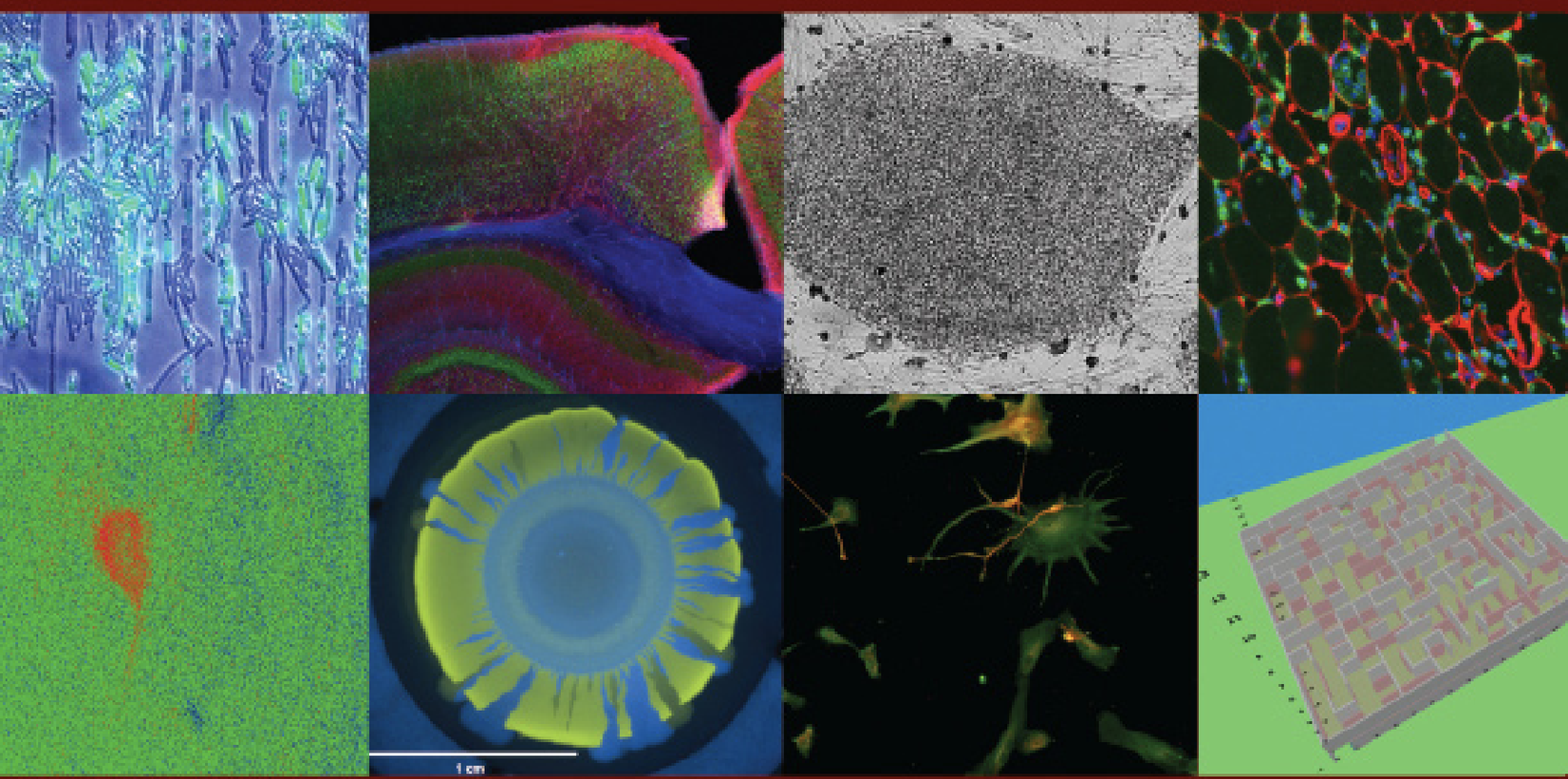
HARVARD COLLEGE

PRISE

PROGRAM FOR RESEARCH IN SCIENCE AND ENGINEERING



ABSTRACTS

2010

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Letter from the Director

I am pleased to once again write this letter of introduction for the Abstract Book of the 2010 Harvard College Program for Research in Science and Engineering, PRISE. During this fifth summer of PRISE, our undergraduate Fellows have been affiliated with over one hundred Harvard faculty across the Faculty of Arts and Sciences, the Harvard Medical, the Harvard School of Public Health, the affiliated teaching hospitals, and other allied research enterprises. Truly interdisciplinary as the PRISE community itself, the topics herein comprise a broad spectrum of scientific research conducted in Cambridge and Boston. The PRISE Fellows' research experience has been augmented by participation in our residential community of science scholars at Leverett House, providing a lively and inclusive social environment to interact with each other in a meaningful way.

With great appreciation, I would like to acknowledge the outstanding and dedicated Fellows who have designed and orchestrated this year's Abstract Book, PRISE's fourth edition. I also would like to thank our terrific and tireless Program Assistant Fellows, Francesca Reindel, Senan Ebrahim, James Pelletier, and Denise Xu, who have provided wise counsel and stewardship to fellow-initiated activities throughout the summer

The PRISE Fellows of 2010 have been a terrific and enthusiastic group throughout a hot summer that flew by far too quickly. I wish you all the best of success in your academic and research pursuits going forward, and hope that the relationships you have cultivated during PRISE continue through the rest of your time at Harvard and beyond. Thank you for making this summer so enjoyable and memorable!

Gregory A. Llacer, Director
Harvard College Program for Research in Science and Engineering (PRISE)

Letter from the Editors

Dear PRISE fellows,

During the school year, we are so consumed by challenging courses and extracurricular activities that it can often be difficult to slow down and appreciate our environment. Summer gave us that invaluable gift of free time, and PRISE brought 115 of us together, provided food and shelter, remarkable professors to talk to, and resources to support our fun and educational escapades. It proved to be the perfect recipe to make for a productive, intellectually stimulating, and memorable experience. Through baseball games, concerts, roller coaster rides, World Cup-watching parties, and other wholesome summer activities, we have forged new bonds that will undoubtedly endure summers from now.

For some of us, the past ten weeks were the first time we ventured into a lab; for others, it was a chance to delve deeper into research, or even develop a thesis project. Regardless, we've all spent significant amounts of time in our laboratories, and the expression "going to lab" now holds so many meanings, whether it's perusing journals, encountering dead-ends and defective equipment, sifting through data, or g-chatting our way through the downtime that is so characteristic of research. Many of us have also felt the the long awaited moments of satisfaction following long hours of preparation and repetition, when our experiments finally yielded successful results or when our data finally "made sense."

The 2010 PRISE Abstract book that you hold in your hands is the culmination of the past ten weeks of dedication and a representation of our wide range of intellectual pursuits. We're very proud of this book, for it took many hours in the Leverett computer lab and participation from every single one of you to complete. We hope that you enjoy reading it and learning about your fellow students' work, and we look forward to seeing the great things that will follow this one memorable summer.

Sincerely,

Helen Yang '12, Anugraha Raman '12, and Timothy Kotin '11
Editors-in-chief

The PRISE 2010 Abstract Book Editorial Staff:

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Chemistry and Biochemistry

Serena Bai *Undeclared*
Dunster 2013

Characterizing surface chemistry and molecular interactions of HCA II

The Whitesides Laboratory,
Department of Chemistry and Chemical Biology,
Harvard University

The study of molecular recognition, the structure-specific, non-covalent interactions among organic molecules, is one of the most important and fundamental problems in biochemistry. Currently, there are gaps in our understanding that prevent us from developing not only a fundamental structural characterization of proteins in a rational way, but also biomedicinal applications such as in silico drug design. In particular, we aim to study the effect of hydrophobic ligands interacting with hydrophobic surfaces of globular proteins and to shed light on protein surface chemistry, specifically the aqueous layer within ~1 nm of the protein that plays an integral role in crystal packing. In this study, we use and develop the model system of human carbonic anhydrase II (HCA II), a protein with remarkably stable tertiary structure despite modifications to its surface chemistry. We have discovered that the hydrophobic surface of benzenesulfonamide ligands interacting with the hydrophobic shelf of HCA II is favorable due to the release of non-optimally hydrogen-bonded water molecules that hydrate the hydrophobic wall of HCA II. In terms of protein surface chemistry, X-ray crystallography of traditional proteins yields structures containing ions commonly found in the aqueous buffers used for crystallization. None of these ions localize clearly in resulting crystal structures. To better understand the surface chemistry of proteins, we use ion exchange to load crystals of HCA II with higher atomic weight ions, specifically Cs+ and I-, in order to explore systems in which tight, localized binding is possible. This project will continue to use a combination of X-ray crystallography, isothermal calorimetry (ITC), and other tools to shed light on the physical organic basis of molecular interactions of protein-ligand and protein-protein systems.

Dan Cahoon *Chemical and Physical Biology*
Winthrop 2011

Effect of conductive minerals on carbon uptake and extracellular electron transfer in deep sea sediment

The Girguis Laboratory,
Department of Organismic Evolutionary Biology,
Harvard University

Redox gradients in deep sea sediment provide a driving force for electron transfer from the underlying anoxic zone to the above oxic layer. Benthic microbial fuel cells have been successful in harnessing bacterial extracellular electron transfer in these sediments by bridging these two layers with an electrical circuit including solid electrodes for bacteria to interface with. It has been shown, however, that even without an added electrical circuit, extracellular electron transfer occurs over several centimeters between the anoxic and oxic zones, with sulfide and organic carbon oxidation in the anoxic zone coupled electrically with oxygen reduction in the overlying oxic zone. In our experiment, we are testing the hypothesis that conductive minerals like pyrite can stimulate extracellular electron transfer and lead to increased carbon uptake relative to a control with no environmental alterations. Our approach entails incubating samples of sediment retrieved from 1,000 meters below the ocean surface in Monterey Bay, CA with varying amounts of conductors naturally found in more metal-rich sediments. To test carbon uptake, we are incubating with 13C-labeled acetate as an organic carbon source and are analyzing how much is fixed, respired, and unused over a series of time points. 16S rRNA PCR will be done on samples followed by 454 pyrosequencing to track the microbial community, and will allow us to see whether communities change significantly to adapt to a more metaliferous environment, perhaps with selection for bacteria that are good at extracellular electron transfer.

Kevin Chen *Chemistry*
Currier 2012

Identification of anti-infective, immunomodulatory compounds active against vancomycin-resistant Enterococcus faecalis infection

Read Pukkila-Worley, MD and Frederick M. Ausubel, PhD.,
Massachusetts General Hospital,
Harvard Medical School

The increasing prevalence of antibiotic-resistant bacterial pathogens has created an urgent need to identify novel antimicrobial therapies. Here, we use a live-animal infection model to discover new therapies for drug-resistant bacteria. The nematode Caenorhabditis elegans can be infected with multiple nosocomial pathogens, including Enterococcus faecalis. In an earlier study, members of the Ausubel laboratory conducted a high-throughput screen of 37,200 compounds and natural products for those that promoted survival of worms infected with E. faecalis. 28 compounds without previously reported antimicrobial activity were identified, including six structural classes that cured animals in vivo at concentrations significantly lower than that required to inhibit bacterial growth in vitro. We therefore hypothesized that a subset of these “anti-infective” compounds promoted survival in our assay by directly modulating the nematode immune system. Using a quantitative, real-time PCR-based approach, we tested these 28 compounds for their ability to activate immune response genes in the worm and identified five candidate immunomodulators. Two compounds were of particular interest. Compound 14 strongly induced the transcription of several genes (C17H12.8, T24B8.5, F56D6.2 and K08D8.5) that act downstream of PMK-1, the p38 Mitogen Acitvated Protein kinase homolog and central regulator of nematode immunity. Interestingly, reporter activation was abrogated in pmk-1(km25) mutant animals, suggesting that this compound directly stimulates this pathway. Ad-

ditionally, both compound 14 and a second candidate immunomodulator, Compound 24, activate immune response genes independently of the PMK-1 pathway. Compound 24 induced a 145-fold change in the PMK-1 independent gene F35E12.5 in both wildtype and pmk-1(km25) mutant animals. Because these compounds appear to act on the host rather than the pathogen, we hypothesized that these agents would have activity against antibiotic-resistant bacteria. We adapted the C. elegans assay to study the vancomycin-resistant E. faecalis strain V583 and found that four of the five candidate immunomodulatory compounds cured nematode infection with this strain. From dose-response experiments, we observed potent rescue with compounds 14 and 24 at a concentration as low as 1.57 mg/mL. Thus, our data suggest that compounds indentified using a live-animal infection model have a distinctive mechanism of action that involves directly augmenting the host innate immune response.

Logan Clark *Chemistry and Physics*
Quincy 2012

Effects of energy input on electrostatic self-assembly of polymer spheres

Whitesides Group,
Department of Chemistry,
Harvard University

Self-assembly provides an efficient method for bottom-up construction of systems with large-scale order from simple constituents. In electrostatic self-assembly we take advantage of contact electrification to cause spheres of two different polymers to charge oppositely when vigorously agitated together in a metal dish. As the spheres are agitated—and once they become sufficiently charged—they will spontaneously assemble into two-dimensional crystals with a variety of structures governed by electrostatic interactions and the force of agitation. We have observed hexagonal, pentagonal, and square assemblies over the course of agitation. By analyzing videos of these systems and taking careful charge measurements we can learn to better predict and control electrostatic self-assembly. We study the kinetics and energetics of electrostatic self-assembly over a wide range of agitation energies. Our results are applicable to achieving a better understanding self-assembly by electrostatics as well as providing a simple macroscopic model for the electrostatic interactions in ionic crystal formation.

Michael Graham *Chemistry*
Currier 2010

One-dimensional palladium(III) wires

Ritter Group,
Department of Chemistry,
Harvard University

Superconductors are an important class of compounds which, below a critical temperature, have the ability to conduct electricity with exactly zero resistance. This unique property has led to a number of applications, although their widespread use is currently limited as even the best superconductors must be cooled by liquid nitrogen in order to reach their critical temperature. Consequently, much effort has been put into creating superconductors with higher and higher critical temperatures.

Although the standard theory of superconductivity (Bardeen-Cooper-Schrieffer, or BCS theory) places a material-specific limit on the maximum possible critical temperature, an alternate theory proposed in 1964 by W.A. Little and later expanded by J.P. Collman requires no such limit. Thus, under Little's theory, materials that superconduct at room temperature and higher are both conceivable and expected to exist. To date, this theory has been neither verified nor disproved.

In order to explore this theory of superconductivity and create novel nanomaterials, one-dimensional extended metal-atom chains (EMACs) have been synthesized that, according to Little's theory, have the potential to superconduct. Although no conducting wires have yet been found, it is hoped that by continuing to modify the ligand scaffold and electronic environment of these wires, their conductivity and stability can be enhanced, perhaps eventually leading to the creation of a room-temperature superconductor.

Daniel Haldar *Chemistry*
Kirkland 2013

Identification of protective metabolite pathways in Pseudomonas aeruginosa via a liquid chromotagraphy-mass spectrometry profiling approach

The Saghatelian Laboratory,
Department of Chemistry and Chemical Biology,
Harvard University

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen that poses a significant health risk to immunocompromised cancer, AIDS, and cystic fibrosis patients. Case fatality rates of patients infected by P. aeruginosa are disproportionately large due to the bacteria's high level of antibiotic resistance. In this project, we employ liquid chromatography-mass spectrometry (LC-MS) to identify metabolites in P. aeruginosa that play a key role in the bacteria's responsive pathway to antimicrobial agents. As an important tool in the field of metabolomics, LC-MS integrates the separation power of chromotagraphy with the identification capabilities of mass spectrometry. In order to establish that known metabolites can be measured from P. aeruginosa using the LC-MS technique, preliminary profiling experiments were conducted to compare samples of a wild-type PA14 strain against samples of a mutant PA14 strain lacking acyl homoserine lactone (aHSL), a signaling molecule associated with bacterial quorum sensing. After the metabolite detection abilities of LC-MS in P. aeruginosa were confirmed, experiments were conducted to determine the effect of antibiotics on the production of metabolites within the bacteria. Because antibiotics must be added to the bacteria at sublethal concentrations, a growth curve study was performed to determine the appropriate dosage of azithromycin for experimental use. Profiling studies were then conducted using LC-MS to compare samples of wild-type PA14 with samples of PA14 treated with the appropriate sublethal dosage of azithromycin. By analyzing which metabolites are found at elevated concentrations in the drug-treated PA14 samples, the identity of potential metabolites associated with P. aeruginosa's ability to resist antibiotics was determined.

Future experiments can elucidate the specific metabolic pathway associated with P. aeruginosa's response to drugs by investigating whether knockout strains of the bacteria that lack genes encoding for the identified metabolites are more sensitive to antibiotics. Overall, such information provides valuable insight into drug development by contributing to the identification of new medicinal targets against resistant bacterial strains such as P. aeruginosa.

Chioma Madubata *Molecular and Cellular Biology*
Quincy 2011

Development of a fluorescence assay to characterize the effects of reactive oxygen species on pancreatic cell health

The Schreiber Laboratory,
Chemical Biology Program,
Broad Institute at Harvard and MIT

Type 1 Diabetes (T1D) is a metabolic disorder that results from autoimmune attacks on pancreatic beta-cells. Beta-cell damage and other diabetic symptoms often correlate with elevated levels of reactive oxygen species (ROS). However, previous research only suggests that ROS has a protective role in beta-cell health and has not clearly defined the role of ROS in β-cell dysfunction. Given the current uncertainty about how ROS contributes to beta-cell health, our project aims to characterize the effects of diabetes-associated ROS by developing a fluorescence assay to measure ROS in mouse pancreatic cell lines. After using this assay to measure beta-cell ROS under basal and stressor conditions, I will begin a high-throughput chemical screen to identify compounds that oppose those changes in ROS associated with beta-cell dysfunction.

My current research investigates the effects of diabetes-associated stressors on ROS levels in mouse alpha and beta-cell lines. To mimic the diabetic state, I treat cells with high glucose, free fatty acids such as palmitic acid and oleic acid, and inflammatory cytokines including tumor necrosis-α (TNF-α), interleukin-β (IL-1β), and interferon-γ (IFN-γ). After using the fluorescence-based ROS assay to measure ROS levels in treated cells, I will perform qPCR on proteins whose expression might be affected by modulations in ROS, including proteins regulated by Nuclear Factor-κB apoptosis pathway and proteins involved in the breakdown of ROS.

Allen Shih *Chemical and Physical Biology*
Eliot 2013

Supercharged protein folding

David R. Liu Laboratory,
Department of Chemistry and Chemical Biology,
Harvard University

Protein folding, involving the formation of secondary structures and the subsequent hydrophobic collapse, is very complex and currently not well understood. Since the green fluorescent protein (GFP) fluoresces only when it is correctly folded in the native conformation, it can serve as a reporter for protein folding experiments. Supercharged proteins, proteins synthesized with a high or low theoretical charge to mass ratio, exhibit unusual stability against aggregation and misfolding to the extent that some refold after heat denaturation. Two such supercharged proteins, +36 GFP and His39 GFP, demonstrate sensitivity to temperature, pH, concentration, salt, and metal ions. +36 GFP contains numerous lysine and arginine residues that create a theoretical charge of +36. His39 GFP, with 39 amino acid changes to histidine, has the special property of being resistant to aggregation and stable at a narrow pH range between 5.5 and 6.5, which makes His39 especially useful in understanding protein folding in specific environments. While GFP tends to aggregate at higher concentrations, His39 and +36 GFP still retain its ability to fold into the native proteins. Through further characterization of +36 GFP and His39 GFP, the mechanisms for protein folding can be better understood. Supercharged proteins also have been shown to penetrate mammalian cells effectively. When this property is combined with their unique stability and refolding capability, supercharged proteins may become potent vectors for protein and nucleic acid delivery.

Brandon Silverman *Chemistry*
Eliot 2012

Enzyne ring-closing metathesis to produce small chiral building blocks in diversity-oriented synthesis

The Schreiber Laboratory,
Chemical Biology Program,
Broad Institute at Harvard and MIT

Diversity-oriented synthesis (DOS) aims to introduce diversity into the space of organic molecules. Molecular diversity, measured in terms of structural, skeletal, and stereochemical diversity, is introduced through a build-couple-pair strategy in which small building-block molecules are synthesized and reacted in a combinatorial fashion. In this project, we elucidate a novel DOS pathway featuring enyne ring-closing metathesis (RCM), a process in which a tethered alkyne and alkene react to form a ring containing a 1,3-diene. The ring products of RCM, containing the 1,3-diene, can be oxidized to give an α,β-unsaturated ketone, a reactive group that readily allows for post-metathesis transformations promoting skeletal diversity. In order to incorporate stereochemical diversity into the ring-closed heterocycles, we utilize chiral amino acids as starting materials, which allows for retention of optical activity throughout the DOS pathway. Additional stereogenic centers are introduced post-metathesis on the growing molecular scaffolds.

Akansha Tarun *Chemistry*
Cabot 2013

Interacting domains of phosphatidylcholine transfer protein (PC-TP)/starD2 and thioesterase superfamily member 2 (Them2): physiological importance of PC-TP polymorphisms

Dr. David E. Cohen,
Gastroenterology Department,
Harvard Institutes of Medicine

The interacting proteins PC-TP and Them2 are highly expressed in the liver and other oxidative tissues. Suggestive of the importance of their interaction in metabolic regulation, mice lacking expression of either PC-TP or Them2 exhibit improved lipid and glucose homeostasis. Single nucleotide polymorphisms (SNPs) of PC-TP that yield to non-synonymous amino acid substitutions in humans and mice result in larger low-density lipoprotein (LDL) particle size and protection against high-fat diet induced diabetes. Aim: The objective of this study was to determine the interacting domains of PC-TP and Them2 and to study whether PC-TP polymorphisms in human and mice disrupt Them2 and TSC2 binding.

Glutathione-S-transferase (GST) pull-down and mammalian two-hybrid assays were utilized to assess PC-TP-Them2 interactions. Guided by the crystal structure of PC-TP, deletion constructs of recombinant human PC-TP were engineered by truncating of five, ten, fifteen, and twenty-two amino acids from both the N- and C-termini. SNPs were inserted in PC-TP by site directed mutagenesis.

Truncations of recombinant PC-TP by five amino acids at both the N-terminus and the C-terminus did not disrupt interactions with Them2 or TSC2. Analysis of the remaining deletion constructs are in progress, as are constructs containing two human SNPs. The use of the mammalian two-hybrid system for the study of PC-TP-Them2 interactions has been validated.

Neither the initial nor final five amino acids of PC-TP are required for interactions with Them2. Testing of additional deletion constructs and SNPs in GST pull down and mammalian two-hybrid assays should reveal the interaction domain of PC-TP. We speculate that molecules designed to disrupt PC-TP-Them2 interactions could prove valuable in the management of cardiovascular disease and diabete

Matt Zapf *Social and Cognitive Neuroscience*
Lowell 2011

Ube3a and autism: a gene dosage disorder

Anderson Lab,
Department of Pathology,
Beth Israel Deaconess Medical Center

Autism is a neurological disorder characterized both by impaired communication and social abilities and by repetitive and stereotyped behaviors. In a recent genome wide association study (GWAS) that sought to identify gene copy number variations (CNVs) in an autistic cohort, researchers found a significant association between the disorder and duplications of the gene E3 Ubiquitin Ligase (Ube3a). Additionally, mutations that cause Ube3a to lose function cause Angelman Syndrome (AS), a neurological disorder that has some symptoms in common with autism. To investigate the function of Ube3a, we BAC recombineered mice to have one or two extra copies of Ube3a on their maternal allele. These mice displayed autism-like traits in three behavioral paradigms that measured social interaction, stereotyped behaviors, and communication. These findings support the gene-dosage dependent model of autism that arose from GWAS human CNVs. However, the effects of extra copies of Ube3a in creating the autism phenotype are not fully yet elucidated. Ube3a is a member of the ubiquitin ligase family of proteins that are known to add ubiquitin moeities to substrates. This ubiquination usually leads to the degradation of that substrate, but it can also cause modification of the substrate to change its function or cellular trafficking properties.

In order to better understand the function of all the protein's isoforms, two of which contain ubiquination sequences (Long-form) and one of which does not (Short-form), we are using various methods to determine where each isoform is expressed in neurons. Additionally, we are studying the gene-dosage effects on the electropysiological and morphological properties of cortical pyramidal neurons. Understanding the cellular response caused by Ube3a gene-dosage differences in the autism mouse model will allow us to better understand how circuitry is disrupted in AS and this model of autism. Studying Ube3a on the genomic, molecular, circuitry and behavioral levels will hopefully provide insight into the devastating disorders of autism and AS.

