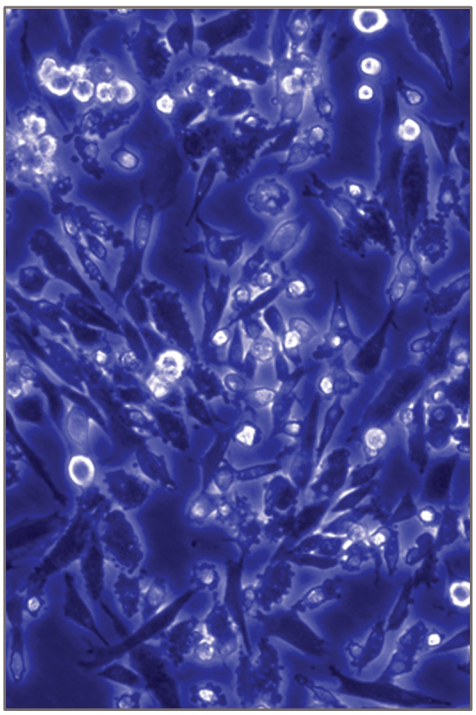
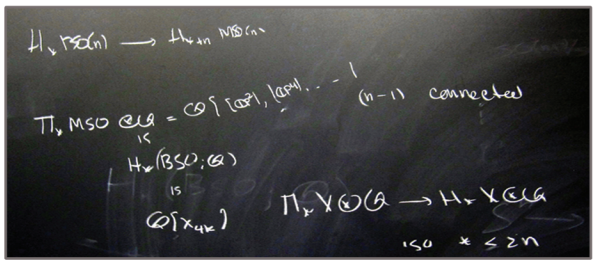
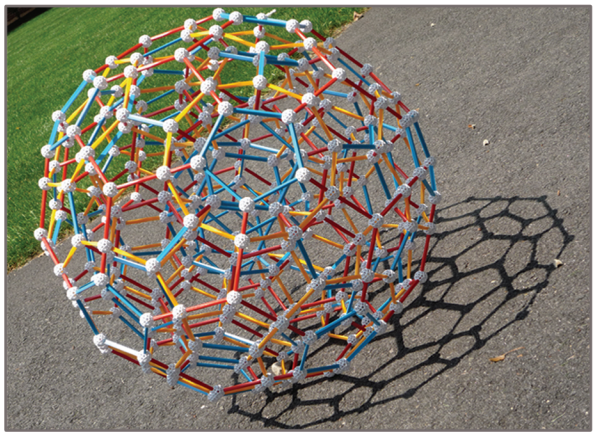
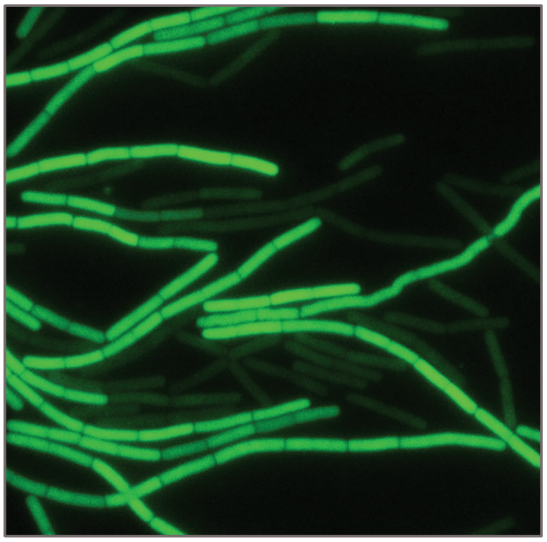
PRISE 2009

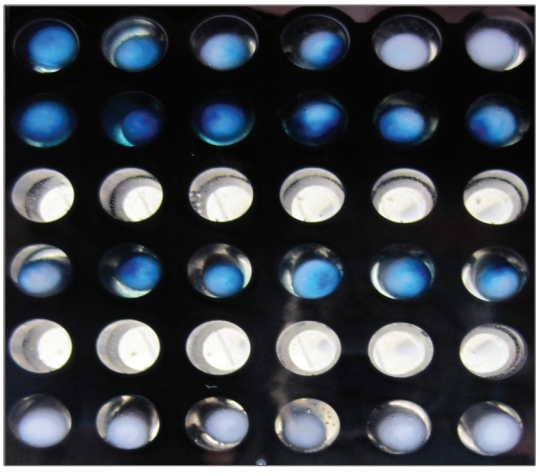






Abstracts





Harvard College Program for Research in Science and Engineering

HARVARD COLLEGE

PRISE

PROGRAM FOR RESEARCH IN SCIENCE AND ENGINEERING

ABSTRACTS

2009

prise.fas.harvard.edu

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Cover Design: Kristen Hunter

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Letter from the Director

Once again, it is a privilege and great pleasure to introduce the research abstracts of the 2009 Harvard College Program for Research in Science and Engineering, PRISE. During the summer, the PRISE 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 affiliated hospitals, the Harvard School of Public Health, and other allied research enterprises. As you will see in these abstracts, the research spans innumerable permutations of life, physical, and engineering sciences, a testimony to the diverse and interdisciplinary community PRISE intends to create. This scientific experience has been augmented by participation in PRISE's indefatigable residential community at Leverett House, providing a social environment and the opportunity to interact with peers in a meaningful way.

For each of the last three years, a group of dedicated PRISE fellows has taken as a project the effort to collect research summaries from the participants. Along with the PRISE Program Assistant Fellows who have helped with all of the Fellow-initiated projects, I would like to thank this group especially for taking on this great project recording the research efforts of the PRISE community this summer.

Clearly, the 2009 PRISE fellows have exceeded both the salient and secondary goals of PRISE with their collective energy, enthusiasm, and inclusivity. To all of you I wish you the greatest success as you continue pursuing your academic and career paths. I hope you remember your time in PRISE fondly, and that the personal and collegial relationships you've made this summer last long past your Harvard years.

Gregory A. Llacer, Director
Harvard College Program for Research in Science and Engineering (http://prise.harvard.edu)

Letter from the Editors

Dear fellow PRISE Fellows,

It seems like a brief time ago that we were all hauling boxes to the basement of Leverett, and in a few days, we'll be repeating the process again. The summer's coming to an end, but when we all descend down the concrete steps heaving our belongings, it will be different: we'll all have gained innumerable friendships, explored more of Boston and Cambridge together, and yes, we'll have forged a strong scientific community in these unbelievably short ten weeks.

Looking back, we've had so many experiences together as a group – whether dodging the next water balloon coming our way, enjoying cheesecake and boxed lunches at talks, gasping in awe at the whales breaching, laughing at the insanely unscientific methods in some movies, or enjoying the heated scientific debates on PRISE-list, the bonds we've made will stay with us forever, even as we return to the hustle of classes and activities in the fall. Just as importantly, we've also all spent significant time in the lab, exploring everything from stars to viruses and from computer programs to rat brains – over 100 research projects have developed this summer, and our own scientific wisdom, ideas, and perceptions have been widened by the massive compendium of knowledge from our community.

We hope that this abstract book will not only serve as a collection of the amazing work all of you have done this summer, but will also serve as a reminder of the fun times we've had and the unbelievable power of a scientific community that will be separated by location in the upcoming years but will hopefully remain together in spirit.

Best wishes for the future.

Sincerely,
Jeremy Hsu and the PRISE 2009 Abstract Book Editorial Staff

Kristen Hunter '12 • Gerald Tiu '10
Kyle Chauvin '10 • David Gootenberg '11 • Jeremy Hsu '11 • Vijay Jain '11
Kimberly Murdaugh '11 • Koning Shen '10 • Laura Starkston '10 • Denise Xu '11

ASTROPHYSICS

Kenneth Gotlieb *Astrophysics*
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Characterization of water vapor variability for astrophotometric calibration

Chris Stubbs
Dept. of Physics

All ground-based astronomical observations are subject to various atmospheric conditions, some of which are spatially and temporally variable. Many atmospheric sources of extinction have not been properly dealt with as their impact has been relatively minor. However, as improving CCD technology promises increasingly precise photometry, modeling of these small sources of error becomes necessary. Water vapor absorbs very strongly at 950 nm and therefore the water vapor content of the atmosphere determines how much flux is lost in photometric measurements in a narrow passband surrounding this wavelength. Not only would this extinction display air mass dependence, but potentially considerable spatial and temporal variability hurt precision in single-image differential photometric measurements on large field-of-view survey telescopes such as Pan-STARRS and LSST. Bright stars were tracked on a CCD imager looking through optical filters at 880 nm (off the water vapor absorption band) and 940 nm (on-band), creating the appearance of two sources on a single image. Conditions at the Cerro Tololo Inter-American Observatory are expected to mimic those at the site of the upcoming LSST. The on-band:off-band flux ratios for the two sources within each image as a star is tracked near the zenith hint at the structure of the water vapor. Despite great differences in absolute flux across images as clouds blew by, the near constant ratio betrays little variability in this case. Meanwhile, similar measurements of a star tracked to the horizon show strong airmass dependence, with the ratio decreasing rapidly as the star sinks in the sky.

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Investigating Supernova 1987A's circumstellar ring

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Supernovae, the death throes of stars, are some of the most violent events in the universe, and also some of the most illuminating. Supernova 1987A, the closest supernova observed in over 350 years, is a peculiar Type II supernova that was discovered in February of 1987. Thousands of years before the star's death, it gently expelled material through a process called stellar wind, leaving three rings of gaseous matter around the star. The innermost ring is about a light year from the former center of the star, so the shock wave from the supernova only hit the ring years after the initial explosion at the star's core. The shock front ionized the gas in the ring, displaying a necklace of spots. Analysis of this ring of spots has allowed astronomers to look into the past by providing information about the composition of the star and the nature of the star's expulsion of mass. In my project, we are specifically examining the velocity and brightness of the ring using 15 years of images from the Hubble Space Telescope. This analysis will provide further information about the interaction of the blast wave from Supernova 1987A with its circumstellar environment, and the shape and density structure of the circumstellar ring.

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Magnetic variability in M dwarf stars

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M dwarf stars are the smallest but most numerous stars in the universe. Unlike the Sun, their internal structure is dominated by convection (the large-scale motion of plasma and liquid) and as a result they cannot create magnetic fields in a similar way. This project uses optical spectroscopy from the Sloan Digital Sky Survey (SDSS) to analyze trends in the magnetic fields of M dwarf stars in an effort to better understand the processes behind their generation. With the largest sample size of over 58,000 stars, both overall magnetic activity and short-term variability will be studied by analyzing the Hydrogen-alpha Balmer emission line. The presence of H-alpha emission in an M dwarf's spectrum is an indicator of magnetic activity and by measuring the line's equivalent width over multiple 15 minute exposures we can search for variability over these time scales. For the first time we can look for dynamic events such as magnetic flares and other short-timescale variations. Initial results show that both magnetic activity and variability increase with decreasing temperature/mass. Spectral classes M5-M8 show particularly active magnetic fields and are also most likely to produce variations in equivalent width greater than a factor of ten. In the long term, such large flares may have severe implications for habitability around M dwarfs.

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Chandra HETGS observation of IRAS 18325-5926: a search for X-Ray winds around a supermassive black hole

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We analyze the Chandra High Energy Transmission Grating Spectrometer (HETGS) observation of the Seyfert 2 active galactic nuclei IRAS 18325-5926. We identify fine features in the high-resolution spectrum, which can help us understand the ionization and kinematics of a surrounding X-ray absorbing material about the source. The best-fit continuum model that describes the observation is a power-law (Gamma=1.99) with a partial covering (covering fraction 0.93). We detect a slightly blueshifted warm absorber in the X-ray spectrum, indicated by the presence of several hydrogen- and helium-like absorption features. The warm absorber has velocity -360 km/s relative to the systematic velocity, a photoionization parameter of log(xi)=2.0, and a hydrogen column density of N_H=1.6x10^{21} cm^{-2}. The absorber may signify the presence of a wind which likely originates in the obscuring torus around the black hole. The outflow velocity is self-consistent with the escape velocity at the predicted distance of the absorber. The estimated mass outflow rate suggests that the supermassive black hole in IRAS 18325-5926 plays a significant role in affecting the large-scale environment of the host galaxy. We also examine the broad Fe K emission line in the spectrum. We find that the inclination of the accretion disk is approximately 25 degrees.

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A finite square well approximation to the multi state Sommerfeld enhancement of dark matter annihilation

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Despite numerous experimental indications of dark matter, this invisible mass that makes up more than 83% of the matter in the Universe remains a mystery. WIMPs, or weakly interacting massive particles, are considered to be the primary candidate for dark matter because they interact with cross sections at or below the weak scale, which includes gravity and the weak force but excludes the electromagnetic and strong forces. Recently, however, there have been a number of unexpected observations in high-energy astrophysics, from excessive positron production to irregular microwave emissions. Each new signal either could be explained independently by some new astrophysical phenomenon or would require significant modifications to the basic WIMP picture if attributed to dark matter physics. However, Arkani-Hamed et al. (2009) proposed a novel WIMP model, where dark matter can be excited from its ground state, scatter inelastically, and annihilate via a new force in the dark sector, which provides a unified explanation for the various observed anomalies. Essential to this model is the Sommerfeld enhancement, a quantum mechanical effect that increases the cross sections of particle interactions at low velocities. Although the Sommerfeld enhancement has been studied in detail for a single dark matter state, the matrix potentials of WIMPs with excited states have been found to be somewhat intractable. To obtain an approximate understanding of the Sommerfeld enhancement's role in multi-state WIMP collisions, we consider a finite square well approximation to the matrix Yukawa-like potentials experienced by the WIMPs. We hope to find a resonance structure bearing some resemblance to the single-state Sommerfeld case.

Chemistry & Biochemistry

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Effects of chemical environment on PDZ domain binding affinity

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PDZ domains are a large family of evolutionarily conserved protein-protein interaction modules that bind to the C-termini of their targets. These domains, which exhibit distinct target sequence specificities, are often involved in scaffolding, adhesion, and other membrane-associated cellular processes. Because PDZ domains are not catalytic, their most important property is binding affinity. For example, since the cell membrane is known to experience localized fluctuations in pH, changes in binding affinity of membrane-localized PDZs and target peptides may be of physiological relevance. Furthermore, depressed pH is known to be a consequence of several disease states; for example, tumor cells' preference for glycolysis over oxidative phosphorylation leads to acidification, which in turn is known to mediate aspects of cell-cell adhesion and directed motility. To date, however, only two studies have directly examined the effects of modified chemical environment on PDZs, and these experiments have involved only a few domains each. Our goal was to examine a broader set of domains and ligands in order to better characterize the role of pH in the activity of the PDZ family as a whole. We used quantitative fluorescence polarization spectroscopy to measure equilibrium constants at a variety of physiologically relevant pH levels for interactions between representative mouse PDZ domains and rhodamine-tagged synthetic peptides corresponding to physiological ligands. For our subset of PDZs and peptide ligands, preliminary results suggest several potential trends in pH-mediated affinity changes. Further studies will involve an expanded set of peptides and characterization of PDZ localization, clustering, and other markers of function in cells subjected to varying environmental acidity.

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Harder, stronger, better, longer: improving cytokine half-life via Sortase-mediated selective transpeptidation

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The Sortase A transpeptidase enzyme is a membrane protein found in Staphylococcus aureus. Sortase recognizes the LPXTG sorting motif; upon cleaving the peptide bond between the threonine and the glycine, it catalyzes the formation of an amide linkage between the free threonine and a pentaglycine cell wall precursor via an acyl enzyme intermediate. If the LPXTG is attached to a protein of interest, a reporter probe can be fused to a pentaglycine peptide and attached to the protein of interest via sortase activity. The sortase mechanism provides a biochemical basis for attaching a non-genetically produced reporter molecule onto proteins of interest.

PEG is an oligomeric unit of ethylene oxide. Cytokines that are attached to PEG molecules, or pegylated, show higher half-life in pharmaceutical studies. However, it is difficult to control both the stoichiometry and site of attachment of a PEG molecule. Although pegylation of protein results in higher half-life, it has an adverse effect on specific bioactivity of the protein in vitro.

We propose an alternative method to produce pegylated cytokines. Four therapeutically relevant cytokines, Interferon-alpha, Interleukin-2, Granulocyte Colony Stimulating Factor, and Erythropoietin were selected for their similarity in structure. A select cytokine will be fused to the LPETG sequence and a PEG-nucleophile covalently attached to this protein via the sortase mediated transpeptidation. This cytokine should have higher bioactivity than commercially available PEG-cytokines due to conservation of the protein's native structure and a higher half-life than the wild-type form of the protein. Thus we propose a general method for increasing activity of minimally modified cytokines.

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Oxazaborolidine-catalyzed Diels-Alder reactions of aromatic compounds

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The expansion and development of catalytic, enantioselective Diels-Alder methodologies is of great importance for the efficient synthesis of stereogenic molecules. Chiral oxazaborolidine catalysts have had much success in both yield and enantioselectivity with a wide variety of substrates in Diels-Alder reactions. While thermal and Lewis acid-catalyzed cycloaddition reactions of polycyclic aromatics and styrene derivatives are precedented, there have been no robust, catalytic, enantioselective methods developed. We hope to explore the use of chiral oxazaborolidines in such reactions. Should the catalyst system prove effective in these transformations, further studies would explore the method's scope and utility in the synthesis of biologically relevant molecules.

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Metabolite profiling via cross-linking assay

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As the global toll of diabetic patients creeps towards two hundred million, and the lifestyle habits of this day and age increasingly predispose individuals to obesity and in turn diabetes, the need for a more solid understanding of the mechanisms underlying the pathology of the disease is tremendous. Neuropeptide Y (NPY), a 36 amino acid neurotransmitter regulating amalgam of biological processes within the human body, is ostensibly linked to the progression of type II diabetes due to the its documented role in the regulation of appetite, the concentration of glucocorticosteriods within plasma, and the development of insulin insensitivity. By utilizing the known interaction between NPY and its receptor in the brain, we aim to develop and optimize a technique to discover novel protein-receptor interactions involving proteins with elevated expression in mouse models of diabetes. In our method, we synthesized NPY derivatives incorporating artificial amino acid resides that promote cross-linking when subjected to UV radiation. By mixing our NPY derivatives with cell lysates from homogenized mouse brain, which presumably contained the known NPY receptor, we induced covalent protein-receptor cross-linking. Western blots of our reaction mixture stained with anti-NPY and anti-receptor antibodies should reveal an interaction by virtue of overlap in fluorescence. Theoretically, this procedure could be used to scan databases of potentially bioactive proteins suspected of playing roles in diabetes pathogenesis.

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Search for small molecule inhibitors of histone demethylase Jmjd2C

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Heritable epigenetic modifications are pieces of information stored in chromatin which effect gene expression without altering the DNA sequence. Specific patterns of epigenetic chromatin modifications contribute to cell fates such as oncogenesis, making epigenetic regulation desirable. The conserved jumanji domain enzymes (Jmjc's), discovered to have histone demethylase activity two years ago, have been implicated in both disease (e.g. cancer and neurodegenerative disorders) as well as in promising therapeutic strategies. Thus, developing small molecule probes that target these specific enzymes may have relevance for both basic research and clinical application. The research goal is to identify a small molecule that interferes with the activity of histone demethylase Jmjd 2C and prevents the removal of the methyl group on the lysine residue. The Schreiber Group focuses on systematizing the discovery of small-molecule probes through the development of stereochemically and skeletally diverse small molecule collections, as well as screening and protein target identification methods. In this study, analysis of the binding activity of this diverse set of compounds to Jmjd 2C, along with rational design based on the mechanism of Jmjd 2C demethylation, have led to the design of a 390 compound library of potential Jmjd 2C inhibitors. Synthesis of this 390 compound library, followed by enzymatic activity assays and binding assays of these compounds toward histone demethylase Jmjd 2C will potentially lead to the development of a chemical probe for related disease biological studies. Further alterations to the compounds of interest to increase specificity could lead to a competitive Jmjd 2C inhibitor which can be used to control epigenetic modifications and gene regulation in cells.

