing the graph, which violates the Markov property but has been proven to converge under mild conditions. The current goal is to adapt limiting results on optimal scaling in the continuous case to the discrete case where computation time increases linearly in graph distance.

# MICROBIOLOGY

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## Explorations in a novel pseudotype for Ebola virus

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Ebola virus (EBOV), a member of the *Filoviridae* family, causes severe hemorrhagic fever with 50-90% human mortality and is classified as biosafety level 4. As such, the majority of EBOV research utilizes a pseudotype virus consisting of a non-pathogenic core, in this case vesicular stomatitis virus (VSV), with a reporter gene and the replacement of the envelope protein (EBOV glycoprotein (EBOV GP)). However, this pseudotype virus has significant background, especially when working with certain cells such as murine embryonic fibroblasts. We believe this background is due to residual VSV G protein, which mediates viral entry into cells, from the fusion of the seed virus to create the pseudotype. By first pseudotyping VSV with avian leukosis virus (ALV) envelope and then using that virus as the seed for EBOV GP pseudotyping, we hope to severely reduce the amount of background. Initial results show that VSV can be successfully pseudotyped with ALV. Ongoing studies include producing the EBOV GP-pseudotyped virus from the ALV-pseudotype seed virus, as well as optimization studies of ALV plasmid purification yield using various bacteria and competency methods and maximizing ALV-pseudotyped virus titer. This research will provide a novel method for an improved EBOV pseudotype virus to aid in EBOV research.

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## Circadian rhythm and the self assembly of the cyanobacterial carboxysome

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Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), the central enzyme of photosynthetic carbon fixation, is notoriously inefficient. Photosynthetic bacteria solve this problem by localizing the enzyme with its substrate. Carboxysomes, known as "bacterial organelles", are compartments that concentrate the enzyme rubisco and its substrate CO<sub>2</sub>, such that carbon fixation can occur efficiently in the photosynthetic bacteria *Synechococcus*. The goal of this research is to develop an understanding of the mechanism of carboxysome self assembly and its role in carbon fixation through *in vivo* observation using single-cell microscopy and fluorescent reporters. To achieve this, the carboxysome shell proteins were labeled with yellow fluorescent protein (YFP). We are currently working on visualizing their assembly process during cell division and circadian rhythm using microscopy. Using YFP-labeled carboxysomes, the interplay between carboxysome number, carbon fixation flux, and

growth rate will be determined. In addition, rubisco will be labeled using cyan fluorescent protein (CFP) and its interaction with the carboxysome shell proteins will be observed *in vivo*. Understanding these processes will have fundamental impact in understanding bacterial physiology and may ultimately lead to the engineering of enhanced photosynthetic organisms.

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## Downregulation of microRNA processing components by HSV-1

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Mature microRNAs (miRNA) are ~22 nucleotide long single-stranded RNA molecules that can induce degradation of mRNA or prevent its translation, thus having a major role in regulating gene expression. Within the Herpes Simplex 1 Virus (HSV-1), miRNAs have been shown to maintain latency by preventing the expression of viral proteins active during productive infection. Analysis of these miRNAs during productive infection has revealed a buildup of viral pre-miRNAs, which are incompletely processed miRNA intermediates. To determine whether this accumulation is caused by a downregulation of processing components, samples from infected cells were taken and analyzed along a time course. Results suggested a downregulation of the processing proteins Dicer and Drosha by an immediate early viral gene and downregulation of Argonaute 2 and Exportin 5 by a late gene. These four proteins are essential in the splicing and transport of immature miRNAs. Therefore, the buildup of viral pre-miRNAs is caused by viral downregulation of several cellular proteins required for complete miRNA processing. This downregulation will be confirmed using a second cell line and then analyzed to determine whether it gives the virus a particular growth advantage, perhaps by preventing the miRNAs from repressing productive genes.

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### **The proteolysis paradigm: Single stringent starvation protein B (SspB) and the search for drug targets in *M. tuberculosis***

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*Mycobacterium tuberculosis* (TB) claims the lives of 2 million people each year, yet currently available treatments are cumbersome to administer and of decreasing efficacy. Current techniques in the study of mycobacteria fail to meet the challenge of studying essential genes which could serve as potential drug targets. A more effective technique would allow the temporary depletion of a gene product through proteolysis to see whether it is essential. The protein degradation pathway in mycobacteria, particularly the ClpXP pathway, is a promising tool to identify these drug targets. In *E. coli*, proteins tagged for degradation are escorted by a chaperone molecule called Stringent starvation protein B (SspB) to the ClpXP proteolysis enzyme. The equivalent in mycobacteria, however, is not currently known. The goal of my research is to find the SspB equivalent in *M. smegmatis*, a non-pathogenic relative of TB. We used glutathione S-transferase (GST) along with an engineered tag that would be recognized by the *M. smegmatis* SspB-equivalent to pull down the target protein from *M. Smegmatis* lysate. Further study of this protein will add to our knowledge of the proteolysis system in mycobacteria and eventually help to engineer an inducible protein

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### **Investigating periplasmic transit of lipopolysaccharide during bacterial outer membrane biogenesis**

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The cell envelope of gram-negative bacteria consists of an inner (IM) and an outer membrane (OM) separated by an aqueous space, the periplasm, containing the peptidoglycan cell wall. The OM is asymmetric, with phospholipids in its inner leaflet and lipopolysaccharide (LPS) in its outer leaflet. LPS plays a crucial role in the OM's barrier function and is essential for survival in most gram-negative bacteria, but until recently little was known about its transport to the OM. Our lab studies the LPS transport pathway, providing insight into the unique process of OM biogenesis and uncovering potential targets for novel antibiotics, which are urgently needed to address the burgeoning global health threat posed by antibiotic-resistant bacterial infections. To better understand periplasmic transit of LPS, we cloned, overexpressed, and purified the periplasmic portion of LptC, an essential IM protein that has been implicated in LPS transport. Currently, this purified fragment is being used to develop LptC-spe-

cific antibodies that will allow us to identify the presence of LptC in protein complexes *in vitro*. We also tested the ability of this LptC fragment to bind LPS. Results to date suggest that LptC alone does not bind LPS; it may be that LptC-LPS interactions require the presence of other proteins in the LPS transport pathway. Future directions include crystallographic analysis of the periplasmic portion of LptC and further studies of its interactions with other proteins and LPS.

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### **Amapari and Tacaribe arenaviruses efficiently enter human cells expressing mutant transferrin receptor**

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The New World (NW) arenaviruses are RNA viruses that can cause severe disease in humans. They spread to humans from their natural reservoirs, which are typically small rodents in South America. Four NW arenaviruses are pathogenic to humans. The Amapari (AMAV) and Tacaribe (TCRV) viruses are closely related to the four pathogenic NW arenaviruses, but AMAV and TCRV do not cause disease in humans. The pathogenic NW arenaviruses use human transferrin receptor 1 (hTfR1), which correlates with their ability to cause hemorrhagic fevers. Recent studies have shown that although AMAV and TCRV cannot use hTfR1 to infect cells, they can use animal forms of TfR1. Here, we used mutational studies to show that variants of hTfR1 can support the infection of viruses bearing the surface proteins of AMAV and TACV (called 'pseudoviruses'). Exchanging only three amino acids in hTfR1 for amino acids found in the same position in animal TfR1 converted hTfR1 into an efficient receptor for TCRV pseudoviruses. Altering eight residues in this region of hTfR1 converted it into an efficient receptor for both AMAV and TCRV pseudoviruses. Our studies shed light on arenavirus evolution and the potential for two nonpathogenic arenaviruses to emerge as human pathogens.

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### **Physical model of the bacterial chromosome and its relation to cell growth in *E. coli***

The Jun Laboratory

FAS Center for Systems Biology

Chromosome segregation influences and is influenced by other growth processes, a part of an incredible integrated network where each process loses its individual reductionist identity to the concept of a unified whole. Cell growth rate provides the ultimate reflection of this coordination of events. For one *E. coli* to become two, replicated strands of the long circular chromosome move towards opposite cell halves before the cell divides. To explain this phenom-



enon, our lab postulates a physical mechanism. In this model, mixed daughter strands explore available entropic conformations and consequently segregate. To test this model experimentally, we employ techniques along an interface of disciplines from single molecule biophysics to microfluidics, optics to computer simulation. We exploit osmosis to lyse the cells and release their chromosomes for experiment. We visited the nanofabrication facility at Cornell to design and fabricate microfluidic chips with features as small as one micron. To confine and compress bacterial DNA *in vitro*, we will use these chips together with hydrodynamic flow or with optical tweezers. We will use other microfluidic chips to measure the growth rate of *E. coli* confined to a channel, and we will continue to design image processing software for high throughput analysis of single cells amidst a growing population. These dual research pursuits will explore the extent to which physical principles underlie and reveal the foundations of prokaryotic life.

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### Significance of EBNA3A association with CtBP for LCL outgrowth

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Viruses, which are dependent on cellular machinery in order to replicate, are only as successful as their ability to co-opt cellular processes for their own benefit. A virus must enter a cell and then hijack its host to achieve its goals. In the case of the Epstein-Barr virus (EBV), infection leads to immortalization of B cells and the outgrowth of lymphoblastoid cell lines (LCL), all of which is accomplished with only six critical viral proteins. One of these six proteins is EBV nuclear antigen 3A (EBNA3A) which is known to repress transactivation of another critical EBV protein, EBNA2, at the Cp promoter. Growth complementation assays studying EBNA3A have identified five domains of interest, one of which encompasses the binding site for the endogenous human co-repressor C-terminal Binding Protein (CtBP). Deletion of this domain results in a reduction of LCL proliferation rate compared to cells expressing wild-type EBNA3A. LCL complemented with a point mutant unable to bind CtBP also grow more slowly than wild type. By comparing differences in gene expression between these two cell lines it should be possible to elucidate the promoters targeted by EBNA3A in complex with CtBP and thus better understand the mechanisms by which EBV immortalizes B cells.

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### Phenotyping unculturability in soil bacteria

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Soil microbes are some of the most important and useful organisms on the planet, affecting humans as pathogens, sources of drugs, and even as symbiotes in our digestive systems. However, it is estimated that less than 1% of microorganisms found in nature can be grown in the laboratory using traditional techniques, let alone identified and studied rigorously. We hope to shed light on this "unculturability" of soil bacteria by studying the phenotypes (i.e. physical appearance and behavior) of presumably unculturable microbes isolated from nature. Bacteria were extracted from soil and grown in a specialized culture apparatus we designed to allow real-time microscopy of microcolonies, or groups of cells that are not visible to the naked eye. Using cell staining, we observed that many microcolonies died or stopped growing under the microscope, suggesting a partial explanation of why macroscopically visible colony counts do not account for most of the microbes isolated from soil. To test whether microcolonies could represent a subset of microbes that are not observed on traditional growth assays, we will perform further experiments such as computing the ratio of microcolony-forming cells to macrocolony-forming cells in different soil samples. The techniques we have developed represent a novel approach to studying microbial phenotypes, and we intend to apply them to investigate other microbial phenomena such as antibiotic resistance, or biochemical interactions in multi-species ecosystems.

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### Molecular evolution of host phage specificity in continuous culture

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The rise of antibiotic-resistant bacteria has cloaked the future treatment of infectious diseases in ambiguity. Consequently, current research probes the use of bacteriophages, viruses that infect bacteria, to combat infections caused by bacterial pathogens. Susceptible to numerous evolutionary forces, the host specificity of bacteriophages rests on an ever-shifting balance. This project aims to elucidate these forces to develop strategies against antibiotic resistance and future epidemics resulting from host specificity mutability. Filamentous bacteriophages infect several bacterial pathogens: *Escherichia coli*, *Vibrio cholerae*, certain *Pseudomonas* species and *Salmonella*. Infection of host cell relies on the phage's minor coat protein, pIII, interacting with the bacterial pili; N-terminal domain identity of pIII determines the phage's host specificity. Experimental evolution of phages to infect bacterial pathogens takes two routes. The first is continuous evolution of phages in a turbidostat-cellstat culture. This required the genetic engineering of two phagemids, one with *E. coli* pIII protein (pIII), the other with the N-terminal domain replaced by that of the pIICTX protein in *Vibrio cholerae* CTXphi bacterio-

phage (N-CTX). Infection efficiency of *E. coli* cells carrying helper plasmid with either pIII or N-CTX was tested on their respective and alternate hosts. Results suggest the convenience of using both constructs in the turbidostat-cellstat system. The second route focuses on the continuous evolution of *E. coli* phage M13KE to infect *Salmonella typhimurium*. Results show decreased M13KE infectivity on original host; events of infection in *Salmonella* are under study to detect mutations in the phage genome. Both routes will culminate to an understanding of host specificity changes in phages under continuous evolution.

