**Poster # 2015.401**

**Presenter** Anna Mukhina (Brandeis / Biology, Neuroscience)

**Title** The Molecular Basis of Cold Sensation and Hygrosensation in Drosophila melanogaster

**Authors** Anna Mukhina, Zach Knecht, Paul Garrity

**Abstract** Thermoregulation is an important process for all animals in order to achieve optimal temperatures for growth and reproduction. Recent work has found several key warm sensing molecules that mediate thermosensation, including TRPA1, Gr28b(D), and Painless. While we know the molecular and cellular processes that govern warmth avoidance in D. melanogaster, the molecular mechanisms that underlie cool avoidance remain unknown. In order to investigate these mechanisms, we performed behavioral testing in dry preference and cold avoidance assays. Our results suggest that three ionotropic receptors (IRs), Ir21a, Ir25a and Ir93a, appear to function together to control cool avoidance. Interestingly, two of these IRs, Ir25a and Ir93a, also control humidity sensation, potentially working with yet another IR, Ir40a. These preliminary findings suggest that Ir25a and Ir93a might form both cool and moisture receptors, with the specificity provided by the third subunit.

**Poster # 2015.403**

**Presenter** Elon R. Mathieson (Brandeis / Neuroscience)

**Title** Taste Experience Enhances Conditioned Taste Aversion Regardless of Palatability

**Authors** Elon R. Mathieson, Veronica L. Flores, Donald B. Katz

**Abstract** Experience influences how an animal perceives and learns about sensory stimuli, thereby controlling behavior. Conditioned taste aversion (CTA) - a paradigm whereby an animal learns to associate the sweet taste of sugar with malaise - offers a tool that we can use to better understand this general truth. It has long been known that experience with sugar prior to training weakens eventual CTA learning; research in our lab, meanwhile, has shown that innocuous exposure to sour (unpalatable) and salty (palatable) tastes has the opposite effect, strengthening sugar CTAs in rats. However, the question of whether the specific palatability of the prior taste experience plays a specific effect on CTA learning has not been examined. In this experiment, we directly compared learned aversions between rats that had experience with salty taste versus rats that had experience with sour taste. Results to date suggest that the palatability of taste experience does not influence CTA—that is, exposure to sour and salty tastes have similar impact on later CTA. The implications of this study suggest that the palatability of a taste has no bearing on aversion learning; it is the experience of different tastes in recognition with one another that influences learned aversions.

**Support** STEM Posse Summer Funding Award
Title MutS, XonA, and XseA and their Effect on Spontaneous Mutations in *Escherichia coli*

Authors Ariana Traub, Vincent Sutera, Alexander Ferrazzoli and Susan T. Lovett

Abstract Spontaneous mutations naturally occur for a variety of reasons, predominantly for evolution in successive generations of organisms. In this experiment we examine spontaneous mutations accumulated over a multitude of generations in wild-type *Escherichia coli* and three derivatives: *mutS*, *xonA*, *xseA*. All three of these genes, *mutS*, *xonA*, and *xseA*, play important roles in DNA mismatch repair, the major method for repairing replication errors. MutS recognizes the mismatch and recruits MutL and MutH to form the Mismatch Repair System (MMRS), and both xonA and xseA are exonucleases responsible for the excision step of mismatch repair. We sought to obtain, sequence, and analyze genomic DNA at the beginning (day 0), middle (day 10) and end (day 21) of the growth days. The overall number and distinct types of mutations were determined for each strain. We hypothesized that the triple deficient mutant (STL19689 ΔxonA ΔxseA mutS::FRT Kan) would result in the highest number of mutations and mutations would increase considerably from day 0 to day 21.

Support Provost's Undergraduate Research Fund

Poster # 2015.405

Presenter Michelle W. Kim (Brandeis / Psychology)

Title Do Inadequate Anti-Inflammatory Responses Underlie Exaggerated IL-6 Responses to Repeated Stress in Overweight Individuals?

Authors Michelle W. Kim, Christine McInnis, Dena S. Goldblatt, Danielle Gianferante, Luke Hanlin, Xuejie Chen, Myriam Thoma, Nicolas Rohleder

Abstract Approximately two-thirds of Americans either overweight or obese, which increases risk of inflammatory-mediated diseases such as atherosclerosis and type 2 diabetes. We have recently shown that these individuals also have exaggerated pro-inflammatory responses to repeated stress, therefore potentially putting them at even greater risk these diseases. Therefore, we sought to investigate how pro-inflammatory responses are exaggerated in overweight and obese individuals. We subjected 40 healthy adults with a range of BMIs to the Trier Social Stress Test (TSST) two consecutive afternoons. We collected blood and measured IL-6 at baseline, 30 and 120 minutes post-stress. We measured IL-10 at baseline, 60 and 120 minutes post-stress.