Computer Science

Matthew Chartier *Computer Science & Math*
Kirkland 2012

Procedural generation and cooperative robotic search of three-dimensional environments

Professor Matthew Welsh,
School of Engineering and Applied Sciences,
Harvard University

Throughout the world, structural collapses as the result of earthquakes or other disasters continue to create tragic situations in which people are left trapped beneath piles of rubble, unable to free themselves, their continued survival contingent upon the speed with which would-be rescuers are able to exhaustively search through and clear the debris. My project involves development of a 3D modeling environment for subterranean exploration using a swarm of micro-sized aerial vehicles, called RoboBees. RoboBees, swarms of autonomous flying insect robots, may prove invaluable in optimizing the process of detecting the locations of such individuals in situations where they are trapped beyond the reach of humans but not necessarily beyond that of an artificial bee.

The development of some basic tools and algorithms was necessary in order to begin implementing a "search and rescue" behavior for a simulated swarm of RoboBees. This involved creating methods for procedurally generating virtual three-dimensional environments modelling both mazes and realistic underground mine structures, replicating sensor functionality related to the detection of human individuals, and creating search algorithms tailored specifically to the unique capabilities and limitations of a swarm of flying robots.

Much of the challenge of enabling a swarm of autonomous robots to search a bounded region is involved in simply navigating the space efficiently, a skill the testing of which requires a set of environments. Procedural generation, a process by which content is produced algorithmically, was employed in the creation of programs which output pseudo-random examples of varying types of environments based on a fixed set of parameters.

Thermographic sensors were emulated as a means by which a RoboBee could identify human individuals by detecting heat given off by a human body in its line of sight. Algorithms were also developed and tested in order to maximize the efficiency with which RoboBees were collectively able to navigate a simulated mine structure, detect randomly-placed individuals and relay their locations within said structure.

Ashok Cutkosky *Mathematics*
Kirkland 2013

Lie algebra representations

Professor Joseph Harris,
Dept. of Mathematics,
Harvard University

Representation theory is the study of maps between algebraic structures and linear transformations of vector spaces. One important class of representations are those of Lie groups. A Lie group possesses a manifold structure in addition to its algebraic structure in such a way that the two are compatible. Since the linear maps on a vector space also have a certain geometrical structure, representations of Lie groups are inherently interesting. The set of vector fields on a Lie group invariant under the group action forms a powerful object called the Lie algebra which can be used to probe the representations of the original group. These objects are also of interest in physics, where representations of Lie algebras can provide information about different particles. The idea of a Lie algebra can be made general and the representations of Lie algebras can be decomposed in turn into simpler representations, involving so-called semisimple Lie algebras. This project focuses the classification of these fundamental Lie algebras in order to understand the over-arching representations.

Jerry Kung *Applied Math (Computer Science)*
Winthrop 2011

Reward selection for reinforcement learning agents

EconCS Group,
School of Engineering and Applied Sciences,
Harvard Univeristy

Reinforcement learning (RL) agents learn to act in an environment through repeated observations and inputs that stem from their programmed internal reward functions, a process usually modeled as a Markov Decision Process. These internal reward functions affect the learning behavior of the agent and dictate the optimal policy to which RL agents will eventually converge in their environment. Within this process, the determination of how to set the agent's internal reward function is a critical step. An RL agent will invariably converge to the optimal policy when it is given the agent designer's objective utility function, but the time required for this convergence varies. Our goal is to understand when the environment and longevity of the agent cause the designer to prefer the agent to acquire a more naïve policy.

We consider the relative "complexity" or sophistication of different policies to better understand the amount of time an agent needs to acquire these policies. Using empirical results of specific instances in a single environment, we attempt to generalize to other environments. We try to answer the following question: given features of an environment and a time horizon for the RL agent, what is the best

way to set the agent's internal reward in order to maximize realized performance?

Nitish Lakhanpal *Physics*
Dunster 2013

Properties of credit networks

The Parkes Group,
School of Engineering and Applied Sciences,
Harvard University

Understanding economic systems is of inherent interest since it can enable tasks as diverse as constructing fair systems and averting crises by providing a degree of predictive power: connecting the outcome of interventions with properties of the system. In this project: we study an abstracted economic system in the form of a particular type of transactions game over directed graphs. Developed as a model of credit networks, the framework used in this research envisions a graph of vertices representing economic agents connected by directed edges of weight corresponding to the quantity of credit available from one agent to another. Two agents are selected to attempt a credit transaction at each time step, with transactions taking place via the routing of a unit of credit through the graph (a unit flow) from one agent (the creditor) to another (the recipient). A repayment of this "credit extension" is then carried out by routing unit flows from the recipient back to the creditor. Under certain assumptions, such a credit network displays robustness to attack from malicious agents and allows for straightforward calculation of such important properties as bankruptcy probability. The first part of our work involves relaxing these assumptions and examining, via simulation of the system in C++, how properties such as robustness change as a result. We also examine how strategic play on the part of the agents affects robustness. In addition to examining the basic properties of the model we would like to extend it, perhaps by linking the distribution of transactions with the underlying network structure. Additional topics of interest are the potential for collusion between agents, whether equilibria involving collusion exist, and how quickly bystanders can determine whether collusion is occurring.

Lucia Mocz *Computer Science*
Quincy 2013

Optical flow-based navigation of robotic bees

Professor Matthew Welsh,
School of Engineering and Applied Sciences,
Harvard University

Motion perception and interpretation are important aspects of study in a robot's vision system. Understanding motion can allow one to infer the structure of the environment, detect objects—both moving and stationary—or anticipate the subsequent movement of the robot. Therefore, the focus of this work is to develop algorithms to simulate, understand, and utilize the information obtained from optical flow, which may subsequently be useful for the autonomous navigation of a robotic bee. There is considerable evidence to suggest that bees use visual motion as a navigational tool, something that optical flow attempts to imitate. The algorithms in this project were developed in the Java programming language in an existing physical simulation environment known as Simbeeotic that utilized the JBullet physics library and was visualized in Java3D. Three versions of optical flow were implemented based on the works of Horn, Zuffrey, and Humbert. The original model observed the optical flow of a single point in the environment using a pinhole projection for the camera. A spherical projection of the bee's surroundings was then observed to more closely parallel the actual eyesight of insects, which are able to use perception to fly with six degrees of freedom. The third sensor, named the Centeye Motion Sensor (CMS), was adapted to resemble the output of the optical flow hardware from Centeye, which are to be used on the physical robot bees in the RoboBees project. CMS generates a square planar image of a motion vector field based on 25–81 points in its view field and computes a scaled velocity reading of the bee's motion in its environment. CMS was then further used to develop navigation strategies for the bees, including hover, cruise control, wall-centering, take-off and landing, obstacle avoidance, height-control, and turning, as well as an odometry reading based on optical flow outputs. Note that very little processing is required in the encoding and decoding of information in optical flow, making it an optimum choice to develop a cost- and time-efficient navigation method. The navigation method was based on a simple closed-loop control scheme created by the present author and various environments were tested to observe the robustness of the algorithm.

Tyler Zou *Computer Science and Math*
Mather 2012

A theoretical representation of the MapReduce paradigm for distributed computing

Professor Leslie Valiant,
School of Engineering and Applied Science,
Harvard University

Though computer architecture has progressed at astronomical rates in recent decades, the software that makes the hardware operational has sometimes lagged behind. Computers have transitioned from an age when a single processor controlled the operations to one in which every motherboard contains two or more processors. However, computer engineers have not yet learned how to reach the full potential of this advancement. In fact, even many "simple" problems, such as sorting a list of numbers most efficiently on multi-core computers, still remain unsolved. Under the guidance of Prof. Leslie Valiant, we studied the theoretical models for parallel computing such as the BulkSynchronous model and the Parallel Random Access Machine paradigm Wecalculated the fundamental parameters governing the widely implemented but commonly misunderstood MapReduce paradigm for distributed computing. A clear theoretical representation of MapReduce will enable computer scientists to fully exploit the potential of parallel machines.

Earth and Planetary Science

Jane Baldwin *Earth and Planetary Science*
Adams 2011

Interactions between temperature and precipitation in determining the equilibrium of glaciers

Huybers Lab,
Earth and Planetary Science Department,
Harvard University

A glacier's response to climate is commonly characterized by a change in the position of its equilibrium-line altitude (ELA) over time– ELA being the elevation at which the accumulation and ablation rates of a glacier are equal. ELA is generally regarded as the most representative altitude on a glacier as it provides a good proxy for glacier extent. Many studies have inferred past earth temperatures and levels of precipitation by examining glacier ELAs and assuming linear relationships between ELA and these climate factors.

However, such assumptions appear to inaccurately oversimplify the interactions between temperature and precipitation in relation to glacier ELAs. In our research, we simulate glacier ELA on a mountain by coupling a positive-degree-day ablation model with a model of precipitation on a mountain. Even this basic model reveals many complex, non-linear relationships between temperature and precipitation in determining ELA, with implications for trends in sensitivity of different altitude ELAs.

Recently a dataset has been compiled of worldwide mountain glacier ELAs at the present and Last Glacial Maximum, which occurred approximately 20,000 years ago. By calibrating our model to recreate the observed data trends in ELA change, we can gain insight into how temperature and precipitation have varied since the Last Glacial Maximum. We can also attempt to determine the relative importance of temperature versus precipitation in setting the mass-balance and extent of a glacier. If successful, our research should allow more accurate predictions to be made of how mountain glacier mass-balance will respond to the current period of anthropogenic global warming, with important consequences for sea-level rise and world hydrological cycles.

Lester Kim *Physics and Math*
Eliot 2011

Modeling the magnetic field of Jupiter

Professor Jeremy Bloxham,
Department of Earth and Planetary Sciences,
Harvard University

The study of Jupiter's magnetic field is of great importance for two reasons. First, Jupiter makes up most of our Solar System's mass outside of the Sun. Analyzing its magnetic properties would give us insight into the composition of Jupiter and how our star system originated. Second, analyzing the geometry of its magnetic field would shed much light onto the subject of interplanetary magnetism as a whole since it is still not yet fully understood. Unlike the Earth, Jupiter is a gas giant that lacks a solid outer shell. This allows us to more directly study the dynamo mechanism, caused by the magnetohydrodynamics (or MHD) of the conductive fluid near the core. Dynamo action is responsible for generating the magnetic dipole that is characteristic of planets such as the Earth and Jupiter (also Saturn, Neptune, and Uranus). On August 2011, NASA will launch a spacecraft named Juno to orbit around Jupiter, gathering data about its magnetic field. It is expected to arrive in 2016, but we can start creating theoretical data in advance to predict the expected field values. We are creating the interpolation algorithm to map out Juno's orbits completely from its arrival in 2016 until the end of 2017. We are also developing codes for predicting the magnetic field values based on the spherical harmonics as given by the associated Legendre polynomials. Another project includes mapping out the spatial coverage of field values that Juno will accomplish during its mission.

Matthew Newman *Mechanical Engineering*
Leverett 2013

Shock thermodynamics experiments in icy mixtures and hydrated minerals

Shock laboratory,
Department of Earth and Planetary Science,
Harvard University

In the Shock Lab we are conducting research on the properties of impact craters and the conditions under which they are formed. These craters offer a useful way to study the properties of near-surface material layers on other planets, moons, and even comets. The phenomena of cratering is an especially useful way to study the surface layers of extraterrestrial objects (and even terrestrial ones) as collisions pervade the solar system. This means we can use the same method to study the surface of mars, the surface of the moon, and the surface of comets. The experiments being done add to a very limited pool of data available that shows how the surface composition of Mars affects how the surface of the planet responds to impacts. How these variables affect cratering is an extremely poorly understood concept even at a theoretical level, and further knowledge on the subject will certainly be important as we search for new knowledge about alien worlds.

In this experiment we work specifically with nontronite, as it is a well studied clay whose presence has been identified on Mars. Constructing the experiment physically has been a major part of my job this summer. This means creating workable sample "disks" of nontronite as well as constructing the electromagnetic gauges used to collect data. These copper gauges are embedded in the nontronite samples which are then placed in a magnetic field. This allows us to measure an induced current in the gauges when the sample is shocked. This process directly gives us the particle velocity of the shocked nontronite which is used to determine shock loading and unloading paths.

Corinne Tu
Cabot 2012

Astrophysics and Statistics

Do not try this at home: the violent variablity of boisterous Blazars

Professor Xiao-Li Meng & Dr. Aneta Siemiginowska,
Center for Astrophysics,
Harvard University

Blazars are a type of high-energy radio-loud active galactic nuclei with relativistic jets that point close to the line of sight. The emission spectrum of a blazar covers all wavelengths, and the radiation is highly variable on all time scales, ranging from parts of a day to years. In this summer project, we examine the variability in high-energy gamma-ray emissions, because they provide the strongest constraints on unknown blazar characteristics. This summer project employs daily and weekly gamma-ray light curves of 59 blazar sources collected by the Fermi Gamma-Ray Space Telescope. We first perform some preliminary statistical analysis of the weekly blazar light curves collected by the Fermi Gamma-Ray Space Telescope, using statistical methods commonly used in astronomy: structure function analysis and variability index. We then apply a statistical method that has heretofore never been used before in the gamma-ray wavelengths, that involves Metropolis-Hastings (a Markov Chain Monte Carlo algorithm coded in R) to randomly sample from complex probability distributions. The MCMC helps to determine the parameters for use in an autoregressive time series analysis method, which fits the light curves by a random process. The purpose of applying statistical analysis to the light curves of blazars is to discover any existing patterns, such as characteristic variation, amplitudes, or time scales.

Engineering & Bioengineering

Kidus Asfaw *Engineering Sciences*
Eliot 2013

In vitro release study of rhodamine-labeled BSA and rhodamine B in methacrylated-alginate hydrogels

Edwards Lab,
School of Engineering and Applied Sciences,
Harvard University

One important aspect of drug delivery is the controlled release of the drugs our bodies need, preferably over long periods of time. It has been found that we can engineer synthetic gels to achieve any desired rate of drug release. One problem with such synthetic gels, however, is that they are not always biodegradable or biocompatible, and hence have some limitations for in vivo applications.

Currently, we are performing a release study of drug models from photopolymerized gels (nanoporous) and spongy gels (macroporous) that are made of Alginate, a naturally occurring degradable polymer distributed widely in the cell walls of brown algae. The release profile of drugs can be fine tuned through the gel preparation method and drug size, among other controllable parameters. We are using two fluorescent proteins of different molecular weights (MWs) to model drugs that are encapsulated inside the Alginate gels: Rhodamine B (479 g/mol) and Rhodamine-labeled BSA (66x103 g/mol). Moreover, we are studying which gel polymerization method best suits a steady release of our proteins.

A total of four different types of gels were investigated to determine the release profile from our Alginate-based gels:

1) Rhodamine-BSA encapsulated in 1% MA-Alginate Spongy Gels

2) Rhodamine B encapsulated in 1% MA-Alginate Spongy Gels

3) Rhodamine-BSA encapsulated in 1% MA-Alginate Photo Gels

4) Rhod B absorbed overnight in 1% MA-Alginate Spongy Gels

In order to monitor drug release, fluorescence emission readings are being taken every 3 days under the physiological conditions. The readings indicate a slow and steady release for the Spongy vs. Photo gels in which Rhodamine-labeled BSA was incorporated (up to 2 months). A faster release was observed for low MW Rhod B. The experiment is still in progress, however, because most gels still bear the pink color of the fluorescent dye. Thus, the drug release rate or burst when the gels start to degrade is yet to be seen.

Also, a series of Alginate-based magnetic spongy gel nanocomposites have been used to illustrate remote ontrolled drug delivery. By applying magnetic fields, we were able to switch the on-demand drug release profile of the smart gels between ''on'' and ''off'' mode. As a result, an efficient and effective drug therapy can potentially be administered at the right time with the right dosage.

Rose Cao *Engineering Sciences*
Quincy 2011

Analyzing the viability of harmonic radar for tracking and disambiguation of robot bees

Professor Matt Welsh,
School of Engineering and Applied Sciences,
Harvard University

Global honey bee populations have been declining since 2006, with hundreds of thousands of colonies vanishing due to a phenomenon known as "Colony Collapse Disorder." Since honey bees pollinate about a third of everything we eat, their disappearance threatens many crops like apples, oranges, carrots, onions, and coffee. If nothing is done, not only will our diet be significantly limited, the global economy and natural ecosystem will also be adversely affected.

Enter RoboBees, a collaboration of different departments and universities working on a swarm of robotic bees-to-be. Pollination is one of its many applications, including search and rescue, climate mapping, and traffic monitoring. In order to efficiently pollinate a field of flowers, however, there needs to be effective communication and coordination among the bees and with the hive. One possible method for this is harmonic radar, which involves sending out electromagnetic waves that will reflect off the bees (equipped with transponders) at a harmonic, or multiple, of the original frequency. As this has mostly been used with small numbers before, its feasibility with many, closely spaced, individuals needs to be tested. While the physical bees are in production, tests are being carried out through a simulator programmed to mimic the behavior of real-world objects and situations. The radar sensor is currently programmed as a sweeping wedge able to detect un-obscured bees in its beam. Once the bees are detected, their locations are recorded for tracking, which involves an estimation technique called a Kalman Filter that allows the sensor to predict where the individual bees are likely to be next based on their previous velocity and position, keeping an error covariance for better future predictions. The radar is simulated by empirical models for the antenna pattern, wave propagation, and path loss. By programming these, tweaking the models, and using previously observed numbers, we hope to determine the practicality of actually using harmonic radar to track robotic bees in the field.

Aaron Deardon *Physics*
Cabot 2011

Genetically engineered iGarden

Harvard iGEM,
Northwest Labs,
Harvard University

Genetic engineering of plants promises to be a technology that could help to feed the world's growing population. Large companies have developed genetically engineered seeds for commercial use, and today approximately 80% of processed foods in the USA contain some genetically modified (GM) products. Despite its widespread use in North America, the genetic engineering of plants has remained a controversial issue around the world. Two major concerns are the uncontrollable spread of GM plants and the untested long-term effects of eating GM products.

We are designing a system to genetically engineer plants and overcome the previously mentioned concerns. Our approach is to make genetically engineered plants suitable for smaller scale cultivation, allowing those who want to eat GM produce to grow their own. To reassure those who are concerned about the health effects of consuming GM food that their produce is not transgenic, we are working on implementing safeguards that will prevent the spread of GM plants. To demonstrate possible characteristics of plants that individuals might want to grow in their own gardens, we are working on changing the flavor of plants, knocking down common allergens and changing the color of petals. We are using RNA interference to achieve allergen knockdown and to change petal color.

To prevent the spread of transgenic plants we are working on a "genetic fence". We are inserting the gene that codes for barnase, a protein with ribonuclease activity, which kills the plant. We are also inserting a system that prevents the activity of barnase in the presence of methoxyfenozide. In the absence of methoxyfenozide the plant dies, so the plant will only grow in areas that are treated with the chemical.

My work has focused on developing the vector to insert our constructs into plants. We created six vectors containing the Silver Standard cloning site, which allows for simple insertion of constructs conforming to the same standard. The construct is inserted into the plant using agrobacterium, which infect the plant and transfer part of their Ti plasmid semi-randomly into the plant's genome. Some of the resulting seeds contain the inserted gene and produce transgenic plants.

Jonathan DeWerd *Physics*
Quincy 2013

iGEM: gene containment and allergen knockdown in *Arabidopsis thaliana*

Harvard iGEM,
Northwest Labs,
Harvard University

Every year the international Genetically Engineered Machines (iGEM) contest challenges contestants to imagine and realize novel biological constructs by building and expanding upon emerging standard practices for genetic engineering (namely the BioBrick standard and accompanying parts registry).

This year, the allergy subteam of the Harvard iGEM team aims to knock down allergens in A. thaliana (and eventually strawberries) using RNA interference (RNAi) pathways. These pathways originally evolved to defend cells against overexpression and viral attacks by way of targeted RNA destruction using a series of proteins collectively known as the RNA-Induced Silencing Complex (RISC). Certain patterns of RNA (hpRNA and amiRNA) form hairpins that incorporate segments of themselves into the RISC and give it specificity to a certain RNA sequence which the RISC will then target and degrade, preventing translation (potentially to a degree of 95-100%). By targeting segments of RNA common to several characterized allergens we hope to suppress the level of allergen present in the aforementioned plants.

Other subteams include Team Flavor (Miraculin (Miracle Berry Protein), Valencene (orange), banana scent, and wintergreen scent), Team Fence (responsible for a genetic construct which will prevent the spread of our modifications into the wild), and Team Vector, which will insert these genes into Arabidopsis with the help of Agrobacterium Tumefaciens. This species of bacteria usually operates by inserting tumor-inducing genes into plants in order to create vulnerable growths of plant flesh on which it can feast without risk of being attacked by a healthy plant immune system. Fortunately, the tumor-inducing behavior is specific to a plasmid within the bacterium that can be swapped out for arbitrary genetic material. Team Vector will swap the tumor-inducing plasmid for the other subteams' modifications, creating an all-around more fascinating version of the common Arabidopsis plant.

Juan Hernandez-Campos *Engineering Sciences*
Leverett 2012

Nanoporous platinum

Aziz Group,
Gordon McKay Laboratory,
School of Engineering and Applied Science,
Harvard University

A fuel cell is a device that generates electricity from reactions between chemicals that are supplied continuously. A hydrogen-oxygen fuel cell is an example of a fuel cell where hydrogen and oxygen react to produce electricity and water. Since chemical reactions govern the generation of electricity in a fuel cell, catalysts are essential because they facilitate the reactions in the fuel cell. Nanoporous platinum is a desirable catalyst due to its stability and its potential to have a thousandfold increase in surface area when compared to a piece of regular platinum of similar size. One way to make nanoporous platinum is to sputter platinum and copper on a substrate to create a platinum-copper alloy that is then put on a reverse galvanic cell to remove the copper. This leaves nanopores where the copper used to reside producing a sponge-like structure. Other techniques involve submerging a platinum-copper alloy in strong acids to dissolve copper and leave nano-sized features on the surface of the alloy. Ultimately, the resulting samples are examined using a scanning electron microscope to determine if the morphology of the nanoporous platinum is desirable.

Kelly Holt *Engineering Sciences*
Pforzheimer 2012

Flight performance and predatory fitness in odonates

Combes Laboratory,
Department of Organismic and Evolutionary Biology,
Harvard University

Although it is not immediately apparent, birds, bees, dragonflies, and other flying animals use very different mechanisms to accomplish flight. The differences in anatomy between these animals have been thoroughly studied, as have the corresponding changes in flight performance. However, most studies of flight performance have occurred in wind tunnels or similarly controlled environments and have aimed to use flight speed, turning radius, or other similarly arbitrary variables as a way to characterize the flight performance. What is not known is how well changes in these variables correlate to changes in the insects' fitness in the wild. By combining traditional wind-tunnel tests of flight performance with trials of the ability to catch drosophila, we aim to characterize which traditionally measured variables are important to the dragonflies' ability to feed themselves in the wild. In order to assure that feeding trials in a greenhouse provide a valid insight into behavior in the wild, we are also engaging in observational studies in the woods surrounding the Concord Field Station in Bedford, MA. An additional goal is to analyze if and how dragonflies can compensate for wing damage both by including individuals with natural wing damage in the feeding trials and by artificially damaging individuals' wings upon capture in order to discern their immediate and long-term reactions to this change.

George Huang *Engineering Science*
Winthrop 2012

Directed differentiation of juxtaposed mineralized tissues

Professor David J. Mooney; Praveen Arany PhD,
Laboratory for Cell and Tissue Engineering,
School of Engineering and Applied Sciences,
Harvard University

A challenge that faces current scaffold-based techniques for tissue regeneration is accurately recreating the complex 3D apposition of different tissues found within the body. This project seeks to develop a method for engineering the formation of juxtaposed mineralized tissues within a scaffold that has been seeded with a single undifferentiated cell type. Specifically, we aim to spatially and temporally tailor biochemical cues within a tri-layered PLGA scaffold that has been seeded with D1 mesenchymal stem cells in order to direct the differentiation of precisely juxtaposed bone and dentin.