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### Microaerobic conditions and virulence expression in *Vibrio cholerae*

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The bacterium *Vibrio cholerae* is the causative agent of cholera, a disease characterized by severe watery diarrhea. *Vibrio* produces two major virulence factors: toxin-coregulated pilus (TCP), which allows *Vibrio* to colonize the small intestine, and cholera toxin (CT), which binds to cell receptors and leads to the secretion of water and electrolytes. Though *in vitro* virulence inducing conditions have been determined empirically, it is unclear what and how environmental signals trigger the expression of virulence factors *in vivo*. The Hung lab has recently discovered that virulence expression can be induced under low oxygen conditions, which prompts the questions: is there a microaerobic signaling pathway which turns on virulence expression, and is this how virulence expression is regulated *in vivo*? Dr. Deb Hung had previously identified 15 small molecules which inhibit virulence expression in *Vibrio*. One molecule, hypoxistatin, inhibits expression only under these microaerobic conditions. We are using several approaches to identify hypoxistatin's target(s). First, we have fused a zeocin resistance gene to the CT promoter, and will use a zeocin-based selection to identify hypoxistatin-resistant mutants. (Zeocin is an antibiotic.) Second, we will use affinity-based methods, such as protein arrays and affinity chromatography, to identify hypoxistatin's target. Though these projects are in their early stages, the ultimate goal of understanding how certain signals trigger the production of *Vibrio*'s virulence factors is to find ways of disrupting this process—thus preventing *Vibrio cholerae* from causing disease.

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### Regulation of the cell wall hydrolase, RipA, in *Mycobacterium tuberculosis*

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Tuberculosis, the world's deadliest bacterial disease, kills 2 million people annually, and is caused by the bacterium *Mycobacterium tuberculosis*. To combat widespread drug resistance, new antibiotics are desperately needed. One potential target is RipA, an essential cell wall hydrolase. RipA degrades a cell wall component called peptidoglycan, and is required for division and growth of *M. tuberculosis*. Recent work has demonstrated that RipA binds to two cell wall remodeling enzymes, the lysozyme RpfB and peptidoglycan synthase Pbp1. While the RipA-RpfB complex synergistically degrades the cell wall, this synergy is blocked by the presence of Pbp1. We hypothesize that the RpfB-RipA and Pbp1-RipA interactions are mutually exclusive, and that such a relationship may represent a model for post-translational regulation of RipA activity during growth. To test this hypothesis, we employed *in vitro* biochemical and *in vivo* genetic approaches. In the biochemical approach, we utilized purified RipA, RpfB, and Pbp1. By titrating in increasing concentrations of Pbp1 and RpfB, we assessed by Western blot whether we can compete apart RipA-RpfB and RipA-Pbp1 interactions, respectively. In the genetic approach, we used a modified yeast-3-hybrid system, which detects interactions by the activation of reporter genes. We created yeast strains expressing RipA and Pbp1 or RipA and RpfB. We then induced expression of additional Pbp1 and RpfB to assess whether Pbp1 can compete apart the RipA-RpfB interaction or vice versa. A further understanding of RipA activity will not only open avenues for tuberculosis drug development, but may also serve as a model for general mycobacterial cell wall regulation, a poorly understood process.

# MOLECULAR AND CELLULAR BIOLOGY

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## **HIV preventive therapy: engineering recombinase enzymes to recreate the CCR5 $\Delta$ 32 mutation conferring resistance to HIV infection**

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We are working to create recombinase enzymes capable of excising the end of gene CCR5, thus mimicking the naturally occurring CCR5 $\Delta$ 32 deletion mutant conferring resistance to HIV infection. This mutant yields a premature stop codon in the CCR5 coding region, producing a truncated receptor that no longer facilitates entry of HIV into cells. We intend to engineer recombinase enzymes which will specifically target CCR5 to generate an analogous mutation for people having the normal-length gene product. Based on known zinc finger binding affinities, we have identified promising target sites on the end of CCR5 and engineered the sites in four separate zinc finger recombinase enzymes. Although the four recombinases will work as a tetramer to excise part of CCR5, we tested each individually for activity on their respective sites and found them to be varied. We will thus evolve some for further activity and subsequently test all four together, in bacteria, on the relevant segment of CCR5. Should little heterotetramer activity be found, further directed evolution will be necessary. Finally, after concluding that all four recombinases are active, we will test the recombinases in mammalian cells—their ultimate objective for mimicking the CCR5 $\Delta$ 32 deletion mutant and preventing HIV infection.

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## **Chromosomal recombination during meiosis in yeast**

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In times of environmental stress, *Saccharomyces cerevisiae* undergo a process called meiosis, in which the yeast divide and cut their chromosome number in half to produce haploid spores from diploid cells. A necessary step in meiosis is the linking of homologous chromosomes; this often results in the exchange of genetic material through a physical swap of chromosome arms, called crossing over. Intermediates in this process are well known: double strand breaks, single end invasions, double Holliday junctions, crossovers, and non-crossovers. The aim of current research is to understand the roles of certain proteins in this process; this is achieved by forcing yeast strains in which a protein of interest is deleted to undergo meiosis. Samples are taken during early meiosis, when crossing over is known to occur; DNA is purified from each sample, and crosslinked to preserve multi-chromosomal structures. After enzyme digestion, the DNA is run on a conventional, one-dimensional gel to separate and quantify double strand breaks, crossovers, and non-crossovers.

Two-dimensional gels are used to quantify the various intermediates; in one dimension DNA is separated by size, while in the other dimension it is separated by size and structure. This allows for the migration of each intermediate to a different location on the gel. Preliminary results for tel1-deletion yeast show the slightly delayed formation of more double strand breaks and the overall resolution of fewer crossovers, when compared to wildtype.

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## **A novel technique for regenerating rod photoreceptors: use of an electrospun poly( $\epsilon$ -caprolactone) scaffold to deliver predifferentiated retinal progenitor cells to the subretinal space**

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Given the mammalian retina's lack of spontaneous regeneration and the prevalence of retinal degenerative diseases such as Retinitis Pigmentosa and Age-Related Macular Degeneration, a goal of recent research has been the transplantation of retinal stem/progenitor cells into the diseased retina. Previous studies have established the capacity of transplanted retinal progenitor cells to differentiate into the various mature retinal cell types and possibly restore visual function, but the light-receptive sensory neurons that initially die in most retinal degenerations – the rod photoreceptors – have proven particularly difficult to regenerate. This study investigates a novel technique for more efficiently regenerating rod photoreceptors from mouse retinal progenitor cells (RPCs). RPCs are first proliferated on a porous microscale electrospun poly( $\epsilon$ -caprolactone) (PCL) polymer scaffold, which is expected to promote RPC growth and differentiation and facilitate eventual transplantation. RPC-polymer composites are subsequently exposed to differentiation media containing taurine and retinoic acid, molecules that stimulate rod differentiation. Finally, the efficacy of rod photoreceptor regeneration is determined via *in vitro* explants and *in vivo* transplants. Preliminary results confirm the ability of electrospun PCL to facilitate RPC adhesion and proliferation. The responses of RPCs to different concentrations of taurine and retinoic acid are currently being tested, with initial results suggesting that the most effective concentrations are significantly higher than those employed in previous studies. Future steps will involve optimizing differentiation media conditions and evaluating explant/transplant results.



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### GIPC1 silencing in colorectal cancer cells

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The development of several cancers is vitally dependent upon tumor cell proliferation and angiogenesis. Inhibition of these processes could lead to the development of anti-tumor therapies. We have previously shown that silencing of the gene GIPC1 adversely affects multiple angiogenic and proliferative signaling networks in zebrafish, mice, and human MDA-MB231 breast cancer cells. The goal of this project is to examine the effects of GIPC1 silencing in a different type of cancer, using SW620 colorectal cancer cells. Through Western Blot analysis of protein production in normal and GIPC1 knock-down colon cancer cells, we have shown that silencing of GIPC1 in the colorectal cancer cells results in the upregulation of the anti-proliferative protein p27. This upregulation was also seen in the breast cancer line, which indicates that GIPC1 may interact with a complex to degrade p27 in normal cancer cells. If this is the case, GIPC1 silencing may provide an effective way to inhibit the proliferation of cancer cells. We intend to further elucidate the pathways through which p27 is upregulated in these GIPC1 knock-down cells and extend our work to include the development of anti-angiogenic strategies in other cancer models.

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### Identifying correlations between shmoo direction and agglutinin localization in *Saccharomyces cerevisiae* using fluorescence microscopy

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Actin is responsible for cellular functions as varied as motility, division, contraction, and maintaining homeostasis in eukaryotic cells. We study actin in the context of cell polarization. Yeast cells, of which there are two "sexes" ( $\alpha$  and  $\alpha$ ), undergo a series of genetic and physiological changes when exposed to a mating factor from the opposite sex. These changes, including the formation of shmoos (projections towards the source of mating factor) and agglutinins (surface proteins that facilitate cell-cell adhesion), are mediated by the formation of actin tracks for cell polarization. Our key question is whether agglutinins will "shift" in response to changes in the actin network induced by shmooing. We use a recombinant form of  $\alpha$ -type yeast in which the agglutinins are under the control of a different inducer (IPTG, a lactose metabolite) than  $\alpha$  factor; further, they have a surface modification in which antibodies that bind to fluorogens are displayed on the agglutinins. The fluorogens fluoresce when bound to the antibody, thus providing a visual cue of the localization of agglutinins. We can control the time points where the two inducers (mating pheromone and IPTG) are introduced to the cells and then image them to correlate shmoo direction and agglutinin localization. Results are pending, but future directions could include tagging actin with GFP to watch the growth and change in yeast cell actin networks when different inducers are introduced.

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### A chemical suppressor screen of melanoma progenitors in the zebrafish

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Neural crest development and melanocyte biology influence human diseases like melanoma. In zebrafish, mutations in the BRAF oncogene lead to abnormal striping patterns, and when crossed with the knockdown of the p53 tumor suppressor gene, cause these double mutant adults to develop highly aggressive melanoma cancer within the course of a year. Previous studies in the lab using *in situ* hybridization reveal a strong over-expression of crestin in BRAF;p53 zebrafish embryos. Crestin is a pan-neural crest marker that is a relevant marker used to investigate this type of melanocyte biology. Because this gene is also overexpressed in adult tumors, this leads to the notion that crestin progenitors appear during embryogenesis in BRAF;p53 zebrafish embryos and eventually differentiate or develop into entities that contribute to pigmentation abnormalities and tumor formation in adults. This project will evaluate the hypothesis that abnormal crestin gene expression during embryogenesis in BRAF;p53 zebrafish are related to tumorigenesis and pigmentation defects in adult zebrafish. This work will lead into future studies to determine whether the chemical hits also correct pigmentation defects and melanoma development in adult BRAF;p53 zebrafish utilizing a chemoprevention assay. This research project has important therapeutic implications because it investigates the relationship between embryogenesis and tumorigenesis. It yields particular significance with respect to cancer biology and will help investigate the mechanistic events that transform neural crest progenitors through the BRAF oncogene. The small molecules that result from the screen may also have therapeutic potential to affect human melanoma development.

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### Systematic screening of the kinome-wide activated kinase libraries in neural stem cell proliferation

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Protein kinases are useful targets for combating cancers due to their roles in basic cell functions that are altered in cancerous cells. Widely used kinase inhibitors such as Imatinib (Gleevec®) and Trastuzumab (Herceptin®) already capitalize on abnormal amplification of kinases in leukemia and breast cancer, respectively. This study aims to identify potential druggable kinases in brain cancer. Kinases that are both necessary and sufficient to dysregulate the growth of normal neural progenitor cells will be identified using the human Kinome-wide activated kinase library, which contains constitutively activated mutants of all ~600 kinases in the human genome. Pools of kinases will be transfected into Ink-/- background wildtype neurospheres that recapitulate neural progenitor cells *in vitro*. Those that confer increased capacity to proliferate will be expanded to identify the particular kinase(s) involved. Thus far, culture condition that con-



tains only human bFGF, and no EGF (standard neurosphere culture supplement to induce growth), seemed to result in the greatest difference in growth between the negative and positive control cell lines. Kinases that are identified in the bFGF-only condition will be tested in mice xenograft experiments to assay for their capacity for glioma transformation, in addition to their ability to induce proliferation.

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### Functional characterization of mammalian homologues of the yeast longevity factor Sir2

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Sirtuins are mammalian homologues of the yeast Sir2 protein that regulate lifespan in a large variety of organisms. The mechanisms through which they function are not well understood. There are seven mammalian sirtuins, named SIRT1 through SIRT7. Of these proteins, we are studying SIRT3 and SIRT6. SIRT3 is a mitochondrial protein and mitochondria purified from SIRT3-deficient mice contain large amounts of heavily acetylated proteins, suggesting that SIRT3 plays a role as a deacetylase. However, the effects of mitochondrial acetylation are unclear because SIRT3 mice are apparently phenotypically normal. We attempt to determine the effects of mitochondrial acetylation by using SIRT3 to deacetylate key mitochondrial proteins, including CPS1, a urea cycle protein that may be involved in mediating some of the beneficial effects of calorie restriction, including increased lifespan and lower cancer incidence. Unlike SIRT3 deficiency, however, SIRT6 deficiency results in a severe phenotype of progeria, or premature aging. Mice lacking SIRT6 are physically smaller and develop slower than wild-type mice. In addition, all of them die before reaching one month of age. SIRT6-deficient cells possess high levels of reactive oxygen species that may contribute to DNA instability and the aging phenotype. This suggests that SIRT6 may be important in antioxidant defense. We test whether or not SIRT6 is truly involved in antioxidant defense. Preliminary survival assays reveal that SIRT6 deficient cells exhibit increased sensitivity to oxidizing agents such as methyl methanesulfate and hydrogen peroxide. The next step is to determine whether or not antioxidants such as N-acetylcysteine can rescue such sensitivity.