Baseline IL-10 was unrelated to adiposity. IL-10 responded to TSST2 only when waist circumference was included as a covariate (F(2,66)=3.1, p=0.052). No such relationship existed for TSST1. There was a marginally significant interaction of waist circumference by time in response to TSST2 (F(2,66)=2.7, p=0.07) but not TSST1. IL-10 responses at 60 and 120 minutes post-TSST1, and 60-minutes post-TSST2, were inversely related to adiposity. IL-10 responses were unrelated to IL-6 responses on either day. We found that overweight and obese individuals display inadequate anti-inflammatory IL-10 responses to stress. However, this did not explain exaggerated pro-inflammatory stress responses. This may be due to a relatively small sample size. Our findings are in line with literature reporting deficient anti-inflammatory stress responses in overweight and obese individuals.

Support Provost's Undergraduate Research Fund
Title Enzymatic Transformation "Turns Off" Ligand-Receptor Interactions of Small Molecules

Authors Richard Haburcak, Junfeng Shi, Bing Xu

Abstract Enzyme induced conformational change is ubiquitous in biological systems, used in signal transduction and cellular processes. As an example, during apoptosis formation of the apoptosome is triggered by dephosphorylation of the cytosolic dATP/Apaf-1 complex allowing for subsequent self-assembly. The apoptosome then binds Procaspsase-9 and activates Caspase-3, leading to apoptosis. Inspired by the interplay between enzymatic transformation and specific binding, we introduce the ligand-receptor binding of the antibiotic Vancomycin and the peptide moiety D-Ala-D-Ala to enzyme-instructed self-assembly. Mimicking this fundamental process produces a system which dynamically responds to enzymatic transformation. The peptide 1P (NapFFYpGGaa) binds with Vancomycin; however, after enzymatic conversion of 1P to 1 (NapFFYGGaa), the hydrogelator 1 exhibits a binding too weak to be measured by ITC. Binding to Vancomycin is restored by the addition of a surfactant, Tween-80, which breaks up the nanosheets formed by 1. Investigation of the solution structure of 1 by NMR shows no significant secondary structure which prohibits binding to Vancomycin. By taking advantage of the unprecedented temporal and spatial control provided by both enzymatic transformation and ligand-receptor interactions, the inherently dynamic self-assembly process can be used to target cancer cells which activate the precursor 1P, potentially leading to enhanced selectivity in cancer therapy.

Support MRSEC Summer Research Fellowship

Poster # 2015.406
Presenter Richard Haburcak (Brandeis / Chemistry, Mathematics)
Title Enzymatic Transformation "Turns Off" Ligand-Receptor Interactions of Small Molecules
Authors Richard Haburcak, Junfeng Shi, Bing Xu

Poster # 2015.407
Presenter Anna Bigney (Swarthmore College / Biology)
Title Assessment of Quasi-Palindrome Mutation Rates in E. coli
Authors Anna Bigney, Laura Laranjo, Stephen Gross, and Dr. Susan Lovett

Abstract Quasi-palindromes (QPs) are almost perfect inverted repeats of DNA that form a hairpin and are capable of stalling DNA polymerase. The stalling of the DNA polymerase could cause template switch events and mutations, which are implicated in a wide range of human diseases including cancer and muscular dystrophy. Our goal is to quantify the mutation rate of QPs in E. coli. The Lovett Lab has created a QP reporter in E. coli for the leading and lagging strands by inserting four base pairs into the lacZ gene, which makes the strain lacZ-. When a QP mutation occurs the strain becomes lacZ+ and is identifiable by its blue phenotype on LacMinXI plates. Our strains were treated with 5-azacytidine, an FDA approved cancer drug that stimulates polymerase stalling, for a more robust mutational response. Wild type strains as well as strains lacking the SOS response protein RecA were used in the mutation rate assay. Our results indicate the leading strand has a higher mutation rate than the lagging strand and that 5-azacytidine stimulates further mutations in both the wild type and mutant strains. In the near future the Lovett Lab will begin to quantify the mutation rate in yeast to better understand quasipalindrome mutation avoidance mechanisms in eukaryotes.