We accomplish this by first synthesizing PLGA microspheres within which growth factors (either dentinogenic TGF-β1 or osteogenic BMP-4) or antibodies against these growth factors have been encapsulated. To precisely direct differentiation of the stem cells, parameters were determined for the composition and size of each scaffold layer through mathematical modeling using data collected from examining the release kinetics of proteins from PLGA microspheres and scaffolds as well as concentration effects of the growth factors and uptake/degradation in the presence of media, cells and conditioned media over time. We arrange mixtures of the microspheres in appropriate concentrations together with precisely milled salt particles (to define pore size) in three layers such that one layer contains microspheres in which TGF-β1 or anti-BMP-4 antibodies have been encapsulated, the middle layer contains empty microspheres, and the opposite layer contains microspheres in which BMP-4 or anti-TGF-β1 antibodies have been encapsulated. Following foaming of the layered microspheres together and subsequent removal of salt, we create macroporous scaffolds in which different gradients of varying growth factors can be established in different areas.

To validate the development of the predicted morphogen fields, we have uniformly seeded these tri-layered scaffolds with TGF-β1 (p3TP luciferase) or BMP (C2C12 BRE) reporter cell lines and verified the presentation of the growth factors to growing cells by luciferase imaging. We are presently seeding the D1 cells in these tri-layered scaffolds for 7, 14 or 21 days and we plan to assay for specific mineralized tissue formation by cryosectioning and immunostaining or RT-PCR for expression of tissue-specific markers.

Timothy Kotin *Engineering Sciences*
Kirkland 2011

Distributed neural network algorithms and applications: motion classification in the programmable second skin project

Professor Matthew D. Welsh,
Wyss Institiute for Biologically Inspired Engineering,
Harvard University

Several scenarios arise when normal neuromotor and neuromuscular function in individuals is lost, significantly impaired or abnormally developed resulting in serious challenges to locomotion, and thus other normal human activities. Diagnosis and treatment of such disorders often involves careful monitoring and analysis of gait in affected individuals. Novel anticipatory medical devices are being developed, which also depend on careful analysis of a wearer's gait. For instance, our lab has also developed MERCURY: a wearable sensor network platform for high-fidelity motion analysis to be employed in patients affected with epilepsy or Parkinson's disease. The current work forms part of a collaborative effort with research labs at Harvard Medical School to develop one such an assistive orthotic device. We are specifically exploring the use of a distributed implementation of neural networks for motion classification using the motion data of body sensor networks (BSNs) such as MERCURY. Eventually, by distributing the neural network computations of unto the individual motes comprising BSNs, rather than relaying accumulated motion data to a base station, we hope to support real-time applications like anticipatory medical devices. Ongoing clinical research, particularly regarding sensorimotor development in infants, suggests the possibility of 'educating' the nervous system through an appropriate control system for novel orthotic devices. Our neural network approach thus seeks to develop a biologically inspired framework for collaborative performance of the distributed components of body sensor networks or special orthotic devices. In the long term, we hope to develop algorithms by which the embedded sensors of a body sensor network or actuators of a prosthetic device may adaptively assist nervous function development in in-

fants or recovery in brain-injured patients. However, beyond direct relevance to these medical innovations, a robust adaptive distributed neural network framework offers potential applications in other systems which may employ a network of sensor components for data acquisition, processing and interpretation. The sensory inputs here could be optical, for instance in a system of cameras to track some complex series of events, as even electrical, mechanical or chemical to mention a few.

Katerina Mantzavinou *Engineering Sciences*
Currier 2011

Investigating the effect of losartan on tumor collagen I

The Edwin L. Steele Laboratory,
Harvard Medical School,
Massachusetts General Hospital

Many solid tumors have a high content of interstitial proteins surrounding the tumor cells. This interstitial content consists mostly of collagen I fibers and is the result of a stromal reaction that seeks to hinder tumor growth and progression. The collagen fibers form a dense matrix that obstructs the transport of therapeutic macromolecules in tumors. The fiber density and orientation affect macromolecular diffusion through the extracellular matrix (ECM). We can image tumor collagen non-invasively by second harmonic generation (SHG). SHG is a non-linear optical imaging method based on signal generated by non-centrosymmetric structures like collagen I.

We are using HSTS26T human fibrosarcomas in the dorsal skinfold chamber of immunodeficient mice as a model to study the changes in tumor collagen levels. Using SHG, we obtain images of fibrillar collagen surrounding tumors in vivo in mice. SHG images allow us to correlate collagen content and macromolecular diffusion through the ECM, and to investigate the modification in collagen content and structure after treatment with losartan. Losartan, an angiotensin II receptor antagonist drug, inhibits collagen production in the ECM and so increases macromolecular diffusion. We use the SHG signal to predict the diffusion pattern through the imaging area in the chamber and to investigate the effect of losartan administration to collagen dynamics in the tumor-surrounding capsule.

We investigate the kinetics of collagen remodeling by losartan by imaging tumor regions of interest (ROIs) over a period of two weeks. We use tumor vessel markers to locate and return to the ROIs. SHG signal intensity is kept constant during the experiment by maintaining a constant laser power at 700mW. We also use a constant photomultiplier tube (PMT) voltage of 80V.

As a final step in the experiment, we will examine the influence of losartan on oncolytic Herpes Simplex Virus (HSV) efficacy in HSTS26T and MU89 tumors.

Sonia Pernia *Engineering Sciences*
Quincy 2011

Bisphosphonate-related osteonecrosis of the jaw

Mooney Laboratory,
School of Engineering and Applied Sciences,
Harvard University

BRONJ, or bisphosphonate-related osteonecrosis of the jaw, is a condition that causes cell death in the jaw as a result of bisphosphonate treatment. Though biphosphonates, drugs commonly prescribed both orally and intravenously for patients with osteoporosis or bone cancer, are generally safe and efficacious for therapeutic use, they can sometimes lead to necrosis of the jaw bone. In fact, approximately 12% of intravenous bisphosphonates patients and 1 in 100,000 patients who take the drug orally can develop BRONJ. Intriguingly, the bone necrosis is limited to the jaw bone, and is not known to affect any other bones in the skeletal system. The goal of this project is to elucidate the mechanisms of BRONJ, specifically by studying the effects on osteoblasts and osteoclasts, which are cells that maintain normal bone physiology by forming and resorbing bone.

In order to study the effect of these drugs on differentiation, pre-osteoblast and pre-osteoclast cells are treated with various combinations of growth factors and drugs. Following treatment, signaling molecules are detected and differentiation markers are measured. An additional factor that must be considered is the unique mechanical environment of the jaw and the effect of repetitive stretch on cellular differentiation. Differentiation experiments are carried out in elastomeric scaffolds and subjected to mechanical stretch to mimic the timing and duration of jaw movement in the human mouth during chewing. Thus, differentiation is tested at the same time as mechanical stretch, further approximating human activity in the jaw. Based on the results of signaling and differentiation experiments, BRONJ can be understood more comprehensively. Eventually, a mouse study may be developed, thus linking the laboratory results to an animal model.

Jackie Quinn *Engineering Sciences*
Currier 2012

The Harvard iGarden

Harvard iGEM,
Northwest Labs,
Harvard University

As a part of the 2010 Harvard iGEM team, I am working on the implementation of synthetic biology's Biobrick system in plants. Our ultimate goal is to create a toolkit for growing a personalized, genetically engineered garden, titled "The iGarden." We hope that the iGarden can become a platform for public awareness of synthetic biology and genetic engineering.

iGEM (International Genetically Engineered Machines) is a competition in which each team designs a project that uses the Biobrick system to create a new "biological machine." Biobricks are sequences of genetic material flanked by standardized multiple cloning sites that can be easily cut out of vectors with restriction enzymes and reassembled with other digested biobricks to create new constructs. The idea is to create "molecular legos" out of sequences coding for anything from promoters to proteins, and then assemble those parts to create functional units that can be expressed in various organisms to complete specific tasks.

This year, the Harvard team aims to create an array of parts to be expressed in garden plants. We are currently working on four sub-projects: expression of taste inverters such as miraculin and other flavor modifications, knockdown of allergens in strawberries and arabidopsis using RNA interference, alteration of carotenoid metabolism to produce novel pigmentation, and construction of a

genetic fence to prevent unwanted spread of transgenic material. We plan to use agrobacterium-mediated transformation to integrate our constructs into the plant's genetic material.

I am a member of "Team Color," the pigmentation group. We are attempting to express red and orange coloration in the ordinarily white arabidopsis flowers by knocking down specific enzymes in the carotenoid metabolic pathway via artificial microRNA interference. By knocking down these steps in the pathway, we hope to induce the accumulation of the metabolic intermediates lycopene and beta-carotene, which are colored red and orange, respectively.

Anugraha Raman *Human Developmental and Regenerative Biology*
Pforzheimer 2012

iGarden: Creating Personalized Genetically Engineered Hypo-Allergenic Foods

Harvard iGEM,
Northwest Laboratory,
Harvard University

In order to deal with the growing problem of food allergies worldwide, scientists are currently making efforts to design hypoallergenic foods. Hypoallergenic foods cause fewer allergic reactions because the presence of the allergy-causing protein is significantly reduced. Scientists have demonstrated that common food allergens can be knocked down through RNA interference in plants without hindering the plant's natural growth and development. As an extension of this work, this project aims to create an "iGarden"—a personalized garden containing genetically engineered plants that are tailored to the owner's allergies and flavor preferences.

To create our hypoallergenic plants, we are designing ihpRNA (intron-containing self-complementary hairpin forming RNA) and amiRNA (artificial micro RNA) constructs to knockdown proteins that are similar to allergens found in strawberry and arabidopsis plants. By targeting the following proteins: Bet v1 (a pathogenesis-related protein and homologue to the allergen involved in the birch allergy), Lipid Transfer Proteins, Germins (stress proteins), and Profilins (actin-binding proteins), we will test for gene knockdown by inserting our constructs into agrobacterium, transforming into their respective plants (arabidopsis/strawberries), and running quantitative PCR to look for a decrease in the expression of allergens with an increase of expression of the flavor-altering proteins . First, we will test knockdown of GFP (green fluorescent protein) in GFP-containing arabidopsis as a proof of-principle study followed by an experiment to test the knockdown of our chosen allergens in arabidopsis and strawberries. We are also designing more ihpRNA constructs for allergens in other plants, such as tomatoes, celery, and carrots. The flavors of the plants will be modified and monitored by the expression of Miraculin and Brazzein proteins attached to the fluorescent reporter (YFP).

By demonstrating knockdown of these allergens and expressing these flavor proteins, we can successfully create an iGarden prototype. The standardization and addition of our various constructs into the openly accessible BioBricks registry will allow us to lay the foundations for future experimentation upon other allergens. Ultimately, we hope to expand upon the variety of plants present in our iGarden and commercialize this model to allow individuals, regardless of scientific background, to create their own personalized gardens of hypoallergenic foods.

Lisa Rotenstein *Chemical and Physical Biology*
Eliot 2011

Design of polyvinyl alcohol hydrogels for the adhesion of endothelial cells

Auguste Lab ,
School of Engineering and Applied Science,
Harvard University

Atherosclerotic cardiovascular disease results in loss of function, hemorrhage, or aneurysm formation in the vasculature. Current endovascular treatment methods are limited by post-treatment restenosis and fibrosis. Endothelial cell delivery may mitigate these issues by providing a barrier between the substrate and the damaged vessel. Active biomaterials may allow for endothelial cell adhesion and migration, encourage vessel formation, and discourage fibrosis and thrombosis. Previous work has exploited conjugation of arginine-glycine-aspartic acid (RGD) (a binding motif from fibronectin) to hydrogels in order to encourage cell adhesion and migration. An alternate method involves conjugating antibodies against cell-specific markers to gels in order to encourage adhesion of cells of interest.

HDECs will be derived from hESCs through application of shear stress or exogenous growth factors. We will then explore the gene expression responses of HUVECs, HDECs, and hESCs when exposed to the inflammatory cytokines TNF-α and IL-1. Upregulation of Vascular Cell Adhesion Molecule (VCAM-1), Intercellular Adhesion Molecule-1 (ICAM-1), Endothelial Leukocyte Adhesion Molecule (ELAM-1) Matrix Metallopeptidase 2 (MMP-2) and Platelet Cell Adhesion Molecule (PECAM-1) will be assessed on HDECs and hESCs and expression of Rex1, Nanog, Sox2, and Oct4 will be assessed for hESCs and HDECs. Antibody presenting poly(vinyl alcohol) (PVA) gels for binding human embryonic stem cell(hESC)-derived endothelial cells (HDECs) and human umbilical vein endothelial cells (HUVEC) will be synthesized. Biocompatible and non-degradable PVA gels will be modified to include amine groups for the conjugation of biological molecules to promote cell adhesion and differentiation. Based on preliminary upregulation data, anti-ICAM-1, anti-ELAM-1, and anti-VCAM-1 will be conjugated to gels. We will characterize the adhesion, migration, and proliferation of these cells on PVA gels of composition (weight percent of PVA), stiffness, and antibody presentation (antibody ratio, and density).

Barthalomew (B.A) Sillah *Bioengineering/Biophysics*
Eliot 2012

Optogenetics approaches in cardiomyocytes

Parker Laboratory,
School of Engineering and Applied Sciences,
Harvard University

Several biological systems rely on action potentials of excitable cells. These rapid increases and decreases of cell membrane potential regulate intracellular processes such as neuron firing and muscle contraction. The ability to control action potentials reliably and repeatedly without the aid of stimulating electrodes remains a challenge of our field of research.

The emerging field of optogenetics has provided neuroscientists the optical control of cellular action potentials through photoexcit-

able proteins called opsins. One such opsin is channelrhodopsin-2 (ChR2), a light-gated ion channel found in green algae. Upon excitation by blue light, ChR2 channels open, facilitating the influx of sodium cations that change the membrane potential of cells on the millisecond scale. Recent experiments with mammalian neurons transfected with ChR2 suggest that light-sensitive opsins can be used to control the electrical impulses of neuron subpopulations in these transgenic animals.

In the Disease Biophysics Group, we are investigating the potential use of optogenetic systems in cells of the cardiovascular system. This application could offer a new method of controlling action potentials in the heart and contraction of the myocardium. This project is anticipated to offer a viable new experime

Michelle Vhudzijena *Engineering Sciences*
Cabot 2012

Neuronal induction in human mesenchymal cells on patterned microstructures

Aizenberg Laboratory,
Wyss Institute for Biologically Inspired Engineering,
Harvard University

Biomaterials are known to induce proliferation and differentiation of human mesenchymal cells into neurogenic, myogenic and osteogenic cells. Tailoring microstructures that specify adult human mesenchymal cells to the neuronal lineage is crucial in developing novel stem cell therapeutics for neurological diseases. Neurological diseases account for one percent of the deaths in the world and eleven percent of the global burden of disease. In this study, we investigate how adult stem cells respond to matrix elasticity and express neuron- specific proteins, exhibiting polarized morphologies indicative of neuronal induction in stem cells. Research suggests that substrates with lower stiffness constants mimic the elasticity of the soft matter in the brain. Physical and surface modification of biomaterials influence cell growth, proliferation and differentiation. Recent studies have shown that designing structures from materials that are biocompatible, non-toxic and stable on a nano and micro scale can result in high proliferation and differentiation toward desired lineages of human mesenchymal cells. It is known that matrix elasticity directs stem cell lineage specification in polyacrylamide gels. Applying this knowledge to other substrates will result in a better understanding of proliferation and differentiation of stems cells on different biomaterials.

Jake Weatherly *Engineering Sciences, AB*
Dunster 2012

Smart Sock

Professor Radhika Nagpa, Leia Stirling PhD,
Wyss Institute for Biologically Inspired Engineering,
Harvard Medical School

Drop foot syndrome is a condition in which the muscles on the front of the lower leg are weak, making ankle dorsiflexion difficult.1 An affected person has trouble bending the ankle and toes upward. Causes of drop foot syndrome include injury to the peroneal nerve at the top of the calf, and many diseases, including multiple sclerosis, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Lou Gehrig's disease, muscular dystrophy, cerebral palsy, and Down syndrome.1 Traditionally, rigid orthotics have been used to help children with drop foot syndrome, but these are cumbersome and do not adapt to changes in a child's motion, such as a change from straight-line gait to a turn. As an alternative, we are developing an active soft orthotic, a device worn as a sock that uses sensors to gather information about the wearer's motion and employs actuators to assist the motion. A major challenge for such a device is to anticipate when the wearer is going to turn during gait, so a study is being performed in healthy five-year-old children to learn how they adjust their gait before turning. If we can understand the differences in step length, step width, and center of mass trajectory before a turn, then we can develop a control system to anticipate a turn.

The study uses eight Vicon cameras that send near-infrared light to reflective markers placed at forty-eight joint locations on the subject. The Vicon software tracks the marker trajectories as the child walks down a platform and makes a turn. The kinematics (displacement, velocity, and acceleration) of the hip, knee, and ankle can be calculated from the marker trajectories. FlexiForce sensors are inserted into the child's shoes to keep track of heel-strike and toe-off so that step length and width can be calculated. Center of mass trajectory is analyzed in the open-source motion simulation software OpenSim, which takes the child's measurements as input and outputs a three-dimensional model of the motion. There are few studies of turning in children in the literature, but studies of adults indicate that step length decreases, step width remains the same, and speed of the center of mass decreases before a turn.2,3,4 The present study seeks to determine whether the same trends are seen in children, and will lead us on our way toward developing a control system for our "smart sock."

Fiona Wood *Computer Science*
Leverett 2013

From local divisions to global network topology: exploring space-dividing networks in a wide variety of systems

Professor Radhika Nagpal,
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School of Engineering and Applied Sciences,
Harvard University

Complex networks can develop and self-regulate in myriad ways. One type of self-organizing system, the so-called space-dividing network, develops and grows based on certain proliferation rules. Instead of divisions that propagate simultaneously through a medium, space-dividing networks rely on the formation of temporally distinct divisions. This project is an examination of how space-dividing networks form through such proliferation. Nature and human activity are rich with examples. Epithelial cells, leaf venation, crack patterns in glazes, as well as maps of political boundaries are all examples of space-dividing networks. Each is composed of dividing cells that feature minimal rearrangement. We seek to determine if these net-

works share any common properties and whether those properties can provide insight into how fundamental proliferation rules that regulate local cell behavior on a small scale are able to produce larger structures on a more global scale throughout the entire network.

Exploring space-dividing networks of various types required us to develop image analysis tools to extract information from images of the space-dividing networks in question. With these tools we can quantitatively measure various properties such as neighborhood relationships, cell area, the probability a cell will divide, and each cell's lineage in the hierarchy of cell divisions that form with successive divisions over time. We find that cell lineage is important to examine because it represents a crucial difference between space-dividing networks and other types of networks for which rearrangement, not proliferation, plays a more central role in network development. We plan to use the information gathered about the properties of space-dividing networks as they change and grow to develop computational models to test our understanding of the nature of these networks and predict the ways in which proliferation affects their development.

A better understanding of the development of proliferation networks is important for many applications; for example, in studying the abnormal cell proliferation that is characteristic of many forms of cancer.

Mathematics, Statistics & Economics

Paula Bu *Comparitive Religion*
Quincy 2012

Seasonal energy intake by the Pumé Foragers in Venezuela

Conklin-Brittain Lab,
Department of Human Evolutionary Biology,
Harvard University

Though anthropologists have long been interested in the diet of hunter gatherer societies, such research seems particularly relevant in a day and age where obesity has become an epidemic and there is a growing public awareness in the importance of nutrition. It is interesting to examine the diet of these societies, especially when it seems as though as they continue to live in nutritionally stressful environments. In the case of the Pumé foragers, who live in the savannas of southern Venezuela, they not only have no access to modern health care or supplemental food programs, but they also have annual fluctuations in food supply and seasonal undernutrition. Their diet consists primarily of roots, tubers, manioc, mangoes, and fish seasonally. But studies have shown that in spite of living in harsh conditions, Pumé girls still mature quickly and bear children in their midteens. This may be due to the higher foraging returns of older women and men being shared out to younger women and girls who are less productive (Kramer KL et al., 2009). The data for this study shows the foraging return rates for roots and mangoes in terms of kilograms of food per hour spent foraging. Through various lipid, free sugar, crude protein, insoluble fiber, crude ash, and starch assays, macronutrient analyses are being done on these foods and the previous data will be converted into terms of kilocalories. Furthermore, we will also compare the results to pre-existing data on the diet of the Hadza, a hunter gatherer society in Tanzania. Questions regarding food allocation also arise for the Hadza, as they choose to engage in the riskier endeavor of hunting big game over smaller game (Hawkes K et al., 1991) and turn to tubers as fallback foods (Marlowe FW et al., 2009).

Sumit Malik *Applied Mathematics*
Kirkland 2013

Mathematical equality: erasing poverty with quantitatively optimal corporate governance

Professor Mihir Desai,
Department of Finance,
Harvard Buisness School

The current state of international income inequality is alarming. The world's poorer half collectively controls only 1% of global assets, whereas the 3 richest individuals are wealthier than the bottom 600 million combined. The neoclassical Solow, Mankiw-Romer-Weil, and Schumpeterian models, among others, attribute per capita income differences to immediate disparities in physical capital, human capital, and technology without identifying suboptimal incentive structures that underlie deficiencies in capital accumulation and technological innovation.

To assess institutional incentives in heterogeneous, profit-maximizing firms, we develop and examine analytical and statistical models for efficient corporate governance and financial legal structure. In particular, we consider firm behavior in two capacities: first, by exploring financial consequences of imperfect property rights, and second, by computationally replicating tax effects on corporate debt policy.

Insecure property rights imply a high probability of expropriation of minority shareholders by corporate insiders, thus depressing expected profits for potential financiers. Unless sufficiently high upside revenue compensates for risk exposure, weak shareholder protections discourage external investment and obstruct capital accumulation necessary for firm maintenance and growth. Ideal legal structure minimizes profits attained from exploitation of social and political infrastructure rather than by productivity. This analysis is consistent with Legal Origins Theory, which emphasizes the persistence of centuries-old legal traditions transmitted largely via colonization and conquest, as a fundamental source of present global inequality.

Furthermore, comparatively high corporate tax rates incentivize accounting schemes to represent corporate income as personal income while maintaining nontax advantages of the corporate organizational form. Heavier reliance on debt over equity financing evades high corporate taxes because of tax deductibility of interest on debt but not of dividends on equity. The associated model quantifies corporate debt policy with controls for firm size, business cycles, and asset composition. Mathematical assessment of public policy implications for firm behavior provides insight on financial structure conducive to equality, efficiency, and economic prosperity.

Adrian Sanborn *Mathematics*
Adams 2011

Self-Intersection of Space-Filling Curves

Aiden Lab,
Department of Mathematics,
Harvard University

Intuitively, one may think that a "curve" has no volume because it is infinitesimally thin. However, in 1890, Giuseppe Peano discovered the first "space filling curve": a curve that maps from a one dimensional domain and fully covers a higher-dimensional region, such as a square or cube. Evidence has recently emerged suggesting that such "space-filling" curves may help describe how the two-meter-long human genome folds up inside the six-micron-wide cellular nucleus. In particular, it was found that the genome's tendency to collide with itself at various distances closely resembles a similar behavior that is seen in space filling curves.

The purpose of my project is to characterize the frequency of

"collisions", or self-intersection, in space-filling curves by exploiting their self-similar fractal structure. How often do two points in the domain map to the same point in the range? We rigorously compute the Hausdorff dimension of the set of collision points, present a method for extracting collision points, and study how the frequency of collisions changes as a function of the distance between the two points. These results elucidate a new property of fractal curves and will help place the interpretation of ongoing experiments on a solid theoretical foundation.

Adam Sealfon *Mathematics/Computer Science*
Dunster 2013

Approximating entropy of polynomials over small finite fields

Vadhan Group,
School of Engineering and Applied Sciences,
Harvard University

Entropy is a fundamental information-theoretic concept that measures the amount of randomness in data. Approximating the information entropy of low-degree polynomials appears to be a difficult general problem. Many cryptosystems implicitly rely on the difficulty of determining the entropy of polynomials, in the sense that an efficient algorithm for determining or approximating the entropy of polynomials could allow the cryptosystem to be broken. We explore approximating the entropy of a list of low-degree polynomials in many variables over a small finite field. We investigated whether the Jacobian rank is a good approximation of entropy in this instance. Previous results by Dvir, Gabizon, and Widgerson demonstrated a correlation between rank and min-entropy over sufficiently large fields. We search for similar relationships for entropy and min-entropy for polynomials over small finite fields. In order to gain insight into this problem, we also run simulations in the computer algebra systems SAGE and Macaulay2. We also explore which cryptographic problems can be reduced to the problem of approximating entropy of polynomials. Two problems we consider are the problem of learning with errors (LWE) and its special case of learning parity with noise (LWN). Questions about approximating entropy and determining an entropy gap relate to the concept of statistical zero-knowledge proofs. A statistical zero-knowledge proof is an interactive proof by which one party can convince another party of the truth of an assertion while conveying statistically no additional information other than the fact that the statement is true. The problem of determining through sampling which of two distributions has higher entropy is HVSZK-complete, meaning that it is computationally as hard as any honest-verifier statistical zero-knowledge promise problem. Statistical zero-knowledge is intimately linked to cryptography. It has been shown that an average-case hardness result for SZKP would imply the existence of one-way functions, which would have strong cryptographic consequences.