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### Lipid chaperones and their effect on lipid and systemic metabolism

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In recent decades, global obesity levels have grown at an alarming rate reaching epidemic proportions. Obesity is strongly correlated with the cluster of diseases and risk factors known as metabolic syndrome, including type 2 diabetes and atherosclerosis. It has also been linked with a chronic state of low-level inflammation. Adipose tissue is a central organizing site of key cytokines involved in both metabolic and inflammatory responses. The lipid chaperones, a group of adipocyte and macrophage fatty acid binding proteins (or FABPs), play a significant regulatory role in metabolic processes via their ability to direct lipid activity and to modulate various inflammatory pathways. Mice deficient in the two FABPs, aP2 and mal1 that are genetically protected from nearly all aspects of metabolic syndrome. Through a systemic lipid analysis and molecular approaches, Hotamisligil's lab has identified a unique lipid profile in FABP-deficient mice and established that this lipid milieu might be responsible for the metabolic output of these mice. We investigated the *in vivo* effects of the unique lipid profiles especially on insulin signal transduction. Using lipid infusion into conscious mice in combination with phospho-specific immunoblotting, we confirmed that modulating serum lipid composition can efficiently regulate metabolic responses in liver and muscle tissues. We also found that generating lipid profiles similar to FABP-deficient mice improved systemic glucose metabolism. Ultimately, these findings suggest that targeting specific serum lipids could serve as potential sites for therapeutic intervention in metabolic diseases.

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### Diabetes Mellitus Type II: Understanding the pathogenesis of low birth weight associated diabetes through its effects on brown adipose tissue

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Previous studies have shown that low birth weight (LBW) infants are at significantly higher risk for obesity and type II diabetes. Through 50% maternal caloric restriction in the final third week of gestation, our lab has developed a LBW-associated diabetes mouse model, which shows a 24% weight reduction, followed by postnatal rapid increase in amounts of adipose tissue and higher risk for obesity and diabetes. Brown adipose tissue (BAT) is found in substantial amounts in infants, contributing to energy expenditure by dissipating energy as heat (thermogenesis). In our LBW model we have detected a decrease in expression of genes that promote thermogenesis, indicating that functional differences in BAT may contribute to the development of obesity and diabetes. Current experiments focus on identifying a phenotype reflecting decreased thermogenesis gene expression. Mice from LBW model are cold exposed (5°C) for a period of four hours and body temperatures are measured at differ-

ent time intervals in order to access their thermogenesis. In addition, mice from the same cohort are injected with CL 316,243, a drug that stimulates the release of fatty acids into blood and induces thermogenesis in BAT, which oxidizes free fatty acids. Temperatures are measured at different time points within a four hour period after injection to assess thermogenesis and blood is collected to assess blood free fatty acid levels at the selected time points. These experiments are currently still underway and may highly contribute to understanding the pathogenesis of LBW-associated diabetes through its effects on BAT.

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### **Molecular regulation and modulation of pancreatic cellular identity and function**

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In Type 1 Diabetes, a patient's immune system destroys his or her insulin-producing beta cells, forcing the patient to become completely dependent on exogenously administered insulin. However, this therapeutic strategy fails to provide the exquisite regulation of blood glucose levels afforded by unimpaired beta cells. To address this issue, we seek to discover small molecules, potential precursors to human therapeutics, that promote the regeneration of beta cell function and amplification of beta cell number. Because chromatin structure is critically implicated in establishing gene expression programs, we believe the families of chromatin modifying enzymes represent viable therapeutic targets for manipulating pancreatic cellular identity and function. In conjunction with these small molecule studies, we are characterizing the genome-wide contributions of chromatin structure in establishing glucose-responsiveness and cellular identity in human pancreatic endocrine tissue. Such studies will allow us to explore the potential of chromatin biology and epigenetics as means of drug discovery in human metabolic disorders.

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### **GIPC1 as a potential therapeutic target for breast cancer treatment**

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Cancers are among the leading causes of morbidity and mortality in industrialized societies. GIPC1 is a scaffold protein that plays an integral role in the TGF $\beta$  signaling, and as such is an important player in the regulation of cell proliferation, differentiation, apoptosis, cell migration, and blood vessel formation (angiogenesis). As a result, the perturbation of cell proliferation and angiogenesis essential for tumor growth and metastasis via GIPC1 hold great potential as a therapeutic target for cancer treatment. To evaluate the role of GIPC1 in signal transduction we systematically knocked down (KD) the expression of GIPC1 in both MDA-MB231 breast cancer

cells and healthy Human Mammary Epithelial Cells (HMEC) using a lentiviral vector to insert a DNA fragment coding for a shRNA molecule, which binds to GIPC1 mRNA and targets it for degradation. The cells were then stimulated with TGF $\beta$  for varying time points, and their protein lysates were analyzed by Western-blot analysis. Our results indicated a significant over-expression of the cyclin dependent kinase inhibitor p27 responsible for G1 cell cycle arrest in the MDA-MB231 GIPC1 KD cells (as compared to their control counterparts), which was not observed in the HMEC GIPC1 KD cell line. These results suggest the existence of a GIPC1-dependent cancer-specific mechanism for the aberrations in cell cycle progression characteristic of most cancers. Consequently, inhibition of GIPC1 holds great promise in furthering the advancement of breast cancer treatment and future therapies. However, further investigation into the physiological effects of GIPC1 KD in healthy HMEC cells is necessary before definitive conclusions can be reached.

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### **The effect of mutant K-ras oncogene on Hypoxia-Inducible Factor 1 $\alpha$**

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When a cancerous tumor develops, it outstrips its blood supply and induces the formation of new blood vessels through the activation of various transcription factors, such as Hypoxia-Inducible Factor (HIF) 1 $\alpha$ . HIF-1 $\alpha$  can upregulate genes responsible for cell survival, such as Vascular Endothelial Growth Factor (VEGF) that promotes angiogenesis. Many pathways have been shown to induce HIF-1 $\alpha$ . But the effect of the mutant, K-ras oncogene on HIF-1 $\alpha$  has not been closely examined. K-ras is a membrane protein that recruits growth factors to relay a signal to stimulate the cell. However, a mutant K-ras is constitutively turned on and constantly induces cell growth. The purpose of this study is to determine the effect of mutant K-ras on HIF-1 $\alpha$ . The established cell lines used in this study included two controls (mutant K-ras) and two knockouts (wildtype K-ras). All cell lines were subjected to hypoxic (1% O<sub>2</sub>) or normoxic (ambient air) gas treatments for 6 hours. The cells were then harvested for western blotting and VEGF luciferase assays. Western blots showed that HIF-1 $\alpha$  protein expression in controls was much higher than that in knockouts under hypoxia, suggesting that mutant K-ras induces HIF-1 $\alpha$ . The VEGF luciferase assay showed that under hypoxic conditions, VEGF transcriptional levels increased about 3-fold in controls while only about 1.5-fold in knockouts. These findings are consistent since knockouts have lower HIF-1 $\alpha$  levels and are therefore expected to have lower VEGF transcriptional activity. In order to further investigate the role of K-ras, other transcriptional factors such as HIF-2 $\alpha$  and c-Myc must be examined to determine their relationships with HIF-1 $\alpha$ . Moreover, the finding that mutant K-ras upregulates HIF-1 $\alpha$  necessitates the study of the specific pathway of this mechanism.

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### Structural and biochemical analysis of the *Bacillus cereus* Mini-Chromosome Maintenance protein complex

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Mini-Chromosome Maintenance protein complexes are essential to DNA replication in eukaryotes and archaea and are believed to act as the replicative DNA helicase. However, the exact mechanism by which MCM proteins unwind DNA is unknown, and a number of mysteries and paradoxes still remain unsolved. Recently, during a database search for homologs of the MCM complex, a gene was identified encoding an MCM-related protein within a prophage integrated in the *Bacillus cereus* bacterium genome. This *Bacillus cereus* MCM (*BcMCM*) gene provides a novel system to study the MCM family, and we hope to solve the structure of the amino-terminal region of the protein through X-ray crystallography as well as study its biochemical properties. Limited proteolysis was used to determine several candidate amino-terminal *BcMCM* truncations for crystallization. These truncations were subcloned from the full-length gene and expressed in *E. coli* cells. Induction trials of these amino-terminal fragments revealed that most of the candidates were both expressible and soluble. These proteins were subsequently purified by affinity and ion exchange chromatography. Crystallization trials will be set up for the purified proteins, and if successful, we will then seek to determine their structure by means of X-ray diffraction analysis. A number of assays such as a DNA-binding assay and an ATPase assay will also be used to help us determine the biochemical function of the region. This work is expected to help us move forward in fully understanding the protein assemblies that catalyze the replication of DNA, a central process to all living organisms.

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### Effects of the Renin –Angiotensin-Aldosterone System on cardiac progenitor cells

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After suffering a myocardial infarction (heart attack) the heart undergoes a series of pathophysiological changes due to neurohormonal responses. In this research project, we are focusing on one main response system: the Renin –Angiotensin-Aldosterone System (RAAS). Under the RAAS, the heart undergoes a remodeling process to compensate for the loss of cell mass due to the myocardial infarction. Although this remodeling process is an initial attempt to repair itself, it ultimately leads to heart failure. By studying the effects of Angiotensin II (ATII), our aim is to discover and improve upon the mechanisms that will help boost the heart's potential for repair. For this study, endogenous cardiac progenitor cells (cardiac side population (CSP) cells) isolated from mouse hearts were incubated with ATII in a dose-dependent manner. The viability of these cells was assessed by measuring the ATP content, the total protein and total DNA, and by assays for apoptosis and differentiation.

### Modulation of adaptive immune responses to prototype HIV vaccines by Toll-like receptor-ligand adjuvants in mice

The Barouch Laboratory  
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A successful Human Immunodeficiency Virus (HIV) vaccine will need to induce a significant and robust cytotoxic T lymphocyte (CTL) response. To this end, three HIV vaccine strategies are being pursued: recombinant adenoviral vectors and plasmid DNA that express HIV gene(s), and purified HIV protein(s) themselves. The immunogenicity of all three of these vaccines has the potential to be modulated by Toll-like receptor (TLR) ligand adjuvants, which can define the early cytokine milieu and induce maturation of antigen-presenting cells. In this study, Simian Immunodeficiency Virus Gag-expressing plasmid DNA and adenoviral vaccines adjuvanted with a variety of TLR ligands were injected into wildtype mice in order to screen for those TLR ligands that modulate the adaptive immune response. Epitope-specific tetramer staining was used to measure the kinetics and magnitude of the CTL responses to the vaccine formulations; interferon- $\gamma$  ELISPOT and intracellular cytokine staining were used to measure their functionality. The cytokine milieu was analyzed with ELISA on serum drawn at early timepoints following injection. Preliminary results suggest that a number of different TLR ligand adjuvants can alter response kinetics. Most interestingly, stimulation of TLR3 appears to suppress the CTL response to both plasmid DNA and adenoviral vaccines. Further experiments are needed to confirm this modulation and elucidate its mechanism of action. A greater understanding of the interaction between TLR signaling and vaccine immunogenicity should prove useful in the design of future TLR ligand-adjuvanted vaccination protocols.

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### The Sonic Hedgehog Relay: Clarifying Cdo and Boc receptor function in the sonic hedgehog signaling pathway

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The Sonic Hedgehog (Shh) protein plays a significant role in directing the formation of tissues and organs in the development of the embryo. By establishing a concentration gradient across tissues, Shh influences the fate of progenitor cells in developmental processes such as digit specification and neural cell differentiation. Therefore, investigating the cellular response to Shh levels reveals how Shh regulates such developmental processes and how they go awry in birth defects and cancer. To this end, it is important to explore the interactions between cell surface receptor proteins and the Shh protein. In this project, we focus on two of the various Shh receptors – Cdo and Boc. Efforts are needed to clarify their mechanism of function, their relative contribution to signaling, and their interactions with Shh. Through transfections, receptor expression is knocked down and/or overexpressed in cells, whose response is quantified by the



luminescence of a reporter gene. So far, knockdown results have confirmed the up-regulatory functions of both receptors; overexpression experiments have suggested the possibility of Cdo and Boc as having additive rather than redundant roles. In future experiments, live imagery will be used to observe changes in cell surface distributions of the receptors as they bind to Shh.

