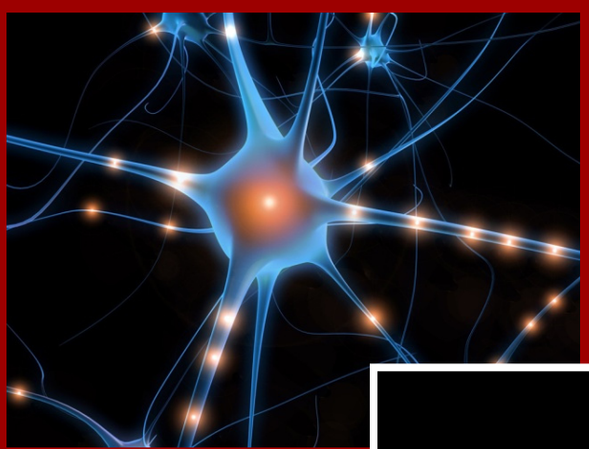
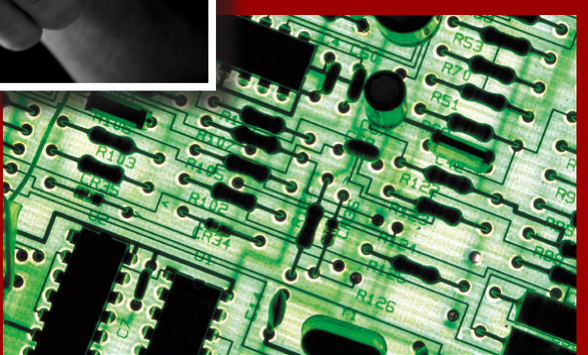
The 3rd Annual

Harvard Undergraduate Research Symposium

In Memory of Peter Cai





ABSTRACTS

November 1st, 2008

Harvard College Undergraduate

HCURA

Research Association

The 3rd Annual Harvard Undergraduate Research Symposium

Abstracts

November 1, 2008

www.hcura.org

In Memory of
Peter Cai

Peter Cai, a junior in Adams House, was an active and dedicated member of the Harvard College Undergraduate Research Association. Since sophomore year, Peter served as the Seminar committee chair and organized numerous seminars and workshops led by distinguished faculty. Not only was he a treasured member of HCURA, but he was also a brilliant leader in the science community and a role model for us all. He was a great student, colleague, and friend. We are all devastated by this sudden loss and will miss him dearly.

Yours Always,

The Harvard College Undergraduate Research Association

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HARVARD COLLEGE

OFFICE OF THE DEAN

UNIVERSITY HALL, FIRST FLOOR
CAMBRIDGE, MASSACHUSETTS 02138

November 1, 2008

Dear HURS Participants,

Welcome to the 2008 Harvard Undergraduate Research Symposium (HURS). We're delighted that you have decided to join us for this exciting showcase of undergraduate research.

This year's symposium is the culmination of countless hours of scientific research and represents some of the most exciting and intriguing analysis of those results. You'll find poster presentations, plenary talks, abstracts on cutting edge questions, and engaging dialogue with our students, faculty members, and staff who are deeply engaged in the enterprise of science. This symposium allows you the opportunity to forge relationships with scientists at Harvard from across the University at various stages in their career and your peers with whom you share the passion of research and discovery. I hope you'll spend this Saturday deeply engaged in interdisciplinary cross talk with the assembled community of scholars, educators, and researchers.

I also wanted to take this opportunity to commend the student organizers of the second annual symposium, the Harvard Undergraduate Research Association. They deserve our deepest gratitude for pulling together this symposium and attending to countless logistics while also being deeply involved in the research you are about to see. Please take the time to thank these organizers for their incredible efforts.

From my earlier days as a researcher at MIT and Harvard, I know the excitement that comes from sharing one's own research with colleagues, peers, and scientific leaders. To share your results with others who feel as passionate about discovery is a great feeling. Congratulations on the hard work you have done and, for our undergraduates, I hope this is just the beginning of the contributions you'll make to scientific knowledge.

Yours sincerely,

Evelynn M. Hammonds

Evelynn M. Hammonds
Barbara Gutmann Rosenkrantz Professor of the History of
Science and of African and African American Studies
Dean of Harvard College

Harvard College Undergraduate Research Association
HCURA

Saturday, November 1st, 2008

To the Undergraduate Researchers at Harvard,

On behalf of the Harvard College Undergraduate Research Association, we would like to welcome you to the third annual Harvard Undergraduate Research Symposium! It is amazing how quickly HURS has grown since its inception in 2006. This year HURS features over 50 poster presenters, six student plenary speakers, an outstanding keynote speaker, and a new addition this year – faculty discussion tables. It is both encouraging and inspiring to have renowned professors interested in the advanced research that we undergraduates are doing!

HCURA is continuing its mission of fostering a community of undergraduate researchers by sustaining an expansive advising program and hosting monthly faculty talks, annual symposia, various developmental workshops and social events. Though these events draw an extremely diverse crowd of students, there is one commonality that each of the students share: a passion for discovery. This year HCURA aims to facilitate this enthusiasm by organizing the first ever Boston Undergraduate Research Symposium (BURS), which will be held in April 2009. BURS is a collaboration between many Boston-area colleges, including Harvard, MIT, BU, BC, Northeastern, among others. Harvard will be pioneering this collaboration by hosting the inaugural BURS.

We are fortunate to attend a university that is so committed to research and development. This year alone we have seen hundreds of millions of dollars poured into science initiatives. The largest individual gift Harvard has ever received, $125 million, is going towards the interdisciplinary Wyss Institute for Biologically Inspired Engineering! Additionally, the multidisciplinary Northwest Science Building opened its doors to students this fall. This center will become a central part of undergraduate education in the coming years, bringing together research and students like never before. Harvard is also in the process of building the massive Allston Science Complex which aims to facilitate interdisciplinary research and collaboration between the Harvard Schools: FAS, HMS, HSPH, and others. Last year we noted that it is an exciting time to be an undergraduate researcher at Harvard. We believe that this excitement and the opportunities available to us will only increase exponentially in the next few years.

Sincerely,

Lev Shaket
President, HCURA

Shiv Gaglani
Founding President, HCURA

Student Organization Center at Hilles (SOCH), Box 374
59 Shepard Street, Cambridge, MA 02138

www.hcura.org
www.hcs.harvard.edu/hurs

Keynote Speaker

Dr. Steven E. Hyman, M.D.

Provost, Harvard University
Professor of Neurobiology, Harvard Medical School

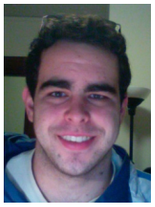
Dr. Steven E. Hyman, M.D. is currently the Provost of Harvard University and a Professor of Neurobiology at Harvard Medical School. He was the director of the National Institute of Mental Health (NIMH) from 1996-2001, focusing on integrating multiple disciplines to generate the knowledge needed to treat and understand mental diseases. Dr. Hyman graduated from Yale College summa cum laude in philosophy and the humanities, in 1974. He then became a Mellon fellow in philosophy of science, and received the B. A. (first class honors) and M.A. degress from the University of Cambridge in 1976 and earned his M.D. at Harvard Medical School. After receiving his medical degree, he quickly rose through the ranks at the Medical School, receiving numerous awards and eventually being promoted to professor of psychiatry and also the Director of Psychiatry Research at Massachusetts General Hospital. His research focused on mechanisms by which dopamine produced long-term changes in brain function which led to a better understanding of how therapeutic psychotropic drugs produced beneficial effects. Then from 1994-1996, Dr. Hyman became the first faculty director of Harvard's Initiative on Mind/Brain/Behavior (MBB). Dr. Hyman is a leading authority on how various internal and external stimuli affect mind, brain, and behavior and has served on the editorial boards of various journals and on many advisory boards for organizations such as the Howard Hughes Medical Institute and is a member of the Institute of Medicine of the National Academy of Sciences.

Plenary Speakers

William Jones

William Jones, a senior in Winthrop house, is an Organismic and Evolutionary biology concentrator from Fremont, Mich. In 2007, he spent his summer in Bonn, Germany where he observed the immune response elicited by malaria in mice. This past summer, he designed his own senior thesis research project with the help of his twin brother Dan to study fish diversity and resource partitioning in the Charles River under the guidance of Prof. James McCarthy and Prof. Robert Woollacott. Away from the river, William is the Vice President of the Harvard Undergraduate Bioethics Society; a group he helped found last spring and is currently the webmaster. Additionally, he is a member of the Harvard Men's Swimming and Diving Team. As a member of the team, he competed at the NCAA championships in the 200 and 100 butterfly events last spring. After graduation, he is plans to attend graduate school where he hopes to make an impact studying climate change and the future of fisheries.

David Levary



David Levary, a prospective Chemistry & Physics concentrator, is a freshman at Harvard College. He has conducted research in areas ranging from plant lipidomics to protein engineering. This past summer, Levary traveled to the Weizmann Institute in Rehovot, Israel where he worked on developing computer algorithms for brain computer interfaces. A United States Presidential Scholar, David recently returned to the Wittrup Lab at MIT, having first joined the group through the Research Science Institute in 2007. His current project involves the design of chemo-enzymatic methods for post-translational protein fusion. An advocate for the general accessibility of science, Levary works on peer review at the Harvard Undergraduate Research Journal and helps coordinate outreach to MIT for Seeding Labs, an organization committed to equipping scientists in the developing world.

Whitney Muhlestein

Whitney Muhlestein was born and raised in South Pasadena, California, and is a junior concentrating in Molecular and Cellular Biology. She disliked science all through high school, but fell in love with it in college thanks to Life Sciences 1a and Prof. Robert Lue. Whitney wanted to see if her love for science was true, so she decided to put it to the ultimate test – a whole summer doing only research. She worked in David Baltimore's lab at Caltech and, much to her surprise, found that she loved research. She began working in Doug Melton's lab in the fall of 2007 and has been stuck at the same bench, which she is honored to share with her post-doc, Dr. Danwei Huangfu, ever since. Whitney doesn't really know what she wants to do with her life after college, but suspects it will involve at least a decade more of school.

Sophie Rengarajan

Sophie Rengarajan is a junior in Eliot House concentrating in Neurobiology. She currently studies zebrafish habituation in the Engert lab. Sophie has been involved with the Radcliffe Choral Society and Ghungroo Tech and is currently a board member for Harvard Dharma and a mentor at the Harvard-Allston Portal. A southern California native, Sophie also enjoys singing, exploring the beauty of New England on her bike, and drinking Greenhouse coffee.

Anna Shneidman

Anna Shneidman is a senior at Harvard University, concentrating in Chemistry and Physics. Most of her earlier research has been motivated by biological questions. She has studied the aging of human skin cells from a materials science perspective, probed the role of molecular motor protein activity and regulation in cell spreading, and worked on the development of a new method to study a family of enzymes famous for their function in modulating DNA expression. In her junior year she joined the laboratory of Professor David Weitz at the Harvard Physics Department and School of Engineering and Applied Sciences to study the non-equilibrium glassy state. Through her research, she intends to gain insight into the connections between the microscopic motions of particles and the macroscopic behavior of materials by using microscopy, image processing, and statistical methods. Anna is a recipient of the HCRP and Summer Undergraduate Herchel Smith fellowships, and was a participant in the Summer 2008 Harvard NSF-REU MRSEC program. Besides research, she enjoys traveling, outdoor sports, reading, photography, and dance lessons.

Chiamaka Nwakeze

Chiamaka Nwakeze, born in 1988 in New York, has been interested in science research from an early age. In high school, she met Aristea Galanopoulou, M.D., Ph.D and conducted research at the Albert Einstein College of Medicine on interactions between KCC2, MeCP2 and DNA methylation. For this work, she was named a semifinalist in the 2006 Intel Science Talent Search. This past summer (2008), she worked in the Davisson Lab at the Weil Cornell Medical School through the Weill Cornell/Rockefeller/Sloan-Kettering Gateways to the Laboratory Summer Program. There, she studied potential inflammatory mechanisms involved in angiotensin-II-dependent hypertension. She is currently in the Yellen Lab, working to develop an adenosine monophosphate (AMP) detecting protein.

Faculty Discussion Tables

David Charbonneau, Ph.D.

Dr. David Charbonneau '01 is currently a Thomas D. Cabot Associate Professor of astronomy. His research currently focuses on looking at planets outside of our solar system, known as transiting exoplanets. He studied math, physics, and astronomy at the University of Toronto, where he received his bachelor's degree in 1996 and earned a doctorate in astronomy at Harvard in 2001. He continued to complete his post-doctoral work at the California Institute of Technology. Dr. Charbonneau was recently named the 2007 Scientist of the Year by Discovery Magazine for his work on exoplanets.

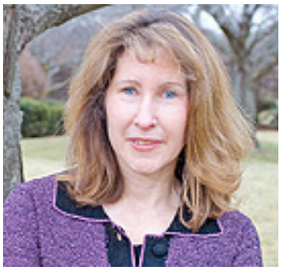
David E. Clapham, M.D., Ph.D.