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Analyzing gene essentiality in the capsular polysaccharide pathway in *Streptococcus pneumoniae* D39

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Streptococcus pneumoniae is a Gram-positive pathogen that causes several diseases, including pneumonia, meningitis, and otitis. Rising antibiotic resistance in S. pneumoniae to current antibiotics has prompted research to find new treatments for infections caused by S. pneumoniae. Capsular polysaccharide (CPS) is a polymer on the surface of S. pneumoniae and is a virulence factor. Virulence factors are needed to infect a host, but they are not needed to survive. We postulate that a common feature of several virulence pathways is that downstream genes are conditionally essential: the downstream genes can only be deleted in a background where the first gene of the pathway has been deleted. We will test this hypothesis by studying the CPS pathway. Unmarked deletions of the first gene in the pathway will be created via a double crossover event with a 5-fluroorotate counterselection plasmid containing constructs created by overlap extension polymerase chain reaction (PCR). Downstream genes will be placed on a maltose inducible promoter, and their essentiality will be tested in wild-type and knockout backgrounds. If the downstream genes prove to be conditionally essential, this pathway can then be screened for molecules that will inhibit the production of CPS, which would prevent S. pneumoniae infections. Because the bacteria are not killed, there is less evolutionary pressure to develop resistance to these molecules.

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Magnetic nanostructures in radical chemistry catalysis

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Magnetic field effects (MFE) on the kinetics of radical pair reactions are well-established phenomena in molecular photochemistry. Pairs of electrons, each with spin 1/2, can exist in singlet (S) or triplet (T_0,T_-,T_+) states. The fate of radical pairs is governed by their spin multiplicity: singlet pairs may recombine, and triplet pairs, by Pauli's exclusion principle, cannot. Conversion between singlet and triplet states is called intersystem crossing (ISC). The goal of our project is to demonstrate magnetic field effects on chemical processes via magnetic field gradient-induced ISC, a phenomenon requiring magnetic nanostructures.

Though the magnetic energy of a single electronic spin is orders of magnitude smaller than the thermal energy, MFEs have been observed in bulk and micellar systems. A weak external field has been shown to promote radicalization and consequently to increase polymerization rates.

The gradient-enhanced magnetic field effect requires a field that varies over a distance comparable to the spin pair separation. Achieving this gradient is possible with magnetic nanostructures, such as magnetic nanoparticles and ferromagnetic domain boundaries. Magnetic Fe_3O_4 nanoparticles (10nm diameter) can produce surface field gradients of about 1000G/nm; such a strong gradient would directly alter the precession rates of electronic spins and thereby affect ISC.

We have both characterized polymerizations by FTIR and observed delayed fluorescence in excimeric complexes. Enhanced polymerization rates of styrene and butylmethacrylate have been developed with external fields. The delayed fluorescence of 2,5-Bis(5-tert-butyl-benzoxazol-2-yl)thiophene (TPD) -N,N'-Bis(3-methylphenyl)-N,N'-diphenylbenzidine (BBOT) complexes in poly(methyl methacrylate) has also been observed in bulk and on the ferromagnetic domains of computer harddisks. These high field gradients may potentially be implemented in heterogeneous catalysis of radical chemical reactions.

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Bio-medical diagnostics on paper-demonstrating DNA gel electrophoresis on paper

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Gel electrophoresis is commonly used in biochemistry and molecular biology to separate nucleic acids or proteins based on their molecular weights. Additionally, miniaturized gel electrophoresis is one of the key components of point-of-care diagnostic devices, and has broad applications in identifying genotypes, detecting and identifying epidemiological diseases, and sequencing DNA fragments. In order to reduce cost and improve efficiency for applications to developing countries, as well as allowing compatibility with other diagnostic systems developed by the Whitesides research group at Harvard University in paper diagnostics, we demonstrated the applications of DNA electrophoresis techniques to a paper substrate, while meanwhile developing a process from low-cost materials to perform the same DNA separations in a manner affordable to developing countries. To achieve our goal, we first identified the proper gel material, either agarose or polyacrylamide gel, as well as the practical voltage for building a portable device. Compared with the typical electrophoretic voltage of 140 V, it was then success to separate DNA on a paper substrate through using a lower voltage. Further applications include developing techniques in paper-based DNA amplification that would allow this paper-based genetic diagnostic process a more complete utility. These results, we believe, have a wide range of applications, such as low-cost portable devices for biomedical applications, paper diagnostic systems for developing countries as well as building new tools for molecular biology.

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Structural studies of the C-terminal domain of the O protein in bacteriophage λ

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Dept. of Molecular and Cellular Biology

Bacteriophage λ's system of DNA replication initiation bears similarities to its prokaryotic and eukaryotic counterparts. The "O-some" complex in phage λ, consisting of eight monomers of the O protein, is thought to initiate DNA replication through melting double-stranded phage DNA at the oriλ region, thus facilitating the formation of further initiation complexes in preparation for DNA replication. The rough shape of the "O-some" bears resemblance to DnaA and ORC replication initiation complexes in E. coli and eukaryotes, respectively, though the molecular structure of the "O-some" is not known. The structure of the O monomer is being parsed: O consists of two domains, of which the structure of the N-terminal portion has been solved. I work on elucidating the structure of C-terminal domain of O through X-ray crystallography, and on determining its intermolecular interactions through DNA binding assays. The C-terminal O truncation (hereafter known as λOC) was first subcloned from the whole O gene with an N-terminal histidine tag into an expression vector, then expressed in E. Coli cells. Cells were lysed to obtain λOC in the supernatant, from which the protein of interest was purified using nickel-nitrilotriacetic acid agarose batch purification and Q column ion-exchange chromatography. Size exclusion chromatography and laser light scattering revealed λOC in solution to be a monomer of about 20 kilodaltons. Crystallization trials are in progress to determine optimal conditions for λOC crystal formation.

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2D peptidomics profiling for bioactive gut peptides to elucidate biological pathways

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The term hormone refers to a chemical that is released from a tissue to regulate a physiological process. In metazoans, bioactive peptides serve as a major class of hormones and regulate diverse functions, including glucose homeostasis (insulin), hypertension (angiotensin), and social behavior (oxytocin). Global peptide profiling (peptidomics) has emerged as a powerful tool to directly probe physiological peptide levels, including those of bioactive peptides. However, studies using this technique have yet to focus on peptides in the gut, a tissue thought to contain numerous bioactive peptides. We are interested in profiling the gut specifically for peptides involved in signaling pathways related to feeding and hunger. To that end, we profiled the guts of mice in fed vs. fasted conditions to detect changes in individual peptide levels using a two dimensional peptidomics profiling workflow centered around a liquid chromatography-mass spectrometry platform for in-Dept.h coverage of the gut peptidome. Following the gut peptide profiling, we plan to inject the individual peptides we discover into our mouse models to detect phenotypes with the hope of assigning specific physiological roles to the peptides. The long-term goal of this endeavor is to elucidate the biological pathways that involve the bioactive peptides we discover in our profiling studies to give insight into the biomedically relevant physiological processes that underlie feeding, hunger, and satiety.

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A cell model for studying CGRP metabolism

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Calcitonin gene related peptide (CGRP), a bioactive peptide involved in vasodilation, is produced by the central nervous system. Like other neuropeptides, CGRP is released from neurons and binds to cell surface receptors throughout the body. Recent peptidomics experiments performed in the Saghatelian lab indicate that CGRP might be regulated by insulin degrading enzyme (IDE), a metalloprotease. My goal was to create a cell model using TT cells and PC12 cells in order to elucidate whether IDE regulates CGRP. TT cells, which are derived from human medullary thyroid carcinoma, were selected because they are known to express CGRP. PC12 cells, which originate from a neuroendocrine tumor of the rat adrenal medulla, normally produce IDE. To study the relationship between CGRP and IDE, I aimed to identify whether TT cells also produce IDE, and to express CGRP in PC12 cells so that each of the cell lines would have both the enzyme and its potential neuropeptide substrate. The CGRP DNA can be cloned and transfected into the PC12 cells, allowing them to overexpress the neuropeptide. Once both cell lines express CGRP and IDE, I can establish an assay to screen for IDE inhibitors. Small interfering RNAs (siRNAs) can be used to silence the gene that codes for IDE so that I can further study the relationship between IDE and CGRP. If this pathway is relevant, it could offer a way to regulate CGRP in vivo.

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Discovery of biologically active small molecules via *in vitro* selections of a DNA-templated macrocycle library

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DNA-templated organic synthesis (DTS) enables scientists to use biological principles to both synthesize diverse organic compounds and discover biologically active small molecules. Liu and co-workers have used DTS technology to synthesize a 13,000-member DNA-conjugated small molecule library consisting of peptide-based macrocycles. These macrocycles are labeled with unique, amplifiable, information-carrying DNA barcodes that allow for selection of biologically active compounds. By taking advantage of the DNA-labeled molecules, researchers can perform single-pot selections against an entire library without having to carry out thousands of individual assays for each unique compound as in a traditional small molecule screen. In this study, I will attempt to discover biologically active small molecules by selecting for binding to several protein classes using the 13,000-member macrocycle library. Current studies in the Liu group using this library have already uncovered several micromolar kinase inhibitors, including molecules that inhibit kinases implicated in several different types of cancer. This project will expand upon this work and will attempt to select for macrocycle inhibitors of proteases, histone deacetylases, and histone demethylases. Given the relatively large size of the library and the peptide-based structure of the macrocycles, it is the hope that selections will uncover potent inhibitors for these enzymes and will in the long-term reveal molecules with potential biomedical applications.

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Asymmetric synthesis and application of bis(phenylthio)cyclohexane and related ligands

Elias James Corey
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The total synthesis of natural products and pharmaceutical compounds depends on developing a wide variety of efficient, enantioselective transformations. Most of these transformations utilize either an enatiopure catalyst or a transition metal catalyst with enatiopure ligands to induce selectivity. Compared to chiral phosphines, amines and oxides, chiral sulfides and sulfoxides constitute an underdeveloped ligand set for asymmetric catalysis, despite sulfur's generally strong bond to common catalytic metals. Bis(thioether)cyclohexane derivatives represent an attractive class of C2-symmetric ligands for further research because they should be readily accessible in enantiopure form, are less susceptible to oxidation than corresponding phosphines and posses different bonding properties than amines. Existing syntheses of bis(phenylthio) cyclohexane either depend on chiral starting materials or proceed with poor yield and enantioselectivity. In this research, a highly selective route from inexpensive, readily available, racemic starting materials such as cyclohexene oxide and cyclohexene was sought. Enzymatic routes were initially pursued to separate the enantiomers of key alcohol intermediates prior to displacement with the appropriate thiol. Poor resolution with a variety of lipases turned our attention to kinetic resolutions using organocatalysts and research continues on an efficient route to the enantiopure alcohols. Once in hand, these intermediates should allow ready access to a variety of bis-thioethers and sulfoxides, with potential applications to many areas of asymmetric catalysis.

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Scope of palladium-catalyzed, substrate-directed C-H bond functionalizations

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Palladium(II) complexes can catalyze substrate-directed C-H bond functionalizations, in which a C-H bond is broken and a C-C or C-heteroatom bond is formed. The specific C-H bond broken is controlled by a directing group—usually a nitrogen-containing functional group—on the substrate itself. For example, in the presence of benzo[h]quinoline and $PhI(Cl)_2$, an oxidant, $Pd(OAc)_2$ can catalyze C-Cl bond formation, giving 10-chlorobenzo[h] quinoline as product. Previously, this reaction was thought to occur through a monometallic, Pd II/IV pathway, in which one metal center is oxidized by two electrons to give a Pd (IV) hexacoordinate intermediate, which is then thought to reductively eliminate to give the chlorinated product. However, the Ritter group has recently isolated a bimetallic Pd (III) intermediate from the above reaction, supporting a bimetallic Pd II/III pathway as a mechanistic alternative. We are now using this mechanistic knowledge to guide reaction discovery. Currently, C-Cl, C-Br, and C-OAc bond formations are possible, and we are working on developing $C\text{-}CF_3$ and $C\text{-}OCF_3$ bond formation reactions.

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Enhancing the interface between silicon nanowire field effect transistors and biological systems

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Silicon nanowire field-effect transistors (Si-NWFETs) have previously been used for recording signals from electroactive biological systems such as hearts, cultured cardiomyocytes and neurons. NWFETs are particularly useful due to their cell-relevant size scale and three-dimensional architecture; however, these signals are still limited in magnitude by the interface between the cell and device itself. We have devised a method to fabricate devices that takes advantage of the inherent flexibility of nanowires in order to minimize the spacing between cultured cardiomyocytes and devices. Devices are positioned in dense arrays and can be measured simultaneously and analyzed to calculate signal propagation rates. We found a significant decrease in device performance after prolonged exposure to cell culture, and therefore have adopted a technique developed in our lab of bringing cells cultured on another substrate, polydimethylsiloxane (PDMS), into close contact with our devices using an x-y-z micromanipulator. This method has made it easier to measure extracellular potentials of cardiomyocytes at cellular and sub-cellular resolution.

Computer Science

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Lattice cryptography: complexity considerations

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Cryptography based on lattices has two distinct advantages over standard number-theoretic methods: First, lattice techniques often lead to surprisingly efficient schemes due to lattices' simple additive structure (such as NTRU, or more recent LWE-based schemes). Perhaps more importantly, many lattice schemes enjoy very strong security proofs based on the worst-case (as opposed to average-case) hardness of standard lattice problems-a phenomenon so far unseen in other branches of Cryptography. This strongly motivates the study of the complexity of the underlying worst-case lattice problems. For example, a result of Lyubashevsky and Micciancio ('09) shows that the Unique Shortest Vector Problem (uSVP), previously thought to be "easier" than its unrestricted variants (such as GapSVP), is in fact just as hard up to a small polynomial factor, and thus the Ajtai-Dwork cryptosystem ('97) is secure under a worst-case GapSVP hardness assumption.

We seek similar hardness results relating to other standard lattice problems. As a concrete example, we seek to improve the methods of Liu et al. ('06) to show the (randomized) NP-hardness for Bounded Distance Decoding for approximation factors smaller than the current best result of $1 / \sqrt(2)$.

We also study the cryptographic implications of the relationship between lattices and their natural generalization, quadratic forms. Quadratic form cryptography has the added benefit of requiring only constant-dimensional forms, whereas lattice problems only become hard as the dimension grows. We seek to combine these two bodies of work, combining the efficiency benefits of quadratic forms with worst-case hardness techniques from lattices.

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From MIDI to cycles: analyzing the music of Franz Schubert

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Transformational music theory emerged in the late twentieth century as a way of explaining musical passages that elude analysis by traditional diatonic methods. The objects in this theory are not harmonies, but rather the operations, or transformations, between harmonies. My project focuses on the basic Neo-Riemannian transformations P, L, and R, which form mathematical groups under function composition over the major and minor triads. Due to group closure, repeated application of some combination of P, L, and R on a chord generates a sequence of chords beginning and ending with that chord, known as a "cycle". Although music theorists have identified harmonic cycles of this kind in music by hand, I am not aware of any comprehensive study; my goal is to accurately and efficiently find all cycles in the music of Schubert through automation. Because music, like natural language, is not governed by a fixed set of rules, this problem is computationally expensive--and intractable in the general formulation. Past efforts in music information retrieval have used various artificial intelligence models to generate harmonic analyses. My approach uses graph search, hidden Markov models, and dynamic programming to find harmonic cycles by exploiting their musical properties.

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3-dimensional stereoscopic special effects

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Special effects in traditional 2-dimensional cinema are well understood. However, aside from studies on adjusting baseline and convergence, effects in 3-dimensional stereoscopic movies are largely uninvestigated. This project created the framework for studying various stereoscopic special effects by using a PBRT-based renderer with photorealistic scenes and pairs of controlled cameras. This project is interested in how varying conventional parameters (e.g. focal length, aperture, shutter speed) impacts the stereoscopic experience. In addition to those parameters, the framework also allows adjustments to the image space (resolution, color space, shifted color bands, contrast curve, frequency), scene space (contrasting frequencies, partially visible objects), scene time (motion blur, time offsets), and stereo geometry of each individual camera in a scene. We are most interested in how varying those parameters between cameras impacts the stereoscopic experience. The framework will be used to conduct perceptual studies on stereoscopic 3-dimensional scenes and animations with varying parameters between the left and right cameras.

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Exploring the capabilities of network monitoring of CitySense

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CitySense is a planned 100-node wireless sensor network being deployed in Cambridge, MA. It consists of embedded computers with sensors measuring weather, air quality, and noise pollution, each equipped with dual 802.11 radios and mounted on streetlights and rooftops around the city. CitySense nodes are connected to the Internet via a wireless mesh network that allows for rapid node deployment and network scalability without incurring the high cost of a wired or cellular network connection. In addition to pollution monitoring, CitySense is also capable of passive wireless network monitoring. To date, the majority of network monitoring has focused on indoor settings. This study explores network monitoring on a complex, outdoor urban environment using eight fixed sniffer nodes; specifically, the study attempts to evaluate the capability of locating a single device within this noisy environment with a low number of sniffer nodes. The study uses a single device to transmit 802.11 radio packets at various locations while the passively sniffing CitySense nodes record the packets. The metrics used to evaluate the nodes' capability include the ratio of captured packets to transmitted packets, the received signal strength indication according to 802.11 protocol, and the difference in time between sending and receiving each packet. The initial results confirm the noisy nature of the environment, additional analysis will offer more insight on outdoor urban wireless network monitoring. Further studies are planned to explore the rate of transmission on the quality of monitoring.

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The evolution of group solidarity

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Our study seeks to investigate how individuals who interact over time form notions of groups. Group solidarity is the propensity to favor one's own group members and disfavor members of other groups. Past studies of group solidarity have imposed group affiliations at the beginning of experiments, based either on existing preferences (e.g. Democrat or Republican) or on arbitrary differences (e.g overestimating or underestimating the number of dots in an image). However, in real life, groups can evolve dynamically over time, as a function of different sorts of interaction (e.g. meeting people on a plane, soccer practice). Our research therefore attempts to gauge the development of group solidarity in the absence of advance divisions.

We investigate this using Colored Trails, a general framework for studying human and computer decision making that provides an analogue to task settings. In our setup, we create a board game with a 10x10 grid and icons representing six participants and two goals. We then simulate a weak form of interaction by asking the subjects to follow either of the two randomly-moving goals around the board. At the end of the movement task, we use standard forms of measurement from the social science literature (e.g. dictator games) to evaluate group solidarity. We hypothesize that solidarity will increase in proportion to the duration of the movement task, and will be strongest amongst players who remain near each other as they move around on the board.

The study has implications for cognitive science and for the development of AI agents that need to interact realistically in group situations.