Jonathan Wang *Mathematics*
Adams 2011

The moduli stack g-bundles on an algebraic curve

Gaitsgory Group,
Department of Mathematics,
Harvard University

In algebraic geometry, a scheme can be loosely thought of as a subset of common zeros of polynomials in n variables in Cn. A moduli problem studies the functor sending an arbitrary scheme T to a class of schemes over T . In certain nice cases this functor is itself representable by a scheme, but this is impossible when the classes of objects being studied admit automorphisms. Instead, a very useful notion is when the functor in question is an algebraic stack – a generalization of the notion of a scheme. The particular class of objects we study are G-bundles on X ×T , where X is a smooth projective curve over C and G is a linear algebraic group (e.g., GLn(C), the group of invertible n×n matrices). Over a scheme Y , a G-bundle is defined to be a scheme P with a map to Y such that P is isomorphic to G×Y locally on Y . The functor BunG, which sends T to G-bundles over X ×T , is in fact an algebraic stack. We study the proof of this fact and explore other properties of G-bundles. The study of G-bundles is important in the subject of geometric representation theory, which has applications to number theory and physics, in particular gauge theory and quantum field theory.

Xiaomeng Jessica Zeng *Economics*
Kirkland 2012

Extending behavioral economic insights to government pensions plans and the health sector

Professor David Laibson,
National Bureau of Economic Research

Behavioral economics is a branch of economics that incorporates observations of bounded rationality in humans into classical economic frameworks in order to more accurately model human behavior. My research group, led by professors David Laibson, Brigitte Madrian, and James Choi, has used retirement savings data to further behavioral economics and apply its ideas to nudge household investment behavior. We have studied 401(k) plans, which are retirement savings plans primarily provided by employers in the private sector. The group has examined the effects of defaults and "active decisions" on 401(k) participation. Defaults are statuses in which participants are automatically placed and would have to actively "opt-out" if they prefer an alternative. If individuals were completely rational, then defaults should not affect individual choices. However, research has found that defaults do have a powerful influence on outcomes (i.e. individuals overwhelmingly stay with a defaulted choice). "Active decisions" are situations in which individuals are required to explicitly make a choice between different options. This group has found that active decisions also increase employee participation in 401(k) plans.

One project involved examining public pension plans and potential improvements on their designs. There is much heterogeneity in the structures of these plans, and we are interested in whether

these differences are changing the amount that individuals are saving through their pensions.

Our insights from the 401(k) domain can also be applied to the health sector. We are working on a project that aims to help a company improve the health of its employees by using an active choice mechanism to increase biometric screenings and subsequent treatments and services. We plan to analyze employees' participation rates and health outcomes.

We are also analyzing data from an experiment implemented by a pharmacy benefit manager that aims at improving drug adherence among prescription drug plan members. The randomized experiment involved individuals who had diabetes, high blood pressure, or high cholesterol levels and who failed to frequently take medication. We are interested in analyzing the data to see if the different treatments would increase the drug adherence of the recipients. This has broad policy implications in illuminating cost-effective channels to improve health.

MICROBIOLOGY

Peter Freese *Applied Mathematics (Biology)*
Leverett 2012

Bacteriophages and conjugation on solid growth medium

Irene Chen PhD and Kirill Korolev PhD,
FAS Center for Systems Biology,
Harvard University

Conjugation is a mechanism of horizontal gene transfer by which bacterial cells transfer genetic material by direct cell-to-cell contact or via a physical contact through a conjugative pilus. The spread of antibiotic resistance genes often occurs through bacterial conjugation; thus, one potential strategy to stop or reduce the spread of antibiotic resistance is to control the mechanism by which conjugation occurs. One way in which conjugation can be inhibited is by introducing bacteriophages (viruses that infect bacteria). The effect of bacteriophages on conjugation is not fully understood, and the aim of this project is to develop a quantitative model to describe such systems.

My project will investigate the bacteriophage M13 and the host cell Escherichia coli, which readily conjugates using the well-studied F-plasmid. M13, like some other bacteriophages, infects host cells via attachment at the tip of the pilus encoded by the F-plasmid. Previous research of this system in liquid culture indicates that infected cells conjugate at a significantly slower rate than uninfected cells and that a high concentration of the bacteriophage in the immediate environment is sufficient to completely inhibit conjugation. However, a homogeneously mixed liquid environment fails to provide a realistic view of the conjugative process as most conjugation takes place on surfaces or within biofilms. In such populations with spatial structure, bacteria can only interact with their neighbors rather than every other individual within the population. My research will examine the conjugative process on plates of solid agar, a medium more similar to bacteria's natural environment. The goal of the project is to develop a qualitative understanding of conjugation and the effects of phages on the process through measurement and modeling of conjugation rates in different environments.

Nina Jain *Molecular and Cellular Biology*
Winthrop 2011

Quantifying mistranslation in *Mycobacterium smegmatis*

Rubin Laboratory,
Department of Immunology and Infectious Diseases,
Harvard School of Public Health

Tuberculosis is a worldwide plague. With more than two billion people infected world-wide, it causes approximately ten million cases of acute disease per year, resulting in nearly two million deaths annually – more than any other single infectious disease agent. Patient responses to antituberculous agents vary considerably, even among those infected with confirmed drug-sensitive microorganisms. The possibility that these microorganisms could respond to antibiotics via modulation of translational fidelity has not been fully explored. Previous work in our laboratory has indicated that mistranslation, by means of physiological misacylation and/or ribosomal codon misreading, does contribute to mycobacterial drug tolerance, but this phenomenon has not been adequately quantified. We developed a quantitative assay to measure mistranslation rates in Mycobacterium smegmatis, a model organism for the disease-causing agent of tuberculosis. We used a construct containing the Renilla (sea pansy) luciferase gene mutated at a critical residue. The construct included the firefly luciferase gene to serve as the activity benchmark to which Renilla luminescence was compared. The construct was successfully cloned into the pMC1S expression vector and tested in M. smegmatis. The assay will be used to measure mistranslation rates in mycobacteria subject to stresses including exposure to a number of antibiotics, temperature fluctuations, high salt conditions, and pH variations.

Sesheta Mwanza *Undecided*
Winthrop 2013

Isolation of bacteriophages with high affinity for mycobactin using phage display technology

Rubin Laboratory,
Department of Immunology and Infecious Diseases,
Harvard School of Public Health

Mycobacterium tuberculosis is one of the leading causes of death worldwide. With at least one third of the world's population infected with the bacterium and at least 2 million deaths every year, it is extremely critical that quick and effective detection methods be developed to detect the bacterium as soon as possible. Current methods for detecting tuberculosis—skin tests and chest X-rays—are slow and lack accuracy. Previous studies show that phage display technology could potentially be used as a reliable diagnostic tool for screening the presence of M. paratuberculosis in bulk milk samples. This technique could possibly be applied for detecting the presence of M. tuberculosis in human serum. The principal objective of this project is to isolate bacteriophages that bind to mycobactin, a cell associated iron-binding compound, or siderophore, of M. tuberculosis. This siderophore is needed to transport iron into the cell because the environment present in the host cells is rather iron deficient. The phages are to be isolated from two commercial phage-peptide libraries that encode both c7c and 12 mer peptides respectively. In order to pinpoint the bacteriophages that bind most specifically to mycobactin from the library of ~ 2.7 x 10^9 phages, phage display technology is being used. This technique involves biopanning, in which mycobactin covered beads are incubated with a library of phages and then washed to remove any non-specific binding phages. The remaining specific binding phages are eluted and then amplified for another round of biopanning. This process is repeated 3 times and then the most selectively binding phages are sent for nucleotide se-

quencing. This experiment is still in the early stages of biopanning. If successful, phage display technology could potentially be used as a diagnostic tool.

Lauren Onofrey *Molecular and Cellular Biology*
Cabot 2012

Viral evolution of HIV-1 subtype C

Essex Lab,
Immunology and Infectious Disease,
Harvard School of Public Health

To infect host cells, an HIV virion fuses with the cell membrane, releasing its viral RNA genome and reverse transcriptase; inside the cell, reverse transcriptase converts the RNA genome into proviral DNA for incorporation into the DNA genome of the host. This stage of the viral life cycle is extremely error-prone, such that the DNA copy of HIV's genetic information can be slightly different from the original RNA template. As a consequence of the accumulation of the errors during viral replication, remarkable viral diversity develops within the individual patient from the virus of initial infection. When the HIV is subsequently transmitted, many factors constrain the viral variant that infects the new host, but the possibility that the transmitted virus is different from the virus of initial infection follows naturally from the existence of such impressive viral diversity. More broadly, HIV considered on a global scale is even more diverse, with many subtypes and innumerable quasispecies within each subtype. This diversity and diversification creates great challenges for clinical treatment and vaccine design.

The work of the Essex Lab focuses on HIV-1 subtype C, the most prevalent HIV-1 subtype, not only in Botswana, but also in the global epidemic. In Tsedimoso, the acute and early infection study, 42 patients with estimated time of seroconversion, or development of antibodies, were identified very soon after infection and followed for up to 5 years. Recent analysis of samples from these patients has revealed that a high initial load of viral RNA and slower decline from this peak viremia is strongly associated with high initial viral diversity; and current research further tests this finding. Utilizing single genome amplification and direct sequencing, samples from these acute and early infection cases are analyzed to determine the impact of early viral RNA load and diversity on later viral RNA load and CD4 T-cell count. Analysis addresses potential associations between viral replication and viral diversity and diversification within HIV-1 genes gag and env can confirm early conclusions for four- and five-year clinical outcomes, inform further interventions and treatments, and provide valuable data to vaccine research.

Marianne Walwema *Chemistry*
Adams 2012

Studying DNA translocation by SpoIIIE in *Bacillus subtilis*

Burton Laboratory,
Department of Molecular and Cellular Biology,
Harvard University

In every organism the accurate segregation of replicated DNA from one cell to its daughter cells is a crucial event. The ability of an organism to perform this process with high efficacy will often guarantee its survival. However, in bacteria the transfer of DNA is often not completed before cytokinesis occurs, and as a result, the DNA needs to be translocated from one cell to another. This process requires DNA transporters, which are motor proteins that actively pump the DNA from the original cell to the newly-formed daughter cell. Similar to cytokinesis, during sporulation in Bacillus subtilis, a motor protein SpoIIIE, pumps the chromosome across a division septum. The formation of the division septum results in an asymmetric division of the cell, which traps 70% of the replicated chromosome in the incorrect daughter cell. By ensuring the translocation of the entire chromosome into the correct daughter cell or forespore, this protein plays an essential role in sporulation. In our lab we have designed a system, which allows us to track the movement of the chromosome from the mother cell to the forespore in real time. This assay uses a combination of GFP-fused Tet repressors, which bind to tet operators integrated into the genome, and fluorescence microscopy that enable us to visualize the translocation of a specific point on the chromosome. From this we can study the effects of mutated SpoIIIE by visualizing the effects on DNA pumping in the cell. Since nucleic acid transport using a motor protein is a system, which exists ubiquitously in cells of many living organisms, the information learned through this research can potentially help us understand a large variety of chromosomal translocation in different biological systems.

Molecular and Cellular Biology

Rajarshi Banerjee *Neurobiology*
Currier 2011

Understanding the B-cell immune response in meningiomas

O' Connor Group, Hafler Laboratory,
Center for Neurologic Diseases,
Brigham and Women's Hospital

Meningiomas account for nearly a third of all primary brain tumors in the United States. These tumors trigger a humoral immune response in patients—humoral immunity is the aspect of immunity that involves the transformation of B cells into plasma cells that then produce and secrete antibodies. The nature of this response, however, remains to be elucidated: While it is known that B cells appear in the immune cell infiltrates in meningiomas, for example, it was even recently still unclear if these cells are recruited to the tumors by specific antigens. By cloning and analyzing the variable regions of antibodies in meningiomas, we have revealed the occurence of clonal expansion and somatic hypermutations in these antibodies, supporting the hypothesis that B cells appear in meningiomas as a result of an antigen-driven, antibody-mediated immune response in those tumors. Furthermore, we have reconstructed several B antibodies present in meningiomas, and are in the process of identifying antigens in these tumors that these antibodies bind with. Such antigens can serve as diagnostic markers, and may be used for tumor-specific immunotherapies, which can reduce the need for radiation therapy in meningioma patients.

Kayla Berry *Chemical and Physical Biology*
Currier 2013

Maintenance of polycomb group proteins on DNA templates during replication

Francis Laboratory,
Department of Molecular and Cellular Biology,
Harvard University

Polycomb Group (PcG) proteins in Drosophila melanogaster are epigenetic regulators that maintain silencing of homeotic (Hox) genes through numerous cycles of cell division, long after initial transcriptional repressors have retired. PcG proteins alter chromatin by modifying histones and compacting chromatin. However, little is known about the mechanism by which these proteins are maintained on the DNA template when disruptive processes such as replication and transcription disturb chromatin conformation. Maintenance of PcG proteins through DNA replication and cell division may be essential to maintain patterns of gene silencing. Polycomb proteins are well conserved in Drosophila to mammals. Understanding the mechanism by which PcG proteins are inherited through replication will illuminate epigenetic mechanisms in mammals.

Polycomb Repressive Complexes (PRC) 1 and 2 are two major complexes of the PcG proteins. PRC1 alters chromatin folding and inhibits chromatin remodeling and transcription. PRC2 has been found to methylate histone H3, thereby contributing to silencing of Hox genes. In this study, we focus on PCC, PRC1 Core Complex, consisting of Posterior Sex Combs (PSC), Polycomb (Pc) and dRING, and how it might be maintained through DNA replication. There are two potential models to explain how PcG proteins are maintained through DNA replication: 1) PcG proteins remain bound to DNA during replication and recruit new PcG proteins to maintain silencing, or 2) PcG proteins dissociate and subsequently re-associate after replication. We are testing these two models in vitro using the simple T7 bacteriophage system. Previous experiments indicate that PCC binds to double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) before and after replication. To determine whether PCC remains bound to DNA during replication in vitro, we will add competitor DNA to capture dislodged PCC. The results of these experiments will provide insight into possible mechanisms for inheritance PcG proteins through DNA replication.

Alyssa Botelho *Chemistry*
Leverett 2013

Structural and biochemical studies of DNA lesion recognition by UvrAB complex in *Bacillus stearothermophilus*

Jeruzalmi Laboratory,
Department of Molecular and Cellular Biology,
Harvard University

Cells maintain the integrity of their genetic information by constantly repairing DNA under attack by mutagenic agents in their environment. Though a myriad of DNA repair pathways have evolved to perform this task, proteins involved in most repair pathways have highly specific binding and correct only one type of DNA lesion. In this sense, nucleotide excision repair (NER) proteins are unique in their versatility: they can detect a vast range of structurally unrelated DNA lesions and employ the same repair mechanism on all damaged sites. Though NER proteins in prokaryotes and eukaryotes do not seem to be evolutionarily related, the principle of their repair mechanism is the same: an elegant three-step pathway that 1) recognizes DNA damage, 2) cuts and removes damaged DNA bases, and 3) recruits DNA replication machinery to fill the gap with a corrected sequence. Two such NER proteins are UvrA and UvrB from the thermophilic bacterium Bacillus stearothermophilus. The first two damage-recognition proteins in the NER pathway, UvrA and UvrB bind together as UvrAB complex to target a DNA lesion and initiate repair. The goal of our research is to better understand how this UvrAB complex recognizes and binds to a wide spectrum of DNA lesions. Our first approach is to determine the atomic structure of a complex between UvrA and truncated UvrB through x-ray crystallographic studies. Our second approach is to elucidate where the UvrAB complex binds in relation to a DNA lesion through biochemical experiments. We will synthesize modified DNA with an artificial

fluorescein lesion and place diazirine cross-linkers at different positions relative to the damaged site. These reactive cross-linkers could then trap UvrA and UvrB as they interact with the fluorescein lesion and surrounding DNA. In this way, diazirine cross-linking may provide insight to UvrA and UvrB's initial detection of a DNA lesion and their recruitment of other NER proteins downstream in the DNA repair pathway.

Ryan Christ *Chemistry and Physics*
Lowell 2013

A Drosophila model of golgi dysfunction in Alzheimer's Disease

Feany Lab,
Brigham and Women's Hospital Department of Pathology,
Harvard Medical School

Tauopathies are a class of terminal neurodegenerative diseases characterized pathologically by the aggregation of microtubule-associated protein tau; one such aggregate is the intraneuronal inclusion called a neurofibrilary tangle (NFT) found in Alzheimer's disease (AD). AD is a devastating tauopathy that impacts approximately 10% of Americans over the age of 70. Patients typically present progressive memory loss, motor ataxia, and language impairment. By over expressing different pathogenic versions of human tau within the neurons of Drosophila, the Feany Lab has developed a tauopathy model that recapitulates several characteristics of AD including adult onset neurodegeneration and motor dysfunction. Samples taken from AD patients during autopsy have implicated the fragmentation of the Golgi apparatus in AD pathology. Using immunofluorescence and other techniques, we are studying Golgi morphology and function in the neurons of our tauopathy flies. Furthermore, we are investigating the role of several Golgi-associated proteins; for example, Rab6, a GTPase that facilitates retrograde transport and has been connected to NFT formation in human brain samples, is a focus of our efforts. One preliminary result suggests that the overexpression of Rab6 reduces the toxicity of human tau. We hope to show that the activity of such Golgi proteins impacts the tau-induced neurodegeneration in our fly model.

Francis Deng *Human Developmental and Regenerative Biology*
Lowell 2012

Generation of pancreatic endocrine lineage-specific human embryonic stem cell reporter lines

Melton Laboratory,
Department of Stem Cell and Regenerative Biology,
Harvard Stem Cell Institute,
Harvard University

Transplantation of pancreatic endocrine islets, especially the beta cells that make the hormone insulin, holds therapeutic promise for type 1 diabetics who require insulin. Because human embryonic stem cells (hESCs) are a virtually unlimited cell source, we want to direct the differentiation of hESCs first into the endocrine lineage and then into beta cells, and we are working to optimize the in vitro production of this cell type. To aid directed differentiation and cell purification efforts, I am generating hESC reporter cell lines using three strategies: bacterial artificial chromosome (BAC)-mediated transgenesis, zinc finger nuclease (ZFN)-mediated gene targeting, and lentiviral vector-mediated transgenesis. In particular, I am interested in the gene neurogenin 3 (ngn3), a transcription factor that is necessary for specifying the endocrine lineage in mouse. Cells that transiently express ngn3 in the embryonic mouse pancreas are endocrine progenitors; consequently, isolation of a population of human ngn3+ cells would further our long-term efforts in directed differentiation, transplantation, and beta cell development. In addition to optimizing the production of mature beta cells, we are interested in using hESCs to study aspects such as cell lineage, fate decisions, and signaling factors in the development of the human endocrine pancreas.

Aaron Deutsch *Chemical and Physical Biology*
Kirkland 2013

miR-34a: a micro-tumor suppressor

Lieberman Laboratory,
Immune Disease Institute,
Harvard Medical School

Recent evidence has uncovered the role of micro-RNAs (miRNAs) as tumor suppressors, or genes that inhibit cell proliferation. Because such genes are often deleted in cancer cells, understanding how they prevent cell division is crucial to stopping tumor growth.

Micro-RNAs are an instance of the larger family of RNA silencing. Unlike the commonly known small interfering RNAs (siRNAs), miRNAs are generated from shorter transcripts that form hairpin structures. These structures are cleaved by the Dicer protein, creating a short fragment of approximately 23 nucleotides. This fragment, in turn, binds to a target gene via a partially complementary "seed" site, often found in the 3' untranslated region (3' UTR) of the mRNA. Such binding inhibits translation of the mRNA and thereby lowers expression of the target gene.

One particular miRNA, miR-34a, is a known tumor suppressor, but its mechanism of inhibiting cell division remains unclear. MiR-34a is especially intriguing because it is linked to p53, a protein that has been considered "the guardian of the genome" because it is mutated in over 50% of all human cancers. Overexpression of miR-34a causes an increase in p53 levels, while activation of p53 leads to miR-34a transcription, suggesting a positive feedback loop between these two genes. This loop culminates in cell cycle arrest, in part because miR-34a downregulates crucial cell-cylce genes, including various cyclin-dependent kinases (Cdks) and members of the E2F family.

My project investigates the mechanism by which miR-34a regulates the cell cycle. In particular, it focuses on members of the Fanconi anemia (FA) family, which are involved in DNA damage repair. Overexpression of miR-34a appears to downregulate the expression of various FA proteins. In addition, computer prediction software has identified multiple potential targets of miR-34a within the 3'UTRs of these genes. We are investigating whether the downregulation of FA genes is due to direct binding by miR-34a, or whether it is an indirect effect as part of the larger p53/E2F pathway. By analyzing the FA

genes, we will hopefully learn more about the role of miR-34a as a tumor suppressor within the cell.

Xuezhi Dong *Molecular and Cellular Biology*
Mather 2012

Investigating the clonality of Ptch+/- and Ptch+/-;P53-/- derived medulloblastoma

The Jackson-Grusby Laboratory,
Department of Pathology,
Children's Hospital Boston

Medulloblastomas are the most common malignant brain tumor in children. These developmental tumors are thought to arise from neural progenitors for the cerebellar granule cell. Recent research has found that variants of medulloblastomas can exhibit cellular heterogeneity, and subpopulations of tumor cells may possess stem-cell like properties including self-renewal and multipotency. It has been further hypothesized that these cells are capable of tumor initiation and may lead to relapse and metastasis while the bulk of tumor cells are destroyed by traditional therapy that nonspecifically targets rapidly dividing cells. However, it is still currently unknown whether medulloblastomas originate from a single or multiple tumorigenic cells, a key piece of information helpful for the assessment of the cancer stem cell theory in medulloblastoma. My project seeks to elucidate the clonality of Ptch+/- and Ptch+/-;P53-/- derived brain tumors in mice by creating a reporter system using the combinatorial expression of fluorescent proteins strategy, also known as Brainbow, developed by Livet et al. (2007). This strategy employs the Cre/lox recombination system to stochastically express different fluorescent proteins in individual cells, which allows for the labeling and tracing of single cell lineages in early tumor development. Understanding the clonality of Ptch+/- and Ptch+/-;P53-/- derived brain tumors allows for further evaluation of the cancer stem cell theory in the context of medulloblastoma and may have future implications for diagnostics and treatment of the disease.

Veda Eswarappa *Biomedical Sciences and Engineering (S.B.)*
Currier 2012

Disrupting the expression of immune-response genes via NFAT and Fos

Dr. Anjana Rao,
Immune Disease Institute,
Harvard Medical School

Though a relatively recent discovery, the nuclear factor of activated T cell (NFAT) protein, has immense functions and potential applications are immense. Despite its name, NFAT protein and its target genes are expressed by both immune and non-immune cells, ranging from interleukin cytokines to genes that control the slow twitch program of skeletal muscles.

The primary immunosuppressive drugs in use today - FK506 and cyclosporin A (CsA) – inhibit calcineurin, a phosphatase that helps activate NFAT. Such immunosuppression is needed following medical procedures such as skin grafts or organ transplants, in which the recipient's immune system might otherwise react unfavorably to the procedure. However, FK506 and CsA are not selective and often inhibit other immune processes as well, potentially resulting in side effects like infection, nephrotoxicity, or carcinogenesis.

NFAT proteins can be divided into five groups, each of which has a highly conserved DNA-binding domain (DBD). I spent the first half of the summer working with NFAT1. Transformed E. coli bacteria were induced with isopropyl-beta-D-thiogalactopyranoside (IPTG) to express NFAT1-DBD. This protein was then purified using affinity chromatography. Elutions collected from the binding column were run on SDS-PAGE to confirm that the protein had been effectively purified.