Dr. David E. Clapham is Aldo R. Castañeda Professor of Cardiovascular Research at Children's Hospital Boston and a professor of Neurobiology and Pediatrics at Harvard Medical School. He studies the signal transduction control of ion channel activity and the role of calcium as an intracellular messenger. Dr. Clapham is a Howard Hughes Medical Institute Investigator and a member of the American and National Academies of Arts and Sciences. He is a recipient of numerous awards, including the Cole Award from the Biophysical Society, the Basic Science Prize from the American Heart Association, and the Bristol-Myers Squibb Award for Distinguished Achievement in Neuroscience Research. Dr. Clapham was trained in Electrical Engineering at the Georgia Institute of Technology and obtained his M.D. and Ph.D. degrees from Emory University.

David E. Golan, M.D., Ph.D.

Dr. David E. Golan is a professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School. He is a physician at Brigham and Women's Hospital and the Dana Farber Cancer Institute. He is a former co-director of the Harvard Medical School M.D. Ph.D. Program. Currently, Dr. Golan studies the molecular interactions controlling protein and lipid mobility and distribution in blood cell membranes. Dr. Golan holds an A.B. in Chemistry from Harvard University, an M.D. from the Yale University School of Medicine, and a Ph.D. in Molecular Biophysics and Biochemistry from Yale University.

Mary Lynne Hedley, Ph.D.



Dr. Mary Lynne Hedley serves as Chief Scientific Officer and Executive Vice President of MGI PharmaBiologics. MGI Pharma develops and commercializes products critical to the acute care and cancer treatment markets. The company's Lexington-based Biologics subsidiary focuses specifically on immunotherapy. Current research is directed towards the development of a therapeutic for cervical dysplasia and cancer associated with human papillomavirus. Dr. Hedley serves on the board of tutors in the Harvard MCB department in the area of biochemical sciences. Dr. Hedley received her Ph.D. in molecular immunology from the University of Texas Southwestern Medical Center and her A.B. from Purdue University. In addition to her professional interests, Dr. Hedley has performed humanitarian work, helping to build health clinics in Nicaragua.

Qing Liu, Ph.D.

Dr. Qing Liu is on the Board of Tutors in Biochemical Sciences at Harvard University. She works for the High Content Bioimaging Group of Sanofi-Aventis, the world's third largest pharmaceutical company. She is currently working to develop an image-based assay for tumor angiogenesis as well as to identify the target of Xaliproden, a proposed treatment for several neurodegenerative conditions including amyotrophic lateral sclerosis (ALS) and Alzheimer's disease.

Faculty Discussion Tables

Joseph Loscalzo, M.D., Ph.D.

Dr. Loscalzo has served as Chairman of the Department of Medicine of Brigham and Women's Hospital in Boston since 2005. His current research is focused on the vascular biology of endothelial cells and platelets and their role in atherosclerosis and thrombosis. Dr. Loscalzocompleted his clinical training simultaneously at Brigham and Women's and the Harvard Medical School. While at HMS, Dr. Loscalzo served as Resident and Chief Resident in medicine and Fellow in cardiovascular medicine. He was a member of the faculty at Harvard and staff at Brigham and Women's from 1984 to 1994, when he moved to the Boston University School of Medicine. Currently, he serves as Editor-in-Chief of the journal Circulation and the Hershey Professor of Theory and Practice of Physic at HMS. He received his A.B., M.A., and Ph.D. from the University of Pennsylvania.

Richard Losick, Ph.D.

Dr. Losick is Maria Moors Cabot Professor of Biology at Harvard. His primary research interests include RNA polymerase, gene transcription, and microorganismicdevelopment. Dr. Losick is a member of the National Academy of Sciences, a fellow of the American Academy of Arts and Sciences, a fellow of the American Association for the Advancement of Science, a fellow of the American Academy of Microbiology, a former visiting scholarof the Phi Beta Kappa Society, and a Howard Hughes Medical Institute Professor. He is the recipient of numerous awards in recognition of his work, including the Selman A. Waksman Award in Microbiology from the National Academy of Sciences. In addition, he is on the editorial boards of Science and Cell. Dr. Losick received his Ph.D. in biochemistry from the Massachusetts Institute of Technology and B.A. in chemistry from Princeton University.

Dr. Thomas Michel, M.D., Ph.D.

Dr. Thomas Michel is currently a Professor of Medicine at Harvard Medical School (HMS). In June 2008, he became the very first Dean of Education at Harvard Medical School. He is a senior physician at the Department of Medicine at Brigham and Women's Hospital. Dr. Michel's current research focuses on studying signal transduction pathways in the cardiovascular system using biochemical and cell biological approaches. His group focuses particularly on the targeting and regulation of nitric oxide synthesis. Dr. Michel earned his AB in Biochemical Sciences from Harvard College and received his MD and PhD degrees from Duke University, followed by clinical training in internal medicine and cardiology at Brigham and Women's Hospital. He has received numerous awards and honors including the Eugene Braunwald Award for Excellence in Teaching Clinical Cardiology in 2005 and was named a Scholar of the Academy at Harvard Medical School.

Manuel A. Navia, Ph.D.

Dr. Manuel A. Navia is a founder of Altus Pharmaceuticals, and has served on its Board of Directors since 1992. He served as Vice President and Senior Scientist at Vertex Pharmaceuticals from 1989 to 1997. He was a founder of The Althexis Company, Inc., and served as its President and Chief Executive Officer until 2001. He was then Executive Vice President for Research at the biotechnology company Essential Therapeutics, Inc. from 2001 to 2003. Dr. Navia is currently an Executive-in-Residence at Oxford Bioscience Partners, a venture capital firm that finances emerging companies in the life sciences and healthcare sectors. Dr. Navia holds a B.A. in Physics from New York University, as well as a Ph.D. and an M.S. in Biophysics from the University of Chicago.

James Olesen, Ph.D.

As a postdoctoral fellow at Harvard, Dr. James Olesenperformed cutting-edge research into the role chromatin plays in the regulation of gene expression. While working in the lab of Dr. Tom Maniatis, Dr. Olesen explored the possibility that a protein called High Mobility Group 1 can stimulate the functioning of conventional transcriptional activators. Dr. Olesen's research helped to create the foundation for further research into the way gene expression is affected by the packaging and location of chromatin in the nucleus. Dr. Olesen now works as a patent attorney with the Boston law firm Wilmer Cutler Pickering Hale and Dorr LLP, defending patent applications from local universities and biotechnology companies. In addition, he works with the firm's litigation department as an associate on patent infringement cases.

Faculty Discussion Tables

Jeff Tong, Ph.D.

Dr. Jeff Tong, who earned his A.B., M.M.S., A.M., and Ph.D. degrees from Harvard,serves as Vice President of Corporate and Product Development of Infinity Pharmaceuticals. Under his leadership, Infinity's product development team is researching the potential applications of the inhibitors IPI-504 and IPI-609in the treatment of cancer. Before joining Infinity, Dr. Tong served as a consultant with McKinsey and Company, working with leading biotechnology companies in the areas of new business building, venture investing, and technology spinouts. Dr. Tong was also a founding member of the Bauer Center for Genomics Research. In addition to his work with Infinity Pharmaceuticals, Dr. Tong's professional research interests have included histone deacytelase biology, particularly with respect to histone deacytelase identification and characterization.

Gregory Tucci, PhD.

Dr. Gregory Tucci is currently the assistant director of undergraduate studies for Harvard's Department of Chemistry and Chemical Biology and a concentration adviser. He is also a Senior Lecturer in Chemistry. He received his PhD., in Chemistry at Harvard University.

David van Vactor, Ph.D.

Dr. David van Vactor is a professor of Cell Biology at Harvard Medical School. He studies the mechanisms that guide neuronal processes to proper targets within the developing nervous system. David Van Vactor received his undergraduate degree in Behavioral Biology from Johns Hopkings University in 1985. He received his Ph.D. from the University of California, Los Angeles in 1991. Dr. Van Vactor is now an Assistant Professor in Cell Biology at Harvard and received his professorship after completing his postdoctoral research at the University of California, Berkeley.

Chemistry and Biochemistry

Leslie Beh — *Chemical and Physical Biology*
Pforzheimer 2011 — *Lbeh@fas.harvard.edu*

Characterization of the SUMO E2 activity of the polycomb repressive complex 2 (PRC2)

Department of Molecular and Cellular Biology, Harvard University

Polycomb Repressive Complex 2 (PRC2) is a highly conserved Polycomb Group (PcG) multiprotein complex that is involved in the faithful maintenance of the repression of Hox genes through repeated cell divisions. We have shown that the fruit fly (Drosophila melanogaster) PRC2 can act as a SUMO (Small Ubiquitin-related MOdifier) conjugating enzyme, being able to catalyze the attachment of a SUMO protein modifier to its target. This is supported by in vitro SUMOylation assays which show that the SUMOylation activity of PRC2 is dependent on substrate, SUMO activating enzyme, and ATP, but not on the canonical SUMO conjugating enzyme ubc9. Turnover ratio measurements yielded 3.59 molecules of SUMOylated substrate per molecule of PRC2, suggesting that PRC2 possesses catalytic SUMOylation activity, characteristic of an enzyme. Current experiments are focused at identifying the protein subunit within PRC2 that possesses this SUMO "E2" activity. SUMOylation has important functional consequences, ranging from an alteration of nuclear localization to transcriptional repression. The characterization of PRC2 as a novel SUMO E2 conjugating enzyme would expand existing knowledge of SUMOylation mechanisms and provide new insight into the role of SUMOylation in Polycomb-mediated silencing.

Joshua Green — *Chemistry*
Cabot 2010 — *jtgreen@fas.harvard.edu*

Development of a reaction discovery methodology employing mass spectrometry

Chemistry and Chemical Biology Department

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

Xin Guan — *Chemical and Physical Biology*
2012 — *guan@fas.harvard.edu*

Novel far-red monomeric and dimeric fluorescent protein pairs for improved FRET

National High Magnetic Field Laboratory

Metabolic FRET biosensors derived from cyan and yellow fluorescent protein variants have proven useful for a large number of in vivo detection strategies, ranging from intracellular pH and Calcium measurements to monitoring protease cleavage, phosphorylation, and the visualization of protein-protein interactions. Unfortunately, many of these sensors are hampered by relatively low dynamic range and the requirement for illumination wavelengths below 450 nm, a region where phototoxicity can seriously limit the useful lifetime of imaging experiments. In recent years, the fluorescent protein color palette has been expanded to include variants featuring excitation and emission wavelengths spanning almost the entire visible light spectrum. In this study, combinations of new monomeric and dimeric reef coral proteins with GFP derivatives as potential FRET pairs with the potential to display increased levels of sensitized emission in the orange, red, and deep red wavelengths were explored. Combinations of cyan, green, and yellow fluorescent proteins, derived from both corals and jellyfish, were paired with coral protein derivatives absorbing in the orange and red wavelength regions. In parallel with the FRET studies, investigations on comparing the efficiency of new fluorescent protein color variants in terms of performance in fusion constructs were compared to GFP derivatives.

Philip Petrou — *Chemistry*
Currier 2009 — *ppetrou@fas.harvard.edu*

One-pot synthesis of alpha-carbolines via Pd-mediated Heck coupling reaction

Lab for Drug Discovery in Neurodegeneration

A one-pot synthesis of alpha-carbolines via a Pd-mediated Heck coupling reaction was optimized to produce alpha-carbolines in high yield. Few methods have been established using palladium chemistry to form alpha-carbolines in one or two steps. Recently, a domino reaction has been reported that allows a one-step construction of annulated heterocycles by means of an amination and direct arylation via a palladium-mediated Heck coupling reaction. Optimization experiments were undergone to synthesize alpha-carboline in high yield from aniline and pyridine. The versatility of the developed methodology was tested using various substituted anilines and pyridines. Results indicated that electron-rich anilines and electron-poor pyridines favored heterocyclization. The results are evaluated in the context of a total synthesis of mescengricin, a reported neuroprotectant.

Mariama Runci *Molecular Cellular Biology*
2012 *meruncie@fas.harvard.edu*

Free radical scavengers arrest yeast cells prior to DNA replication

Stephen Kron Lab, Center for Molecular Oncology, University of Chicago

Reactive oxygen species (ROS) are free radicals that are byproducts from redox reactions. Although excessive amounts of free radicals can damage DNA, free radical production in the body is a normal and essential component of cellular metabolism. Recently, Dowdy and colleagues reported that human cells treated with a radical scavenger, Tempol, arrest prior to DNA replication. Additionally, preliminary studies indicated that yeast cells may also be affected in a similar way. Indeed, when we treated yeast cells with Tempol they arrested prior to DNA replication in 4 hours and resumed the cell cycle within one hour of removing Tempol. In order to determine if the arrest was caused by a lack of radicals, we added reagents that produce radicals to the arrested yeast and observed their effect on the arrest. Our preliminary results indicate that addition of Pyrogallol and Paraquat to Tempol arrested yeast slightly improved recovery from arrest. Based on these results, we propose that there is a sensor in yeast that detects the amount of radicals present. When the cell senses low amounts of radicals it receives a signal to arrest, and therefore does not replicate its DNA. We have identified several candidates that could be involved in sensing of radicals such as ribonuclease reductase (RNR), superoxide dismutase, and mitochondrial oxidative phosphorylation. We are in the process of examining the effect of Tempol on mutant strains of yeast lacking the possible radical sensor. Our results show that free radical scavengers arrest yeast prior to DNA replication in a reversible manner.