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### **Collaboration between mutation and epimutation in medulloblastoma**

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Medulloblastoma is the most common malignant pediatric brain tumor. Deregulation of imprinted gene expression [loss of imprinting (LOI)] has been shown to be the earliest and most abundantly observed alteration in various human cancers. It has been suggested that a loss of Patched (of the Sonic Hedgehog/Patched signaling pathway) function contributes to medulloblastoma formation and LOI reduces p53 expression and thus may accelerate tumorigenesis. As such, the research aim is to conduct rescue experiments where the defective pathway is restored to test for phenotype correction. LOI Ptch mutant embryonic stem (ES) cells will be generated through transient demethylation and remethylation achieved with a reactivatable DNA methyltransferase 1 (Dnmt1) knockout construct. To study the correlation between genetic and epigenetic mutations, the methylation statuses of regions of interest will be examined with bisulfite sequencing technology. In addition, changes in imprinting with the induction of transient demethylation will be revealed. Preliminary data has identified several interesting regions, including Nnat, Gnas, Sgce, Mest, Nap115, Usp29, H19, Kcnq1, Kcnq10t1, Plagl1, Grb10, Commd1, Gtl2, Peg13, Igf2r, and Impact. The conventional treatment for medulloblastoma, which may involve surgical resection, aggressive chemotherapy, and radiation therapy, has many deleterious and long-term side effects such as slowed growth or mental retardation. With increased understanding of medulloblastoma development, new diagnoses and treatments could severely alter the course of disease.

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### **Understanding the Sonic hedgehog signaling pathway's role in muscle development**

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Adult skeletal muscle regeneration is mediated by myogenic precursor cells, which are a subset of myofiber-associated mononuclear satellite cells located beneath the basal lamina of multinucleated mature muscle fibers. The Wagers lab recently demonstrated the presence of self-renewing muscle stem cells in adult skeletal muscle and showed that transplantation of these cells could be used therapeutically to enhance muscle function in a mouse model of Duch-

enne muscular dystrophy. Yet, the molecular pathways that regulate these skeletal muscle stem cells and control their function in muscle growth and repair remain unclear. Understanding these regulatory mechanisms could suggest strategies to improve their expansion potential and regenerative activity and may clarify what goes wrong in abnormal muscle development such as tumor formation. Recent research implicates a role for the conserved developmental morphogen Sonic hedgehog (Shh) in skeletal muscle development. This project aims to clarify the role of Shh signaling in adult muscle regeneration by (i) qPCR to determine which components of the Shh pathway are expressed in injured/regenerating vs. uninjured muscle; (ii) *in vitro* culture of muscle stem cells to determine what effect(s) Shh pathway agonists and antagonists have on their differentiation, proliferation, and apoptosis; (iii) fluorescence-activated cell sorting (FACS) and immunohistochemistry of transgenic reporter mice to determine which cell populations in muscle produce and receive Shh signaling; and (iv) lentiviral transfection and transplantation of muscle stem cells to determine whether these cells can trigger neoplastic or otherwise abnormal muscle development.

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### **RNA-based sensor modules for RNAi-capable molecular automata**

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Molecular automata that exhibit input-output behavior can enable programmable manipulation of biological systems. Recently, we constructed a biomolecular computing core that can evaluate Boolean logic using RNA interference (RNAi) in mammalian cells. However, the state of endogenous inputs needed to drive the computing core could not yet be detected. Here, we construct sensor modules that are composed entirely of synthetic RNAs that can 1) detect the presence of disease-specific mRNA signals *in vitro* and 2) respond to the signals by initiating RNAi through the spontaneous formation of siRNAs. Using radiolabeled cleavage assays, we demonstrate that the sensor modules can each detect mRNA signals, including the proto-oncogenes Bcl2 and PLK1, with high specificity and minimal cross-talk. Through fluorescence resonance energy transfer (FRET) analysis, we also show that the chemical kinetics of siRNA formation, which is triggered by the activation of the sensors, are robust against both the concentration and length of input molecules. RNAi efficiency was confirmed using specific arrangements of siRNA targets, including those representing "OR" and "AND" gates, in radiolabeled cleavage assays. Implementation of these RNA-based sensors will greatly facilitate autonomous decision-making in a system that could diagnose and treat diseases such as cancer on a molecular scale, and do so all within an individual cell.

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### The roles of *C. elegans* FCI-1 gene in early embryonic DNA damage response

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Fanconi anemia (FA) is a cancer susceptibility disorder in humans that is characterized by sensitivity to DNA cross-linking agents. Usually DNA damage will trigger a DNA damage checkpoint. Previous work from the Michael lab has shown that the checkpoint response is actively silenced in *Caenorhabditis elegans* embryos. In humans, the complexity of the FA genetic network makes it difficult to understand. *C. elegans* represents a simpler system to get at the mechanism of action of these genes. Recently, FANCI has been identified as a FA gene in humans. A putative homolog, fci-1, is found in *C. elegans*. However, this gene is completely uncharacterized. We hypothesized that this gene may play a role in early embryonic DNA damage response. To test this, we measured the embryonic lethality of worms that had fci-1 knocked down by RNA interference (RNAi). While RNAi embryos did not show increased sensitivity to Mitomycin C, a DNA cross-linking agent, when compared to wild type embryos, they did show increased sensitivity to other non cross-linking mutagens like hydroxyurea and methanesulfonate. This suggests that fci-1 may indeed play a larger role in DNA damage response. However, time lapse microscopy of the pronuclear migration did not reveal any delay in the S phase of the RNAi embryos, which suggests that fci-1 does not participate in actively silencing the aforementioned DNA damage checkpoint. In order to better understand fci-1 and checkpoint silencing, we are currently improving and adapting to *C. elegans* an assay that quantifies the DNA gaps created by translesion DNA polymerases in *Xenopus*. Translesion DNA polymerases skip over damaged DNA, leaving it to be fixed later, which creates gaps. Combined with RNAi, quantifying these gaps will not only help us understand fci-1 but also a multitude of other genes.

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### Temperature compensation in the three-protein cyanobacterial circadian clock

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Global gene expression in the cyanobacterium *Synechococcus elongatus* PCC 7942 is tightly regulated by a circadian clock organized around a core protein oscillator comprised of three proteins, KaiA, KaiB and KaiC. In 2005, circadian oscillation in KaiC phosphorylation was reconstituted *in vitro* with just the Kai proteins and ATP. A defining feature of circadian clocks is temperature compensation: the period of oscillation remains nearly constant over a broad range of physiologically relevant temperatures. Remarkably, the *in vitro* Kai protein oscillator is similarly temperature-compensated.

This research project aims to understand the mechanism by which the temperature compensation of the *in vitro* oscillator occurs. Two possible methods of temperature compensation are apparent. All individual phosphorylation and dephosphorylation rates could be compensated, or the rates could vary such that they would mutu-

ally offset one another. Measurement would be taken of the rates of partial reactions at different temperatures to isolate which rates and their corresponding interactions form the basis for temperature compensation. Preliminary data from the partial reactions appear to support a model of mutual compensation. A mathematical model for the oscillator would be fitted to complete set of experimental data to isolate the rates of each individual phosphorylation and dephosphorylation reaction. Further work would proceed by hijacking the circadian cycles at critical points to observe the effect of temperature changes on well-understood protein interactions to propose a molecular basis for the compensation.

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### Signal transduction pathways in cancer cells

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Cell growth and division is regulated through signal transduction pathways that involve kinase proteins and cascade reactions. Mutations in these signaling pathways can lead to cancer. Different cancer cells can show more sensitivity to certain therapies, and the goal is to understand the underlying genetic factors that give these varying sensitivities. We are currently studying a specific lung cancer cell, which has a mutated epidermal growth factor receptor (EGFR) that increases its sensitivity to EGFR inhibitory drugs such as erlotinib. Some of the experiments we have done involve growing the cells in media with low glucose concentrations. The lack of glucose appears to prevent EGFR from being transported to the cell membrane; western blots seem to indicate that the cells have a constitutively active EGFR even when the receptor is not present in the cell membrane. Other tests seem to indicate that the cells remain sensitive to erlotinib even if EGFR remains inside the cell. It would be useful in the future to know why these cells remain sensitive to erlotinib when EGFR is retained in the cytoplasm, in order to better understand how cancer cells respond to treatment and to develop targeted therapies.

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### Interactions between VEGF and forkhead proteins

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Endothelial cells form the inner-most cellular lining of blood vessels. Among their functions, they: control vasomotor tone, blood cell trafficking, hemostatic balance, etc. Angiogenesis is the term used to describe the physiological process of the growth of new blood vessels from pre-existing vessels. Our lab is studying the chemical stimulation of angiogenesis through Vascular Endothelial Growth Factor (VEGF) pathway. More specifically, we are doing research on the transient phosphorylation and inhibition of forkhead proteins by VEGF. This is an interesting area of research because recent studies have shown that the interactions between VEGF and forkhead are essential for endothelial cell growth and proliferation. We are looking

at these interactions *in vitro*, as well as *in vivo*. In our experiments *in vitro*, human coronary artery endothelial cells are transduced with adenovirus overexpressing constitutively active phosphorylation-resistant triple mutant FKHR and then treated with VEGF. Through Northern blot analyses and PCR, we looked at which VEGF-responsive genes require FKHR activity for optimal expression in endothelial cells. We now hope to eventually do the same *in vivo*. Our results are still pending but there is much excitement surrounding these results. The research that my lab conducts derives its importance from the essential role of angiogenesis in vascular injury and cancer. By investigating the pathways that trigger angiogenesis, we can better understand and treat cancer and heart disease.

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### **How cells think: modulation of the hyperosmotic stress response in *Saccharomyces cerevisiae* by carbon source**

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Like humans, cells face stressful situations. In order to survive, cells must detect the stress, "think" about it, and respond. Cellular thinking, or processing of external cues, can happen in the form of signal transduction where protein signaling results in changes in gene expression. The Hog1-mediated pathway and the PKA-mediated pathway are two signal transduction pathways that regulate the cellular response to high osmolarity and nutrient deprivation, respectively. Hog1 induces genes and counterbalances the high concentration of external osmolyte by promoting glycerol synthesis. Glycerol synthesis requires carbon from sugar fermentation, suggesting that cells integrate signals about nutrient availability and osmotic stress. Indeed, PKA has been found to function with members of the Hog1 pathway in the osmotic stress response. Our work aims to identify how carbon source modulates the hyperosmotic stress response mediated by Hog1 and PKA. We use microarray transcriptome profiling, immunoblotting and immunoprecipitation to test the hypothesis that Hog1 and PKA activate different transcription programs in salt stressed cells grown in glucose, galactose, and ethanol and glycerol. We anticipate that these experiments will provide a model for how gene expression in response to osmolarity and carbon source is regulated from the bottom up by exploring gene induction, promoter binding, transcription factor activity, and kinase activation state.

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### **Reprogramming mouse embryonic fibroblasts into pancreatic progenitors through the use of defined factors**

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Embryonic stem cells are the precursors of every cell in the human body. The ability of these cells to differentiate into any cell type, combined with the ability to grow indefinitely while maintaining this pluripotency, makes a stem cell-based therapy a prime target for the treatment of conditions like Alzheimer's disease, spinal cord injury, or diabetes. The goal of the Melton lab is to use stem cells to make beta cells, the insulin-secreting cells of the pancreas that are non-functional in patients with Type I diabetes. It has been shown that human and mouse fibroblasts can be reprogrammed to a stem-cell like state through the viral introduction of several transcription factors that are highly upregulated in human and mouse stem cells. Theoretically, a diabetic patient's fibroblast can be reprogrammed into a stem cell, differentiated into a functioning beta cell, and then implanted in the patient. We propose that these same principles can allow us to bypass the stem cell step, allowing us to reprogram directly between terminally differentiated cell types. By introducing various transcription factors that are important in pancreatic development into mouse embryonic fibroblasts, we hope to be able to reprogram fibroblasts directly into mouse pancreatic precursor cells.

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### **Tumor suppressor and oncogenic gene identification in human and mouse melanomas**

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Cancer is often a result of mutations that lead to aberrant expression levels or function of regulatory proteins whose expression and function are otherwise tightly controlled in the cell. Many such proteins, important for various control mechanisms in the cell, have been documented and their pathway scheme hypothesized and generally understood. Despite such findings, there are various aspects of cell cycle regulation and cancer biology that are not fully understood. It is conjectured that many other proteins that play essential roles in such pathways have yet to be found, and their roles must be explored in order to fully understand the mechanism underlying cancer biology. It is the focus of this research to identify potential targets for drugs to treat cancer. Cloning technologies are used to insert genes of interest from a cDNA library into viral vectors, which are inserted into a virus capable of infecting mice, and used to induce aberrant expression of such genes in mice. Tumor tissue from such mice is analyzed to identify the proteins that potentially caused cancer. Such analysis consists of confirming the presence of genes of interest in the cells and identifying aberrant levels of protein expression.