Support Cell and Molecular Visualization REU
**Poster # 2015.408**

**Presenter**  Martha Garcia, Connie Lee, William Lenh, Dadmaly Olmedo, Harry Rankin, & Kyle Tan  
(Waltham High School)

**Title**  In 3D - Molecules of life: a pilot outreach course

**Authors**  Students: Martha Garcia(1), Connie Lee(1), William Lenh(1), Dadmaly Olmedo(1), Harry Rankin (1), & Kyle Tan(1)  
Developers: Daniel Pomeranz Krummel(2), PhD.; Anique Olivier-Mason(3), PhD; Eduardo Beltrame(4); Vivekanand Pandey Vimal(5)  
(1)Waltham High School; (2)Dept. Biochemistry; (3)BioInspired Soft Materials MRSEC Director of Education, Outreach and Diversity; (4)Federal University of Santa Catarina, Brazil; (5)Neuroscience Program, PhD candidate

**Abstract**  This summer six Waltham High School students participated in a pilot K-12 outreach, attending morning lectures and afternoon practicums. The motivation for this outreach was to inspire K-12 students to pursue careers in the sciences, prepare graduate students in the physical and life sciences to be the next generation of teacher scholars, and teach students the physiochemical principles that govern molecular structure by having them visualize, manipulate, create, and print molecular models using state of the art 3D printing technologies.

**Support**  MRSEC Summer Research Fellowship, National Science Foundation (Award No. 1157892 to D.A. P.K.), BioInspired Soft Materials MRSEC, (Award No. 402430), SPARK Grant

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**Poster # 2015.409**

**Presenter**  Urann Chan (Brandeis / Biology, Neuroscience)

**Title**  Investigating the effects of Rem2 on neuronal morphology in vivo

**Authors**  Urann Chan, Anna R. Moore, Sarah Richards, Stephen D. Van Hooser, Suzanne Paradis

**Abstract**  Neural plasticity plays a key role in the development of the mammalian CNS, where neurons undergo morphological changes in response to different sensory experiences to maintain proper neuronal networks (Zillies, 1992; Fuchs and Flügge, 2014). Previous research from our lab has identified Rem2, a member of the RGK family of small Ras-like GTPases, as an activity regulated gene important for regulating neuronal morphology. Specifically, knockdown of Rem2 results in a decreased number of dendritic spines but an increased number of dendritic branches, suggesting that Rem2 is a positive regulator of synapse formation and a negative regulator of dendritic complexity (Ghiretti and Paradis, 2011; Moore et al., 2013). While we are interested in replicating our previous results, we want to examine the effects of Rem2 in a more intact system, directly in the mouse visual cortex. When mice are deprived of visual experience (e.g. raised in the dark), they exhibit decreased spine density. We therefore hypothesize that in the absence of Rem2, where activity can no longer promote spine formation, there should not be a difference in spine density between dark-reared and typically-reared mice (Valverde, 1971). Using a program called, "Reconstruct," in combination with Golgi Cox labeling, we measure the lengths and widths of each spine along various segments of apical dendrites and characterize the morphology based on an equation that takes into consideration the length to width ratio of the spine (Risher et al. 2014). As an additional way to determine the effects of Rem2 on morphology in vivo, we used a second mouse line, (Rem2flx/flx;EMX1Cre) in combination with viral injection of AAV-flex-eGFP to label a subpopulation of neurons where we then measured dendritic complexity using Sholl Analysis. We hypothesize that in the absence of Rem2, neurons will have more complex dendritic arbors when compared to neurons from wildtype mice (Moore et al., 2013).

**Support**  Computational Neuroscience Traineeship
**Poster # 2015.410**

**Presenter** Kimberly Montano *(Brandeis / Biology)*

**Title** Development of a Human Papilloma Virus Assay using LATE-PCR

**Authors** Kimberly Montano, J. Aquiles Sanchez, Nicky Sirianni, Lawrence Wangh

**Abstract** Human Papilloma Virus (HPV), the most common sexually transmitted infection, is a risk factor for cervical cancer. Of those who are sexually active, 70% may become infected with HPV and are unaware due to a lack of symptoms. The best way to detect HPV is through a diagnostic test that identifies carriers and is useful for early-stage intervention of cervical cancer. However, current diagnostic methods of 14 High-Risk HPV types can only individually identify two types, HPV 16 and 18. The use of Linear-After-The Exponential Polymerase Chain Reaction (LATE-PCR) to design an HPV diagnostic assay, would distinctively detect and identify all 14 High-Risk HPV types. This development would be useful as a cervical cancer diagnostic application.