Elizabeth Ryznar *Chemistry and Physics*
Dunster 2010 *eryznar@fas.harvard.edu*

Discovery of new antibiotics in photorhabdus luminescens

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

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

Robyn Thom *Chemical and Physical Biology*
Eliot 2011 *rthom@fas.harvard.edu*

Characterization of epoxidixed carthamus tinctorius oil (ECTO) as a novel polyvinyl chloride plasticizer

Burt Lab, University of British Columbia

Polyvinyl chloride (PVC) is among the top three most widely used plastics. Di(2-ethylhexyl) phthalate (DEHP) is often added as a plasticizer by up to 40 wt% to impart flexibility. Plasticizers are low molecular weight compounds which are added to plastics to impart characteristics such as flexibility or strength. DEHP, a known toxin and carcinogen, can leach into the atmosphere, causing environmental contamination. This study investigates the suitability of epoxidized Carthamus tinctorius oil (ECTO) as a PVC plasticizer. The physical properties of PVC films with DEHP and ECTO were compared. ECTO was found to be a possible alternative for DEHP at up to 30 wt%, displaying statistically similar physical properties in elastic modulus, resilience modulus, and toughness. For glass transition temperature, ECTO was of significantly higher performance than DEHP at all wt%. ECTO has potential in replacing DEHP, as it exhibits similar plasticizing effects. With the inclusion of epoxy groups, this plasticizer should also have the added advantage of being a heat stabilizer. Previous studies have shown that epoxides are effective PVC thermal stabilizers. The use of ECTO not only has the potential to reduce the production of DEHP but may also minimize the manufacture of heat stabilizers, chemicals that are also often hazardous to the environment. ECTO, the derivative of a renewable natural vegetable oil, may be more environmentally friendly than DEHP and other petroleum-based plasticizers. If ECTO were implemented on a broad scale basis, this could alleviate the health and environmental concerns caused by widespread use of DEHP.

Nwamaka Uzoh *Engineering Sciences*
Leverett 2011 *amaka.uzoh@gmail.com*

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

Children's Hospital, Boston, Harvard Medical School

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

Computer Science

Brad Seiler — *Computer Science*
Lowell 2010 — *seiler@fas.harvard.edu*

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

The Zickler Lab, Graphics, Vision and Interaction Group, SEAS

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

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Seeing and sensing: visualization of the CitySense wireless sensor network

Sensor Networks Lab, Electrical Engineering and Computer Science, SEAS

Research in wireless networking has advanced rapidly, ranging from systems that gather sensor data from remote wilderness sites to globally distributed systems that provide Internet services. Most research-focused wireless networks are small; the larger ones are deployed in enclosed environments like office buildings and laboratories. To study the challenges of building an urban-scale, outdoor wireless network, we deploy a 100-node sensor network, called CitySense, in Cambridge, MA. The nodes, mounted on rooftops and streetlights, will contain sensors that collect weather, pollution and noise data. As an open testbed on which researchers may run data collection and network programs, CitySense addresses the question of how to manage applications and services over a network of environmentally exposed nodes with unstable connections. To analyze connection quality over the CitySense network, I have created software that aggregates data and visualizes the state of wireless links. My application analyzes variables like data transmission rate (TCP throughput) and loss rate of transmitted packets temporally, spatially and summarily. Preliminary analysis has shown that TCP throughput across outdoor nodes varies by time of day and correlates negatively with distance between nodes. The high noise levels of measurements and apparent leakages in TCP throughput over time remain unexplained; further development and use of my visualization tool should help answer such questions.

Earth and Planetary Sciences

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Shock waves in samples of icy sand

Shock Compression Laboratory

The analysis of crater formation on planets requires studies of high-velocity impact events; shock wave experiments on the materials that make up these craters give data similar to such events. By hitting a sample at a known speed (2.1 km/s), we can find the resulting temperatures and peak after-shock pressure, parameters required for crater modeling. The current sample under analysis is a mixture of ice and quartz. The radiance of the sample during and after the shock is recorded for four infrared and one visible wavelength; voltage measurements are converted into radiance temperatures by Planck's law for blackbody radiation. By fitting plots of the change in temperature over time to a model of theoretical radiance, we can find the absorption coefficients of the shocked and unshocked sample; we can then examine the post-shock radiance to find the relative proportion of extra hot spots to shocked sample and their respective temperatures. Our data showed us the progression of the shock through the sample, but our final temperature conclusions are uncertain due to the presence of air. We've also shot a sample of porous ice. Next we plan to design a way to create an ice-quartz sample that avoids air bubbles, and we're also looking at ways to measure the velocity of the shock front during the shock itself. We hope to then understand the effects of shocking icy ground.

Education

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Characteristics of ideal applicants to Boston-based residency programs

Beth Israel Deaconess Medical Center

There are currently 67,000 medical students who apply each year to 8,000 ACGME-accredited residency programs in various specialties. This application process places tremendous pressure on students to stand out from fellow applicants. There is no clear source of data that informs students about what characteristics are valued the most by residency directors in specific clinical disciplines. The purpose of this study is to provide quantifiable data that students can use to guide their medical school pathway towards choice residencies. Materials and Methods: 350 students at Harvard Medical School were asked which questions they had regarding residency acceptance criteria. A validated questionnaire was then generated targeting Boston ACGME-accredited residency programs. 51 residency directors verbally consented and were asked 9-22 questions, including questions about board scores, research, and second degrees. Data were analyzed based on all programs. In an open-ended question about evaluating an applicant's superiority, 78% of programs discussed clerkship grades as an influential factor ($p<0.05$) and 61% stated the Dean's letter ($p<0.05$), versus 49% that mentioned USMLE Step 1. This data didn't differ between surgical and non-surgical programs. When data were analyzed based on surgical (15) versus non-surgical specialties (36), surgical specialties had a mean board score of 217 (sd=16.3) below which they looked negatively at applicants, while non-surgical specialties had a mean of 196 (sd=37.4), $p<0.05$. There were 81% of non-surgical programs that reported having a second degree as beneficial, versus only 33% of surgical programs that did, $p<0.01$. Conclusion: The literature currently available regarding residency criteria talks only in qualitative terms. Our data go beyond which factors are important to state the importance of each variable. Students can use this data to become better prepared to apply to desired residencies.

Engineering and Bioengineering

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Inter-trial spacing increases retention in motor adaptation by specifically raising the level of a slow learning process

Harvard Neuromotor Control Lab

Over 2.5 million Americans suffer from motor disability due to strokes, accidents or neurological disorders. The rehabilitation process necessary for recuperation or management of such disabilities relies greatly on the ability to retain the training from session to session. A good understanding of the neural processes involved in this retention can serve to increase the effectiveness of these therapy sessions. We attempt to understand these underlying neural processes and try to elucidate the effect these mechanisms have on motor learning retention. Previous research has shown that there are at least two different neural mechanisms that contribute to learning and retention. We can separate the relative contributions of these processes to overall learning and retention by increasing the time delay between each individual trial of a motor task such as a reaching movement. Consequently, we are able to selectively alter the contribution from one of the processes, which has been shown to be responsible for retention. By having different groups of subjects perform a learning task with different inter-trial times and by measuring their retention after 60 second and 24-hour delays, we show that the group with the longest spacing between trials has the best retention, whereas the group with the shortest spacing has the worst retention.

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Anti-foaming without defoaming agents

Stone Lab

We study the entrainment of air bubbles as a result of multiple drop impacts on a liquid/air interface. Previous studies from the literature have focused almost exclusively on the mechanism by which a single drop impacting a flat liquid-air interface entrains an air bubble. For sufficiently small droplets at low velocities, the existing literature predicts that no air bubbles will be entrained, but we often observe air entrainment if two drops impact sequentially. We qualitatively identify different entrainment behaviors following the sequential impact of two drops, and we present experimental data quantifying the critical crater depth and the time interval between successive drops necessary to entrain bubbles. We apply this approach to 1 mm diameter drops impacting a liquid surface with speed $u\approx 1$ m/s (We ≈ 10) and find that a critical separation time $t< 5$ ms is necessary for bubble entrainment. This critical time agrees with a dimensional estimate of the time necessary for an impact crater to close owing to capillary effects. Using these ideas we demonstrate a rotating-nozzle apparatus which prevents sequential drop impacts and consequently suppresses foam formation. The key implication of this technology is the development of liquid-into-liquid dispensers that suppress foam without requiring the use of chemical defoaming agents.

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In Silico model of acetate production in S. cerevisiae

Silver lab

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

Isabelle Dortonne
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Biomechanical response of the in-situ primate lens

University of Miami Ophthalmic Biophysics Laboratory

Presbyopia, the most common refractive disorder of the elderly, is the age-related loss of accommodative ability, or near vision. Lens and capsule-based theories of presbyopia assert that the decrease in accommodative amplitude can be attributed to increased hardening of the lens substance and decreased elasticity of the lens capsular bag with age. One novel technique in the restoration of accommodation is Phaco-Ersatz, or lens refilling. The promise of Phaco-Ersatz can be assessed by characterizing the biomechanical properties of the lens in its natural versus empty state. Postmortem cynomolgus monkey, rhesus monkey, and human eyes of varying ages were stretched in their natural and empty states in an Ex-Vivo Accommodation Simulator in eight, 0.25mm steps, mimicking the changes in zonular tension that occur in-vivo. The diameter-force relationship of the natural and empty lens was characterized and compared. There was no relationship between the empty-bag diameter slope and age, indicating that the lens capsule's mechanical properties do not change the setting of accommodation. Moreover, the ratio of the empty capsule to natural lens load-diameter slope decreased significantly with age, proving that it rnis the lens material, and not the elasticity of the capsular bag, that contributes to presbyopia. The rnresults confirm the postulation that accommodation can be efficiently conducted as long as the lens rncontents have proper viscoelastic properties. Thus, Phaco-Ersatz is a viable future treatment for rnpresbyopia.

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Proliferation of osteoblast cells on biodegradable scaffolds

Van Dyke Lab, Boston University School of Dental Medicine

Bone loss or fractures often require the substitution of bone with grafting materials. Several methods have been tested to treat bone loss in patients. Tissue-engineering bone is a novel process being researched to grow osteoblast cells in vitro on biodegradable scaffolds, with the aim of later using these scaffolds in vivo. The purpose of this experiment is to study the proliferation of osteoblast cells in the presence of varying supplements on biodegradable scaffolds to facilitate their growth. By using an established cell culture model, we investigated the development of human bone cells on a biodegradable scaffold. Cells were tested for the presence of alkaline phosphatase and mineralization to ensure that the cells were indeed osteoblasts and were viable. In addition, we were able to conclude that supplements of fibrinogen and thrombin are necessary for the cells to become embedded within the scaffolds, and therefore improve the effectiveness of the scaffolds compared to samples not treated with these additions.

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Fabrication of single atom nano wires for applications toward quantum computation

Keio University, Tokyo, Japan

Developments in nanotechnology over the last two decades have opened up the possibility of unparalleled computing power, such as the theoretical development of a quantum computer. The realization of quantum computers depends on our ability to specifically place quantum bits (qubits) in positions where they can be initialized and measured. In our all-silicon model of a quantum computer, we utilize silicon 29 isotopes as our qubits due to their inherent nuclear spin. Phosphorous 31 must be deposited at regular intervals between 29Si atoms so that its spin-polarized valence electron can initialize the silicon atoms' nuclear spin states via spin diffusion. In this study we aimed to fabricate single atom-wide 29Si nanowires with regular spaced 31P atoms using molecular beam epitaxy (MBE). rnIt is clear that a bottom-up approach is the most feasible and efficient way to fabricate single atom nanowires. In this method we use MBE to deposit 29Si and 31P atoms on a surface upon which the atoms self assemble into nanowire structures. Silicon(111) wafers were polished and heated to form the relatively stable 7x7 DAS Reconstructed surface in either U- or F-step conformations. Intentional kinks were made at regular intervals along the steps so that there were high energy corners with many dangling bonds. Low concentrations of 31P were first deposited on the surface and migrated to the high-energy corners. Silicon 29 was deposited next to form neat rows along the step edges. Proper fabrication of 29Si nanowires with regularly spaced 31P atoms was assessed using scanning tunneling microscopy (STM) and spectroscopy (STS). Fabrication of qubit arrays represents the first step towards realization of a quantum computer.

David Levary *Chemistry and Physics*
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A high-throughput, rapid, and highly sensitive selection method for de novo protein engineering

Wittrup Lab

The ability to create proteins with predefined properties would revolutionize fields ranging from medicine to environmental engineering, accomplishing tasks from tissue-specific drug delivery to bioremediation. While rational design of proteins remains difficult due to a lack of quantitative structure-function relationships, directed evolution through random mutagenesis and selection from combinatorial protein libraries has proven highly successful and is ideal for improving existing protein-target interactions. Isolation of novel protein interactions from naïve libraries (e.g. the human antibody library), however, is complicated due to their relative weakness before in vitro evolution. Using a yeast-based platform, we have developed a highly sensitive, rapid, and economical method for isolation of de novo protein interactions based on multivalent antigen presentation. This system allows microgram quantities of target to identify and select even extremely weak binders from libraries of billions of proteins, combining the advantages of yeast as a protein engineering host and complimenting the subsequent use of directed evolution to isolate variants with improved properties. Our method has already been adopted by several protein engineering groups, facilitating de novo design projects that were nearly impossible using traditional in vitro selection methods. We are currently using multivalent antigen display to develop antibodies for cancer radioimmunotherapy.

