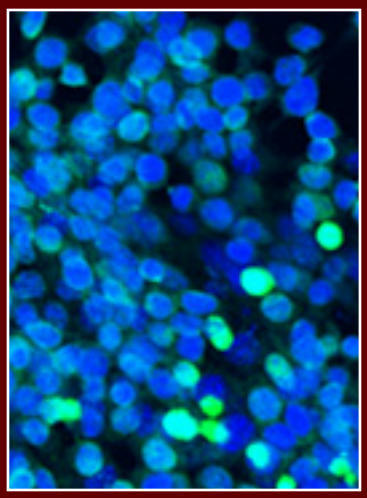
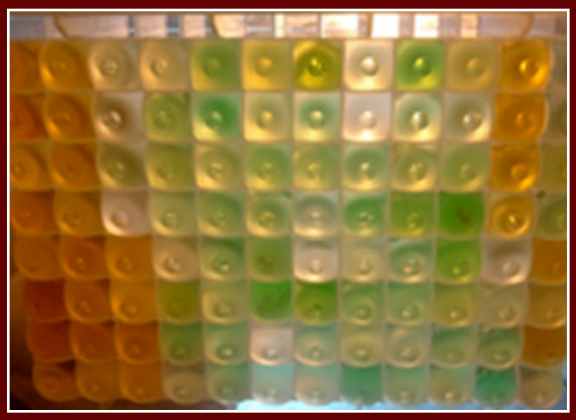
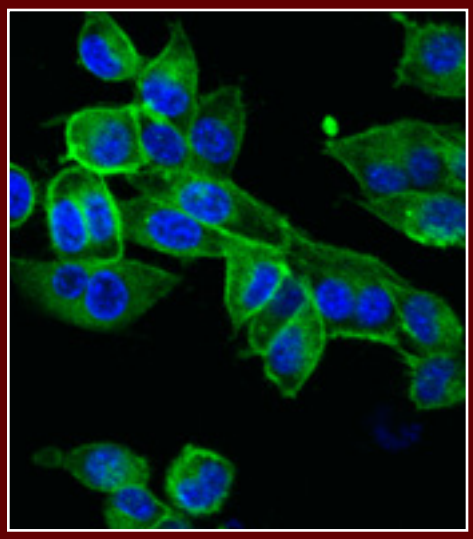
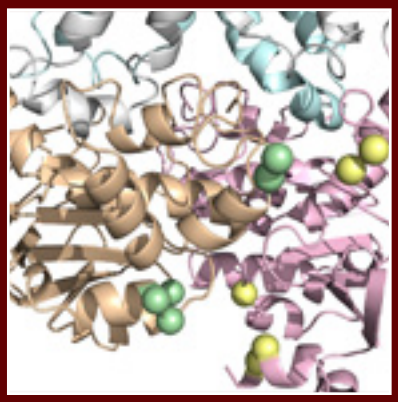
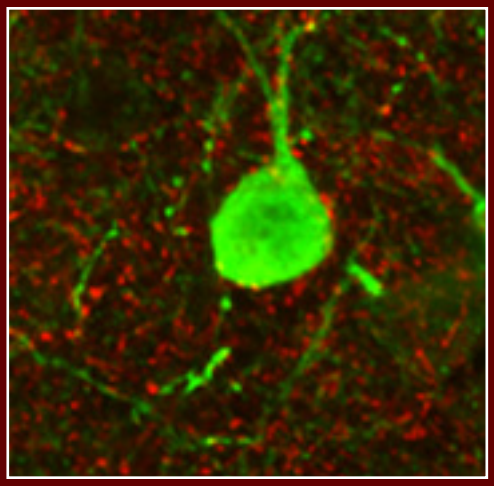
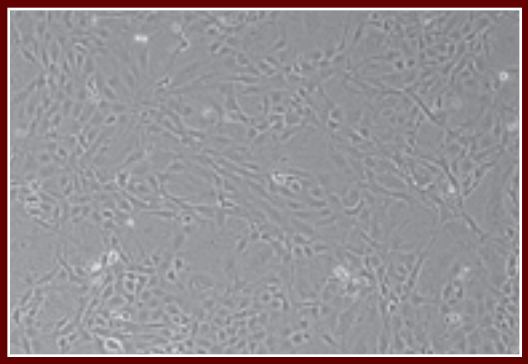
HARVARD COLLEGE

PRISE

PROGRAM FOR RESEARCH IN SCIENCE AND ENGINEERING

ABSTRACTS

2011

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Photographs courtesy of Ally Freedy, Michael Lindeborg,
Kevin Ni, Jesus Mario Luevano Jr., and Hillary Singer.

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Letter from the Director

I am delighted to introduce this collection of research abstracts from the 2011 Harvard College Program for Research in Science and Engineering. 2011 is the sixth year of PRISE, and once again the Fellows have done exceptional work guided by Harvard faculty and investigators in the Faculty of Arts and Sciences, the Harvard Medical School and affiliated teaching hospitals, and other allied research enterprises across Cambridge and Boston. The research as described herein reflect the full range of breadth and diversity among scientific interests and pursuits of the Fellows this summer in the life, physical, engineering and applied sciences. Combined with the lively and energetic residential community at Leverett House, the PRISE experience this summer once again is a testimony to the compelling value of developing community among emerging scientists across a full range of disciplines (including the new social sciences programs BLISS and PRIMO, with whom we shared the summer intellectually and socially).

This historical collection of abstracts could not have been possible without the outstanding effort of the group of editors whose mission it has been to collect, organize, and publish the work of the PRISE Fellows. Along with the PRISE Program Assistant Fellows, who have helped with all of the Fellow-initiated activities, I would like to thank this group especially for taking on the particular challenge to record the research projects of the PRISE community this summer.

To the PRISE Fellows of 2011, I wish you every success in your further growth and trajectory as scientists. Your engagement, enthusiasm, and inclusiveness are inspiring, and I hope the personal and collegial relationships you have cultivated these past ten weeks continue to grow and flourish long after your PRISE summer.

With all best wishes,

Gregory A. Llacer
Director, Harvard College Office for Undergraduate Research Initiatives
Director, Harvard College Program for Research in Science and Engineering

Letter from the Editors

Dear PRISE fellows,

It's hard to believe that after nearly ten weeks, PRISE is already coming to a close. Over this time, we have spent hours conducting tireless research, foraging for scientific explanations beneath mounds of data, and combing through endless research articles. Yet for all of us, PRISE has signified more than just an acronym associated with an undergraduate summer research program. PRISE has adopted an even broader definition that extends beyond research in the lab, and, rather, has derived most of its meaning from the community of its fellows.
The PRISE fellows, in becoming close to one another, have defined the program through their love of the sciences and spontaneity in creatively planning activities together. PRISE has become an environment in which fellows could infuse their diverse scientific perspectives with inevitable quirkiness. Whether it was in the Leverett dining hall after a long day in lab, during a pick-up Ultimate Frisbee Game, participating in late night games of Mafia, or during one of the weekend activities, the PRISE community has provided us a unique opportunity to broaden our own outlook in the sciences and build new relationships with others.

We hope that this abstract book will rekindle those memories and unexpected moments in which, as a tight-knit group of scientists and friends, we have become close enough to call ourselves a PRISE family. Let this book be a memento that signifies the relentless work that fellows put into their respective labs, but also a reminder to never forget the friendships and experiences that were shaped while members of PRISE. We are proud to present the collective enthusiasm and work from all of the members in the PRISE community. Among the memorable trips to six flags, Gloucester beach, or whale watch, we have all contributed to the close bonds that will undoubtedly last beyond the end of the summer. On behalf of the PRISE community, thank you to everyone who made this summer experience unforgettable and invaluable in fulfilling the potential of such a wonderful group of future scientists.

Sincerely,
Nick Perkons '14, Matt Abrams '14, and Michael Lindeborg '14
Editors-in-Chief

The PRISE 2011 Abstract Book Design Staff:

Yun Jee Kang '14

The PRISE 2011 Abstract Book Editorial Staff:

Andrea Brettler '14 • Roxana Feier '12
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Richard Sima '12 • Anji Tang '13 • Bing Fang Edna Wang '14 • Linda Xia '13

Astronomy & Physics

John Capodilupo *Computer Science*
Quincy House *Class of 2014*

Stella Offner
Center for Astrophysics
Harvard University

The origin of the observed stellar multiplicity is an unsolved problem in star formation theory. In this work, we compare numeric simulations and observations of structure in starless cores which may go on to form multiple star systems. Observations with the CARMA interferometer suggest that cores in low-mass star forming regions lack substructure. Whether these results are an artifact of the limitations of detector technology or reflect a physical reality will have immense consequences for explaining the origin of stellar multiplicity. Using the simulations of Offner et. al 2009, we identify gravitationally bound stars within cores and then create simulated observations of these images using the CASA software suite. In the project, we analyze the ability to resolve the cores including blurring caused by noise and detector effects in order to evaluate the accuracy of such ground-based observations.

Jeremy Cushman *Physics*
Pforzheimer House *Class of 2012*

John Doyle
Center for Ultracold Atoms
Harvard University

A Search for the Electric Dipole Moment of the Electron with Thorium Monoxide

The electron electric dipole moment (EDM) is an intrinsic property of an electron that relates its potential energy to the strength of an external electric field. For over 50 years experiments have searched for non-zero electric dipole moments in elementary particles. Although no non-zero EDM has yet been detected, various experiments have placed upper limits on its value. The current experimental limit is 13 orders of magnitude greater than the value predicted by the Standard Model, but it is widely believed that an observation of a non-zero EDM is likely if the experimental sensitivity can be improved by just a few orders of magnitude. The observation of a non-zero EDM would imply a violation of parity and time-reversal invariance, and its specific value would eliminate or require modification in certain theories that lie beyond the Standard Model to match the experimental result.

The ACME experiment uses a cryogenic beam of the heavily polar molecule ThO (thorium monoxide) to attempt a measurement of the electron EDM. A non-zero EDM would cause slight energy shifts in particular molecular states in the presence of an electric field. These energy shifts would flip sign when the electric field is reversed, so by flipping the electric field and observing the change in the resulting energy levels we hope to determine the size of the electron EDM.

Aisha Down *Physics & English*
Eliot House *Class of 2014*

Baryon Acoustic Oscillations and Dark Energy

Models of inflation usually hold that inflation stopped at slightly different times in different places in the primordial universe, leaving it with density perturbations that began to propagate outwards in a manner we model as sound waves—baryon acoustic oscillations—during the early stages of the universe's reheating. At this time, baryonic matter was ionized, and so the mean free path of a photon was much smaller than the size of the horizon. Therefore, information (or baryon acoustic oscillations—primordial density perturbations) could travel, and propagated outwards from the sites of the initial density perturbations in waves. However, when the universe had expanded and cooled sufficiently, matter began to de-ionize and so photons and matter were de-coupled; the photons shot outwards towards the horizon and the matter fell out (because photons decoupled, radiation pressure went down and the 'sound waves' were no longer able to travel). Now, using comprehensive telescope surveys such as the Sloan Digital Sky Survey (SDSS) to record the positions of faraway galaxies, we can observationally determine a peak in the autocorrelation function of baryonic matter at around 100 megaparsecs (a peak in the autocorrelation function at 100 megaparsecs means that, given any galaxy, we are statistically more likely to find another galaxy at a distance of 100 megaparsecs away than at other distances where the autocorrelation function has a lower value). Wading through the measurement uncertainties associated with finding distance from redshift (and from the different peculiar velocities that can affect redshift measurements), our goal is to fit our observational value for the baryon acoustic peak to a theoretical model derived using simulations of clustering of N-halo galaxies.

Our particular research has involved finding our autocorrelation function using Fourier analysis and a density grid, instead of pair- counting, which enables us to compare the power spectra we get from different survey morphologies (i.e., radial shells of the sky, versus octant slices, versus something totally different), and is a slightly faster algorithm to run. Once familiar with how the autocorrelation function looks in different survey morphologies, we will move to the more theoretical side of things—that is, we will be taking a random Gaussian density grid, and applying the Zel'dovich approximation for structure development (which models the change in position as a function of time as the product of the growth function, D(t), and the displacement from the gravitational force) to attempt to reproduce in our model the same sort of large-scale structure—the 'cosmic web' and superstructure—that we find in our sky surveys. Should we find a way to relate our observations with these simulations, we will be able to constrain some

of the initial parameters of the primordial density field, and the development and propagation of baryonic acoustic oscillations. If we combine this data with equations for the Hubble constant and angular distance as a function of redshift, (H(z) and dA(z), we can solve for the constant Ωλ, which is the ratio of the energy density due to Einstein's cosmological constant and the critical density of the universe. Knowing this constant will enable us to better understand the topology and potential future of our universe—different energy densities due to the cosmological constant can imply that the universe will expand exponentially forever or collapse in on itself (or something else entirely).

Caroline Huang *Astronomy & Astrophysics*
Eliot House *Class of 2013*

Edo Berger
Center for Astrophysics
Harvard University

Gamma-ray bursts are extremely luminous explosions thought to result from the formation of a stellar black hole either by the collapse of a massive star or the merger of two binary stars. There are short and long gamma-ray bursts. Short-duration gamma-ray bursts are believed to originate from the merger of two binary stars and long-duration gamma-ray bursts are believed to come from the collapse of a single massive star. Gamma-ray bursts are the most luminous and distant objects detected in the universe, which allows us to learn more about the universe not long after the big bang; one gamma-ray burst is typically brighter than the rest of the universe in gamma-rays. Since gamma-ray bursts typically last from 0.1 to 100 seconds, astronomers usually study their afterglows and emissions from their host galaxies rather than the bursts themselves. By studying the host galaxies, we can gain insight into the mechanisms that lead to the formation of gamma-ray bursts, and at the same time improve our understanding of the most distant galaxies.

The most massive stars emit the majority of their radiation in the ultraviolet range. Thus, galaxies with high output in the ultraviolet range tend to be locations with a high rate of massive star formation. For extremely distant galaxies, the rest-frame ultraviolet emission is redshifted into the optical range. In this project, optical observations of high-redshift gamma-ray bursts from the Magellan telescopes will be studied. The focus will be on the rate of formation of young massive stars in these galaxies with the hope that it will shed light on the relation between gamma-ray bursts and massive stars.

Mark Martinez *Undeclared*
Adams House *Class of 2014*

Gerald Gabrielse
Department of Physics
Harvard University

Search for the Electric Dipole Moment of The Electron with Thorium Oxide

According to the Standard Model, there is a present, but infinitesimally small, positive lump on the electron. However, the Standard Model does not fully describe all of the forces in the correct magnitude, in particular, gravity. That is why other theories such as Super Symmetry are actively trying to become proven experimentally. One of the limiting factors of many of the current theories in physics is the magnitude of the electric dipole on the electron. By experimentally finding a lower limit on the magnitude of the electron electric dipole moment (EDM), many theories can be disproven. One of the byproducts of the electron EDM experiment is that it can also possibly explain the matter-antimatter asymmetry by observing time violation, which cannot be explained by the Standard Model alone.

In order to test the magnitude of the EDM, Thorium Oxide is raised to a more suitable energy state using lasers and is placed in a magnetic field so the ions orient. Using the strong attractive forces in the ionic compound, electrons are placed between the two ions. The precession of the electron is measured and the electron EDM is then calculated from those measurements.

Samuel Meyer *Astrophysics*
Currier House *Class of 2013*

Robert Kirshner
Center for Astrophysics
Harvard University

Hierarchical Bayesian Modeling of Type Ia Supernova Light Curves

Type Ia supernovae are noted both for their interesting explosion physics and their cosmological usefulness, where they are used as standard candles to probe the depths of the universe and study cosmic acceleration and dark energy. Our group studies all types of supernovae, but these are of particular interest given the dual nature of their usefulness. My work utilizes the hierarchical Bayes method, a rigorous method of Bayesian statistical modeling that accounts for noise, to model the light curves of these supernovae as a Gaussian process.. This Bayesian method is a powerful way of determining the covariance matrix of the parameters of interest for us, and enables us to utilize the light curves more fully (e.g., in the study of dust or in cosmology) than we would be able to do with a simpler frequentist curve-fitting approach. The covariance matrix in this Gaussian process enables us to infer the distribution of possible curves, rather than simply choosing a curve to use while fitting the data. By enabling us to accurately model the maximal magnitude and the shape of the supernovae, among other things, we can infer parameters such as colors at given times, which are themselves very useful for the study of dust.

Hamsa Sridhar *Physics*
Dunster House *Class of 2012*

Federico Capasso
School of Engineering and Applied Sciences

Harvard University

Quantum Levitation Using the Casimir Effect

The Casimir effect was first formulated in quantum field theory as a generalized Van der Waals force, resulting from quantum fluctuations of the electromagnetic field in vacuum. This theory predicts many surprising results, such as an attractive force between two non-interacting metal plates in vacuum, defying the intuition of classical physics. Recent calculations have shown that the Casimir force can also be made repulsive in certain cases: for example, between a free particle and a substrate submersed in fluid. Furthermore, one can achieve 'quantum levitation' of free particles above a substrate, by counteracting the gravitational force with Casimir repulsion. This effect can be extremely useful in the actuation of Micro-Electro-Mechanical Systems (MEMS) in order to overcome problems such as friction and adhesion between movable parts at the submicrometer scale.

My project focuses on achieving quantum levitation of a glass microsphere above a gold substrate in ethanol. In order to measure such a force, I built a Total Internal Reflection Microscope (TIRM), which creates an exponentially-decaying evanescent wave field at the sample through total internal reflection in a prism. When the evanescent wave encounters an optically trapped particle, it scatters light, and the resulting signal is collected by an avalanche photodiode. I then wrote a MATLAB program that combines the statistics of Brownian motion and the scattered intensity data, to reconstruct the potential function of the particle above the substrate. This potential is a combination of many forces, including gravity, Casimir repulsion, and electrostatics. Through careful controls and theoretical models, we hope to isolate the Casimir effect and demonstrate the existence of quantum levitation.

Jason Wien *Physics & Mathematics*
Quincy House *Class of 2013*

Charles Marcus
Department of Physics
Harvard University

The Hunt for Majorana Fermions

In the mid 1930s, an obscure Italian physicist named Ettore Majorana proposed the existence of a yet unobserved class of particles. Dubbed Majorana particles, these particles are fermions in that they have spin 1/2 like electrons, but have no charge and are their own anti-particle. Mathematically they show up as zero energy modes of a quantum system. Recently, it was proposed that these particles could be used as quantum bits in a topological quantum computer. Physicists are now looking to create and detect these particles in a variety of condensed matter systems.

Specifically, we are trying to produce Majorana modes in a Josephson junction with high spin-orbit coupling and an external magnetic field. A Josephson junction consists of a superconductor-semiconductor-superconductor interface with a supercurrent passing through it. The superconducting proximity effect drives the semiconductor into a topological superconducting phase, allowing for the flow of a zero resistance current across the whole junction. According to theory, a perpendicular magnetic field applied across the junction creates a set of conditions that allow for zero energy fermionic modes, which are precisely Majorana fermions.

Creating this setup proves to be nontrivial, as superconductivity vanishes above a critical temperature and a critical magnetic field. The experimental process starts with etching a junction between two superconducting pads of niobium across the semiconductor indium arsenide. Next, gates and probes are added to control and measure the electron density across the junction. Finally, the whole device goes into a fridge that cools it down to a few miliKelvin and the measuring process begins. Reading a characteristic signature in the Josephson current or probing the local density of states will tell us that we have found these elusive particles.

Chemistry & Biochemistry

Sophie Arlow *Chemistry & Physics*
Quincy House *Class of 2013*

Tobias Ritter
Department of Chemistry and Chemical Biology
Harvard University

Carbon-Fluorine Bond Formation for PET Tracer Synthesis

Selective carbon-fluorine bond formation is a difficult transformation relevant to the synthesis of pharmaceuticals, materials, agrochemicals, and tracers for positron emission tomography (PET). The positron-emitting isotope fluorine-18 is the most prevalent nucleus in PET imaging, and radiotracers labeled with fluorine-18 are used in clinical oncology and medical research for in vivo molecular imaging. However, the short half-life of fluorine-18 (109.7 minutes) severely restricts the diversity of available PET tracers, as fluorine introduction must occur at a late stage in tracer synthesis to limit isotopic decay. Despite recent advances in the field of late-stage fluorination, a practical, efficient, and functional group-tolerant method for carbon-fluorine bond formation in complex molecules remains elusive. Previous work in the Ritter group has identified a reagent capable of promoting the deoxyfluorination of phenols to afford aryl fluorides. This reagent is capable of fluorinating a variety of both electron-poor and electron-rich substrates. The proposed deoxyfluorination mechanism proceeds via a 2-phenoxyimidazolium bifluoride salt, in which the bifluoride counteranion engages in hydrogen bonding with one hydrogen atom of the imidazolium heterocycle. However, the potential utility of this fluorinating reagent in PET chemistry is limited by multi-hour reaction times and by the presence of exchangeable fluoride in the reagent's bifluoride counteranion, which could reduce the radiochemical yield of the reaction. Current work seeks to introduce electron-withdrawing functionality onto the fluorinating reagent to increase reactivity and decrease reaction times. In addition, exchange of bifluoride for a different hydrogen-bond-accepting counteranion which does not contain exchangeable fluoride is currently under investigation.

Abhishek Chintapalli *Mathematics*
Leverett House *Class of 2014*

Eugene Shakhnovich
Department of Chemistry and Chemical Biology
Harvard University

In this work, two primary theoretical models were developed and used in order to establish a relationship between the catalytic and thermodynamic parameters of the DHFR (dihydrofolate reductase) protein in vitro and its corresponding effects on the model organism's (E. coli) fitness. Parameters defining maximal growth rate, lag time, and saturation level of the DHFR growth curves were extracted, fitting the data using a modified Gomertz logarithmic model. With these parameters, smooth function curves resembling Michaelis–Menten kinetics were derived along with Vmax and Km values for the respective mutants as a measure for in vivo DHFR activity. Furthermore, as a test of whether the fitness of our model organism is a function of its growth curve, the outcomes of bacterial growth competitions were simulated using a system of differential equations exploiting the individual growth dynamics. Running these simulations on Mathematica, we observe in the case of several mutants the inadequacy between the predicted competition outcome and the factual fitness data measured in vivo by competing wild type and mutant DHFR strains. That is, real competition data could not be explained unless death rates of the organism were considered. Indeed, thermodynamic data from in vitro measurements of the purified DHFR mutants and data from the growth experiments performed in vivo suggest the central role that protein aggregation propensity and stability play in determining fitness. This is in direct contrast to the widely held notion of fitness in phenomenological models where the growth rate of a population is higher for the more fit species. Whereas these types of approaches lack a fundamental microscopic connection between fitness and quantities of proteins that are both easily justifiable on biological grounds and measureable (e.g., structure/stability, function, or regulation), in this study we take a first step in systematically making a clear connection between the effects of mutations on molecular properties of proteins and the organismal fitness.