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## Understanding the transcriptional regulation of the melanoma oncogene MITF

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Melanoma, the deadliest form of skin cancer, is caused by the unregulated proliferation of melanocytes, the skin cells that are normally responsible for pigmentation. Microphthalmia-associated transcription factor (MITF) is a key regulator of melanocyte development, and improper expression of this oncogene is a critical step in the development of cancer. Our research aims to understand the pathways that aberrantly activate MITF in melanoma. Several genes were previously identified in large-scale RNA interference (RNAi) screens as necessary for MITF activity. We are now investigating the most promising of these “hits” to first confirm their regulation of MITF and then elucidate their mechanism. Current assays involve employing RNAi to assess the effect of each hit on MITF itself. For instance, we seek to identify the particular regions of the MITF promoter affected by these putative upstream regulators. We also aim to understand why some family members of a protein class have an effect on MITF expression, while others have none. We hope that this research will illuminate some of the key regulatory pathways that govern MITF expression in melanoma and thus identify potential drug targets.

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## The beta cell niche: promoting beta cell proliferation

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Diabetes, which affects over 180 million people worldwide, stems from pancreatic beta cells' inability to produce enough insulin to regulate blood sugar. The disease could be treated by encouraging proliferation of beta cells. Proteins released by the pancreatic mesenchyme cells, which surround beta cells during pancreatic development, may promote beta cell proliferation. This effect would be useful both *in vitro*, to improve beta cell culture for screens, stem cell differentiation and transplant therapies, and *in vivo*, as a therapeutic measure to cure diabetes. Recent work done by a postdoctoral fellow in the laboratory identified three candidate proteins that are secreted by pancreatic mesenchyme isolated at embryonic day E15.5, during organogenesis of the pancreas. These three proteins are: frizzled homolog 2 (FZD2), follistatin-like 1 (FSTL1), and ephrin B1 (EFNB1). RNA interference using lentiviral vectors will be used to determine the effects of knockdown of each of the corresponding three genes in a co-culture of pancreatic mesenchyme and beta cells. The beta cells are isolated from transgenic mice which express GFP in conjunction with the insulin-producing transcription factor PDX1, and beta cell health can be monitored through GFP fluorescence. Optimization experiments have so far provided information on transfection of lentiviral packaging cells and on optimal puromycin selection mesenchymal cells. Future experiments will include transduction of the pancreatic mesenchyme and co-culture with beta cells. Knockdown of gene expression will be measured by quantitative rtPCR, and ef-

fects of the transduced mesenchyme on beta cells in coculture will be measured by quantitation of beta cell fluorescence. Follow-up experiments include administering these proteins *in vitro* to beta cells or beta cell precursors, and studying their effects on beta cell proliferation.

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## Regulation of stem cell fate by the ubiquitin-proteasome system

The Scadden Laboratory

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During development, differentiation of cells into separate lineages is controlled by tissue-specific gene expression patterns that are regulated by a combination of transcriptional, epigenetic, and translational events. However, the regulation of protein degradation, controlled by the ubiquitin-proteasome system (UPS), is now suspected to play an important role in controlling transcription and differentiation. In order to understand the role of ubiquitination in regulating stem cell differentiation, human embryonic stem cell derived mesenchymal stem cells (hES-MSCs) were used as a model system. MSCs have great therapeutic potential and can differentiate into osteoblasts, chondrocytes, adipocytes, and neurons. By using a set of genetic and pharmacological tools, we will develop a novel system to identify nuclear protein degradation sites. Initially we will focus on analyzing protein degradation in the vicinity of loci encoding for transcription factors that are required for MSC maintenance and differentiation. Western blots and immunohistochemical analyses were conducted to validate our system. Later we want to employ chromatin immuno-precipitation (ChIP)-chip assays to identify nuclear degradation sites on a whole genome level. Understanding the role of ubiquitination in MSC development may uncover new genetic sites for inducing differentiation and controlling cellular maintenance.

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## ABCB5 expression in neurofibromatosis1- associated tumors

The Frank Laboratory

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Neurofibromatosis1 (NF1) is one of the most common genetic diseases with a prevalence of 1 in 3,500 people. It is caused by germline mutations in neurofibromin (*Nf1*), a tumor suppressor gene, and is inherited in an autosomal dominant fashion. When a second inactivating *Nf1* mutation occurs in somatic cells they give rise to multiple tumors, which include dermal and plexiform neurofibromas. 4-13% of plexiform neurofibromas undergo malignant transformation and develop into malignant peripheral nerve sheath tumors (MPNST). MPNST are known to originate from Schwann cells within plexiform neurofibromas, however the important question regarding the potential role of stem cells as a driving force of tumor progression and resistance in this malignancy remains to be elucidated. This project aims to determine whether a novel melanoma stem cell marker ABCB5, which belongs to the family of multidrug resistance transporters, identifies a similar stem-like cell population within MPNST. As the first step, we analyzed ABCB5 expression in the human CRL-2885 MPNST cell line derived from a patient af-

The retinal pigment epithelium (RPE), a pigmented monolayer of cells behind the retina, is essential to proper eye functioning and development. Despite the importance of the RPE, there have been limited studies of its development because it is difficult to isolate from embryos. A recent microarray profiling study identified seventy-eight genes that are differentially expressed in zebrafish embryo RPE. We are currently characterizing the functions of two of these genes, *stra6* and *silverb*, during RPE development. Mutations

Tandem repeats, DNA units of 2 or more nucleotides repeating head-to-tail, are regions of genomic instability. During DNA replication, there is often change in number of repeat units. In humans, some of these variations lead to neurodegenerative diseases such as Huntington's chorea. Although prior research has shown that repeat mutation rates are often more than 2 orders of magnitude greater than in non-repetitive DNA, the specific molecular mechanism underlying repeat instability is still largely unknown. Several DNA-repair proteins, however, may be involved in these rearrangements. Using *S. cerevisiae* as a model organism, the aim of this project is to investigate the roles and relative significance of such repair proteins in this phenomenon. In our protocol, an artificial tandem repeat sequence was inserted in the 5' end of a specific reporter gene, URA3. Variation in this repeat leads to frameshift mutations that turn the gene ON or OFF. Alternating cell growth between media that selects for the ON or OFF state of the URA3 gene therefore allows us to select for cells that have undergone repeat variation (URA3 from ON to OFF), and measurement of the number of cells that do so determines the exact repeat mutation rates. Using this system, the mutation rates of cells in which selected genes encoding DNA-repair proteins were deleted allows us to investigate the importance and possible roles of these proteins. Thus far, several deletion strains show significant changes in mutation rates, indication that they could be involved in the repeat mutation process.



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## **Metformin and AMP-activated protein kinase in regulation of endothelial nitric oxide synthase**

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These studies have explored the effect of metformin, an anti-diabetic drug, on signaling responses in endothelial cells. Metformin is a commonly prescribed oral drug for type 2 diabetes mellitus; the endothelium is the innermost layer of cells that line blood vessels. Recently, metformin has been shown to enhance specific pathways critical for vascular homeostasis in the endothelium. Research has suggested that metformin activates endothelial nitric oxide synthase (eNOS), an enzyme that synthesizes nitric oxide (NO). Both a metabolite of nitroglycerin and a signaling molecule naturally found in the body, NO is a vasodilator. However, the pathways through which metformin induces NO production have not been clearly defined. Using cultured bovine aortic endothelial cells as a model, initial studies have reaffirmed that metformin may activate pathways resulting in the phosphorylation of AMP Kinase (AMPK), an enzyme that may serve as an energy gauge and has been shown to activate eNOS. Through a combination of biochemical and immunochemical techniques, including siRNA-mediated knockdown of specific signaling proteins, the planned studies will further explore and elucidate the role of metformin in the modulation of endothelial molecular mechanisms.

# NEUROSCIENCE AND PSYCHOLOGY

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## **Who's the fairest of them all?: A study on fairness in 3-9 year old children and cotton-top tamarin monkeys**

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William James Hall

Fairness, the preference for equitable outcomes or an aversion to inequitable outcomes, is a central aspect of human interaction and is crucial in mediating cooperative decision-making. A sense of fairness has been shown to exist cross-culturally in humans, with people everywhere rejecting unfair offers even if they would be rewarded for accepting them. Fairness studies in animals are less conclusive, with some experiments suggesting that animals simply care about their own share and others indicating that animals can be other-regarding and averse to inequities. This summer project has two goals: a) to explore how and when a sense of fairness develops in humans, and b) to determine whether cotton-top tamarin monkeys have a sense of fairness. In experiments with both children and tamarins, there is an "actor" and a "recipient." We present inequitable and equitable quantities of food to actors and recipients. The actor is the decision-maker. If the actor accepts the offer, both subjects receive the food. If the actor rejects the offer, both subjects receive nothing. To date we have found that under the age of 5, most children will accept all offers, while older children have a significant preference for equitable outcomes. Most of the male tamarins show inequity aversion, while most of the females do not. These studies contribute to our understanding of the evolution of inequity aversion in primates and humans.

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## **Organization of odor information during different brain states in the rodent olfactory system**

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The human brain is arguably the most complex organ and one that is being heavily researched. However, the olfactory system is still one of the least explored areas of the brain. In the Murthy laboratory, we strive to explore the unexplored. Odors can induce long-term memories that guide behavior in many mammals, as well as accommodate new experiences by changing synapses, called synaptic plasticity. The rodent olfactory system consists of odor receptors, two olfactory bulbs, and the cortex, which share millions of synapses between them. This project focuses on two questions: How is information organized in the early stages of the olfactory system, and how is information processed alternately during different brain states of sleep

and wakefulness? To investigate these two questions, we have developed and implemented novel optical methods to image the olfactory bulb when stimulated with different odors, under anesthesia, and *in vivo*. One such method has been to incorporate Channelrhodopsin2 (ChR2), a light-gated ion channel protein that can photo-stimulate the olfactory bulb. With these methods and further studies we hope to examine whether odor information is organized by the molecular structures of the odors, or the memories associated with them.

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## **Effect of site-specific mutations on enzymatic and biological activity in SAD-A and SAD-B kinases**

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Neurons receive signals on their cell bodies and dendrites and transmit this information through their axons to other neurons at synapses. During development, they typically polarize to form one long, slender axon and several shorter, thicker dendrites. Using *in vitro* and *in vivo* studies, our lab previously determined that two protein kinases, SAD-A and SAD-B, which are known to phosphorylate the microtubule-associated protein tau, are important components of the pathway required for cortical neuronal development and migration. This project involved creating site-specific mutations in SAD-A and SAD-B kinases using bioinformatics approaches, and then testing these mutated kinases for enzymatic and biological activity. We spent the majority of the summer generating these constructs, and are now transfecting the mutated versions into cultured epithelial cells to check for tau phosphorylation. We will also insert the mutated versions into bacterial plasmid vectors to generate large amounts of the protein to purify and assay for activity. In the future, we will use electroporation to introduce the mutated SAD kinases into primary neuronal cultures and observe the effects on neuronal polarization. By studying the effect of external modifications on SAD kinases, we eventually hope to understand upstream cues and pathways that may be regulating SAD kinases.

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### **Morphological characterization of the axonal arbors of retinal ganglion cell in mouse**

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Retinal ganglion cells (RGCs) send visual information from eye to brain. Careful studies of the “receiving ends” of RGCs in the retina have allowed for these cells to be grouped into 10-15 classes based on electrophysiological response properties and morphological characteristics. Different classes are thought to process different visual information. The “sending ends” of mouse RGCs, their axonal arbors in the brain, have not been well-described. It is not known whether there are several classes of murine axonal arbors and whether RGCs of the same retinal class have similar axonal arbors. To answer this question, RGCs must be labeled in a manner that allows for imaging of individual cells in detail in both retina and brain. We use two methods to express fluorescent proteins sparsely among RGCs. To label a random subpopulation of RGCs, we inject virus into the mouse retina. To label distinct subpopulations of RGCs, we use mouse reporter lines. We then use confocal microscopy to image labeled cells both in the retina and the brain. Preliminary qualitative results suggest that there is diversity in axonal arbor types and that the axonal arbors of at least one defined retinal class share many morphological characteristics. Continuation of these studies and quantification of the morphological characteristics of the RGC arbors is still needed.

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### **Seeing the forest o'er trees – The effect of slow-wave sleep on semantic memory**

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How do brains process memories? Our brains are not camcorders, recording every sight and sound for posterity. Rather, we condense, adapt and transform events into pieces of evolutionarily helpful information before committing them to memory. How and when does all this memory processing occur? Past research has shown that sleep plays a key role in the consolidation of memories, aiding in the solidification of events in our minds. This summer, we further examined this relationship in an overnight sleep and memory study. Participants listened to a list of words, went to bed while being monitored using polysomnography and then were asked to recall as many of these words as possible the following morning. We believe that subjects who remain in slow-wave sleep (SWS), a stage of very deep sleep, for a longer period will perform worse on this task, and instead falsely recall a greater number of “gist” words (words not included in the list but that are closely related). This finding would expand our understanding of memory processing in sleep, the results indicat-

ing SWS allows us to remember general concepts at the expense of details. Consequently, we can see the forest o'er the trees! Because so little is known about the functions of sleep, further research of the purpose of various sleep stages and their relationship with memory will be necessary in the future.