John Liu *Chemical and Physical Biology*
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RNA-based molecular sensors for RNA transcripts

FAS Center for Systems Biology

Molecular automata that combine sensing, computation, and actuation enable programmable manipulation of biological systems. Here, we construct and implement molecular sensors for detecting RNA transcripts in vitro. Composed entirely of small RNA strands, the sensors can switch from siRNA-inactive to siRNA-active states upon sensing input mRNA transcripts in buffer by strand displacement. In the siRNA-active state, siRNA-like duplexes are formed and trigger RNA interference upon target transcripts. We show that the RNA sensors are capable of detecting several different input RNAs, following 2nd order reaction kinetics. The sensors can rapidly detect transcripts of different lengths (140 to 2157nt) and different concentrations (50 nM to 750 nM). Hence, this study provides a potential sensory mechanism to detect endogenous RNA expression patterns in cells.

Mathematics

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Omnibus Sequences

Godbole Lab

Consider locating words of length k as subsequences of a length n random string. How large should n be in order to ensure that all a^k words are present, where a is the alphabet size? In this project we prove necessary and sufficient conditions for such to occur, and we provide probabilistic and statistical analyses of their frequency. Efficient listings of words have been previously studied using universal cycles; however, the method we present requires a significantly shorter string to encode the same number of words. Several potential applications are presented. For example, this project demonstrates how Tolstoy's *War and Peace* contains this abstract, or any other abstract of this length.

Kelley Harris — *Mathematics*
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On integers n that divide $\varphi(n) + \sigma(n)$

Gallian Lab

The expressions $\varphi(n) + \sigma(n) - 3n$ and $\varphi(n) + \sigma(n) - 4n$ are unusual among linear combinations of arithmetic functions in that they each vanish on a nonempty set of composite numbers. In 1966, Nicol proved that the set $A := \{n \mid (\varphi(n) + \sigma(n))/n \text{ in } \mathbf{N}_{\geq 3}\}$ contains $2^a \cdot 3 \cdot (2^{a-2} \cdot 7 - 1)$ if and only if $2^{a-2} \cdot 7 - 1$ is prime and conjectured that A contains no odd integers. In this paper, we let A_K denote the set of n in A with exactly K prime factors and present a computer-implementable algorithm that decides whether Nicol's conjecture holds for a given A_K. We verify Nicol's conjecture for numbers with fewer than seven prime factors, and completely classify the elements of A that have fewer than five prime factors. In addition, we prove that every A_K is contained in a finite union of sequences that each have the form $\{p_1^{a(1i)} \cdot \ldots \cdot p_k^{a(ki)} \cdot u \cdot w_i\}$, where $p_1, \ldots, p_k$ are distinct primes and each w_i is relatively prime to $p_1 \cdot \ldots \cdot p_k \cdot u$. More specifically, we prove that all but finitely many n in A_4 have the form $2^a \cdot 3 \cdot p_3 \cdot p_4$, and that all but finitely many n in A_5 have the form $2^a \cdot 3 \cdot p_3^{a(3)} \cdot p_4^{a(4)} \cdot p_5^{a(5)}$.

Xiaoqi Zhu — *Applied Mathematics*
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Gram Determinant of States in Planar Surface

Przytycki Lab, GWU

An application of the Catalan numbers, a standard combinatorial sequence, arises when computing the number of graphs that can be drawn in a disk with n non-crossing chords connecting $2n$ points along the outer boundary of the disk. This problem is closely related to computing the number of graphs in an annulus with n non-crossing chords. Rodica Simion proposed a number of questions regarding the Gram determinant of a bilinear form over these graphs, which have since been researched. My research investigated a generalization of the bilinear form based on curves in an annulus. Roughly speaking, I investigated the Gram determinant of the bilinear form over the set of graphs in a disk with two holes, and proved several results regarding this determinant. My research concludes that the determinant based on n-1 curves divides the determinant based on n curves. I also proved explicit formulas for certain terms in the determinant. Additionally, a number of symmetries were identified in the Gram matrix and provide algorithms for simplifying the calculation of the Gram determinant. The results are the first step toward calculating a complete factorization of the Gram determinant. Based on calculations and observations, I advance a number of conjectures regarding said factorization and provide future direction for the project. The project is also related to topics in knot theory, which will be further investigated at a later time.

Microbiology

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A Novel Approach to the Detection of Pathogenic Bacteria from the Classroom to the Clinic

Harber Lab, Oxnard and Ventura Colleges with CCBC

Currently, illnesses caused by rampant bacterial infections of *Methicillin-Resistant Staphylococcus Aureus* (MRSA), *Bacillus Anthracis* (Anthrax), and *E. coli*, among others, are extremely difficult to diagnose and treat due to the current reliance on outdated biochemical tests and culturing procedures taking days to weeks for identification. In the midst of reports of tomatoes, jalapenos, and spinach tainted with unknown E coli strands, the world needs systems and protocols in place that can detect strains of bacteria within minutes that depend on cutting-edge genetic identification, not staining and culturing methods that are grounded in technology from the 1800's. With this problem in mind, our lab set out to bring cost effective and convenient protocols involving the Polymerase Chain Reaction method to the general public through optimizing a Cepheid Real-Time PCR system to successfully gauge the ability to detect bacteria in 15 minutes using a lyophilized primer set. From there, our goal was to discover an alternate (and lower cost route) to achieve the same goal so that students or any clinician could simply and rapidly duplicate this experiment using PCR equipment. Using a degenerate PCR primer set (targeting the gene for the 16s ribosomal subunit) whose sequence had had been previously published, our lab optimized another protocol to amplify the DNA of 32 different genera of bacteria using this single primer set. After running the DNA on an agarose gel, we verified how each sample would produce its own uniquely identifiable banding pattern, allowing virtually anyone to discern one bacterium from another in a matter of hours and for pennies per reaction.

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

Harvard School of Public Health

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

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Evolution of Marine Cyanobacteria in the Red Sea

Post Lab, Marine Biological Laboratory

The cyanobacteria *Prochlorococcus* and *Synechococcus* are the smallest photosynthetic organisms on Earth yet account for two-thirds of the oceans' photosynthetic reactions. Their abilities to survive in diverse environments make them ideal for studies of genetic diversity. This research investigated the genetic compositions of multiple *Prochlorococcus* and *Synechococcus* species isolated from the Red Sea to determine how the organisms have evolved to nitrogen stress conditions and therefore differ from cyanobacteria found in other bodies of water. PCR-based sequencing of cyanobacterial DNA was following by computer-based analyses to determine the functions of sequenced genes. A phylogenetic analysis compared the sequences of Prochlorococcus marinus strains 8390-C9 and 13A3 to Synechococcus species 4320-C2 and 4320-C3. The alignments were used to observe genotypic adaptations to nitrogen stress, causing sequences to differ from *P. marinus* strain HOT0M-8F9, isolated from the Pacific Ocean.rn One hundred ninety-two open reading frames, corresponding to possible genes, were identified through sequencing and assembly. Of these, 95 - 100% had highest-significance matches to other cyanobacterial genes, indicating that the geographic boundaries separating species do not cause extreme evolutionary divergence. Twenty-six nitrogen assimilating and metabolizing genes were identified in the two *Synechococcus* sequences. Only one such gene was found in *Prochlorococcus* DNA. This lack of nitrogen related genes was surprising due to high levels of nitrogen stress found in the Red Sea.rn The ability of *Prochlorococcus* to survive in nitrogen-stressed environments despite an apparent lack of necessary genes for nitrogen metabolism should be further investigated.

Charles Liu *Chemical and Physical Biology*
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Investigating periplasmic transit of lipopolysaccharide during bacterial outer membrane biogenesis

FAS Department of Chemistry and Chemical Biology,
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The cell envelope of gram-negative bacteria consists of an inner (IM) and an outer membrane (OM) separated by an aqueous space, the periplasm, containing the peptidoglycan cell wall. The OM is asymmetric, with phospholipids in its inner leaflet and lipopolysaccharide (LPS) in its outer leaflet. LPS plays a crucial role in the OM's barrier function and is essential for survival in most gram-negative bacteria, but until recently little was known about its transport to the OM. Our lab studies the LPS transport pathway, providing insight into the unique process of OM biogenesis and uncovering potential targets for novel antibiotics, which are urgently needed to address the burgeoning global health threat posed by antibiotic-resistant bacterial infections.rnrnTo better understand periplasmic transit of LPS, we cloned, overexpressed, and purified the periplasmic portion of LptC, an essential IM protein that has been implicated in LPS transport. Currently, this purified fragment is being used to develop LptC-specific antibodies that will allow us to identify the presence of LptC in protein complexes in vitro. We also tested the ability of this LptC fragment to bind LPS. Results to date suggest that LptC alone does not bind LPS; it may be that LptC-LPS interactions require the presence of other proteins in the LPS transport pathway. Future directions include crystallographic analysis of the periplasmic portion of LptC and further studies of its interactions with other proteins and LPS.

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Arenaviruses utilize variants of human transferrin receptor 1

Children's Hospital

The New World (NW) arenaviruses are RNA viruses that can cause severe disease in humans. They spread to humans from their natural reservoirs, which are typically small rodents in South America. Four NW arenaviruses are pathogenic to humans. The Amapari (AMAV) and Tacaribe (TCRV) viruses are closely related to the four pathogenic NW arenaviruses, but AMAV and TCRV do not cause disease in humans.rnrnThe pathogenic NW arenaviruses use human transferrin receptor 1 (hTfR1), and this correlates with their ability to cause hemorrhagic fevers. Recent studies have shown that although AMAV and TCRV cannot use hTfR1 to infect cells, they can use animal forms of TfR1. Here, we used mutational studies to show that variants of hTfR1 can support the infection of viruses bearing the surface proteins of AMAV and TACV (called 'pseudoviruses'). Exchanging only three amino acids in hTfR1 for amino acids found in the same position in animal TfR1 converted hfR1 into an efficient receptor for TCRV pseudoviruses. Altering eight residues in this region of hTfR1 converted it into an efficient receptor for both AMAV and TCRV pseudoviruses. Our studies shed light on arenavirus evolution and the potential for two nonpathogenic arenaviruses to emerge as human pathogens.

James Pelletier *Chemical and Physical Biology*
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Physical model of the bacterial chromosome and its relation to cell growth in E. coli

Jun Lab, FAS Center for Systems Biology

Chromosome segregation influences and is influenced by other growth processes, a part of an incredible integrated network where each process loses its individual reductionist identity to the concept of a unified whole. Cell growth rate provides the ultimate reflection of this coordination of events. For one E. coli to become two, replicated strands of the long circular chromosome move towards opposite cell halves before the cell divides. To explain this phenomenon, our lab postulates a physical mechanism. In this model, mixed daughter strands explore available entropic conformations and consequently segregate. To test this model experimentally, we employ techniques along an interface of disciplines from single molecule biophysics to microfluidics, optics to computer simulation. We exploit osmosis to lyse the cells and release their chromosomes for experiment. We visited the nanofabrication facility at Cornell to design and fabricate microfluidic chips with features as small as one micron. To confine and compress bacterial DNA in vitro, we will use these chips together with hydrodynamic flow or with optical tweezers. We will use other microfluidic chips to measure the growth rate of E. coli confined to a channel, and we will continue to design image processing software for high throughput analysis of single cells amidst a growing population. These dual research pursuits will explore the extent to which physical principles underlie and reveal the foundations of prokaryotic life.

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siRNA-induced depletion of cellular factors impedes mRNA degradation by the Virion Host Shutoff (VHS) protein of Herpes Simplex

University of Missouri-Kansas City

Herpes Simplex Virus (HSV) is a highly contagious infectious virus that affects approximately 80% of humans. The Virion Host Shutoff (Vhs) protein is an essential component of HSV that initiates host mRNA degradation, causing cell death, during infection. In phase I of this study, the importance of cellular translation factors eIF4H and eIF4B on Vhs function was shown. siRNA-transfected HeLa cells were infected with WT HSV-1 and analyzed by Real-Time rtPCR targeting 18S-rRNA and ACTB-mRNA. The results showed that in contrast to all other samples, HSV-infected cells with depleted eIF4H had up to 99% of the gene expression of normal, non-infected cells suggesting that eIF4H is essential to Vhs function. In phase II, the eIF4AI, eIF4AII, and eIF4AIII factors were studied. The results showed decreased Vhs binding with depleted eIF4AIII, suggesting that the exon junction complex is a key mRNA marker for Vhs binding. This study also revealed that the eIF4AI and eIF4AII factors possibly tradeoff function in the cell and have partially regulatory effects on Vhs function, both of which are novel findings. These findings expose the Vhs pathway and mechanism of HSV infections, and have potential for the development of effective antiviral treatments or possible cures for HSV and other viral infections.

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Gut reactions: the interplay of Pseudomonas entomophila and gut microbiota in Drosophila melanogaster

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As evidence accumulates for the significant involvement of native gut microbiota in various conditions of human health and disease, the need for an easily manipulated animal model intensifies. Recent studies have identified *Drosophila* as a model organism for studying the interactions of host and commensal bacteria during pathogenic challenge, and here, we examine the specific impact of the microbiota on adult fly oral infection by *Pseudomonas entomophila* (Pe), an entomopathogen that persists in the gut and stimulates local and systemic immunity via the Imd pathway. Initially, we characterize the resting state gut microbial composition. Comparing Pe infection outcomes in axenic (lacking gut bacteria) and non-axenic flies, we then find that the presence of indigenous bacteria reduces host survival and increases systemic Imd activation in response to Pe. Pe-infected axenic flies conversely show diminished expression of Imd and stress genes, as well as decreased reactive oxygen species production. Since sustained Imd activity has been observed to have deleterious effects in *Drosophila*, we postulate that microbiota-mediated overstimulation of the immune system may contribute to Pe pathogenesis. Furthermore, the *Drosophila*/Pe system, in which the host gut bacterial communities can be methodically controlled, may prove useful for generalized studies of crosstalk between host, pathogen, and microbiota.