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Swarming algorithm

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Swarming is a phenomenon widely observed in nature in populations of insects, birds, and fish, who self-regulate group movement in order to explore terrain efficiently in search of nutrients and defense against predators. Artificial swarm literature focuses on elementary computer and robotic models using spring-like linear forces or otherwise rough assumptions. Such assumptions include identical agents and unbounded velocities. Limitations of current models include large discontinuities in particle accelerations, the implicit assumption that distance perception is Euclidean, and neglect of frictions. Biological literature has shown that swarming populations frequently exhibit non-visual communication (e.g., chemical recognition). I propose a model with more realistic forces that uses non-Euclidean distance perception. I use a logarithmic distance model to mimic chemical perception. In order to ensure physical plausibility of the model, I take into account frictions with the environment, as well as limitations on acceleration, speed, and angle of turn. Friction force ensures asymptotic stability of the system, which is not guaranteed by other models. I present the results that several neighborhood criteria and single and multiple swarm leadership produce. These swarm models have the ability to better follow evading prey while performing movements much closer to those of swarms in nature. Therefore, they are better suited both for biological swarm modeling and for robotic swarm applications.

Engineering & Bioengineering

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Constructing light-switchable display and communication in *Saccharomyces cerevisiae*

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The goal of this project is to genetically engineer a photointerconvertible system of optical communication in the yeast *Saccharomyces cerevisiae*. To build such a pathway we combine a light-detecting component and a light-emitting component within the framework of the yeast two-hybrid system. Light detection occurs at the phytochrome PhyB, a plant photoreceptor sensitive to both red and infrared light. When treated with red light in the presence of the small molecule phycocyanobilin (PCB), PhyB binds to the protein PIF3, causing a conformational change that promotes the expression of the gene firefly luciferase. When subsequently treated with infrared light, the phytochrome reverts to its inactive conformation, halting the production of the gene. Light emission results from the luciferase-catalyzed oxidation of the pigment luciferin and can be tuned to emit in the same wavelength range as the phytochrome's absorption spectrum. It is necessary to supplement the system with PCB, either exogenously or by directly integrating the PCB biosynthetic pathway, normally found in lower plants and cyanobacteria, into the yeast genome. One proof-of-concept application of such a system is a light-switchable display, or "cellular blackboard," wherein a red laser pointer functions as the writing utensil and an infrared lamp as the eraser. Another potential application is a system of optical communication among a population of *S. cerevisiae*, not unlike fiberoptics. Using red light as a means of communication, yeast populations can be induced to perform tasks such as mating and interacting with other species of organisms.

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Spatial guidance of *in vitro* angiogenesis via induction of pH gradient

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Angiogenesis, the process by which new blood vessels are formed from pre-existing blood vessels, is vital for the development, maintenance and repair of vascular tissues and networks. Furthermore, this process is a fundamental step in tumorigenesis, where a tumor transitions from dormant to malignant state. The first step in angiogenesis is the sprouting of endothelial cells from a cell wall. There has been research on how changes in extracellular and intracellular pH have the capability to control certain cell behaviors such as protein synthesis and cell cycle. Therefore, by examining the extracellular pH environment in a sprouting endothelial cell, there is a gain in understanding how to control and restrict angiogenesis, which will have positive ramifications on the many diseases that are in question. It has been indicated that there is higher binding of vascular endothelial growth factor (VEGF) at slightly acidic conditions. This study investigates the feasibility of using a pH gradient to guide vessel formation. A sustainable pH gradient was established in an in vitro 3D fibrin gel-sprouting assay using Human Umblical Vascular Endothelial Cells (HUVECs). Maintenance of the pH was secure for at least 2 hours with buffering by sodium bicarbonate and HEPES. By placing media of differing pH across a fibrin gel, we created a pH gradient that may also result in a VEGF gradient. In our preliminary results, we have indicated that the pH gradient is able to directionally guide endothelial sprouting, possibly due to its relationship to VEGF gradient.

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Swarm behavior in bristle-bots

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It has long been known that a vibrating surface can induce movement. Spread millions of sand particles across a vibrating plate and they will move into a harmonic pattern; replace the sand with a heavy box of grain and its contents will settle and compress. If the surface is tilted at an incline or covered with angled bristles, a directional bias can be introduced and even very large particles can be controlled. Applications are far-reaching in both industry and nature, in many cases using a different source of vibration and bias than those outlined above. Manufacturing processes often use a vibrating bristled surface for sorting and conveying parts; snakes and worms use their own surface bias (i.e. scales or bristles called chaetae) and internally driven undulations to move across the ground; certain carnivorous plants exploit an insect's frantic movement to trap it through a bristled funnel. Recent inventions include a self-propelled vibrating pipe cleaner and the viral "bristle-bot" made from a toothbrush head and vibrating pager motor.

Our research focuses on the bristle-bot, first as an individual particle and then as a member of a swarm. We resolve the direction and velocity of the bristle-bot as a function of (a) bristle angle, (b) motor chirality and (c) its center of mass with respect to the center of vibration. The efficiency and load characteristics are then found

as a function of (i) bristle height, (ii) density and (iii) stiffness, as well as (iv) bristle-bot mass and (v) surface friction. An attempt at characterizing bristle oscillation is made. Finally, a multi-particle system is introduced as a macro-scale model of the fluctuation-dissipation theorem in statistical thermodynamics.

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Novel user interface for lab-on-a-chip technology

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We created a touch screen interface for a chip that allows users to click and drag single living biological cells and chemical droplets in real time with their fingers. The hardware and user interface to perform lab-on-a-chip experiments have been almost exclusively accessible to specialized physicists. This interface will make such technology practical for doctors, medical researchers, biologists, and chemists. The user places a droplet of cells or chemicals onto the chip and can immediately start controlling their movement. The size, shape, number, and function of the cell traps can be instantly manipulated and customized by the user. To our knowledge this is the first application of multi-touch technology to interact with cells. Further developments might allow for programmable automated experiments performed on a microfluidic chip.

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Inability to learn two conflicting motor tasks even when aided by contextual cues

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Our active interactions with the surrounding environment rely on the output of the motor division of the central nervous system. Previous studies have shown that we are able to adapt to changes in the environment where our motor system operates, but despite this, our ability to adapt to rapidly changing (within seconds) dynamic environments has not yet been fully ascertained. To elucidate this, we asked healthy human subjects to perform planar reaching arm movements with quick and straight trajectories based on visual cues while grasping a robotic manipulandum. After becoming acquainted with the passive dynamics of the manipulandum, the subjects were then perturbed by a velocity-dependent curl force-field that pushed their movements in a direction perpendicular to their reach. The direction of the force-field randomly alternated between clockwise to counterclockwise, but subjects were made aware of the upcoming directions by corresponding audiovisual cues. When learning these conflicting FF in neighboring – though not identical – directions, the subjects were able to adapt, compensating with a comparable but opposite force. Moreover, we found that in a condition of low-interference (the FF-induced disruptions did not overlap in movement space) subjects exhibited significantly higher generalization – transfer of learning to adjacent directions – than in a condition of high-interference. When the corresponding directional and audiovisual cues were swapped, subjects typically compensated for the FF appropriate for the direction, suggesting that the target's position acted as a cue that outweighed the non-spatial information. Furthermore, when subjects were later presented the conflicting FFs along the same movement directions, they were not able to adapt, even when given the aforementioned predictive cues.

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Use of alginate hydrogels to direct dendritic cell fate towards antigen specific tolerance

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Dendritic cells are the key link between the innate and adaptive immune system. They are particularly significant due to their role in activating T cell mediated immune responses. An important challenge is to direct dendritic cell fate in response to specific antigens, thereby directing T cell fate downstream. The overall objective of these studies is to guide dendritic cell fate in vivo towards antigen specific tolerance. Previously, in vitro studies were performed on bone marrow derived dendritic cells to determine the effects of dexamethasone and LPS on activation. Flow cytometry showed increases in MHCII, CD80, and CD86 expression in response to culture with LPS. Dexamethasone, however, was found to decrease expression of these surface proteins with sufficient efficacy to counteract LPS stimulation. Based on this work, design and testing of a novel mechanism for antigen specific treatment for autoimmunity in mice was undertaken. The proposed treatment consists of implantation of alginate hydrogels containing antigen, dexamethasone, and GMCSF, prior to attempted induction of disease. Preliminary results of in vitro studies have shown alginate alone to have negligible effect on dendritic cell activation. In vivo studies of the treatment's efficacy are currently underway. It is expected that implantation of this scaffold will reduce the severity or entirely prevent induction of disease in murine models of autoimmunity as compared to controls.

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Probing biofilm formation of pseudomonas aeruginosa PA14 on abiotic surfaces

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Mature biofilms engender a relatively secure and robust environment for individual bacterium cells, in essence, because they provide a framework for microbial adhesion, reproduction, and communication. The biofilm constitutes of cells enclosed by a matrix of exopolysaccharides which has both structural significance by enclosing individual cells and functional significance by facilitating signaling and communication, both factors which greatly increase bacterial pathogenicity. Biofilms have been studied frequently in the last few decades on agar and nutrient-rich solutions, but in recent years, unwanted biofilm growth on prostheses, industrial pipelines, dentures, and other abiotic surfaces has inspired new interdisciplinary questions. With regard to the stages of biofilm development, initial microbial adhesion has been assumed to be precisely a surface chemistry and surface topography problem. Here, this project has observed for PA14 Pseudomonas aeruginosa, self-assembled monolayers of alkanethiols rather than monolayers with hydrophilic head groups induce more rapid biofilm growth as revealed by crystal violet staining for general biomass as well as selective matrix and fluorescence cell staining. Furthermore, nanopost arrays of critical dimensions and orientations have been observed to encourage optimal cell packing for bacterial adhesion rather than serving as mechanical inhibition. The project will continue to seek such combined surface chemistry and surface topography variables for further P. aeruginosa and E. coli strains, particularly mutants with enhanced quorum-sensing genes.

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Developing tools for *in vivo* measurement of the properties of the mitral annulus

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Mitral valve prolapse is a heart disorder estimated to occur in 2-3% of the U.S. population. Surgical repair of the mitral valve typically involves opening the chest and placing the patient on cardio-pulmonary bypass, but these procedures can increase infection risk and patient recovery time and lead to tissue damage and decreased post-operative cognitive function. The Harvard Biorobotics Laboratory is developing an instrument for minimally invasive, beating-heart mitral valve surgery that will avoid these adverse effects. However, it is necessary to first understand the properties of the mitral annulus and the forces the instrument will encounter in the heart environment. The focus of this project is the design of the tools necessary to measure the properties of the heart tissue at the mitral annulus in vivo. We evaluated various methods of tracking the position of fast-moving heart tissue and instruments, choosing 3D ultrasound (3DUS) for its low cost, ease of use, and ability to rapidly image soft tissue. To facilitate pulling on the tissue around the annulus, several methods of attaching to the heart tissue were developed and evaluated for strength, stability, and ease of insertion. We also created various fiducial designs to improve the visibility of these attachments under 3DUS, exploiting certain artificial geometries that stand out in a noisy 3DUS environment. This additionally allows image segmentation for finding the attachment location and displacement to be automated. Upcoming work will focus on developing a force sensor to complement the displacement measurements enabled by the attachments and 3DUS.

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Designing a scaffold-based prophylactic vaccine for breast cancer

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Cancer cells often display mutated proteins on their surface that allow them to become targets of immune response. The first step of this immune response involves activation of dendritic cells (DCs), which sample antigens from tissues, migrate to lymph nodes, and then go on to activate other immune cells such as B and T cells against these antigens. One approach to treating cancer is targeted induction of an immune response against cancer-specific antigens. While manipulation of DCs ex vivo has proved unsuccessful thus far, an in vivo vaccine that recruits DCs into a polymer scaffold containing tumor lysate has shown promising prophylactic results in murine melanoma models. Here we aim to design a similar scaffold-based vaccine for breast cancer. Tumor size data indicated that scaffolds incorporating breast cancer lysate induced smaller tumors than blank scaffolds. Interestingly, scaffolds extracted from vaccine mice were enlarged as compared with blank scaffold, suggesting scaffolds successfully recruited DCs. Continued study into the effect of tumor lysate on dendritic cell activation in vitro will enable better manipulation of DCs in in vivo murine models, and could ultimately have application in the generation of patient-specific immune responses.

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Engineering memory in complex eukaryotic cells

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The ability to logically engineer constructs to perform novel cellular functions provides deeper understanding of biological systems and has potential applications including drug treatment and disease discovery. Ajo-Franklin, et al. (2007) has designed a galactose-in-

ducible memory loop in yeast cells in which galactose triggers the activation of a positive feedback loop. Each 'trigger' gene consists of a DNA-binding domain, a red fluorescent protein mCherry, the viral activator VP64, and a nuclear localization sequence (NLS), all under control of a GAL1/10 promoter. Each reporter gene consists of DNA-binding sites corresponding to its given transcription factor, and its protein coding region encodes a yellow fluorescent protein Venus. Upon transfection into larger mammalian cells (i.e. U20S cells), however, the memory loop failed to initiate despite attempts at optimization. Three potential explanations are premature degradation within the cell, dimerization failure of lexA, and transgene blocking. Our project involves reengineering this memory loop to account for these three factors, implementing both different 'triggers' and different transcription factors/binding domains. Firstly, the redesigned circuit will respond to a wide range of inducers: doxycycline, histone methylation, or damage to cells. Secondly, the circuit will test a few transcription factors: full lexA to allow for required dimerization at the binding domain, full lexA linker to remove potential steric hindrance at the binding site, and zinc finger proteins native to mammalian cells. In conclusion, this project aims to predictably engineer large eukaryotic networks, in order to pave the way for complex synthetic devices that will have positive long-run applications.

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Limitations on methane release and production in terrestrial wetlands

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Methane is an extremely effective greenhouse gas, with 20 times the heat trapping effect of an equivalent amount of carbon dioxide. As Arctic permafrost thaws, the release of methane could cause drastic increases in the rate of climate change. Wetlands are the largest natural source of methane, yet scientists lack a quantitative understanding of how methane emissions from wetlands change over time. We believe anaerobic methane production is oxygen limited, and tested this hypothesis by combining fieldwork at Sallie's Fen in New Hampshire with direct laboratory manipulation. After collecting peat samples from a New Hampshire wetland, we created parallel incubations with air atmospheres and nitrogen atmospheres completely lacking oxygen. We compared methane production rates using a flame-ionization detector on a gas chromatograph. We also tested microbes as limiting reactants by setting up incubations lacking various fungi or bacteria and comparing methane production rates. Additionally, we made high time-resolution measurements of carbon dioxide and methane across the wetland surface over a period of 24 hours. We cryogenically separated the gases using a vacuum line with liquid nitrogen, a capillary, and a water trap. We compared the covariance between these concentrations and the isotropic composition of carbon dioxide to understand if methane is produced at the surface boundary layer.

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Multiplex automated genome engineering

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Multiplex automated genome engineering (MAGE) is a technology for rapidly generating targeted genomic mutations in parallel. Our technique utilizes single-stranded oligo directed mutagenesis mediated by the lambda-red recombination system in E. coli. We constructed a prototype device that can cyclically carry out this procedure and utilized it to metabolically engineer the 1-deoxy-d-xylulose-5-phosphate (DXP) biosynthesis pathway for lycopene synthesis in E. coli by directed evolution through simultaneous mutagenesis of twenty-four sites. After three days, we created over 12.6 billion variants and were able to isolate clones that had over fivefold greater lycopene production. The MAGE technology enables rapid creation of phenotypic variants for the engineering of biological organisms.

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Engineering macroporous biocompatible hydrogels

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Emerging from the junctions of material, biological, and physiochemical science, biosynthetic scaffolds are recreating in vivo micro-environments that aid the process of tissue regeneration. These engineered structures promote cellular attachment and migration, present soluble signals for cell-scaffold mediated interactions, and allow for nutrient diffusion. Fibrous scaffolds, with their highly interconnected void volumes (>90% air), have been successfully used in several tissue engineering applications because they provide a large surface area that facilitates both nutrient diffusion and cellular infiltration. Hydrogel materials typically have negligible void volume which prevents cellular infiltration, but offer precise control over soluble signal delivery, enabling easy manipulation of cell-scaffold interactions.

Our study aims to develop a two-phase alginate hydrogel system that contains both the micro-environmental control of a traditional hydrogel and the interconnected voids characteristic of fibrous scaffolds. We developed a system that consists of rapidly degrading porogens (oxidized alginate beads or sucrose crystals) embedded within a non-degrading bulk alginate hydrogel. With a high porogen to bulk gel ratio, interconnected macropores develop within the bulk alginate hydrogel when the porogen degrade. As evidenced by cell migration assays, cell infiltration occurred within one week in our newly developed system. With water-wicking assays and Environmental Scanning Electron Microscopy, we have started to characterize the physical and biological properties of our scaffolding system and plan to continue exploring how porogen composition, size, and degradation rate, affect cell infiltration. Once fully characterized, this system will be highly significant to the field of bioengineering as it can potentially serve as a non-invasive, injectable delivery mechanism for clinical cell therapies.

Mathematics, Statistics, & Economics

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401(k) savings

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Retirement savings in the United States are funded principally through tax protected accounts known as 401(k) plans. 401(k) participation and contribution rates are known to be significantly affected by factors such as default status, that are ignored in classical economic analysis. The work of professors David Laibson, Brigitte Madrian, and James Choi has focused on identifying which of these behavioral factors are most important for savings outcomes and suggesting policy measures in response. Among recent findings is the gap in contribution rates between employees who contribute an absolute amount to their savings plan and those who contribute a fixed fraction of their salary. Our group has designed a field experiment that will test the framing effect of absolutes versus percentages through a randomized mailing of different types of encouragement letters to employees of a particular company. Also in progress is the development of an online tool which allows employees to see the participation and contribution rates of others in their company, as categorized by demographics such as age and income level. By analyzing use of the tool and changes in employee behavior after implementation, we will be able to test the magnitude of 'peer effects' on savings decisions. Finally, we are beginning to study the dynamics of membership in the still relatively underutilized Roth 401(k) pension scheme, which is ideal for individuals who expect their tax income brackets to have risen by retirement.

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Cobordism and homotopy theory

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Cobordism theory calls two n-dimensional manifolds cobordant if they make up the boundary of an (n+1)-dimensional manifold. Under this equivalence relation, we attempt to classify manifolds by identifying invariants of the cobordism classes. Cobordism was first introduced by H. Poincaré in 1895. L. S. Pontryagin demonstrated in 1938 that the cobordism groups for framed manifolds are isomorphic to certain homotopy groups of spheres. Calculating the homotopy groups of spheres was and remains one of the major open problems in algebraic topology, and Pontryagin's result suggested that the tools from cobordism could be used to examine the homotopy classes of maps between spheres. R. Thom's 1954 paper laid the framework for identifying the cobordism ring for a given class of manifolds to the homotopy ring of a particular spectrum. For many important classes of manifolds, the resulting homotopy problem proves easier to solve than the original cobordism problem. Rather than applying cobordism to homotopy theory, we can turn the approach around and apply homotopy theory to the cobordism problem. We explore this technique and explain the calculations for several standard classes of compact manifolds, including the cobordism classes of framed, unoriented, oriented, complex, and spin manifolds.