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### **Implications of neuronal oscillations for cognitive function and disease**

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During the last two decades, researchers have studied the synchronization of fluctuations in membrane potential among specific subsets of neurons. It has been proposed that these oscillations may underlie a sort of “Morse Code” in the brain by allowing different brain regions to telegraph complex information as neurons fire together in specific patterns that link relevant information from separate circuits. This logic is also supported by the breakdown in oscillations that is seen in many neurological disorders, such as Alzheimer's, epilepsy and schizophrenia. Our research is directed toward establishing a causal connection between neuronal oscillations and cognitive functions by utilizing a pharmacological imitation of schizophrenia in rats. We have used *in vivo* electrophysiological recordings of the limbic system because the presence of oscillations is well established in the hippocampus and the system's relevance to learning, memory, and cognition is also well accepted. The selective death of hippocampal parvalbumin neurons is reliably reported in human patients, and by recording from various locations of the rats' brains at various time points, we will be able to determine whether this damage affects neuronal oscillations. These findings, once fully compiled, will help provide further insight into whether neuronal oscillations are inherently responsible for the breakdown of cognitive functions in schizophrenia.

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### **Using stem cells to model spinal muscular atrophy**

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Spinal Muscular Atrophy, or SMA, is a devastating neurodegenerative disorder that affects about 1 in 6,000 people, many of whom are young children. It is caused by a mutation in the SMN (survival motor neuron) gene which leads to the death of motor neurons in the spinal column. There is a wide range of symptoms depending on the severity of the disorder, but those with infantile SMA exhibit muscle weakness and limpness, and the disease is often fatal. Stem cells are being used to model this disease because they have the remarkable property of self-renewal, and they can also differentiate into any spe-

cialized cell types. This lab directed the differentiation of stem cells into motor neurons, which were created with a deficient SMN gene. These motor neurons were then treated with different compounds to see how the levels of SMN protein could be increased. Antibody staining was used to visualize levels of SMN. Another goal was to direct the stem cells to differentiate into a more caudal motor neuron identity because the disease impairs these motor neurons the most. Future steps will involve a large-scale chemical screen to test many more compounds that could be involved in regulating SMN levels, working towards a cure for this debilitating disease.

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### Search for downstream genetic program of Ctip2

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Corticospinal motor neuron (CSMN) degeneration is involved in amyotrophic lateral sclerosis and spinal cord injury and medium spiny neuron (MSN) degeneration in Huntington's disease. Previously, the Macklis lab has worked to identify a program of transcription factor controls over the specification and the differentiation of cortical projection neurons from progenitor cells. One transcription factor, COUP-TF interacting protein 2 (Ctip2), plays a critical role in differentiation of both CSMN in the neocortex and MSN in the striatum. Unfortunately, little is known about the function of Ctip2 in the execution of these important neuron types. In order to search for the downstream genetic program on Ctip2, we chose to look for key target genes in both CSMN and MSN by using a microarray followed by confirming expression patterns through *in situ* hybridization. In the future, we hope to confirm the expression in CSMN or MSN through backlabeling and possibly using an *in vitro* rescue. Thus, we hope to identify the mechanisms by which Ctip2, a central regulator of CSMN and MSN identity, acts to facilitate the development of CSMN and MSN. These mechanisms will play an important role in understanding the genetic controls regulating the generation and maturation of these cells, which is crucial for the development of cellular repair strategies.

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### Neural circuits underlying learned motor sequences in mice

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How does the mammalian brain encode learned motor behaviors? Much is known about the hierarchy and basic circuitry responsible for innate behaviors, but little is known about how the brain accommodates new, learned motor behaviors. We propose to study the neural circuits underlying learned motor sequence learning in the mouse. The advantage of doing this in the mouse is that it allows us to use a wide array of genetic tools for measuring from and manipulating

neural circuits. To allow for optical measurements of neural activity in the motor cortex during behavior, we aim to establish a behavioral assay for examining motor sequence learning in head-fixed mice. We train head-fixed and semi-restrained mice to perform a "morse" code of lever presses: mice are rewarded with water or medial forebrain bundle stimulation (which evokes a sensation of pleasure/reward) for tapping a lever multiple times within precise time intervals, while its head is fixed in a setup. We aim to explore the feasibility of such an assay within reasonable time constraints, and will ask how well and how complex a motor sequence mice thus trained are capable of performing. Preliminary results have been mixed and when compared to freely behaving mice, which are capable of learning quite precise and complex "morse" codes, head-fixing proves problematic because of physical stress on the subject. Furthermore, there seems to be great variability in the ability of different mice to learn. Other alternatives yet to be explored include the same procedure using rats, which are known to be more docile and disposed to learning behavior.

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### Investigating the role of 5-HT1B serotonin receptors on aggression in *Drosophila melanogaster*

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From the Serengeti plains where survival motivates animals to attack one another, to the streets of Broadway where rush hour road rage is as common as the Starbucks on every corner, aggression is an unavoidable phenomenon. Aggression, as with other behaviors, is intricately tied to the nervous system. By studying this behavior in *Drosophila melanogaster*, we are able to examine the complex neural circuitry that controls this behavior. The relationship between brain serotonin (5-HT) and aggressive behavior has been well studied in mammals, but little is known about this relationship in *Drosophila*. In previous studies, the manipulation of the entire serotonin circuitry has yielded a mild phenotype. Thus, studying the effects of one specific type of serotonin receptor seems to be a valuable approach. The closest ortholog of the mammalian receptor shown to be correlated to some psychological disorders is the d5-HT1B serotonin receptor in *Drosophila*. To assess the role of 5-HT receptors in the modulation of aggression, the GAL4/UAS system, a common genetic tool, was used to over-express the 5-HT1B serotonin receptors specifically in the 5-HT1B-positive neurons. When an aggression assay was conducted, the two feuding male experimental flies displayed abnormal courtship behavior (singing, mounting), but then proceeded to aggressive behavior. Preliminary analysis of classical courtship and locomotion assays have not shown many phenotypic differences between experimental and control males. A more detailed analysis of the experiments conducted will confirm these initial conclusions and guide future studies.



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### Characterizing the neuronal basis of habituation to electric shocks in zebrafish (*Danio rerio*) larvae

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Habituation, a form of non-associative learning, occurs when the behavioral response of an organism to a repeated stimulus decreases or ceases completely. Here, habituation to electric shocks is explored at the behavioral and neuronal level in 5-7 day post-fertilization larval zebrafish in order to establish a potential neural mechanism for habituation. Presentation of an electric shock results in the escape response: a robust and stereotypical behavioral response where both freely swimming and head embedded fish dramatically bend their tail to swim away from any aversive stimulus. The behavior of head embedded larval zebrafish was monitored during presentation of electric shocks of different intensities at several frequencies to study the onset of habituation. In a different set of experiments designed to seek and study a neuronal correlate for this habituation, fish injected spinally with a calcium green dextran dye, which retroactively labeled reticulo-spinal neurons, were imaged under an epifluorescence microscope. Activity from the Mauthner cell, a reticulo-spinal neuron known to play an important role during the escape response, and other neurons was recorded during shocking. Through analysis of habituation at the behavioral and neuronal level, this research seeks to understand how neural processing after presentation of a stimulus affects how an organism responds to its environment.

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### *In vivo* selection of adeno-associated viral vectors for brain tropism

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In this study, we examine the possibility of targeting intravenously (i.v.) administered viral vectors to the brain with the aim of developing a non-invasive and effective therapy for several neurological diseases. Gene therapy utilizes vectors, including viruses, to insert therapeutic genes into cells and tissues of a diseased patient. The blood-brain barrier prevents most gene therapy vectors from entering the brain after i.v. injection, including adeno-associated virus (AAV) vectors, which are the most effective vehicles for *in vivo* gene delivery. The ability of these vectors to enter specific tissues *in vivo* is determined by their protein shell (capsid). Up to ~100 different AAV capsids have been cloned mostly from humans and macaques, but thus far none seems to target the brain effectively after i.v. injection. Here we have generated a novel AAV capsid library by genetically engineering capsid genes from different AAV variants, effectively mixing the different genes to create unique capsids. We hypothesize that *in vivo* selection of this library may yield novel brain-targeted AAV capsids. Following i.v. injection of the capsid library in mice, genomic DNA was isolated from the brain, and AAV capsid genes were amplified from the genome by PCR. Sequence analysis indicated that some of the selected capsids are comprised of several AAV

variants. Subsequent rounds of selection and amplification will be performed until we have enriched for a small number of viruses isolated from brain. Then we will ascertain if the novel capsids can efficiently deliver therapeutic genes to the mouse brain.

Veronica Shi  
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### Using mesenchymal stromal cells as a therapy for glioblastoma

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Glioblastoma multiforme (GBM) is a devastating brain tumor, and current therapies do not provide sufficient treatment, as the tumors are inevitably lethal. The major issue is the recurrence and invasiveness of the tumor due to the migration and scattering of tumor cells into the brain. A promising therapeutic solution is the direct targeting and eradication of these tumor cells by genetically modified cellular vectors. Human bone marrow-derived mesenchymal stromal cells (hMSC) possess the unique and remarkable ability to do this, as they target and migrate towards tumor cells. In this study, we use genetically modified hMSC as therapeutic delivery vehicles to produce biological agents at the GBM tumor site. The hMSC were transduced with a lentiviral vector to express PEX (hMSC-PEX), a protein that acts as an inhibitor of tumor angiogenesis, proliferation, and migration. Cytotoxicity assays and co-cultures of GBM cells with the hMSC-PEX resulted in the inhibition of tumor cell growth. Migration assays *in vitro* confirmed that hMSC-PEX cells retain their tumor tropism. *In vivo* experiments in mice are currently being conducted to evaluate the migration of hMSC-PEX cells and their therapeutic efficacy. Labeling of hMSC with Feridex, a superparamagnetic iron oxide contrast agent, allows for monitoring and tracking of these cells in real time by MRI. Future directions include translation of this approach for use in a clinical trial in GBM patients. This research provides promise for an effective treatment that can improve patients' survival and quality of life.

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### A possible molecular mechanism for reproductive suppression of mice under predator-induced stress

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One of the most observable behavioral phenomena that occurs under predatory odor-induced stress is the suppression of reproductive activity. It has been documented that female rodents chronically exposed to predator odor display clear changes in normative reproductive behavior, such as refusal to mate and alteration of estrous cycles. In mice, the vomeronasal organ (VNO) plays a role in the modulation of reproductive behaviors, since both major histocompatibility complex identity and major urinary protein composition, chemosensory information associated with mate selection, are received by the organ. The VNO itself is comprised of approximately 300 distinct G-protein coupled receptors (GPCR), each of which binds to a specific

Behavioral Inhibition to the Unfamiliar (BI) is a highly heritable temperament trait observed in young children. Children with BI typically exhibit behavioral restraint as well as increased autonomic and limbic reactivity in response to unfamiliar situations. BI is considered a trait of clinical importance because of its close relationship with several psychiatric conditions including social phobia, generalized anxiety disorder, obsessive-compulsive disorder, and panic disorder. Previous studies have identified some of the genetic determinants of BI, including several genes involved in neurodevelopment, limbic function, and regulation of the HPA-axis, but much is still not known about the genetic basis of this trait. The present study aims to detect new candidate genes for BI, characterize the distribution of these gene variants in children who have undergone neuroimaging and behavioral assessments, and identify significant associations between specific genotypes and behavioral or neurological phenotypes related to fear and anxiety. We include both case-control and family-based project designs, with over 1,500 individuals enrolled and recruitment ongoing. Subject DNA is being extracted from saliva samples so that relevant single nucleotide polymorphisms (SNPs) can be genotyped. These SNPs were chosen based off of a comprehensive literature and HapMap search. Multiplex PCR and genotyping is currently underway, and results are expected by mid August. These results will be analyzed through case-control association and transmission disequilibrium testing. Neuroimaging data, including functional magnetic resonance imaging (fMRI), will be included in the final analysis.

The olfactory bulb (OB) is the first and primary relay station for odor information; olfactory receptor neurons in the nasal epithelium, upon odor stimulation, send action potentials to mitral cells in the OB. Different odors result in unique patterns of mitral cell excitation modified by surrounding interneurons (granule and periglomerular cells). However, the electrical and functional circuitry of these interneurons remains largely unknown. By labeling the cells with a GFP-lentivirus and visualizing them *in vivo* using two-photon microscopy, one can potentially delineate their circuitry, including even the complex granule cell basal dendrites. Also, by labeling the interneurons using a split-GFP lentivirus coupled with synaptophysin and G-CaMP, one can visualize live calcium responses of granule cells upon odor stimulation. In addition, the interneurons are continuously born during adulthood in the subventricular zone and migrate

through the rostral migratory stream to the OB, where less than half survive. However, not much is known concerning their functional integration into the existing circuitry. Again using lentiviral labeling, this time with ChR2, a light-activated ion channel, and two-photon microscopy, it would be possible to stimulate the cells using light and study the effect of activity on cellular migration, differentiation and integration.