Jue Wang — *Chemical and Physical Biology*
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Phenotyping unculturable soil bacteria

Kishony Lab

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

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Molecular Evolution of Host Phage Specificity in Serial Passage

Chen Lab

The rise of antibiotic-resistant bacteria has cloaked the future treatment of infectious diseases in ambiguity. As a result, there is resurging interest in phage therapy, the use of bacteriophages to combat bacterial pathogens; in principle, the main advantage of phages is their potential evolvability. This project aims to understand the evolutionary adaptability of the host specificity of phages under serial passage evolution. The eventual goal is to engineer phages to target specific bacterial pathogens, such as *S. typhimurium*. The serial passage evolution of phages to infect S. typhimurium takes two routes: weak and strong selection. For both selection processes, *S. typhimurium* is transformed with the M13 phage that normally infects *E. coli*. Weak selection selects for *S .typhimurium* infected with the M13 phage, while strong selection only selects for the evolving M13 phage. Preliminary results show decreased phage infectivity on *E. coli* and maintained infectivity on *S. typhimurium*; further studies are underway. Simultaneously, mutations in the phage genome will be monitored to better understand the phage's evolution on a molecular level. In time, the evolving genome will be mapped onto a fitness landscape for comprehensive assessment of genotype variants at the peaks of high fitness and *S. typhimurium* infectivity.

Molecular and Cellular Biology

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Engineering Recombinase Enzymes to Emulate the CCR5-delta32 Mutation Conferring Resistance to HIV Infection

Liu Lab

We are working to create recombinase enzymes capable of excising the end of gene CCR5, thus emulating the naturally occurring CCR5Δ32 deletion mutation conferring resistance to HIV infection. This mutation yields a premature stop codon in the CCR5 coding region, producing a truncated receptor that no longer facilitates entry of HIV into cells. I intend to engineer recombinase enzymes which will specifically target CCR5 to generate an analogous mutation for people having the normal-length gene product.rnrn We have identified promising target sites on the end of CCR5 based on known zinc finger binding affinities and engineered the sites in four separate zinc finger recombinase enzymes. Although the four recombinase enzymes will work as a tetramer to excise part of CCR5, their individual activity on their respective sites was found to be varied. We will thus evolve some for further activity and subsequently test all four together, in bacteria, on the relevant segment of CCR5. Should little heterotetramer activity be found, further directed evolution may be necessary. Finally, after concluding that all four recombinases are active, we will test the recombinases in mammalian cells—their ultimate objective for mimicking the CCR5Δ32 deletion mutant and furthering developments towards HIV preventive therapies.

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Transcriptional Dynamics of Endodermal Organ Formation

Melton Lab

Although endodermal organs including the liver, pancreas, and intestine are of significant therapeutic interest, the mechanism by which the endoderm is divided into organ domains during embryogenesis is not well understood. To better understand this process, global gene expression profiling was performed on early endodermal organ domains. This global analysis was followed up by dynamic immunofluorescence analysis of key transcription factors, uncovering novel expression patterns as well as cell surface proteins that allow prospective isolation of specific endodermal organ domains. Additionally, a repressive interaction between Cdx2 and Sox2 was found to occur at the prospective stomach-intestine border, with the hepatic and pancreatic domains forming at this boundary, and Hlxb9 was revealed to have graded expression along the dorsal-ventral axis. These results contribute to understanding the mechanism of endodermal organogenesis and should assist efforts to replicate this process using pluripotent stem cells.

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Functional Characterization of Mammalian Homologues of the Yeast Longevity Factor Sir2

Alt Lab, HMS, Children's Hospital Boston, HHMI

Sirtuins are mammalian homologs of the yeast Sir2 protein that regulate lifespan in a large variety of organisms. The mechanisms through which they function are not well understood. There are seven mammalian sirtuins, named SIRT1 through SIRT7, of which SIRT3 is the focus of this study. SIRT3 is a mitochondrial protein and mitochondria purified from SIRT3-deficient mice contain large amounts of heavily acetylated proteins, suggesting that SIRT3 plays a role as a deacetylase. However, the effects of mitochondrial acetylation are unclear because SIRT3 mice are apparently phenotypically normal. Using SIRT3 to deacetylate key mitochondrial proteins, including CPS1, a urea cycle protein, the effects of mitochondrial acetylation were sought to be determined. Unlike SIRT3 deficiency, however, SIRT6 deficiency results in a severe phenotype of progeria, or premature aging. Mice lacking SIRT6 are physically smaller and develop slower than wild-type mice. In addition, all die before reaching one month of age. SIRT6-deficient cells possess high levels of reactive oxygen species that may contribute to DNA instability and the aging phenotype. This suggests that SIRT6 may be important in antioxidant defense. SIRT6's role in antioxidant defense was also assayed. Preliminary survival assays reveal that SIRT6 deficient cells exhibit increased sensitivity to oxidizing agents such as methyl methanosulfate and hydrogen peroxide. The next step is to determine whether or not antioxidants such as N-acetylcysteine can rescue such sensitivity.

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Diabetes Mellitus Type II: Understanding the Pathogenesis of Low Birth Weight Associated Diabetes through its effects on Brown Adipose tissue

Patti Lab

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

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

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The Role of DNA-Repair Proteins in Rates of Tandem Repeat Recombination

Verstrepen Lab, FAS Center for Systems Biology

Tandem repeats, units of 2 or more nucleotides repeating head-to-tail, are regions of genomic instability. Although prior research has shown the variation in number of repeat units to be more than 2 orders of magnitude greater than in non-repetitive DNA, the specific mechanism by which this repeat mutation, or recombination, occurs is still unknown. Several well-known DNA-repairing proteins, however, may be involved in this complex series of rearrangements during DNA replication. Using S. cerevisiae, or Brewer's Yeast, as a model organism, the aim of this project therefore, is to investigate the roles and relative significance of such repair proteins in this phenomenon. By inserting artificial tandem repeats into the coding region of a gene, any variation in the number of repeat units would cause a frameshift mutation that turns the gene on or off. Alternating between media that selects for the expression or non-expression of the gene, only cells that mutate can survive. By experimenting with cells in which selected DNA-repairing proteins have been deleted, relative mutation rates can be compared, and the importance of these proteins to tandem repeat recombination assessed. Thus far, several deletion strains have shown promisingly high mutation rates, and inspire further investigation of the specific mechanisms of these proteins with relation to tandem repeat recombination.

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Bone growth in zebrafish occurs via multiple pulses of cell proliferation

Iovine Lab, Lehigh University

Fin length in the zebrafish is achieved by the distal addition of bony segments of the correct length. Genetic and molecular data provided evidence that segment growth utilizes a single pulse of growth, followed by a period of stasis. Examination of cell proliferation during segment growth was predicted to expose a graphical model consistent with a single burst of cell division (e.g. constant, parabolic, or exponential decay) during the lengthening of the distal-most segment. Cell proliferation was detected either by labeling animals with BrdU (during S-phase) or monitoring histone3-phosphate (mitosis). Results from both methods revealed that the number of proliferating cells fluctuates in apparent pulses as a segment grows (i.e. during the growth phase). Thus, rather than segment size being the result of a single burst of proliferation, it appears that segment growth is the result of several pulses of cell division that occur about every 60 microns (average segment length ~ 250 microns). These results indicate that segment lengthening requires multiple pulses of cell proliferation. In addition,the gap junction protein Connexin43 was found to regulate mesenchymal cell proliferation of osteoblast precursors. For more info see publication: http://www.lehigh.edu/bio/pdf/Iovine/Jain%20et%20al.%202007.pdf.

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The Effect of Hypertrophic Stimuli Upon Cardiac Progenitor Cells

Liao Lab, Cardiology Division: Brigham and Women's Hospital, Harvard Medical School

Heart failure is the leading cause of death in the United States. It affects more than 5 million people and many of them will succumb within the next 5 years. Heart failure is a clinical symptom defined by the inability of the heart to pump an adequate amount of blood to the body to support the normal workload. After suffering a myocardial infarction (heart attack), to maintain cardiac function, the heart will undergo a series of molecular and structural remodeling mechanisms. Typically, this remodeling is done through a phase called cardiac hypertrophy, whereby individual cardiomyocytes increase in size in order to compensate for the loss of cell mass due to the infarction. Although this remodeling process is an initial attempt to repair itself, it ultimately leads to heart failure.rnCardiomyocytes enlarge in response to several known inducers of hypertrophy, including phenylephrine (PE), endothelin-1 (ET-1), and Angiotensin II (ATII). One type of resident cardiac progenitor cell, the Cardiac Side Population (CSP) cell, has been shown to proliferate in vitro and differentiate into several cardiac cell lineages, including functional cardiomyocytes, upon proper stimulation (Pfister, et al., 2005 and Mouquet et al., 2005). It still remains unclear what role the hypertrophic stimuli (AT II, ET-1, and PE) play on CSP cells. Whether these cardiac progenitor cells also respond to the hypertrophic stimuli (AT II, ET-1, and PE) as do the cardiomyocytes is unknown. This project investigates the effects of these inducers on murine CSP cells.

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

Barouch Lab, Beth Israel Deaconess Medical Center

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

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

McMahon Lab, Harvard University

The Sonic Hedgehog (Shh) protein plays a significant role in directing the formation of tissues and organs in the development of the embryo. By establishing a concentration gradient across tissues, Shh influences the fate of progenitor cells in developmental processes such as digit specification and neural cell differentiation. Therefore, investigating the cellular response to Shh levels reveals how Shh regulates such developmental processes and how they go awry in birth defects and cancer. To this end, it is important to explore the interactions between cell surface receptor proteins and the Shh protein. In this project, we focus on two of the Shh receptors – Cdo and Boc. Efforts are needed to clarify their mechanism of function, their relative contribution to signaling, and their interactions with Shh. Through transfections, receptor expression is knocked down and/or overexpressed in cells, whose response is quantified by the luminescence of a reporter gene. So far, knockdown results have confirmed the inductive functions of both receptors; overexpression experiments have suggested the possibility of Cdo and Boc as having additive rather than redundant roles. In future experiments, live imagery will be used to observe changes in cell surface distributions of the receptors as they bind to Shh.

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Collaboration Between Mutation and Epimutation in Medulloblastoma

Jackson-Grusby Lab, Harvard Stem Cell Institute, Longwood

Medulloblastoma is a devastating pediatric brain tumor that arises in the cerebellum. The development of medulloblastoma has been linked to the Sonic Hedgehog/Patched (Shh/Ptch) signaling pathway through genetic analysis. A number of inactivating mutations in human Ptch, often accompanied by the deletion of the second allele, have been found in 8-12% of medulloblastoma, suggesting that a loss of Ptch function contributes to tumor formation. In addition to genetic mutations seen in tumors, epigenetic alterations often play key roles in tumor initiation and progression. For example, deregulation of imprinted gene expression [loss of imprinting (LOI)] has been shown to be an early and abundantly observed alteration in various types of human cancers. Our lab has shown that through this epigenetic mutation, the Igf2 pathway is deregulated in LOI murine embryos, leading to an overgrowth phenotype, which can be restored by reactivating Igf2R. Given the corroboration of all these findings, my hypothesis is that there is a synergistic collaboration between the genetic Ptch mutation and the epigenetic mutation seen through LOI in the development of medulloblastoma. In order to test my hypothesis, I plan to address the following questions: (1) in the mutated Ptch mouse, is tumorigenesis modified by induced epigenetic changes such as LOI?; (2) which imprinted loci are involved in this change of phenotype?; (3) and more specifically, does Igf2 signaling in the LOI/Ptch medulloblastoma model potentiate tumorigenesis?

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

Wagers Lab, Joslin Diabetes Center

Adult skeletal muscle regeneration is mediated by myogenic precursor cells, which are a subset of myofiber-associated mononuclear satellite cells located beneath the basal lamina of multinucleated mature muscle fibers. The Wagers lab recently demonstrated the presence of self-renewing muscle stem cells in adult skeletal muscle and showed that transplantation of these cells could be used therapeutically to enhance muscle function in a mouse model of Duchenne muscular dystrophy. Yet, the molecular pathways that regulate these skeletal muscle stem cells and control their function in muscle growth and repair remain unclear. Understanding these regulatory mechanisms could suggest strategies to improve their expansion potential and regenerative activity and may clarify what goes wrong in abnormal muscle development such as tumor formation. Recent research implicates a role for the conserved developmental morphogen Sonic hedgehog (Shh) in skeletal muscle development. This project aims to clarify the role of Shh signaling in adult muscle regeneration by (i) qPCR to determine which components of the Shh pathway are expressed in injured/regenerating vs. uninjured muscle; (ii) in vitro culture of muscle stem cells to determine what effect(s) Shh pathway agonists and antagonists have on their differentiation, proliferation, and apoptosis; (iii) fluorescence-activated cell sorting (FACS) and immunohistochemistry of transgenic reporter mice to determine which cell populations in muscle produce and receive Shh signaling; and (iv) lentiviral transfection and transplantation of muscle stem cells to determine whether these cells can trigger neoplastic or otherwise abnormal muscle development.