Another protein called Fos is crucial to NFAT function. As a dimer with the Jun protein, Fos forms Activator Protein 1 (AP-1), which combines with NFAT to form a ternary complex that binds with DNA. Many genes like GM-CSF and CD25 cannot be regulated without both NFAT and AP-1, though NFAT binding to DNA is generally much stronger with AP-1. I spent the second half of the summer purifying the bZIP (basic leucine zipper) region of a mutated Fos protein, where Isoleucine146 had been changed to a cysteine.

The purified Fos protein w will be used in fluorescence screens along with Jun, ARRE2 (a site of the murine promoter for the cytokine IL-2 which contains binding sites for both NFAT1 and AP-1) and a variety of compounds and inhibitors. Hopefully, the results will help determine an effective way of disrupting this complex in order to selectively interfere with the formation of this quaternary complex to inhibit a particular immune response thus providing a better alternative to current immunosuppressants.

Chris Goldstein *Human Developmental and Regenerative Biology*
Currier 2013

Directed differentiation of cortical progenitors from mouse embryonic stem cells as a system for developmental screens

Rubin Laboratory,
Department of Stem Cell and Regenerative Biology,
Harvard University

The long-term goal of this project is to assess the effects of bioactive compounds on subtype specific differentiation of cortical progenitors derived from mouse embryonic stem cells (mESC). When looking in vivo, neocortical progenitors—located within the rostral and dorsal-most region of the neural tube—undergo corticogenesis to generate a rich diversity of cortical neuronal subtypes, which include classes of projection neurons (PN). These PNs, when improperly developed, have been implicated in a variety of disorders. Callosal PNs, which project across the corpus callosum, have been implicated in autism, while subcerebral PNs, which project to the midbrain, hindbrain, and spinal cord, have been implicated in ALS. Due to the disorders associated with the development of these neural subtypes, it is very important to create an in vitro model of corticogenesis. As demonstrated by Gaspard et. al. (Nature 2008), cortical progenitors can be derived from mESCs in vitro by manipulating the activity of morphogens, which normally pattern the neural tube in vivo along the rostrocaudal and dorsoventral signaling axes. By expanding upon this protocol we have been identifying and manip-

ulating extrinsic and intrinsic factors to enrich regionally specific progenitors in hopes of directing their differentiation to various pure populations of cortical subtypes. We have extrinsically manipulated the Sonic Hedgehog (Shh) signaling pathway, which directs dorsoventral patterning. We plan to further optimize this system in order to conduct high throughput screens, which will allow us to assay the effects extrinsic and intrinsic factors have upon neural regionalization and development. This analysis will potentially provide insight into the causes of many neurodegenerative diseases and the neural signaling pathways these factors affect.

Peter Hadar *Chemical and Physical Biology*
Eliot 2013

Effect of codon choice on protein misfolding in yeast

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Synonymous codons encode the same amino acid but may have different error rates during the process of translation. To determine the effect of codon usage on protein folding, two different DNA sequences encoding the yellow fluorescent protein (YFP) variant NP1 will be analyzed by microscopy. NP1, a destabilized yellow fluorescent protein (YFP), was transformed into yeast under the control of either the inducible pGal1 promoter or the constitutive pPGK1 promoter. The NP1 coding sequence is either denoted "opp," in which high-accuracy codons are used at evolutionarily variable sites and low-accuracy codons are used at evolutionarily conserved sites believed to be important for protein folding, or "cor," in which accuracy and conservation are correlated. Our experimental hypothesis is that evolutionarily variable sites are less sensitive to translation errors than conserved sites, and therefore that translation errors will more frequently induce misfolding in the opp variant.

To visualize destabilized proteins in the cell, NP1-mCherry fusions were created. These red-fluorescent variants glow even when misfolding compromises the fluorescence of YFP, allowing subcellular localization of misfolded variants to be determined. We have established that misfolding of YFP imposes a fitness cost on yeast due to the intrinsic toxicity of the misfolded molecule, and are presently examining possible causes for that toxicity as a model for the general toxicity of protein misfolding in cells, including in human diseases such as Alzheimer's and Huntington's diseases. We are also beginning to investigate a relatively unexplored hypothesis, which suggests that hydrophobic residues exposed during protein misfolding disrupt the structure of water within the cell, altering molecular transport and membrane stability in ways that reduce cellular fitness.

Nikola Ivica *Chemical and Physical Biology*
Adams 2012

The effect of RNA secondary structure on the rate of template-directed non-enzymatic polymerization of activated mononucleotides

Chen Lab,
FAS Center for Systems Biology,
Harvard University

The 'RNA world' theory of the origin of life suggests that RNA could serve as both a template for faithful replication and a chemical catalyst for a primitive living system. Templating requires RNA to have a relatively open structure, making it available to base pair with a short RNA primer to form a double-stranded structure capable of polymerization. However, catalytic ability is usually dependent on stable folding to form a specific intramolecular structure. To understand the tradeoff between these opposing properties, we use a system of non-enzymatic, template-directed polymerization of 5'-phosphorimidazole nucleotides (ImpN) on an RNA polymer template. We designed a series of RNA sequences that can form hairpin structures with different minimum free-energies to mimic folds of varying stability in catalytic RNAs. We then determine the templating ability of each sequence by estimating the rate of ImpN incorporation into a complementary RNA primer. The two measurements can then be used to deduce whether an RNA molecule can serve as a template and a catalyst at the same time.

Lynn Jiang *Molecular and Cellular Biology*
Adams 2011

Long-range intrachromosomal repair of DNA double strand breaks in different cell types and loci

Monica Gostissa PhD, Dr. Frederick W. PhD,
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Class switch recombination (CSR) is the mechanism by which B cells can produce different antibody classes. Each class is characterized by a specific "constant region" encoded by a set of so-called CH exons. During CSR, the first set of CH exons of the immunoglobulin heavy chain (IgH) constant region can be replaced by downstream constant exons. More specifically, the enzyme activation-induced cytidine deaminase (AID) results in the introduction of double strand breaks (DSBs) into switch (S) regions - large sequences that flank each constant region.When DSBs in the upstream S region fuse to breaks in a downstream S region, CSR occurs. Such joining occurs quite efficiently and accurately over distances of around 100kb. However, the mechanism by which such long range (LR) intra-chromosomal joining occurs still remains unknown. While it was initially believed the specific sequence of S regions played an essential role, previous studies determined that S regions are not required in order to get LR rejoining. My goal is to further examine the mechanism by which such LR joining can occur. Specifically, I

hope to determine whether it is specific to B cells or if LR joining can be seen in other types of cells. I will also determine whether LR joining is limited to the IgH locus, suggesting that specifics about the locus structure may be involved, or if can also be observed in other loci. Tumor cells often harbor chromosomal abnormalities that result from fusion of genes located far apart on the same chromosomes. These translocations likely arise from LR joining of DSB with deletion of the intervening sequences and they are often major pathogenetic factors in the disease. These studies will therefore help elucidate factors that play an integral role not only in the physiological process of CSR, but also in the generation of oncogenic translocations in tumors.

Geon Woo (Nathan) Kim *Neurobiology*
Cabot 2013

Treatment of glioma stem cells with oncolytic herpes simplex virus and g-secretase inhibitors

The Martuza Laboratory,
Brain Tumor Research Center,
Massachusetts General Hospital

Glioblastoma is the most common malignant primary brain tumor and is fatal despite surgery, radiotherapy, and chemotherapy. It is thought that one reason for glioblastoma's resistance to traditional treatment is due to the existence of cancer stem cells—undifferentiated cells that can survive standard chemotherapy and radiation therapies and regenerate a tumor after treatment. The Martuza Lab focuses on treatment using oncolytic viruses, such as G47D. G47 D is a herpes simplex virus engineered to selectively replicate in and kill tumor cells.

The Notch signaling pathway is a highly conserved developmental pathway that has been linked to a wide range of cancers. It is believed that this pathway is specifically involved with the survival and proliferation of cancer stem cells. I am interested in how Notch blockade by g-secretase inhibitors (GSI) can affect glioma stem cells and established cell lines. Using two different GSIs (GSI-XX and GSI-I), we show a dose-response relationship with U87 (a human glioma cell line), and BT74 and GBM8 (human glioma stem cells). When the cancer cells are treated with both G47D and GSI, we see additive cytotoxic effects in all three cell lines, particularly at lower viral doses. We also find that BT74 cells are more susceptible to GSI-I than U87 cells, which merits interest due to glioma stem cells' generally stronger resistance to treatment. We plan to ensure the specificity of GSI-I at the doses used by analyzing levels of key representative proteins of the Notch pathway (Notch1 and Notch2 receptor, Hes1) using Western blotting and quantitative PCR. We hypothesize that Notch pathway inhibition with GSIs can be effective in inhibiting glioma stem cell growth and tumor growth overall.

Phoebe Kuo *Molecular and Cellular Biology*
Eliot 2011

At the interface of blood and muscle: Inhibiting the immune response in Muscular Dystrophy

Wagers Lab,
Department of Stem Cells & Regenerative Biology,
Harvard University

In skeletal muscle, a population of satellite cells is activated upon injury to repair the tissue. The regenerative capacity of the satellite cells seems to become exhausted in muscular dystrophies, and the disease is marked by muscle atrophy and necrosis. In one subset of dystrophies, the dysferlin protein is impaired and is not able to perform its normal role of resealing disruptions in the muscle membrane; the muscle becomes progressively less able to respond to injuries that arise either from everyday use or from acute damage. Both clinically and in mouse models, muscle atrophy in dysferlinopathies is accompanied by a heightened inflammatory response. How these immune cells affect muscle regeneration is not clear, although immune cells in normal muscle tend to help regeneration. To investigate the role of the immune cell infiltration, we have bred dysferlin-deficient mice where CCR2, a receptor that recruits immune cells to muscle, has been knocked out. With this model, it is possible to evaluate the contribution of immune cells to muscle regeneration in dysferlinopathies.

Shimwoo Lee *Chemical and Physical Biology*
Mather 2013

Investigating the effect of sirtuins on friedrich's ataxia

The Haigis Laboratory,
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Harvard Medical School

Friedrich's Ataxia (FRDA) is a recessively inherited neurodegenerative disease characterized by degeneration of sensory neurons in the dorsal root ganglia and spinocerebellar tracts, muscular defects that lead to wheelchair dependency, slurred speech, cardiomyopathy, and increased incidence of diabetes mellitus. FRDA is caused by reduced expression of an essential protein called frataxin, which provides iron during iron-sulfur cluster (ISC) biosynthesis in the mitochondria. When there is a deficiency in frataxin, the ISC biosynthesis is disrupted, leading to decreased activity of ISC-containing enzymes, which play major role in the citric acid cycle and the electron transport chain. Moreover, mitochondria exhibit excess iron accumulation and increased susceptibility to oxidative stress, and as a consequence, cannot function normally. Currently, there is no cure for FRDA, and the majority of the patients die an early death around the age of 40 to 50.

The goal of this project is to investigate whether overexpressing proteins called SIRT1 and SIRT3 will rescue a murine cellular model of FRDA from mitochondrial defects. SIRT1 and SIRT3 are part of a family of highly conserved proteins called sirtuins, whose roles include promoting longevity, cell survival, and importantly, mitochondrial metabolism and stress tolerance. Sirtuins have shown

potential to protect against mitochondrial diseases. SIRT1 is localized in the nucleus and acts to increase mitochondrial biogenesis while SIRT3 is localized in the mitochondria and regulates mitochondrial oxidative capacity. Both may also reduce harmful reactive oxygen species produced in the mitochondria. To determine whether the sirtuins protect against FRDA, we will measure various markers of mitochondrial activity in the overexpressed cell lines, such as cell proliferation, lactic acid production, reactive oxygen species production and iron levels in the mitochondria. The studies may lead to a new area of therapy for FRDA.

Debbie Lin *History of Science*
Currier 2011

Titin missense variants' contribution to familial dilated cardiomyopathy

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Department of Genetics,
Harvard Medical School

Dilated cardiomyopathy (DCM) is characterized by enlargement and weakening of the heart, and is directly implicated in 1 out of every 3 cases of heart failure, making it the most common non-ischemic cardiomyopathy and a leading cause of cardiac mortality and morbidity. Although injury and disease are often associated with DCM, 20-40% of cases occur in otherwise healthy patients with affected family members. Known contributory mutations primarily affect contractile and cytoskeletal protein-encoding genes of the heart. One such gene, which codes for the giant protein titin (TTN), has been of particular interest for over a decade as a potential culprit. We have recently developed a filter assay for titin, which we have applied to 213 samples (68 DCM+, 89 control, 1 glycogen storage disease +, 85 hypertrophic cardiomyopathy +), through which we have identified 1,048 titin variants. It has been tentatively estimated that titin changes may be responsible for approximately 20% of the familial cases without other known mutations. Most of the nonsense mutation-causing variants are presumptively pathogenic (pending confirmation in familial analyses) but the majority of the variants found are missense mutations that pose a greater analytical challenge. We first develop an algorithm for ranking missense variants and prioritizing for study based on number of informative meioses (affected individuals and siblings, confirmed through separate clinical evaluation) available for study in the family, prevalence in general population and in controls, conservation through evolution, consistency of change in genome, position in titin protein, and nature of the amino acids affected. Next, using either restriction enzyme digest or sequencing, we determine whether each variant of interest is present in samples from family members. Then, we perform segregation analysis to determine whether the variant does indeed correspond to affected family members. Finally, we synthesize genetic and clinical data to further characterize the pathogenic nature of variants confirmed to have a correlation with DCM.

Mengyuan (Marion) Liu *Molecular and Cellular Biology*
Dunster 2011

Investigating the domains responsible for RNA discrimination in RIG-I and MDA5

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Harvard Medical School

The innate immune system confers the body's first line of defense against pathogens by recognizing common motifs or pathogen-associated molecular patterns (PAMPs) on foreign invaders. Viral nucleic acids are a category of PAMPs detected by the innate immune system; in particular, retinoic acid-inducible gene-1 (RIG-1) and melanoma differentiation-associated gene-5 (MDA5) are two cytosolic receptors for viral RNA. RIG-I and MDA5 share sequence homology and belong to the same RIG-I like receptor (RLR) family, but have different known ligands. RIG-I is activated by short double stranded RNA (dsRNA) with 5' triphosphate, as well as single stranded RNA (ssRNA) from negative (-) RNA viruses. Since most host RNA is capped and single stranded, the immune system recognizes dsRNA with 5' triphosphate as foreign. On the other hand, MDA-5 does not need a 5'triphosphate and responds to long dsRNA, presumably produced by complement strand binding during viral transcription. My project hopes to address how RIG-I and MDA5 are able to differentiate between these different types of RNA and the domains responsible for this discrimination. RIG-I and MDA5 have three domains: a tandem caspase activating and recruitment domain (CARD), a DExH box RNA helicase with ATPase activity and a C-terminus regulatory domain (RD). To study the domains of RIG-I and MDA5, I made chimeric domain mutants in which the CARD and RD are interchanged between the two proteins. Though these chimeric proteins are not physiologically relevant, they are a good vehicle to study the intermolecular interactions between domains because the full-length protein is retained and any gain of function activity can be compared to mutants with single domains. Since crystal structures of these two proteins are currently unavailable, understanding the role of each domain in the event of RNA recognition and molecular binding will be helpful in elucidating the mechanism of their activation.

Lisa Ma *Human Developmental and Regenerative Biology*
Quincy 2012

Expression of Asb2 in Mouse Embryos

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Stem cells have the potential to revolutionize the treatment of many diseases such as cardiovascular disease. Heart attacks often kill portions of heart muscle, which can lead to heart failure. Instead of requiring heart transplant, with induced pluripotent stem cells, patients could potentially simply regenerate the damaged heart muscle without complications with taking immunosuppressant drugs to prevent rejection. However before direction of stem cell differentiation is possible, the development of a normal heart must first be

understood.

The ankyrin repeat-containing protein with a suppressor of cytokine signaling box 2 (Asb2) is the specificity subunit of the E3-ubiquitin ligase complex, and thereby marks specific proteins for destruction by the proteasome. One of these proteins is actin-binding protein filamin B (Flnb), whose degradation regulates muscle differentiation. The Asb2 gene has an α and β isoform. Asb2α has been shown to induce differentiation in myeloid leukemia cells, while previous studies indicate that Asb2β is expressed in the hearts of adult mice. We have recently shown that Asb2α is expressed in the hearts of mouse embryos. We hypothesize that this isoform switch may play an important role in the differentiation of heart muscle cells. In order to more closely examine the expression patterns of Asb2, RNA probes that bound to regions of mRNA transcript specific to each isoform were made, as well as a probe that bound a section of mRNA common to both the α and β isoforms. The probes were used for whole mount in situ hybridization of mouse embryos, which were then sectioned to provide better visualization of expression patterns in the heart structure.

Daniel Mark *Chemical and Physical Biology*
Adams 2011

Investigating lipocalins as mouse pheromones

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Department of Cell Biology,
Harvard Medical School

Animals emit pheromones, chemicals that affect the behavior and physiology of other members of the same species. In the mouse, some pheromones have been identified that affect mate choice, aggression, reproductive physiology and individual identification, but the pheromones that mediate other odor-driven social behaviors of the mouse are unknown. Molecules ranging from small chemicals to large peptides can act as pheromones. Major urinary proteins (MUPs) represent one major class of mouse pheromones and they constitute 99% of the protein mass in urine, a source of social odors. MUPs are part of the lipocalin protein family and other types of lipocalins function as pheromones in different rodent species. Here, I will analyze the biosynthesis patterns of all lipocalins encoded in the mouse genome, using a qPCR approach to examine production in secretory tissues of mice of different gender, age, and physiological state. These studies should identify lipocalins that have potential to function as mouse pheromones.

James Meixiong *Human Developmental and Regenerative Biology*
Pforzheimer 2013

Elucidating the mechanisms of the Guided entry for TA proteins (GET) pathway

Denic Lab,
Northwest Labs,
Harvard University

Tail-anchored (TA) proteins are integral membrane proteins which are involved in many different cellular processes including apoptosis, protein translocation, and vesicular transport. These proteins are characterized by having a single transmembrane helix at their extreme c-terminus. Because of this positioning, TA proteins are not able to use the signal recognition particle dependent cotranslational pathway that many other integral membrane proteins use to get inserted into the membrane. As a result, TA proteins must somehow find their own target membranes and be post-translationally inserted. This is a very interesting problem as many TA proteins can spontaneously insert in different membranes and these erroneous insertions can lead to drastically different protein function. Recently, the guided entry for TA proteins (GET) pathway has been characterized as a major decision maker in the targeting and insertion of TA proteins in yeast. GET3, an ATPase in the pathway, has been shown to associate with TA proteins in the cytosol and target them to the endoplasmic reticulum. However, the way in which GET3 binds and releases its protein substrates is still unknown. My summer project focuses on the ATPase arsenite pump (ArsA), a homolog of GET3, in the archaea species Methanocaldococcus Jannaschii. This archaeal strain was chosen for study because its ArsA is a viable homolog of GET3. My primary interest is in testing how well ArsA is able to bind to TA proteins. By showing that ArsA is able to associate with TA proteins in a manner that is comparable to GET3, the unsolved problems of how GET3 binds and releases its substrate would become clearer. ArsA could be utilized as a template for sequence comparisons to identify conserved amino acid sequences, evolutionary mapping of the GET3 pathway, locating conserved binding aspects of the protein, and finding other homologs of GET proteins and protein interactions in the bacterial and archaeal strains. Because TA are such a diverse and important group of proteins in the cell, understanding how TA are targeted and inserted into membranes would have a huge impact on our understanding and ability to manipulate many cell processes.

David Orozco *Statistics*
Cabot 2012

Estimating transcription rates *in vivo*

Springer Lab,
Harvard Medical School

Genome-wide rates of mRNA processing in vivo are unknown. Using RNA sequencing we can extract these rates by comparing the number of reads from the 5' and 3' end of genes. The biological data has a lot of variability from underlying differences in mRNA processing between genes and noise in our experimental measurement. Determining the amount of noise experimentally and improving the measurements is time-consuming and expensive. To pinpoint the relative magnitude of each of these variations, we simulate RNA sequencing, and use idealized, stochastic computer-generated data.

Sarvagna Patel *Neurobiology*
Kirkland 2013

Elucidating the mechanism of the Alzheimer's Disease relevant γ-secretase complex: interactions between α- and γ-secretase

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Center for Neurologic Diseases,
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Amyloid precursor protein (APP) is a type I transmembrane protein that is processed by β- and then γ-secretase to produce the neurotoxic amyloid-β (Aβ) peptide, which is believed to play a central role in the pathogenesis of Alzheimer's Disease (AD). Alternatively, APP may be processed by α- and then γ-secretase, which precludes Aβ generation. Due to its role in Aβ synthesis, γ-secretase has emerged as an attractive target for the treatment of AD; however, the exact mechanisms of how γ-secretase cleaves and how the protease is regulated remain to be elucidated. It is currently believed that α- and γ-secretase cleavages occur as separate events at two distinct locations. Previous data from the Selkoe lab has shown that full length substrates, which are not the immediate targets of γ-secretase, interact with the enzyme, suggesting that the α- or β- cleavages may be coupled with γ-secretase and occur in the same location. We hypothesize a mechanism in which α- and γ-secretase form a complex that can accept substrates and process them sequentially. My project deals with optimizing conditions in which to isolate and observe this complex using various α-secretase modulators and purification techniques for the enrichment of γ- and α-secretase. I have performed immunoprecipitations in an overexpression system with a γ- specific resin and shown robust co-immunoprecipitation of ADAM10 and ADAM17, the predominant α-secretases which process APP. This suggests that α- and γ-secretase do indeed form a complex during processing. Currently, I am treating cells with various α- and γ-secretase activators and inhibitors to determine whether they induce complex formation. In the future, I will perform immunoprecipitations with different resins to obtain optimal enrichment of this complex consisting of α- and γ-secretase. Eventually, I hope to validate this interaction under endogenous expression levels.

André Pineda *Neurobiology*
Currier 2011

Assessing the role of GEFs in Mst3b-dependent axon outgrowth and regeneration

Nina Irwin PhD,
Children's Hospital Boston

The protein kinase Mst3b was recently discovered to play a crucial role in axonal outgrowth and regeneration in vivo and in vitro. Mst3b appears to be involved in the well-established Ras/Raf/MEK/ERK signaling pathway, downstream of Rap1, a Ras-family small GTPase. Rap1 can be activated by a variety of GEFs (guanine nucleotide exchange factors), such as Epac, C3G and PDZ-GEF1, in different cell types and contexts. Our project investigates the role of Rap1 GEFs in Mst3b-dependent axonal outgrowth and regeneration in embryonic rat cortical cells. To characterize these processes, we employ in vitro assays for axonal outgrowth and axonal regeneration after injury as well as Western blotting to detect activating phosphorylation of members of the Mst3b pathway in response to GEF stimulation. Depending on the results of these experiments, future experiments may use GFP-fusion proteins to localize the GEFs that activate this pathway and then use a mouse injury model to activate these GEFs.

Shwinn Ricci *Chemistry*
Eliot 2013

Modulating extra-cellular conditions in determining effects on skeletal muscle precursor cell activity

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Skeletal Muscle Precursor (SMP) cells are believed to be involved in the repair and maintenance of muscle tissue in response to injuries and aging. As a subset of adult muscle stem cells, SMPs are a candidate for advancing cell therapy for engraftment into humans. As a result, several in vitro and in vivo studies have been carried out to determine the rate and extent to which SMPs proliferate into multiple colonies and/or differentiate into myofibers.

In order to understand the effect of the extracellular matrix on these cells, various collagen/laminin coating methods were investigated for their effects on survival, proliferation, and differentiation of skeletal muscle precursors (SMPs) after single-cell sorts. Coating of cell plates was broken down into separate 37oC incubation times: 1 minute and 60 minutes. A single-cell sort was performed in order to place one SMP cell into each well. After 5 days of feeding with basic fibroblast growth factor (bFGF) at a 25 μg/ml concentration, each plate were examined under a microscope. Total number of cells were counted.

An instantaneous coating technique exhibited greater colony formation, and whereas an hour-long incubation of the coat resulted in increased differentiation into myocytes. There was no significant trend in cell survival using either method.

These results demonstrate that the extra-cellular matrix affects the rate SMP differentiation. These results warrant further investigation on the collagen/laminin ratio to determine any remarkable changes based on adjusting another factor involved in the application of extra-cellular conditions.