Aidan Daly *Computer Science*
Quincy House *Class of 2013*

Alan Aspuru-Guzik
Department of Chemistry and Chemical Biology
Harvard University

Developing Binary Classification and Nonlinear Regression Models to Screen for Potential High-Efficiency Electron Donor Molecules for Use in Organic Photovoltaic Solar Cells

Organic photovoltaic (OPV) cells are plastic solar cells with a bulk heterojunction architecture made with organic molecules. Although cheaper and more environmentally friendly than traditional silicon-based cells, they have yet to exceed the efficiency of their inorganic counterparts. The short circuit current (JSC) and open circuit voltage (VOC) are two properties of great importance to the efficiency of these devices, but these properties are very hard to determine without producing the device – a time and resource-intensive process that makes experimental high-throughput screening impractical. We sought to generate predictive models that could estimate the Jsc and Voc of a device based on single molecular properties calculated from the structure of the electron donor compound. This model will allow us to predict the most ef-

ficient donors in our database of over two million combinatorially generated molecules, producing a smaller subset for which experimental validation is tractable. We gathered a training set of OPV electron donors reported in the literature and used the DRAGON software to calculate over 4,000 molecular descriptors for each molecule. After eliminating highly correlated or low varying descriptors, we applied a binary classification algorithm to determine which descriptors could most accurately predict whether or not the given molecule could reach a Jsc*Voc value over a certain threshold. We then applied genetic algorithms, binary integration, and a limited exhaustive search to solve for the optimal and sparsest subset of descriptors with which to build a binary classification model, while additionally applying Gaussian processes to generate a nonlinear regression model based on the reduced descriptor space. We have generated promising models that are able to classify independent training sets well, and we hope to additionally solve our binary classification problem using quantum hardware in the near future.

Richard Ebright *Chemistry*
Cabot House *Class of 2014*

Stuart Schreiber
Department of Chemistry
Harvard University

Tiny Solutions to a Huge Problem: Using Small Molecules to Identify Novel Therapeutic Targets in Cancer

In the late 1990s, scientists developed the small molecule imatinib. Marketed as Gleevec, imatinib halts chronic myelogenous leukemia by selectively binding to the kinase responsible for the aberrant cell growth. Since then, a handful of small molecule drugs have been developed, effectively targeting and halting specific cancers. Such small molecules continue to be a promising route for cancer treatment. However, more needs to be known about how various cancers work for small molecule development to be efficient.

As we learn more and more about the various pathways that lead to cancer and uncontrolled cell proliferation, it is becoming clear that there are numerous, complex interactions between proteins involved in distinct cancers. The Cancer Target Discovery and Development (CTD2) team of the Schreiber lab seeks to develop novel small molecule cancer treatments by shedding light on these complex protein interactions.

We screen small molecules that have been identified as having very specific, known targets in normal cells against hundreds of various cancer cell lines that have had their mutations and abnormalities characterized. We then determine the effectiveness (or lack thereof) of the small molecules in killing, or otherwise halting growth, of the cancer lines. The effectiveness of each small molecule is then correlated with the cancer line's lineage, as well as its specific mutations.

By comparing a cancer line's response to the effects of a small molecule and the cancer line's specific mutations, we are able to draw connections between the target of the small molecule and the mutation. Through analyzing thousands of such responses, we are able to predict previously unknown interactions between various cancer-inducing factors within the cell. We then develop and carry out experiments to confirm these predictions.

A thorough understanding of these interactions will lead to novel pathways and targets for anti-cancer drug development, allowing for the creation and identification of new small molecules to effectively combat cancer.

Sam Yanting Jiang *Chemistry*
Winthrop House *Class of 2014*

Gregory Verdine
Department of Stem Cell and Regenerative Biology
Harvard University

Directed Evolution of a MYC/MAX Transcription Factor Complex Inhibitor Through Yeast Display

MYC is a basic helix-loop-helix leucine zipper (bHLHZip) transcription factor that is essential in the growth and development of somatic cells and is also present in a small subset of adult cells. MYC heterodimerization with bHLHZip partner protein MAX is required before transcription factor activity can be initiated, whereby the protein-complex binds to DNA. However, in many cancerous cells, MYC is deregulated and overexpressed, leading to MYC-induced proliferation and oncogenesis. The goal of this project is to develop an inhibitor of the assembly of the MYC/MAX heterodimer, which binds to DNA and acts as a transcription factor. The approach taken is to evolve a variant of MAX that binds MYC with higher affinity than the natural protein, and then use the variant to design peptide inhibitors. Early results have shown that yeast display systems can produce improved versions of MAX, and that binding with 1nM affinity is possible, compared with the hundreds of nM affinity of the wildtype.

Samantha Keyser *Chemistry*
Eliot House *Class of 2012*

Jon Clardy
Department of Biological Chemistry and Molecular Pharmacology
Harvard Medical School

Regulation of Antialgal Compounds Produced by Algal-Associated Bacteria of the Roseobacter Clade

Although in the past, discovery of small molecules has focused on single bacterial species, bacteria interact with a variety of organisms in their natural setting. However, the way in which bacteria sense and respond to these organisms is not yet understood, so deeper study of symbioses is necessary to examine the molecular basis of the interactions and to discover the novel metabolites involved. One such symbiosis exists between the environmentally significant marine algae Emiliania huxleyi and the algal-associated

bacterium Phaeobacter gallaeciensis. Recently, it was determined that when E. huxleyi senesces, it releases cell wall breakdown products such as p-coumaric acid (pCA) into the surrounding medium. In response to these products, P. gallaeciensis produces novel potent antialgal compounds ("roseobacticides"), indicating a shift from a mutualistic relationship between the algae and the bacteria to a pathogenic one. Here, the regulation of antialgal production was examined by activity-guided fractionation and Nuclear Magnetic Resonance (NMR) structure elucidation techniques.

Namhun Scott Kim *Chemistry*
Adams House *Class of 2014*

Pamela Silver & Jeffrey Way
Department of Systems Biology
Harvard Medical School & Wyss Institute for Biologically Inspired Engineering

Path to Sustainability: Production of Fatty Acid-based Biofuels

Rising environmental concerns and fossil fuel costs have emphasized America's need to transition from fossil fuel to a renewable alternative. Through Department of Energy's ARPA-E grant, Silver Lab in collaboration with other labs at Harvard is investigating a method for efficient production of biofuels, alcohols in this particular case. Silver Lab's approach to the problem involves three main steps: electron uptake, carbon fixation, and biofuel production. This system will have E.colitake in electrons through its membrane from an electric source. The E.coli will then be engineered to carbon fixate without glycolysis, ultimately making CO2 the primary carbon source for metabolism rather than sugar. Then, bacteria will be further engineered to produce a variety of biofuels, including alcohols, ethyl-esters, and alkanes.

The subgroup that I'm currently involved with is Group 3 – Biofuel Production. In terms of biofuel energy density and compatibility with the current fuel systems, octanol and dodecanol are currently deemed promising targets. Production of these alcohols in E.coli involves meticulously engineering the fatty acid pathway then expressing genes from other organisms in E.coli in order to produce alcohols.

The primary precursor of alcohol is free fatty acid in the cytosol. Production free fatty acid is the phase in the fatty acid pathway that determines the chain length of the subsequent organic compounds. Because the number of carbons in the chain is crucial to biofuel compatibility, we utilize a series of different thioesterases from plants to produce the fatty acid with the optimal carbon chain length. Then, overexpression of fatty-acyl reductase genes is able to reduce the fatty acids into alcohols. Although we can produce specified chain-length alcohols, 10-fold increase in yield of alcohols is necessary in order to make this metabolic pathway industrially competitive.

Michael Rizzo, Jr. *Chemical and Physical Biology*
Kirkland House *Class of 2014*

Eric N. Jacobsen
Department of Chemistry and Chemical Biology
Harvard University

Enantioselective, Nucleophilic Addition to N,N-Dialkyliminium Ions

Direct nucleophilic addition to N,N-dialkyliminium ions to form chiral tertiary amine products, while relatively facile racemically, has proved very challenging enantioselectively. Many biologically active compounds, like the widely used anti-clotting drug Plavix, contain chiral tertiary amines, yet current methods require highly inefficient procedures for synthesizing them with the desired stereochemistry—processes which often form the product racemically then use a classic resolution to separate the enantiomers. We aim to develop a chiral thiourea catalyst which will not only catalyze the direct addition of nucleophiles to N,N-dialkyliminium ions, but also do so enantioselectively. We have preliminarily observed catalysis with moderate ee (enantiomeric excess) for the addition of cyanide using catalysts with bulky, electron-rich aryl groups. We are therefore following this trend and designing catalysts with even larger, electron-rich rings to optimize the reaction further. While the main nucleophile that has been examined thus far has been cyanide, we are developing the reaction with other nucleophiles such as (trifluoromethyl)trimethylsilane, isopropenyl acetate, and (isopropenyloxy)trimethylsilane. Overall, our work will lead to a more efficient, and correlatively, more cost effective syntheses of molecules containing chiral tertiary amines.

Laszlo Seress *Chemistry & Physics & Mathematics*
Cabot House *Class of 2014*

Theodore Betley
Department of Chemistry and Chemical Biology
Harvard University

C-H Bond Functionalization Using Manganese and Iron Complexes with Metal-Ligand Multiply-Bonded Intermediates

Functionalizing unactivated C-H bonds remains a challenge in both complex molecule synthesis and the transformation of hydrocarbons into commodity chemicals. State of the art methods promote C-H functionalization using late-transition metal catalysts. However, these methods are inherently limited due to the forcing conditions required for reactivity, wherein oxidation processes often become nonselective and subject to over-oxidation. Alternatively, C–H bond activation may occur proximal to substrate-directing groups. New inorganic/organometallic catalysts can reduce the reliance on directing groups and minimize waste generation. Recently, a novel strategy using transiently formed metal-ligand multiple bonds has achieved atom- and group- transfer processes. The goal of the proposed research is to improve upon this process by increasing yields and catalyst lifetimes and to extend the reactivity to encompass oxygen atom transfer.

The new catalysts should last longer, exhibit higher turnover,and consequently increase the yields of the oxidation reactions. The

formation of amines via nitrene intermediates from azides has been explored, but this proposal seeks to improve upon the present state-of-the-art by increasing yields. Furthermore, introduction of oxygen has heretofore only been successful with highly oxophilic metal centers. The extension of this reactivity to the less oxophilic mid-to-late transition metals is an additional goal of this research.

The applications of these techniques are considerable. Selective functionalization would be a valuable tool for any synthetic chemist. Industrial applications of this technique could streamline current methods of converting hydrocarbons into commercial chemicals; namely, cracking, separation, and then functional conversion of fossil fuels.

Rohini Shivamoggi *Chemistry & Physics*
Pforzheimer House *Class of 2013*

Adam Cohen
Department of Chemistry and Chemical Biology
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Magnetic Field Effects in Chained Electron Donor-Acceptor Pairs

Recent studies on migratory birds have indicated that these birds appear to navigate by using the earth's magnetic field lines as a reference. Given the magnitude of the earth's field strength, the energy associated with a magnetic field is much smaller than thermal energy, which would lead one to expect that a chemical reaction would have an almost vanishingly small sensitivity to an applied magnetic field. It is interesting, then, that birds can apparently use some chemical means to characterize local field lines. One theory holds that the cryptochromes, or light-sensitive proteins, in a bird's eye play the crucial role in this process. It has been conjectured that when exposed to light, cryptochromes enter an excited radical state in which the protein is sensitive to the configuration of its radical electron pairs. A radical electron pair can exist in one of two spin states: either a singlet state, in which the spins are antiparallel, or a triplet state, in which the two spins are parallel. The orientation of these spins dictates the behavior of the compound.

While it would be difficult to investigate the magnetic field dependence of photochemical reactions in a compound as large as a protein, it is possible to investigate similar effects in smaller molecules consisting of a donor-acceptor pair, tethered together by an alkyl chain. Since aromatic groups have low-lying antibonding orbitals, they are ideal candidates for donor and acceptor complexes because they are easily excited when exposed to light, forming a radical pair. In the absence of a magnetic field, the three triplet states of the radical ion pair are degenerate in energy with the singlet state and exchange freely in a process known as intersystem crossing. In a biradical compound, such as those that are considered in this project, if the two radicals are both in the singlet state, then they can form a bond to create a transient intermediate known as an exciplex, whose decay back to the ground state results in fluorescence. The application of an external magnetic field perturbs the energies of the three triplet states, which interfere with intersystem crossing and result in diminished exciplex formation and an observable change in the compound's fluorescence.

The focus of this project is to synthesize compounds that display strong magnetic field effects. The goal for such compounds is twofold. Firstly, it is important to find a compound whose magnetic field effect is large in magnitude because such effects can be observed more accurately. Secondly, it is important to create a compound that can be excited in the visible range of wavelengths rather than the ultraviolet range because current optical technology is much more reliable when observing fluorescence in the visible range. With these two objectives in mind, both rigid and non-rigid chained donor-acceptor pair compounds will be synthesized for further study.

Isabel Vogt *Chemistry*
Lowell House *Class of 2014*

Jack Szostak
Center for Computational and Integrative Biology
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The RNA world hypothesis gains much of its support from the ability to artificially evolve a RNA molecule with catalytic properties. The current state-of-the-art polymerizing ribozyme, selected from the Bartel class 1 ligase has reasonably good polymerizing capabilities; however, it has an affinity for the molecule to be polymerized only by virtue of base-pair complementarity. As this is unrealistic for the origin of life, the RNA world hypothesis would be strengthened by a polymerizing ribozyme that is selected to necessitate affinity for the target primer/template molecule. In order to select for RNA molecules that can add a nucleotide to a primer/template complex, it is necessary to couple the RNA to the primer if and only if it has this catalytic property. Thus a selection scheme was developed to link an RNA oligomer to an activated monomer via a flexible polyethylene glycol (PEG) linker. If the RNA oligomer is catalytic, it will, in the process of polymerizing, attach itself to the primer, labeled with biotin. Thus artificial enrichment and selection can be achieved.

In order to couple RNA transcripts to PEG, a universal initiator nucleotide was synthesized, which can be incorporated only in the first position during in vitro transcription. This nucleotide, functionalized with a terminal alkynyl side chain, can then be coupled with an azido-PEG through Cu(I) catalyzed Huisgen cycloaddition. Both the incorporation and the Huisgen coupling have the high efficiency needed to propagate a catalytic RNA molecule through multiple rounds of selection. In addition, the initiator nucleotide developed may be applied to link other useful molecules to RNA oligomers with high efficiency.

David Yang *Chemical and Physical Biology*
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David R. Liu
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A Laboratory Approach to Exploring the

Relationship Between Protein Stability and Evolvability

A protein's ability to evolve to perform various biochemical tasks is of great interest. A greater comprehension of it would help us both develop a better understanding of how life evolved and more effectively engineer proteins with various activities. One current hypothesis states that stable proteins can more readily evolve than less stable variants as increased stability allows proteins to accept a wider number and range of mutations, many of which are destabilizing. Experimental evidence for this hypothesis, though, has been rather limited as traditional laboratory evolution methods are both time- and labor-intensive, often requiring days or longer with frequent human intervention for each round of evolution. Consequently, a detailed understanding of the relationship between protein stability and evolvability remains largely unknown.

Recently, researchers in the Liu laboratory have developed a method for laboratory evolution that allows for dozens of rounds of evolution to occur in a single day without human intervention. This method, phage-assisted continuous evolution (PACE), utilizes a modified life cycle of the M13 filamentous phage and Escherichia coli to rapidly evolve proteins by linking the activity of the protein to the reproduction of the phage. I am using PACE to experimentally explore the hypothesis that more stable proteins are more evolvable than their less stable cousins by using wild-type and thermostable variants of T7 RNA polymerase with very similar activities as starting points in evolution. Exploring the outcomes that each starting point provides after hundreds of generations has provided insights into the relationship between stability and evolvability.

I hope that this work will enlarge the current pool of knowledge concerning protein evolution. With this additional information, we can both more successfully engineer proteins and move one step closer toward understanding how life evolved.

Michael Zhang *Economics*
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Structural Characterization of Residues Involved in Lesion Recognition by MutM

MutM is a bifunctional bacterial DNA glycosylase that searches millions of undamaged DNA base pairs in order to identify, extrude, and cleave the mutagenic 8-oxoguanine (oxoG) associated with many cancers. We used X-ray crystallographic and disulfide-crosslinking techniques to elicit the individual impact of key residues along the protein-DNA interface. Four structures, solved to 1.85-1.95 Å resolution, capture the unique changes at the target sites of point mutation constructs bound to normal and mutated DNA. These complexes and supporting biochemical assays elicit the specific residues that offer kinetic control for the oxoG extrusion necessary prior to its excision. The results give insight into the accurate, energy efficient oxoG recognition methods developed by MutM that may be potentially generalized among other repair enzymes.

Computer Science

Spencer Chan *Computer Science*
Eliot House *Class of 2012*

Barbara Grosz & Ya'akov Gal
School of Engineering and Applied Sciences
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Cooperative Strategies in Multi-Agent Games

The annual Lemonade Stand Game Tournament was originally inspired by Repeated Prisoner's Dilemma tournaments. In the Lemonade Stand Game, three players simultaneously choose from twelve locations around the edge of a circular island and place lemonade stands at their chosen locations. Customers on the island go to the nearest stand. This process repeats with every player trying to place their stand and to draw the highest average number of customers per round. There are many ways in which two players could cooperate in this game, so agents must decide not only who to cooperate with but also how to do so. The 2011 Lemonade Stand Game generalizes the previously used format by specifying a distribution of customer density maps rather than a single map.

On uniform maps, the most successful form of cooperation was for two players to play directly across the map from each other. Our NewAcross agent was designed by taking key properties of this successful collaboration strategy and attempting to generalize them to the new non-uniform distributions.

One possible explanation for the success of the across strategy in previous tournaments is that it guarantees that both cooperators will do at least as well as the third player, regardless of what moves the third player makes. In the 2011 version of the game, playing geometrically across from a partner does not necessarily have this effect, but on any given map there are often several other configurations where two players may guarantee that they will do at least as well as the third player. By aiming for these configurations, NewAcross won 2nd place in the 2011 tournament this July.

The next step in our research is to evaluate how humans play the Lemonade Stand Game, both against each other and against computer agents.

Louise Hindal *Computer Science*
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One of the challenges for teachers in the classroom is keeping track of their students' learning. In a classroom of 30 students, it is practically impossible for a teacher to get a sense of what every student is doing, and this makes it harder to provide appropriate assistance and guidance. Computers and educational software in classrooms provide a promising avenue for addressing this issue, but are not immediately helpful without the appropriate feedback to teachers about their students' actions.

This project is meant to aid teachers by providing them with automatic analysis of students' activities using such software. Within open-ended educational software, students can solve problems in many different ways, performing different steps in different orders to get to the same desired answer. The algorithms we are developing take logs of individual student actions within such open-ended pedagogical software, and analyze their steps to report to teachers the methods used to solve problems.

Specifically, the project is working with an open-ended chemistry program, Virtual Lab created by The ChemCollective and researchers at Carnegie Mellon University. I am looking at specific chemistry problems, attempting to determine whether a computer can accurately determine distinctions in the ways we have observed students go about solving these problems. I am implementing algorithms to compile high-level features from the collected data of the student interactions. I hope to utilize these features when using techniques from the field of machine learning to cluster and classify what students are doing. I also hypothesize that displaying these features themselves will help teachers understand their students' interactions with the software more completely.

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Verified Analysis of Untrusted Code

Increasingly, companies are creating websites that offer features that once existed only in installed programs. These applications offer many advantages, such as being accessible from any computer that has an internet connection. However, they suffer from two key disadvantages. First, they are often not as fast as more traditional programs. Second, preexisting software libraries are typically incompatible and must be rewritten. These issues stem from the fact that these web applications are not written in the low level machine code that the user's processor can execute directly. Instead, they are written in a higher level language that the web browser then interprets and runs. This interpretation process is not as efficient as direct execution of machine code. Although executing machine code directly would be faster, it poses security risks because such code would have more direct access to the computer it runs on.

Previous attempts to have web browsers that can download and run machine code have typically relied on asking users whether they trust the website they are visiting. This has historically been a source of many security compromises, because users have trouble figuring out what websites to trust. More recent efforts to remedy this issue include validators in the browser that can analyze the machine code of a program and determine if it adheres to certain security policies. However, it is often difficult to ensure the correctness of these validators because of their complexity.

This work seeks to provide strong guarantees about the correctness of such validators. To that end, we are constructing a formal semantics of Intel x86 machine code in the Coq programming language. The Coq programming environment allows one to construct software and prove theorems about the properties of this software. Using this functionality, we are proving the correctness of validator programs with respect to our formal model of the processor.

Earth and Planetary Sciences

Richard Alt *Earth and Planetary Sciences*
Mather House *Class of 2012*

John Shaw
Department of Earth and Planetary Sciences
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Mechanical Modeling of Thrust Faults and Characterization of Fault Propagation Folds

Thrust faults localize brittle deformation of the earth's crust in an environment of compressive tectonic stress. Under intense stress, rocks fracture and fail at local points of weakness, eventually forming a fault plane along which older rock strata are forced upward over top of younger rock strata. Uplift along networks of thrust faults over geologic time is the process by which most of the highest and most expansive mountain ranges on earth formed, including the Himalayas, the Rocky Mountains, and the Andes. Because many of these regions remain tectonically active, there is an important human interest in identifying and quantifying the earthquake risks associated with these faults. Economically valuable oil and natural gas deposits are often trapped inside subsurface folded structures that formed with ancient thrust belts and these natural reservoirs can be ideal sites for CO2 sequestration.

Broadly speaking, fault-fold systems are divided into two distinct structural styles: fault bend folds and fault propagation folds. These two styles of deformation define very different relationships between the fold geometry and the behavior of the underlying fault. Fault bend folds form when a change in the dip angle of an underlying fault ramp localizes strain at the apex of the bend. Fault propagation folds form when the slip on a fault ramp dies off and the fault cannot penetrate overlying rock strata. This type of fault is known as blind thrust, and its presence is not obvious to an observer at the surface. When the fault slip falls to zero, the continuing upward propagation of rock from below results in an accumulation of compressed and folded material near and above the fault tip. To effectively identify and analyze blind thrusts, it is necessary to quantify and characterize this folding behavior with numerical models and geophysical measurements of the subsurface, such as seismic sections. In the Shaw Lab, we are modeling the formation of these folds with a two-dimensional Discrete Element Method (DEM) mechanical model. The goal is to use data from the surface-exposed fold to interpolate the behavior of the deeper fault underlying the fold. Our codes simulate the evolution of a thrust fault over geologic time on a scale of kilometers, with the intent of generating both types of structures in the model and identifying the physical properties that control the transition between them. The simulated physical conditions of the model can be used to evaluate existing kinematic models and theories of stress fields and fold development, which have proven insufficient to describe the full range of structures observed in the field. The advantage of a mechanical model, like our DEM simulation, is that it allows for a robust treatment of the forces acting on each parcel of rock as it is stressed, sheared, and resettled during the incremental compression process. Our experiments are intended to improve the quality of seismic hazard assessments for blind thrusts and aid in economic decisions for oil and natural gas exploration by better defining the geometry of subsurface fossil fuel traps.