**Support** Hain Lifesciences

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**Poster # 2015.411**

**Presenter** Devon Powell *(New College of Florida / Chemistry)*

**Title** Are You Gellin’? Exploring Cancer Therapy with Hydrogels

**Authors** Devon Powell, Jie Li, Borui Li, Xuewen Du, Junfeng Shi, Bing Xu

**Abstract** Small-peptide hydrogels have been investigated as effective alternatives to conventional cancer therapy, however an ideal gel has not yet been developed. In this work, we investigate the properties of the hydrogelators NapFFHFNPR (1), NapFFE (2), and NapFFN (3) in hopes of discovering a compound effective at killing cancer cells. We report that 1 and 3 alone as well as mixtures of 1, 2, and/or 3 form hydrogels in water. The cytotoxicity of each compound alone to HeLa cells was not significant, and that of the mixtures is currently in testing. The addition of one equivalent of lactose to the solutions effectively strengthens some hydrogels while leaving others unchanged. Overall, this work not only illustrates some of the properties of these hydrogelators, but also reveals some of the effects of mixing multiple compounds.

**Support** MRSEC REU
Inhibition of microRNA-190 in neurons affects sleep regulation in *Drosophila*

Authors: Emily R. Daigle, Patricia R. Goodwin, Leslie C. Griffith

Abstract: Sleep causes changes in gene expression which result in improved cognition and overall better health [1]. Because micro-RNAs (miRs) are important regulators of gene expression, a screen for miRs involved in sleep was performed by the Griffith Lab using the fruit fly *Drosophila melanogaster*. This screen implicated miR-190 as an important regulator of sleep. To verify that miR-190 is required in neurons for its effects on sleep, I used the GAL4/UAS system to express a miR-190 inhibitor in neurons only. I looked at mean sleep episode duration to evaluate sleep consolidation. Fewer, longer sleep episodes indicate consolidated sleep, and many shorter sleep episodes point to fragmentation of sleep. Flies expressing the miR-190 inhibitor in all neurons displayed significantly less sleep than controls and had shorter mean sleep episode duration, indicating that miR-190 is required in neurons for consolidated sleep. Because multiple brain structures, including the fan-shaped body[2] and dopaminergic neurons [3], have been implicated in sleep regulation, I next investigated where specifically miR-190 is required. I found that miR-190 plays a role in sleep regulation in the fan-shaped bodies, but not in dopaminergic neurons.

References:

Support: Provost’s Undergraduate Research Fund

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Blocking and Distinguishing Reflections in the ATLAS Muon Spectrometer Alignment System

Authors: David Matthews, James Bensinger, Craig Blocker, Andrey Dushkin, Kevan Hashemi, Richard Studley, Forrest Webler, Hermann Wellensteine

Abstract: The ATLAS Experiment at the Large Hadron Collider observes high-energy proton-proton collisions in order to deepen our understanding of the fundamental structure of the universe. One part of the ATLAS detector is the Muon Spectrometer, which can only provide useful data if we can account for any changes in the positions and shapes of its various components. The Alignment System keeps track of these changes using a network of cameras, light sources, and temperature sensors. The current Muon Spectrometer will be upgraded soon, along with the Alignment System. In order to test for any problems that this system might encounter in the upgrade, we have built a mock-up pair of detector platforms with a 37 mm narrow gap and light sources placed along its 1940 mm length. One problem we found was that the analysis software could confuse a reflection of a light source with the direct light source. We have developed a reliable method for determining appropriate sizes and locations for small blockers which will eliminate these reflections without obscuring the direct light sources. We have also found that the total intensity of a direct light spot is at least 1.5 times greater than that of a reflected light spot. This can be used to distinguish between the two as a fail-safe technique if a reflection is not blocked.
**Poster # 2015.414**

**Presenter** Luke James Sisto (Brandeis / Chemistry)

**Title** Towards a New Cinchona Based Asymmetric Phase Transfer Catalyst

**Authors** Luke James Sisto, Li Deng

**Abstract** This project uses alkaloids from the cinchona family of plants to provide the asymmetric chirality for a phase transfer catalyst to engage in unprecedented enantioselective chemistry. The chemistry in question is the deprotonation of 4 nitrophenyl benzyl imines at the benzylic position and then the addition of Michael accepters to the imine’s carbon. These catalysts are notable due to their ability to change the imine from the usually electrophilic role to that of a nucleophile, as well as alter the expected regioselectivity of the addition. The end product can be easily transformed into a highly enantioenriched amine, like those found commonly in drugs and natural products. In addition to the establish catalysts there is one structural variant that has yet to be made. This variant, known as the 3,5 diphenylethynel, is the objective of this project. The intended result is a green asymmetric catalyst without the use of toxic metals, the tedious perpetration of enantiopure precursors or chiral chromatography. Members of the current cinchona based catalyst line used by Deng Group routinely provide ratios of enantiomers over 19:1 or 90% enantiomeric excess.