Konlin Shen *Physics*
Dunster 2013

Turning behavior of Drosophila larvae during thermotaxis

The Samuel Laboratory,
Department of Physics,
Harvard University

Drosophila melanogaster is widely used in research due to its ease in genetic manipulation, as well as its complex, but quantifiable be-

havior. However, the neural circuitry in Drosophila that allows the animal to sense and respond to temperature changes remains to be discovered. Drosophila melanogaster larvae are extremely sensitive to temperature changes – 1st instar larvae can sense temperature gradients as small as 0.005ºC/s1. When placed in an environment with an uncomfortably climate, larvae will make turns in order to orient itself in a direction that will allow itself to move to a more suitable temperature. In particular, during thermotaxis, larvae regulate the frequency, size, and direction of turning events. During each turning event, the larva will sweep its head from side to side. These head sweeping movements utilize distinct muscle segments. We are interested in knowing how the larva selects specific muscle segments to execute each turning event. We use transgenic Drosophila larvae with GFP-tagged muscles in order to observe which muscles are recruited during each movement during thermotaxis. We analyze videographic records of larval movement using a MATLAB code designed to pick out the headsweeps, locate muscle segments, and quantify the lengths and fluorescence intensity of each body segment. We can then quantify the utilization of specific muscles during each turning event. We hope to obtain an understanding of thermotactic behavior all the way from sensory input to motor control.

Eric Shieh *Molecular and Cellular Biology*
Leverett 2011

The role of TRPC6's carboxyl-terminal domain in mediating focal segmental glomerulosclerosis

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Mutations in transient receptor potential channel TRPC6 are a cause of an autosomal dominant pattern of glomerular kidney disease called focal segmental glomerulosclerosis (FSGS). Three of the FSGS-associated mutations, K874*, R895C, and E897K, that display clear gain-of-function effects through enhanced current amplitude and diminished inactivation, map to a 20 amino-acid sequence in the carboxyl-terminal domain that is predicted to form a coiled-coil domain. Coiled coils are structural motifs known to facilitate protein-protein interactions and serve as important regulatory domains. However, the role of TRPC6's putative coiled-coil domain and these mutations in FSGS induction remain largely undetermined. Elucidating the function of this domain has the potential to further our understanding of TRPC6 regulation in the pathophysiology of FSGS and other TRPC6-mediated diseases, such as cardiac hypertrophy. The aim of this study is to determine whether the coiled coil is necessary and sufficient for assembly of the functional tetrameric TRPC6 channel. First, standard subcloning techniques will be employed to create fusion proteins comprised of wild-type and mutant carboxyl-terminal TRPC6 containing the coiled-coil domain and linked to sequences that bring the fusion protein closer in proximity to full-length TRPC6. If TRPC6's coiled coil is capable of tetramer formation, the fusion protein will likely exhibit dominant-negative effects on TRPC6 signaling and channel activity. To test this hypothesis, cells will be transiently transfected with the fusion proteins and full-length wild-type and mutant TRPC6. Co-immunoprecipitation and dual-luciferase reporter assays will be used to examine the fusion protein's ability to inhibit downstream targets of TRPC6 signaling, including kinase ERK1/2 and transcription factor NFAT. These studies will be followed by calcium imaging studies and electrophysiological patch-clamp recordings that assess basal calcium levels and TRPC6 channel activity.

Alicia Smart *Molecular and Cellular Biology*
Lowell 2013

Hepatocyte differentiation for drug toxicity screening

Rubin Laboratory,
Department of Stem Cell and Regenerative Biology,
Harvard University

The most common function of stem cells in drug development research is to produce, in vitro, the cell type affected by the disease being studied. If large quantities of a specific type of cell can be manufactured efficiently, then many compounds can be tested on the cells for therapeutic properties. Often a library of several thousand small molecules can be screened on stem cells that have been differentiated to the desired cell type. Compounds that cause cell proliferation or prolong survival can then be studied for therapeutic use. After identifying a potential drug molecule, studies must be conducted to determine possible side effects. Most commonly, side effects occur in cells of the heart and liver. Liver cells (hepatocytes) are affected because the liver functions in metabolizing various toxins that enter the body. These toxins may accumulate in the liver at higher concentrations than in other tissues and lead to cell death. Successful hepatocyte generation would allow for the testing of drug compounds in vitro and may eliminate undesirable compounds in earlier testing stages of drug development. However, functional, mature hepatocytes have not been successfully differentiated in vitro, nor can biopsied hepatocytes be maintained in culture. In this study, mouse embryonic stem cells were subjected to sequential differentiation directed to produce hepatocytes. The cells progressed through several endodermal and hepatic progenitor states through the addition of several growth factors to produce hepatocyte-like cells. RNA analysis and immunohistochemistry will be used to characterize these cells based on the presence of known hepatocyte markers and additional growth factors will be tested to drive further hepatocyte maturation. The development of a protocol for the differentiation of functional hepatocytes would improve drug development research by allowing the assessment of side effects in an in vitro model.

Cynthia Tsai *Undecided*
Cabot 2013

Discriminating right from wrong: examining human 8-oxoguanine DNA glycosylase I's interaction with unmutated double-stranded DNA through disulfide bond DNA-protein cross-linking

Verdine Laboratory,
Department of Stem Cell and Regenerative Biology,
Harvard University

The oxidation of guanine by intracellular agents yields 8-oxoguanine (oxoG), which pairs with adenine. Unless repaired, this oxoG mutation in DNA will bring about a guanine-cytosine to thymine-

adenine base pair transversion that can cause cancer. 8-oxoguanine DNA glycosylase I (hOGG1) recognizes oxoG and works to excise this mutated base from DNA in human cells. However, minimal information presently explains how hOGG1 effectively discriminates within double-stranded human DNA between the normal guanine nucleotide and the mutated oxoG lesion, which differ from one another structurally at only two positions.

This project therefore seeks to better understand hOGG1's ability to recognize and subsequently bypass normal guanine nucleotides in the cell by cross-linking hOGG1 with unmutated double-stranded DNA. Particularly, chemical modifications of both DNA bases and hOGG1 can facilitate the creation of disulfide bonds between the DNA and hOGG1 that ensure that the DNA and protein remain held together for structural analyses. Current studies focus on variations, including the location of the disulfide bond along either the major or minor groove of the DNA and the length of hydrocarbon chains alongside this bond, that can circumvent the unnatural contortion of the DNA-protein complex through this disulfide bonding process. Consequently, this project then seeks to optimize conditions that will facilitate the crystallization of the complex to allow for final structural examinations of hOGG1 bound to normal double-stranded DNA that can shed light on the specificity of this protein for only oxoG lesions in human DNA.

Afoma Umeano *Biomedical Engineering*
Lowell 2013

IPS cell disease modeling for neutral lipid storage disease-M subtype

Wu Laboratory,
Massachusetts General Hospital

The ability for iPS cell to function as disease models has attracted scientist since its generation by Yamanaka in 2006 at Kyoto University and confirmed in 2007 by researchers at Massachusetts General Hospital and MIT.

The Wu lab focuses primarily on the study of heart development and understanding heart disease on a cellular level through the use of induced pluripotent stem (iPS) cells and embryonic stem (ES) cells as in vitro models. During the spring and summer of 2010, I worked primarily on a project focusing on disease modeling with IPS cells that carry the genetic mutation for Neutral Lipid Storage Disease-M subtype (NLSD-M). NLSD-M is a neutral lipid storage disease characterized by skeletal and cardiac myopathy due to mutation in the gene for adipocyte triglyceride lipase (ATGL). In mice, ATGL null mice are cold sensitive, energy starved, and experience increased cell death in the heart and the liver. Their hearts have increased mass due to lipid accumulation, and subsequently decreased contractility resulting in severe cardiac inefficiency.

The three objectives of the project were: (1) Can IPS cells successfully model the ATGL mutation effectively in vitro, (2) If so, is there a compound that reduced lipid accumulation in the cardiac cells, and (3) What do these drugs reveal about the altered metabolic pathway of a ATGL null mouse? The merit of the project is that it provides a clear example in which IPS cells, when compared with in vivo systems with the same mutation, having the same phenotypic expression: an accumulation of triglycerides in the cell. The impact of such a study is that it confirms the use of iPS cells in disease modeling and further provides a platform for discovery new drugs or cellular pathways that may help to understand the mechanism of the disease. We hope that through such study we can better explain the metabolic consequences that occur in the diseased cells and also when these cells are treated with new drugs that affect lipid accumulation. Analysis of the iPS cell-derived "disease-in-a-dish" will hope to further our understanding of the mechanisms of heart diseases.

Akachi Uzosike *Molecular and Cellular Biology*
Cabot 2013

A new experimental paradigm for extending telomere length without tumorigenic genetic recombination

Rossi Lab,
Harvard Medical School

In the absence of telomerase activity, telomeres of human cells shorten with each successive cell division, eventually reaching a critical length that triggers growth arrest, a phenomenon referred to as the Hayflick limit. If allowed, continued cell division at this point can lead to chromosomal instability, apoptosis, and tumorigenesis. The limit to cellular proliferation imposed by telomere shortening is believed to underlie the loss of regenerative capacity with aging. Therefore, restoration of telomere length could be a viable therapeutic strategy toward ameliorating or reverting aging associated deterioration. Furthermore, telomere shortening must be overcome if patient derived cells were to be expanded in large quantities for generating replacement tissues. Experiments have shown that ectopic expression of telomerase reverse transcriptase (TERT) in telomerase-deficient cells can restore telomerase activity and maintain or extend telomere length, even allowing for the Hayflick limit to be bypassed. Such techniques however have classically utilized viral methods of gene transduction, which are therapeutically undesirable.

To address this therapeutic need, I propose to restore telomere length in human fibroblasts by introducing in vitro transcribed mRNA encoding TERT. This mRNA is produced from the double-stranded DNA open reading frame of TERT with RNA polymerase. Telomerase activity will be quantified with the telomeric repeat amplification protocol, and telomere length will be measured using quantitative telomerase fluorescent in-situ hybridization.

This approach allows for gene therapy without the risk of inducing potentially tumorigenic genetic recombination. We hypothesize that ectopic expression of TERT-encoding mRNA will allow cultured human fibroblasts to divide past the Hayflick Limit, while retaining telomerase activity and telomere integrity. Our approach of mRNA transfection of the TERT gene into cells could have broad applications both for regenerative medicine and treatment of genetic disorders such as aplastic anemia, dyskeratosis congenital, and idiopathic pulmonary fibrosis.

Joshua Wortzel *Neurobiology*
Pforzheimer 2013

A new microcephaly gene and its impact on brain development

Walsh Laboratory,
Department of Genetics, Center for Life Sciences

Although the brain is largely what makes us human, relatively little is known about the key steps of neurodevelopment and the causes of neuropathology. Microcephaly, or "small head," is a neurological condition that affects 1% of the population. Recently our lab diagnosed a consanguineous Arab-Israeli family in which 5 of 7 children were affected with severe microcephaly (<4 SD) and simplified gyri (minimal brain folding). Sequence analysis performed on the family revealed a mutation in the gene Znf335 located on chromosome 20. Relatively little is currently known about Znf335, especially concerning its role in brain development. Our work focuses on gaining a better understanding of the expression and function of Znf335. Specifically, when and in what brain tissues is Znf335 expressed and is Znf335 essential for progenitor cell proliferation and maintenance? To answer these questions, we use both a mouse model system and in vitro cell culture systems.

To assay when Znf335 protein is normally expressed, we performed immunoblotting analysis on brain lysates over a developmental time course. From this we learned that Znf335 is expressed in all brain tissues tested and peaks at the height of neurogenesis. To further characterize Znf335 expression in specific regions of the brain, we used a mouse model in which β-gal expression is driven by the Znf335 promoter. X-gal staining for β-gal expression is subsequently used as an indicator of Znf335 expression. To determine whether Znf335 is essential for cell proliferation and survival, we utilized an in vitro shRNA knockdown system. We cultured neurospheres and granule cells both in the presence and knockdown of Znf335 to monitor how cell division and cell death are affected, respectively. A better understanding of the role of Znf335 in neurogenesis may shed light on fundamental molecular mechanisms governing brain development and enable the development of future treatments.

Helen Yang *History of Science*
Adams 2012

Phenotypic characterization of live homing T-cells during chronic Hepatitis C infection

The Lauer Laboratory,
Gastrointestinal Research,
Massachusetts General Hospital

Nearly 170 million people worldwide are infected with the Hepatitis C virus (HCV), making it a serious global health problem. Because few patients are able to spontaneously clear the virus during the acute phase of infection, the majority develops chronic infection and, consequently the risk of liver failure and hepatocellular carcinoma. Several studies in mouse models have been published demonstrating that intrahepatic T-cells express the inhibitory receptor programmed death 1 (PD-1) protein at higher levels than do PBMCs (Peripheral blood mononuclear cells). In order to define the phenotypic profile of liver-homing T-cells in healthy and HCV positive individuals, we performed immunohistochemistry stains for the surface proteins CD3, CD4 and CD8, as well as for PD1 and its ligand PD-L1, on antigen-presenting cells. We hope to gain a better understanding of these inhibitory interactions, in order to eventually develop drugs meant to boost the immune systems of chronic HCV patients.

As we expected, there is a correlation between the later stages of HCV infection and a greater number of liver-infiltrating T-cells. On one hand, we may detect that T-cells of HCV end-stage diseased liver disease tend to cluster around the portal tracts rather than in the parenchyma. The T-cells also appeared to withdraw from the parenchyma as the infection progressed to late chronic stages. On the other hand, PD-L1 could be found in both parenchyma and portal tracts. All our results are preliminary, however, and further staining of more HCV positive and healthy livers needs to be analyzed to draw conclusions about the PD-1 positive T-cell/PD-L1 interactions.

Paul Yarabe *Chemical and Physical Biology*
Pforzheimer 2013

Potential synergistic effects of p73 pathway activation with poly(ADP-ribose) polymerase inhibition in treating refractory breast cancers

Ellisen Laboratory,
Gillette Center for Breast Cancer,
Massachusetts General Hospital

Poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors have been proved to be effective in treating breast and ovarian cancers arising in patients with germline BRCA1 or BRCA2 mutations. Recent clinical trials have shown promising clinical responses by combining PARP inhibitors with DNA-damaging agents, such as gemcitabine and cisplatin, in treating sporadic triple negative breast cancers (TNBC). However, traditional chemotherapy is toxic to normal cells; thus, the pairing of PARP inhibitors with targeted therapies is a promising research endeavor. The lab discovered that the p63/p73 pathway is a therapeutic target in TNBC, and we hypothesize that activation of a p73-mediated pathway will have synergistic effects with PARP inhibition in cancer cell killing. We had conducted a genome-wide screen to identify gene targets ("hits") whose inhibition can activate the p73-mediated apoptotic program. We predict that the inhibition of these hits will synergistically potentiate cell death of TNBC cells in combination with PARP inhibition. Using cell viability assays and siRNA transfection to inactivate target genes, we are currently screening to determine which hits can potentiate cell killing by a PARP inhibitor ABT-888, in both BRCA-1 deficient HCC1937 cells and sporadic TNBC MDA-MB-468 cells. This screen will have important mechanistic and therapeutic implications that may ultimately lead to an effective therapy against this treatment-refractory breast cancer subtype.

Caleb Yeung *Molecular and Cellular Biology*
Quincy 2012

Identification of STAT3 gene targets in EGFRvIII-expressing glioblastoma

Bonni Lab,
Department of Pathology,
Harvard Medical School

Glioblastoma is the most common and aggressive primary brain tumor. It arises from the uncontrolled proliferation of astrocytes and their precursors, the neural stem cells. Despite conventional multimodal therapy, glioblastoma has one of the worst prognoses of any tumor type. An understanding of the specific cellular signaling pathways responsible for the transformation of astrocytes is crucial to developing new methods of treatment. It was previously shown that Signal Transducer and Activator of Transcription 3 (STAT3) plays a dual role as a tumor suppressor and as an oncogene in glioblastoma in a mutational profile dependent manner. Within the context of astrocytes with Phosphatase and Tensin Homolog (PTEN) deficiency, STAT3 acts as a tumor suppressor. In astrocytes expressing a truncated version of Epidermal Growth Factor Receptor variant III (EGFRvIII), STAT3 acts as an oncogene, forming a physical complex with EGFRvIII in the nucleus to drive transcription of downstream targets. Within the context of EGFRvIII-expressing astrocytes, investigating the mechanisms by which STAT3 upregulates transcription of downstream genes might reveal potentially novel therapeutic approaches. We found that Inducible Nitric Oxide Synthase (iNOS), which catalyzes the production of nitric oxide (a key diffusible signaling molecule), is an upregulated downstream target of STAT3 in EGFRvIII-expressing astrocytes. Inhibition of iNOS with the water soluble, FDA-approved small molecule inhibitor 1400W dihydrochloride reduced cell proliferation of EGFRvIII-expressing astrocytes in a STAT3-dependent manner in vitro. Further investigation of the ability of 1400W to reduce tumor growth in vivo may reveal a promising new targeted therapy for glioblastoma tumors expressing EGFRvIII.

Chi Zhang *Molecular and Cellular Biology*
Winthrop 2012

Construction and functional characterization of ATPase deficient Nod3 mutants

Department of Cancer Immunology and AIDS,
Dana Farber Cancer Institute

The interplay between innate and adaptive immunity is crucial in protecting the host from microbes and viruses and combating infection. A group of proteins known as the nucleotide binding domain-leucine rich repeat/Nod-like receptor (NBD-LRR/ NLR) family of cytoplasmic pathogen sensors is evolutionary conserved from plants to humans and plays a prominent role in antigen recognition and initiation of the innate immune response. This class of proteins possesses a central oligomerization domain, the nucleotide binding domain (NBD) critical for activation and leucine-rich repeats (LRRs) for sensing PAMPs. NLR proteins derive diverse functionality from their variable N-terminal effector domains which mediate signaling with downstream factors. The Nod subfamily of NLR proteins contain N-terminal caspase-recruitment (CARD) domains that form CARD-CARD interactions in the signaling pathway to activate immune responses.

Previous studies conducted in the Kobayashi lab have confirmed Nod3 (NLRC3) as a novel member of the NLR family. Like other Nod proteins, Nod3 contains the characteristic CARD domain, central NBD, and C-terminal LRRs. But unlike Nod 1 and Nod2, which are found in antigen presenting cells, Nod3 expression is restricted primarily to T cells and lymphoid organs such as the spleen and thymus. The generation of ATPase deficient Nod3 mutants via site-directed mutagenesis of the Walker A and B motifs of the NBD region would provide optimal controls in gauging the functional effect of Nod3. Mutating Lysine-->Alanine on the 151st amino acid in the Walker A motif would inhibit nucleotide binding while a Glutamate-->Glutamine shift on the 223rd amino acid of the Walker B motif would prevent ATP hydrolysis, serving as a substrate trap. We have generated stably transfected HEK293T and Jurkat cell lines of wild-type (WT), Walker A, Walker B, and Walker A/B Nod3 mutants tagged with GFP. These cells will help us answer the questions of whether Nod3 oligomerizes and with which partners, if the Walker motifs contribute to localization of Nod3 to the cytosol, and how Nod3 affects T cell function.

Yuemei (Amy) Zhang *Human Developmental and Regenerative Biology*
Mather 2012

Function of Mafb in pancreatic reprogramming

Zhou Laboratory,
Bauer Labs,
Harvard University

Reprogramming is the process by which fully differentiated cells are converted to another cell type. Usually, this is done with transcription factors, as in the case of iPS cells. My project involves the reprogramming of pancreatic exocrine cells into endocrine cells, i.e. glucagon-producing α cells, insulin-producing β cells, etc. Studying this will not only provide more information on pancreatic development and maintenance of cell fate, but may also have a medical application to treating diseases such as type I diabetes.

Previously, it has been established that a combination of three transcription factors, Ngn3, Pdx1, and Mafa could reprogram pancreatic exocrine cells into β cells and that Ngn3 by itself could reprogram pancreatic exocrine cells to δ cells.

My project is studying how the transcription factor Mafb functions in pancreatic reprogramming. Mafb is expressed in β cells during pancreatic development, and is expressed in α cells in adults. In humans, it is also expressed in adult β cells. Our recent data suggests that a combination of Ngn3, Pdx1, and Mafb can also reprogram pancreatic exocrine cells into β cells. Currently, I am working on creating different constructs with Mafb and other transcription factors and later studying how these will reprogram exocrine cells.

Han (Angela) Zhang *Molecular and Cellular Biology*
Dunster 2011

Exploring anti-viral memory CD8+ T cell response

Von Andrian Laboratory,
Immune Disease Institute and Department of Pathology,
Harvard Medical School

One of the hallmarks of adaptive immunity is the generation of immunological memory. Immunological memory is characterized by increased rapidity of lymphocyte response upon host rechallenge with a pathogen that has already been encountered. It is also the basis for preventative vaccination. Recent studies have begun to elucidate the characteristics that differentiate cells identified with memory responses from cells primarily associated with the response to primary infection.

Jung Hwan Sung in the von Andrian laboratory observed that a subset of memory cells in the lymph node, CD8+ central memory (TCM) T cells, relocalize from the T cell zone to the subcapsular sinus (SCS), interfollicular area, and medulla in the secondary response to lymphocytic choriomeningitis virus (LCMV) challenge. Sung found that more TCM relocalize to the lymph node SCS, interfollicular area, and medulla than non-memory, naïve CD8+ T cells that have not previously encountered cognate antigen. Chtanova et al. (2009) also noted in their study of parasitic infection that memory cells relocalize to the periphery of the lymph node. Importantly, the functional significance of TCM relocalization during the recall response remains unknown. The connection, if any, between TCM relocalization and the ability of the memory response to clear virus is still to be determined.

I worked to determine the functional significance of TCM relocalization to the SCS, interfollicular area, and medulla during recall response by pursuing the following: (1) Checking whether TCM relocalization is associated with faster rate of TCM activation and earlier onset of proliferation. (2) Testing whether TCM kill infected cells in the SCS, interfollicular area, and medulla. (3) Determining whether TCM relocalization affects rate of viral clearance in the lymph nodes draining infected tissues.

Neuroscience and Psychology

Lawrence Benjamin *Neurobiology*
Winthrop 2011

Characterization of Ab production in neurons with wild-type APP and APP gene variants linked to Alzheimer's Disease

Young-Pearse Lab,
Department of Neurology,
Harvard University

Advancements in medical treatment increasing the human lifespan have allowed for the increased prevalence of Alzheimer's disease (AD), a debilitating condition that carries substantial morbidity and economic burden for millions of people worldwide. The pathogenesis of AD has been linked to Ab, a protein secreted by neurons (though it is secreted by all cell types) that was found to be the primary component of the hallmark plaques that develop in the brains of AD patients. Cleavage of amyloid precursor protein (APP) gives rise to Ab, and rare genetic mutations in the APP gene have been linked to early onset Alzheimer's disease. Likewise, individuals with Down's syndrome also often develop early onset Alzheimer's disease, suggesting that the triplication of the APP gene found on chromosome 21 might contribute to its pathogenesis. Ab has been studied using a number of model systems and post-mortem biopsies of AD patients, but there has been little opportunity to characterize Ab production directly from living human neurons. Thanks to a technique developed by Shinya Yamanaka and colleagues in Japan, it is now possible to derive pluripotent stem cells from already differentiated cells. Because these induced pluripotent stem cells (iPSCs) behave like stem cells, they can be reprogrammed into neurons, opening new avenues into understanding neurodegenerative diseases including AD. In collaboration with the Daheron laboratory in the iPSC core at the Harvard Stem Cell Institute, we seek to derive iPSCs from healthy patients, AD patients with Down's syndrome, and AD patients with rare predisposing genetic mutation. These iPSCs will then be differentiated to a cortical neuronal fate. By characterizing the forms of Ab produced by these neurons, we hope to be able to further elucidate just how the function of neurons of healthy individuals could potentially differ from the function of the neurons of individuals with AD.

Lauren Carvalho *Neurobiology*
Quincy 2011

From sensory input to behavioral output: decoding the language of the brain

Professor John Maunsell,
Harvard Medical School

The decoding of neural activity into behavior remains one of the great frontiers of neuroscience. Though much is known about the average behavior of neurons during repeated behavior, it is not well understood how single actions are controlled. In uncontrolled environments the brain must be able to generate specific responses to single stimuli; however, in controlled environments, such as in a laboratory, repetitive behavior is observed as averages are taken over repeated trials. Evidence suggests that many neurons are involved in any such behavior. Until the advent of optical micro-stimulation and its capacity both to monitor and to control the activity of single neurons in vivo, however, research has remained stagnant in this area. Using this method, we hope first to correlate mouse neuronal firing with features of visual stimuli, and later to activate key groups of neurons directly and observe resultant behavioral responses. Our project ultimately seeks to understand the role of specific areas in the pathway from sensory input to behavioral output.