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An evaluation of improvements on the sequential parallel comparison design

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Recent research has shown that the placebo response is a growing problem in clinical trials, especially for psychiatric disorders. As more patients respond positively to placebo, it has become difficult for investigators to determine the efficacy of a particular treatment in the traditional double-blind, placebo-controlled clinical trial. Researchers have tried to address the problem with novel clinical trials, which have not had much success. Most recently, a group led by Dr. Maurizio Fava at the Harvard Medical School proposed in 2005 the Sequential Parallel Comparison Design (SPCD). In SPCD, there are two phases in the trial, and patients are reallocated to drug or placebo in phase two based on their response in phase one. The goal is to reduce the overall placebo response rate and the number of patients needed. The design has yet to be widely accepted, and only one trial is currently using SPCD. This project aims to improve upon SPCD through an interim analysis to reduce sample size and duration of trials. Computer models are created to test empirically the new design. Preliminary results show that the interim analysis design match the power of SPCD and reduces the length of the trial for many patients, which is a savings in resources. Further testing needs to done to generalize the results to broader sets of parameters, and other criteria will be used for the interim analysis as well.

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A statistical approach to protein folding

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Predicting a protein's tertiary structure from its primary amino acid sequence is an important problem in biology with applications to the design of drugs and vaccines. Computationally, this amounts to finding the conformation with the lowest energy. However, finding the ground state is an intractable problem, and approximation algorithms must be sought. Traditional approaches have typically focused on determining local structures first and then inferring the global structure from the local structures. We instead propose a Markov chain Monte Carlo (MCMC) approach that uses fragment regrowth via energy-guided sequential sampling (FRESS). At each iteration, a fragment of varying length is cut out of the protein and regrown. Residues are regrown one-by-one by sampling torsion angles, bond lengths, etc. from a database of proteins whose structures are known. The energy of the new conformation is calculated by considering the interaction energies of the atoms, and this proposed conformation is then accepted or rejected according to a probability rule favoring conformations with lower energy. Previously, the FRESS algorithm was applied to 2D and 3D lattice models, where it matched or outperformed other algorithms in finding lower energy levels. The task now is to extend the FRESS model beyond the basic lattice model.

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The Khovanov homology of pretzel knots

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Knot theory seeks to distinguish knots (closed tied up loops embedded in 3-space) into isotopy classes. Two knots are isotopy equivalent if they can be stretched and moved around so that they look identical, without needing to tear open the knot. Knot theorists prove that two knots are not equivalent by calculating isotopy invariants, mathematical objects whose values are the same for knots that are isotopy equivalent. One classical knot invariant is the Jones polynomial, which distinguishes knots up to 9 crossings but is not as effective for more complex knots. In order to capture more information about a knot, Mikhail Khovanov found a way to categorify the Jones polynomial in the 1990s. His invariant is now known as Khovanov homology. It contains the information of the Jones polynomial, as well as additional information about the knot, making it a stronger invariant. Although Khovanov homology can be computed algorithmically, the direct computation grows exponentially with the complexity of the knot. It is known how to compute the Khovanov homology of a quasi-alternating knot over rational coefficients from the Jones polynomial and the knot signature. We consider infinite classes of pretzel knots that are not known to be quasi-alternating. We use techniques from algebraic topology and apply some results of Eun Soo Lee and Jacob Rasmussen to calculate the Khovanov homology over rational coefficients for these infinite classes.

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On the representation theory of Lie algebras

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Representation theory is a branch of mathematics dealing with the study of algebraic structures by identifying their elements with linear transformations of vector spaces. One such algebraic structure to which representation theory is applied is the Lie Algebra. Lie Algebras were first introduced by Sophus Lie in an attempt to study infinitesimal transformations. In particular, Lie discovered that studying certain linear structures arising from vector fields corresponding to so-called Lie groups could give insight into the theory of continuous transformations. These linear structures are now said to possess the properties of a Lie algebra. Lie algebras are used to study differential manifolds and Lie groups. They are also important in physics, where they explain the properties of various particles and correspond to symmetries of the universe. The goal of this project is to study in detail the representation theory of Lie algebras, in particular, a special class of Lie algebras known as semisimple Lie algebras. In particular, we answer questions regarding the geometry of this representation theory.

MICROBIOLOGY

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Trichomonas vaginalis virus: a little-studied virus with a big impact

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The parasite Trichomonas vaginalis causes Trichomoniasis in humans, the most common STD in the world. The WHO has estimated T. vaginalis infects approximately 180 million people a year worldwide, resulting in preterm delivery, pneumonia, oral lesions, and increased mortality. In addition, recent studies have shown that the parasite predisposes humans to HIV infection and AIDS.

About half of all T. vaginalis parasites are infected with a non-enveloped dsRNA virus called Trichomonas vaginalis virus (TVV), of which there are three known types (TVV1, TVV2, and TVV3). Studies have shown the virus increases the pathogenicity of the parasite, making the virus's study pertinent to the global health community.

Currently, I am working on identifying more TVV viruses (potentially even a TVV4) from lysed T. vaginalis cells and analyzing their genomes for putative ribosomal slippery sequences and pseudoknots. Additionally, we will begin examining the frameshifting mechanisms TVV uses to translate its two viral proteins: the capsid protein and RNA-dependent RNA polymerase. Of special interest is why two different frameshifting mechanisms (+1 for TVV1, -1 for TVV2 and TVV3) exist in a single virus. Other areas to be investigated include whether multiple TVV types can infect a single parasite, and if so, how this affects the parasite's overall pathogenicity.

Although the Nibert Lab's projects are in their early stages, our hope is that by understanding more about the virus and how it infects T. vaginalis, it may be ultimately possible to find a way to reduce the pathogenicity of the parasite and prevent it from causing Trichomoniasis.

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Recognizing and treating fungal growth in culture heritage items

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The microbial deterioration of cultural heritage items can destroy them if not caught and treated early, resulting in the loss of important historical information. This summer, we focused on biodeterioration: specifically, the fungal growth and deterioration that is often stimulated when these items are kept in high temperature and humid conditions. We are further developing a novel fungal assay that uses fluoroscopy to detect early fungal growth before it is visible to the naked eye, which works by fungal chitinases cleaving the fluorochrome from N-acetylglucosamine. The result is a greater fluorescence related to a greater fungal biomass. One aspect of this project involves working with old daguerreotypes, which are suspected to have some deterioration due to previous fungal growths on the surface. Using this assay and an SEM, we were able to determine that the deterioration in question was most likely from fungus, something which was previously unknown in the conservation world. Another aspect of this project was how to effectively kill the fungus once its presence is known. Of special interest was how to do this with paper, such as is found in old drawings and books. We looked at a few different biocides, eventually building a chamber that we filled with methanol vapor, so as to penetrate the paper without damage. Current and future research is focused on lower detection limits for the assay and ways to treat larger areas and quantities of paper. We are also looking at different areas where our fungal assay could be applied.

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Gene expression and scout behavior in *Mycobacterium smegmatis* persisters

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Approximately one-third of the world's population is infected with *Mycobacterium tuberculosis (M. tb)*, but ninety percent of those infections are latent or inactive. One mechanism behind latency is bacterial persistence, where a fraction of the population avoids elimination by antibiotics, host defenses, or both. Persistence is a transient phenotypic state; unlike genetic mutants, these cells become fully drug sensitive when regrown in the absence of antibiotics and exposed again. The non-inherited antibiotic resistance of persisters is linked to their slower growth rates. Although persistence is a particularly well-known phenomenon in tuberculosis, it exists across a range of bacterial species and is a key contributor in biofilm formation and recurring infections. We seek to elucidate the genes and regulatory systems responsible for persistence in *M. smegmatis*, a non-pathogenic environmental relative of *M. tb*. We are creating a promoter trap library with Green Fluorescent Protein (GFP) reporter to identify persister-specific genes. A second goal is to understand the relationship between cell division and death in 'scout cells,' or persisters that revert back to wild-type susceptibility. A combination of inducible systems, based on the tetracycline repressor and a recently adapted nitrile-inducible construct, will allow us to study cell turnover and gene expression simultaneously in single cells.

Molecular & Cellular biology

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Validating positive hits in a genome-wide siRNA screen for loss of SAC (Spindle Assembly Checkpoint) function in human HeLa cells

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Aneuploidy, abnormal numbers of chromosomes, is a hallmark of cancer that has been directly linked to deregulation of the Spindle Assembly Checkpoint (SAC) in the cell cyle. The SAC proteins suspend the cell in metaphase until all chromosomes are properly attached to spindle microtubules and oriented for anaphase. MAD2 is a well-known component of the mammalian SAC, which binds to Cdc20 and inhibits APC (Anaphase Promoting Complex) activity. In hopes of identifying new genes and proteins involved in the SAC, the King Lab previously performed a genome-wide siRNA screen of human HeLa cells. Positive hits were defined by the ability to exit metaphase and complete mitosis in the presence of taxol (a microtubule inhibitor). Interestingly, upon western blot analysis, it was found that the majority of positive phenotype siRNA hits showed decreased mRNA and protein levels of the critical SAC component MAD2. Many of these hits are false positives due to off-targeting effects of siRNAs, where an siRNA contains a seed match to the 3'Untranslated Region of MAD2 mRNA, resulting in degradation of the MAD2 message through miRNA type function. However, it was also hypothesized that some of the positive hits may encode proteins that affect MAD2 mRNA stability and protein levels through a different mechanism. Our goal for the next stage in this investigation is to use four new siRNAs for some of the most promising selected genes to validate these hits by reproduction of the aforementioned positive phenotype. HeLa cells are transfected with these new siRNAs, treated with taxol, and imaged. Next, western blotting is performed to quantify MAD2 mRNA levels in the affected cells. Ultimately, we hope to identify a protein of interest that has 2 or more siRNAs (without seed matches to MAD2 3'UTR) that give a reproducible positive phenotype and characterize its possible role in regulating MAD2 mRNA stability and the mammalian SAC.

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The generation of an ERAD retrotranslocation intermediate

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Because the accumulation of misfolded proteins is toxic to living cells, cellular quality control pathways function to degrade proteins that fail to attain a proper three dimensional conformation. During Endoplasmic Reticulum Associated Degradation (ERAD), proteins that misfold in the lumen of the endoplasmic reticulum are recognized, translocated back into the cytosol and degraded by the ubiquitin-proteasome mechanism. The process of retrotranslocation is hypothesized to involve a protein-conducting channel, but the nature of this channel remains controversial. In the past, a fruitful approach to identifying such protein channels has been to trap a translocation intermediate, which is what we are attempting to accomplish. Our approach is to covalently attach a biotin molecule to a model ERAD substrate *in vivo*, and then co-express this construct with avidin/streptavidin. The extremely tight interaction between avidin and biotin and the very tight folding of the avidin molecule will then stall retrotranslocation. Thus far, we have succeeded in biotinylating the ERAD substrate and driving its import into the ER, but in doing so we have somehow stabilized the substrate to the point that it is apparently no longer degraded. The next issue to address is driving ERAD-dependent degradation of the chimeric substrate, after which we will be able to generate an intermediate by co-expressing avidin. We will then be able to use this intermediate to discover the identity of the channel by carrying out *in vivo* cross-linking experiments.

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Oncolytic Virus: a new immunotherapy for malignant gliomas

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A glioma is a malignant primary tumor of the central nervous system. The prognosis for malignant gliomas is extremely poor. Currently patients with glioblastoma have a median survival duration of 12-15 months, thus new treatments are desperately needed. One such treatment developed by the Martuza Lab uses oncolytic viruses such as G47delta. Oncolytic viruses are mutated so that they replicate and kill selectively in tumor cells. G47delta has also been shown to elicit a specific and durable antitumor response. Using a murine glioma cell line, CT-2A, I am attempting to use the virus to pulse dendritic cells (DCs) to vaccinate against the tumor. *In vitro* I have observed that the DCs can be activated by the virus and CT-2A cells. DCs will be extracted from the mice and pulsed with tumor antigens using the virus. The DCs will then be injected into the mice, and the mice will be challenged intracranially with a lethal dose of CT-2A cells. Their survival will then be followed.

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Investigating the activity of ECT2 during cleavage furrow formation in response to shYAP knockdowns

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Yes-associated Protein (YAP) has been recently found to have oncogenic properties. While it normally functions as a tumor suppressor in untransformed mammary cells, the overexpression of YAP causes phenotypic alterations like growth factor independent proliferation, suppression of apoptosis, and invasion into matrigel. YAP is in the Hippo pathway that was originally defined in Drosophila melanogaster to include core components Hippo, Sav, Mats, Wts, and transcriptional coactivator Yki. The Hippo pathway is conserved in mammals and includes the corresponding proteins Mst1/2, WW45, Mob1, LATS1/2, and transcriptional coactivator YAP. This pathway negatively regulates growth by simultaneously inhibiting proliferation and promoting apoptosis. Our lab found that this YAP is localized in mitotic structures in the midbody like other upstream Hippo Pathway proteins. Our findings suggest that shYAP knockdowns cause abnormal division, longer periods of mitosis, and multi-nucleation in cells. Ongoing research includes investigating the effects of short hairpin RNA knockdown of YAP in MCF 10A cells during cytokinesis, and looking at the activity of ECT-2. ECT-2 catalyzes guanine nucleotide exchange on Rho, the protein responsible for cleavage furrow formation. In interphase cells, ECT-2 is mainly localized in the nucleus but in mitotic cells, ECT-2 is localized in the midzone where the formation of cleavage furrow starts. The inhibition of ECT-2 by microinjection of anti ECT-2 antibody specifically blocks the completion of cytokinesis, resulting in multinucleated cells.

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Assessing stimulus-dependent transcription in neurons with single-cell resolution

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Many cells transcribe certain genes in response to extracellular signals. In particular, neurons express a set of genes known as immediate early genes (IEGs) within 30 minutes of an electrical stimulus such as depolarization of the plasma membrane. Protein products of IEGs are required for the development and function of the nervous system, and mutations in IEGs are known to cause some forms of epilepsy, autism, and mental retardation. To better understand the mechanisms that activate transcription of IEGs, we asked whether all neurons express the same genes with the same kinetics in response to membrane depolarization. Using RNA *in situ* hybridization to detect IEG transcription with single-neuron resolution, we found that some IEGs are highly likely to be expressed in the same cells, while others may be expressed alone. We also noted that at a given time after depolarization, many neurons transcribe an IEG from only a single allele. This likely reflects a less stimulated cell state, since higher-voltage depolarization caused more neurons to transcribe both alleles of an IEG. Finally, in many neurons IEGs were transcribed at the nuclear periphery, a domain normally thought to be involved with transcriptional repression. All three observations may represent general mechanisms of regulating the level or timing of stimulus-dependent IEG transcription. These results confirm that *in situ* hybridization can be used to detect patterns of IEG expression with single-cell resolution.

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Elucidating the role of Lkb1 in hematopoiesis

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Lkb1 is a multitasking kinase that regulates a varied array of cellular processes and which has been shown to be a tumor suppressor. This project involves assessing the role of Lkb1 in hematopoiesis. We used the Mx-Cre-pIpC inducible Cre/lox system to knockdown Lkb1 in mice. We then isolated bone marrow and peripheral blood and analyzed the lymphocyte population through Flow Cytometry. Five days following Lkb1 deletion, we find a significant decrease in both mature and progenitor B and T cells, and more than a 50% decrease in thymus size. To assess the causes of leukopenia, we analyzed the mice at earlier time points, finding that at 1 day post-deletion the leucyte population is only 15% decreased. Further analysis revealed that this less significant decrease is due to a sharp decrease in pre-B cells and an increase in the pro-B population, suggesting a block in B-cell maturation. We are currently analyzing the effect of Lkb1 deletion in T-cell maturation. Furthermore, we will use RNAi to study the upstream signaling pathways perturbed by the loss of Lkb1.

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mTert-GFP mice as a novel model for the isolation and identification of intestinal stem cells

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The enzyme telomerase (Tert) delays cellular senescence by preventing chromosomal telomeres from shortening. Since a hallmark of stem cells is their ability to escape senescence, telomerase may function as a biomarker for stem cells. Our lab has created a transgenic mouse line, mTert-GFP, that marks progenitor cells in multiple organs, including the testes, bone marrow, and intestine. Using a Cre/Lox recombination system, we have tested whether mTert-positive cells can give rise to lineage-specific cells over long

periods of time in the intestine. Our results indicate that these cells function as stem cells and give rise to all the different lineages in the intestine. The identification of mTert as a stem cell marker may provide a model for future isolation of stem cells in other organs.

Recently, Barker et al. (2008) demonstrated that the intestine also contains a population of rapidly-cycling stem cells which express Lgr5. We are interested in comparing mTert-positive cells with these Lgr5-expressing cells, which appear to mark a related but distinct population of intestinal stem cells. Specifically, one current project is to generate a telomerase reporting mouse using a red fluorescent reporter in order to determine the relationship between $mTert^+$ cells and $Lgr5^+$ cells.

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Reprogramming muscle satellite stem cells into cardiac progenitors

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Ischemic cardiac disease is the leading cause of death in the developed world. The inability of the adult heart to adequately repair itself has motivated stem cell researchers to explore cell-based treatments aimed at transplanting *in vitro* derived cardiac cells into patients after heart injury. One promising approach strives to accomplish this by reprogramming somatic cells using master cardiac transcription factors such as mesoderm posterior 1 (Mesp1). Mesp1, a crucial regulator of cardiovascular progenitor cell development, has been shown to induce cardiac commitment in differentiating murine embryonic stem cells. My project goal is to determine whether the induction of Mesp1 expression in muscle satellite stem cells - a set of multi-potent cells that are autologous, abundant, and accessible - can reprogram these cells into cardiac progenitor cells. Satellite cells may be ideal multi-potent starting materials as they are closer in cell developmental lineage to cardiac cells than other types of multi-potent cells currently under investigation. We plan to introduce Mesp1 along with other transcription factors involved in nuclear reprogramming, plus chemical modifiers of epigenetic states, to determine if these cells can be reprogrammed preferentially into cardiac cells. The intent is to produce cells with beating cardiac phenotypes and/or increased expression of cardiac marker genes. The successful reprogramming of satellite cells into cardiac or cardiac-like progenitors could bring us one step closer towards developing feasible methods of generating heart cells for patient treatment.

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The role of microRNA in the induction and kinetics of Th17 differentiation

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T helper 17 (Th17) is a recently identified subset of T helper cells that has been linked to autoimmune conditions like multiple sclerosis, diabetes, and arthritis. Th17 with self-antigen specificity has been shown to cause autoimmunity in animals and IL-17, a pro-inflammatory cytokine produced by Th17, has been detected in human tissues affected by those autoimmune diseases as well. Th17 development can be divided into three sequential steps: differentiation, expansion, and stabilization. Each phase can be distinguished by specific transcription factors and cytokines.

MicroRNA (miRNA) are short noncoding RNAs that regulate gene expression post-transcriptionally by binding to target RNA to cause degradation or repression of translation. Growing data supports the expanding role of miRNA in the immune system, particularly in cell differentiation, immune response, and inflammation.

The role of miRNA in the kinetics and induction of Th17 development however has not yet been studied and thus is the focus of the project. First, a set of candidate miRNAs was identified through microarray and then quantitative PCR was used to verify their specificity and variance in relation to time. By comparing the miRNA time course data with those of major transcription factors and cytokines in the Th17 differentiation, clusters of target genes were predicted. The candidate pathways can then be studied by over-expression or knockdown of the specific miRNA and monitoring the cytokine repertoire and cell differentiation of the cell lineage. Understanding the role of miRNAs can elucidate more insight on Th17 plasticity and its developmental pathway and offer a potential therapeutic mechanism for many autoimmune conditions.