**Support** Jordan-Dreyer Summer Research Assistantship

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**Poster # 2015.415**

**Presenter** Karina Wagenpfeil (Brandeis / Biology, Neuroscience)

**Title** Isolation of a Single Metal Bound Form of Acireductone Dioxygenase in Klebsiella Oxytoca for Crystallographic Study

**Authors** Karina Wagenpfeil, Aditi Deshpande, Gregory Petsko, and Dagmar Ringe

**Abstract** Acireductone dioxygenase from Klebsiella oxytoca (KoARD) is an example of the only known metalloenzyme in literature that exhibits dual chemistry depending solely on the metal cofactor. KoARD catalyzes the on-pathway reaction when bound to Fe2+ and catalyzes the off-pathway reaction when bound to Ni2+. The on-pathway reaction recycles methionine in the methionine salvage pathway and the off-pathway reaction leads to the formation of butyric acid, formate, and carbon monoxide. The structures for these two proteins have been solved by solution NMR spectroscopy but due to the paramagnetic nature of the metal center, the active site is modeled using XAS methods. A crystal structure of both NiKoARD and FeKoARD should provide the structure of the active site and determine how these metal cofactors allow for dual pathway activity. Isolation of a single metal bound form of KoARD will improve the chance of crystallization due to increased homogeneity. KoARD was overexpressed in BL21-CodonPlus(DE3)-RIPL cells and transformed and grown in minimal media. A single metal cofactor was added during induction of protein expression. Preliminary results indicate that the nickel bound form of KoARD was successfully isolated. The KoARD purified using this method is folded and displays off-pathway activity consistent with previous studies. Metal analysis will be performed using ICP-MS to confirm the identity of the metal in the active site. The Fe bound protein will be expressed and purified as N-terminal Strep tagged KoARD using anaerobic conditions. Crystal screening trials of both metal bound forms will be performed to help solve the X-ray crystal structure of the resting protein and the protein bound to product and substrate analogs.

**Support** Cell and Molecular Visualization REU
Kinases are responsible for protein phosphorylation, which regulates myriad cellular activities pertaining to cell proliferation, growth and communication. The uncontrolled activity of these enzymes leads to development of tumor cells, which has motivated extensive research aimed at elucidating the molecular mechanisms of kinase regulation. Src and Abl are the two non-receptor tyrosine kinases that have similar structures but different activation and inhibition mechanisms. Here, we assess the regulation of these enzymes by introducing point mutations that involve substitution of individual tyrosine residues with phenylalanine. Kinetic assays performed on unphosphorylated and phosphorylated proteins, as well as Western blots, were used to examine the effects of phosphorylation of the regulatory tyrosines on the catalytic activity of the kinases. Our results demonstrate that phosphorylation increases catalytic activity of both wild type and mutant proteins, with an exception of Src Y418F mutant. To augment our understanding of regulation, we studied the effect of combining regulatory modifications, where Src Y418F mutant was used in combination with the SH2-binding peptide. The rate after addition of SH2-binding peptide was roughly 2.5 folds greater than the mutant’s initial rate. For phosphorylated and unphosphorylated Abl, we observed that incubating with myristoyl group adversely affects the enzymatic activities by approximately 7 and 6 folds respectively.
**Poster # 2015.417**

**Presenter** Molly Srour (St. Olaf College / Biology, Music)

**Title** Reconstitution of accelerated steady-state actin treadmilling *in vitro*

**Authors** Molly Srour, Adam Johnston, Bruce L. Goode

**Abstract** The actin cytoskeleton is a dynamic polymer network crucial for a diverse range of cellular processes such as cell motility, endocytosis, and cytokinesis. Actin filament networks continuously assemble and disassemble, consuming ATP in the process to generate force and drive movement. As such, it represents a form of active matter, and is ideal for use in bio-inspired soft materials that would utilize similar polymerization and depolymerization dynamics to generate polymers with unique physical properties. In order to reconstitute the dynamic nature of the actin cytoskeleton *in vitro*, one must be able to manipulate and speed up the process of treadmilling. Treadmilling is an inherent property of actin filaments, and occurs when a filament simultaneously undergoes assembly at its barbed end and disassembly at its pointed end. However, the spontaneous rate of pointed end depolymerization is much slower than the rate of barbed end assembly. At steady state, when monomer and filament concentrations remain fixed, the rate of assembly is limited by the rate at which actin subunits can be removed from pointed ends and recycled for new growth. Therefore, disassembly must be sped up significantly to increase treadmilling rates *in vitro*. Existing cellular machinery can be utilized to serve this purpose, with two main potential candidates being Twinfilin and Srv2. Twinfilin (Twf1) is an actin disassembly factor (ADF) in the Cofilin superfamily that greatly accelerates pointed end depolymerization in concert with Srv2, a multifunctional hexameric actin-binding protein. We used Total Internal Reflection Fluorescence (TIRF) microscopy assays to test whether these disassembly factors accelerate actin treadmilling at steady state. At low concentrations of Twf1 and Srv2, we were able to modestly increase pointed end disassembly and accelerate treadmilling. However, Twf1 and Srv2 also antagonize barbed end growth and at high concentrations can cause disassembly from the barbed end as well as the pointed end, so visualization of treadmilling often proved difficult in assays involving high levels of the two proteins. Attempts were made to protect the barbed end and restore fast growth using the formin Bnr1, an actin assembly-promoting factor which sits at the barbed end of growing filaments, but it was found that Twf1 and Srv2 can cause the formin to be displaced from actin. Further steps to visualize accelerated treadmilling should focus on the network level rather than individual filaments, and will include a procedure to test actin network fluorescence recovery after targeted photobleaching (FRAP).