Matt Mulroy *Earth and Planetary Sciences &*
Mather House *Environmental Science and Public Policy*

Steven Wofsy *Class of 2012*
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The measurement of atmospheric carbon dioxide (or CO2) concentrations represents a major subject in the field of atmospheric sciences because of its relevance towards global warming. Instruments for precise CO2 measurement have been available for decades but temporal and spatial variations complicate the estimation of CO2 surface fluxes. Photosynthesis and respiration from the biosphere create diurnal variations in CO2 levels much larger than anthropogenic contributions, and these variations depend on spatially-variant vegetation type (i.e. forests, grasslands). Additionally, the atmosphere transports CO2 which makes it difficult for researchers to locate and quantify CO2 emissions from point sources (i.e. power plants, factories).

Particle transport and biosphere models are employed to estimate CO2 surface fluxes. For my research, I use the Stochastic Time-Inverted Lagrangian Transport (STILT) Model which releases imaginary air parcels backwards in time from a receptor point (location of interest). The model incorporates wind data to stochastically send parcels back to their sources, and it maps the CO2 footprint (fraction of CO2 emissions reaching the receptor point) of locations upwind. I will use the Vegetation Photosynthesis and Respiration Model to account for the biosphere's influences on the STILT footprints. The model calculates photosynthesis and respiration rates based on vegetation type, the latter which can be classified into 1 of 12 types through use of satellite image data (based on landscape color). Datasets for modeled CO2 emissions are also available which I use alongside the calculated CO2 footprints to estimate the CO2 fluxes from different locations. The above models and data are validated by comparing modeled CO2 surface fluxes with observed CO2 measurements (minus background CO2 conditions).

My area of focus is CO2 fluxes in the Los Angeles Basin. I will be using CO2 and meteorological data from a large-scale atmospheric measurement campaign called CalNex 2010. My summer project is to calculate CO2 surface fluxes in the basin and map out sources of emission on a high-resolution latitude/longitude grid.

Engineering & Bioengineering

Daniel Bruder *Engineering Sciences*
Cabot House *Class of 2012*

Rob Howe
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Mitral valve prolapse, also known as "floppy mitral valve", is a condition that impedes the proper function of the heart muscle. For those with severe prolapse, surgery is essential. In most cases, mitral repair surgery is done to fix the pathological valve. Generally, mitral repairs require the heart to be stopped during the procedure, while the patient's blood is circulated artificially by a bypass machine. However, stopping the heart for extended periods of time, even with induced circulation by the bypass machine, can cause brain damage and other long term complications. The goal of this project is to create a handheld surgical tool that can be used to quickly and reliably deliver a small clip to the mitral valve in a beating heart, tightening it and restoring its functionality.

Jimmy Huang *Engineering Sciences*
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David A. Weitz
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Nanocrystallization with Colloidal Templates

Colloidal templates are tightly packed molds of spherical particles that allow nanocrystal fabrication to occur within the interstitial spaces of the template with the degree as well as size of crystallization to be controlled through parameters such as temperature and cooling rate. To produce crystals within these interstitial spaces requires a solid understanding on how a sample's state of matter behaves at certain temperature ranges as well as the underlying physical and chemical interactions occurring between the template and drug surfaces. A little known problem in the pharmaceutical world is that many orally ingested drugs do not optimally break down within the watery environment of the human stomach which thus delays treatment. Colloidal templating technology can be applied to current pharmaceutical drugs to enhance their performance by methodically engineering the size of the nanocrystalline drug during the drug encapsulation stage as well as to optimize their delivery in the human body by increasing the surface of the drug. However crystallization within such tight confinements is not perfectly understood and is not completely uniform. These imperfections within our template, which limits our control over the template's structural integrity, impede our goal to use this technique in the pharmaceutical industry. The intent of our research is to delve further into this technique of nanofabrication in order to understand the physical and chemical interactions during the crystallization phase and hopefully discover the reason and a solution to the imperfections observed in our earlier attempts of nanocrystallization with colloidal templates.

Danielle Ithier *Engineering Sciences*
Cabot House *Class of 2014*

Robert Wood
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Micro Air Vehicles (MAVs) have numerous future applications, including search and rescue missions and exploration of environments that are unsafe for humans. Their size and maneuverability give them advantages over robots currently used for such jobs because they are able to enter and operate in extremely small spaces. However, the small size of MAVs implies a low Reynolds number with increased boundary layer effects, resulting in an unfavorable aerodynamic flight regime.

We hypothesize that airfoils resembling the shape of butterfly wings result in MAVs being able to mimic the aerial locomotion abilities exhibited by butterflies, therefore leading to a more energy-efficient flight. Studies of wing shape and insect flight kinematics have been done in the past, but such studies have not systematically explored wing shape variations. In addition, previous studies have typically treated the forewing and hindwing as a single body and have largely focused on insects other than butterflies.

The objective of this study is to understand how butterflies hold their wings while flapping and gliding by studying the orientation of the forewings and hindwings. This is achieved through analysis of high-speed footage taken during tethered flight of Monarch butterflies (Danaus plexippus) inside a low-speed wind tunnel and by examining 3D trajectories of Monarchs in free flight. Based on the results, we hope to gain insight about the optimal wing shape for devices such as MAVs and to further biologists' understanding of butterfly flight.

Christopher Madl *Engineering Sciences*
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David Mooney
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Directing Mesenchymal Stem Cell Fate with Peptide Mimics of Soluble Growth Factors

One of the major challenges facing regenerative tissue therapies is maintaining the ability to locally direct the fate of transplanted stem cells in vivo. Most often, soluble chemical signals, such as growth factors, are delivered along with the stem cells to influence

their differentiation. However, such soluble growth factors have significant drawbacks, including the possibility of undesirable secondary effects in surrounding tissues. Recent work has focused on the development of peptide mimics of many soluble growth factors, which have shown comparable bioactivity to the recombinant growth factors and are significantly less expensive to manufacture. Furthermore, these peptides may be conjugated to materials-based delivery systems to localize the signaling cues to the site of interest. This project aims to decrease the healing time required for critical sized bone defects by transplanting mesenchymal stem cells (MSCs) to the injury site on macroporous alginate hydrogel scaffolds to which peptide mimics of bone morphogenetic proteins (BMPs) have been covalently bound. Peptides derived from BMP-2 are synthesized using solid-phase Fmoc chemistry and coupled to alginate using carbodiimide and "click" chemistries. In vitroexperiments focus on identifying the role of the apparent valency of the peptide and the effects of crosstalk with integrin signaling on osteogenesis. Differentiation of the MSCs in vitro is quantified by measuring alkaline phosphatase activity and confirmed by staining for mineralization. Future in vivo studies may include subcutaneous implantation of the scaffolds in mice to assess ectopic bone formation and an injury model, such as a femoral defect in rats, to assess wound healing.

Terrence Barry McKenna *Mechanical Engineering*
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Robert Howe
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Robust Robotic Grasper via Shape Deposition Manufacturing

Robotic systems have already found widespread use in industrial applications; however, their use in domestic applications has been limited. This transition requires many obstacles to be surmounted, in particular effective and robust robotic grasping in unstructured environments.

To date, researchers have successfully built several sophisticated and versatile robotic hands that feature many degrees of freedom and precision control. However, these are extremely delicate, require full knowledge of one's working environment, andcost hundreds of thousands of dollars each making them unpractical for domestic use. Our research aims to design and prototype a robust, inexpensive robotic hand for use in unstructured environments. To achieve this we are employing a technique known as shape deposition manufacturing (SDM).

SDM is an iterative and incremental process involving the overlay of various polymers in a mold to create complex, monopartite apparatuses with embedded components. The resulting system is extremely robust as it is a single part; it requires no fasteners, significantly improving its durability and range of elastic deformation. The internal components are embedded incrementally directly into the part's structure, offering precision placement, long-term position stability, and reducing the likelihood of damage. Furthermore, the process enables a single part to exhibit several material properties as different polymers can be used for various iterations; the use of polymers also decreases material cost.

Nicholas Perkons *Biomedical Engineering*
Kirkland House *Class of 2014*

Peng Yin & William Shih
Wyss Institute for Biologically Inspired Engineering

Engineering DNA Nano-Structures that Contain and Controllably Release Cargo

Nanotechnology is a rapidly expanding field, especially with respect to the subcategory of DNA nanotechnology. Using ssDNA extracted from various bacteria, scientists have the ability to form virtually any two or three-dimensional structure that uses this ssDNA source as a "scaffold" path for a desired structure. Complex structures and curvatures can be induced through the engineered addition of many short, 20-80 base-long, oligonucleotides, or "staple sequences," complementary to the scaffold and capable of bending the single-stranded DNA through a defined two or three dimensional path. The complexities and curvatures achieved are a result of the relatively short persistence length of the single-stranded scaffold, or its ability to change direction over a short distance. While the process of engineering the appropriate staples for the formation of a specific structure is extremely tedious when done by hand, an open source software program called caDNAno that was released in 2009 has largely helped to automate this procedure. As a subfield of DNA nanotechnology, the above-described process of engineering structures using a single scaffold and many staples is known as DNA origami.

Given the ability to make nearly any structure using the techniques of DNA origami, my team and I are working towards making this robust process medically relevant. We have both replicated the design for a spherical origami structure and have designed our own DNA origami Box. Our project aims to develop a process for controllably opening and closing our structures using a combination of strand-displacement and photo-cleavable locks, which are opened upon either the introduction of a key strand that binds to a DNA toehold or the introduction of visible light, respectively. As a secondary goal, we hope to be able to attach cargo to the inside of our structures upon initial folding, solubilize them within our structures after folding, and then release them upon controlled opening of our structures. Similar to the mechanisms for opening our structures, we have designed a mechanism to attach and later solubilize cargo inside of our structures that is dependent upon the use of strand-displacement and photo-cleavable locks. While our process for attaching cargo could theoretically accommodate cargo as small as a DNA or RNA strand, we are currently using a 5nm gold particle coated with a DNA oligonucleotide sequence as cargo for ease of classifying our results. This is because a 5nm gold particle is extremely visible under TEM. It is our goal to be able to image the attachment of our cargo within our folded structures, and then image the controlled release of this cargo upon the introduction of the appropriate key signals. Furthermore, we believe these processes will help the DNA nanotechnology community come closer to their goal of a in-vivo application of DNA

origami that may one day include an origami-based pathogen detection system or drug delivery mechanism.

Hillary Singer *Undeclared*
Mather House *Class of 2014*

Donald Ingber
Wyss Institute for Biologically Inspired Engineering

Impact of Gel Stiffness on Growth of Lewis Lung Carcinoma Cells

It is has recently been established that tensional changes in the extracellular matrix, which surrounds cells, alter cell shape and behavior. This finding is one of the emerging fields of mechanical biology that examines how mechanical forces govern cell behavior. These forces include tension, sheer stress, and traction. Still, many of the changes in cell behavior remain unstudied. In this study, we aim to characterize the differences in cell proliferation, mRNA levels, and endothelial responses among Lewis Lung Carcinoma (LLC) cells grown on varying stiffnesses of gels in vitro and in vivo. We are interested both in cell cycle and structural changes of the LLC cells and in the resulting effects on the growth factors involved in angiogenesis and the appearance of blood vessels around tumors developed from gels in vivo. Specifically, we quantify the relative amounts of the angiogenic growth factor VEGF and the angiogenic inhibitory factors TSP1 and TSP2. We also observe the histology of the gels grown in vivo for evidence of tumors and vascular development. Finally, we count the differences in proliferating cells and observe the cytoskeleton differences in shape and tension. An additional goal of the study is to map the tumor growth and mRNA levels of LLC cells grown in vivo as a function of time.

Carol Tran *Undelcared*
Kirkland House *Class of 2014*

Guillermo García-Cardeña
Department of Pathology
Harvard Medical School

Biomechanical Forces in the Specification of Human Hemogenic Endothelial Cells

Further understanding the critical steps of targeted stem cell differentiation is necessary to gain insights into fundamental developmental processes and advance the potential of regenerative medicine. It is now clear that both intrinsic genetic factors and environmental cues are important for stem cell differentiation. In particular, Runx1is a transcription factor that serves as a master regulator of hematopoiesis, and its expression is tightly regulated by different factors. Our laboratory and others have previously established that the onset of the heartbeat in vertebrates stimulates Runx1 expression in hemogenic endothelial cells located on several sites of the developing cardiovascular system such as the ventral aspect of the dorsal aorta. Nevertheless, the precise role exerted by biomechanical forces on the vascular wall in determining hematopoietic potential is not well understood. Therefore, we are interested in examining the effects of hemodynamic shear stress, the frictional force that blood tangentially imposes on the endothelium as it flows through vasculature, on hematopoiesis. Our previous in vitrostudies with mouse embryonic stem cells demonstrate that hemodynamic shear stress increases both Runx1expression and hematopoietic colony-forming potential in CD41+c-Kit+ hematopoietic progenitor cells.

In order to begin translating these findings to the human system, we have begun studying the effects of hemodynamic shear stress on hemogenic endothelium derived from embryoid bodies (EBs) generated from human induced pluripotent stem (iPS) cells. To this end, we first characterized the presence of hemogenic endothelium in these EBs by performing immunoflourescent staining for VE-cadherin, an endothelial cell adhesion molecule, and CD45, a protein tyrosine phosphate present in hematopoietic cells at days 15, 16, 17, 18, 19, and 21 into differentiation. Using two-photon excitation microscopy, confocal microscopy, and fluorescence-activated cell sorting (FACS) analysis, we detected VE-cadherin+CD45+cells in day 18 EBs. We also detected single-positive cells with either cell marker including VE-cadherin+ cells that have begun to segregate into network-like structures by day 17. Next, we used the same techniques to characterize both the presence of Runx1, which is known to precede the expression of CD45, and its colocalization with VE-cadherin. Taken together, the presence of VE-cadherin+CD45+ cells documents the existence of endothelial cells with hemogenic identities within EBs. Future experiments will include exposing VE-cadherin+CD45+ cells to hemodynamic shear stress using the Dynamic Flow System previously developed in our laboratory. Our investigation of the role of biomechanical forces in hematopoietic development may be critical to fully understanding the process of hematopoiesis and potentially improving hematopoietic stem cell transplantation therapy used for patients with hematologic cancers and diseases.

Mathematics & Statistics

Levent Alpoge *Mathematics*
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Benedict Gross
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Fourier Analysis on the Adèles: Tate's thesis and Generalizations

In algebraic number theory, associated to each global field k is its ring of adèles A_k, the restricted direct product of all completions of k. One can view this ring as a generalization of $Z \subset R$, in that k embeds as a discrete and cocompact subset of A_k. A_k turns out to be a locally compact group that is Pontryagin self-dual, allowing for an interesting Fourier theory. Tate, in his thesis, was the first to follow this through, and was able to prove the analytic continuation and functional equation of Hecke's L-functions by considering both A_k and the associated group of idèles I_k for any number field k. We study this proof, as well as the structure of the adèles and idèles, with an eye towards generalizations in the vein of the Langlands philosophy. The results of Tate's thesis, as well as the development of the idèlic viewpoint in class field theory, are viewed as the beginnings of the Langlands program, which gives a non-abelian class field theory via a conjectural connection between Artin L-functions and automorphic representations.

Katie Banks *Mathematics*
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Joe Harris
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We frequently need to determine properties of some object based on a limited set of measurements we can take of its outside. The best possible situation is that the values of some function on the outside determine its values everywhere on the object completely – a situation known as the Dirichlet problem. This happens, for example, with electric charge: the distribution of charge on the boundary determines completely the electric potential inside the object. In other applications, the measured scattering of light or sound off an object can tell us about its shape, and geologists use recorded echoes to map oil, bedrock, and other crustal features. The starting point of this project is an explanation of the solution to one such inverse problem: whether we can determine the shape of a membrane in the plane, or a more general manifold, knowing only the overtone series it produces when struck. (This problem became famous when appealingly posed as "can you hear the shape of a drum?" – a nice case study in how good PR can revitalize whole research programs.) It turns out we can't; but on the way to solving this problem, mathematicians established several interesting results on what can be determined from the overtone series. We explain some of the acoustical implications of these results, while also touching on the surprising connections between number theory and physics that this problem illustrate well.

Osbert Bastani *Mathematics*
Dunster House *Class of 2012*

Barry Mazur
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Elliptic Curves: Structure and Applications

Elliptic curves are defined to be the set of points (x,y) satisfying y^2 = x^3 + ax + b, where x, y, a, and b are complex, real, or rational numbers, or numbers in some finite field. Such mathematical objects have applications ranging from algebraic geometry and number theory in pure mathematics to cryptography and coding theory in theoretical computer science. In this group, P+Q+R = 0 for any three collinear points P, Q, and R in the curve. This gives the curve interesting symmetries and arithmetic properties.

In particular, I am studying the Mordell-Weil theorem, which shows that the group of rational points on an elliptic curve is a finitely generated abelian group. Mazur's theorem additionally demonstrates that the order of the torsion of the abelian group is bounded. Another important notion related to elliptic curves is the theory of modular curves, which parametrize the space of elliptic curves. This is an algebraic variety with interesting properties, and the study of complex functions on modular curves, called modular forms, plays a central role in number theory.

I am also studying the applications of elliptic curves to cryptography and to coding theory. Cryptography is an important field attempting to create and break encryption schemes that allow people to share information over insecure channels. Elliptic curve cryptography takes advantage of the difficulty of the discrete logarithm problem, i.e. inverting "exponentiation" on the group of rational points in an elliptic curve. These curves also have applications in coding theory, where the modular curves are used to construct good codes. These generalize the famous Reed-Solomon codes that are important in information theory and communication.

Roxana Feier *Mathematics*
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Network Reconstruction of the Wnt

Pathway Using Discrete Dynamical Systems

The Wnt signalling pathway is a complex biological network involved both in embryogenesis and adult tissue homeostasis, whose components are often mutated in various types of cancer. Understanding how perturbations to this system affect its function is thus crucial for drug design, and requires an accurate determination of both the topological structure and the dynamics of the pathway. In our project, we use tools from algebraic geometry and optimization to create a quantitative model.

Given time-series data for some of the pathway components, the first goal is to produce the dependency graph of the network, which describes the proteins and complexes that interact with one another. This reverse engineering problem is approached by fitting the observed data to polynomials over finite fields, using techniques from computational algebraic geometry. If the polynomial evolution of the concentration of one species depends on the concentration of another, the two components are connected in the dependency graph and presumably interact at the chemical level. Prior information about the network, such as pairs of species that are known to be interacting, can be incorporated into the model. To further improve the accuracy of this method, we apply the main algorithm several times under slightly different conditions, sampled from a combinatorial structure known as a Gröbner fan. Only the edges that appear in a significant fraction of these samples are included in the final dependency graph.

Our second goal is to use the time-series data for parameter estimation, assuming that the pathway can be described by a system of ordinary differential equations (ODEs). Specifically, we need to find the values of the reaction rate constants and total concentrations of the different species that best reproduce the observed data. This is approached as an optimization problem, and leads to the calibration of the model, which then can be used to study the response of the pathway under new perturbations.

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Modular Curves and Modular Forms

In 1637, Fermat famously conjectured that there are no positive integer solutions to the equation $a^n + b^n = c^n$ for $n > 2$. This seemingly innocuous problem resisted the best efforts of mathematicians until the mid 1990s, when it was finally resolved by the celebrated Modularity Theorem, which expresses a deep relationship between important number-theoretic objects: modular forms, elliptic curves, and Galois representations. One form of the theorem asserts that all rational elliptic curves are parametrized by certain comlex manifolds called modular curves.

This project seeks to understand the properties of these modular curves and their connections to other aspects of number theory. In particular, we studied the problem of realizing algebraic models for these curves and computing the representations of certain matrix groups on their spaces of differential forms.

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Exploring Degeneracy in Statistical Models

Statistics is inherently cross-disciplinary, and physics lies at the heart of many statistical problems. Particularly exciting is the field of computational physics, which implements numerical algorithms to solve complex problems. Applications of computational physics include Markov Chain Monte Carlo techniques, which are used in a variety of fields – from physics to economics and even to literature.

This project lies at the interface of statistics, physics, and computing. We try to address the common degeneracy problem in statistics, a phenomenon described below which arises in many of the most frequently used models for biological, social, and technological networks. Often, statisticians use a Bayesian technique for model checking called posterior predictive checking to generate "replicated data" from the estimated model and then assess whether the real data resemble the replicated data. When degeneracy occurs, the replicated data look absurdly extreme, suggesting that another model should be used instead – which is hard to anticipate in advance with current knowledge, especially since these kinds of models have many good statistical properties. Not much research has been done to determine for what models degeneracy will occur; this is the main focus of our research this summer. The degeneracy problem has direct applications to physics and computer science, as studying interactions between particles and within networks closely depends on finding models that fit the data well.

We use Markov Chain Monte Carlo techniques to simulate and estimate models which are fundamental to computational physics. We specifically work with the Exponential Random Graph Model (ERGM), which is widely used for complex networks and is closely related to Gibbs models in statistical physics. We explore the degeneracy problem further by working with the Ising model, which describes interactions of magnets through modeling the behavior of the spins that define them. Through our research, we hope to gain a thorough understanding of the natural connections between statistics and physics. On a day to day basis, we conduct background research, construct models, and run simulations in an attempt to uncover the models for which degeneracy occurs. Hopefully, our research will lead to a better understanding of degeneracy and its causes.

Molecular and Cellular Biology

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Mechanism of Programmed Ribosomal Frameshifting in HIV-1

Research into disease-causing retroviruses focuses on vulnerable steps in the viral lifecycle. Programmed ribosomal frameshifting controls a crucial step in the lifecycle of the Human Immunodeficiency Virus type I (HIV-1). HIV-1, which causes the Acquired Immune Deficiency Syndrome (AIDS), destroys cells by mass replication using the cells' own machinery, including ribosomes, which are enzymes that translate mRNA into a polypeptide. mRNA is usually read sequentially in consecutive codons of three bases, but under certain conditions the ribosome can frameshift, jump back or ahead by one or two bases, shifting the sequence of codons read. In HIV-1 and other viruses frameshifting occurs due to the presence of two sequences in mRNA: the slippery sequence and frameshift signal, a folded tertiary RNA structure. HIV-1 produces two essential poly-proteins, Gag and Gag-Pol at a level of about twenty to one, tightly controlled by frameshifting; an imbalance reduces viral infectivity. Both gag and pol are on the same mRNA transcript, but in different reading frames, thus the ribosome must frameshift in order to avoid a stop codon and translate pol after gag, producing the fusion poly-protein Gag-Pol. Previous work in the lab on the Murine Leukemia Virus (MLV) which has a similar mechanism for regulating its viral proteins provided a basis on which to study HIV-1 -- frameshifting could be regulated solely by the structure formed by the RNA frameshift element, working as a type of mRNA switch. I am researching the different structures that the HIV-1 RNA frameshift element can form to determine the mechanism for frameshifting, to provide a new basis for therapeutic drugs.