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Creating a humanized mouse model of type 1 diabetes

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Type 1 diabetes (T1D) is an autoimmune disease characterized by a depletion of insulin-producing pancreatic beta cells. Little is currently known about the pathophysiology of the disease, and treatment is limited to the introduction of exogenous insulin. In order for new therapeutic strategies to be developed, the underlying autoimmune mechanism must first be elucidated. Because of the destruction of beta cells occurs prior to the symptoms, we must rely on rodent models to understand onset and progression of the disease. However, only a few rodent models exist, and findings from these models have had limited reproducibility in humans. Hence, a functional model that more closely resemble humans is

needed to understand the pathophysiology of T1D. We therefore propose to reconstruct T1D in a humanized model possessing relevant cell populations, namely effector immune, target pancreatic beta, and T cell-educating thymic epithelial cells, derived from patient-specific pluripotent stem cells. There is much ongoing research investigating the development of the first two cell lineages. However, the directed differentiation of ES cells to thymic cells has not been studied.

My project is to generate means to identify intermediate cell populations in directed differentiation efforts. In order to do so, we aim to generate fluorescence reporter ES cell lines to mark the differentiation status of stem cells to thymic epithelial cells. To this end, we have created reporter constructs to modify specific genes, namely PAX9 and FOXN1 that mark the intermediates cell types in the thymic epithelial lineage, the pharyngeal foregut and the thymic epithelial progenitor, respectively. Currently, we employ bacterial artificial chromosome recombineering approaches to generate gene targeting constructs for the human and mouse genes. The two would together allow for isolation and characterization of target populations along with high-throughput screening to identify factors beneficial for directed differentiation efforts.

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Characterization of a J5-specific T-cell line

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Multiple Sclerosis (MS) is an autoimmune disease in which the immune system attacks the myelin sheaths coating neurons. Some current therapies available to alleviate the symptoms of MS, such as the drug copaxone, are believed to function by raising a population of regulatory T-cells that modulate the immune response to myelin. This project seeks to characterize the T-cell response generated in mice by J5, a potential MS therapeutic agent, to see if J5 encourages the development of a regulatory T-cell population. SJL mice were immunized with J5 and their splenocytes were harvested and cultured in IL-2-supplemented media. The splenocytes were periodically restimulated using antigen-presenting cells and J5. After 3 restimulations, the splenocytes will be analyzed for specificity and reactivity to J5 by a ^{3}H-thymidine proliferation assay. The cells will also be stained for the lineage-specific transcription factor FoxP3, which characterizes a specific subset of regulatory T-cells. The T-cell receptor repertoire of the generated cell line will be determined by antibody staining and flow cytometry analysis for the Vβ chain and RT-PCR for the Vα chain. The supernatants collected from T cells after restimulation will be tested by ELISA for relevant secreted cytokines such as IL-10 (an immunomodulatory cytokine), IFN-γ and TNFα (inflammatory cytokines).

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Reprogramming hepatocytes to beta cells: role of mechanisms that protect hepatocyte identity

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In type I diabetes, patients suffer from an inability to metabolize glucose properly due to an absence of or insufficient number of insulin-producing pancreatic β cells. One potential therapy to correct this deficiency is to reprogram patient-specific cells into β cells that can then be implanted back into the patient. It has been shown that pancreatic exocrine cells can be reprogrammed into β-like cells *in vivo* through the expression of pancreatic developmental regulators. However, it is difficult to use this technique in the clinical setting because the *in vivo* approach or removal of pancreatic tissue to attempt reprogramming *in vitro* poses significant risks to patients. Therefore, we are attempting to reprogram *in vitro* other cell types which are more easily accessible. In addition, we hypothesize that the barrier to direct reprogramming of cells is lowest between cells that share a close lineage. We therefore have focused on reprogramming hepatocytes, the parenchymal cells of the liver, which like the pancreas is derived from the endoderm during development and can be more easily harvested than pancreatic exocrine cells. So far, there has been little success reprogramming hepatocytes, so we are looking specifically at how hepatocytes protect their identity in the terminally differentiated state, and ways of knocking down these barriers to make hepatocytes more amenable to being reprogrammed directly into pancreatic β cells.

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Programming human induced pluripotent cells (hiPS) into adipocytes using an inducible lentiviral system

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Afflicting one in every three Americans, obesity is one of the most common causes of morbidity due to its implications in cardiovascular disease and type II diabetes. Although adipocytes have been found to play a central role in energy homeostasis, current *in vitro* models of adipocytes do not provide a suitable model of obesity. Despite the well-established protocols involving the differentiation of adipose derived mesenchymal stem cells (ADMSCs) into adipocytes, the *in vitro* lifespan of ADMSCs are limited and unsuitable for generating knock-out or reporter lines. Conversely, in addition to being capable of generating reporter lines that can expand indefinitely *in vitro*, human induced pluripotent stem cells (hiPS) can be generated as a disease model directly from patients or through homologous recombination. By introducing the green

fluorescent protein under the control of adipocyte specific promoters, we can examine the influence of small molecules in the adipogenic differentiation medium and assess their potential to increase or inhibit adipogenesis. Because of the inefficiency of current adipogenic differentiation protocols, a doxycycline induced lentiviral system was used to overexpress the key adipogenic transcription factors PPARγ, C/EBPα, C/EBPβ, and C/EBPδ, resulting in a highly efficient differentiation of hIPS and hES into mature adipocytes. These *in vitro* derived adipocytes will be then analyzed comparatively via quantitative RT-PCR and microarray gene expression profiles with primary human adipocytes as well as those differentiated from ADMSCs. Further, functional levels will be compared using immunocytochemistry and assays that measure the ability to respond to insulin, induce lypolysis, and synthesize and incorporate fatty acids.

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Exploring the role of the yuk proteins in *Bacillus subtilis* as a potential type VII protein secretion system

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More than one-third of the world's population has been exposed to the tuberculosis pathogen Mycobacterium tuberculosis, and two million people worldwide die annually from the disease. Despite the prevalence of this bacterium, its mechanisms of virulence are still largely undefined. Recent studies suggest that the bacterium utilizes a novel secretion system called the ESX-1 secretion system to transport virulent factors across the cell membrane. The ESX-1 secretion system is distinct from the well-characterized Type I – Type VI protein secretion systems, so it has recently been named a Type VII protein secretion system. Similar Type VII protein secretion systems have also been identified in other gram-positive bacteria, including a protein secretion system encoded by the yuk locus in the non-pathogenic bacteria Bacillus subtilis. Studying the yuk secretion system provides an interesting opportunity to investigate the mechanisms of a potential Type VII secretion system in a well-studied, non-pathogenic bacterium.

This project aims to address several fundamental questions about the role of the Yuk proteins in B. subtilis. First, I will characterize the proteins that compose the transmembrane domain in order to gain a better understanding of the structure of the channel. Secondly, I will characterize the proteins that are exported from the cell and study the conditions under which they are expressed and secreted by this pathway. Finally, I will study the interactions of the transmembrane proteins and the secreted proteins to gain a better understanding of the mechanism of protein export. These studies will help to elucidate the role of this unique protein channel in Bacillus subtilis and provide insight into Type VII secretion systems in a wide variety of bacteria, including pathogenic mycobacterium.

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Circadian transcriptome analysis in cyanobacteria

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Approximately 64% of predicted genes in *Synechococcus elongatus* PCC 7942 oscillate with 24-hour period. Each of these circadian genes is either maximally activated at dawn or dusk in order to coordinate physiology with the day-night cycle. Recent bioinformatic and experimental evidence suggests that circadian oscillations in chromosome superhelicity play a primary role in dictating oscillating expression.

To further investigate the role of supercoiling and the transcriptional architecture of *S. elongatus*, we performed whole genome tiling microarrays at 12 nt. resolution to interrogate strand-specific transcriptional units at various times during the circadian cycle. We mapped the 3' and 5' UTR for hundreds of annotated and non-annotated transcripts. We find the median 5' UTR length to be 180 nt. with 80% of UTRs less than 1000 nt. In addition, we find that several genes appear to have multiple 5' transcription start sites, including start sites within the predicted coding region. As expected, we find that several genes are organized on operons, and that these operons' predictions are highly correlated with previous computational and experimental models. Apart from expected transcripts, we find antisense transcriptional segments with a median size of 60 nt. for approximately 25% of predicted coding regions.

To improve the resolution of transcriptional architecture determination, current work focuses on using a novel strand-specific RNA-seq technique to determine transcript sequences at a theoretical resolution of 1 nt. We hope to combine results from RNA-seq with high-density tiling microarrays to produce a comprehensive picture of the *S. elongatus* transcriptome. In the future, we hope this analysis will help us understand the sequence features of transcripts which determine their sensitivity to supercoiling, and thereby their phase of oscillation in the circadian cycle.

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Human embryonic stem cells as a model for spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disorder and is the leading inherited cause of infant mortality. It is characterized by the degeneration of lower α-motor neurons and its genetic basis has been isolated to the Survival Motor Neuron 1 (SMN1) gene from chromosome 5q13. In addition to the SMN1 gene, the locus also contains a centromeric SMN2 copy, which is almost identical to SMN1, except for a single nucleotide transition; this results in only 10-20% of the full-length SMN pro-

tein being produced. Mutations or deletions of SMN1 thus lead to significantly decreased levels of full-length SMN, and are thought to be responsible for SMA. Animal models have been used extensively to study SMA, but they pose certain limitations—primarily, the lack of an SMN2 gene; thus, they often rely on complicated overexpression and knockdown strategies to obtain an accurate phenotype. As a result, human-based models for SMA would be invaluable, especially for screening potential therapies. Although induced pluripotent stem (iPS) cells have recently been used as a disease model with promising results, no robust human models currently exist. We used human embryonic stem (hES) cells derived from *in vitro* fertilized embryos with the SMA genotype, as well as SMN1 RNA interference knockdown hES lines to model SMA. These lines will be used to derive a population of purified motor neurons by induction with Sonic Hedgehog and Retinoic Acid; this will allow us to characterize the mechanism of neuronal death in SMA patients as either self-autonomous or environmentally-triggered.

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Determining the mechanism for Lkb1 mediated regulation of hematopoiesis

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Lkb1 is a tumor suppressor kinase that regulates a variety of cell processes, such as cell metabolism, proliferation, and polarity. The gene is also inactivated in several sporadic cancers. Lkb1 mediates its activities by phosphorylation of the AMPK family of kinases. To study the role of Lkb1 in hematopoiesis, we engineered the loss of Lkb1 in the hematopoietic compartment using the inducible Mx-Cre mouse model. Inactivation of Lkb1 leads to rapid loss of hematopoietic stem and progenitor cells resulting in bone marrow failure, indicating that Lkb1 is essential for maintenance of hematopoiesis. I am investigating signaling pathways regulated by Lkb1 that are essential for hematopoiesis by western blot analysis of wild type and mutant bone marrow, spleen and thymus tissues. We find that wild type, but not mutant, tissues show Lkb1 mediated activation of the canonical AMP-kinase leading to suppression of the mTOR pathway. This suggests that elevated mTOR activity in mutant cells may contribute to bone marrow failure. However, we find that blocking the mTOR pathway with the drug rapamycin in Lkb1 mutant mice does not rescue bone marrow failure, indicating that Lkb1 control of hematopoiesis does not function through the mTOR signaling pathway. I am continuing to investigate Lkb1 regulation of hematopoiesis by looking at the role of different kinases downstream of Lkb1. I will continue to analyze the status of different downstream kinases as well as other growth and apoptotic pathways by western blot analysis. Further, I will use chemical and genetic inhibitors of these pathways in Lkb1 mutant mice to identify the mechanism of hematopoietic regulation by Lkb1.

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A molecular characterization of the pancreatic mesenchyme during embryonic development

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Type 1 Diabetes mellitus is an autoimmune disease that results in the destruction of the insulin-producing beta cells of the pancreas. Currently, a core objective of diabetes research is to generate new beta cells for cell replacement therapy for diabetic patients. To reach this goal, the molecular mechanism of beta cell development in vivo must first be clearly elucidated so that this process can be effectively recapitulated both in vivo and in vitro to create new beta cells. While it has been known for decades that the development of the pancreas is dependent on epithelial-mesenchymal interactions, the exact molecular nature of these interactions has yet to be characterized. Furthermore, it has not yet been concretely established whether the mesenchyme provides permissive or instructive signals to the pancreatic epithelium and if these signals specifically induce exocrine or endocrine differentiation.

We hypothesized that the pancreatic mesenchyme is made of multiple subpopulations that then have distinct interactions with the epithelium over the course of development. Previous gene expression studies in our laboratory found that the transcription factors Dermo1 and Prrx1, both of which are crucial in limb development, are highly expressed in the pancreatic mesenchyme. Using transgenic mouse lines, we examined the expression of Dermo1 and Prrx1 at days 13.5, 14.5, and 15.5 post fertilization, a key time period of pancreatic organogenesis during which there is a rapid expansion of endocrine progenitors. We found that the expression of both genes is restricted to the mesenchyme. While Dermo1 expression is pan-mesenchymal, expression of Prrx1 is restricted to a specific subpopulation of the pancreatic mesenchyme that lies adjacent to the tips of the branching epithelium. This provides evidence that there is, in fact, differential gene expression within the pancreatic mesenchyme.

Currently, we are utilizing fluorescence activated cell sorting (FACS) to isolate the Dermo1-positive, Prrx1-positive, and Prrx1-negative mesenchymal populations at E13.5 and E15.5. Microarrays of these distinct populations will be used to reveal further differential gene expression of transcription factors as well as components of signaling pathways. Future functional assays will include co-culture of the identified mesenchymal subpopulations with embryonic stem cells, embryonic pancreatic progenitors, and adult islets to assay their ability to induce endocrine differentiation and to support beta cell survival and proliferation.

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Sortassembly: *in vitro* protein-protein fusion catalyzed by sortase A

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Chimeric proteins boast widespread use in areas ranging from cell biology to drug delivery. Post-translational protein fusion using the bacterial transpeptidase sortase A provides an attractive alternative when traditional genetic level fusion fails. We describe optimization of conditions for this in vitro protein ligation and report the successful fusion of 10 pairs of protein domains with preserved functionality demonstrating the robust and facile nature of this reaction.

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Characterization of an HIV clinical isolate with high-level resistance to small molecule CCR5 antagonists

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The prognosis for HIV-infected patients has significantly improved in recent years thanks to the development of multi-drug regimens known as highly active antiretroviral therapy (HAART). However, some strains of HIV have become resistant to conventional HAART drugs. Therefore, new drugs with new targets are being developed to block HIV infection. One such class of drugs blocks HIV interaction with CCR5, an immune cell co-receptor that HIV uses for entry into its target cells. By binding to CCR5 and disrupting its structure, these drugs (called small-molecule CCR5 antagonists) prevent HIV entry into its target cells.

During a phase IIb clinical trial of one of these drugs, an HIV strain was identified that had high-level resistance to this class of drugs. This resistant virus is able to use both wildtype CCR5 and drug-bound CCR5, which suggests it has evolved flexibility in its interaction with CCR5 extracellular domains. The objective of my project is to characterize the extent of this flexibility and identify any domains of CCR5 which the resistant virus uses differently than wildtype virus. I am attempting this using monoclonal antibodies against specific domains of CCR5, and using chimeric versions of CCR5 in which specific domains have been substituted for the analogous domains of CXCR4, a related receptor. This project will explain the mechanism of HIV resistance to small-molecule CCR5 antagonists, but it will also provide new information regarding HIV use of CCR5 in general.

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Reparative processes in tissue and immune-based injuries

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When tissue undergoes damage, a complex network of cellular and molecular processes is necessary for proper wound formation and repair. Ideal regeneration results in the replacement of injured or lost cells with the restoration of original tissue structure. Despite the potential application to a variety of clinical settings, the involvement of immune cell subsets in the regenerative process is unclear. To visualize cells of mesenchymal lineage and stromal cell networks, we utilized collagen type-I-GFP reporter mice. This also enabled us to develop models of virus-mediated destruction and mechanical skin tissue injury to examine the loss and restoration of stromal cells. Following virus infection in both the lymph nodes and ear skin, we observed marked destruction of collagen-GFP+ stromal network. In the lymph node, destruction of the stromal network was followed by the appearance of a collagen-GFP+ population within the node as well as an expansion of collagen-GFP+ cells in bone marrow and circulation. Similarly in the skin, the loss of the collagen-GFP+ network was accompanied by collagen-GFP+ cell populations emanating from hair follicles. Using bone marrow chimeras we aim to assess the origin of these populations to determine whether they originate from circulating hematopoietic bone marrow-derived progenitors or are generated by local expansion of resident mesenchymal stem cells. Moreover, by immunofluorescence microscopy, flow-cytometry, and intravital imaging, we are investigating the phenotype and function of this collagen-GFP+ population, as well as the kinetics and trafficking molecules involved in the reparative process.

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Imaging neuronal metabolism

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Glucose is the primary energy substrate for brain metabolism. Glycolysis breaks down each molecule of glucose to generate two molecules of adenosine triphosphate (ATP), two molecules of pyruvate and two molecules of NADH. Levels of cytoplasmic NADH can thus provide information on the state of glycolysis in cells. Currently, researchers rely on the preparation of cellular homogenates or NADH autofluorescence to study cellular metabolism. The main disadvantage of preparing cellular homogenates is dynamic changes in cellular metabolism cannot be studied. Furthermore, NADH autofluorescence is not specific between NADH and NADPH, measuring mostly NAD(P)H bound to proteins of which the strongest signals come from the mitochondria. It is therefore a nonspecific reporter for glycolysis in cells. Recently,

our lab has developed a genetically encoded fluorescent biosensor for NADH. In our present study, we tested if the NADH biosensor can record changes in metabolism due to changes in fuel source in differentiated, cultured Neuro2A cells using fluorescent microscopy. Our data thus far demonstrates that the NADH biosensor can report changes in the NADH levels of cells during metabolic challenges. A second aspect of our present study is to develop a new fluorescent biosensor for adenosine monophosphate (AMP). We will engineer this biosensor by using the published ATP sensor as a template to conduct error-prone PCR mutagenesis. Because AMP level is a good indicator of cellular energy status, this biosensor can record changes in cellular metabolism. Though preliminary, our studies suggest that our biosensors will be important tools for tacking questions regarding neuronal metabolism.

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Using retinoic acid in the differentiation of A9 dopamine-producing neurons

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Since drugs such as Levodopa do not manage to revive the A9 dopamine-producing neurons that die in Parkinson's Disease, and therefore do not truly correct the problem, we have turned to human embryonic stem (hES) cells to find an actual cure for the disease. We use hES cells because, although transplantation of fetal A9 cells into affected human brains has proven effective, it is unfortunately not a viable option for future treatment due to the immense demand and the multiple fetal brains necessary per affected adult brain. hES cells, however, are pluripotent, so through cocktails of different proteins, they can be turned into other different types of cells. However, A9 dopaminergic neurons have proven very difficult to make. The current standard protocol, while effective at turning 1% of all hES cells into dopaminergic neurons, has not been successful at producing any meaningful number of A9 dopaminergic neurons. The standard protocol, however, does not include any addition of retinoic acid. Considering research that shows the role of retinoic acid in the caudalization and induction of neural differentiation, we are optimizing the amount of retinoic acid at the correct times in the differentiation protocol to yield midbrain, rather than other anterior or posterior dopaminergic neurons. By putting the hES cells through the differentiation protocol in vitro and then injecting them into the striatum of the rat brain, we can look at where the axons of the grafted cells grow to in vivo and surmise what type of neurons have been produced.