**Support** MRSEC REU

**Poster # 2015.418**

**Presenter** Natsuko Nina Yamagata (Brandeis / Chemistry)

**Title** A novel approach to cancer therapy: sequencing cytotoxic peptides based on Immunoreceptor Tyrosine-Based Inhibitory Motif (ITIM)

**Authors** Natsuko Nina Yamagata, Jie Zhou, Xuewen Du, Bing Xu

**Abstract** Overexpression of cell surface phosphatases is an underrated yet important generic difference that makes cancer cells unique from normal cells. Utilizing this property of cancer cells, we design, synthesize, and analyze small peptides that self-assemble to form gels upon dephosphorylation. Three sequences (L, D and retro inverso peptides) of LYYpYYL have been thoroughly examined. We have found so far that although all three peptides form gels, interestingly, they do not exhibit cell cytotoxicity. We will continue investigating the effects and properties of this sequence on cancer cells, particularly its interdependence with lymphocytes.

**Support** Jordan-Dreyer Summer Research Assistantship
**Poster # 2015.420**

**Presenter** Dena Goldblatt (Brandeis / Biology, Neuroscience, Psychology)

**Title** Association of stress-induced increases of inhibitory kappa-B gene expression with depressive symptoms

**Authors** Dena S. Goldblatt, Christine McInnis, Myriam V. Thoma, Danielle Gianferante, Luke Hanlin, Xuejie Chen, Diana Wang, Nicolas Rohleder

**Abstract** Depressive symptoms are associated with exaggerated inflammatory and altered cortisol stress responses. Cortisol has been shown to stimulate inhibitory kappa-B (IκB) gene expression, which counteracts pro-inflammatory cytokine production. Increased inflammation is a risk factor for disease, highlighting the need to understand factors controlling inflammation. No studies have examined IκB expression in response to acute stress in relation with depressive symptoms. We recruited n=48 healthy individuals and exposed them to the Trier Social Stress Test (TSST). Stress responses of plasma Interleukin-6, salivary cortisol, as well as IκB gene expression in peripheral blood, were measured before, as well as 30, and 120 minutes after stress. We also assessed self-reported depressive symptoms, post-stress mood, and stress appraisals. Stress exposure induced increases in IL-6 and cortisol (IL-6: F=42.41, p<0.001; cortisol: F=15.98, p<0.001). IκB expression increased after stress with a peak at 30 min and recovery at 120 min (F=13.11, p<0.001). Depressive symptoms were positively related with IκB expression 30 min after stress (beta=0.30, p=0.05), and this relationship was mediated by decreases in positive affect. These show that in individuals with more depressive symptoms, acute stress translates into upregulated gene expression of inhibitory kappa-B, which might be mediated through stronger decreases in mood. Higher IκB might allow better down regulation of inflammation, but could also be a response to stronger inflammatory reactivity in general.

**Support** Division of Science Summer Research Fellowship, Dr. Ilene Gordon Wittels Fellowship in Social Psychology

**Poster # 2015.421**

**Presenter** Daniel Kats (Brandeis / Biochemistry)

**Title** Moonlighting Interactions of IMPDH Affect *Escherichia coli* Growth

**Authors** Daniel J. Kats, Deviprasad Gollapalli, Yifan Dang, and Lizbeth Hedstrom

**Abstract** Inosine-5'-monophosphate dehydrogenase (IMPDH), which is part of the guanine nucleotide biosynthesis pathway, is a target for anticancer and antibiotic drug development. Additionally, mutations in IMPDH are correlated to inherited retinal degeneration. Previously, the Hedstrom Laboratory discovered binding interactions between IMPDH and both ribosome and RNA polymerase in *Escherichia coli*. I designed several IMPDH mutants to disrupt the binding between IMPDH and ribosome in order to locate the binding site. I have also developed a method to phenotypically screen IMPDH mutants for the presence of these interactions. Future experiments will screen many mutants, and then use affinity chromatography to purify selected mutant IMPDHs. Those IMPDHs will be checked by western blotting for interactions with RNA polymerase and ribosome.