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Temperature Compensation in the Three-Protein Cyanobacterial Circadian Clock

O'Shea Lab

Global gene expression in the cyanobacterium Synechococcus elongatus PCC 7942 is tightly regulated by a circadian clock organized around a core protein oscillator comprised of three proteins, KaiA, KaiB and KaiC. In 2005, circadian oscillation in KaiC phosphorylation was reconstituted in vitro with just the Kai proteins and ATP. rnrnA defining feature of circadian clocks is temperature compensation: the period of oscillation remains nearly constant over a broad range of physiologically relevant temperatures. Remarkably, the in vitro Kai protein oscillator is similarly temperature-compensated. This research project aims to understand the mechanism by which the temperature compensation of the in vitro oscillator occurs. Two possible methods of temperature compensation are apparent. All individual phosphorylation and dephosphorylation rates could be compensated, or the rates could vary such that they would mutually offset one another.rnrnMeasurement would be taken of the rates of partial reactions at different temperatures to isolate which rates and their corresponding interactions form the basis for temperature compensation. Preliminary data from the partial reactions appear to support a model of mutual compensation. A mathematical model for the oscillator would be fitted to complete set of experimental data to isolate the rates of each individual phosphorylation and dephosphorylation reaction. rnrnFurther work would proceed by hijacking the circadian cycles at critical points to observe the effect of temperature changes on well-understood protein interactions to propose a molecular basis for the compensation.

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Identifying Components of Complexes Containing the Polycomb Group Proteins Bmi1 and Mel18 During Mitosis

Francis Lab

The Polycomb Group (PcG) proteins mediate transcriptional repression which is stable through rounds of cell division. PcG proteins are implicated in many types of cancer and play a key role in development. PcG proteins also silence a subset of developmental genes in order to maintain pluripotency in embryonic stem (ES) cells. Mitosis presents a challenge to PcG-mediated gene silencing. During mitosis, chromatin undergoes roughly 500-fold compaction; this gross structural rearrangement is accompanied by many changes in the chromatin, including the identity of proteins bound. It is not known whether PcG proteins remain associated with chromatin during mitosis. If they are stripped from the DNA, how do they return to genes which are supposed to be silenced? If PcG proteins remain bound to DNA, what factors aid in this process? PcG complexes might interact with different proteins at different stages in the cell cycle--such proteins might disrupt their interaction with chromatin, or help maintain them on chromatin during DNA replication or mitosis. This project aims at identifying proteins that interact with Bmi1 and Mel18 specifically during mitosis. The identity and function of these proteins or complexes may help elucidate the mechanism by which PcG silencing is maintained through rounds of cell division. Thus far, an experimental model in which to study Bmi1 and Mel18 has been developed; both pluripotent and committed cell lines expressing epitope-tagged Bmi1 or Mel18 have been created.

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Studying NRAMP Protein Structure via X-ray Crystallography

Gaudet Lab

The NRAMP family of transporters (Natural Resistance Associated Macrophage Proteins) pumps divalent metal ions (Mn2+, Fe2+ or Co2+) across biological membranes. In humans, NRAMPs are located in macrophages, where they help to defend against bacterial infections by robbing invading bacteria of essential metal ions. A second NRAMP isoform, expressed at the epithelial tissue of the intestine, mediates uptake of dietary iron. To discover how NRAMPs work, we sought to determine the 3-dimensional structure at near atomic resolution. Protein crystallography requires the protein in question to stack as a crystal, which diffracts X-rays and the resulting pattern is analyzed to deduce the protein structure. We began by cloning various bacterial NRAMP genes into so-called vectors for expression in E. coli. To pull the NRAMP protein out of the crude mixture of bacterial proteins, we used a histidine tag before the sequence that binds to Ni2+ coated beads. The purified protein is used to grow crystals in various solutions of salts and buffers. In addition to the crystal studies, we set up an assay to determine residues that are essential for protein activity and possible ion specificity. I identified conserved residues reported to line the active site and mediate ion transport (deduced from other studies) and mutated those to non-conserved residues. The mutated NRAMP genes were expressed in *E. coli* and used in cobalt uptake assays. The amount of cobalt in the cells corresponds to the level of function of the mutated transporter.

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Reprogramming Mouse Embryonic Fibroblasts into Pancreatic Progenitors Through the Use of Defined Factors

Melton Lab

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

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Proteolytic Removal of Tandem Affinity Tags for Absolute Protein Quantification

Martin Lab, Institute for Systems Biology

The Prostate Specific Antigen (PSA) test employs ELISA to quantify PSA and is commonly used to assess a male's risk for prostate cancer. Early detection can improve outcomes if interventions are available. Unfortunately, the antibodies needed for ELISA are expensive and time-consuming to produce. Novel methods for absolute protein quantification will undoubtedly create a surge of new diagnostics as biomarkers can be more effectively analyzed. Here we introduce a mass spectrometry-based method utilizing Stable-Isotope Labeling by Amino Acids in Cell Culture (SILAC), a way to create heavy protein standards by metabolically incorporating heavy amino acids into proteins produced by auxotrophic yeast. Protein construct plasmids were transfected into S. cerevisiae. The yeast were grown in CSM-Leu-Ura, induced, and lysed. The protein constructs were purified with tandem affinity tags, precipitated with trichloro acetic acid and digested with Tobacco Etch Virus (TEV) to cleave off the tags. Constructs with PSA and QCAT, a concatemeter of tryptic peptides specific for several proteins of interest, were successfully expressed and purified. The tandem affinity tags were successfully cleaved to achieve both the same size and isoelectric point (pI) as the endogenous protein. 2-D gel electrophoresis will further purify the heavy standards, which will then be quantified with BCA assay, "spiked" into cell lines expressing the desired endogenous protein, processed, and analyzed by Multiple Reaction Monitoring Mass Spectrometer to quantify the endogenous protein. A robust absolute protein quantification method is the first step in a systems biology approach toward truly elucidating the role of proteins in disease processes.

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A potential role for nucleoprotein phosphorylation in RNA synthesis control

Doudna Lab, UC Berkeley

Influenza A nucleoprotein (NP) is the major protein component of viral ribonucleoprotein complexes (RNPs) and is known to be phosphorylated by cellular kinases. The predominant sites of phosphorylation are at serine residues, with the phosphorylation pattern of NP changing throughout the replicative cycle and potentially affecting polymerase activity. Nucleoprotein has also been characterized as a phosphoprotein pivotal at multiple stages of the influenza viral life cycle and is now believed to mediate a switch from RNA synthesis in the replication mode to synthesis in the transcription mode. We have investigated twenty, externally exposed serine residues using site-directed mutagenesis to characterize the effects of serine to alanine mutations at these positions. Mutagenesis analysis using a viral polymerase activity assay has implicated three serine residues, amino acids 9, 407 and 486 as potential sites of phosphorylation essential to polymerase-mediated RNA synthesis. Primer extension analysis further implicated these same amino acids as potential phosphorylation sites important for viral replication. Knowledge of site-specific phosphorylation events on influenza nucleoprotein may aid in novel approaches to anti-viral design.

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Examination of potential inflammatory mechanism involved in angiotensin-II-dependent neurogenic hypertension.

Davisson Lab, Weil Cornell Medical School, Department of Cell and Developmental Biology

Angiotensin II (Ang-II) affects cardiovascular function by activating CNS neurons located in specialized regions termed circumventricular organs (CVOs). While it is well-established that Ang-II dependent hypertension involves the subfornical organ (SFO), a prominent forebrain CVO, the precise molecular mechanisms involved are unknown. Recent studies have suggested a role for cyclooxygenase (COX)-derived prostaglandins in Ang-II signaling in peripheral cardiovascular sites. COX enzymes (COX-1 and COX-2) catalyze the first step of the conversion of arachidonic acid into various prostaglandins that each act on one or more receptors. Evidence in our lab has suggested that COX-1-derived PGE2-acting on EP1R is involved in the actions of Ang-II in SFO neurons. In this study, we tested if COX-1 in the SFO is important for the development of chronic Ang-II hypertension. First, immunohistochemistry studies revealed robust COX-1 expression in the SFO. Next, after determining the optimum parameters for measuring blood pressure in mice using radiotelemetry, we monitored mean arterial pressure in COX-1-/- and wild-type controls before, during, and after chronic slow-pressor infusion of Ang-II, known to be an excellent model of human essential hypertension. Our data demonstrates that Ang-II-induced increases in pressure are inhibited in COX-1-/- mice at these early time points. These studies suggest that COX-1-derived prostaglandins play an important role in the pathogenesis of Ang-II-dependent hypertension, and will be important for understanding the mechanisms of neurogenic hypertension.

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Pancreatic Mesenchyme Proteins involved in Long-term Islet Culture

Melton Lab

Diabetes, which affects over 180 million people worldwide, stems from pancreatic beta cells' inability to produce enough insulin to regulate blood sugar. The disease can be treated by encouraging proliferation of beta cells. Proteins released by the pancreatic mesenchyme cells, which surround beta cells during pancreatic development, may promote beta cell maintenance and proliferation. Current beta cell culture methods are only capable of maintaining cells in culture for approximately a week. Better beta cell culture methods would be useful both in vitro, for screens, stem cell differentiation and transplant therapies, and in vivo, as a therapeutic measure to cure diabetes. Recent work done by the Melton lab has identified three candidate proteins that are secreted by pancreatic mesenchyme isolated at embryonic day E15.5, during organogenesis of the pancreas. RNA interference using lentiviral vectors has been used to determine the effects of knockdown of each of the corresponding three genes in a co-culture of pancreatic mesenchyme and beta cells. The beta cells are isolated from transgenic mice which express GFP in conjunction with the insulin-producing transcription factor PDX1, and beta cell health can be monitored through GFP fluorescence. Optimization experiments have so far provided information on transfection of lentiviral packaging cells and on optimal puromycin selection mesenchymal cells. Future experiments will include transduction of the pancreatic mesenchyme and co-culture with beta cells. Knockdown of gene expression will be measured by quantitative rtPCR, and effects of the transduced mesenchyme on beta cells in coculture will be measured by quantitation of beta cell fluorescence. Follow-up experiments include administering these proteins in vitro to beta cells or beta cell precursors, and studying their effects on beta cell proliferation.

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A Novel Pathway Potentially Contributory to the Pathophysiology of Prostate Cancer

Strahlendorf Lab, Texas Tech Health Sciences Center

Adenocarcinoma of the prostate is the most common cancer in men. The most popular current treatment, androgen ablation, is effective in early stages but is invariably followed by aggressive,metastatic and drug-resistant disease. At this stage the efficacy of chemotherapy is low. It is clear that there is much need for the new treatments for prostate cancer and differentiation therapy could provide an alternative solution. The objective of this study was to determine whether caspase-3, a molecule important to the differentiation of Bergmann glia, plays a similar role in prostate cancer cells. PC3 prostate cancer cells were treated with combinations of vitamin D3 (VD3), retinoic acid (RA), sodium phenylacetate, and sodium butyrate. VD3 and RA induced intermediate stages of differentiation, while sodium phenylacetate induced a late stage of differentiation. I found that caspase-3 levels increased with differentiation and that caspase-3 inhibitor, FK025 reversed differentiation of the PC3 cells, which was measured by the morphology, proliferation, and the expression of differentiation markers keratin 8 and 19. We concluded that caspase-3 is critical for the differentiation of prostate cells. An increase in cell proliferation caused by FK025 cells suggests that caspase-3 may aid the cell in exiting cell cycle. Our results demonstrate a novel function of caspase-3 in cell differentiation and point to its potential use for the differentiation therapy against prostate cancer.

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Regulation of Stem Cell Fate by the Ubiquitin-Proteasome System

Scadden Lab, Harvard Stem Cell Institute, MGH

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

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Investigating ABCB5 Expression in Neurofibromatosis 1

Frank Lab, Transplantation Research Center, Children's Hospital Boston

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

man MPNST cell lines, CRL-2884, CRL-2885, and CRL-2886, derived from patients affected with NF1. Using FACS analysis we identified that ABCB5 is expressed on average by 8-18% of cells. Reverse-Transcriptase PCR confirmed ABCB5 expression on the mRNA level. rnrnIn the future we are planning to further dissect the functional role of ABCB5-positive cells within MPNST with rapamycin, which is known to inhibit growth of these tumors in vitro and in vivo as well as examine the role of ABCB5-positive MPNST cells in tumor progression and resistance in vivo. We hope that specific targeting of these cells will help to develop effective therapies against NF1 associated tumors.

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Effect of N-acetylcysteine on ventilation-induced inflammation and apoptosis in human lung epithelial cells

MGH Pulmonary and Critical Care Dept.