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### **Exploring the potential of the NAD<sup>+</sup> biosynthetic pathway in axon protection**

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Multiple sclerosis (MS) is a neurological disorder in which the body's immune system attacks the myelin sheath, impairing signal propagation down axons. Current treatments for MS are primarily designed to suppress the immune system, while no therapy targets repair of the damaged axons. By focusing on this largely overlooked aspect of the disease, we hope to gain the information that may one day lead to an alternative or complimentary treatment for MS patients. High nicotinamide (NAD<sup>+</sup>) levels have been shown as one possible therapy against axonal damage. The method by which NAD<sup>+</sup> affects MS disease course is still ambiguous. To illuminate upon this mechanism, we are inducing an MS-like disease in transgenic mice with selective cellular overexpression of an enzyme (PBEF) involved in the NAD<sup>+</sup> biosynthetic pathway. With this experiment, we will to observe if selective overexpression of PBEF in neurons lessens the symptoms of the disease, and isolate where this neuroprotective effect might originate. So far, we have viewed preliminary differences that suggest mice that overexpress PBEF in their neurons suffer less severe symptoms than the littermate controls. After the 60 day time-course of the animal experiments, the mice will be dissected and analyzed to see if any morphological differences are apparent in their brain tissue. Our lab plans to continue to explore the mechanism by which PBEF operates by setting up further transgenic mice that express PBEF in both neurons and glial cells, as well as globally in all cells.

# ORGANISMIC AND EVOLUTIONARY BIOLOGY

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## Evolution of burrowing in *Peromyscus*

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The genetic basis of behavior in mammals is not currently well-understood. One obstacle to understanding is the lack of quantitative data, which is difficult to obtain for many behaviors. One common mammalian behavior, burrowing, can be easily quantified by its physical dimensions. We studied burrowing behavior in the old-field mouse *Peromyscus*, which is believed to be completely inheritable, specifically comparing two sister subspecies, *polionotus* and *maniculatus*. *Polionotus* is a beach-dwelling species which recently evolved a set of burrowing behaviors which set it apart significantly from its cousin, *maniculatus*. Burrow measurements, in concert with DNA sequencing, can be used to perform a quantitative trait loci (QTL) analysis, which uses statistical methods to find “hotspots” in the genome where genetic control over the behavior is localized. The mice were bred in captivity for several generations, at which point a back-cross was performed between second-generation hybrids and *maniculatus*. The back-cross mice were placed separately in sand-filled burrowing chambers for two-day trials, after which their burrows were measured for physical traits. The back-cross mice’s burrows ranged from simple *maniculatus* burrows to the large, complex burrows of *polionotus*, and all varieties in-between. As of writing, we have not yet generated a large enough sample size to perform the QTL, which will hopefully pinpoint those portions of the genome which contain the gene or genes of interest. From there, future research may shed light on the genetic basis of the evolution of burrowing behavior in *polionotus*, namely, whether the gene evolved through a mutation or mutations in its regulatory regions and/or coding regions.

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## Wingless expression and color pattern in *Heliconius* butterflies

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Mimicry is one of the most fascinating aspects of adaptive evolution in organisms. In particular, the Kronforst lab works with the distasteful tropical butterfly genus, *Heliconius*, which have employed Mullerian mimicry to share the costs of predator education. Main goals of the lab include identifying and understanding the genes responsible for color pattern variation in the wings. As a whole, these projects give us a better grasp on the mechanisms behind speciation in natural populations. We are currently investigating the transcription of a color pattern candidate gene, wingless, in two color morphs of one butterfly species: the white morph, *H. cydno galan-*

*thus*, and the yellow morph, *H. cydno alithea*. After extracting RNA from specimens at different developmental stages, messenger RNA (mRNA) was isolated and transcribed into complementary DNA (cDNA) using an altered PCR protocol with reverse transcriptase replacing DNA polymerase. Using RACE protocol (Rapid Amplification of cDNA Ends), the transcripts were amplified and inserted into bacterial clones to separate different products. PCR was then conducted on the bacterial clones to reproduce pools of transcripts for sequencing. The sequenced wingless transcripts were different sizes and incomplete in both species. The process also picked up a large amount of ribosomal RNA and some unidentifiable genes. In order to clarify our results, future work includes using suppressed subtraction hybridization (SSH) to find the most abundant transcripts in each color morph. Then, perhaps we can determine if there are indeed fixed genetic differences between the two morphs.

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## An assessment of select Charles River biota

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The Charles River has had a long history of anthropogenic modification, from the introduction of exotic species to the dumping of industrial pollution. Over 375 years of modern human settlement along the Charles River has caused severe water quality and aquatic habitat impairment. While the Charles has seen improvements in water quality in the last few decades, the river’s natural ecosystem has been fundamentally altered forever. This study focuses on the biodiversity, species dominance, and species composition of zooplankton caught in the lower Charles River by a 150µm net. Additionally, phytoplankton abundance was monitored using chlorophyll  $\alpha$  measurements and fish community structure was assessed through a series of seining. Phytoplankton provide food for zooplankton, which in turn support fish populations. Because pollutants enrich the Charles River in nitrogen and phosphorus and deteriorate water quality, phytoplankton and zooplankton communities are structured such that they cannot support fish specialized in feeding habits. As a result, fish species that are macrohabitat generalists, meaning that they adaptively feed on a wide variety of prey, dominate the Charles River. To understand the health of fish populations in the Charles River, it is essential to understand the structure of the lower trophic orders. With a better understanding of plankton community structure in the Charles River and how it is influenced by nutrients in the river, fisheries management strategies can be improved.



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### Resource partitioning between bluegill, pumpkinseed, and perch in the Charles River

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This study examines the diet and habitat use of three of the most common fish found in the Lower Charles River littoral (near shore) zone. In the first part of this study, 105 fish were collected by seining between June and July. Seven different fish species were observed, with three species; bluegill sunfish (*Lepomis macrochirus*), pumpkinseed sunfish (*Lepomis gibbosus*), and yellow perch (*Perca flavescens*), accounting for >92% of the total fish catch. The second part of this study focuses on the partitioning of resources in the littoral zone of the Charles River through the examination of the gut contents of 20 bluegill, 38 perch, and 40 pumpkinseed. Traditionally, in environments where bluegill and pumpkinseed coexist naturally, bluegills forage primarily on open-water zooplankton, while pumpkinseeds specialize on vegetation-dwelling gastropods. In the lower Charles River littoral zone, however, where the bluegill is not native, the diets of bluegill and pumpkinseed overlapped and were dominated by benthic invertebrates. Future research will investigate the hypothesis that this deviance from the traditional feeding patterns reflects different resource availability and a more complex interaction between the introduced bluegill and native pumpkinseed for available resources.

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### Testing Buller's convective hypothesis through fluid dynamic modeling in *Basidiomycete* mushrooms

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Numerous species of *Basidiomycete* fungi use mushrooms to aid dispersion of millions of spores for reproduction in response to environmental cues. In the book *On the Researches of Fungi*, Reginald Buller hypothesized that the heat produced from fruiting bodies warmer than the surrounding air would create convection currents to help the spores disperse from the sporulating mushroom. The purpose of this research is to test Bueller's convective hypothesis in *Basidiomycetes* by exposing fruiting bodies to various environmental conditions and testing temperature gradients in four different parts of the mushrooms. We will approach this problem by constructing a mushroom model using the principles of fluid dynamics to understand the necessary temperature gradients required for Bueller's hypothesis. Individual movement of spores from *Ganoderma lucidum* will be compared to the model to test the relevance of convection currents in spore distribution. By measuring the temperature differences between different parts of a fruiting body, we will test the existence and intensity of the temperature gradients in different parts of the mushroom in an ecologically relevant context. Our current results suggest that *Basidiomycete* fruiting bodies are colder than the air surrounding them. The most likely candidate to demonstrate

Bueller's convective hypothesis, if at all, would be *Ganoderma lucidum* exposed to direct sunlight. The results of our research would help elucidate the physical mechanisms of fungal reproduction and the biophysical implications, if any, of heat and fluid dynamics in this system.

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### Using bite force scaling to understand the role of incisors in feeding of fossil hominins

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This study estimates how much bite force hominins could produce, and tests some hypotheses about the relationship between bite forces and bite stresses. Humans produce comparatively low bite forces and have small teeth, but many early fossil human ancestors (hominins) from the genus *Australopithecus* have extremely large teeth. Soon after the origin of meat eating, in the genus *Homo*, molars and premolars became smaller. One longstanding hypothesis is that molars became smaller because bite forces declined, in large part because molar bite force scales strongly with molar surface area, thus keeping stresses constant (stress is force/area). While there is a consensus that molar bite force has declined over time, there are few quantitative studies that have examined molar bite force in large part because it is difficult to estimate bite forces from skulls. There has also been little research on how incisal bite forces scale with incisor size. Using cranial measurements, we estimated how much bite force various ape and hominin species could produce during molar and incisal chews by summing the torques generated by the three principle muscles of mastication: temporalis, masseter, and medial pterygoid. We then calculated both molar and incisal stresses using data on tooth size. We validated the model by comparing our estimates with published values on maximum bite forces produced by humans and non-human primates.

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### Evaluation of the Acoustic Niche Hypothesis: sound spectrum partitioning in tropical and temperate communities

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Before we can begin to alleviate the current trend of rapid biodiversity loss, we must develop methods to quickly and reliably assess species diversity and ecosystem health. Bioacoustics—the study of the sounds produced by organisms—offers a rapid, inexpensive, and noninvasive solution. Since mating calls are species-specific, insect, frog, and bird songs can be used to count the number of species present in a given area. Not only can song serve as a measure of biodiversity, but the Acoustic Niche Hypothesis suggests that bioacous-

tics can also provide an index for ecosystem health. The hypothesis predicts that, in a mature ecosystem, species will avoid competition by singing at unique pitches at unique times. It follows that mature, healthy ecosystems will exhibit more pronounced partitioning of the sound spectrum, while young or disturbed ecosystems will exhibit more song overlap or silent gaps. To test this hypothesis, recordings were taken at seven sites in Massachusetts. Every site was sampled four times. Each time, recordings were taken at sunrise, noon, sunset, and midnight. In order to compare the bioacoustics of temperate and tropical ecosystems, recordings were also taken in the Dominican Republic. Sites were chosen to represent a spectrum of human disturbance. Preliminary results indicate that sound spectrum partitioning is indeed occurring. We are currently developing a statistical method to quantify the degree of this partitioning. Once we have developed this method, our next goal will be to test whether the degree of partitioning does in fact correlate with ecosystem health.

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### **Chicken legs: Exploring the effect of strain on bone growth and microstructure biomechanics**

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Studying the bone structure of different animals allows researchers to learn about the function and kinetics of an animal's way of life. In order to further our knowledge of the effect of strains on bone microstructure, we explored the difference between flight and running strains on the guinea fowl's tibia tarsus and humerus bones. Twenty four guinea fowl were assigned to three different groups – a control group, a flying group, and a running group. The flying and running groups were exercised daily to maximize their use of their wings or legs, respectively. After a month of exercise, strain gauges were attached to the humerus bone of the flyers and the tibia tarsus of the runners. Strain measurements were recorded for all groups under their trained exercise conditions. We expect to find that the strains recorded for the running group's tibia tarsus will be lower than those for the control group because the bones of the birds that exercised will have the appropriate structure to support the exercise routine. Similarly, the strains for the flying group's humerus are expected to be lower than those for the controls. The bones of the guinea fowl will be collected to analyze their microstructure and growth patterns.

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### **You run what on a treadmill?!?: Guinea fowl locomotion disruption and recovery**

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The guinea fowl is a rather robust bird native to Africa. This lab seeks to discover how guinea fowl locomotion is affected in the simultaneous presence of motor control and absence of sensory input. During the experiment, the lateral gastrocnemius (LG) leg muscle nerve is cut then reattached. This disturbs both the sensory function, so that the muscle receives no instructions from the brain or spinal cord, and motor ability, which is the ability for the muscle to participate in leg movement. It is expected that the guinea fowl will regain quality motor ability even in the absence of sensory input. While this nerve reinnervation experiment had previously been performed on cats, the guinea fowl's bipedal movement and greater willingness to run allow for a more appropriate animal model whose performance may provide further insight into the development of human prosthetics in the future. Six joints of the guinea fowl's hip and leg region were marked for tracking purposes. EMGs (electromyographs) were implanted into the LG of healthy guinea fowl to detect the presence or absence of muscle function. The birds were videotaped and their EMG recordings were documented while running on a treadmill for control data. In surgery, the nerve that attaches to the LG was cut then immediately reattached. The animals recovered for a 2.5 week minimum. The treadmill procedure was repeated for post surgery testing data and at a later testing date. Data analysis is still in progress but it was noted that the guinea fowl are still able to run well even after surgery. Joint angles will be calculated and a method to ensure that sensory input was eliminated will be created to compare the differences in the control versus experimental data.