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The role of TopBP1-dependent Polymerase α hyperloading in the activation of the ATR-dependent checkpoint in Xenopus laevis

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The DNA replication checkpoint in eukaryotic cells is a critical pathway designed to protect the cell and the integrity of its genome. A checkpoint response to DNA damage requires recruitment of the Ataxia telangiectasia mutated and Rad3 related (ATR) kinase to stalled replication forks, as well as other critical factors on the DNA. Topoisomerase IIβ binding protein I (TopBP1) and Polymerase α (pol α) are two known components in the checkpoint complex. TopBP1 is necessary for ATR activation and polymerase α binding prior to checkpoint activation. Polymerase α has also been seen to hyperload on DNA in a TopBP1-dependent manner. If checkpoint activation is independent of pol α hyperloading, it should be possible to localize a separation of function mutant of TopBP1 that can activate the DNA checkpoint in the absence of hyperloading. The aim of this research is to understand the role of pol α hyperloading in the activation of the ATR-dependent checkpoint. To accomplish this, TopBP1 mutants will be created and mixed with damaged DNA in Xenopus laevis egg extract. Analysis of the chromatin bound proteins and of checkpoint activation will further explain the relationship between TopBP1, pol α and activation of the ATR checkpoint.

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A novel technique for investigating the cell cycle dynamics of the Polycomb Group (PcG)

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The Polycomb Group (PcG), initially characterized in Drosophila, represents a highly conserved group of proteins involved in the stable maintenance of patterns of expression of the homeotic genes during development. These proteins modify chromatin structure to repress gene expression through multiple rounds of cell division. Several PcG protein complexes have been identified—two of which included Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2) which each alter chromatin in unique ways. Elucidating the nature of epigenetic regulation by PcG proteins requires an understanding of how these proteins function during cell division, since events in the cell cycle are disruptive to chromatin structure. To study PcG protein function during specific parts of the cell cycle, it would be useful to be able to manipulate PcG protein levels within a single cell cycle (several hours). Neither stable deletions nor RNAi-mediated knock-down are feasible—the former is lethal, and RNA interference may require several cell cycle to achieve the desired effect. To achieve this tuning of protein function, we will

fuse a "destabilizing domain" (DD) to a PcG protein. The DD triggers proteolytic degradation of the protein of interest within four hours. The fusion protein can be rescued through the addition of a small molecule ligand, Shield-1. By inducing RNAi-mediated knock-down of an endogenous PcG protein while simultaneously expressing the PcG protein as a DD-protein fusion, it will be possible to tune protein levels within a matter of hours. This should make it possible to test functions of PcG proteins at specific points in the cell cycle. Our initial studies will focus on components of PRC1 in Drosophila Schneider (S2) cells.

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Viral control in elite controllers with non-protective HLA alleles

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For the last century, Human Leukocyte Antigen (HLA) alleles have been known to correlate to the development of certain autoimmune diseases. Recent data has shown a correlation between HLA alleles and delayed progression to AIDS. In extreme cases, certain HLA alleles, specifically B57 correspond to elite controllers, patients infected with HIV who are able to suppress viral replication to an undetectable level without antiretroviral treatment. Although the mechanism of such control is not fully defined, past studies suggest CD8 T cell involvement due to the enrichment of certain HLA class I alleles. This study examines elite controllers who do not express noted protective alleles in order to characterize how they are able to sustain low viral replication and high CD4 T cell counts. We hypothesize that while HIV-specific CD8 T cells are involved in viral control in patients with non-protective HLA alleles, CD4 T cells are critical. We first test the overall magnitude and breadth of T cells reactivity to specific epitopes of each patient's HIV protein using Enzyme-linked immunosorbet spot (ELISPOT) assays. We then deplete CD4 and CD8 T cells separately to distinguish the epitope responses. We find a strong CD8 T cell response. Furthermore we find, in contrast to previous studies, a narrowly targeted T cell response. In order to fully characterize the immune response in these elite controllers, we must further test the infectivity levels of the HIV virus, patient HLA class II alleles, and the involvement of other immune response components such as B-lymphocytes and NK cells. A greater understanding of how patients naturally fight HIV infection will steer the development of new antiretroviral treatment.

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26S proteasome expression in HIV-infected cells

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Several lines of evidence indicate that HIV-specific CD8+ T cells play a critical role in reducing viral load during acute HIV infection and in controllers- persons who are infected with HIV but maintain a healthy CD4 count and an undetectable viral load without the use of therapy. Thus, generating an effective CD8 response is an essential goal in HIV vaccine design. HIV-specific CD8+ T cells specifically recognize and kill HIV-infected cells because infected cells display fragments of HIV proteins (epitpoes) bound to the major histocompatibility complex class I (MHC-I) molecule at their surface. These epitopes arise from the degradation of HIV proteins in a multistep degradation pathway called antigen processing. The first step is degradation of proteins by the 26S proteasome, a large protein complex that degrades proteins into shorter amino acid sequences that are further processed for presentation by MHC-I. Whether HIV infection affects the expression and function of the proteasome is currently unknown. In our experiments, we have studied the expression of proteasome subunits in PBMC (peripheral blood monocytic cells) in healthy donors, chronically infected donors, and controllers. We measured the level of expression of various proteasome subunits in these patient subsets by Western blot. Our preliminary data suggest that the expression patter of some proteasome subunits is altered in HIV-infected persons. We are conducting additional experiments in order to understand if and how the virus may affect the expression of proteasome subunits in HIV-infected cells.

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Mapping antibody Agr86

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Agrin is a protein involved in the maturation and maintenance of the postsynaptic apparatus in the vertebrate neuromuscular junction (NMJ). The C-terminal region of agrin interacts with receptors on the muscle surface and induces AChR aggregation. Various agrin isoforms are generated by alternative splicing at the X, Y and Z sites in the C-terminal regions of agrin. In particular, insertion of an 8aa peptide (Z+) at splicing site Z increases the clustering activity. To assess the functional consequences of agrin depletion at the NMJ, conditional mouse mutants will be used. We have bred a new transgenic line in which a Cre-estrogen receptor fusion (Cre-er) and Yellow Fluorescence Protein (YFP) are expressed in a subset of NMJs. This system allows us to assess the function of agrin in both synaptic maintenance and aging by injecting tamoxifen at distinct ages to delete agrin from motor neurons in marked, YFP positive cell subsets. My project has focused on mapping the binding region of a Z+ specific antibody, anti-agrin 86, that reacts with rat but not mouse Z+ agrin. Since the Z+ insertion is identi-

cal between rat and mouse, Ab 86 may bind either outside the Z region and/or recognize a Z+ dependent protein conformation. Its mapping may provide insight both into methods for generating additional Z+ specific antibodies as well as the mechanism by which agrin causes clustering of AChRs.

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Investigation of tail anchored protein insertion into the ER membrane

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Tail-attached (TA) proteins are a class of membrane-bound proteins that possess a single transmembrane domain near the C-terminus and a functional domain near the N-terminus. Therefore, TA protein insertion cannot be achieved via the traditional SRP translocon that operates translocationally. The GET (Guided Entry of Tail-Anchored Proteins) protein complex has recently been implicated in an alternate pathway responsible for the TA insertion into the endoplasmic reticulum (ER) membrane, which then allows for transport to other membranes of the secretory pathway. As the TA protein class encompasses many integral proteins, the characterization of this insertion pathway is very important. Our research has focused on Get3, a soluble ATPase homodimer believed to function as a chaperone for TA proteins, and its two ER receptor proteins, Get1 and Get2. In order to better understand the complexities of the interaction between these three proteins, it is necessary to first better characterize the binding between them. To this end, we have tagged Get3 with a hexahistidine tag and employed a nickel pull-down assay. Preliminary results suggest that Get1 and Get2 bind independent sites on Get3, supporting the current model that Get1 and Get2 form a heterodimeric receptor on the ER membrane. We have also compared eukaryotic Get3 amino acid sequences with those of its prokaryotic homologue, ArsA, which serves a different function in bacteria as a soluble subunit of an arsenic pump. Analysis has revealed several motifs that differ between bacteria and eukaryotes but are highly conserved within each kingdom. These motifs are prime candidates for residues linked to each protein's distinct function. We have begun to create mutations in these conserved regions and plan to proceed with in vivo screening for loss of Get3 function. Once the subtle interactions between the Get proteins are understood, we will be in a better position to fully characterize this important insertion pathway.

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EDD involvement in autocrine TNFα production

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Programmed cell death plays an important role in an organism's development and continual upkeep. One of the main forms of programmed cell death is apoptosis, a pathway that involves cysteine proteases called caspases that cleave the cell. Caspase function can be blocked by inhibitor of apoptosis proteins (IAPs) which can in turn be inhibited by Smac/Diablo. The other main form of programmed cell death is necroptosis, a more recently defined pathway that resembles necrosis but is a programmed pathway rather than an unspecific response to cell stress. An important protein in the necroptosis pathway is EDD, an HECT E3 ubiquitin ligase that is involved in autocrine tumour necrosis factor (TNFα) production. TNFα is an important player in inflammation as well as a death factor that plays a significant role in the signaling of both apoptotic and necroptotic forms of cell death. Past studies have shown that treating cells with a Smac mimetic can cause autocrine TNFα release. It has also been shown that in Drosophila, HYD and DIAP1, orthologs to the mammalian EDD and IAP respectively, bind to one another. We are currently researching the possibility that through the binding of EDD with IAP, EDD plays a part in TNFα release when Smac/Diablo is added. Our experiments have shown that EDD binds to cIAP1 and XIAP, two IAPs, and we are currently studying the effect of EDD on Smac-induced TNFα production.

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Characterizing the interactions between the protein disulfide isomerase family of enzymes and redox proteins of the endoplasmic reticulum

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Protein disulfide isomerases (PDIs) are a family of 20 enzymes that catalyze disulfide bond formation and isomerization within newly synthesized proteins in the endoplasmic reticulum (ER). PDIs donate disulfide bonds to nascent proteins, becoming reduced to the dithiol form in the process. Alone, PDIs do not have oxidase activity- they must be reoxidized to complete the catalytic cycle. However, the redox partners of individual PDIs remain largely undetermined. Knowledge of the mechanism of action of PDIs is critical to the understanding of native disulfide bond formation, while information concerning their interactions with redox enzymes of the ER could have significant clinical implications. The aim of current research is to characterize the interaction of members of the PDI family of proteins with four redox systems of the ER (Ero1α, Ero1β, VKOR, and VKORL). This is achieved by utilizing active-site mutagenesis to trap mixed disulfide protein partners. Cells are transfected with the PDI and redox enzyme of interest. Proteins are then harvested under conditions that preserve existing disulfide bonds and inhibit the formation of new disulfide bonds. After immunoprecipitation, protein samples are analyzed via western blot under non-reducing conditions. To measure the ability of the four redox enzymes to reoxidize each PDI family member, pegylated maleimide, a thiol-alkylating reagent, is used to quantify reduced Cys-SH content in PDIs that are exposed to varied amounts of redox enzyme. Preliminary results support the hypothesis that each redox Ero1α, Ero1β, VKOR, and VKORL each interact with a specific subset of PDI proteins.

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Indirect regulation of gene expression by RsmA through intermediate transcription factors in *Pseudomonas aeruginosa*

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In the opportunistic pathogen *Pseudomonas aeruginosa*, the protein RsmA acts as a post-transcriptional regulator by binding to messenger RNAs at the conserved Shine-Dalgarno sequence. RsmA prevents translation initiation by blocking ribosome binding at this site. Orthologs to RsmA are found in many other Gram-negative bacteria, including CsrA in *Escherichia coli*. In a previous microarray analysis performed in the Lory lab, an *rsmA* mutation was shown to cause changes in mRNA expression levels of approximately 500 genes compared to the wild type strain. It is thought that some of these genes are indirectly affected through RsmA regulated translation of intermediate transcription factors. This summer, we attempted to identify regulatory genes whose expression is directly affected by RsmA. We investigated eight regulatory genes detected in the *rsmA* microarray analysis, fusing each gene to a *lacZ* reporter and placing the resulting translational fusion under a constitutively active placUV promoter. The fusions levels were measured by performing Miller Assays. Significant differences in expression levels were observed between the wild type and Δ*rsmA* strains for the gene encoding the ribose operon repressor (RbsR). RsmA had a positive effect on its expression, suggesting that this gene is directly and positively regulated by RsmA binding. Potential techniques for further experiments include *in vitro* confirmation of direct interaction between RsmA and *rbsR* mRNA and microarrays to determine the regulon of RbsR.

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Microtubule-based cargo transport in *Aspergillus nidulans*

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The mechanics behind cytoplasmic microtubule (MT)-based cargo transport on motor proteins (particularly dynein and kinesin) have been generally hard to examine due to the lack of simple genetic systems capable of identifying the molecules required for cargo attachment using genetic screens. One of the major difficulties is that information cannot be derived from genetic screens of model organisms such as Saccharomyces cerevisiae, unlike other basic processes in cell biology. This problem arises from the fact that cytoplasmic MTs serve very few functions in yeast and its genome only encodes for kinesins that are not associated with cargo-transport. Like humans, Aspergillus nidulans relies more on the MT cytoskeleton for transport and utilizes both kinesin 1 and kinesin 3, among other motor proteins. Kinesins are linked to mitochondrial transport, anterograde axonal transport, and lysosomal transport. Dynein is associated with chromosomal movement, spindle assembly checkpoint inactivation and endocytic transport. By screening and comparing wild-type and motor knockout strains with fluorescently-labeled organelle markers such as PexK (peroxisome), SNARE proteins (endosome) and histone H1 (nucleus), we can start to understand how these motors are capable of moving along the MT cytoskeleton and binding to and transporting a variety of cargo with spatial and temporal precision. We are also in the process of creating an A. nidulans deletion collection, which will allow us to identify and characterize novel genes required for MT-based transport. Further understanding of these molecular motors and their interactions may help facilitate the development of medical treatment for diseases caused by a defective MT-based organelle transport.

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Peripheral to decidual: T cell differentiation

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The immunological paradox of pregnancy was first proposed by Peter Medawar in 1953. The fetus is partially paternally encoded for and therefore an allograft which is not rejected by the mother. Current research suggests immune regulation may be responsible for maternal tolerance to the fetus. Because T cells are a main cause of allograft transplant rejections, one interest of our group is to identify their role in the decidua. Previously CD4+CD25bright Regulatory T cells and CD8+CD28- effector-memory T cells have been shown in decidual tissue. To understand what factors influence T cell differentiation in the decidua, peripheral blood T cells were isolated and cultured with supernatants of decidual stromal cells. Stromal cells which line the decidua have been shown to be important in the differentiation of peripheral Natural Killer (NK) cells to decidual NK cells. Our research focuses on how cytokines released by stromal cells influence T cell differentiation. We perform flow cytometry to determine the T cell proliferation and induction of CD4+ T regulatory cells and effector-memory T cells.

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Derivation of engraftable skeletal muscle progenitors from mouse embryonic stem cells

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The skeletal muscle harbors a remarkable ability to regenerate and repair itself after injury, facilitated by a pool of skeletal muscle stem cells located beneath the basal lamina of muscle fibers. There has recently been interest in the directed differentiation of embryonic stem cells (ES cells) into myogenic progenitors; however, the efficient isolation of a pure population of skeletal muscle stem cells derived from embryonic stem cells and is able to undergo self-renewal in vivo has been elusive. This study aims to generate an enriched population of engraftable myogenic progenitors

from mouse embryonic stem cells that can efficiently form myofibers in vivo. It is known that the transcription factor Pax3 plays an important role in the migration and specification of myogenic precursors in the dermomyotome during embryonic development. Additionally, in the adult animal, the transcription factor Pax7 is crucial for the specification of satellite cells, which have the ability to be activated upon muscle injury and contribute to myofiber repair. Transgenic ES cells expressing the early myogenic markers Pax3 and Pax7 were created, and a myogenic population was isolated via FACS. The ability of this population to differentiate in vitro will be assayed through immunofluorescence staining for terminal differentiation markers. Furthermore, engraftment studies will be performed to determine the capacity of these cells to undergo proliferation and differentiation when transplanted into injured leg muscle of mice. Lastly, the functional contribution of this population of transgenic ES cells to the skeletal muscle stem cell compartment will be assessed.

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Functional screening of microRNA mimic and inhibitor libraries to identify repressors of mTORC1 signaling in a cell-based model of PTEN-null prostate cancer

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The majority of human prostate cancers (~75%) exhibit loss of PTEN, a lipid phosphatase that antagonizes the function of phosphatidylinositol-3-kinase (PI3K). Loss of PTEN leads to aberrant activation of the kinase Akt, an upstream activator of the mammalian target of rapamycin complex 1 (mTORC1). mTORC1 gain-of-function leads to enhanced cellular anabolism, giving malignant cells a growth advantage that contributes to tumor formation/progression. The role of microRNAs (miRNAs) as upstream regulators of mTORC1 function is not known. miRNAs are endogenous small RNAs that control gene expression by repressing the translation of target mRNAs. Widespread loss of miRNA expression in cancer suggests that most miRNAs are tumor suppressors; however, several miRNA species also act as oncogenes. Exogenous delivery of miRNAs or miRNA inhibitors represents a novel therapeutic strategy for cancers with mTORC1 hyperactivation. To identify miRNAs or miRNA inhibitors capable of repressing mTORC1 signaling in a PTEN -/- genetic background, we will perform a high-throughput functional screen using miRNA mimic/inhibitor libraries in PC-3 prostate cancer cells. The screen will utilize a novel fluorescent In-Cell Western (ICW) assay developed in our laboratory to monitor ribosomal protein S6 Ser-235/236 phosphorylation, a well-established mTORC1 biomarker. In preparation for this screen we performed extensive optimization experiments to validate the endpoint assay described above, selected the appropriate small RNA transfection conditions, chose the correct positive/negative siRNA/miRNA controls and established a formal Z-score enacting these parameters. Future work will focus on execution of the primary screen and characterization of "hits" that elicit the greatest repression of mTORC1 signaling.

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Regulation of the Fanconi Anemia pathway by microRNAs

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During the normal lifespan of a human cell, the cell's DNA will inevitably be damaged. Whether it be from UV radiation or DNA damaging chemicals, the cell must use mechanisms to fix these potentially lethal injuries. These DNA repair pathways involve protein complexes that either cut out and replace the damaged nucleotides, or, should the damage be unfixable, direct the cell to undergo apoptosis. Although these pathways' importance suggests a tendency for high conservation in normal cells, many cancer cells are shown to be defunct in one repair pathway, possibly to increase their mutation rate and adaptability; however, cancer cells cannot survive when two or more of these pathways are knocked out. Our group focuses in particular on the Fanconi Anemia (FA) pathway involved in homologous recombination repair. Consisting of thirteen known protein-encoding genes, this pathway allows the cell to repair DNA crosslinks. Our recent project investigates the post-transcriptional regulation of these genes via interactions with microRNAs. As endogenous small non-protein-coding RNAs that degrade mRNA in a similar fashion to siRNA, microRNAs can be overexpressed and potentially drop levels of FA protein translation. By inducing FA pathway blockage via microRNA overexpression, one could potentially use these microRNAs as therapeutic agents against cancer cells deficient in a non-FA repair pathway. Although normal cells will survive with a partial knockdown of the FA pathway, combining this knockdown with an existing repair pathway deficiency in cancer cells will invariably kill these cells. In this manner, specific microRNAs could be used as a novel treatment tool for a particular set of cancers.