Ainsley Faux *Biomedical Engineering*
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Microbial Approach to the Extraction and Recovery of Tellurium and Indium

As advancements in clean energy technology are made, solar cells are likely to play a larger role in satisfying the world's energy needs. Two of the primary materials used in producing thin film photovoltaic cells that ultimately find their way into solar cells are copper-indium diselenide (CIS) and cadmium telluride (CdTe). Both indium and tellurium are among the rarest elements in the earth's crust; thus, the extraction of these metals presents a critical obstacle in the proliferation of these devices. Furthermore, current methods of refining these elements, which rely on acid leaching and cementation, are environmentally damaging, energy intensive, and inefficient.

The goal of the project is to develop a method of using deep sea hydrothermal vent microbes to concentrate the metals from unrefined sources, such as mine tailings and copper anode slime.Before this is possible, however, we must first understand the nature of the interaction of the microbes with the metals. This includes, but is not limited to, determining how well the microbes can survive in high concentrations of the metals, whether or not the microbes can safely metabolize the metals, whether or not the microbes are capable of converting the metals from one form to another (oxidation, reduction, methylation, etc.), and what proteins are likely involved in these processes. As these questions are answered, we come closer and closer to developing a solution to this problem.

Catherine Gu *Human Developmental and*
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PD-1 (Programmed Death-1) is a coinhibitory receptor expressed on T cells, and suppresses immune responses when activated by its ligands PD-L1 and PD-L2; this pathway plays an important role in maintaining immune homeostasis andpreventing autoimmunity. Treatment with antibody that blocks PD-1 from binding with its ligands, removing the brakes from the immune response, has shown promise in clinical trials for the treatment of melanoma. Though PD-1 is mainly expressed on T cells, found constitutively on CD4 T-regulatory cells, it and its ligands, PD-L1 and PD-L2, are also expressed on various other cell types of the immune system, including APCs (antigen presenting cells) such as dendritic cells and macrophages, and little is known about the role of PD-1 on these cells. The goal of this project was to characterize this pathway on dendritic cells. First, we demonstrated PD-1 upregulation upon stimulation of dendritic cells with poly I:C (polyinosinic: polycytidylic acid). Next, we developed a conditional knockout mouse by flanking the PD-1 locus with loxP recombination sites and crossing them with mice that had Cre recombinase expressed in CD11c positive cells (a dendritic cell marker), effectively deleting PD-1 in all dendritic cells. Dendritic cells from these CKO mice were isolated and compared to dendritic cells from wild-type mice, using flow cytometry to compare cytokine production andupregulation of activation markers in order to find differences in immune response activation. We intend to analyze these mice in vivo experiments involving infection and autoimmune disease. We anticipate that the PD-1 conditional knockout mice will be more resistant to infection due to the removal of this immunosuppresion pathway. These results should shed light on the role of the PD-1 pathway in APC function and in the innate immune response, and determine whether PD-1 is a viable therapeutic target for acute infection and its complications, such as sepsis.

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Microtubule (MT)-based transport is the basis for all long distance intracellular transport in eukaryotic organisms, making it integral for most eukaryote's survival and proliferation. MT-based transport is driven by two classes of motor proteins, dynein and kinesin. Dyneins walk towards the minus-end of MTs and thus are used for retrograde transport. The majority of kinesins walk towards the plus-end of MTs and function mostly in anterograde transport. MT-based transport is responsible for the correct localization of proteins, mRNAs and many organelles, including the nucleus, preoxisomes, mitochodria, and endosomes. MT-based transport is particularity important for neuronal development in humans and other animals. As neurons develop the nucleus must migrate from one end of the cell and back again, a process known as interkinetic nuclear migration. MT- based transport is responsible for this migration making it a critical component of brain development. Defects in the MT-based transport system, specifically in dynein and dynein-associated proteins, have been implicated in neurodegeneration including such diseases as Alzheimer, Parkinson's and ALS. Dynein and kinesins are motors responsible for the transport of multiple cargoes to many locations at specific times within the cell cycle. Currently the mechanism through which dynein and kinesin achieve such specificity is unknown. We hypothesize that there are other proteins associated with dynein, kinesin and/or their cargo that are responsible for this regulation. In order to identify these proteins we have set up a screen in the model organism, Aspergillus nidulans. Mutants with phenotypes similar to dynein and kinesin knock-out phenotypes will have mutations in proteins important in the MT-based transport system.

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Peripheral arterial disease affects about 12 million people in the United States, and this number is expected to more than double by the year 2050. Vein grafts remain the therapeutic mainstay treatment for peripheral vascular disease. However, recent clinical trials have shown a high rate (~40%) of failure of these conduits within the first year alone. There is growing evidence supporting an "outside-in" hypothesis of vascular inflammation, whereby changes initiated on the vessel wall periphery play an important role in mediating the progression of vascular disease. Adipose tissue is an important component of this peri-graft environment. Using a murine model of standard surgical trauma, RT-PCR analysis of unregulated pro and anti-inflammatory cytokines and immune cell markers including TNF-a, Il1b, Mmp2, Mmp9, Tgfb1, Il10, etc and immunohistochemistry, we characterize the adaptive response of adipose tissue to standard surgical trauma in the setting of dietary manipulations that can alter the fat phenotype. The magnitude and duration of the induced response to injury under these conditions may have an impact on vein graft adaptations.

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Visualization of Histone-Like proteins in Synechococcus elongatus using FISH

To optimize their survival, many organisms have evolved timing mechanisms. These organisms use internal timepieces, circadian clocks, to sense the natural, predictable rhythms of the earth (sunlight, temperature, etc.). In cyanobacteria, specifically Synechococcus elongatus, the circadian clock drives rhythmic expression of over 30% of the genome. Chromosome compaction is regulated by a circadian clock in S. Elongatus. Since histones (proteins responsible for packaging DNA in eukaryotes) are not present in bacteria, 'histone'-like proteins are responsible for assisting chromosome compaction in prokaryotes. Until now, chromosomes have been looked at using fluorescence microscopy. As is the problem with conventional microscopy, resolution and the clarity of small components within the cell is lacking. Spatial resolution is limited by the diffraction limit of light, so the spatial dynamic of the chromosome compaction is masked. Using Fluorescence in situ hybridization (FISH), we will visualize the localization to histone like proteins hup-B1 and hup-B2 fused to fluorescent protein CFP.

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Gary Ruvkun
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Constructing a System for Tissue-Specific Ribosomal Profiling in *C. elegans*

The study of protein translation provides a deeper understanding of the regulatory mechanisms, such as microRNAs and a number of stress responses, controlling the phenotypic levels of translation. Ribosome profiling has recently emerged as a method for genome-wide examination of these levels. It was previously known that the position of a translating ribosome could be precisely determined by the fact that a ribosome protects a fragment (~30 nt), or footprint, as they are called, on its mRNA template from nuclease digestion. By deep-sequencing tens of millions of these ribosome footprints, we can analyze the global translational profile with nucleotide precision.

We are now working on constructing a system for tissue-specific ribosomal profiling in *C. elegans,* an organism not previously employed for this purpose. Our approach entails building a transgenic *C. elegans* line expressing the ribosomal protein L-18 tagged with FLAG, driven by a tissue-specific promoter. Following the affinity-purification of poly-ribosomes (polysomes) by beads conjugated with anti-FLAG antibody, the polysomes are treated with nuclease. After the nuclease

digestion, the ribosome footprints are isolated and converted into a DNA library, which will be subjected to the deep-sequencing analysis to generate a comprehensive view of protein translation.

Ribosomal profiling has formerly only been performed in yeast, a unicellular organism, therefore the use of *C. elegans* will present us with the opportunity to see tissue interaction and communication at the translational level. In addition, we are collaborating with another lab to study the plasticity of neurons using this system. We are specifically interested in the dynamics of the global translation profile during the neural development.

Andrew Kennard *Applied Mathematics*
Quincy House *Class of 2013*

Erin O'Shea
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Systematic Mapping of Interactions in the *Synechococcus elongatus* Two-Component System Regulatory Network

Two-Component Systems (TCSs) form a class of signal transduction pathways found throughout the bacterial kingdom, as well as in some fungal and plant species. A TCS is comprised of two proteins: a Histidine Kinase (HK), which is phosphorylated on a conserved Histidine residue in response to environmental signal; and a Response Regulator (RR), which receives the phosphate from the HK on a conserved Aspartate. The RR is activated by this phosphorylation and proceeds to effect the cellular response to the signal, typically through transcriptional regulation. It has been shown that HKs phosphorylate a specific subset of the RRs in a genome, referred to as the cognate RRs of that HK. A complete understanding of an organism's TCS network requires a complete list of its cognate HKs and RRs. Fortunately, HKs show significant in vitro preference for their in vivo cognate RRs, opening this problem to biochemical analysis. Using a system-wide technique that compares the rates at which each HK phosphorylates each RR, one can create a comprehensive map of all the two-component interactions occurring in a particular organism. This summer I applied these methods to study the TCSs of the cyanobacterium Synechococcus elongatus, an organism amenable to this work due to the manageable number of TCSs in its genome. Beyond further characterizing the activity and lifestyle of S. elongatus, this project could contribute to a better understanding of the composition and organizing principles of signal transduction networks in general, shedding light on how cells can efficiently and robustly respond to their perilous, ever-changing environments.

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Role of Transcription Factor T-bet in Inducing Pathogenic Th17 Cells

IL-17-producing TH17 cells are often present at the sites of tissue inflammation in autoimmune diseases, implying that TH17 cells are one of the main drivers of autoimmune tissue injury. Cytokines such as IL-17 produced by TH17 cells act on tissue cells to promote tissue inflammation by inducing pro-inflammatory cytokines such as IL-1, IL-6, TNF-a, IL-8 and matrix metaloproteinases. Th17 cells express an orphan nuclear receptor protein called RoRgt, which acts as a master transcription factor for the generation of Th17 cells. Loss of RoRgt in RoRc (the gene encoding RoRgt) knock-out mice (RoRc-/-) results in complete loss of TH17 cells, and these RoRc-/- mice are resistant to development of a number of autoimmune diseases. Accumulating data, however, shows that not all IL-17 producing TH17 cells are pathogenic. In addition to RoRgt, pathogenic TH17 cells express another transcription factor called T-bet whose role in TH17 pathogenicity is currently unclear. Using whole genome Chromatin immunoprecipitation followed by the sequencing (ChIP-seq) technique in TH17 cells, we began to identify the genetic elements that are bound and transactivated by T-bet in Th17 cells. The long-term goal of this project was to study the role of each of the T-bet dependent genes in inducing pathogenic TH17 cells. In order to accomplish this, we identified particular genes that were either over-expressed or under-expressed from the ChIP-seq analysis. We used bacterial clones to introduce the gene of interest into the T-bet knock out T-cells and tested them for pathogenicity to help determine the role of T-bet in TH17 cells. Our work on investigating these pertinent genes will continue using the same cloning technology to identify the gene that could be critical in autoimmune diseases such as Multiple Sclerosis.

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Cell Lineage Analysis of a Human Pancreatic Tumor

The mechanism for cancer development and progression remains not fully understood. Specifically, genetic instability is prevalent in cancer, but the role of such instability in driving tumor progression is unclear. Furthermore, genetic heterogeneity is a prominent feature of tumorigenesis, but the extent of mutations between cells within a tumor has not been thoroughly characterized.

Single cell whole genome amplification allows us to observe the genetic differences among a heterogeneous population of cells. Notably, this technique enables us to construct a lineage tree indicating the hereditary relationship between single cells. In addition, deeper genetic analysis can more completely capture the degree of genetic heterogeneity among single cells. Lineage analysis of single cells has potential applications in the study of tumor progression as well as in the study of embryogenesis and development.

This project aims to create a lineage tree for single cells in a human pancreatic tumor. Laser capture microdissection and whole genome amplification are used to isolate and amplify the genome of single cells. Microsatellite regions, which exhibit higher rates of mutation due to

replication slippage, are examined to differentiate between single cells. A small-scale lineage analysis is conducted, which may reveal the general level of genetic heterogeneity in pancreatic cancer tissue. We ultimately strive to perform a large-scale single cell lineage analysis of a human tumor. In the future, single cell lineage analysis may be used further clarify aspects of carcinogenesis such as spatial development, tumor initiation, the origin of metastasis, and genomic instability.

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Peter Turnbaugh
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Studying the Interaction between Host Diet and Microbiota Composition

The mammalian gastrointestinal tract contains trillions of microorganisms, the gut 'microbiota'. Previous research has implicated these organisms in both sides of the energy balance equation: gut microbes enable the digestion of complex polysaccharides that are otherwise inaccessible to the host, in addition to regulating key host genes and proteins that increase the storage of adipose tissue. However, unlike our human genome, the microbiota has incredible plasticity, and can be rapidly shaped by a variety of factors, such as the composition of the host's diet.

My project will be utilizing mouse models to explore the complex interrelationship between diet, the gut microbiota, and energy balance. These studies involve culture-independent 16S rRNA gene sequencing. In brief, fecal samples (time course) or intestinal axis samples (mucousal scrapings and luminal flushes) are collected from mice, extracted by mechanical lysis (bead beating), and PCR amplified with bar-coded primers targeting conserved regions of the 16S rRNA gene (variable region 4). PCR products are checked by gel electrophoresis, cleaned with magnetic beads, and quantified by fluorescence analysis relative to a standard curve. Samples are then pooled to normalize their concentrations to ~60ng/ul, and sequenced on the Illumina Hi-Seq platform.

To date, I have prepared and submitted over 200 samples for sequencing, and intend to submit another 500. In the coming weeks, I plan to learn how to analyze these complex datasets, with the goal of shedding new light on the gut microbiota and its role in energy balance.

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Characterizing the role of LSD1 in DNA Damage Response

The threat to cellular genomic stability from both endogenous (e.g. free radicals) and exogenous sources (e.g. ultraviolet or ionizing radiation) is omnipresent. In particular, chromosomal double-strand breaks (DSBs) formed in response to DNA damage are especially cytotoxic and must be quickly repaired to avoid tumorigenesis. To counter this, cells have evolved a canonical DNA damage response (DDR) pathway involving multiple chromatin modifications to repair lesions incurred by the genome. This is clinically relevant because numerous epidemiological studies have linked mutations in the DDR pathway to human cancer-susceptibility diseases. Recently, epigenetic players have been identified in this pathway. LSD1, the first histone demethylase discovered (by the Shi lab), changed the conception of histone methylation as a "permanent" modification. Unpublished data has shown that LSD1 is also mobilized to sites of DNA damage in vivo, correlating also with histone demethylation. Interestingly, loss of LSD1 disrupts localization of other DNA damage repair factors. I am interested in exactly how LSD1 impacts the two distinct DNA repair pathways that cells have evolved to maintain genomic integrity: homologous recombination (HR) and non-homologous end joining (NHEJ). In addition, I am investigating whether LSD1 has a cell-cycle linked role since these repair pathways are preferentially utilized at different cell cycle stages.

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Susan Mango
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Translational regulation is necessary to prevent the translation of incorrect mRNA transcripts. One method of regulation is nonsense-mediated decay (NMD), in which transcripts with premature stop codons are marked for degradation. Current models suggest that a surveillance complex associates with the ribosome, identifying nonsense mutations and preventing translation of mutant transcripts. However, not all of the proteins that form this complex have been identified. In this study, we focus on SAP-1, a protein that might be involved in the NMD pathway.

We examine SAP-1 activity in Caenorhabditis elegans, a nematode species with well-characterized anatomy throughout development. In these experiments, I use a mutant strain that is homozygous for an unc-54 allele with a premature stop codon. As unc-54 codes for a myosin heavy chain, homozygous mutants typically display limited movement. In the absence of other mutations, mRNA transcripts from this allele are reduced to a fraction of wildtype levels. When the NMD pathway is also disrupted, allowing translation of the unc-54(r293) transcripts, the movement of unc-54(r293) mutants is close to that of wildtype animals. To assess SAP-1 in relation to the NMD pathway, I am breeding a sap-1;unc-54 double mutant strain examining animal movement. If SAP-1 is involved in the NMD pathway, sap-1 loss-of-function mutations should partially rescue the phenotype of unc-54(r293) mutants. We hope to gain more information on the significance of SAP-1 in C. elegans.

Yoon Mun *Molecular and Cellular Biology*
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Glenn Dranoff
Dana-Farber Cancer Institute

One serious drawback of most cancer therapies being used today

is that they tend to target proliferating cells in general and are not tumor specific. This lack of specificity results in unintentional destruction of normal cells, which in turn leads to the toxicity effects often associated with such treatments as chemotherapy. In contrast, cancer vaccines are able to selectively target tumor cells in cancer patients by inducing the immune system's own anti-tumor mechanisms, thus abrogating treatment toxicity. Dranoff Lab has had particular success with GM-CSF vaccines, which present tumor antigens alongside cytokines like GM-CSF to induce strong immune response specifically against the tumor antigens. Our lab has shown that cancer vaccines can induce therapeutically significant antibody and T-cell response against tumors in advanced cancer patients, resulting in better disease prognosis. We are currently investigating the specific mechanisms by which successful cancer vaccine treatments operate. Of particular interest is the identification of key antigens against which successful immune activation may lead to selective tumor destruction.

In order to identify the key tumor antigens, we have screened bacteria culture plates expressing cDNA derived from melanoma cells with sera from melanoma patients who responded favorably to vaccination. These patients should have elevated antibody levels in their serum against specific tumor antigens, which can be detected through the cDNA expression library screen. The goal is to isolate genes whose products are critical to both tumor growth and effective anti-tumor immune response. Isolating these target antigens will provide insight into the mechanisms of successful cancer vaccine treatment, which may help guide the development of more effective therapy.

Kevin Ni *Chemical and Physical Biology*
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Rachelle Gaudet
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Studying ABC Transporter Structure by X-ray Crystallography

ATP-binding cassette (ABC) transporters represent a family of integral membrane proteins that use the energy of ATP hydrolysis to shuttle a variety of substrates across biological membranes of cells and cellular organelles. They function by coupling conformational changes in the nucleotide-binding domains (NBDs) induced by ATP binding and hydrolysis events to substrate transport. The two NBDs of ABC transporters dimerize to sandwich ATP at two ATPase sites in the NBD interface. Heterodimeric NBDs associate to form non-equivalent ATPase sites, and the consequences of this asymmetry on the transport cycle remain unclear. To examine and to understand how functional asymmetry powers substrate transport, we sought to determine the first high resolution structure of an isolated heterodimeric NBD system in the closed-ATP bound conformation. In order to obtain high yields of NBD heterodimer suitable for crystallization trials, we introduced C-terminal leucine zippers to promote preferential heterodimer formation of the homologous NBD subunits and C-terminal affinity tags to facilitate purification. These leucine zippers and affinity tags typically hinder crystallization, so we used targeted di-sulfide cross-linking to covalently stabilize the NBD heterodimers before cleaving the C-terminal tags. Our optimized strategy achieved high yield and purity, which allows us to efficiently screen crystals for high-resolution diffraction; while a structure is not yet available, we expect to obtain one in the near future.

Ellen Young-soo Rim *Human Developmental and*
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Gary Ruvkun
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MicroRNAs (miRNAs) are 21-25nt long, single-stranded RNAs that function as endogenous post-transcriptional regulators by targeting mRNAs of protein-coding genes. These small RNA molecules are transcribed from introns and then loaded into an Ago-clade Argonaute protein to generate the RNA-induced silencing complex (RISC). Complementary base-pairing of the RISC miRNA to a target mRNA results in inhibition of its translation or its degradation. While miRNAs are involved in regulation of more than half of all protein-coding genes in humans, relatively little is known about the factors that process and assemble miRNAs into RISCs and the mechanism by which these complexes induce negative regulation of target genes.

C. elegans has many known small RNAs and small RNA targets, as well as the ability to absorb complementary RNA molecules through the digestive system for convenient feeding RNAi, making it an excellent model for identifying the roles that two Ago-clade Argonaute proteins, ALG-1 and ALG-2, play in the miRNA pathway. Previous biochemical assays have demonstrated interaction between these Argonautes and miRNAs, and phylogenetic analyses have also implicated their involvement in biogenesis and/or activity of miRNAs. Furthermore, it has been suggested that ALG-1 and ALG-2 play nonredundant functions in biogenesis or activity of miRNAs despite their 88% identity in the protein-coding region; alg-1 and alg-2exhibit have different knockout phenotypes. However, the exact functions of these Argonaute proteins and how their roles in the pathway may differ remain unknown.

We hypothesize that ALG-1 and ALG-2 have indispensible and distinct functions in the C. elegans miRNA pathway, due either to different temporal or spatial expression patterns, or to association with different groups of miRNAs or protein cofactors. As part of a widespread, conserved gene regulation mechanism, miRNAs are involved in many biological processes including development, cell differentiation, metabolic control, and cell proliferation. Studying the roles of widely conserved miRNA effector proteins would help elucidate the mechanism of this key biological pathway.

Mariama Runcie *Molecular and Cellular Biology*
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The Target of the Rapamycin Complex 2 (TORC2) acts as a serine/ threonine kinase. In C. elegans TORC2 regulates fat metabolism, feeding, growth and lifespan. The predominant known biochemical

role of TORC2 is to phosphorylate and thereby activate the AGC (cyclic AMP, cyclic GMP, and Protein kinase C) family of kinases on their hydrophobic motif. In C. elegans, AGC family kinases such as Akt may be activated by the Insulin like Growth Factor (IGF) pathway in parallel to TORC2. In C. elegans, TORC2 is thought to operate in parallel to the IGF pathway because the effect of TORC2 and IGF on fat accumulation is additive. However, in C. elegans, the effect of TORC2 and IGF on lifespan is not additive. This paradoxical effect suggests that TORC2 is involved in nutrient sensing, and regulates proteins differently in the presence or absence of different nutrients. Because the TORC2 pathway is conserved between C. elegans and humans, I will use C. elegans as a model organism to study this pathway. By investigating lifespan phenotypes of TORC2 mutants, I will conduct a directed search for new proteins in the TORC2 pathway.