Mechanical ventilation has been used to support patients for decades, but this assistance has potential complications. One of these is ventilation-induced lung injury (VILI). A549 human lung cancer cells, type II-like alveolar epithelial cells, have been used as an in vitro model of VILI. NAC has been identified as an antioxidant agent. I hypothesized that NAC would inhibit lung inflammation and apoptosis in this in vitro VILI model. rnA549 cells were seeded on fibronectin coated silicone membrane. A cell stretch device produced biaxial protein levels by ELISA and uniform strain. The measurements of IL-8 and TNF-α apoptotic index by Hoechst staining were used. Unstretched controls and stretched cells were treated for 24 hours prior to stretch NAC, 1 mM. The cells were divided into groups: control, control + NAC, stretch, and stretch + NAC.rnStretch for 4 hours and wait 2 hours produced significantly higher level of IL-8 (2.35 ± 0.03pg/ml in stretch group, compared to 0.42 ± 0.03 pg/ml in control group) and TNF-α (10.47±7.9 pg/ml in control, 34.05±7.5 stretch). Pretreatment with NAC blocked release of IL-8 (0.85 ±0.13 pg/ml) and TNF-α (11.61±1.04 in stretch + NAC). Stretch for 4 hours produced noticeably higher percentage positive stained cells, 35.40% ± 4.54 in stretch and 5.33% ± 2.37 in control. Pretreatment with antioxidant significantly reduced the percentage of apoptotic cells, 6.75% ± 3.60 in stretch + NAC vs. stretch.rnThis study suggests that NAC may protect lung cells against stretch-induced injury, maybe leading to new approaches in management of VILI.

Christina Tartaglia *Molecular and Cellular Biology*
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Potential mechanism for the role of adjuvants using a murine mouse model of multiple sclerosis.

Strominger Lab

The role of adjuvants is to stimulate the immune system to increase the response to a vaccine. My research goals are investigate the efficiency of each adjuvant as a potential carrier for poly(FYAK)n or FYAK, which may have implications for improving therapy of the disease. More broadly, a study of the mechanistic role of these adjuvants in stimulating the immune system has valuable implications for the development of vaccines, which have been recently highlighted for their therapeutic as well as preventative capabilities in diseases including HIV, malaria, and cancer. In this study it was found that novel copolymer FYAK, when administered in conjunction with alum, resulted in a delayed onset of disease. These results suggest that Imject Alum® effectively aided the copolymer, FYAK, in suppression and delayed onset of EAE in SJL/J mice. This suggests that an adjuvant may be necessary in order to see the effects of the copolymer.

Hemali Thakkar *Human Evolutionary Biology*
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Construction of New Human Monoclonal Antibodies by Combination of IgG Variable Domain and IgA Constant Domain for Defense Against HIV Infection

Cavacini Lab, Beth Israel Deaconess Medical Center

IgA and IgG antibodies, found in secretions of the genital tract, protect the body from foreign substances through the adaptive immune system. Studies show that specific human monoclonal antibodies inhibit HIV infection when administered alone or in combination. IgA, however, is more effective on a molar basis at mucosal defense than IgG. Therefore, our goal is to combine each of the IgG variable domains of antibodies 7B2, A1g8, and B4e8 with the constant domain of antibody IgA in order to produce new human monoclonal antibodies for defense against HIV infection. The procedure requires isolation of the total RNA from the monoclonal antibody hybridoma cell lines, immediately followed by reverse transcription PCR to synthesize cDNA and amplify Ab variable domain. Furthermore, there is amplification of the IgA2 constant domain from IgA2 antibody vector, combination of constant and variable domain using gene splicing by overlap extension (SOE), double digestion of the new fragments with restriction endonucleases NheI/NotI, and the insertion of the digested fragments into pIRESpuro3 expression vector. After successfully constructing the desired recombinant antibodies, they are expressed in the Chinese hamster ovary (CHO) mammalian cells for further analysis of any binding and/or neutralizing activity against HIV.

Caterina Yuan *Molecular and Cellular Biology*
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Regulation of the cell wall hydrolase, RipA, in *Mycobacterium tuberculosis*.

Rubin Lab, Harvard School of Public Health/Harvard Medical School

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

Neuroscience and Psychology

Alissa D'Gama — *Molecular and Cell Biology*
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Effect of site-specific mutations on enzymatic and biological activity in SAD-A and SAD-B kinases

Department of Molecular and Cell Biology and Center for Brain Science, Harvard University

Neurons receive signals on their cell bodies and dendrites and transmit this information through their axons to other neurons at synapses. During development they typically polarize to form one long, slender axon and several shorter, thicker dendrites. Errors in the formation of these processes and the subsequent formation of synapses often result in the premature termination of electrical signals and can lead to neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's disease. Using in vitro and in vivo studies, our lab previously determined that two protein kinases, SAD-A and SAD-B, which normally phosphorylate the microtubule associated protein tau, are important components of the pathway required for cortical neuronal development and migration. My project involved creating site-specific mutations in SAD-A and SAD-B kinases using bioinformatics approaches, and then testing these mutated kinases for enzymatic and biological activity. I spent the last few months generating these constructs, and am now transfecting the mutated versions into cultured epithelial cells to check for tau phosphorylation. I will also insert the mutated versions into bacterial plasmid vectors to generate large amounts of the protein to purify and assay for activity. In the future I will use electroporation to introduce the mutated SAD kinases into primary neuronal cultures and observe the effects on neuronal polarization. By studying the effect of external modifications on SAD kinases, I eventually hope to understand upstream cues and pathways that may be regulating SAD kinases.

Kyle Gibler — *Economics*
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Seeing the forest o'er the trees

Center for Sleep and Conition

How do brains process memories? Our brains are not camcorders, recording every sight and sound for posterity. Rather, we condense, adapt and transform events into pieces of evolutionarily helpful information before committing them to memory. How and when does all this memory processing occur?rnrnPast research has shown that sleep plays a key role in the consolidation of memories, aiding in the solidification of events in our mind. This summer, we further examined this relationship in an overnight sleep and memory study. Participants listened to a list of words, went to bed while being monitored using polysomnography and then were asked to recall as many of these words as possible the following morning. We believe that subjects who remain in slow-wave sleep (SWS), a stage of very deep sleep, for a longer period will perform worse on this task, and instead falsely recall a greater number of "gist" words (words not included in the list but that are closely related). This finding would expand our understanding of memory processing in sleep, the results indicating SWS allows us to remember general concepts at the expense of details. Consequently, we can see the forest o'er the trees!rnrnBecause so little is known about the functions of sleep, further research of the purpose of various sleep stages and their relationship with memory will be necessary in the future.

Yuliya Nikolova — *Psychology*
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Blunted hedonic capacity in dysphoric subjects: cross-cultural validation of a probabilistic reward task in a bulgarian sample

Affective Neuroscience Lab

Depression is a heterogeneous disorder, the understanding of which may be facilitated by an endophenotypic approach. One of depression's promising endophenotypes is anhedonia, the loss of pleasure or lack of reactivity to pleasurable stimuli. To objectively assess hedonic capacity, we used a probabilistic reward task which provides a measure of reward responsiveness, i.e., an individual's ability to modify behavior in accordance with reinforcement history. Previous research has shown that dysphoric participants, particularly those with elevated anhedonic symptoms, as well as subjects with major depression have blunted reward responsiveness. As part of a larger study, we assessed the cross-cultural generalizability of these findings by measuring reward responsiveness in 110 Bulgarian students attending high school in Yambol, Bulgaria. Participants were divided into groups based on their Beck Depression Inventory (BDI) scores. Consistent with previous research, participants with high BDI scores ($\geq$16, n = 38) displayed blunted reward responsiveness relative to participants with low BDI scores ($\leq$7, n = 35). These findings provide cross-cultural evidence for the validity of the probabilistic reward task as a tool for objectively assessing reward responsiveness. In ongoing analyses, we are assessing the effects of naturalistic stress (final school exams) and candidate genotypes on reward responsiveness.

Francesca Reindel — *Neurobiology-Mind/Brain/Behavior*
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Investigating the role of 5-HT1B serotonin receptors on aggression in Drosophilia melanogaster

Kravitz Lab, Harvard Medical School

From the Serengeti plains where survival motivates animals to attack one another, to the streets of Broadway where rush hour road rage is as common as the Starbucks on every corner, aggression is an unavoidable phenomenon. Aggression, as with other behaviors, is intricately tied to the nervous system. By studying this behavior in Drosophila melanogaster, we are able to examine the complex neural circuitry that controls this behavior. The relationship between brain serotonin (5-HT) and aggressive behavior has been well studied in mammals, but little is known about this relationship in *Drosophila*. In previous studies, the manipulation of the entire serotonin circuitry has yielded a mild phenotype. Thus, studying the effects of one specific type of serotonin receptor seems to be a valuable approach. The closest ortholog of the mammalian receptor shown to be correlated to some psychological disorders is the d5-HT1B serotonin

receptor in Drosophila. To assess the role of 5-HT receptors in the modulation of aggression, the GAL4/UAS system, a common genetic tool, was used to over-express the 5-HT1B serotonin receptors specifically in the 5-HT1B-positive neurons. When an aggression assay was conducted, the two feuding male experimental flies displayed abnormal courtship behavior (singing, mounting), but then proceeded to aggressive behavior. Preliminary analysis of classical courtship and locomotion assays have not shown many phenotypic differences between experimental and control males. A more detailed analysis of the experiments conducted will confirm these initial conclusions and guide future studies.

Sophie Rengarajan — *Neurobiology, Mind/Brain/Behavior*
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Characterizing the neuronal basis of habituation to electric shocks in zebrafish (Danio rerio) larvae

Engert lab

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

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The roles of potassium and calcium in ischemic insult

Stanford University School of Medicine

Stroke remains a leading cause of morbidity and mortality in the United States and currently available treatments are only palliative. A lack of effective treatment is due partly to our limited understanding of the mechanisms that producing neuronal damage associated with brain ischemia. The present study used electrophysiological recording in brain slices from the rat hippocampal CA1 region, a region known to be important for learning and memory, and which has been shown to be strongly impacted by ischemia. We investigated the short-term physiological effects of traditional oxygen glucose deprivation (OGD), and newer, more physiologically relevant ODG solutions with altered ionic compositions that reflect changes seen in vivo during stroke (ODG + low sodium and high potassium; OGD+HiK), or high potassium alone (90 mM; HiK); either in the presence or absence of calcium (low Ca + high Mg; CaMg). A five minute exposure to ODG solution produced a complete depression of synaptic transmission, measured using Schaffer-collateral to CA1 neuron evoked population spike (PS) amplitudes. Full recovery from this depression occurred within 10 min following reperfusion with normal solution. OGD+HiK solution exposure also completely depressed PS amplitudes, and only partial recover of response amplitudes were seen on reperfusion. HiK solution completely mimicked the OGD+HiK effect, indicating that the elevated potassium alone was sufficient to produce irreversible damage. Removing Ca from the reperfusion solution improved the speed of recovery from HiK exposure, but did not improve the degree of recovery. These results indicate that the increase in potassium concentrations during ischemia appears to be the major contributing factor to neuronal damage. The lack of a protective effect of CaMg solution during reperfusion suggests that the flow of calcium into cells during ischemia, including excitotoxic entry via NMDA receptor activation, do not contribute substantially to neuronal damage – this is consistent with the lack of protection seen in clinical trails of NMDA and calcium antagonists.

Lev Shaket — *Molecular and Cell Biology*
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In vivo selection of adeno-associated viral vectors for brain tropism

Neuroscience Center, Massachusetts General Hospital

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

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Using mesenchymal stromal cells as a therapy for glioblastoma

Brigham and Women's Hospital

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

Erica Tsacoyeanes *Neurobiology and Chemistry*
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Prevention of pediatric medulloblastoma through regulation of miRNA and p53

Children's Hospital, Boston

Medulloblastomas are the most common malignant brain tumors of childhood. They occur exclusively in the cerebellum and are thought to arise from granule neuron precursor cells. Thus, genes and pathways important in cerebellar development have been implicated in medulloblastoma pathogenesis. The p53 protein is a known tumor suppressor encoded by a gene whose disruption is associated with approximately 50 to 55 percent of human cancers, including medulloblastoma. p53's role is critical in cancer prevention since it acts as a checkpoint in the cell cycle, either preventing or initiating programmed cell death. Our lab has identified a candidate miRNA (a single-stranded RNA molecules of about 21–23 nucleotides in length, which downregulate gene expression) that we feel directly effects p53, and increases chemosensitivity in medulloblastoma. It is our belief that if we can knockdown the miRNA Boris, then p53 expression will increase, thus resulting in a better response to chemotherapuetic agents.

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Using a multiple sclerosis disease model to study the therapeutic potential of the NAD biosynthetic pathway

He Lab, Children's Hopsital

Multiple sclerosis (MS) is a neurological disorder in which the body's immune system attacks the myelin sheath, impairing signal propagation down axons. While current treatments for MS are primarily designed to suppress the immune system, no therapy treats the long-term effects of axonal damage. By focusing on this largely overlooked aspect of the disease, we hope to gain the information that may one day lead to an alternative or complimentary treatment for MS. One possible avenue of protecting against axonal damage is the administration of high nicotinamide (NAD) levels. In order to illuminate upon the mechanism by which high NAD levels affects MS disease course, a MS-like disease was induced in wildtype and transgenic mice with selective cellular overexpression of the rate-limiting enzyme (PBEF) involved in the NAD biosynthetic pathway. It was observed that selective overexpression of PBEF in just neurons lessens the symptoms of the disease, as preliminary differences suggest that PBEF overexpressing mice suffer a less severe disease than wildtype controls. Future experiments will continue to explore the efficacy of PBEF as a therapeutic and further isolate where PBEF must necessarily be overexpressed to provide its protective effects. Experiments using transgenic mice that express PBEF in both neurons and glial cells, as well as globally in all cells have been planned to accomplish these goals.