Neuroscience & Psychology

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Investigating the assumptions of generalizations

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We study directional generalization, the idea that training received in one direction affects performance in subsequent untrained directions. In previous studies, subjects trained and learned to produce a velocity-dependent force pattern while performing point-to-point movements in one direction and were subsequently tested at 34 equally spaced directions around the unit circle. To quantify the effect of learning on the non-trained directions, they compared subjects' performance in each of the 34 non-trained directions to the trained direction. Highest learning was measured near the trained direction and the level of learning rapidly decreased away from it; this pattern is represented by the directional generalization function (GF). However, this GF is based on data that is collected too tediously per untrained direction; about 12 testing trials for each untrained direction, proving time consuming and costly.

We have designed a novel paradigm that allows to collect data more efficiently such that 12 testing trials under our new design can collect learning data for all 360 degrees. With more data, we hope to obtain a finer, more detailed and reliable GF otherwise unobtainable from current methods. Preliminary data is encouraging and the overall shape of our GF obtained thus far resembles that previous GF's.

Natalie Cameron — *Psychology: Social and Cognitive Neuroscience*
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Rockabye baby: the therapeutic components of music for newborn infants undergoing painful procedures

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All infants undergo at least one painful procedure following birth: a heel-stick is performed to test for phenyketonuria (PKU). This lab recently found that controlled auditory stimulation in the form of lullabies reduced heart rate by 16% in newborns ten minutes after a heel-stick. However, the question remains as to which components of controlled auditory stimulation contribute to its therapeutic effects. Current research explores the roles of harmony and rhythm in attenuating pain in newborns undergoing painful procedures. The first 30 newborns will be randomly assigned to the harmonic-rich (traditional Western lullabies played on piano) or harmonic-poor condition (the same lullabies digitally edited so that the fundamental frequencies (F0s) of notes embedded within the chord are separated from adjacent F0s by a factor of 21/12). The next 30 infants will be randomly assigned to the rhythmic (simulated heart beat) or arrhythmic (random beats) condition. Heart rate and rhythm, oxygen saturation, salivary cortisol, and behavior will be monitored and compared across groups. Results will provide insight into the innateness of harmony and rhythm perception.

Alicia Cowley — *Neurobiology*
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Characterization and identification of a midbrain dopaminergic neuron reporter

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Degeneration of midbrain dopaminergic (mDA) neurons in the substantia nigra is a hallmark of Parkinson's disease. To date, the most commonly used marker for DA neurons is tyrosine hydroxylase (TH), a rate-limiting enzyme in the biosynthesis of catecholamines. Previous studies suggest that the homeodomain-containing transcription factor Pitx3 is required for the regulation of TH expression in midbrain DA neurons as well as the generation and maintenance of these progenitors. Pitx3 is a more specific marker for midbrain DA neurons, unlike TH, which is a general, non-specific marker for DA neurons. Pitx3's ability to be cloned into a lentiviral packaging system can facilitate the study of the differentiation and maintenance of this population of DA neurons both in vivo and in vitro. To take advantage of the size and specificity of Pitx3 we are creating a midbrain DN reporter construct using the Pitx3-Promoter expressing GFP and iresGFP. We are introducing the construct into zebrafish embryos to check if it is functional in vivo. GFP expression would establish Pitx3 expression as a suitable midbrain DA reporter. Such a finding, in addition to further characterization via stem cell transfections, could lead to this reporter's employment in cell replacement strategies for Parkinson's disease – be it in clinical trials (to characterize the number of surviving DA neurons during medication-administration and drug screens) or in differentiation assays (to verify the time points of DA neuron differentiation in living cells or to describe the efficiency of different differentiation strategies).

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Transcription Factors as Critical Controls of Corticothalamic Projection Neuron Development

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Corticothalamic projection neuron (CThPN) development is critically controlled by combinatorial expression of select transcription factors. Previous microarray data from the Macklis lab identified transcription factors that are highly expressed uniquely in CThPN when compared to other cortical projection neurons (such as corticospinal motor neurons and callosal projection neurons). FOG2 and MGFM4 are two such transcription factors with high expression in CThPN populations and they have been previously described as important controls for specification and differentiation in other cell types. The exact role of these transcription factors in the differentiation of cortical progenitors has not yet been determined. Expression patterns of both proteins in wild-type mice were studied through immunocytochemistry and in situ hybridization. FOG2 and MGFM4 expression were also comparatively assessed in the cortex, mesencephalon, and heart through a Western blot assay. The CThPN population is being studied in FOG2 conditional knockout mice using axonal tracing techniques and immunocytochemical markers to assess changes in the connectivity and molecular phenotype. The effects of loss of function and gain of function mutations of MGFM4 will be studied by analyzing the morphology, migration, connectivity, and expression of subtype-specific molecular markers in the CThPN population. Continued study will help further the understanding of the exact function of two molecular-genetic controls over CThPN development.

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Characterization of metabolic and immunologic changes in the brain following HIV infection

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Despite the advent of effective anti-retroviral drug regiments, penetration into the central nervous system (CNS) is poor, and HIV infection in the brain remains poorly understood. Magnetic resonance spectroscopy (MRS) is a non-invasive tool used to assess metabolic changes within the brain. Previously, the Lentz lab has worked with diffusion and spectroscopic images from rhesus macaques and HIV-positive humans to identify and characterize the underlying metabolic and structural changes that occur during acute and chronic stages of the disease. In accordance with the literature, our acute data suggests that an elevation of choline (Cho), a molecule involved in membrane synthesis, precedes a decrease in N-acetyl-aspartate (NAA), a marker of neuronal integrity. While many studies have explored cognitive and immunologic correlations with decreased NAA levels and have provided increased understanding of NAA, the altered changes in other brain metabolites (choline, myo-inositol, creatine) following HIV infection need further characterization. To address this issue, we designed a MRS experiment to verify the cellular metabolism of brain and immune cells in their dormant, activated, and infected states. Specifically, the purpose of this project is to perform MRS imaging on in vitro cell assays to identify the nature of brain metabolism changes due to HIV-1 infection in the central nervous system. Ultimately, we aim to provide the necessary preliminary data to construct an accurate cell culture model of neuroAIDS that will allow for raid testing of therapeutic and neuropathogenic hypotheses.

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Intrinsic noise in cognition: motion effects on the representation of object location

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Phenomenally, the visual perception of low-level object properties (e.g. size, location, orientation) is compellingly unambiguous. Similarly, we interact with the physical world in discrete, all-or-none terms (quantum funny stuff aside): faced with a fork in the road, we can only choose one tine. Yet an outstanding problem in cognitive psychology remains in understanding how such information is actually represented in the mind, given specific neural constraints. Recent theoretical and empirical work has profited from advancing the possibility that the internal representation accessed when generating a perceptual report may actually be probabilistic. This model postulates that the brain expresses its uncertainty by weighting multiple simultaneously held hypotheses with their own truth-probabilities. In a remarkable 2008 study by Vul and Pashler, it was found that taking the mean of participants' first and second best guesses on several trivia questions yielded an answer that was closer to the correct response than either guess alone, providing support for the idea that even high-level semantic information may be stored probabilistically. Extending these findings, the present study is motivated by the intuition that reporting a moving object's perceived location among other moving objects may also reveal how mental representations can be 'noisy,' or uncertain. We hope to test predictions of a probabilistic model of spatial location by collecting multiple responses on each trial, as well as by presenting each trajectory multiple times. A secondary goal will be to systematically perturb the probabilistic representation by adjusting stimulus parameters, such as item number, velocity, and tracking duration.

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An increase in learning rate as a possible explanation for savings observed in motor adaptation

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It is remarkable that humans are able to learn to perform novel tasks on a daily basis; however, our understanding of how our motor control system is able to quickly learn these tasks has proven difficult. Savings, a property of memory in which prior learning enhances subsequent relearning of a given paradigm, has been demonstrated in several motor learning tasks. Savings can be demonstrated in one of two ways: the A--> -A--> A paradigm in which learning of a task is followed by the negative of that task and then subsequent relearning; or the A--> washout--> A paradigm in which a task is learned, then followed by baseline trials and then the relearning of the task. In both cases, motor performance returns to baseline before relearning. As such, the washout phase is significantly longer than the negative phase in the former paradigm. A recent experiment has demonstrated a two state model which accounts for the savings in one of the paradigms, while another similar experiment has found a model that explains the savings noted in both cases. However, recent findings have shown that in a consistent environment in which a force field is learned, the learning rate of a subject may increase. In this project, subjects will perform the positive and negative of a task and be tested for an increase in learning rate. This increase will be illustrated as a significant increase in the learning of the positive task and decrease in the learning of the negative task. If there is indeed an increase in the learning rate, this would suggest that the two state model does account for the savings noted in both paradigms.

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Molecular definition of anatomical subpopulations of callosal projection neurons

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The corpus callosum is composed of axons of callosal projection neurons (CPN), which are responsible for the integration of and information transfer between the two hemispheres of the brain. CPN are anatomically distinguishable from corticofugal projection neurons (CFuPN), which do not cross the midline of the brain. Recent evidence from the Macklis lab indicates that there are both anatomical and molecular subpopulations of CPN (Mitchell and Macklis, 2005; Molyneuax, Arlotta, et al., submitted). In this study, we investigate whether the specification of the unique subpopulation of CPN with frontal collaterals is determined by an identifiable combination of temporally regulated molecular-genetic controls already identified as enriched in CPN. To anatomically identify CPN with dual frontal projections, we will retrogradely label neurons from the contralateral sensory-motor cortex and ipsilateral premotor cortex. To identify specific genes important for the development of these sub-populations, we will perform immunocytochemistry and fluorescent in situ hybridization on the retrogradely labeled tissue.

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Projection neurons and interneurons' interaction in the developing cerebral cortex of Fezf2 null-mutant mice model

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In the mammalian cerebral cortex, the interaction between glutamatergic excitatory projection neurons (PN) and GABAergic inhibitory interneurons (IN) is critical for the maintenance of balanced microcircuitry. The connections between these two populations of cortical neurons are established during embryonic corticogenesis, and unbalanced cortical activity can cause neurological disorders, including epilepsy. There exist many different subtypes of cortical PN and IN, which during development are fate specified from different pools of neural progenitors. From the ventral telencephalon, IN migrate a long distance to the cortex where they synaptically interact with PN that have been born the same day from the dorsal progenitor pool. The mechanisms that determine the ultimate relative position and pairing of PN and IN in the cortex are largely undefined. Our specific question is whether PN directly instruct the final positioning of their IN partners. In order to answer this question, we have selected a mutant mouse model in which the loss of the transcription factor Fezf2 results in the specific loss of one PN population (e.g all the subcerebral projection neurons), without defects in IN fate specification and/or tangential migration. The main aim is to study whether the selective absence of a specific population of PN in the Fezf2 null-mutant cortex affects the final distribution of the IN. Interestingly, Fezf2 null-mutant mice exhibit epileptic-like behavior when handled or stimulated, which might result from unbalanced cortical activity. Understanding the mechanisms that establish and maintain proper PN-IN microcircuitry in the cerebral cortex may aid efforts to modulate this circuitry in the context of neurological disorders like epilepsy.

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Can you hear me now?: the effect of early auditory environment on perception and the cortical representation of sounds

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Individuals with Autism Spectrum Disorders (ASD) suffer an awareness of environmental sounds, abnormal loudness perception, and difficulty in filtering important auditory information from background noise. In vivo studies of rats indicated that white noise or selective frequency rearing can drastically alter cortical patterning and produce abnormal mapping of tones onto the auditory cortex. I hypothesize that such aberrant wiring leads to improper cortical activation, which may underlie the inability to filter select sounds from the environment. However, currently, little is known about the effects of abnormal auditory cortical development on behavior and sound perception. I developed a Go/NoGo operant conditioning task to test the ability of mice to differentiate between two tones. Mice reared in pulse trains of 7kHz pure tone were compared to age-matched controls raised in a normal acoustic environment. Our findings suggest that mice exposed to pure tone during their auditory critical period are worse at perceiving fine differences between two tones close to the 7kHz region compared to the controls. To better understand the cortical development, I have begun immunohistochemistry of c-Fos, a marker for neuronal activity, to locate the regions activated by the tones.

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Neurochemical and behavioral effects of postnatal maternal separation in mice: a model of early life adversity

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During critical periods in human development, early experiences drive and shape emerging neurological functions from motor skills to sensory perception to higher-level cognition. Just as monocular deprivation may permanently damage visual acuity, psychological stressors encountered in early life, from poverty to abuse and neglect, can shape the development of neural circuits that control adult behavior, increasing the risk of anxiety disorders, depression and other psychological impairments. Maternal separation (MS) during the first two postnatal weeks (3 hrs/day, from p2–14) is an established animal model of early psychosocial deprivation. Past studies of the MS paradigm and related treatments show that stressed mice display hyperactive hypothalamic-pituitary-adrenal (HPA) responses and increased anxiety in behavioral tests such as the elevated plus maze and open field. However, less is known about the impact of MS stress on the maturation of inhibitory circuits. This study follows the expression of inhibitory markers such as parvalbumin, GABA, VGAT, and GAD65 in the amygdala and frontal cortex from the period immediately following separation through adulthood. In order to test the hypothesis that stress can cause early aging, adults will also be examined for prematurely decreased telomerase activity. To compare the impact of MS on sensory function and inhibitory circuit formation, optomotor responses will be measured to determine whether MS stress affects the trajectory of visual development. Concurrently, behavioral testing will seek to confirm previous research on the increase in anxiety-related behaviors.

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The effect of creative writing on health as indicated by changes in sIgA levels

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Previous studies have found that there are health benefits to expressive writing about traumatic events. Interestingly, researchers have found that subjects who benefited the most were those who created a personal narrative or story through their expressive writing. Supporting this idea that writing stories can be therapeutic is the field of creative writing therapy, which has benefited a wide variety of patients. In my study, I aim to see whether short story writing has a positive effect on levels of salivary immunoglobulin A (sIgA), a protein which has been used by researchers as an indicator of overall immune system health. To summarize, research has shown that creative activity or writing can create a positive mood. Other studies have shown that positive mood is correlated with increases in salivary immunoglobulin A. While a link has been drawn from creative writing to positive mood, and from positive mood to health, the point of my project is to make the direct link between creative writing and health by showing an increase in a creative writer's sIgA levels. For the project, I recruited 48 subjects and collected their saliva before and after a writing exercise. The experimental group wrote a short story, and the control group wrote about what they did that day. I will assay their saliva for sIgA. If creative writing increases sIgA levels, then creative writing may improve one's health and serve as a tool to help people with a wide range of physical ailments and immune disorders.

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Investigating the interaction between UBQLN1 and DACH1 and their role in APP processing

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Alzheimer's disease (AD) is the leading cause of dementia and the sixth-leading cause of death in the US. The sequential cleavage of amyloid precursor protein (APP) by members of the β- and γ-secretases produces the toxic beta-amyloid (Aβ) peptides that accumulate to create both synapto-toxic Aβ oligomers and amyloid plaques. Variants of the UBQLN1 gene are associated with increased susceptibility of AD, and the risk allele is associated with increased production of a UBQLN1 transcript lacking exon 8 (UBQLN1-TV2). We were interested in elucidating the mechanism of UBQLN1-mediated neurodegeneration. By employing Drosophila genetics, we found that UBQLN1-TV2 interacts with DACH1, a protein responsible for mushroom body and retinal eye development in the fly. Based on this genetic interaction, we became interested in both the mechanism of their interaction and the effect of DACH1 overexpression on APP processing. First, we transfected naïve H4 neuroglioma cells with UBQLN1-TV2 and performed a co-immunoprecipitation (IP) experiment with anti-UBQLN1 and anti-DACH1 antibodies. The co-IP did not detect a direct interaction between UBQLN1 and DACH1, though we have not ruled out an indirect interaction mechanism. We have also begun to analyze the effect of DACH1 overexpression on APP processing by transiently transfecting DACH1 in H4 cells stably expressing full length APP. Specifically, we are using western blots and enzyme-linked immunosorbent assays (ELISA) to determine whether DACH1 increases the secretion of APP fragments and Aβ. The results of these experiments could provide valuable insight into the mechanisms involved in AD.

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An investigation of pathways involved with neuroregeneration: a study of expression levels in the mTOR Pathway

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The inability of neurons in the central nervous system (CNS) to regenerate after injury remains a serious concern for neurobiologists. Various hypotheses have been proposed to explain this lack of regrowth, such as an inhibitory environment or a reduction in the neurons' intrinsic growth ability. We are exploring the hypothesis that CNS neurons have an intrinsic inability to reenter the growth state in any environment as the result of the previously-observed downregulation of the mTOR pathway, which regulates proteins involved with translational activity and inhibits apoptosis, thus promoting cell growth. We performed in situ hybridization to measure the expression levels of three molecules involved with the mTOR pathway (Rheb, mTOR and P70S6K) in the retina during mouse development and at different intervals after injury to the optic nerve. Our results did not show a significant amount of mRNA downregulation for these molecules during development or after retinal injury. This suggests that if any of these molecules are downregulated, the downregulation may occur either at the level of translation or post-translational modification. The downregulation of the mTOR pathway may also be due to the downregulation of molecules in the pathway other than those tested. We plan to further study the mechanisms behind the downregulation of the mTOR pathway through immunostaining to measure protein levels of mTOR, P70S6K, phospho-mTOR, and phospho-P70S6K during development and after injury, and perform in situ hybridization or immunostaining for other inhibitors or activators of mTOR. We hope that by better understanding the mechanism by which mTOR downregulation occurs, we may determine how to most effectively manipulate the pathway to achieve CNS neuron regrowth.

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Piecing it all together: role of carbonic anhydrase and isochrome 17q in medulloblastoma

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Medulloblastoma, a highly malignant primary brain tumor that originates in the cerebellum, is the most common pediatric brain tumor comprising 14.5% of newly diagnosed cases (Ries et al., 1999). The most frequent chromosomal rearrangement in medulloblastoma is isochromosome 17, or i(17q) (Mendrzyk et al., 2006). Genetic analysis of primary medulloblastomas show upregulation of several genes located on the q arm of i17q, one of these genes of interest encodes CA4, carbonic anhydrase IV, which is commonly upregulated in medulloblastoma and other types of cancers. To investigate the link between CA4 and medulloblastoma tumorigenesis, carbonic anhydrase inhibitors, Sulpride and Hydrochlorothiazide (HCTZ), were used to examine the effect they had on specific medulloblastoma cell lines harboring the i17q rearrangement. Tumor viability decreased with increasing concentrations of both the CA4 inhibitors, implicating CA4 in the pathogenesis of this common, recurrent genetic rearrangement and in medulloblastoma.