Ketsia Saint-Armand *History and Science*
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Harvard Medical School

Comparison Study on Tumor Regression Mediated by Adipose-Derived Invariant Natural Killer T Cells

Invariant Natural Killer T Cells, which are abundant in the lymphocyte populations of the murine liver and adipose disuse and predominantly the adipose tissue in humans (the population in the liver is very small at 0.05%), are important in immunosurveillance and mediating antitumor immunity. Capable of Th1 and Th2 responses, iNKT cells can be stimulated with an exogeneous antigen, α-Galactosylceramide (α-GalCer), to release the cytokines interferon-γ (IFN-γ) and interleukin-4 (Il-4). Together, these cytokines activate other immune cells and cause them to home in on the tumor, thus enabling the immune system to recognize and defend against tumor cells, which normally remain undetectable because they render immune cells responsible for immunosurveillance anergic. In this study, we are investigating the effectiveness of iNKT cells derived from adipose tissue versus liver tissue in mice through a sarcoma model involving TRAMP-GMCSF cells and a lung cancer model using B16F10 cells. Black mice – C57BL/6 females and knockout Jα18-/- females that do not have functional iNKT cells – receive the tumor cells intraveneously, followed by treatment with iNKT cells harvested from wildtype donors. Afterwards, the mice are treated with 2μg of α-GalCer at regular intervals. The results of this study will help us further understand the functions of different subpopulations of iNKT cells and advance our ability to apply our knowledge of the killing abilities of iNKT cells to human cancer therapies.

Jean Shiao *Engineering Sciences*
Kirkland House *Class of 2013*

Roberto Kolter
Department of Microbiology and Immunobiology
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Building Reporter Strains in the Developmental Model Organism Streptomyces coelicolor

Streptomyces Coelicolor is a Gram-positive mycelial Actinobacterium responsible for producing secondary metabolites that contribute to more than half of the world's natural antibiotics. Its morphological differentiation is a subject of great interest, with its initiation catalyzed by the transformation of vegetative substrate mycelium into aerial hyphae that subdivide into unigenomic prespore compartments through synchronized multiple septation; the result is the formation of long chains of spores that disperse to new environments. The complicated process behind cell differentiation and subsequent sporulation involves a highly coordinated program of gene expression, resulting in changes in genetic activation and the visible biosynthesis of four chemically distinct antibiotics over various growth phases. Previous studies have identified some genes required for morphological and physiological differentiation in S. Coelicolor in order to understand the regulatory mechanisms that control each. Through use of green fluorescent protein as a reporter for gene expression and analysis under fluorescence microscopy, this technique enables a thorough analysis of temporal gene expression and regulation in S. Coelicolor. However, the mechanism and details behind this complex network of spatial and temporal gene expression is not fully understood.

The principal objective of my project is to investigate expression of specific reporter genes prior to aerial hyphae formation: desA to direct desferrioxamine and siderophore biosynthesis as a reporter for iron starvation; ramC to produce lantibiotic-like surfactant peptide in decreasing water tension and permitting soil breakage of branching aerial hyphae; various starvation response and active growth genes; actI ORF1, cdaPS1, and redL as antibiotic-producing or immunity-signaling genes; rpmJ2 as the gene activator for ppGpp in the antibiotic biosynthesis pathway; cellulose- or matrix- producing genes at the tip of vegetative mycelium filaments; and genes responding to oxidative stresses. The GFP gene is inserted into plasmids to replicate in Escherichia coli, which then undergo cloning with reporter gene inserts. Vectors will then be used to analyze the spatial and temporal expression of these genes under fluorescence microscopy. Simultaneously, the direction of my project involves investigating interactions between S. Coelicolor and other Actinomycetes by plating soil cultures from across the world with Actinomycete-selecting mediums. Since the complex morphogenetic program culminating in the development of aerial hyphae and spore chains is not well-understood, the information learned through this research can potentially help bridge gaps between genetic triggers and antibiotic production in developmental stages while better understanding reciprocal communication between S. Coelicolor and other Actinobacteria.

Nick Stanford *Chemical and Physical Biology*
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Fred Ausubel
Department of Molecular Biology
Massachusetts General Hospital

The opportunistic pathogen P. aeruginosa and the nematode C. elegans have recently been developed as a model of host pathogen in-

teractions. The lack of adaptive immune elements of higher organisms makes it ideal to study innate immune response. Classically, an innate immune response has been associated with the recognition of specific pathogenic structural features (cell wall fragments, flagella, etc), called PAMPs (Pathogen-associated molecular patterns); however, research has of yet failed to elucidate a PAMP that triggers C. elegans immunity. A likely explanation is that C. elegans is recognizing the effects of pathogens such as damage to the cell or interference with vital cellular processes rather than the pathogens themselves (ie the PAMPs). Our lab has recently demonstrated that a specific Pseudomonas toxin, exotoxin A, which inhibits translation, activates an immune response, and that inhibiting translation by other means, such as the drug Hygromycin, produces the same response. Expanding on these results, I am investigating whether exposing the animal to these translational inhibitors can lead to more effective pathogen resistance when infection occurs.

Michael Sun *Chemical and Physical Biology*
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Navigating through an environment involves a diverse set of strategies that include hard-coded rules linking sensory input to motor output or more complex relationships between sensory stimuli and the resultant behavior of an animal. Previous work in invertebrates showed hard-coded locomotor responses to changes in temperature, but the navigational strategies of vertebrates remain mostly unknown. To examine these strategies in a model vertebrate system, we have developed a real-time behavioral tracking system of freely swimming larval zebrafish and a transgenic line capable of single-cell recordings from the entire larval zebrafish brain. These novel tools will allow us to determine both how zebrafish navigate a thermal gradient and what regions of the brain are involved in processing themal stimuli and navigation. Preliminary data suggest that two structures called the habenula and its downstream target, the interpeduncular nucleus (IPN), are responsible for thermal navigation in zebrafish. By ablating the habenula, it is possible to test this hypothesis by assaying thermal navigation of a larval zebrafish in the absence of the habenula and other implicated structures. Furthermore, experiments are currently underway to elucidate the connections between the habenula and other parts of the brain. These experiments help to establish a new behavioral paradigm of thermal navigation. Various components of neural function, from sensory processing andmotor function to spatial learning and memory, can be investigated using our approach.

Lillian Tsai *Molecular and Cellular Biology*
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Edward T. Ryan
Division of Infectious Diseases
Massachusetts General Hospital

Identification of Immunogenic Salmonella Paratyphi A Proteins Uniquely Expressed in Humans and Not Under Standard Laboratory Conditions

Enteric fever is caused by invasive Salmonella infection including Salmonella Typhi and Paratyphi. Enteric fever affects 20 million individuals each year, killing approximately 200,000. Salmonella Typhi andParatyphi are human-restricted infections. There are limited data on host pathogen events during these infections, especially with Paratyphi. In an attempt to identify immunogenic S. Paratyphi antigens expressed uniquely in infected humans, I will apply a technology called In Vivo Induced Antigen Technology (IVIAT) to S. Paratyphi A, the major global cause of paratyphoid fever. In South Asia, 1 in 5 cases of enteric typhoid fever is caused by S. Paratyphi A. Identification of such antigens could lead to improved diagnostics, vaccine development, and understanding of host-pathogen events during this significant global infection.

Shelun Tsai *Neurobiology*
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The Role of TorsinA in Secretion and Implications to Neurological and Peripheral Human Disorders

TorsinA is a chaperone protein located in the lumen of the endoplasmic reticulum (ER) and the nuclear envelope (NE). A glutamic acid deletion in the carboxyl terminus of torsinA (DE303) is associated with the manifestation of early-onset dystonia (EOD). EOD is characterized by sustained, involuntary muscle contractions that typically begin in a lower limb and gradually progress up the body. This leads to severe twisting, painful body postures, and significant disability. The pathophysiology of EOD has been linked to altered brain development and plasticity within the dopaminergic system of the basal ganglia in the absence of apparent neurodegeneration. However, the exact mechanism connecting torsinA to the manifestation of EOD remains unknown. We have previously shown the role of torsinA in degrading misfolded proteins in the ER and processing normal proteins through the secretory pathway. Our recent preliminary data support a critical role for torsinA in three different biological pathways: (i) regulating the intracellular levels of a1-antitrypsin (A1AT), a secreted protein implicated in emphysema and liver diseases, (ii) promoting cell surface trafficking of the dopamine 2 receptors (D2Rs) implicated in dopaminergic neurological conditions, and (iii) affecting the outcome of the Wnt/b-catenin pathway, which determines various aspects of the ventral midbrain during embryonic development. The common connection between the above is the secretory pathway. A1AT and D2R exit the ER through the secretory pathway to reach the cell surface. Similarly, a co-receptor called low-density lipoprotein proteins 5/6 (LDRP5/6) in the Wnt/b-catenin pathway also needs to traffic to the cell surface. Thus the effects of torsinA on the three different biological pathways could reflect its role in secretion. Our work should shed light on not only the mechanisms underlying dystonia but also other neurological diseases such as Parkinson's disease, as well as neurodevelopment diseases, emphysema, and liver diseases.

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Construction of recombinant proteins for the structural studies of the capsid and RNA dependent RNA polymerase of Trichomonas Vaginalis Virus

Trichomonasvirus is a newly proposed genus of viruses in the family Totiviridae that infects the protozoan Trichomonas Vaginalis (TV), which in turn causes the sexually transmitted disease Trichomoniasis. TV infection is not only associated with low birth weight and premature delivery but also elevates both susceptibility to HIV transmission and the risk of cervical cancer. Previous research by Alderete et al. indicated that the presence of Trichomonas Vaginalis Virus' (TVV) in its host (hereby referred to as trichomonas) was found to change the expression of the protozoa's virulence factors such as P270, a highly immunogenic surface protein, and cysteine proteinases. According to recent data from Dr. Raina Fichorova's laboratory, our collaborative counterpart at the Brigham and Women's Hospital, TVV infected trichomonas elicited a heightened inflammatory response compared to non-infected ones. Some immediate results of TVV's presence in trichomonas are upregulation of interleukin 8, which serves to attract white blood cells such as neutrophils to the site of infection, and an increase in interferon beta, which mobilizes the immune system by recruiting T cells and macrophages.

In order to better characterize the effect of TVV in trichomonas, structural and functional studies of its main proteins, capsid and RNA dependent RNA polymerase (RdRp), must be done. The species studied in this project are Trichomonas Vaginalis Virus (TVV) 2, 3 and 4, which are isolated from the UR1 and OC3 strain of the TV protozoa. The primary part of the project will aim to obtain a substantial amount of purified capsid and RdRp proteins to generate antibodies. Capsid and RdRp sequences from each strain of TVV will be cloned into protein expression vectors such as pET16b and pET28ac+ to make sizeable isolates. Since each virus species is marked by unique protein sequences, antibodies against these proteins can be used in western blots to determine not only the presence of TVV in trichomonas but also the exact species of TVV. A more long-term goal of this project aims to use purified capsid and RdRp proteins to form virus-like particles for structural characterization using cryoelectron microscopy. Combined with crystallography, which will hopefully be done in the future, this preliminary data will help in extrapolating the active domain of the protein, potential interaction or binding sites with other cellular components, and key residues.

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Leonard Zon
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The role of chromatin-remodeling factor Arid4b in tumor suppresion and the regulation of hematopoiesis

Chromatin-remodeling factors alter gene expression by modifying histone tails. Many, including Arid 4b, are aberrant during cancer. Arid4b recruits histone deacetylase (HDAC), which represses gene expression. Recently, the Zon lab found that knocking down Arid4b increases the number of hematopoietic stem cells (HSCs) and the proliferation of blood progenitors. As the molecular networks that are frequently aberrant in leukemia are also utilized by normal HSCs, elucidating the role of Arid4b in HSC formation could lead to better understanding the disruptions that occur in leukemia.

Knocking down Arid4b increases HSC formation and progenitor proliferation, suggesting that Arid4b may suppress tumors. At present, we are developing a technique to knockdown Arid4b in the adult zebrafish. We eventually aim to perform competitive HSC transplantation assays whereby HSCs from kidney of Arid4b knockdown fish (labeled with green fluorescent protein) are transplanted, along with red fluorescent protein-labeled wildtype marrow, into adult fish whose immune systems have been ablated by irradiation. We expect that Arid4b morphant HSCs will repopulate the fish's immune system to a greater extent than wildtype marrow despite the fact that less morphant marrow will be transplanted. Such a finding would suggest that a lack of Arid4b confers a growth advantage to the transplanted cells, supporting the hypothesis that Arid4b may function as a tumor suppressor.

Lynn Yi *Physics*
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5'UTRs in Translation Regulation

Despite being a universal and fundamental process, translation is not well understood on a systems level. Recent advances in deep sequencing technology reveal that translation efficiencies of mRNAs vary greatly across the yeast genome. Because initiation is the rate-limiting step of translation, the 5' untranslated region (5'UTR) is thought to be the main regulator of translation. However, regression models show that known factors in the 5'UTR can explain only 40% of the variation in translational efficiency. This project tackles two questions: (1) how much of the variation in translational efficiency can be attributed to the 5'UTR, and if the 5'UTR plays as important of a role as expected, and (2) what elements in the 5'UTR account for the 60% unexplained variance in translational efficiency? To address the first question, I use dual reporter constructs preceded by what deep sequencing suggests are the best and worst translating 5'UTRs and test whether the large differences in translational efficiencies can be reproduced. To address the second question, I generate random mutation libraries, using endogenous 5'UTRs in yeast as templates, to screen for new factors in the 5'UTR that affect translation. To explore the mechanism by which the 5'UTR regulates translation, I have some simulations on how the ribosome scans along the 5'UTR.

Neuroscience & Psychology

Ally Freedy
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Takao Hensch
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Investigating Critical Periods: Determining the Effect of Experience on Visual Cortex Circuitry during Neuronal Development

Critical periods are defined as times of heightened brain plasticity during normal development. One of the best studied critical period models is that of ocular dominance plasticity, which is when visual experience determines a visual cortex cell's response to sensory input through each eye. Although the details responsible for triggering and regulating this critical period are still unknown, it has been proven that the excitatory/inhibitory balance is essential for the initiation of this critical period. Specifically, work by our lab has shown that a subclass of interneurons, parvalbumin positive cells, are crucial for this initiation. Parvalbumin positive interneurons also receive direct thalamic input through terminals expressing a specific glutamate transporter, vGlut 2 (different from the glutamate transporter used in intracortical connections). In mice, the critical period for ocular dominance plasticity is from post-natal day 22 to 30 (p22-p30). In this project, we stain for parvalbumin positive cells and vGlut2 at the peak of the critical period (p27 mice). By staining for parvalbumin positive cells and vGlut2, we can quantify approximately how many inputs from the thalamus surround any given parvalbumin positive cell. Primarily, we look at the effect of monocular deprivation on visual circuitry during the mouse's critical period by comparing synapses of deprived mice to control mice. We can also observe the mouse brain before and after the critical period to observe any developmentally dependent changes in the thalamocortical synapses.

If successful, this method would provide us with more information about how interneurons are wired into circuits. It's known that in many diseases like schizophrenia and autism, there is dysfunction within the inhibitory interneuron's circuitry. Since critical periods rely on the excitatory/inhibitory balance, a future direction for research could be to use this method to search for a correlation between an abnormal critical period and autistic-like symptoms.

Joy Qiyue He
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Ole Isacson
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Patient-Specific Modeling of Mitochondrial Dysfunction in Parkinson's Disease

Parkinson's disease (PD) is a complex, progressive neurodegenerative disorder affecting about 2% of Americans over 60 years-of-age. The disease is characterized primarily by the deterioration of dopaminergic neurons in the brain, especially from the substantia nigra, which causes a loss of motor control and coordination, and results in patients developing tremors, instability and dyskinesia. Posthumously, PD can be identified in patients by the presence of protein aggregates in the brain called Lewy bodies.

Research has suggested that some chemicals such as paraquat and MPTP can induce parkinsonian symptoms in humans. Paraquat has since been shown to disrupt mitochondrial complex I, an important enzyme of the ATP-producing electron transport chain, by producing harmful superoxides into the mitochondrial matrix. Similarly, studies of MPTP have also found that, when metabolized, MPTP becomes MPP+, an inhibitor of complex I. Following these associations, PD has been connected with high pesticide/herbicide use as well as close proximity to industrial plants and quarries. However, since PD is usually a late-onset disease, it is sometimes difficult to ascertain if it is caused or simply exacerbated by those conditions.

Indeed, the majority of PD cases have no identifiable cause. However, while most cases of PD are idiopathic in nature, some forms of the disease have a clear genetic basis. Study of PD-causing mutations has led to the establishment of known genetic risks associated with certain genes, such as PINK1, PARK2, LRRK2, SNCA, and ATP13A2. Like paraquat and MPTP, many of these genes are also involved in regulation of mitochondrial function. For example, more than 20 mutations are connected with PINK1 (PTEN induced putative kinase 1)-associated PD. These missense and nonsense mutations result in abnormally truncated proteins that disrupt the kinase and mitochondrial targeting domains of the protein, leading to reduced ability to regulate mitochondria during cellular stress. Previous research has established that these mitochondrial protein mutations contribute to the pathology of PD.

To this end, understanding quality control of mitochondrial integrity – how cells respond and adapt to the lower energy environment which results from mitochondrial damage – becomes of high interest for researchers seeking to mitigate, reverse, or prevent the effects of PD. Specifically, my work at the Isacson laboratory at McLean Hospital has included identifying mitochondrial phenotypes in neural cells differentiated from PD patient-derived induced pluripotent stem cells. The PD-iPSC experimental platform builds upon existing genetic data to potentially improve understanding of how PD affects cells in the early stages of disease, before any outward phenotypic indicators of PD are detectable in patients. This is done by studying different types of patient cells. Fibroblasts (skin cells) from patients with high-risk mutations for PD are reprogrammed to become pluripotent stem cells, and then differentiated into neurons and neural precursor cells much like those affected by PD. This provides experimental flexibility to better understand how PD affects human cells by comparing different human cell type-specific vulnerabilities before any physical symptoms are ever presented by the patients.

In the context of mitochondria, we can examine quantitatively how PD cells and healthy individual cells differ in their cellular

and mitochondrial responses to stress by treating these different types of cells with subtoxic levels of mitochondrial stressors and then staining for mitochondria. Moreover, we may also gain additional insight into the specific vulnerability of already genetically compromised PD patient-derived neurons when treated with low dose cellular stressors, compared to that of other similarly treated cell types such as patient derived fibroblasts. By seeking to characterize mitochondrial stress responses using this 'double hit' model, our research seeks to lay the foundation for mitochondria-related drug discovery in the future.

Charlotte Lee *Undeclared*
Adams House *Class of 2014*

Michael E. Greenberg
Department of Neurobiology
Harvard Medical School

Searching for the MEF2 Transcription Factor's Targets and Understanding Its Activity in the Hippocampus

During development, excess synapses are formed, so the elimination of synapses through neuronal activity is crucial for the adult organism. These synapses can be eliminated in an activity-regulated manner that can depend on our sensory experiences, which throughout our lifetime affects synaptic connectivity and plasticity and, in turn, learning and memory.

I am studying a particular transcription factor called myocyte enhancer factor 2 (MEF2) in the hippocampus, an area associated with memory storage and consolidation. When activated, this transcription factor is known to restrict the number of excitatory synapses by inducing the expression of certain genes. Through mice behavior tests such as the water maze, it has also been shown that MEF2 function is correlated with spatial memory formation, fear conditioning, and decreases in spine density. I'm currently confirming and looking for direct transcriptional targets of MEF2 (specifically MEF2A and MEF2D), which is highly present in the whole brain, and also the biochemical mechanism by which these transcriptional programs are activated by MEF2, through the use of conditional knock-out mice. We have currently been working with P21-28 mice (21-28 days old). To induce genes, we either use kainic acid to induce seizures in normal mice (and, later on, conditional MEF2 knockout mice), or place the mice into an "enriched environment" by giving them new and colorful toys to play with for a set time, observing them, and then doing RT-qPCR. Ultimately, we are trying to see what genes have been induced (undergone a fold increase) after being seized or given new sensory experiences, and what the expression levels of these genes are. Some potential targets of MEF2 are Arc, synGAP, cfos, BDNF (brain-derived neurotophin factor), Homer1a, and c-jun; some of these targets also regulate postsynaptic excitatory strength. To map out DNA targets and find binding regions, we are using ChIP. From the research done on MEF2 thus far, it has been found that many of the transcriptional targets are mutated in epilepsy, autism, Angelman Syndrome, and other neurological disorders, so it is interesting to see how these mutations may be related to MEF2's control of certain genes.

Yvette Leung *Undeclared*
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Ole Isacson
Neuroregeneration Institute
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Using Rodent Models of Parkinson's Disease to Investigate Neurodegeneration and Novel Neuroprotective Therapies

Parkinson's disease (PD) is a progressive and debilitating neurodegenerative disorder that affects nearly a million people in the United States. It is characterized by degeneration of dopamine neurons in the substantia nigra, which leads to clinical motor symptoms such as rigidity, tremor, & slowness of movement. However, in addition, a number of non-motor symptoms are frequently experienced by patients with PD, including sleep disorders, gastrointestinal dysfunction, and peripheral neuropathy. A major component of PD is associated with the synaptic protein, alpha-synuclein. Mutations, duplications, and triplications in the alpha-synuclein gene lead to genetic forms of PD. In both sporadic and familial PD, alpha-synuclein is a component of proteinacious Lewy body inclusions and neurites, the major pathological hallmarks of PD, found throughout the central nervous system as well as the peripheral nervous system. We have designed studies to investigate neuronal dysfunction and degeneration in two rodent models of alpha-synuclein overexpression – alpha-synuclein overexpressing transgenic mice (ASO) and viral-mediated overexpression of alpha-synuclein in the substantia nigra of rats.

ASO transgenic mice are particularly interesting models to study since ASO mice exhibit autonomic dysfunction in addition to central nervous system deficits. Specifically, ASO mice show gastrointestinal functional deficits also seen in PD patients. The gastrointestinal tract in ASO mice shows severe distension and blockage of the large intestine. Post-mortem biochemical and histological analyses will be used to analyze neuropathological changes in the gastrointestinal systems in ASO mice. This study will examine ASO mice as a model of peripheral nervous system dysfunction in PD for future preclinical therapeutic development and testing.

Since the cause of neuronal degeneration during PD remains unclear, the rat model of viral vector-mediated alpha-synuclein overexpression has been analyzed to characterize early predegenerative changes in substantia nigra dopamine neurons. This particular model is created by injecting an adeno-associated virus carrying mutant human alpha-synuclein into the substantia nigra. This model is useful for examining neuroprotective candidates, such as the synaptic vesicle protein RAB3B. RAB3B will be coinjected into the substantia nigra along with alpha-synuclein via viral vectors. Histological and biochemical post-mortem analysis will be performed to determine whether RAB3B overexpression can lessen the severity of neuronal pathological changes mediated by accumulation of alpha-synuclein. The ASO transgenic mice and AAV-alpha-synuclein rat model used in the current studies provide opportunities to examine PD-like neuronal dysfunction and de-

generation in vulnerable neurons affected in PD and to test novel therapeutic and neuroprotective paradigms.