**Support** Computational Neuroscience Traineeship
**Poster # 2015.422**

**Presenter** Sara Gelles-Watnick (Brandeis / Biochemistry)

**Title** Creating a functional Src protein kinase construct using Sortase enzyme

**Authors** Sara Gelles-Watnick, Yizhi Sun, Han Yang, Christopher Wilson, and Dorothee Kern

**Abstract** The structural mechanism of C-Src tyrosine kinase, a proto-oncogene ubiquitously expressed in human cells, is largely unknown. To understand exactly how Src mutations contribute to metastasis, we aim to study its dynamics and mode of allosteric regulation. The activity levels of Src depend on which of the four phosphorylation states the protein is currently in. We created constructs corresponding to the kinase domains in the four phosphorylation states. After expression and purification, the Sortase enzyme was used to ligate the kinase domains to an SH3/SH2 construct. To determine if our constructs were folded normally and were active, we used an HPLC-based kinase assay to compare the phosphorylation rates of the ligation product to that of full-length Src. Our back-ligated construct demonstrated normal function in this assay. Future experiments will include NMR studies and activity assays comparing the rates of the other phosphorylation states.

**Poster # 2015.423**

**Presenter** Ariel Morley (Bay Path University / Neuroscience)

**Title** Understanding the relationship between retromer and disease associated proteins at the *Drosophila* neuromuscular junction

**Authors** Ariel Morley, Rylie Walsh, Avital Rodal

**Abstract** Membrane trafficking through the endosomal network allows for movement of protein and other molecules. Retromer is an endosome-associated protein coat complex that sorts cargo and sends it to either the trans-Golgi network or back to the plasma membrane. Amyloid precursor protein (APP) and alpha-synuclein are two disease-associated proteins that are known to be trafficked in endosomes. The Rodal lab has shown that mutations in certain Retromer subunits can increase or decrease the amount of exosomal APP. By using the gene expression system GAL4-UAS in *Drosophila*, immunohistochemistry, and confocal microscopy, we ask whether alpha-synuclein is seen in exosomes at the NMJ like APP has been shown to. We also visualize where Retromer localizes in the muscle tissue in an attempt to understand their relationship to exosomes. We were unable to visualize alpha-synuclein in exosomes at the NMJ. The vps35 subunit of Retromer localizes closer to the NMJ than SNX-3 and SNX-1, possibly showing that it might be close enough to be involved in transport of exosome cargo. Further work will include the use of antibody staining and biochemistry to confirm synuclein expression in *Drosophila*, and also further imaging will be done to expand the dataset.

**Support** MRSEC REU
**Abstract**
Sleep fragmentation (SF) is a periodic disruption of sleep in humans and other animals that is associated with disorders such as sleep apnea, narcolepsy, and restless leg syndrome. One of the well-known functions correlated with sleep is memory consolidation, which has been shown to be impaired in chronic SF models with rodents (Ramesh V et al. 2012). However, the mechanism is not clear. *Drosophila melanogaster*, with its relatively simple brain system, and its powerful genetic tools, has been widely used to understand basic processes at different levels, from behavior to genes, as well as establish different disorder models. However, there is no model to be used to understand SF. Here we are establishing a fragmented sleep model by two methods: mechanical shaking and light pulse at night. The subsequent effects of discontinuous sleep including total sleep, number of sleep episodes, as well as mean sleep episode length were measured. We found that, significant fragmentation (more episode numbers and shorter episode length) in both individual females and males can be induced by 5 seconds of shaking every 1-2 hours at night, but statistically significant total night sleep loss was also detected. Applying 5 minutes of light stimulus every hour at night to individual flies, both female and male flies slept fragmented, and kept their total sleep at night constant. In addition, males seemed to exhibit an increased sleep in the recovery day. In populations of flies, fragmentation is easier to induce in female populations but total sleep at night is still reduced. Thus, parameters that can generate fragmented sleep without disturbing total sleep still need to be explored.

**Support**
Cell and Molecular Visualization REU

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**Abstract**
Members of the genus of Naegleria are characterized by their ability to change from an amoeba to a flagellate, but species differentiation is difficult due to a lack of morphological differences. A set of nine Lights on/Lights off probes were designed in order to distinguish and establish a rapid closed tube identification system for Naegleria. As each probe is mismatch tolerant, each strain of Naegleria binds to each probe at a different temperature, which can be visualized into a unique fluorescent signal.