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Teaching self-restraint: steps towards neuroimaging in awake and behaving rats

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Much of the current knowledge of the neural representation of motor sequences in mammals comes from studies of nonhuman primates. However, these studies are costly, involve extensive training, and the ultimate behaviors observed are overtrained. Therefore, we propose to study neuronal basis of motor learning in rodents, which have demonstrated the ability to perform sequential motor tasks such as lever-pressing. A limitation of current electrophysiological and imaging techniques is their inability to record from awake and behaving animals due to movement of the animal. One way to stabilize the animal is to physically secure its head. Previous studies have involved a head-fixed rat preparation in order to remove confounds due to locomotion and isolate whisker movement. Such experiments involve forced animal constraint, and are therefore limited by the stability of the head restraint on the animal. Our goal is to develop a method to chronically image neuronal activity in the rat motor cortex while they perform a timed lever tapping sequence. To do so, we have designed a modified home cage that can head-fix the animal and permit imaging, in parallel with a method of shaping voluntary head-fixation. Currently, our rats are willing to subject to head fixation for several minutes, suggesting that chronic neuronal imaging is not only feasible. Furthermore, our method of head-fixation may be stable enough to permit 2-photon imaging. These methods are easily adaptable to other behavioral assays, such as whisking behavior or odor perception.

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No pain, no gain: examining the neuroimmune response in the mouse spinal cord after peripheral nerve injury

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Pain is a necessary part of life. Without nociceptors—the subset of primary sensory neurons that respond to and process noxious stimuli—organisms would be severely disadvantaged by their inability to detect harmful external elements. In contrast to this physiological pain, there are several clinical conditions associated with exaggerated pain (hyperalgesia) or pain felt in the absence of noxious stimuli (allodynia). One group of these conditions comprises diseases or lesions of the nervous system that consequently produce pain (neuropathic pain). Our research uses the spared nerve injury (SNI) model in the mouse, which results in symptoms equivalent to hyperalgesia and mechanical or cold allodynia, the hallmarks of neuropathic pain in humans. We employ this model to better understand the neuroimmune response in the spinal cord to peripheral nerve injury and how it contributes to the development of chronic pain. Using immunohistochemistry and flow cytometry, we have examined the activation of resident microglia and peripheral macrophages, both of which are involved in this immune response. We have looked specifically at how activation of these cells is modulated over time in the spinal cord of SNI animals. We hope that this research will contribute to the elucidation of mechanisms underlying neuropathic pain, so that we may more effectively target the molecular and cellular causes of chronic pain in diseases of the nervous

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Development of the tonotopic organization of the auditory cortex in mice

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The formation of functional properties and connectivity in many neural systems is influenced and guided by experience. In particular, a critical period can exist when the shaping influence of experience is exceptionally powerful and crucial. The primary auditory cortex of mice is organized in a well-defined tonotopic manner. This is not present at birth, suggesting that the connections may be modified by postnatal experience. We have been studying the development of the tonotopic arrangement in the mouse auditory cortex by focusing on the thalamocortical connection, the last segment of the auditory pathway. The tonotopic organization is strongly influenced by the sound environment the mice is in, though this influence seems to be limited to a critical period from postnatal day 12 to postnatal day 15. Exposing mice during this period to an abnormal environment dominated by 7-kilohertz frequency sounds resulted in a tonotopic organization with an over representation for that frequency. But exposure to 7-kilohertz frequency sounds prior to or after the critical period did not alter the normal tonotopy development. Exposing mice during the critical period to white noise (unstructured sounds) seems to prevent tonotopy from developing while keeping the system in a plastic state. When returned to a normal environment, the mice are able to formal normal tontopic organization despite being pass the critical period. Continued study of this system can help us understand neuronal plasticity and the development of functional connectivity, perhaps applicable to human hearing and language learning.

Organismic & Evolutionary Biology

Jeremy Lutan Hsu — *Organismic and Evolutionary Biology*
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Examining the genetic basis of migratory behavior in Monarch butterflies

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Monarch butterflies are widespread throughout the New World and show a divergence in migratory behavior. The nominate North American subspecies (*Danaus plexippus plexippus*) migrates from Canada to northern Mexico and back, while the subspecies *Danaus plexippus megalippe* (Hübner 1826) is nonmigratory and concentrated in the southern U.S. and Central and South America. Previous research has demonstrated that genes associated with the circadian clock, sun compass orientation, and juvenile hormone pathway are involved in migration in *D. p. plexippus*; however, these genes have never been studied in *D. p. megalippe*, with the genetic differences responsible for their disparate migratory behaviors remaining unclear. To elucidate specific genes that contribute to differences in migration, we designed primers for five candidate genes – *Vrille, Takeout, Will die slowly, Allatotropin, and CAPA receptor* - involved in these pathways, as well as several nuclear and mitochondrial control genes. Cloning was performed via polymerase chain reaction (PCR) on 27 migratory monarch samples collected in Boston and Minnesota (*D. p. plexippus*) and 14 specimens from the non-migratory population in Ecuador (*D. p. megalippe*). Following amplification, the genes were sequenced and screened for polymorphisms. Allele frequencies were compared between populations for both candidate and control genes, with the goal of identifying evidence of divergent selection between the two populations. Amplified fragment length polymorphisms (AFLPs) were also generated from the samples, allowing for the detection of genome-wide polymorphisms for comparison to the candidate genes screened.

Krzysztof M. Kozak — *Organismic and Evolutionary Biology*
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Evolution of tail length variation in the deer mouse (*Peromyscus maniculatus*)

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Evolutionary biology attempts to explain the history of natural variation. The deer mouse (*Peromyscus maniculatus*) is an excellent subject for such studies, as seventy morphologically distinct subspecies of *P. maniculatus* occur in a range of habitats throughout North America. Mice in the forested North West typically have longer tails as an adaptation for climbing. In order to study the evolutionary history of this simple trait I have worked at the Hoekstra Lab in the Department of OEB creating a phylogeny (i.e. a systematic tree) of the recognised subspecies. Genetic material of *P. maniculatus* from various populations of the major subspecies distributed at seventy-seven locations throughout the continent was extracted from tissue samples acquired from museum collections. Five genes were selected based on their suitability for gene sequencing in *P. maniculatus*. I am currently determining nucleotide sequences of the chosen genes in individual mice. Using standard methods (e.g. Parsimony, Bayesian Inference) I will reconstruct the phylogenetic relationships among the subspecies based on the numbers of differences between the sequences. I expect all the individuals from long-tailed subspecies to cluster together on the trees, demonstrating that this adaptive morphology evolved once. Alternatively, it is possible that the adaptation arose multiple times independently. Either outcome will provide a starting point for studying the genetic mechanisms regulating formation of elongated tails.

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Replaying life's tape in a microcosm: massive parallel evolution in the bacterium *Pseudomonas fluorescens*

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The famous evolutionary biologist Stephen Jay Gould argued that natural selection is not a predictable process. If we were to rewind and replay the "tape" of evolution, the new version of life today would be unrecognizable. Here we show that, at least for short time scales, evolution by natural selection can be exquisitely predictable. When grown in rich solid media, wild-type colonies of the proteobacterium *Pseudomonas fluorescens* Pf0-1 are outcompeted by naturally occurring mutant variants that produce a mucoid polymeric substance. The mucoid variant has a higher relative fitness than the wild-type and can expand over the original wild-type colony within a few days. Previous work on one of the mutant variants revealed that a point mutation in the *rsmE* gene – which codes for a translational mRNA regulator – could result in the mucoid phenotype. This begs the question: are mutations in *rsmE* always responsible for the mucoid phenotype and associated fitness advantage? Is evolution in Petri dishes repeatable and predictable? We isolated 25 independently evolved mucoid colonies that arose from different wild-type populations and sequenced the regulatory and coding regions of the *rsmE* gene of each colony. This revealed that evolution was completely repeatable: all of the independently evolved mucoid variants had acquired mutations within the target region. Moreover, the changes at base-pair level were highly predictable. These included mutations that simply truncate the RsmE protein and, most strikingly, a large number of amino acid changes that destabilize key sites for target mRNA recognition. Additional competition experiments confirmed that the independently evolved mucoid variants also had the predicted fitness advantage. Our next step will be to determine the mechanistic basis of this advantage over the wild-type strain.

OTHER

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Modelling multilevel selection in plasmid evolution

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Genes that control plasmid replication are subject to two opposing levels of selection that are interesting to observe in steady state and limiting situations. When considering only evolution occurring within a cell, mutant plasmids that tend to overreplicate seem evolutionarily advantageous. However, taking into account intercellular selection shows us that the higher fitness of these mutant plasmids also puts more of a metabolic burden on the cell, slowing down cell growth. Thus, while the mutants may reach fixation in the cell more quickly, the normal plasmids are more likely to reach fixation within the entire population. To investigate what happens under these opposing evolutionary pressures, we use computer simulations under specified parameters such as the number of plasmids per cell, population size, and plasmid and cell fitness. Using a useful limit called the Moran process to model this selection conflict allows us to consider the probabilities of fixation of one type of plasmid both within the cell and within the entire population under assumptions such as complete time-separation, a constant number of plasmids within each cell, and a constant population size. However, the exact plasmid-cell selection conflict is more interesting than this approximate model. By removing the approximations used in the Moran process and varying the set parameters, we hope to discover more about different influences on the intracellular and intercellular selection conflict and resulting changes in probabilities of fixation. We are also interested in how adding mutation and migration to this system will alter our previous observations.

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Recognizing and treating fungal growth in culture heritage items

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Hemorrhage is the leading cause of preventable death following trauma, and improved treatment strategies within the first few hours of hemorrhagic shock will improve survival, especially in the battlefield, or during mass casualties and other resource-constrained environments. Several recent studies have shown that early administration of plasma can improve trauma-associated coagulopathy. Other studies have demonstrated that Valproic Acid (VPA), a common anti-epileptic drug, protects cultured neurons from hypoxia-induced apoptosis and also improves survival during lethal hemorrhages in rats. In order to improve knowledge of resuscitation techniques, we have designed several experiments to determine if VPA and spray-dried plasma can improve survival in a clinically realistic swine model of poly-trauma and hemorrhagic shock. Our treatment groups are as follows: Hextend (control), spray-dried plasma, VPA, and spray-dried plasma + VPA.

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Analysis of the correlations between atmospheric boundary layer moist static energy and temperatures in the free troposphere

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The goal of this project is to compare vertical correlations between atmospheric boundary layer moist static energy and temperatures in the free troposphere for humid rainy periods with those for dry rainy periods. Moist static energy refers to the sum of dry enthalpy, latent heat, and gravitational potential energy released by water vapor that would condense from a parcel of air if that parcel were lifted adiabatically to the top of the atmosphere. Radiosonde data from the Integrated Global Radiosonde Archive (IGRA) containing daily records of temperature and humidity at different pressure levels of the troposphere is used, and the region around the Philippines (5 N – 11 N, 110 E -130 E) is selected for this study because it receives significant rainfall during an extended period yearly . The hypothesis we make is that the Dept.h of convection is limited by the humidity of the free troposphere. This implies that our results should indicate a weaker correlation of moist static energy at the boundary layer with temperatures in the free troposphere in the drier rainy months than in the more humid rainy months. Our results will be confirmed by doing the same study for the Micronesia region, for which there is more refined atmospheric data.

Physics & Biophysics

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Bubble dynamics

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Drop a rough or creviced, spheroid, slightly negatively buoyant rigid body into a gaseous liquid. The body will fall slowly, then rise, lifted by the buoyant force generated from attached bubbles nucleated from its creviced surface. Surface bubble diameters are seen to double over short distances (0.1-0.2m), empirically suggesting that bubble growth is dominated not by a pressure-volume relationship--for such a hypothesis requires a rise height of 10m--but by accruing gas during lift. The subsequent oscillations appear quixotic, as the scattered periodicities and diverse helical, spiral, even zigzag trajectories observed defy simple explanation. Yet this phenomenon is relevant to a rich class of problems, in particular, the dynamics of gas-driven lake, and possibly ocean, explosions. It is well established that geometrical cavities of a minimum radius lower the free energy barrier, allowing nucleation to occur in supersaturated liquids. We will estimate bubble growth inside a crevice of unknown geometry, and establish a mean bubble nucleation frequency as a function of gas concentration, temperature, and pressure. The minimum bubble growth rate during ascent will be determined, enabling us to determine a critical supersaturating ratio that is related to the radius of curvature of the crevice. Thus, we can determine the geometry of the surface cavity in which bubble production initiated. Finally, we will examine the periodicity of spheroid rigid particles oscillating in carbonated liquids (Reynolds number << 1) to determine if observable oscillation instabilities can be explained through a period-adding route.

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Environment-assisted quantum transport in photosystem I

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Excitation transfer among chlorophyll molecules is the energy transport mechanism of the initial step in photosynthesis. A photon excites part of a chromophoric complex, and the excitation is transferred to a reaction center where biochemical energy storage is initiated. In most types of chromophoric complexes, the excitation energy transfer (EET) is extremely efficient (>95%), but the underlying physics is an ongoing area of research. A previous study focusing on the Fenna-Matthews-Olson complex has shown that fluctuations in the environment can enhance the quantum transport efficiency of the complex. In this work, we study the same phenomenon in cyanobacterial photosystem I (PSI), a relatively large complex with 96 chlorophyll molecules. We implement a wave function method which includes a stochastic element to simulate the time-evolution of the excitation. This method allows for efficient computation even for large systems such as PSI.

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Tweak the beak: from comparative transformations to development

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The shape and size of a finch's beak play a critical role in the ability of the finch to attain food and hence survive. The resulting need for the length and Dept.h of the beak to be easily regulated by natural selection may shed light on the means of beak development. Hence, we are studying finch beaks from both a phenomenological and developmental perspective. It has been found that the beaks of Darwin's finches are related to each other by either simple scaling along two perpendicular axes or shears along one axis. We have sampled several other closely related species both within and outside of the domed nest clade, and we found that shears along one axis continue to relate many pairs of species. However, the affine group, which includes scaling and shears, fails to account for a few of the species sampled. The relative success of affine transformations in relating species is aesthetically pleasing, yet a full understanding of its significance requires us to probe the development of the beak, in which morphogen gradients play a significant yet unclear role. To better understand how these morphogens control growth, we are complementing a model of cell proliferation developed by Hufnagel et. al. with several different time-dependent morphogen profiles. By noting which distributions give rise to simple scaling or shearing of the final shape when parameters are changed, we can narrow down the myriad possibilities of morphogen distribution and functionality.

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B lymphocyte evolution in the germinal center reaction

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A physical insight into antibody optimization is fundamental to our mechanical understanding of adaptive immune disorders, including HIV/AIDS, lymphoma, lymphoid leukemia, and collagen vascular diseases. Germinal centers, transient, oligoclonal substructures of secondary lymphoid organs, are the sites of affinity maturation, the process by which the probability of antibody—antigen interaction is maximized. We represent this evolutionary process by modeling somatic hypermutation of B lymphocytes as the source of affinity diversification and simulating competition among antibodies for antigenic interaction as selection pressure leading to apoptosis. Our model provides for stochastic mutation of explicit gene sequences and compartmentalization of monoclonal expansion and somatic hypermutation in the dark zone and intense selection in the light zone. The iteration of stochastic recycling between the dark and light zones is also examined. Time spent in somatic hypermutation was found to be critically related to probabilities of interaction, as evidenced by decreased affinities in models with monoclonal expansion, mutation rates below a lower bound, or lack of recycling back into the somatic hypermutation phase. Inter—germinal center recycling was found to be unrelated to affinity optimization. Our model provides optimal ranges of mutation rates, flux rates between germinal center compartments, and spatial dynamics of the B lymphocyte trajectory up to 30 days post-immunization. These results offer a novel view of somatic hypermutation as a fundamental force in affinity maturation and guide further investigations of the controversial recycling hypothesis.

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Life at the origin: structural studies of nucleo-protein assemblies at the bacteriophage λ origin of replication

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Accurate DNA replication is essential to maintaining integrity of the genome. The replication machinery can only act on single stranded DNA, but DNA is naturally found double stranded. The cell solves this problem by recruiting replication initiation proteins that melt the duplex DNA to convert it into single stranded DNA. Precise DNA replication relies on this regulation of initiation, in which proteins recognize and bind to the origin of replication, melt the duplex DNA, and recruit other machinery for subsequent steps. Although previous studies have outlined a simple pathway and the molecular players involved, higher resolution structural aspects remain unknown. These aspects include arrangement of protein subunits, location of bound DNA, and overall architecture of large nucleo-protein assemblies. Binding locations and arrangements of protein subunits or DNA dictate mechanistic possibilities, demonstrating the importance of answering these structural questions. The goal of this study is to achieve a clearer picture of the mechanism for the initiation of DNA replication through a structural study of nucleo-protein assemblies at the replication origin. To study this mechanism, a simple system such as bacteriophage λ is ideal. Given the similarities between replication systems, an understanding of initiation in bacteriophage may shed light on eukaryotic analogs. Electron microscopy (EM) is an ideal method to investigate these questions about subunit arrangements and holds many advantages compared to other structural methods. This study uses single-particle electron microscopy to analyze the structures of the helicase-loader and initiator protein-origin sequence nucleo-protein complexes of bacteriophage λ to elucidate mechanistic aspects of DNA replication initiation.

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The effects of solvated ions on DNA overstretching

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Because DNA in cells is often being stretched and bent by various proteins, it is relevant to understand how the molecule responds to various forces. When double-stranded DNA is pulled end-to-end with a magnetic trap, it transitions to an overstretched form that is 1.7 times longer than standard (B-form) DNA. Depending on how the DNA is stretched, it will either over-twist to become more tightly wound or under-twist to become like a flat ladder.

These transitions involve changing the distance between DNA's negatively charged phosphate backbones. The more that cations neutralize (screen) the phosphates, the easier it is to bring the backbones together. Theory suggests that smaller, denser ions like Na+ screen DNA more effectively than large ones like Cs+, but certain experimental surprises (e.g., Rb+ is better than the smaller K+) suggest that we don't fully understand DNA-cation interactions.

My research will focus on hysteresis—when DNA overstretches at a certain applied force F, but then won't relax until the force is diminished well below F. I will measure the dependence of hysteresis on different divalent cations, as well as on DNA sequence. I plan to use my results to modify an existing simulation, so we can better analyze and model the physics of DNA.

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Nanoporous Platinum

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Medical implants have inherent electric power supply limitations. One solution is to generate current from the catalysis of glucose present in the bloodstream by attaching small fuel cell electrodes to the surface of such implants. These fuel cells would require a cathode which reduces oxygen and an anode to carry out the oxidation of glucose to gluconic acid. Nanoporous platinum has been examined as an anode because of its observed ability to generate some current in a blood-like solution even when not surrounded by an oxygen-impermeable membrane. Such platinum can have a surface area up to 1,000 times that of a similarly sized piece of smooth metal. To create and test the generating capacity of such a fuel cell, we examine several fabrication methods for creating a copper-platinum alloy and then removing the copper to leave platinum with holes on the nanometer scale. The primary technique explored is electrodeposition of copper onto platinum foil, annealing at high temperature, and then dealloying in a reverse galvanic cell. Other techniques to be investigated include using a sputter with copper and platinum targets.

ACKNOWLEDGEMENTS

We would like to thank the following people for making PRISE possible:

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Gregory Llacer, Director of PRISE
Georgene Herschbach, Dean of Administration for Harvard College

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Salil Vadhan, Gordon Kay Professor of Computer Science and Applied Mathematics
Dona Lee Wong, Associate Professor of Psychiatry, Harvard Medical School

Consultants

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

Additional thanks to:
Drew Faust – President of Harvard University
Michael D. Smith – Dean of the Faculty of Arts and Sciences
All the Mentors, Principle Investigators, and Lab Members
The PRISE Fellows

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2009 PRISE Fellows
Not in Abstract Book

Harvard College Program for Research in Science and Engineering
Abstracts 2009