Organismic and Evolutionary Biology

Noor Beckwith — *Organismic and Evolutionary Biology*
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Characterizing a larval taxis in a leaf-mining drosophilid fly

Pierce Lab, MCZ

After hatching in the distal part of a leaf, first instar larvae of *Scaptomyza flava (Drosophilidae)* appear to chew a mine towards the mid-vein of the leaf and eventually towards the petiole. When not actively feeding, larvae tend to congregate in the petiole. This petiole-finding behavior remains undescribed and unquantified. Also, the adaptive significance of this behavior remains unknown, and we are testing at least two hypotheses. Herbivorous insects use a variety of strategies to circumvent host plant defenses, including behavioral mechanisms. We are attempting to characterize and quantify this behavior using time-lapse photography on infested leaves in the Pierce Lab greenhouse. We are also conducting experiments to determine (1) the cues used by the larvae to find the mid-vein and petiole and (2) the adaptive significance of the behavior. The first set of experiments relies on Arabidopsis defense signaling mutants and plant hormone treatments to determine if the cue is a component of the host plant defense-signaling repertoire. The second set of experiments is designed to test these hypotheses: (A) Larvae move to the mid-vein and petiole to prevent distal plant defense signaling molecules from reaching the leaf and (B) Larvae move to the mid-vein and petiole to avoid parasitoid wasps, which attack ca. 50% of larvae in nature. Preliminary results quantitatively confirm seeking-behavior, and they also point to interesting new patterns in larval activity rhythmicity; we have not however finished isolating the chemotactic cue or the adaptive significance.

Daniel Jones — *Organismic and Evolutionary Biology*
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What's in that dirty water? An assessment of lower trophic levels in the Charles River

MCZ Labs

The Charles River has had a long history of anthropogenic modification, from the introduction of exotic species to the dumping of industrial pollution. Over 375 years of modern human settlement along the Charles River has caused severe water quality and aquatic habitat impairment. While the Charles has seen improvements in water quality in the last few decades, the river's natural ecosystem has been forever altered.rnrnMy research focuses on the current trophic structure of the lower Charles River. During the summer, I monitored phosphorous, nitrate, and ammonium levels and examined how fluctuations in these nutrients affected phytoplankton biomass through chlorophyll a measurements. Additionally, the biodiversity, species dominance, and species composition of zooplankton was also monitored. Fish community structure was also assessed through a series of seinings. rnrnPhytoplankton provide food for zooplankton, which in tern help support fish populations. Because anthropogenic pollutants enrich the Charles River in nitrogen and phosphorus and deteriorate water quality, phytoplankton biomass is highly variable throughout the summer. The goal of my research is to understand exactly how phytoplankton biomass responds to nutrient fluctuations and how this in turn affects zooplankton diversity and composition. Ultimately, the goal is to make inferences about why the current fish population is structured as it is.

William Jones — *Organismic and Evolutionary Biology*
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Fish biodiversity and resource partitioning in the lower Charles River

McCarthy and Woollacott labs

Over the past several centuries, human settlement along the Charles River has fundamentally altered the natural ecosystem of the river. In the first part of this study, 304 fish were caught by seining from June to October to assess the fish biodiversity of the lower Charles River littoral (near shore) zone. Observations indicated that introduced species account for approximately 40% of the river's biodiversity and nearly 20% of the total fish catch. Additionally, results suggest that fish biodiversity of the lower Charles River has declined by as much as 40% in the last 10 years. The second part of this studies focuses on the partitioning of food resources in the littoral zone of the lower Charles River through the examination of the gut contents of the most common species observed in the biodiversity assessment. Accounting for nearly 90% of the total fish catch, these species include the pumpkinseed sunfish (*Lepomis gibbosus*), yellow perch (*Perca flavescens*), and introduced bluegill sunfish (*Lepomis macrochirus*). Literature suggests that, in environments where bluegill and pumpkinseed coexist naturally, bluegill forage primarily on open-water zooplankton, while pumpkinseeds specialize on vegetation-dwelling gastropods. In the lower Charles River littoral zone, however, where the bluegill is not native, the diets of the bluegill, pumpkinseed, and perch were all dominated by benthic invertebrates including diptera, amphipoda, and to a lesser extent trichotera and gastropodia. Results suggest that these species are in direct competition for food resources. Future research will investigate whether observed feeding patterns reflects resource availability or a more complex interaction between the introduced bluegill and native fish species.

Chioma Madubata — *Molecular and Cellular Biology*
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Evidence for a recent population bottleneck in an Apicomplexan parasite of caribou and reindeer, Besnoitia tarandi

United States Department of Agriculture

The evolutionary history and epidemiology of parasites may be reflected in the extent and geographic distribution of their genetic variation. Among coccidian parasites, the population structure of only Toxoplasma gondii has been extensively examined. Intraspecific variation in other coccidia, for example those assigned to the genus Besnoitia, remains poorly defined. Here, we characterize the extent of genetic variation among populations of Besnoitia tarandi, a parasite whose intermediate hosts include caribou and reindeer (Rangifer tarandus tarandus). Isolates from the Canadian and Finnish Arctic were genotyped at six microsatellite loci, the first internal transcribed spacer region of nuclear rDNA, and the RNA polymerase β subunit (rpoB) encoded in the plastid genome. Remarkably, all isolates exhibited the same multilocus genotype, regardless of the isolate's geographic origin. This utter monomorphism occurred despite the capacity of these loci to vary, as established by evident differentiation between B. tarandi and two other species of Besnoitia. The surprising lack of genetic variation across the sampled range suggests that B. tarandi may have experienced a recent population bottleneck.

Samuel Perez *Organismic and Evolutionary Biology*
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Testing Falck's hypothesis in basidiomycete mushrooms

Undergraduate Research

Numerous species of *basidiomycete* fungi use mushrooms to aid dispersion of millions of spores for reproduction in response to environmental cues. In the book On the Researches of Fungi, Reginald Buller reported Richard Falck's Hypothesis, which said that the heat produced from fruiting bodies warmer than the surrounding air would create convection currents to help the spores disperse from the sporulating mushroom. The purpose of this research is to test Bueller's convective hypothesis in basidiomycetes by exposing fruiting bodies to various environmental conditions and testing temperature gradients in 4 different parts of the mushrooms. We will approach this problem by constructing a mushroom model using the principles of fluid dynamics to understand the necessary temperature gradients required for Falck's hypothesis. Individual movement of spores from Ganoderma lucidum will be compared to the model to test the relevance of convection currents in spore distribution. By measuring the temperature differences between different parts of a fruiting body, we will test the existence and intensity of the temperature gradients in different parts of the mushroom in an ecologically relevant context. Our current results suggest that basidiomycete fruiting bodies are colder than the air surrounding them. The most likely candidate to demonstrate Falck's convective hypothesis, if at all, would be Ganoderma lucidum exposed to direct sunlight. The results of our research would help elucidate the physical mechanisms of fungal reproduction and the biophysical implications, if any, of heat and fluid dynamics in this system.

Michael Peters *Human Evolutionary Biology*
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Estimating biting forces in humans, hominins, and other primates

Lieberman Lab

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

Physics

Masha Baryakhtar — *Physics and Math*
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Development of auxiliary lock acquisition procedures for advanced LIGO

Adhikari Lab, Caltech

A crucial step in LIGO (Laser Interferometer Gravitational-Wave Observatory) is lock acquisition, the process of bringing optical cavities to resonance and locking the detector, making it sensitive to gravitational wave signals. The narrow linear regime of the error signal makes the process of lock acquisition at LIGO a challenge; random motion is largely relied on to bring degrees of freedom into range of the servo. In Advanced LIGO, acquiring lock will become even more challenging with addition of a fifth degree of freedom, so a reliable auxiliary lock acquisition system becomes crucial for operation. This report describes an auxiliary lock method that uses laser light distinct from the main operational beam to control arm cavity lengths via frequency doubled Pound Drever Hall locking. The accuracy required of the system is set by the necessary cavity length precision, and noise limits of components of the system are presented and analyzed in this context. Passing laser light through an optical fiber to the point of injection into detector arms introduces phase noise on the light and is the limiting noise source. Fiber noise levels were measured with a Mach Zehnder interferometer and found to exceed limits set by desired locking precision. A fiber noise cancellation scheme is being implemented, and initial tests indicate it will reduce noise below desired levels.

Christina Grassi — *History and Science*
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Knee Prosthesis Component Wear and Regional Lymph Node Uptake

Weissman Lab, Brigham and Women's Hospital,
Department of Radiology, Harvard Medical School

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

Tim Hsieh — *Physics and Mathematics*
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Calculating Particle Scattering Amplitudes in a Simpler and Faster Way

Cachazo Lab, Perimeter Institute for Theoretical Physics

Scattering amplitudes play a critical role in particle physics; these amplitudes give probabilities that a given set of particles will interact and produce another set of particles. With the advent of the Large Hadron Collider, scattering amplitudes have assumed a central role in allowing experimenters to verify predicted amplitudes through collisions and in doing so, verify the underlying theories that predicted the amplitudes. Recently, much progress has been made in scattering physics. In 2003, Dr. Freddy Cachazo of the Perimeter Institute for Theoretical Physics, along with three other physicists, developed a theoretical technique known as the BCFW deformation. Traditionally, Feynman diagrams are used to calculate scattering amplitudes but they face computational complexity when large numbers of particles are involved. The BCFW deformation is an alternative method that often yields amplitudes in a simpler way and in a simpler form than the Feynman counterpart. We exhibit the versatility of the BCFW relations by applying them in two completely different ways. In one application, we calculate several tree-level scattering amplitudes of interactions involving four and five gravitons, including diagrams containing vertices of three gravitons with the same helicity sign. Though these vertices are ruled out by general relativity, they can potentially be used as effective vertices for loop-level diagrams. We find consistency in many but not all of the scattering diagrams. In the second application, we imitate Steven Weinberg's proof of conservation of electric charge and equality of gravitational charge using the BCFW deformation as opposed to Weinberg's use of polarization tensors and four-vectors.

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An Electron Cryotomographic Analysis of the Hexagonal Lattice in the HIV-1 Capsid (CA) Layer

Jensen Lab, Caltech

Acquired Immunodeficiency Syndrome (AIDS), a life-threatening illness plaguing over 33 million people worldwide is caused by human immunodeficiency virus type 1 (HIV-1), a retrovirus which attacks the human immune system to greatly shorten life expectancy. To design effective anti-HIV therapeutics, structural knowledge is essential. Our aim is to better understand the dynamic changes HIV-1 virions undergo during the proteolytically driven maturation process. Viral maturation is of interest because it activates the lethality of the virus. Of special interest is the structural rearrangement of the capsid (CA) protein shell during Gag polyprotein assembly. Images of the CA shell depict a hexagonally ordered lattice covering the surface along with patches of disordered protein. This finding is unusual because, theoretically, the shell should have an "enclosed" lattice, a geometrically perfect hexagonally ordered lattice pattern across the surface of the particle. We suspect some biological mechanism during the budding process disrupts the formation of an enclosed lattice. Our sample is in vitro assembled Gag particles, chosen to eliminate any possible interfering biological mechanisms. By imaging these particles using electron cryotomography we hope to achieve a more complete picture of the assembly and budding process for HIV-1, information crucial for the development of anti-HIV therapeutics.

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Probing the mechanical response of colloidal glasses with confocal microscopy

Weitz Lab, Department of Physics, and School of Engineering and Applied Sciences

Rapid densification of a colloidal hard-sphere suspension leads to formation of a colloidal glass which is a valuable model system for atomic and molecular glasses. In this study, a shear deformation is applied to a sample at four different rates in order to gain insight into the relationship between the macroscopic behavior and individual particle dynamics under strain conditions. Laser scanning confocal microscopy is used to visualize the 3D motions of several thousands of particles, and their positions are extracted with high precision using digital imaging processing. It is observed that the accumulated strain has a strong effect on the pair correlation function, mean square displacement and the evolution of elastic modulus. Conversely, the shear rate, which determines the rate of the build-up of the strain, has little effect on those features.

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

Jun Lab, FAS Center for Systems Biology

The standard approach to imaging the bacterial chromosome has been to stain the chromosome with fluorescent proteins. Unfortunately, the optical resolution obtained using this method is limited by the diffraction of light to approximately 200-300 nm, which is in the same order of magnitude as the cell diameter. Since direct imaging of the fluorescently-labeled chromosome suffers from this low spatial resolution, we have directed our attention to imaging the complementary space between the nucleoid and the cell membrane. We inserted freely diffusing PEG-coated quantum dots with diameters of 10-20 nm into the cytoplasmic space of E. coli cells using transformation. The quantum dots diffuse in the periphery of the cell and avoid the nucleoid, which is occupied by a dense network of DNA. The quantum dots are fluorescent, emitting light of a given wavelength. By filtering for light of that wavelength, we imaged the individual quantum dots within the cells. We compared our experimental images to the expected intensity distribution for our model, and we used deconvolution software to reconstruct the distribution of quantum dots in the cell. Quantifying the gap between the nucleoid and the cell membrane in E. coli will give us a more detailed view of the inner space of this model organism. Ultimately, this method can be applied to other bacterial species such as Bacilus subtilis or Caulobacter crescentus, for which the existence of a gap between the nucleoid and the cell membrane is still controversial.

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