Jenn Chang *Neurobiology*
Dunster 2011

Assessing the sigma receptor regulation of pre-pulse inhibition and its implications for the development and assessment of antipsychotic drugs

The Caine Laboratory,
Alcohol and Drug Abuse Research Center,
McLean Hospital

When an organism is exposed to a low-intensity pre-stimulus 30-500 milliseconds prior to a high-intensity startling stimulus, the startle response that the organism exhibits to that strong stimulus is reduced. This neurological phenomenon, known as pre-pulse inhibition (PPI), is a normal, unconditioned reaction that reflects the adaptability of the nervous system to external cues and warnings.

Research has shown that patients with certain psychotic disorders, such as schizophrenia, exhibit deficits in both the pre-pulse inhibition of startle and the habituation of startle. These patients are unable to "filter out" irrelevant stimuli, and deficits in PPI may account for the observed attentional deficits that are characteristic of the disease. Though experimentally-induced PPI-deficient rodents are not animal models of diseases such as schizophrenia, they provide an in vivo model for pharmacological analyses of sensorimotor gating deficits. Studying the ability of antipsychotic drugs to recover PPI in animal models that are deficient in PPI may be helpful for predicting the therapeutic effects of drugs for such disorders.

In the identification of neural mechanisms that regulate PPI, very little research has been conducted about the effects of sigma receptors. The antipsychotic medication haloperidol, which binds with high affinity to both dopamine D2 and sigma receptors, has both therapeutic effects and motor side effects in schizophrenic patients. D2 receptor blockade is necessary for antipsychotic effects, suggesting that some of the side effects of typical antipsychotic drugs may be due to greater sigma receptor binding. I am currently assessing the therapeutic and side effect profiles of antipsychotic drugs that have high and low sigma receptor activation. Apomorphine, a dopamine D2 selective agonist in rats, is used to knock down PPI

to make the startle profile of the rats look schizophrenic. Antipsychotic drugs that block the dopamine D2 receptor are given as a pretreatment to the apomorphine, and the ability of the drugs with high sigma receptor activity to recover PPI is compared with that of drugs with low sigma receptor activity to determine the therapeutic profile of the drugs. Rats are also trained on a food-maintained operant behavior task, and a rate assay is performed in order to study the motor decrement effects of the antipsychotic drugs. Isolating the sigma receptor regulation of pre-pulse inhibition may reveal information about the effectiveness of therapeutic strategies and the development of antipsychotic drugs that target sigma receptors.

Justin Chew *Neurobiology*
Leverett 2011

Development of an RGC-5 cell model as an effective tool to study RGC development and regeneration

Dongfeng Chen Lab,
Schepens Eye Research Institute,
Harvard Medical School

Retinal ganglion cells (RGCs) are the neurons whose axons form the optic nerve and relay information from the retina to the visual cortex in the brain. Diseases such as glaucoma involve the death of RGCs, which leads to blindness. Thus, stimulation of RGC regeneration may one day provide a viable treatment for restoring eyesight in patients afflicted with such diseases. Interestingly, RGCs have been shown to regenerate successfully in adult frogs and goldfish to restore functional sight following injury, while RGCs in higher vertebrates lose their regeneration capacity upon maturation. It is thought that certain master genes are switched off in mammalian RGCs during development, which leads to the loss of the capability for RGC regeneration. Preliminary studies have indicated that these genes may involve the insulin-like growth factor 1 (IGF-1) signaling pathway.

Currently, there are many obstacles that complicate the direct study of RGCs, which has led to the creation of a cell model that mirrors the development of RGCs in vivo. My research specifically aims to develop a RGC-5 cell model that will more effectively facilitate the study of genetic mechanisms that regulate axonal growth and elongation in developing RGCs. RGC-5 is a transformed proliferating cell line that expresses markers specific to RGCs. Upon treatment with differentiation factors, RGC-5s undergo morphological changes from fibroblast-like to neuronal cell morphology.

To validate RGC-5s as a good model for RGCs in development, several steps must be taken. First, the optimal differentiation conditions must be determined to allow RGC-5s to most resemble the gene expression profile observed in RGCs. Second, the timeline for differentiation in RGC-5s must be determined in order to map events in differentiating RGC-5s to their corresponding counterparts in developing RGCs. Several techniques will be used towards these two aims, including the measurement of mRNA expression through qRT-PCR, the measurement of protein expression through Western blotting, the observation of morphological changes in differentiating cells, and the observation of protein expression and localization through immunohistochemical staining.

Chris Devine *Neurobiology*
Winthrop 2013

Towards the functional characterization of the neuronal subtype-specific gene Sctf2

Macklis Lab,
MGH-HMS Center for Nervous System Repair,
Departments of Neurosurgery and Neurology,
Massachusetts General Hospital

The six-layered mammalian neocortex, responsible for sensory perception and cognitive function, contains a set of pyramidal projection neurons that extend axons to subcerebral targets (structures located below the cerebrum such as the brainstem and spinal cord). These neurons express different transcription factors, are located in unique cortical areas, and possess varying morphological features. The discovery of genes that are expressed in a subtype-specific manner has made it possible to research the individual specification of projection neuron classes. A particular class of subcerebral projection neurons, corticospinal motor neurons (CSMN), are particularly important since they are required for voluntary motor control, degenerate in amyotrophic lateral sclerosis (ALS), and are damaged in spinal cord injury. Understanding their migration and patterning may help repair proper circuitry in neurodegeneration.

In 2005, members of our lab compared gene expression of purified CSMN from distinct time points in murine development to that of other cortical subtypes using microarray analysis. In this manner, Arlotta et. al identified CSMN-specific genes that act as molecular controls over subtype development. Included here was Sctf2, a gene expressed in subcerebral projection neurons during early and intermediate development that is within a larger class of genes involved in neuronal differentiation and axon pathfinding.

The phenotype of Sctf2 in the cerebral cortex is currently unknown, and I am working to understand its functional significance. I compare Sctf2 knockout and wild-type mice using immunocytochemistry (an antibody labeling technique) and retrograde tracing (injection of a dye into the spinal cord to determine if proper CSMN axon tract formation occurs). Conversely, constructing an Sctf2 overexpression vector and inserting it into embryonic mice will yield gain-of-function data. These cells, which do not normally express CSMN transcription factors, will be analyzed for CSMN-like properties including neuronal positioning and axon formation. Insights toward the functionality of this gene will contribute to the overall understanding of subtype-specific development, and may play a role in future therapies for neural degeneration.

Katherine Dobos *Neurobiology*
Pforzheimer 2012

Cognitive world of *Canis familiaris*

Professor Marc Hauser,
Canine Cognition Laboratory,
Harvard University

Research into the cognitive world of canines is still in its infancy. Currently, only a small group of researchers worldwide are using canines as a model organism in behavioral studies; therefore, the CCL focuses on developing novel techniques that will effectively

answer questions about canines' perception of "fairness," the presence of a general intelligence factor (G) in canines, canines' understanding of aberrant vs. normal physical phenomena, and canines' biases relating to familiar vs. unfamiliar human races and languages, to name a few. The CCL's venture in the realm of canine cognition has provided me with the unique opportunity to learn more about an animal that participates in our daily lives to a far greater degree than do many other model organisms. The CCL incorporates techniques from a variety of fields, including developmental psychology, moral cognition, and primatology, and additionally strives to develop relatively unprecedented methodologies that rely heavily on canines' co-evolution with humans, and are particularly suited for use with *Canis familiaris* in spite of the fact that they may not be as effective for testing other species. As with any complex model organism, canines possess a variety of backgrounds and experiences that can shape their behaviors, and as researchers it is our responsibility to design methods and coding schemes that allow us to correctly determine which results represent relevant, replicable findings. To do this we have implemented a series of novel and strict data coding methods that allow us to accurately remove biases introduced unintentionally by the handler, the experimenters, and any confederates. Nevertheless, in spite of the unique challenges inherent to the use of canines as model organisms, the canines that come into our lab have provided us with profound insight relating to the evolution of cognition and more specifically how domestication may affect the behavior of animals. In addition, the dogs reward us with unceasing adoration and large, slobbery licks of affection in gratitude for all the treats they have received in the process.

Ricky Fegelman *Undeclared*
2013 Cabot

Investigating neurological mechanisms underlying risky decision-making in mice

Uchida Lab,
Department of Molecular and Cellular Biology and The Center for Brain Science,
Harvard University

Many behavioral phenomena rely on making decisions under risky conditions – decisions made between a known number of outcomes of predefined magnitude and probability. While recent theories in behavioral economics have aimed to describe how humans deviate from rational choices under these conditions, the neural underpinning for this remains to be elucidated. We present mice with a task in which they select one of multiple outcomes, each of different magnitude and associated probability of actually obtaining it after selection. We measure their risk preferences: specifically, whether they are risk-averse when choosing among potential rewards and risk-seeking when choosing among potential punishments. We are studying the role of dopaminergic neurons in the midbrain in regulating these decisions. It is thought that these neurons signal reward-prediction error – the disparity between the expected and actual magnitude of reward. Dopamine activity can therefore be related to the degree of uncertainty regarding a particular choice and so lends itself to an understanding of risky behavior. We have used Adeno-Associated Virus to selectively express Channelrhodopsin-2, a light-gated ion channel originally from algae, in dopaminergic neurons in the Ventral Tegmental Area, allowing us to activate them artificially using a fiber optic cable implanted in the brain. We will investigate whether brief bursts of dopamine activity associated with risky (or safe) choices cause the mice to choose (or avoid) risky outcomes. We will simultaneously record activity in dopamine neurons while they perform the task. These results will shed light on how the dopamine system is involved in decision-making and how it may contribute to disease states such as Parkinson's and drug addiction.

Johanna Meehyun Lee *Neurobiology*
Currier 2013

Non-cell autonomous effects of glia with altered levels of SMN on motor neurons

Rubin Laboratory,
Department of Stem Cell and Regenerative Biology,
Harvard University

Spinal Muscular Atrophy (SMA) is characterized by the progressive degeneration of motor neurons and proximal muscle weakness. SMA is an autosomal recessive disorder that is caused by a mutation in the Survival motor neuron 1 gene (SMN1) which results in decreased levels of the Survival Motor Neuron (SMN) protein. Approximately 1 in 6,000 infants are born with SMA, making it the most common genetic cause of infant mortality, and 1 in 40 people are carriers for SMA. Studies have been carried out to alter SMN levels in embryonic stem cell (ESC) derived motor neurons using lentiviral constructs, which preferentially infect neurons, that either overexpress SMN or reduce SMN by small hairpin RNA (shRNA) targeted against the mouse Smn gene. Decreasing SMN levels using a shRNA produced a cell survival defect in motor neurons which resulted in approximately 50% fewer motor neurons as compared to the controls. In contrast, increasing SMN levels using a lentiviral overexpression construct resulted in no change in motor neuron survival. These studies show that reducing SMN in motor neurons negatively affects their survival. However, conversely, elevating SMN levels does not appear to improve motor neuron survival. To address whether altering SMN levels in support cells has an effect on motor neuron survival, we altered the levels of SMN in glial cells rather than in motor neurons and then monitored motor neuron survival over a time period of seven days. This research will address whether altering levels of SMN in glial cells/support cells will have a non-cell autonomous effect on motor neuron survival.

Thomas Zhihao Luo *Neurobiology*
Lowell 2011

Packing wires together: the dense organization ofaxons and dendrites in the cortex

Lichtman Lab,
Northwest Labs,
Harvard University

Behavior arises from electrical activities in a circuit of neurons precisely connected to each other. This precise connectivity between

neurons is established by neuronal projections called axons and dendrites, which form the physical basis of wires in the circuit of neurons. In the brain, axons and dendrites are packed tightly together into a compact volume to minimize space, economize wiring length, and decrease conduction delays. However, unlike a typical circuit, in which the wires have the shape of long, slender rods, the wires of the neuronal circuit are elaborate branching structures with rich arborization and extensive outgrowth. Thus, the brain not only has to solve the difficult problem of assembling precise wiring connections between neurons, but also the problem of packing axonal and dendritic arbors into a dense volume. Understanding the brain's solution to the dense packing problem may shed light on the precise wiring problem and the important properties of neuronal connectivity that gives rise to behavior.

In this initial investigation of the brain's solution to the dense packing problem, the spatial organization of axonal and dendritic segments was examined. Elucidating how segments of axons and dendrites are packed together is an important first step in solving the more general packing problem that takes into account entire axonal and dendritic arbors. A preliminary model describing axonal and dendritic segments as cylinders of uniform radius is currently being constructed. A more complicated model of the segments as curved and tortuous tubes will be devised based on the results of the cylinder model. An apt geometrical model for axonal and dendritic segments will be both constraint on and insight for a model that describes the packing of axonal and dendritic arbors.

Neda Shahriari *Human Developmental and Regenerative Biology*
Mather 2012

In vivo method for chemical screening: using zebrafish as a model

Rubin Laboratory,
Department of Stem Cell and Regenerative Biology,
Harvard University

Neurodegenerative diseases are identified on the basis of the depletion of particular neural populations within the body. Our interest concentrates on Parkinson's disease in which the dopamine secreting neurons—dopaminergic neurons—undergo degeneration to yield symptoms relating to motor system dysfunctionality. Currently, chemical screens are carried out on stem cells, in vitro, in order to discover small molecules that can promote cell differentiation toward certain lineages and, ultimately, cell fate. Since one of the interests in the Rubin lab involves dopaminergic (DA) neurons, the lab has carried out chemical screens using embryonic stem cell-derived neural progenitors. Having identified compounds that were able to promote and increase the number of DA neurons in vitro, the aim of this project is to apply small molecules identified from the ES-based screen to an in vivo system like zebrafish. The aforementioned is significant for verification of results as well as a validation for applicability in the in vivo system. Several screening parameters were assayed before testing compounds in zebrafish, including drug concentration, treatment duration, number of embryos per well, and identification of the positive control. We determined the optimal drug concentration to be 100 μM and the appropriate embryonic developmental stage for drug addition to be 10-14 somites, which is approximately 14 hours post-fertilization. Furthermore, we determined the optimal number of embryos per 96 well-plate to be 6 embryos, on the basis of the embryos' ability to continue maturing normally with no developmental delays or abnormalities. From a chemical screen utilizing 5 potential positive control compounds with known developmental effects, cyclopamine, a Sonic hedgehog inhibitor, was selected. We are currently screening approximately 300 small molecules consisting mostly of kinase inhibitors. All compounds have been treated on the embryos and we are assessing gross morphological changes. We expect to carry out antibody stains against Tyrosinse Hydroxylase (TH), which is an enzyme in DA neurons involved in the dopamine production pathway. By visualizing the number of DA neurons present, we anticipate finding small molecules that can increase the number of DA neurons. Compounds affecting the population of dopaminergic neurons, specifically by increasing the cell count, can further our efforts in seeking better understanding of DA neuron development and ultimately Parkinson's disease.

Adrienne Smallwood *Molecular and Cellular Biology*
Quincy 2013

Autism and the dosage-dependent ubiquitin ligase regulation of glutamatergic circuits

Anderson Laboratory,
Department of Neurology,
Beth Israel Deaconess Medical Center

Autism has been shown to be a heritable, neuropsychiatric disorder. It is one of three psychological conditions included in the term Autism Spectrum Disorders (ASD). These disorders are characterized by a deficit in social interaction, impaired communications, repetitive behavior, and restricted interest. In recent years, researchers have looked to genetics to determine which genes are involved with the disorders, and what goes wrong within the cell to cause ASD. Our lab studies a mouse model of human genetic autism. The most common genetic copy number variation (CNV) associated with Autism Spectrum Disorder is excess copies of chromosomal region 15q11-13 inherited selectively from the mother. We focused on the genes within this chromosomal segment that are expressed from the maternal, but not paternal, allele and added extra full-length gene copies (~250 kb) to transgenic mice. Behavioral studies have already shown defects that resemble the core symptoms of autism spectrum disorder. Another goal of the lab is to study defects in neural circuits. By using immunohistochemistry we can study the changes in synaptic proteins and neuronal activity markers in our mouse model of human genetic autism spectrum disorder. Our lab involves behavioral studies, much protein and DNA work, and sophisticated techniques including BAC gene recombineering, brain whole-cell slice patch-clamp electrophysiology, and proteomic technologies. Recent work in our lab has shown that the regulations of certain circuits in the brain related to Autism are affected by dose-dependent CNV's in the Ube3a gene on 15q11-13.

Michael Stanley *Human Developmental and Regenerative Biology*
Eliot 2013

Neurodegeneration: from bench to bedside

Dr .Ole Isacson, Center for Neurogeneration, MGH; Dr. Merit Cudkowicz,
Neurological Clinical Trial Unit/ALS,
Massachusetts General Hospital

Neurodegenerative diseases such as Parkinson's, Dementia with Lewy Bodies, and Amyotrophic Lateral Sclerosis (ALS) are devastating conditions that rob patients of themselves before robbing them of their lives. Their complex nature and heterogeneous etiologies make their causes as difficult to find as their symptoms are to combat. One of the most progressive elements implemented in finding better treatments has been the interaction between clinicians and scientists. This summer experience dovetailed research into Parkinson's Disease with the coordination of clinical trials for ALS in order to gain a more comprehensive understanding of how best to face the unique challenges of neurodegeneration.

Parkinson's Disease (PD) is principally a movement disorder presenting with bradykinesia, resting tremor, and instability and rigidity in posture. One of the defining features of PD is the presence of particular neuronal inclusions called Lewy Bodies. These inclusions contain aggregates of alpha-synuclein, a protein of unknown function. Recent research demonstrates that both the concentration and mutations of alpha-synuclein may help facilitate aggregation and may play a key role in the disease. This portion of the project focuses upon the splice isoforms of alpha-synuclein, attempting to transfect transcripts into a human neuroblastoma cell line in order to better characterize each isoform's protein product.

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the progressive loss of motor neurons. Striking in mid-life, the diseases progresses at variable rates among patients. Without a cure, all patients will eventually die of the disease—often from choking, pneumonia, or the inability to breathe due to loss of diaphragm function. This summer experience focuses intensely at understanding the clinical diagnosis of patients, the implementation of symptomatic treatments to help assist patients in meeting their changing needs throughout progression, and in the coordination and evaluation of several clinical trials pertinent to ALS.

Ritchell VanDams *Neurobiology*
Leverett 2011

Bipolar and horizontal cell connectivity of the *nof* zebrafish retina

Dowling Laboratory,
Department of Molecular and Cellular Biology,
Biological Laboratories,
Harvard University

Zebrafish are highly visual animals whose visual systems are fundamentally similar to those of humans. Using zebrafish as a model system, my research seeks to characterize the connections made between specific types of neurons in the retina. Specifically, I use imaging techniques which allow me to identify cone photoreceptor (i.e. color vision) cells and the cells that make connections onto these cones. These secondary cells ar ae divided into the two broad categories of horizontal and bipolar, designations which are then further divided into different subtypes. Using transgenic fish, I can visualize the location of some of these cone cells using Green Fluorescent Protein (GFP). The fish I use have been genetically modified so the cone cells that respond to UV light also express GFP when stimulated with laser light. Another dye, DiI, stains horizantal and bipolar cells and fluoresces under laser light, revealing which subtypes of these cells make connections onto different types of cone photoreceptor cells.

My research builds on previous classification work by exploring the connectivity of a specific strain of colorblind mutant zebrafish. These fish are known to be colorblind due to a lack of what is called the optokinetic response (OKR). To test for OKR, zebrafish larvae are presented with a series of moving vertical black and white stripes. As the stripes move across their field of vision, normally sighted fish will track the movement of the stripes with observable eye movements back and forth. Fish that are blind do not make such tracking movements with their eyes. In future studies, I hope to alter the environment of these blind fish and observe effects on vision and retinal connectivity during development.

Ke Xu *Neurobiology*
Quincy 2011

Activity dependent recruitment of Sec5 in *Drosophila* neuromuscular junction

The Schwarz Laboratory,
Dept of Neurobiology,
Children's Hospital Boston

Traffic across cell membrane is a crucial step for many physiological functions, such as cell growth, cytokinesis, and signaling. A protein complex whose activity seems to be closely linked to polarized cell-surface delivery events is known as the exocyst. Sec5, one of the components for the exocyst complex, has been shown to be an integral element for membrane trafficking in Drosophila oocytes. From our earlier data, we hypothesize that calcium activation of the small GTPase Ral leads to its binding of Sec5, which is recruited to the post-synaptic region of the neuromuscular junction (NMJ), causing expansion of the sub-synaptic reticulum (SSR), suggesting that this pathway is intricately linked to neuronal plasticity and activity. We seek to address this hypothesis by using an electrophysiological approach to probe the activity-dependence of plasticity in the SSR of Drosophila larval NMJ.

We used a semi-in vivo 3rd-instar larval preparation to conduct nerve stimulation, electrophysiological recordings, and immuno-cytochemistry. Drosophila larva was dissected and prepared for single electrode stimulation; the effects were confirmed by simultaneous recording of the postsynaptic cell. Then the larva was treated with PFA and underwent standard immunostaining procedures to assess the changes in Sec5 distribution after electrical stimulation.

The results from our experiment will shed light on the regulation and function of the Sec5 protein in post-synaptic development, which will lead to a better understanding of neuronal membrane trafficking and plasticity.

Baltazar Zavala Engineering and Neurobiology
Kirkland 2011

Calcium sensitive imaging of gap-junction coupled networks in the Thalamic Reticular Nucleus

The Landisman Laboratory,
Department of Neurobiology,
Harvard University

Historically, the role of electrically coupled neurons in the mammalian central nervous system has been overlooked. Recently, however, there has been an increase in the number of areas of the CNS known to contain gap junction coupled neurons. One such area, the Thalamic Reticular Nucleus (TRN) is responsible for regulating the gating of signals to and from the thalamus and the cortex. Behaviorally, the TRN has been implicated in the lack of response to sensory input during REM sleep and during cortically generated spike-wave seizures.

In this project, we are using calcium-sensitive dyes in conjunction with fluorescent imaging to study gap junction connections in the TRN. First, we load the TRN with Oregon Green-Bapta dye. By patch clamping a cell in the TRN, we are able to control the membrane potential of the patched cell. We then inject depolarizing current into the cell, which causes the cell to spike and voltage-dependent calcium channels to open. The interaction of the internal calcium with the dye increases the fluorescence of the neuron, which we record with a high-speed camera. By recording the changes in fluorescence when a cell is stimulated, we acquire data that will allow us to model how the change in fluorescence is related to the change in calcium concentration. Once this is done, we can use the same experimental paradigm to determine the changes in fluorescence of the gap junction-coupled neighboring cells when the patched cell is stimulated. This will allow us to visualize which cells are gap junction coupled to the recorded neuron, and thus the size of gap junction networks in the TRN.

During these experiments, we also test the effects of different extracellular potassium concentrations on cells' resting voltage. Because a high level of extra cellular potassium raises the resting potential of neurons, it lowers the amount of current or synaptic input necessary to drive a neuron to spike. By testing different levels of potassium, we hope to find concentrations that facilitate the spiking of neurons that are gap junction coupled to our patched cell and thus allow us to study even weakly coupled networks.

Organismic and Evolutionary Biology

Susan M. Seav *Molecular and Cellular Biology*
Cabot 2012

Developmental bases of sexual shape dimorphism in anole lizards

Thom Sanger (Mentor), PhD & Arkhat Abzhanov (P.I.), PhD, Department of Organismic and Evolutionary Biology, Harvard University

It has been reported that many hormones, like the Growth hormone secreted by the pituitary gland, have a direct effect on bone and cartilage development in vertebrates. However, the identification and characterization of other vital hormonal molecules that play a role in craniofacial development remain elusive. Examination of these hormone-dependent pathways will lead to a more thorough understanding of postnatal skeletal growth and may, in turn, shed light on craniofacial defects in humans, such as Crouzon and Apert syndrome.