Yingna Liu *Neurobiology*
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Takao Hensch
Department of Molecular and Cellular Biology
Harvard University

Behaviorial and Morphological Differences in Maternally Separated Mice Predisposed to Disease Conditions

Early life stress in animal models results in lasting changes within neural circuits. One method that has been used to understand early life stress is maternal separation, whereby rodents are separated from their mothers for a limited period of time per day over a span of a few weeks. Research has shown that maternal separation results in behavioral problems in the form of increased anxietyand fear behavior within both rats and mice. However, conclusions on maternal separation effects on individuals predisposed to disease conditions, such as autism or schizophrenia, have yet to be studied. In Takao Hensch's lab, I am performing maternal separation on Glutamic Acid Decarboxylase 65 kDa isoform (GAD65) knockouts and Glutamate-cysteine ligase catalytic subunit (Gclc) knockouts in the parvalbumin cells of the amygdala. Research indicates that lack of GAD65 results in a decrease in GABA concentration in the amygdala with increased anxiety-like behaviour in light/dark avoidance tests and reduced intermale aggression. Gclc is the first rate-limiting enzyme of glutathione synthesis, which plays a major role in protecting nervous tissue against reactive oxygen species. I hypothesize that these gene knockouts will heighten fear response and anxiety in maternally separated mice, which may indicate the molecular pathways influenced by early life stress.

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Christine Hooker
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Abnormalities in Neural Structures of Attention and their Relation to Impaired Social Function in Schizophrenia

Schizophrenia is a disabling mental illness characterized by deficits in brain structure, cognition, emotional processing, and social functioning. Cognitive impairments often exhibited by individuals with schizophrenia include problems with language, perception, attention, and the recognition and experience of emotions. Social impairments make up another central feature of the disease, with many patients demonstrating difficulty expressing themselves, making social inferences, and establishing and maintaining relationships. Studies have shown that poor social functioning in schizophrenia is strongly correlated with increased symptom severity and worse overall disease outcomes. One cognitive element that plays an important role in social functioning is sustained attention, which has consistently shown impairments in schizophrenia. My thesis research seeks to examine the structural abnormalities that may underlie such attentional and social deficits, which is an important step in both better understanding the etiology of schizophrenia and developing effective methods for treating it.

A brain region of particular interest in schizophrenia research is the anterior cingulate cortex (ACC). The ACC is a bilateral structure located in the medial frontal lobe and has been implicated in executive, social, cognitive, affective, skeletomotor, and viscerometor functions. In addition, the ACC is thought to play a key role in the inhibition and controlled response elements of attention. Studies examining brain structure using magnetic resonance imaging (MRI) have found reduced ACC volume and thickness in patients with schizophrenia, suggesting that structural abnormalities in the ACC may be part of the neuropathology of the disease.

One of the most salient cognitive deficits seen in schizophrenia is impaired attention, particularly on tasks requiring sustained attention. Sustained attention in schizophrenia has been widely assessed using a behavioral paradigm called the identical pairs version of the Continuous Performance Task (CPT-IP). The CPT-IP is a computer-based task that assesses such processes as attentional capacity, verbal working memory, distractibility, reaction time, and speed of processing. Subjects are presented with a rapid series of 2-, 3-, or 4-digit numbers and must respond with a mouse click only when the same number appears twice in sequence. Accuracy and reaction times are measured to specifically test sustained, selective attention.

In addition to the structural and cognitive deficits seen in schizophrenia, the disease is characterized by pervasive impairments in social functioning. Social functioning refers to the ability to effectively and appropriately interact with others. Many schizophrenia patients are unable to recognize facial and verbal expressions of emotion, subtle social cues, or social errors, all of which contribute to the isolation and withdrawal characteristic of the disease. Several studies have indicated that there exists a positive correlation between attentional deficits and poor social functioning among individuals with schizophrenia.

Although extensive research has been conducted on brain structure, cognition, and social functioning in schizophrenia, most studies have only examined one or two of these elements at a time. Few studies have drawn a connection between the underlying neuroanatomical abnormalities in schizophrenia and bothcognitive performance and social outcome. My thesis research seeks to explore this unanswered question, thereby addressing a significant gap in the schizophrenia literature. The first goal of my thesis is to examine the structural differences in the ACC between schizophrenia patients and healthy controls. The second goal of my thesis is to investigate whether impaired performance on the CPT-IP correlates with structural abnormalities in the ACC among schizophrenia patients. The third and final goal of my thesis is to explore whether impaired performance on the CPT-IP andstructural abnormalities in the ACC correlate with social functioning deficits in schizophrenia patients. I will use neuropsychological testing, MRI scanning, and cortical surface analysis to accomplish these three overarching goals.

Carl Malm *Neurobiology*
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Richard T. Born
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A Morphological Analysis of the Effect of Genetically Induced Myelin Disruption on Feedback Connections in the Mouse Visual Cortex

The mammalian visual cortex is composed of an array of areas organized into a hierarchy in which visual inputs enter at "lower" portions and information flows to higher areas--feedforward (FF) connections--with more complex properties represented in successively higher areas. The cortex also anticipates future sensory inputs and adjusts expectations of these inputs based on our actions; this is accomplished using a unique set of connections in which information flows from "higher" to "lower" areas (feedback connections).

Recent experiments in our lab have shown that these FB connections experience a temporal delay in maturation compared to their FF analogues. This difference in timing of development suggests that FB connections could be differentially susceptible to disruption by environmental or genetic factors.

Among the many neurodevelopmental disorders in which cortical circuitry is undermined, schizophrenia provides an example in which impaired FB connections have been shown to be particularly affected. Using transgenic mice in which erbB signaling (a pathway implicated in human schizophrenia) is disrupted in oligodendrocytes we plan to test the hypothesis that FB connections are selectively disturbed in these animals. To this end we are in the process of injecting neural tracers into different areas of the visual cortex. This will enable us to compare the detailed neuronal morphology of FB neurons in the transgenic mice to those of their wild-type littermates, to see what if any effects that their mutated glial environments might have. Further work will involve a comparison of the developmental trajectory of these FB connections in both groups.

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Ronald A. DePinho
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Discovery of Homogygous Deletions and Synthetic Lethal Genetic Interactions In Cancer: Towards a Novel Molecular Directed Therapy

Glioblastoma is an aggressive, malignant type of brain tumor. This deadly cancer is difficult to treat due to its resistance to conventional therapies such as chemotherapy and radiation. The DePinho Laboratory is investigating novel strategies to selectively kill tumor cells by targeting the enzyme, Methylthioadenosine Phosphorylase (MTAP). This enzyme is homozygously deleted in 40% of all glioblastomas. It is critical in the Methionine salvage pathway and in polyamine biosynthesis. It cleaves Methylthioadenosine (MTA) into Adenine and 5-methylthioribose-1-phosphate, the precursor of methionine. Methionine is an essential amino acid, critical for cell growth and survival. Cells with MTAP deleted are more susceptible to deficiencies in Methionine, as they can no longer resynthesize Methionine from other compounds in the cell. My project involves utilizing these homozygous MTAP deletions, inducing cell susceptibility to methionine deficiency, to selectively target tumor cells. This is a promising treatment strategy since MTAP is one of the largest genetically encoded biochemical differences between normal and cancer cells.

Gabriella Paisan *Neurobiology*
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Matthew Anderson
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Effect of Loss of Ube3a's Ubiquitination Function on Autism Spectrum Disorders

Autism is a neurological disorder affecting approximately 1% of the population in the United States. Autism Spectrum Disorders are generally characterized by impaired communication and social skills and repetitive, stereotyped behaviors. Through genome wide association studies (GWAS) of individuals affected with autism, scientists have been able to show that certain small nucleotide polymorphisms (SNPs) and copy-number variations (CNVs) of the gene E3 Ubiquitin Ligase (Ube3a) are strongly associated with autism.

Certain mutant forms of the gene Ube3a, located on Chromosome 15q11-q13, have also been implicated as causes of Angelman's Syndrome (AS), a disorder very similar to the autism spectrum disorders. These mutants typically leave the Ube3a-associated protein, E6-AP, unable to function as a ubiquitylating agent. The ubiquitylation process acts as a form of protein recycling. Ubiquitin Ligases, such as E6-AP, bind to the small protein ubiquitin and then attach the ubiquitin to another substrate protein so that it can be degraded by a proteasome. The mode in which E6-AP undergoes this process occurs through binding of both ubiquitin and the substrate protein to a region on E6-AP called the HECT domain. This functional domain is present in many ubiqiutin ligases. In the middle of this HECT domain is a catalytic cysteine, which forms a thioester bond with ubiquitin so that it may be eventually attached to the protein marked for degradation. Without the effect of this cysteine, E6-AP is not be able to ubiquitinate substrate proteins.

The exact importance that Ube3a has on the nervous system and how its mutant forms can cause AS is not completely understood. To further study the cellular trafficking, localization, and function of common AS-associated mutant versions of the E6-AP, we have developed a DNA construct of Ube3a through BAC recombineering, which contains a point mutation at the catalytic cysteine (CàA) so as to halt ubiquitinating activity. This construct will also contain the sequence for FLAG3 at the 3'end so that the protein

can be directly observed in the cell. The construct will be used in transgenic mice to both directly observe the protein at the cellular level and to observe phenotypic differences in mutant and wild-type mice.

Beverly Pozuelos *Psychology*
Eliot House *Class of 2012*

Christine Hooker
Department of Psychology
Harvard University

Evidence has demonstrated that attentional measures can be markers of vulnerability for schizophrenia. In particular, the attention network test (ANT) is designed to measure attention efficiencies of alerting, orienting, and executive control of attention by asking participants to determine the direction of a central arrow whilst being flanked by both homogeneous and conflicting arrows in different trials. While first-degree relatives of individuals with schizophrenia have been shown to have delayed reaction times as compared to healthy controls, the performance of unrelated, healthy controls with potential vulnerabilities for schizophrenia, based on Chapman scale scores, has not been measured. The Chapman scales are measures of psychosis proneness; they consist of four sub-scales including the Revised Physical Anhedonia Scale, the Revised Social Anhedonia Scale, the Perceptual Aberration Scale and the Magical Ideation Scale. Using the ANT we looked at two groups of healthy controls as gauged by either the Structured Clinical Interview for DSM-IV-TR or the Mini International Neuropsychiatric Interview. The variable between the two groups was the participants' Chapman scores in which the first group consisted of healthy controls with average Chapman scores and the second group consisted of healthy controls with high social anhedonia scores. Furthermore, we adapted the current ANT paradigm and created the ANT-Emotion (ANTE), which is meant to gauge how emotional information affects performance and executive control by flanking the central arrow with either neutral or angry faces. We predict that the second group will demonstrate more difficulty inhibiting the emotional information in the ANTE, consistent with established difficulties in inhibiting negative stimuli in individuals with schizophrenia, suggesting that hindrances with inhibition of emotion may be part of the liability for schizophrenia.

Richard Sima *Neurobiology*
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Edward Kravitz
Department: Neurobiology
Abstract:

A Social Defeat Drosophila Model of Depression

Approximately one in six Americans will suffer from major depression in their lifetime and major depressive disorder is predicted to become a leading cause of disability worldwide by 2020. However, for the past thirty years, the antidepressants drugs introduced into medical practice have been only partially effective for many patients, and are furthermore based on the same biological mechanisms - namely, altering levels of the neurotransmitters dopamine, serotonin and norepinephrine.

Studying animal models is useful in understanding this illness – doing so allows us to use genetically identical organisms to be exposed to closely-tailored stressful events to analyze the effects of adverse experience in isolation from genetic vulnerability. A particularly effective adverse experience is social defeat, which is a relatively new behavioral model system in Drosophila melanogaster. Male fruit flies can reliably be induced to instinctively fight over limited resources such as a cup of food and a potential mate. Of particular relevance to psychiatric research is the finding that the experience of losing changes the defeated fly in a profound and long-lasting manner such that it will virtually never go on to win a subsequent encounter.

My project examines at whether pharmacological interventions found to be effective or relevant in human depression patients may be similarly useful in reversing the effects of social defeat in Drosophila. In essence I'm first testing how well the social defeat paradigm works as a model system for the study of depression and for the discovery of novel antidepressant compounds. I'll then proceed to test the effects of histone deacetylase (HDAC) inhibitors, which produce epigenetic modifications that may underlie antidepressant action, on this social defeat model. The research proposed may ultimately lay the foundations for a screen to identify novel components of the social defeat pathway, and may provide an opportunity for the large scale screening of small molecules.

Christa Simone *Neurobiology*
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Zheng-Yi Chen
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Identification of Key Pathways Involved in Hair Cell Regeneration in Zebrafish

Worldwide, an estimated 250 million people suffer from hearing loss. In the United States, 36 million people are deaf or hearing impaired, making hearing loss the third most common physical ailment, after heart disease and arthritis. This problem is especially prevalent among the elderly population, where one in three people over 65 years of age is affected. Sensorineural hearing loss is the most common form of hearing loss; it is caused by damage to auditory neurons and hair cells, the sensory cells of the inner ear that transduce mechanical signals from sound waves into chemical signals recognizable by the brain. There are very few - about 16,000 - hair cells in the cochlea, and in mammals they lack the capacity for self-renewal. The focus of my lab is on finding ways to induce hair cells to regenerate, by activating certain genes and signaling pathways that are involved in hair cell development but are then repressed after hair cells reach the terminal stage of differentiation. In my study I am using zebrafish as a model system because they have the convenient property of being able to regenerate their hair

cells after they are damaged. We want to study this process of hair cell regeneration in zebrafish in order to identify important pathways that we could utilize in mammalian studies to induce regeneration. Following the destruction of the zebrafish hair cells with an ototoxic drug, we target certain pathways believed to be involved in cell cycle re-entry and hair cell fate determination. By administering certain drugs to inhibit key players in these pathways, we can determine which ones are necessary for hair cell regeneration. We are currently looking at several proteins in the histone deacetylase and DNA methyltransferase families, hypothesizing that blocking these processes should lead to a decreased level of hair cell regeneration. With the identification of key proteins and signaling pathways involved in hair cell regeneration in zebrafish, we are hoping to eventually apply this knowledge to induce hair cell regeneration in the mammalian cochlea.

Anji Tang *Neurobiology*
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Jeff Lichtman
Department of Molecular and Cellular Biology
Harvard University

Spatial Arrangement of Slow Type Motor Neurons in the Mammalian Motor Pool

The neuromuscular junction (NMJ), prized for its large cells and synapses, is a classical model for studying neural circuitry. Confocal microscopy and imaging have revealed that muscles begin multiply innervated; motor neurons make a plethora of connections at birth, but connections become sparser with age until each muscle fiber is innervated by only one motor neuron. Studies have explored the interactions between motor neurons at synapses and factors determining which axons occupy the junction while others recede. Aided by genetically engineered Brainbow colors (the CRE-dependent colors that helped outline axon morphology) — it was determined that neuronal identity played a key role in synapse occupancy. Motor neurons in the spinal cord make frequent pairwise connections to specific partners, and few connections to other motor neurons, as studied in the omohyoid muscle. This partner skewing potentially illuminates circuit level organization in the nervous system. There may exist a relationship between a topographic map in the spinal cord and motor neuron partnering in the spinal cord. It remains to be tested whether such a map exists where a specific spatial domain with a motor pool specifically innervate certain types of muscle fibers. Hebb's postulate — neurons that fire together wire together— suggests that neurons consecutively recruited may have temporally correlated firing patterns and better connectivity. We are interested in how arrangement of motor neurons in the spinal cord relates to innervation of specific muscle fiber types.

Muscle functionality is divided into slow, intermediate, and fast subtypes. Our experiments examine whether a topographic relationship exists for the motor neurons innervating slow muscle fibers in the mouse spinal cord. We will inject a small, superficial muscle— the lumbrical muscle in the mouse's hand—with retrograde tracers cholera toxin and DiI. We will then allow a 48 hr. recovery, perfuse the animal, and image the spinal cord at C8-T1 segments to locate the cell bodies of the motor neurons innervating the lumbrical (motor pool). We will label the slow type motor neurons with SV2A-specific antibody, a synaptic vesicle protein found specifically in slow motor neurons, to determine the existence (or lack thereof) of a topographic arrangement of slow motor neurons in the spinal cord. This will help corroborate or disprove the hypothesis of a spatial-topographical map between the spinal cord and motor neuron subtype.

Amalie Thavikulwat *Neurobiology*
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John Maunsell
Department of Neurobiology
Harvard Medical School

Towards an Understanding of Population Coding for Behavior

Discerning the mechanisms by which the brain controls behavior remains a fundamental aim in systems neuroscience. Research over the past century offers both macroscopic insight into the functions of broad brain regions and microscopic insight into the relationship between neural activity and behavior. Yet, little is understood about how neurons function as populations to reflect sensory input and control behavioral output. Research into this question has largely been confined to the theoretical domain due to technological limitations precluding the simultaneous activation and observation of select neural populations. The Maunsell Lab employs novel optogenetic techniques in conjunction with multielectrode recording to overcome this limitation. We have trained mice to detect the activity of identified neurons by activating those neurons directly via optical methods. Using psychophysics, we quantify how animals learn to detect the firing of these neurons. We are recording the activity of these neurons to determine changes that occur in cortical circuits when the brain reorganizes to detect the activity of specific neurons. The goal of our study is to determine which aspects of population coding in the cortex are most important for behavior.

Eugene Vaios *Neurobiology*
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Rosalind Segal
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A Qualitative Glimpse into the Localization of Bcl-w and Mef2d in the Distal Axons of Mouse Dorsal Root Ganglia Using In Situ Hybridization and Fluorescent In Situ Hybridization

To date, significant progress has been made in uncovering the role of neurotrophins (NTs), a group of proteins synthesized and secreted by post-synaptic cells targeted by neurons. By inducing a retrograde

response, these proteins regulate gene expression and play a pivotal role in ensuring axonal viability of the presynaptic neurons and thus the maintenance of connected neurons. Mef2d and Bcl-w have been identified as among these retrograde response genes (RRGs) which are selectively induced upon NT stimulation of distal axons. In mouse models, the absence of Bcl-w, a member of the Bcl-2 family and highly localized within the mitochondria of axons, has been shown to result in axonal degeneration and progressive sensory neuropathy. Quantitative PCR indicates that Bcl-w and Mef2d are enriched in axons, particularly following neurotrophin stimulation. The current proposed model suggests that NT stimulation of the distal axons initiates a retrograde response which in turn induces transcription of Bcl-w and Mef2d.

Although quantitative PCR has already demonstrated that NT stimulation regulates mRNA levels of the aforementioned genes, we seek to add to the story of neurotrophins and retrograde response genes via qualitative analysis of this process. Through in situ hybridization in vivousing whole mount DRG nerves and fluorescent in situ hybridization (FISH) in vitro using cultured DRGs, we aim to provide a high resolution spatial and qualitative view of the cellular localization of Bcl-w and Mef2d in neurons. The work will add to the larger objective which is to understand in greater depth the coordinated regulation of Bcl-w transcription and translation in neurons with long axons; a process which plays a salient role in ensuring axonal viability and can give rise to future therapies for neurodegenerative diseases.

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Robert Martuza
Brain Tumor Research Center
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Gene therapy, or the insertion, alteration, or removal of genes within an individual's cells and biological tissues in order to treat disease, is a rapidly evolving field with enormous clinical potential, especially for cancer diseases. Our lab applies gene therapy specifically to tumor cells by creating oncolytic herpes simplex virus (oHSV) vectors which can replicate selectively in neoplastic cells and spread within the tumor in vivo, yet are nonpathogenic to normal tissue. Oncolytic vectors are generated by mutating the virus so that it is attenuated for growth in non-dividing cells, but continues to replicate in tumor cells. Since the oHSV vectors specifically target tumor cells, the vectors allow us to insert various genes, which code for anti-angiogenic products, into the tumor cells. My research focuses specifically on treating malignant peripheral nerve sheath tumors (MPNST), which are a form of cancer of the connective tissue surrounding nerves in the brain. MPNST are classified as NF1 tumors since the majority of MPNST cases are diagnosed in individuals with neurofibromatosis (NF1 disease). One of the distinctive features of NF1 disease is plexiform neurofibromas (PNF), or benign nerve sheath tumors that can transform into MPNST. Neurofibromas generally consist of a range of cell types and stromal cells such as fibroblasts, myofibroblasts and mast cells that can significantly enhance NF1 tumor growth. I am currently testing the effects of CD26 on MPNST S462 cells in vitro by using an oHSV vector containing the transgene DPP4 which encodes for CD26, which is a cell surface serine protease expressed on neurons that has been suspected to have anti-tumor properties. CD26 has been shown to have anti-tumor and anti-angiogenic properties through inhibition of the SDF-1/CXCR4 axis. SDF-1 is a small cytokine belonging to the chemokine family which has been shown to be the main agent involved in stimulating cell proliferation, angiogenesis and metastasis through interaction with its receptor CXCR4 through the stimulation of tumor blood vessel growth. Through my research I hope to demonstrate that oHSV vectors in combination with transgenes or small molecule inhibitors that target the SDF-1/CXCR4 pathway are an effective strategy to treat NF1 tumors. Since CD26 inhibits the SDF-1/CXCR4 pathway, it should also inhibit NF1 tumor growth. Testing whether blocking the SDF-1/CXCR4 pathway will inhibit MPNST growth involves titration of HSV, X-Gal staining of the HSV infected cells, and colorimetric tests of the MPNST S462 cells such as MTT and MTS assays, which measure cell viability after the addition of the oHSV vector containing CD26. The use of HSV vectors for cancer therapy and gene delivery in the nervous system is very promising and can aid in the long-term goal of therapeutic application of these vectors to cancer patients.

Yixiao Wang *Undeclared*
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Clifford Woolf
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Direct Detection of Staphylococcus aureus Peptides and Toxins by Nociceptive Neurons

Detection and recognition of bacteria has traditionally been a task associated with the immune system. Pain that occurs during infection is thought of as a downstream effect of the immune response. Staphylococcus aureus is a virulent pathogen involved in painful infections of the skin and other peripheral tissues. Here, we show that S. aureuswhole bacteria and bacterial derived peptides are capable of directly activating a subset of nociceptive neurons in murine dorsal root ganglia (DRG) that mediate pain perception. Specifically, formylated peptides such as formyl-MetLeuPhe (fMLP) and the pore-forming toxin α-Hemolysin trigger acute pain in vivo and calcium flux in neurons in vitro.Recognition of fMLP is known to be mediated by proteins from the formyl peptide receptor (FPR) family, a receptor that causes neutrophil activation and chemotaxis. Through RNA extraction and real time PCR we have for the first time detected expression of this receptor in peripheral sensory neurons. Recognition of α-Hemolysin occurs through the toxin's ability to oligomerize and form a large ion-permeable pore in the cell membrane. While hemolysin is capable of lysing cells, it only activates capsaicin-responsive sensory neurons in the mouse DRG indicating a possible specificity of targets. Our research opens the possibility that sensory neurons have developed methods to detect a variety of bacterial products and may influence how our body responds to infection. The ability of neurons to directly sense bacteria could be a novel mechanism that aids the immune system in defending our body against pathogens.