# PHYSICS AND BIOPHYSICS

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## Knee prosthesis component wear and regional lymph node uptake

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Total knee arthroplasty is used to replace a painful joint in the advanced stages of arthritic degeneration. The articular surface, composed of high molecular weight polyethylene, must tolerate weight comfortably and generate minimal friction without wear debris. Despite best efforts with material design, articular surface wear is one of the most frequent causes of implant failure. Polyethylene wear particles can accumulate in the joint and surrounding tissues creating areas of bone destruction (granulomas). Our hypothesis is that granulomas can be quantified, are proportional to prosthesis wear, and that polyethylene or metal may be identified in lymph nodes near prostheses. Quantitative measurements of granulomas in three-dimensions were obtained with computed tomography (CT), using a 3-D volumetric algorithm from axial, coronal, and sagittal planes. An analysis of lymph node enlargement is performed with density measurements (Hounsfield units, HU). Sixty controls are compared to 104 subjects over an eight year period. Preliminary results indicate that high granuloma number and size are detected with increased prosthesis wear and angulation. Accumulation of polyethylene and/or other high HU density material occurs in the popliteal lymph nodes, causing enlargement. We conclude that quantification using a 3-D algorithm is feasible, and may have value in surgical planning. The finding of lymph node enlargement indicates that wear particles are present systemically more frequently than previously noted.

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## Stretching single DNA molecules: A physical probe into the biological mechanism of RecA

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By mediating the processes of homologous recombination during meiosis (i.e. "crossing over") and the recombinational repair of damaged DNA, the enzyme RecA is crucial for maintaining both genetic diversity and genomic integrity *in vivo*. Structural evidence to date has indicated that bound RecA stretches double strand DNA (dsDNA) locally by a factor of 1.5 length-wise, in order to form a catalytically active species. Nevertheless, the precise physical mechanism that governs this RecA-mediated distortion of dsDNA structure remains to be elucidated. Important to this question is the asymmetry of the double helix which emerges from the anti-parallel nature of the complementary strands. As a result, theoretical models predict that changes in the helical structure of dsDNA induced by stretching should be highly sensitive to the particular ends (3' or 5') from which the dsDNA is pulled. To characterize the preferential binding of RecA to these different structural forms of stretched dsDNA, we use magnetic tweezers to perform single molecule force-induced elonga-

tion experiments on dsDNA in the presence of RecA. By comparing quantitative differences in the relative binding kinetics and polymerization rate of RecA based on dsDNA pulling technique, we demonstrate that RecA-dsDNA filament formation is indeed dependent on the particular structural form of stretched dsDNA. This observation that the force-induced binding of RecA to dsDNA is modulated by pulling technique may shed light on the precise physical mechanism behind the RecA-catalyzed distortion of dsDNA structure *in vivo*.

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## Temperature dependence on DNA's structural preferences

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Biologists hold that at 37° C DNA bases are inaccessible to the surrounding cell environment. However, base rotation can be induced by either overstretching the DNA helix or raising the temperature of the environment to 40° C. Bases in overstretched 5'5' DNA will rotate open at 37° C. In the presence of cyclodextrin, a cyclic sugar that binds exclusively to rotated base pairs, whether this structural change is preserved can be determined with force-elongation curves; a curve showing hysteresis in a strand of overstretched DNA suggests that the system ejected previously bound cyclodextrin and the helix returned to the standard B-form, and an absence of hysteresis is evidence of the opposite case. At lower temperatures, it is more difficult to detect hysteretic behavior. We sought to detect any temperature dependence in 5'5' DNA's preference for the rotated conformation versus the B-form. Using magnetic tweezing, 5'5' DNA was overstretched at 22° C, where we observed notable hysteretic behavior. However, at 20° C, hysteresis in 5'5' DNA disappeared, suggesting that DNA's structural tendencies are influenced by even small variations in temperature. We will explore this relationship further by examining hysteretic behavior in 5'5' and 3'5' DNA at 35° and 40° C.

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## DNA adsorption to solid-state nanopore surfaces and materials

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Current techniques for DNA sequencing are limited both in speed and accuracy, especially for long DNA fragments. A new technique, in which DNA is pulled single-stranded through a nanometer-sized pore and sequenced base by base with a tunneling electron probe, can potentially read long DNA fragments in a fraction of a second. Development of such devices has been hampered by pore clogging, where DNA sticks to the pore instead of translocating through. The purpose of this research is to characterize these sticking interactions,

which are not well understood. Adsorption of single-stranded DNA to nanopore materials (silica and silicon nitride) were studied using a solution depletion method. Silica nanoparticles were mixed into a DNA solution and then removed by centrifugation, simultaneously removing DNA that adhered to the particles. UV-visible spectrophotometry was used to measure the concentration of DNA before and after the introduction of the nanoparticles, and the amount of DNA adsorption was calculated. In another experiment, nanopore chips (silicon nitride on silica) were used in lieu of the powder, and the DNA adsorption to the chip surface was measured using the same principle. The dependence of DNA sticking on ionic strength, pH, and other relevant experimental parameters was studied in order to characterize the driving forces behind the adsorption interaction and develop techniques to reduce pore clogging.

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### Measuring the permeability of Kapton with respect to Radon

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In this experiment, an apparatus for measuring the permeability of different plastics with respect to arbitrary gases was constructed. A gas handling system consisting of a high vacuum region and an atmospheric region was built using ¼" VCR feedthroughs. The high vacuum region houses different vacuum measurement gauges including an Ionization Gauge and a Residual Gas Analyzer. The two pressure regions are separated by a plastic film, which is fixed in place by two Viton O-Rings housed by a leak-tight, metal-metal interface. Using a turbomolecular pump a vacuum of order 10<sup>-6</sup> torr can be achieved in the high vacuum region. An arbitrary gas can then be introduced into the atmospheric region. Due to the pressure gradient across the film, diffusion will occur. By analyzing the subsequent steady states, we can determine the permeability constant. Currently, Kapton, a polyimide, is being studied with noble gases up to Xe. Ultimately this data can be used to extrapolate Kapton's permeability constant with respect to Ra. This measurement is of high significance to the Cryogenic Low Energy Astrophysics with Noble Gases (CLEAN) Experiment, which wishes to use Kapton as a cheap, small size, low temperature gasket that screens Ra.

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### Quantum dots in *E. coli*: A quantitative investigation of the inner space of bacteria

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The standard approach to imaging the bacterial chromosome has been to stain the chromosome with fluorescent proteins. Unfortunately, the optical resolution obtained using this method is limited by the diffraction of light to approximately 200-300 nm, which is in the same order of magnitude as the cell diameter. Since direct imaging of the fluorescently-labeled chromosome suffers from this low spatial resolution, we have directed our attention to imaging the complementary space between the nucleoid and the cell membrane.

We inserted freely diffusing PEG-coated quantum dots with diameters of 10-20 nm into the cytoplasmic space of *E. coli* cells using transformation. The quantum dots diffuse in the periphery of the cell and avoid the nucleoid, which is occupied by a dense network of DNA. The quantum dots are fluorescent, emitting light of a given wavelength. By filtering for light of that wavelength, we imaged the individual quantum dots within the cells. The power of this approach is that we can extract the position of a single particle with higher precision than the diffraction limit. Our resolution is limited by the distance that the diffusing particle traverses during our exposure time rather than by fundamental optical properties. Quantifying the gap between the nucleoid and the cell membrane in *E. coli* will give us a more detailed view of the inner space of this model organism. Ultimately, this method can be applied to other bacterial species such as *Bacillus subtilis* or *Caulobacter crescentus*, for which the existence of a gap between the nucleoid and the cell membrane is still controversial.

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### Silicon nanoparticles for hyperpolarized Magnetic Resonance Imaging

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Magnetic Resonance Imaging (MRI) is a powerful, noninvasive technique for visualizing structures within the body and diagnosing disease that uses radiofrequency signals to excite hydrogen nuclei in tissue. Because of the abundance of hydrogen within the human body, conventional MRI is limited in regions with low signal-to-noise ratios or undergoing constant motion, or if attempting to image a specific feature within the body. Silicon nanoparticles can correct these shortcomings when used as a contrast agent and imaged instead of hydrogen. They are non-toxic and may be hyperpolarized via dynamic nuclear polarization to achieve dramatic MRI signal enhancement. The ideal silicon nanoparticle would have a long spin-lattice relaxation time (T<sub>1</sub>) and be surface-functionalizable with biocompatible ligands. Silicon nanoparticles were fabricated by ball milling high-resistivity crystalline silicon wafers in ethanol and separating by size through successive centrifugations. The resulting particle size distributions were verified by scanning electron microscopy and dynamic light scattering. These particles were then characterized alongside various commercially available amorphous and crystalline silicon nanoparticles synthesized bottom-up to determine the optimal silicon material for use in hyperpolarized MRI. Future studies will investigate techniques for maximizing T<sub>1</sub> and methods of functionalizing the surface of the silicon particles for increased circulation and tumor targeting.



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## **Consultants**

Daryl Chubin, external assessment, American  
Association for the Advancement of Science  
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Association for the Advancement of Science

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Michael D. Smith – Dean of the Faculty of Arts and Sciences  
All the Mentors, Principle Investigators, and Lab Members  
The PRISE Fellows



## Acknowledgements

Scott Kominers  
Kirkland 2009  
Fellow 2006, Program Assistant 2008

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This summer, Scott Kominers has pursued research in economics, mathematics, and ethnomusicology. His economics research has focused on new mathematical approaches to economic geography (supervised by Prof. Edward L. Glaeser, Prof. Andrei Shleifer, and Prof. William R. Kerr) and on theoretical models of position auctions (supervised by Prof. Susan Athey); this work is partially supported by fellowships from the Harvard College Program for Research in Science and Engineering and the Harvard Institute for Quantitative Social Science. Kominers's mathematical research has been predominantly concerned with quadratic form representation theory, weighted weight enumerators for doubly-even self-dual codes (joint with Prof. Noam D. Elkies), and extremal combinatorics (joint with Paul M. Kominers, MIT 2012); this work is supported by a Harvard Mathematics Department Highbridge Fellowship. Finally, his musicological research has applied novel ethnographic methods to children's literature and Gregorian Chant. In August, Kominers spoke in Madison, Wisconsin, at MathFest 2008, on "Configurations of Extremal Even Unimodular Lattices." This talk discussed Kominers's work forthcoming in *International Journal of Number Theory* and *Journal de Theorie des Nombres de Bordeaux* (the latter of which is joint work with Zachary Abel, Harvard 2010). Also in August, one of Kominers's short articles appeared in *Economic Development Quarterly*.

Stephanie Lo  
Cabot 2010  
Fellow 2007, Program Assistant 2008, Photographer 2007 & 2008

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Stephanie Lo has pursued inter-disciplinary research throughout her undergraduate career. After engaging in synthetic biology research for a year and a half, she spent PRISE 2008 continuing her spring semester's work as a research associate with Professor David Moss at the Harvard Business School. Her most substantial HBS project so far has been writing a case study, to be published this fall, on the financing of Australian education. In the case study, which sets up a comparative analysis of educational financing in the United States and Australia, Stephanie elaborates on the considerations and circumstances that eventually led Australia to adopt an income-contingent student loans scheme. Her summer projects have also included investigating the effects on safety legislation on businesses and researching recent attempts to establish federal disaster insurance. Eventually, Stephanie hopes to integrate her experiences in economics and biology in a management-oriented career in the fields of biotechnology or healthcare.

Christina Tartaglia  
Kirkland 2009  
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Christina Tartaglia has immersed herself in the field of molecular and cellular biology both in her studies and her research. She is currently conducting research under Professor Jack Strominger. Her research aims to study the mechanism(s) by which adjuvants, important stimulants of the immune system, act in a murine model of multiple sclerosis (MS). She is examining the suppressive effects of different adjuvants in combination with a new copolymer, an analogue of Copaxone® (a copolymer already in use to prevent relapses of multiple sclerosis). This novel copolymer has been shown to reduce the relapse rate of multiple sclerosis by 30%. The broader goal of this research is to improve the effectiveness of these copolymers and prevent multiple sclerosis. Christina hopes to pursue a career in medicine and medical research.

***Laura Hunter - Administrative Coordinator***

*On behalf of the entire PRISE community, we would like to thank Laura Hunter, administrative coordinator of PRISE for the last two years, for her indispensable contribution to PRISE. Laura has played a key role in helping PRISE Fellows develop and organize their own activities as well as working out logistical details with the Director, the College administration, and science faculty to make PRISE run smoothly. We appreciate her unfailing warmth and cheerful support and wish her well as she leaves Harvard to pursue graduate school studies.*

*Best Wishes,  
The PRISE 2008 Fellows*



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