The Abzhanov lab uses both molecular and developmental techniques to better understand mechanisms of craniofacial development. The goal of my ten-week project in the Abzhanov lab was to elucidate the molecular foundation of sexual shape variation in the snout of the green anole, Anolis carolinensis. To address this question, I have been searching for genes within hormonal and skeletal pathways that are differentially expressed in the snout of juvenile lizards of each sex. This species of lizards serves as a great animal model as it exhibitis both sexual size and shape. Although precise function of these genes is unknown in this species, I have confirmed that several hormonal pathways have an effect on lizard snout outgrowth.

Surprisingly, labeling of proliferating cells with Edu, a molecule that incorporates itself into doubled-stranded DNA due to its nucleotide resemblance, has also shown that snout outgrowth is not the result of cell proliferation, as it is in mammals. Further experiments, such as the correlation of snout growth rates with levels of gene expression, will shed further light on mechanisms of craniofacial development in this unique species. My study is one of the first to combine evolutionary and developmental biology to determine the molecular bases of intra-specific variation.

Shervin Tabrizi *Chemical and Physical Biology*
Quincy 2011

Functional consequences of naturally selected coding polymorphisms in the human genome

Pardis C. Sabeti,
Department of Organismic and Evolutionary Biology, FAS

Recent sequencing efforts of the International HapMap and the 1000 Genomes projects have produced large databases to catalogue human genetic variation. These variations, typically in the form of Single Nucleotide Polymorphisms (SNPs), can be used to detect signals of natural selection in the human genome. Several tests have been developed for search the genome for regions that have been under positive selection, through searching for variants that have reached a high frequency in a particular population, or variants over a large genomic region that seem to be passed on together (large haplotype blocks). Our lab has recently developed a test statistic (CMS – Composite of Multiple Signals) that combines results from multiple tests to create a score that can better-localize the signal of selection and more accurately distinguish the causal variant driving selection at a particular region. Using CMS, we performed a genome-wide scan of the 1000 Genomes database for regions that have been under recent positive selection. We found 22 regions where one of the high-scoring SNPs resulted in a coding change in the protein product of a gene. Using homology modeling software, we created putative models for each of the proteins in order to localize the residue change and determine whether it might have an effect on the function of the protein. We found that a few of the selected coding SNPs have been shown to affect protein function in previous studies. Furthermore, several of the residue changes fall in well-defined functional domains, and are good candidates for functional changes. Once we have completed building protein models and identifying promising coding changes, we will test the ancestral and derived protein variants experimentally to look for functional change. A positive result would provide important new clues at the molecular level on the impact of natural selection on human evolution.

Chung Yao Yu *Chemical and Physical Biology*
Leverett House 2012

Effect of endosymbionts on the metabolism of short-fat and long-skinny Ridgeia piscesae

Girguis Lab,
Department of Organismic Evolutionary Biology,
Harvard University

Ridgeia piscesae is the only vestimentiferan tubeworm species found in the Juan de Fuca Ridge in the northeast Pacific. These tubeworms occupy a great range of sulfide microhabitats and exhibit a wide spectrum of phenotypes that they were first thought to be two or more species based on their distinct morphology. However, due to recent analysis of the cytochrome oxidase I sequence, allozymes, RFLP of nuclear ribosomal genes, and AFLP fingerprinting, these seemingly distinct morphotypes are characterized as one species. This observation suggests a classic case of phenotype plasticity, for which organisms of the same genotype may exhibit different phenotypes in different environmental conditions. However, the mechanism that contributes to this phenomenon is unclear. This finding is further complicated by the observation that all the endosymbionts, on which these tubeworms rely for nutrition, are virtually identical. Though several studies provide strong evidence against the single-taxon endosymbiont hypothesis, the widely accepted notion of a single species belonging to the subdivision g-Proteobacteria raises many questions concerning the host-symbiont interactions that result

in such striking phenotypic differences in the same tubeworm species. As an initial survey, we hypothesize that the basis of phenotype plasticity between the "short-fat" and "long-skinny" tubeworms correlates with the difference in the metabolism of the endosymbionts. We examined the stable carbon isotope ratios of their trophosomes and observed clear differences between these two worm morphotypes. Moreover, since the single-taxon hypothesis is based on 16S rRNA generated from traditional cloning and sequencing, we are exploring differences in endosymbiont populations using 454 pyrosequencing, a newer sequencing technology that generates orders of magnitude more sequencing depth. We suspect that these data will help us parse out the metabolic differences we observed with the carbon isotope data between these two worm morphotypes.

Sarah Zhang *Neurobiology*
Lowell 2011

Phototaxis behavior in "evolution canyon" Drosophila

The DeBivort Laboratory,
Rowland Institute,
Harvard University

Phototaxis is a robust, well-studied behavior in Drosophila, and species-level differences in light response can be artificially selected for in only a few generations. These qualities make Drosophila phototaxis a good model system to study the evolution of behavior. We observe that Drosophila exhibit two types of phototaxis behavior: a "fast" response toward light and a "slow" preference for either light or dark over a longer period of time.

I am investigating the "slow" phototaxis of Drosophila from naturally occurring populations on two sides of "Evolution Canyon" in Haifa, Israel. The two slopes of the canyon are ecologically distinct, as the sunny south-facing slope gets eight times as much solar radiation as the shadier, north-facing slope. Flies can migrate easily between the two slopes, but genetic analyses have shown interslope variation, suggesting incipient sympatric speciation. To investigate differences in the "slow" phototactic response, I optimized a behavioral assay in which flies are given the choice between light and dark. As the slow phototaxis response is of interest, the assay runs for several hours while the flies are periodically agitated and their positions automatically tracked. Once strains with different dark preferences are identified, the eventual goal is to understand the neurobiological mechanisms underlying this evolutionarily relevant behavior, and determine if differential phototactic preference contributes to reproductive isolation in this speciation.

Physics and Biophysics

Sway Chen *Chemistry and Physics*
Kirkland 2012

Undulatory wave propagation in the locomotion of C. elegans

The Samuel Laboratory,
Department of Physics,
Harvard University

The nematode worm Caenorhabditis elegans has been used for decades as a model organism for studying neural circuits, due to its simple body structure and relatively small number of neurons. In C. elegans, forward movement is generated by a sinusoidal undulatory wave that propagates from head to tail (anterior to posterior). In order for this wave to propagate, the activity of all body segments has to be coordinated by neural networks. We are trying to answer the question of how stretch sensors, motor neurons, and body wall muscles are synaptically connected, for body segments to influence nearby segments in wave propagation.

We used novel PDMS microfluidic devices and optogenetics to actively control the bending of particular body segments while measuring the movement of the rest of the body. When a body segment of a worm is confined in a curved microfluidic channel, the curvature of the confined segment causes all posterior segments to follow that curvature. When the curvature of a confined segment is forced to change over time, all anterior segments will follow the curvature change. Since curvature is the effect of muscle contraction on one side of the body and muscle relaxation on the other, we examined worms expressing halorhodopsin in muscle cells and in motor neurons, so we could selectively inhibit certain muscles or neurons. Our experiments showed that stretch sensory feedback is necessary for wave propagation through the worm's body and for determining wave frequency, amplitude, and wavelength. In particular, each body segment appears to receive positive stretch sensory feedback from anterior segments. Further research is being done to confirm our theoretical model of worm locomotion. This model could be a breakthrough in explaining how animal behavior is derived from neural circuits. It may also explain on a neuronal level the continuous-gait phenomenon observed in worms crawling through environments where the external mechanical load (a result of viscosity) is gradually increased.

Kevin Fogarty *Physics and Astrophysics*
Kirkland 2012

Analysis of induced charge and thermal effects in astronomical x-ray detectors

Grindlay Laboratory,
Harvard-Smithsonian Center for Astrophysics

X-ray astronomy of the night sky has been instrumental for the discovery and observation of many new astrophysical phenomena and profoundly impacts our understanding of the cosmos. X-ray spectra from high-energy gamma ray bursts allow us to examine these objects at high red shift and peer back into the early history of the universe. A next-generation X-ray telescope has been proposed by the EXIST team, that will improve on existing space-borne technology, such as the BAT on Swift and the ROSAT observatory, to provide high resolution full sky surveys of the hard X-ray spectrum, detecting light from a minimum noise threshold up to 600 KeV. The first generation prototype detector, protoEXIST I, consists of a detector plane of 8 by 8 closely tiled detector crystal units (DCUs). A DCU is composed of a rectangular piece of cadmium-zinc-telluride (a type of semiconductor) crystal at the base of which is an array of pixel pads that act as anodes that collect charge off the crystal. X-rays incident on the crystal at sufficient energy will force electrons from where the X-ray collides with the crystal lattice over the semiconductor band-gap, creating an electron-hole pair. A 600-volt bias applied as a uniform gradient draws the electrons to the anode for collection.

Currently, we are at the transition stage between protoEXIST I and the next generation prototype, protoEXIST II. As we move to higher resolutions and lower energy consumptions, we will need to more thoroughly understand the properties of the semiconductor we are using. I am currently studying the induced surface charge at the anode that arises when a charge is formed in the crystal, as well as characterizing the change in the semiconductor band-gap due to temperature. Induced charges vary as a function of the depth of interaction as a result of the boundary conditions imposed by the electric potential at the anode. Similar detectors such as the BAT do not observe this effect nearly as dramatically, owing to the thickness of the semiconductor used. These findings are being used in determining how data will be taken in the next generation prototype.

Dmitri Gekhtman *Mathematics and Physics*
Dunster 2013

Wigner crystal melting

Heller Group,
Department of Physics,
Harvard University

In 1934, Eugene Wigner predicted that, at low temperatures, two-dimensional systems of electrons would take on crystalline structure. Since then, Wigner's prediction has been supported experimentally and computationally. However, the melting of these two-dimensional electron solids is not completely understood. To study the melting of the Wigner crystal, we simulate its dynamics directly. That is, we model the system as a collection of classical point charges and integrate numerically to determine their trajectories. We consider two models: one in which the electrons are embedded in a uniform neutralizing charge distribution and one in which the electrons are under the influence of a parallel plane of fixed positive charges. To allow for simulation of large numbers of particles we run our simulations on Harvard's Odyssey cluster. Even with a parallel computing cluster, we cannot simulate particle numbers on the order of what we would have in an actual sample. Therefore, we impose periodic

boundary conditions and use Ewald summation to handle long-range interactions. Eventually, we hope to study the Wigner crystal from a semiclassical perspective and compare the results to those obtained from our current classical analysis.

Rachel Hinman *Physics*
Winthrop 2012

Muon Reconstruction Efficiency in the ATLAS Experiment

ATLAS Group,
Laboratory for Particle Physics and Cosmology,
Harvard University

By colliding protons moving at near-light speeds and detecting the particles that result from these collisions, the ATLAS experiment at the Large Hadron Collider promises to shed new light on a variety of important open questions in particle physics. For instance, the experiment could substantiate or refute theoretical predictions regarding the origin of mass, the possibility of extra spatial dimensions beyond the ones we see, and hypothesized symmetries of nature. The first step in using high-energy collisions to improve our understanding of physics is to measure the performance of the equipment used to identify the products of the quark-antiquark interactions that take place when two beams of protons collide. The goal of my project is to quantify the efficiency of the ATLAS muon detection system at correctly identifying muons by comparing the size of the upsilon resonance peak obtained using two different sets of constraints.

The number of upsilons produced within the ATLAS detector can be inferred from the size of the Gaussian peak centered at the upsilon's invariant mass that results from plotting the combined masses associated with pairs of oppositely charged muons, given that energy and momentum must be conserved when the upsilon decays into two muons. However, if the muon detection system is imperfect, some muons will pass through the inner detector and then fail to form a clean enough track in the muon spectrometer to register as muons. By comparing the size of the resonance peak for two definite muons to the peak for one definite muon and one other particle, it is possible to determine the probability that a muon passing through the detector will produce enough of a track for our software to recognize it as a muon.

Daniel Kim *Chemical and Physical Biology*
Cabot 2013

Sensory integration of C. elegans in linearly-distributed chemical and thermal gradients

The Samuel Laboratory,
Department of Physics,
Harvard University

With the practical benefits of a short life span and a fully sequenced genome, the nematode Caenorhabditis elegans–composed of only 302 neurons and 5000 synaptic connections–offers the optimal workspace for studying neurobiology. By quantifying the animal's locomotion wholly as a function of its individual neural circuits, one can begin to understand the nuances of its underlying neural structure. C. elegans exhibits two fundamental motile behaviors: a conditioned preference for its cultivated temperature (thermotaxis) and cultivated chemical environment (chemotaxis). In past research, these behaviors were often examined separately and, for chemotaxis, under nonlinearly-distributed conditions (i.e. drop assays). To attain a more comprehensive and realistic neurobiological picture, the present study examined C. elegans locomotion under simultaneous presentation of linear chemical and thermal gradients. The steepness and orientation of the gradients were varied, and within each condition, three mutant strains and a wild-type strain were examined. Each mutant strain was ablated for a different neuron within the chemical/thermal pathway: ASE (responsible for chemotaxis), AFD (thermotaxis), and AIY (interneuron postsynaptic to both the ASE and AFD sensory neurons). The animals' drift velocities, reorientation rates, and directional biases were analyzed across these strains to determine how C. elegans integrate multiple channels of information to traverse their environmental gradients. Because the C. elegans nervous system is homologous to that of mammals, the results presented here yield valuable insight into how more complex neural systems might function.

Ruby Lai *Chemistry and Physics*
Kirkland 2012

Coherent spin qubit coupling along carbon nanotube bends

The Marcus Laboratory,
Laboratory for Integrated Science and Engineering,
Harvard University

With a general drive towards miniaturization of electronic devices, great interest has arisen in quantum computation for quantum dot devices. These devices use quantum mechanical phenomena that theoretically allow exponentially faster computation times than classical computers. There are a multitude of material and phenomena systems that are being studied as candidates for the fabrication of quantum dot qubit devices; in particular, this project focuses on coupled electron spin qubits defined on carbon nanotubes.

A central issue in quantum information processing is the design of the interaction between environmental and qubit modes. For efficient information processing, separate processing units must be coherently coupled, while coupling to the random environmental fluctuations must be minimized. Indeed, the use of spin qubits is critical in the rapid dephasing time of electron spins linked to the interaction with the environment. Carbon nanotubes provide a good choice as a substrate material for the spin qubit because carbon-12 has zero nuclear spin, which alleviates the hyperfine interaction spin-spin coupling that can occur between the qubit spin and the nuclear spins of the substrate material.

Recently, it has been theoretically demonstrated that two spin qubits defined on two different segments of a bent carbon nanotube can be coherently coupled by capacitative gating. This coherent coupling is necessary for building devices with more than one processing unit. In my project this summer, I have been fabricating and measuring carbon nanotube devices with sharp bends to begin to experimentally define the effect of the bend in the nanotube on electron spins under a range of magnetic fields.

Nicholas Pomata *Physics/Mathematics*
Dunster 2013

Hysteresis in overstretching of ligated klenow DNA

Prentiss Laboratory,
Department of Physics,
Harvard University

Double-stranded DNA (dsDNA) polymers will undergo a sudden transition to a state about 1.7 times longer than unstretched DNA when pulled at 65 piconewtons. Theories that seek to explain this overstretching suggest that it differs in nature depending on how the DNA is pulled; in particular, the models of Lebrun and Lavery predict that 3'3' DNA (dsDNA for which both strands are attached to a surface from the 3' end), 5'5' DNA (dsDNA for which both strands are attached to a surface from the 5' end) and 3'5' DNA (dsDNA for which one strand is attached to a surface at both the 3' and 5' ends, and the other remains unattached) will each transition into distinct variants of the unstretched B-DNA.

Somewhat less well-explored, however, is DNA that is attached by both ends of both strands, called ligated Klenow DNA after the Klenow fragment, which extends the 3' ends of the split λ-phage DNA used to match the 5' DNA, and the process by which those extended ends are ligated to rest of the strand.

One of the most dramatic differences observable in the stretching of these different forms of DNA is the hysteresis exhibited. When DNA returns from the overstretched state, it will, under certain circumstances, exit the unstretching transition early, not returning to its original length until forces significantly lower than 65 pN (often 40 to 55 pN). This hysteresis has furthermore been shown to vary as the concentration and type of salt present in solution is altered. It is this hysteretic effect (which is often particularly strong in ligated Klenow DNA) subject to these parameters that we have been examining. Through better knowledge of this response to overstretching, we hope to learn more about the overstretched state of ligated Klenow DNA, as well as the interactions of the DNA double helix with dissolved ions and water itself.

Isaac Shivvers *Astrophysics*
Eliot 2010

Working towards high-precision photometry

The Stubbs Laboratory,
Laboratory for Particle Physics and Cosmology,
Harvard University

The brightness of celestial objects is one of their most fundamental characteristics. Astronomical photometry is the science of measuring object brightness (or luminosity) accurately and absolutely. Much of our astronomical knowledge is based upon photometric measurements, from the distance to other galaxies to the rate of expansion of the universe. Ground-based photometry is less precise than we would hope, however, and is often contaminated by significant unknown variation due to atmospheric interference. The current and next generations of sky surveys (like Pan-STARRs or the LSST) are limited by atmospheric interference, not instrumental or statistical noise floors. A better understanding of our cosmos is dependent upon more precise photometric measurements.

Though photometry is a simple idea, it is not so simple in practice. With modern telescopes and cameras on the ground, precision photometry is limited by temporal and spatial variations in the optical transmission of the atmosphere and our current inability to monitor this atmospheric variation accurately. Photometry accurate to the one percent or better level is currently a dream, but we are working to make it achievable.

Our system will use a dedicated secondary telescope and a man-made light source to monitor the atmospheric contribution as a function of both time and angle. These measurements, along with highly accurate instrumental characteristics and atmospheric modeling through MODTRAN, will enable more precise absolute photometry. We are constructing a weather balloon payload with a calibrated light source which is to be flown over the observing telescope at the edge of the atmosphere, along with a ground-based secondary telescope system to accurately track and observe the balloon light source at a resolution of about 1/10 of a second. Simultaneous observation of astronomical objects with a primary telescope, atmospheric observation with the balloon and secondary telescope, coupled with highly accurate instrumental measurements and atmospheric modeling can yield millimagnitude or better absolute photometry.

Nilesh Tripuraneni *Physics*
Dunster 2013

Localization in entangled quantum walks

Hagai Perets,
Harvard-Smithsonian Center for Astrophysics

Quantum walks were first introduced in 1993 and since have been extensively researched within the context of physics, quantum information theory, and probability theory. As a generalization of the classical random walk, the quantum walk allows for superposition of different walk paths resulting in behavior such as constructive and destructive interference. One striking difference between the two walks is that the variance after N steps is proportional to N^2 for a quantum walk, rather than N as in a classical walk on the line. Additionally, several quantum algorithms with optimal efficiency were proposed based on quantum walks, and it was even shown that a continuous-time quantum walk on specific graphs have exponential algorithmic speedups compared to their corresponding classical algorithm.

Here we study multi-particle quantum walks on different lattices. Multi-particle quantum systems have the uniquely, non-classical feature of "entanglement" which allows for an intrinsic correlation between the degrees of freedom of different quantum walks. Simply put, measuring the position of one particular particle has an effect on where the position of another particle on the lattice though there is no "force" between them. Although the simple quantum walks on the line have been extensively studied using a variety of methods from experimental/theoretical physics, mathematics, and computer science, entangled quantum walks are not as well understood. Specifically, we study the influence of spatial disorder and nonlinearity on entangled quantum walks by implementing numerical simulations of their evolution. In simple one-particle quantum walks such disturbances are known to result in the famous Anderson localiza-

tion – a phenomenon in which particles tend to favor certain areas with high probabilities. We attempt to quantify similarities and differences in the entangled multi-particle scenario.

Christopher Wood *Physics-Math*
Lowell 2012

Optimized planar penning traps for quantum information studies

Gabrielse Laboratory,
Department of Physics,
Harvard University

In 2008 the Gabrielse lab measured the magnetic moment of the electron to three parts in 1013, which was fifteen times more accurate than the previous measurement. This was done by trapping a single electron using a Penning trap, which is a cylinder with a harmonic oscillator electrical potential induced at it's center along with a uniform magnetic field. The electron would spin around in the trap and quantum spin flips were detected by a change in frequency, which allowed for the very precise measurement. Building off this, I am assisting an experiment that will use a planar Penning trap to also trap and detect a single electron. The planar trap differs from the standard Penning trap in that it is only a single plane with concentric voltage areas, rather than a cylinder with two planes for manipulating the electron, thus increasing the challenge. The main difficulty with this method is measuring the very small signal created by the oscillation of the electron in the trap compared with the electronic noise. This larger ratio of noise to signal for the planar trap is caused mainly by the impossibility of achieving an exact harmonic oscillator potential because of the geometry of the trap. If the planar trap method succeeds it will lead to increased applications for Penning traps, with the goal of constructing a very small array of traps onto a computer chip which could be used for quantum information studies.

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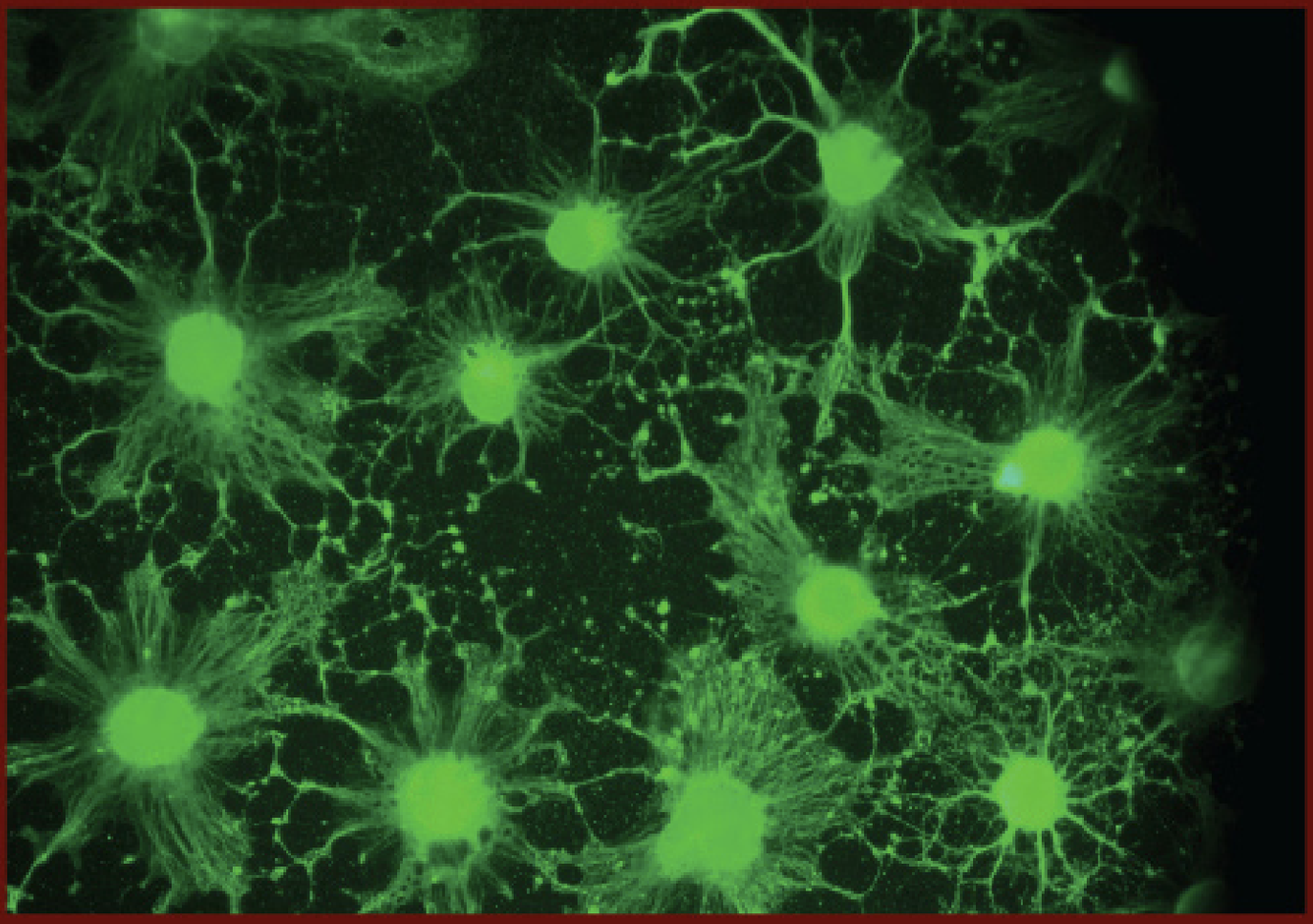

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