Organismic and Evolutionary Biology

Natalie Jacewicz *Organismic and Evolutionary Biology*
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Jonathan Losos & Thom Sanger
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Three hundred sixty-one identified species of Anolis lizards (anoles) inhabit the Americas and the Lesser and Greater Antilles of the Caribbean. Anoles are a classic model system for ecology and evolutionary biology with over 60 years of detailed work on their natural history. Among these species, the ecology, behavior, and body dimensions vary greatly in adults; however, little is known about these characteristics in juvenile lizards. Recent observations of hatchling lizards have shown that juveniles are not simply scaled down adults, but rather have unique body proportions relative to fully mature lizards. Studying the ontogeny of body shape and ecology in juvenile anoles offers the opportunity to better understand the extent to which natural selection has sculpted the life history of these lizards. In order to learn whether or not natural selection has shaped juvenile form and ecology with the same strength that it has in adults, I gathered morphological and behavioral data from a lizard community in the southern Dominincan Republic consisting of four species – Anolis cybotes, Anolis brevirostris, Anolis olssoni, and Anolis coelestinus. I took measurements of over 200 lizards with the goal of identifying whether or not morphological differences that exist in adults also exist in juveniles. In addition, I completed over 100 hours of behavioral observations of adults (male and female) and juveniles for each species. The collected data will shed light on the manner in which a juvenile anole develops into an adult, morphologically and behaviorally. This study is the first to analyze both juvenile behavior and morphology. My findings will elucidate the extent to which juvenile morphology and behavior vary among species and offer greater insight into the natural history of anoles.

William Polachek *Organismic and Evolutionary Biology*
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Christopher Marx
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When life's molecular basis was first unraveled, the difference between the number of protogenic amino acids and the number of three base pair codons was understood as a protection from deleterious mutations by way of redundancy. In fact, many amino acids to be incorporated into a protein's primary sequence, the third base pair of the codon is almost irrelevant. Because of this inherent mechanism that protects a gene's status quo, mutations that did not result in amino acid changes were labeled as synonymous. However, with increase of entire genome sequencing and as well as genetic engineering, it became clear that the choice of codon was not random, and bacterial lineages demonstrated biases for particular codons.

The projects of the Marx Lab focus on the experimental evolution of several species of methylobacteria that possess the ability to subsist on single carbon substrates such as methylamine, formaldehyde and methanol. In order to test the evolutionary results of the introduction of synonymous mutations on Methylobacterium extorquens, several different versions of the gene fae, which codes for formaldehyde activating enzyme were introduced into fae knockouts to create multiple evolutionary strains. As a result of the introduction of the synonymous mutations, the mutated strains demonstrated retarded growth rate in comparison to the wildtype. Over a period of several months, the strains were allowed to adapt to their new alleles and regained a wildtype-like growth rate. Over the evolutionary period, mutation and selection have lead to adaptations which counteract the synonymous mutations, however preliminary sequencing of the fae region shows that changes at that site were not the target of the adaptation.

My project is to carry out further study of the potential sites of adaptation. This includes sequencing the fae locus of all isolates of all populations of all of the strains. I am also carrying out growth rate measurements to confirm the final growth of all populations. To check other regions for potential adaptive mutations, I prepped entire genome samples for sequencing. I also worked on inserting alternate mutations into evolved lines, and preparing N and C tagged mutant alleles into knockouts to measure the protein expression before the evolutionary period. These procedures are to better understand the adaptations in response to synonymous mutations and prepare the next round of experiments.

Guo Xuan Colin Teo *Organismic and Evolutionary Biology*
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Elena Kramer
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Sculpting the Flower: Investigating AGAMOUS, a C-Class Gene, in Aquilegia

Although it is estimated that more than 280,000 species of flowering plants exist, the genetic basis of flower development across widely disparate species has been found to be highly conserved. The ABC model showed how successive whorls of flowers are formed. Prior to the ABC model of flower development, it was believed that flowers were too morphologically varied to be under similar genetic control. Under the ABC model, the outermost floral whorl, composed of the sepals, is regulated by A class genes. Similarly, the next whorl, comprising the petals, is specified by A and B class genes. The B and C class genes regulate the next whorl, which is composed of stamens. Finally, the innermost whorl, comprising the carpels, is specified solely by C class genes.

AGAMOUS is a C-Class gene that controls the development of

carpels and stamens in Arabidopsis thaliana, a widely used model plant. Mutations in AGAMOUS produce phenotypes without reproductive organs because carpels and stamens are converted into petals. The common garden rose is an extreme example of a C-Class mutant, for the stamens are converted to multiple petals. My research at the Kramer lab focuses on understanding the function, regulation, and evolution of the homolog of AGAMOUS in Aquilegia, the Columbine flower. Learning about AGAMOUS in Aquilegia will allow a better understanding of the evolution of flower development across angiosperms. This is because Aquilegia is a basal Eudicots placed in an intermediate position between Monocots and derived Eudicots, both of which have been well investigated in model plants.

The first part of my project involves resolving the phylogeny of Aquilegia AGAMOUS and the larger family of MADS box genes that it belongs to using Rice (Oryza), Grape (Vitis), and Arabidopsis as comparisons. Concurrently, another phylogeny of the Homeobox genes in Aquilegia will be constructed, and the corresponding Aquilegia Homeobox genes will be annotated. The Homeobox genes are important in floral development as they contain many genes that regulate the ABC genes. Then, using Virus-Induced Gene Silencing (VIGS), AGAMOUS will be silenced. At the same time, another candidate regulator gene of AGAMOUS, BELLRINGER, will be silenced using VIGS. Finally, RT-PCR data, and in-situ hybridization will be employed to provide expression level and area data for both AGAMOUS and BELLRINGER.

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Natural Variation Among the Dicer-like 1 (DCL1) Gene in Arabidopsis Arenosa

MicroRNA's (miRNA) have been revealed as fundamentally necessary for the silencing of many genes involved in a large variety of processes. These processes include development and disease resistance. Thus miRNAs and their target gene sequences have been shown to be highly conserved among many eukaryotes. In plants, DCL1 functions in the miRNA-dependent gene silencing pathways by 'dicing' long hairpin RNA structures into miRNA– a near-homologous function to the joint effort of two enzymes found in the better understood, Metazoan counterparts; Dicer and Drosha. Arabidopsis arenosa serves as an important organism for understanding DCL1 natural variation based on its relation to the classical model organism A. thaliana, which has been extensively studied in terms of natural variation and often used as a basis for mutant screenings to determine gene function. Thus information regarding A. arenosa contributes largely to the current findings of natural variation in the Arabidopsis genus. There are two A. arenosa sub-species that appear to be adapted to two different habitats.

Surprisingly, DCL1 in A. arenosa has been shown to include differentiated single nucleotide polymorphism (SNPs) between a small number of individuals from the two subspecies - Two of which cause amino acid changes. My research is to classify these SNPs in terms of their frequency and geographic distribution in a large number of individuals from both subspecies. We hypothesize that such polymorphisms, because of the conserved nature of the enzyme among species that share common ancestors, are unlikely to affect enzyme function, despite their proximity to functional domain coding sequences. Rather, we posit that pathogen targeting has led to variation as an attempt to avoid recognition. A similar case has been documented in metazoan model organisms.

Additionally, we plan to study DCL1 natural variation among other plant species, both those that share recent and ancient common ancestry. Such information could be useful in understanding why A. arenosa stands alone in its specific amino acid variation in the DCL1 gene.

Stem Cell and Regenerative Biology

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The Lin28/let-7 Pathway in Aging and the Regulation of Glucose Metabolism

MicroRNAs are an important method of post-transcriptional regulation of gene expression. Let-7 is one such family of microRNAs and is regulated by Lin28, a highly conserved RNA binding protein. The Lin28/let-7 axis has been implicated in processes of pluripotency, oncogenesis, developmental timing and most recently, regulation of glucose metabolism in mice. Inducible over-expression of both murine Lin28a and human LIN28B led to increased glucose tolerance and insulin sensitivity and resistance to high fat diet induced diabetes. However, the roles of Lin28 and let-7 in metabolism and aging are still unclear. We observed decreased glucose tolerance in muscle-specific Lin28a knock-out mice but also the disappearance of this difference in aged mice, suggesting a role for Lin28 in youth but not older age. Since let-7 expression in human and mouse skeletal muscle increases with age, this project seeks to determine if this is due to a decrease in Lin28 expression in aging muscle and if the Lin28/let-7 pathway may be responsible for impaired glucose uptake and increased insulin sensitivity in aging mice as well as explore the role of Lin28 as a heterochronic gene important in the genetic regulation of metabolism. With metabolic disease and malignancy sharing many biological pathways, and aging being the greatest risk factor for these diseases, this project seeks to better understand the role of Lin28 in aging, metabolism, and growth.

Matthew Abrams
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Comparison of Neuronal Maturation in Cortical Projection Neurons in the Developing Mouse Brain and Cortical-Like Neurons Generated from the Directed Differentiation of Mouse Embryonic Stem Cells

The generation of corticospinal motor neurons (CSMN) from mouse embryonic stem (mES) cells might provide therapeutic benefits for spinal cord injury and neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS / "Lou Gehrig's disease"), either via therapeutic compound screening or via neuronal repopulation. ALS is centrally marked by the progressive and coordinate degeneration of both spinal motor neurons (that use the neurotransmitter acetylcholine) and CSMN in the cerebral cortex (that use the neurotransmitter glutamate), resulting in loss of voluntary motor function and eventually death. CSMN, located in layer V of the mammalian sensorimotor cortex, are large pyramidal neurons that project their axons to the spinal cord (critical for fine motor skills), some with secondary collaterals to the striatum and medulla. Since mES cells are a potentially unlimited cell source with the theoretical potential of differentiating into all neuronal subtypes, we aim to direct the differentiation of large quantities of mES cells first into regionally-specified neural progenitors andthen into the broad class of neurons that include CSMN. Our lab, among others, has elucidated critical components of a combinatorial and multistage molecular program that controls CSMN differentiation; we hypothesize that this molecular program can be applied to direct the differentiation of CSMN from mES cells, but only at the appropriate stages of subtype-specific neuronal maturation. Toward this end, our project aims to: 1) qualitatively define the extent of CSMN neuronal maturation at multiple stages of differentiation in the developing mouse embryo, using multiple markers of neuronal differentiation; and 2) conduct a similar analysis to identify the corresponding developmental stage of mES cell-derived cortical-like neurons. Specifically, we are studying how CTIP2 high-expressing CSMN expresses the following sequence of markers of neuronal maturation, using immunocytochemistry: TuJ1 (immature post-mitotic neurons), DCX (immature migrating neurons), HuC/D (immature and mature neurons), Map2 (somatodentritic neuronal maturation), and NeuN (mature neurons), both in differentiated mES cells and in vivo. The comparison of embryonic CSMN development with mES cell differentiation will potentially enable a more precisely targeted strategy of molecular CSMN programming of mES cells. Ultimately, if large numbers of CSMN are efficiently produced via directed differentiation, potential treatments for ALS might be developed from pharmacologic and small molecule screening using these neurons, or via cell replacement.

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The Compensatory Mechanism of Pancreatic Beta Cells

Diabetes mellitus type 2 is a rapidly growing problem that cost $174 billion dollars for the US in 2007. Type 2 diabetes is characterized by insulin resistance and an inability to secrete insulin in sufficient quantities. This leads to the inability in the maintenance of blood glucose levels. The effects of type 2 diabetes can be reversed by increasing the beta cell replication rate in the pancreas. Here we examined the effect of hypoxia inducible factor 1 alpha (HIF1a) protein on pancreatic beta-cell replication rate of mice.

HIF1a was overexpressed in the liver of mice for a period of 2 to 7 days using tail vein injection of plasmids as well as for a period of 8 weeks using transgenic mice models. This resulted in more than twofold increase in beta cell replication and a significant increase in glucose metabolism. The results indicate that HIF1a is a regulatory protein involved in the beta cell replication pathway, raising possibilities for novel treatments of diabetes.

Andrea Brettler *Human Developmental and Regenerative Biology*
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Jay Rajagopal
Department of Stem Cell and Regenerative Biology
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In Vitro Culture of Mouse Embryonic Lung Buds in Matrigel

The discoveries of embryonic stem cells and induced pluripotent stem (iPS) cells have resulted in an unprecedented opportunity to produce tissue-specific cell types that can be used for disease modeling, drug screening, and possible autologous tissue repair. Currently, the Rajagopal lab has developed a reproducible and an effective step-wise protocol to produce the airway progenitors from both mouse ES cell and human iPS cells from patients with lung diseases. The purpose of my summer project is to develop an effective method to convert these airway stem cells into differentiated epithelial cells for a functional assay. In the lab, we are using embryonic lung in vitro culture as a first step for optimization of differentiation conditions. To do this, I dissected lung buds and trachea from e12.5 Spc-GFP embryos. I then embedded these tissues in Matrigel and cultured them for more than 2 weeks in various media to promote either conducting airway differentiation or pulmonary differentiation. Our results showed that e12.5 embryonic lung differentiated into Foxj1+ ciliated cells, muc5AC+ goblets cells, CCSP+ Clara cells based, T1a+ type I cells and SPC+ type II cells at different conditions. This result demonstrated that our organ culture method can mature early multipotent embryonic lung progenitors into differentiated lung epithelia. Our next steps will be (1) dissociating and re-aggregating embryonic lung and culture in vitro, (2) sorting out the pure epithelial cells from embryonic lung without mesenchyme and examining whether a similar condition can drive the differentiation, and (3) differentiating airway progenitors derived from mES and human iPS by incorporating these cells into either whole embryonic lung cell aggregation or epithelial cell aggregation.

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Lineage Restriction of Nestin-Positive Neural Progenitor Cells (NPCs) in Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis (ALS) is a destructive neurodegenerative disease of the motor neurons which most profoundly affects the spinal cord. Despite a great deal of effort that has been put into learning about the disease, much still remains unclear, including the pathogenesis of ALS. There is currently increased discussion among researchers that ALS may not simply be a disease of terminal cell types such as motor neurons, astrocytes, and oligodendrocytes, the cell types commonly implicated as the cause of the clinical disease process. Instead, the key to understanding the pathogenesis of ALS may lie in the spinal cord progenitor population. There is data to suggest that nestin-positive neural progenitor cells (NPCs) may become lineage-restricted during ALS onset and cannot differentiate into glial fates. Thus, in order to study the differentiation of NPCs to glial lineages in ALS, I am working to create a Nestin-CreER disease model mouse (SOD1-G93A). Using this mouse, I will be able to lineage label nestin-positive cells in these mice when tamoxifen is injected. I hypothesize that an analysis of the spinal cord using immunohistochemistry with markers of glial and neuronal lineages will show that during disease onset, nestin-positive NPCs have a decreased potential to differentiate into glial fates. This finding would lend support to a view of ALS pathogenesis that includes malfunctioning progenitor populations. Also, if my hypothesis is supported, it would contrast with other findings which suggest that NG2-positive oligodendrocyte progenitor cells (OPCs) increase the frequency of differentiation into oligodendrocytes during and after disease onset in the spinal cord. NPCs and OPCs may demonstrate different capabilities to differentiate into oligodendrocyte fates. This understanding from ALS pathogenesis may add to our basic science knowledge of progenitors. As in regions of the brain, there may be at least two distinct progenitor populations present in the spinal cord.

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Harvard University

The Role of Crym in the Protection of Corticospinal Motor Neurons

Neurodegeneration, or the progressive loss of structure or function of neurons, is the general term used to describe a wide range of metabolic defects that eventually result in cellular death. Recent evidence shows that mitochondrial defects and oxidative stress are especially important contributors to neurodegeneration, and common to most neurodegenerative diseases. One major disease in which these metabolic defects have been implicated is Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disease characterized by the selective loss of corticospinal motor neurons (CSMN) and spinal motor neurons (SMN). However, the specific link between oxidative stress and ALS is still unknown. Insights into the effects of oxidative stress on CSMN and SMN may prove useful in understanding the mechanism of ALS pathogenesis and

in devising possible methods of repair.

Interested in the role of CSMN specifically, Arlotta et al identified a series of genes that play a critical role in the development and survival of CSMN. One of these genes wasCrym, which encodes the NADPH dependent thyroid hormone binding protein mu-crystallin. Due to mu-crystallin's biochemical involvement with NADPH and thyroid hormone – two molecules known to regulate cellular levels of oxidative stress – we hypothesized that Crym may provide CSMN with some protection against oxidative stress-induced neurodegeneration. In order to test our hypothesis, we carried out a three-part experiment to (1) compare the behavioral response of wildtype versus knockout mice to chemically induced oxidative stress, (2) compare levels of stress-induced degeneration in wildtype versus knockout brains, and (3) compare the levels of oxidative stress and degeneration due to aging in wildtype versus knockout brains. By understanding the role of Crym in regulating oxidative stress-induced neurodegeneration, we may find a possible link between oxidative stress and the clinically relevant population of CSMN.

Edward Daniel *Human Developmental and Regenerative Biology Class of 2012*
Adams House

Amy Wagers
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Exploring the Role of the Transcription Factor Egr1 in Hematopoietic Stem Cell Proliferation and Mobilization

Homeostasis of the blood and hematopoiesis are complex processes involving numerous interactions between the environment, excreted factors, and various cell types. Of the cell types involved, hematopoietic stem cells (HSCs), a rare, multipotent population, play a crucial role in these processes due to their ability to self-renew and to differentiate into all cells of the hematopoietic lineage. Under normal conditions, HSCs reside primarily in the bone marrow in a specialized microenvironment called the "niche," which, through various cell-signaling mechanisms, maintains these cells in a quiescent state. Additionally, a small, but detectable, population of HSCs normally circulates in the peripheral blood and can reside in the spleen, thymus, and lymph nodes, creating a controlled equilibrium between HSC mobilization to the peripheral blood and other organs and engraftment back to the bone marrow. This equilibrium can be greatly shifted by a massive and rapid proliferation and mobilization of HSCs in the bone marrow niche in response to stress on the hematopoietic system, which can be caused by infection, chemical compounds, or injury.

Previous work in the laboratory has identified the transcription factor Early Growth Response 1 (Egr1) as an important inhibitor of both HSC proliferation and mobilization. I study specifically how Egr1 regulates the retention and proliferation of HSCs in the bone marrow, peripheral blood, and spleen. In order to study the effects of Egr1, I use fluorescent automated cell sorting (FACS) to collect highly purified samples of HSCs and to analyze the frequencies of these populations in the bone marrow, peripheral blood, and spleen between wild-type and Egr1 knockout mice under various conditions. These conditions include comparing young versus old mice, transplanting wild-type or Egr1 knock-out whole bone marrow cells into a lethally irradiated wild-type or Egr1 knock-out mouse, and inhibiting the activity of macrophages, a cell type that has been shown to promote the retention of HSCs in the niche. Additionally, I have attempted to identify and characterize possible downstream targets of Egr1 that could contribute to the phenotype seen in Egr1 knock-out mice.

Ultimately, understanding these pathways and the niche microenvironment can lead to new insights on ways to improve the safety and efficiency of bone marrow transplants.

Theresa Feng *Human Developmental and Regenerative Biology Class of 2013*
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Douglas Melton
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Investigating the Expression of Small Full-Length mRNAs During β Cell Development Using in Situhybridization

Insulin is imperative to maintain glucose homeostasis, as it enables cells to uptake glucose. β cells, found in the islets of Langerhans in the endocrine pancreas, are responsible for insulin production. Diabetes results when these cells are destroyed or malfunction. The focus of the Melton lab is to understand the development of the pancreas with the overall goal of finding a cure for type I diabetes. I am investigating the expression of small full-length mRNAs in the pancreas of 14.5 dpc mouse embryos, a time corresponding to the peak of endocrine genesis. These small mRNAs are less than 1kb and have been largely overlooked. In situ hybridization using anti-sense mRNA probes generated from adult β cells will allow for the characterization of the spatiotemporal expression of these small mRNAs. Endocrine progenitors express the transcription factor Neurogenin 3 (Ngn3). Validation of the in situ protocol involved using Ngn3 as a positive control for specificity and a small mRNA probe for Collagen 9α3 as a positive control for sensitivity. Initial observations unexpectedly revealed greater Ngn3 transcript expression than previously shown. A possible discrepancy between the amount of Ngn3 transcript and protein suggests some form of post-transcriptional regulation, and this will be explored. Identification of a small full-length mRNA co-localized with Ngn3 or miRNA/protein involved in Ngn3post-transcriptional regulation will lead to novel insights into β cell development, could guide the directed differentiation of stem cells into β cells, and eventually be used for cell replacement therapy for diabetics.

Andrea Henricks *Molecular and Cellular Biology Class of 2013*
Cabot House

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Natural Killer (NK) cells are an important component of the innate immune system and perform both cytotoxic and regulatory functions. NK cells are important in recognizing and destroying invaders as well as stimulating the adaptive immune response through the release of cytokines and chemokines. Cytotoxic activity occurs by the secretion of lytic granules containing perforin and granzymes into the immunological synapse (site of contact between NK cell and its target). While most of the perforin and granzymes penetrate the target cell, NK cells must have a method for reabsorbing the excess cytotoxic material, but this method is not well understood.

It has been shown that the vesicle associated membrane proteins, VAMP4 and VAMP7, are both necessary for proper NK cell function and play a role in the release of lytic granules, but the specific function of these proteins has not been elucidated. While VAMP7 appears to play a broader function in NK cell activity, we believe VAMP4 is specifically needed for the reuptake of granzyme B from the immunological synapse and are using degranulation and endocytosis assays as well as imaging to test this theory.

Yannis Kalogirou Valtis *Human Developmental and Regenerative Biology*
Leverett House *Class of 2012*

David Scadden

Center for Regenerative Medicine
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The Role of Mesenchymal Cells in the Neural Stem Cell Niche and in Brain Cancer

Adult multipotent stem cells in various organs, including the bone marrow (BM) and the central nervous system (CNS), reside in specialized microenvironments called niches, which regulate their state and function. In the adult CNS, neural stem cells (NSCs) form clusters with the vascular system, which includes endothelial and perivascular mesenhcymal cells (pericytes). While endothelial cells have been shown to stimulate self-renewal of NSCs in vitro, the functional participation of perivascular populations in the regulation of NSCs remains elusive. Using both in vitro and in vivo systems, we plan to characterize the role of perivascular cells in the mouse CNS and their functional significance as part of the normal and malignant stem cell niche. Our project will utilize genetic model systems developed in our laboratory to selectively ablate pericytes in the CNS, as well as murine glioblastoma models to study the significance of pericytes in tumorigenesis.

Manjinder Kandola *Chemical and Physical Biology*
Leverett House *Class of 2014*

Anthony Rosenzweig
Cardiovascular Division
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Evaluation of the Role of p16 in Senescence of Cardiomyocytes

The heart has been extensively noted for its limited ability to regenerate, often resulting in fibrosis and marked reduction in function following cardiac ischemia or similar stress conditions, contributing to the large health burden of heart failure. Furthermore, aging in both humans and animal models is characterized by diminished cardiac function. Cyclin-dependent kinase inhibitor 2A (CDKN2a), or p16, has been characterized as a tumor suppressor protein due to its role in inhibiting Cdk4 and thereby negatively regulating cell cycle progression. Therefore, p16 also promotes cell senescence, preventing cell proliferation and regeneration. Here, we seek to study the effect of reduced expression of p16 in cardiomyocytes (heart muscle cells) on the heart's regenerativecapacity. Thus, we create a consistent model of p16 knockdown in neonatal rat cardiomyocytes in culture by employing a small interfering RNA (siRNA) transfection method and demonstrate that both mRNA and protein levels of p16 can be significantly reduced for at least forty-eight hours. This model thereby creates a window to explore the effects of such a knockdown, in which we further seek to validate downstream changes in the cell cycle pathway and to characterize phenotypic responses in the hope of yielding valuable insight to cell proliferation in the heart.

Alyssa Klein *Chemistry*
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As Schofield proposed in 1978, the stem cell niche is a specialized microenvironment that regulates stem cell fate in various tissues throughout the body, including the bone marrow (BM). The niche is a dynamic entity enabling both maintenance of stem cell quiescence and stem cell mobilization following application of specific stimuli. By studying the conditions that regulate niche function, we can gain a better understanding of the control of stem cell homeostasis. Recent findings have suggested both that hematopoietic stem cells localize in hypoxic regions and that stem cells localize near blood vessels. However, such studies have been performed separately, and whether these two findings contradict or support each other has not yet been addressed because of the lack of techniques with sufficient resolution to image oxygen tension in the stem cell niche in vivo. The development of a two-photon-enhanced phosphorescent nanoprobe (PtP-C343) allows accurate and high-resolution measurements of oxygen partial pressure (pO2) in the bone marrow vessels and interstitial space, where stem cells reside. Because oxygen quenches phosphorescence emitted by the excited nanoprobe, phosphorescence decay lifetime is inversely proportional to pO2, which allows us to determine the levels of oxygen after excitation with two-photon energy. This study will allow us to understand whether manipulating BM tissue oxygenation could be a novel way to improve stem cell yield during mobilization and engraftment following BM transplantation.

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Directed Differentiation of Human Induced Pluripotent Stem Cells into Corticospinal Motor Neurons

Corticospinal motor neurons (CSMN) are a therapeutically relevant population of subcerebral projection neurons required for motor function. Like other projection neurons, CSMN develop from neural progenitor cells (NPC) in the germinal zone of the dorsal telencephalon. Proneural transcription factors, such as Ngn2 and Mash1, drive the first fate decisions of NPC to a neuronal rather than glial fate. Subsequently, CSMN develop from the glutamatergic subset of NPC under the direction of lineage-specific transcription factors. One such master transcriptional regulator is Fezf2, which directs the birth and specification of CSMN in the cerebral cortex. Here, I plan to draw from this developmental information to attempt the directed differentiation of CSMN fromhuman induced pluripotent stem (hiPS) cells. My strategy is to use Fezf2 along with Ngn2 or Mash1 to drive hiPS cell-derived NPC of dorsal forebrain identity (Pax6+/FoxG1+) to a subcerebral projection neuron/CSMN fate, by way of both lentiviral (LV) vectorsand gene targeting. I have previously contributed to setting up a protocol for differentiating hiPS cells into dorsal telencephalic NPC, optimizing gene delivery methods by viral infection, and generating ongoing strategies to produce CSMN from hiPS cell-derived NPC. Preliminary results from experiments aiming to optimize a full differentiation protocol have shown that the LV transduction efficiency of the hiPS cell-derived NPC is typically very low compared to other cell types in the heterogeneous population. With the understanding that more optimal gene delivery methods are necessary, I have begun preparations for gene targeting in hiPS cells. I aim to make a Cre-recombinase inducible hiPS line that will ensure overexpression of Fezf2 and either Ngn2 or Mash1 at the time of endogenous expression of Pax6. Overall, I hope that my further research will illuminate possible therapies for the degeneration of CSMN due to disease or spinal cord injury.

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Using Patient-Derived Induced Pluripotent Stem Cells (iPSCs) to Model Dunnigan-Type Familial Partial Lipodystrophy (FPLD2)

Dunnigan-type familial partial lipodystrophy type 2 (FPLD2) is a metabolic disorder clinically characterized by the gradual loss of subcutaneous fat from the body's extremities and trunk and the accumulation of adipose tissue on the face and neck regions that begin roughly at the onset of puberty. While it is a relatively rare disease, its further study has great potential to yield invaluable insights into more prevalent metabolic complications such as the growing obesity epidemic. First, FPLD2 recapitulates many of the metabolic complications often associated with obesity, such as insulin resistance and hypertriglyceridemia. Moreover, FPLD2 is convenient to study in a laboratory setting because it is a monogenic disorder unlike obesity, which is a complex, multi-factor condition.

In an effort to study the adipose dysfunction inherent in FPLD2 in greater detail, the overall goal of this project is to develop a cell-based model of FPLD2 using patient-derived induced pluripotent stem cells (iPSCs). These patient-specific iPSC lines will permit a closer examination of adipogenesis and adipocyte function during the overall disease development of FPLD2 in an in vitro setting. Our lab has previously derived iPSCs from FPLD2 patient fibroblasts, and we are currently studying the differentiation of these patient-derived iPSCs into adipocytes and the functional differences between the resulting wild-type and disease-line adipocytes. Furthermore, we will be using several gene-editing strategies to perform rescue experiments to see if the disease phenotypes associated with FPLD2 can be reversed.

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Identification of Small Molecules that Increase Efficiency of Direct Conversion from Old Mouse Fibroblast to Motor Neuron

Direct conversion is the process by which a terminally differentiated cell can be induced to directly convert into another cell type. Recent work has shown that mouse fibroblasts can be treated with a set of transcription factors to induce direct conversion into neurons. However, while fibroblasts isolated from young embryonic mice yield a 20% efficiency, those isolated from old adult mice only yield a 0.2% efficiency. This 100-fold difference is an obstacle to human regenerative medicine because the ultimate goal is to treat adult human patients rather than human embryos. We attempt to increase the efficiency of old direct conversion through a small molecule screen, beginning by isolating tail tip fibroblasts from 2-month-old transgenic mice expressing the Hb9::GFP neuronal marker, so that successfully converted fibroblasts glow green as induced motor neurons (iMNs). We infect the old fibroblasts with 7 lentiviruses to induce conversion, treat with a library of 400 small molecules, and screen for a hit compound that increased iMN number and hence direct conversion efficiency. From there, we attempt to identify and understand the pathway through which the hit compound interacts, allowing for better control over cell fate and leading to important implications for regenerative medicine.

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Congenital heart diseases present in numerous clinical manifestations and occur in 19-75 of every 1,000 live births. The complexity of the molecular interactions in cardiogenesis potentially explains the sensitivity of the heart to perturbations before birth. Despite decades of lineage tracing experiments aimed to identify the origin of the heart, only recently did a more completepicture of cardiogenesis emerge. A novel cell population named the second heart field, marked by expression of theLIM/homeodomain transcription factor Islet1, was found to contribute significantly to the right ventricle and outflow tract. The Isl1+progenitor population initially resides within the pharyngeal mesoderm dorsal-lateral to the primitive heart tube and migrates into theheart during later stages of cardiogenesis. However, the mechanism and regulation of this migration is largely unknown. Preliminary results suggest that the epithelial-to-mesenchymal transition (EMT), a well-characterized phenomenon in development and disease, may play a role in the migration of Isl1+ progenitors. Future work from this project will focus on providing more evidence to support the mechanistic link between EMT and Isl1+ progenitor cell migration, on uncovering the identities of the chemokines that directIsl1+ progenitor cells to their final destination, and on elucidating the role of cell-cell adhesion molecules in defining the microenvironment of Isl1+ progenitor cells in both their pre- and post-migratory states.

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Characterization of Cyclin Dependent Kinase Inhibitor P57 in Hematopoietic Stem Cells

Cyclin Dependent Kinase Inhibitor p57 is a putative tumor suppressor that limits cell proliferation by tightly binding to G1 Cyclin/Cyclin Dependent Kinase complexes. The downregulation of p57 has been implicated in severe childhood diseases such as Beckwith-Wiedemann syndrome and Wilm's tumor. Despite its established role in cell cycle inhibition, the function of p57 has yet to be fully characterized in Hematopoietic Stem Cells (HSCs). The p57 knockout is lethal in mice, making it one of the least characterized and understood members of the CIP/KIP family. As the only member of the CIP/KIP family to demonstrate high levels of expression in HSCs, p57 may play a role in maintaining HSC quiescence or inhibiting their growth. Paradoxically, p57 is upregulated in stem cell types such as embryonic stem cells that exhibit rapid proliferation. Therefore, further characterization of p57 is necessary in order to better understand its function in the context of the stem cell cycle inhibition pathway.

In this study, the function of p57 in HSCs was investigated by first establishing an experimental assay approach with Jurkat and3T3 cell lines. Cell lines were transduced with pHAGE2 lentivirus in order to introduce a p57 and adjacent fluorescent reporter (ZsGreen) vector. In addition, cells were maintained at different concentrations over a defined time course, induced with Doxyclycline utilizing a TRE construct, and assayed after attaining normal post-transduction proliferative activity. With Jurkat and3T3 cell lines, we were able to establish an effective assay system that measured a variety of cell properties. Cells were analyzed for frequency of fluorescence and DNA ploidy distribution using flow cytometry, expression using Western Blots, localization using immunohistochemistry, and phenotype using fluorescent microscopy. It was found by frequency of fluorescence that expression of p57 did not acutely decrease cell proliferation, though it did affect the morphology of 3T3 cells. We plan to translate our established investigative approach to study the absence and upregulation of p57 in primary and hematopoietic stem/progenitor cells. With a better understanding of p57 function in HSCs, we hope to elucidate its effect in stem cells and help better characterize its potential role in other acute diseases.

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Modulation of H3K27me3 in Embryonic Stem Cell Differentiation and Transcription Factor-Based Reprogramming

Chromatin landscape is dynamic and proper regulation is crucial for normal differentiation and development. In embryonicstem cells, bivalent domains are regions with overlapping enrichment in the activating trimethylation of histone H3 at lysine 4 and the repressive trimethylation of histone H3 at lysine 27 (H3K27me3). These epigenetic marks are proposed to contribute to developmental potency by keeping key developmental genes silent while poised for activation. To understand how bivalent domains are resolved during lineage commitment and reconstituted during stem cell reprogramming, I am specifically examining H3K27me3 dynamics and its associated epigenetic modifiers, including polycomb subunits that propagate the mark and demethylases that remove it. I am characterizing global and loci-specific changes in H3K27me3 during initial induction of neuronal differentiation and analyzing how perturbations of the epigenetic modifiers affect the resolution and restructuring of bivalent domains during differentiation and generation of induced pluripotent cells, respectively. I am further interested in exploring whether global H3K27me3 patterns are reversible and influenced by cytosine methylation and characterizing how knockdowns of H3K27me3-specific modifiers affect tissue differentiation in vivo. The mechanistic knowledge would increase current understanding of the role of epigenetics in development and factor-based stem cell reprogramming.

Gina Pan *Human Developmental and Regenerative Biology*
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During mammalian development, the fetal myocardium grows by cardiomyocyte proliferation. In the postnatal stage, cells begin to undergo hypertrophic growth, with cardiomyocyte volume increasing over two-fold between day 3 and day 12. However, the mechanism of the switch from cell proliferation (hyperplasia) to increase in cell size (hypertrophy) is not well understood.

At the root of cell number regulation are signaling pathways that communicate cellular cues to gene transcription. The Hippo (Hpo) signaling pathway, whose components are highly conserved in mammals, plays an important role in organ size control by inhibiting cell proliferation and promoting apoptosis. The Hippo pathway kinase cascade phosphorylates and inhibits Yap, a transcription coactivator downstream in the pathway. Recent research reports that Yap increases organ size, as observed in experiments involving the liver, intestines, and skin.

The purpose of this project is to investigate the Hippo-Yap pathway, in particular the effect of Yap on embryonic and perinatal myocardial growth. We investigate the phenotype, proliferation, and apoptosis of the mouse heart using a Cre-loxP genetic lineage tracing system and genetic models of loss and gain of function of the Yap gene. Our preliminary data has shown that in embryonic hearts, Yap loss of function has resulted in decreased cell proliferation in comparison to control hearts.

Charles Puza *Human Developmental and Regenerative Biology*
Cabot House *Class of 2014*

Rosalind Segal
Department of Cancer Biology
Harvard Medical School & Dana-Farber Cancer Institute

Cell-Autonomous Mutant Phenotype of BDNF Mutant Purkinje Cells

Brain-derived neurotrophic factor (BDNF) is a transcription factor located throughout the brain and central nervous system. BDNF helps to promote the growth, survival, and differentiation of neurons and the synapses connecting them. In particular, BDNF has been proven to be vital for the arborization of the dendrites of Purkinje cells and pruning of the primary dendrites extending from the soma (cell body). Purkinje cells, located in the Purkinje cell layer of the cerebellum, are planar cells that stack on top of each other and are easily distinguishable from other brain cells. Noted for their elaborate arborization and multiple spines, Purkinje cells are among the largest in the brain. While the absence of BDNF has been proven to be dentrimental to the development of Purkinje cells, one can not been sure if the mutant phenotype is cell-autonomous. To test this, a mosaic analysis system was utilized in which it was possible to simultaneously knock-out BDNF and label the mutant cell in an otherwise wildtype environment. The mutant brains will be sagittally sliced using a cryostat and examined using a confocal microscope.

Richard Smith *Chemistry*
Eliot House *Class of 2013*

Joseph Vacanti
Harvard Stem Cell Institute

Engineering Vascularized and Innovated Skeletal Muscle

This project looks to directly tackle the largest problem in reconstructive surgery today - insufficient donor tissue.

Even though the technique to grow monolayers of cells has existed for decades, the complexity involved in synthesizing a complex, multi-cellular structure, such as a human heart, presents many problems. However, a collaborative scientific effort has led to the development of a system that generates tissue specific cells on a multi-dimensional, biodegradable extra cellular matrix (ECM) that is implanted into the patient. The nanometer sized ECM scaffold is dynamic in its design, where ligand rich surfaces interact with cell receptors to coordinate cell fate determination and three-dimensional organization. The Vacanti lab has already utilized this technique to synthesize a human ear on the back of an immunocompromised mouse, an iconic image embodying recent developments in the field of tissue engineering.

Composed mainly from cartilage, the human ear circumvents the complexities involved with a highly vascularized network. Conversely, muscle does not and requires an intricate network of micro-sized blood vessels. Coupled with an elaborate nervous system that must harmoniously control muscular contraction, skeletal muscle synthesis provides many hurdles that we are attempting to overcome. The current project explores ECM designs that lead to precise and optimal cellular patterning, proliferation and differentiation of various cell types, within a biological environment. Human umbilical vein endothelial cells (HUVECs), cells from spinal cord explants and an array of pluripotent stem cells are seeded within the ECM and allowed to develop. The myoid (muscle fiber) that is produced is implanted into immunocompromised mice and rats and studied at various stages of development. Although the system is far from perfect and the project is tackling the long standing challenges in ECM design: the ability to be reengineered by resident cells and to efficiently transfer nutrients andoxygen while removing waste. Working in conjunction with the Armed Forces Institute for Regenerative Medicine (AFIRM) the real value of the project lies in problem solving that could take a closer step toward human tissue regeneration reality.

Janet Song *Chemical and Physical Biology*
Quincy House *Class of 2013*

Jeffrey Macklis
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Characterization of the Function of Pre-synaptic Organizing Protein 1 (POP1) in the Segmental Targeting of Corticospinal Motor Neurons

Corticospinal motor neurons (CSMN) are a specific class of output neurons found in layer V of the neocortex that control voluntary movement by making synaptic connections with multiple different subcerebral target neurons in the hindbrain and cervical and lumbar spinal cord. CSMN degeneration in diseases such as amyotrophic lateral sclerosis (ALS), hereditary spastic paraplegia (HSP), and primary lateral sclerosis (PLS) leads to debilitating, incurable paralysis characterized by well-known anatomic heterogeneity. Previous studies in the Macklis lab have identified developmentally-regulated genes that act together to control the development of CSMN, and more recent work has begun to investigate the molecular controls that govern differential segmental targeting in the spinal cord of cervical-projecting CSMN (CSMNC) and lumbar-projecting CSMN (CSMNL) respectively. Pre-synaptic Organizing Protein 1 (POP1) was identified as a candidate molecular control expressed specifically by CSMNL at a time when their axons are extending toward their targets in the lumbar spinal cord. To examine the effect of POP1 on CSMN segmental targeting, we used an over-expression construct to ectopically express POP1 in CSMNC via in utero electroporation. We hypothesized that POP1 over-expression would result in these axons projecting past their normally-preferred cervical targets to more distal targets in the lumbar spinal cord. Preliminary results have been promising, and we will continue to study POP1 to expand our knowledge of the molecular controls that govern CSMN segmental-targeting specificity.

Will Sun *Chemical and Physical Biology*
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Lee Rubin
Department of Stem Cell and Regenerative Biology
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Characterizing Small-Molecule Regulators of Survival of Motor Neuron Protein

Spinal muscular atrophy (SMA) is a neuromuscular disease that is the leading genetic cause of death among infants. SMA primarily arises from the deletion or mutation of the gene Survival of Motor Neuron 1 (SMN1), resulting in diminished SMN protein expression, motor neuron death, and progressive muscle atrophy. Through the use of high-content screening, we have identified several classes of compounds that effectively increase SMN protein expression. Interestingly, all compounds tested exhibit a similar dose-response, where higher concentrations are increasingly toxic but nonetheless continue to elevate overall SMN in thecell population. Single-cell analysis verified that the compounds do not act in a selective manner, but instead increase SMN in all surviving cells. To further investigate this phenomenon, we explored the expression profiles of human SMA fibroblasts to identify common stress-response pathways implicated in the observed cell toxicity. Though several therapeutics have reached clinical trials, the pathogenesis of SMA has yet to be fully understood. By characterizing particularly compelling pathways produced by the expression analysis, we hope to shed additional light on the molecular mechanisms underlying SMA.

Amy Wang *Neurobiology*
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Clifford Woolf
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Though pain usually protects us by alerting us of harm, when the response becomes maladaptive, pain becomes a disease. Recent advances in stem cell technology may allow for the study of human nociceptors. In the past decade, scientists have differentiated specific cell types from embryonic stem cells, reprogrammed adult cells back into pluripotency (induced pluripotent stem cells, or iPSCs) for further directed differentiation into cell types of interest, and converted fibroblasts into other cell types in a process called transdifferentiation, bypassing the induced pluripotency step. I intend to transdifferentiate pain-sensitive neurons (nociceptors) from mouse embryonic fibroblasts by introducing certain combinations of transcription factors into the fibroblasts. In 2010, Vierbuchen et al. established a triad of factors which convert fibroblasts into non-specific neurons. The literature points to other factors such as Runx1, Islet1, and Drg11 which seem crucial for nociceptor development specifically; I plan to introduce a combination of these factors into fibroblasts with retroviruses. Using calcium imaging and electrophysiology, I will then monitor the fibroblasts for the most salient features of nociceptors, which are the ability to generate action potentials, to respond to capsaicin through the TrpV1 channel, and to express a particular subset of voltage-gated sodium channels. Once a protocol is established, the next step is to apply a similar protocol to transdifferentiate human fibroblasts into nociceptors. This will open up possibilities for more patient-specific treatments, help determine what predisposes certain people to developing pain, and accelerate the process of identifying and screening new targets for pain-relieving drugs.

Bing Fang Edna Wang *Undeclared*
Pforzheimer House *Class of 2014*

Bakhos Tannous
Department of Neurology
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Identification of New Therapeutics for Glioblastoma Multiforme

Glioblastoma multiforme (GBM) is the most common and most malignant primary brain tumor in humans. Infamous for its resistance to treatments including surgery, chemotherapy,

and radiation therapy, the highly invasive GBM is fatal; most patients diagnosed with GBMs experience survival of less than two years. The frighteningly low survival rate from GBM draws significant attention and calls for an urgent need to develop new treatments for the disease.

Dr. Tannous's lab screened over three thousand drugs and compounds using a novel drug screening procedure based on bioluminescence monitoring of the Gaussia Luciferase (Gluc), the expression of which is introduced to tumor cells through a lentivirus vector. By catalyzing light-producing chemical reactions that can be measured in a Luminometer, the naturally secreted Gluc in the media of cultured tumor cells serves as a robust indicator of cell viability, which allows the tracking of tumor cell growthand their response to drug therapy. Employing the Gluc screening assay, Dr. Tannous's lab has identified two drugs that potently kill GBM cells in vitro: Anthotecol and Obtusaquinone.

The goal of my project is to validate these two drugs in vitro and in vivo while investigating the underlying apoptotic mechanism and the signaling pathways induced by these drugs. In vitro, after treating glioma cells with each drug, we confirmed that both Anthotecol and Obtusaquinone are toxic to glioma cells using the Gluc assay. In vivo, we performed a subcutaneous implantation of U87 glioblastoma cells that express Firefly luciferase (Fluc) in nude mice. The expression of Fluc through fluorescence imaging serves as a reporter for tumor growth in mice models. With half of the mice being treated with Obtusaquinone over a period of 14 days while the other half serving as the control, we monitored the weight of the mice over the period of treatment and imaged the mice for Fluc activity to observe the effects of the drug on the tumors in mice models. Another aspect of my project is to study the mechanism of action triggered by these drugs. We looked for the presence of caspase activity, an important indicator of apoptosis, in treated glioma cells. We also treated cultured tumor cells with N-Acetyl Cysteine (NAC), an antioxidant, in combination with the drugs and discovered that NAC inhibits the toxicity induced by both drugs. Since NAC is a scavenger for Reactive Oxygen Species (ROS), which protects cells from hypoxia (characterized by low oxygen concentration in cells), we hypothesize that the apoptotic signaling pathway stimulated by these drugs involves hypoxia. Using tumor cells obtained from excised tumors in mice models, we will probe for proteins involved in the pathway using Western Blot. Results from this study will provide new insights in developing effective therapeutics for GBM.

ACKNOWLEDGEMENTS

Finally, PRISE 2011 would not have been possible without the contributions of the following:

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Gregory Llacer
Director of the Office for Undergraduate Research Initiatives and Harvard College PRISE

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

Additional thanks to:

Drew Faust, President of Harvard University
Michael D. Smith, Dean of the Faculty of Arts and Sciences
All the Mentors, Principal Investigators, and Lab Members
The PRISE Fellows of 2011

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