**Support**
Malaysian Palm Oil Board
Title Chromatin accessibility of circadian regulated genes in *Drosophila*

Authors C Caggiano, Y Sytnikova, K Compton Abruzzi, M Rosbash

Abstract Nearly all organisms have a circadian rhythm, which is a 24-hour period where physical and behavioral states oscillate in a regular sinusoidal manner. These oscillations are controlled by a molecular clock, which is driven by two key heterodimeric transcription factors: Clock (CLK) and Cycle (CYC). CLK/CYC bind to and activate target genes in a rhythmic manner: CLK/CYC binding is highest in the early night and lowest in the early morning. Recent results suggest that the mammalian homolog of CLK, Bmal1, may function to “open” chromatin to facilitate transcription activation. To test whether this hypothesis is true in *Drosophila* brains, ATAC-seq was used to examine how chromatin structure changes throughout the day. Surprisingly, the results indicate that chromatin accessibility on CLK controlled genes is independent of CLK binding and does not change with time of day. This suggests that CLK binding does not influence chromatin accessibility at a level detectable by ATAC-seq. To explore this finding, we tested whether: 1) purified nuclei gave the same results as the cruder preparation initially used 2) broad overexpression of CLK in the brain would alter chromatin accessibility and 3) chromatin isolated from specific sets of circadian cells give similar results to whole brains. Results to date are consistent with the original observation that chromatin accessibility on CLK controlled genes does not change with time of day; rather, it is a fixed factor, underlying general circadian transcription.

Poster # 2015.428

Presenter Harsha Gurnani (Indian Institute of Science / Biology, Mathematics, Neuroscience)

Title How do neurons maintain activity patterns as they grow?

Authors Harsha Gurnani, Timothy O' Leary and Eve Marder

Abstract Neural circuits for important body rhythms often develop early and maintain constant output throughout the growth of the animal, even as the neuronal size and dendritic arborization changes. This constant output cannot be achieved by maintaining the same intrinsic conductances throughout growth. Regulation of channel conductances in an activity-dependent manner to achieve calcium homeostasis helps to maintain activity patterns over short ranges. Introducing local control of channel expression along with (global) somatic calcium homeostasis can compensate for changes in activity due to variable geometry and morphology, and help to maintain burst periods and duty cycles.

Support Khorana Scholars Program
Poster # 2015.429

Presenter Daniel Kutner (Brandeis / Mathematics, Physics)

Title Analytic Solutions to Phase Model of Pulse-Coupled Belousov-Zhabotinsky Oscillators

Authors Daniel J. Kutner, Viktor Horváth, Irving R. Epstein

Abstract We investigate a phase model of two Belousov-Zhabotinsky oscillators reciprocally coupled by inhibitory perturbations. In the experimental system, when one oscillator peaks, inhibitory solution is injected into the reaction mixture of the other following a time delay. Unlike conventional phase models, our model incorporates a variable phase velocity to account for experimentally observed long-term effects of pulse-perturbations. In this model, when one oscillator peaks, the other's phase is modified according to a phase response curve (PRC), and its phase velocity is reduced. Between perturbations, the phase velocity exponentially decays towards a relaxed phase velocity. The model allows for an arbitrary PRC, which can be obtained experimentally using a single pulse-perturbed oscillator. By numerically integrating the differential equations at fixed initial conditions, we can determine the behavior of the system. However, this only gives us a cross-section of the multidimensional space of patterns. The pattern that results depends on initial conditions. To provide more insight, we have developed a method to construct a system of equations and inequalities that must be satisfied by an N:M pattern for a given set of parameters. By finding solutions, we project the multidimensional pattern space down to the phase space of adjustable parameters. This eliminates the need to run simulations with multiple initial conditions. We are able to find solutions for arbitrary N:M patterns with no time delay and for 1:N patterns with time delay. Because phase models are generic, the method may be suitable to predict the temporal behavior of other systems such as networks of neurons.

Support Division of Science Summer Research Fellowship

Poster # 2015.430

Presenter Minh Pham (Brandeis / Biochemistry)

Title Pore Lining Residues in Fluc type F⁺ Ion Channels

Authors Minh Pham, Nicholas Last, Christopher Miller

Abstract Microorganisms are commonly exposed to fluoride toxic environments with concentrations of up to 100 µM that could inhibit cellular pyrophosphatase activity and nucleic acid production. However, prokaryotic, and some eukaryotic, specimens possess fluoride-specific exporting proteins of either the CLC-F or the Fluc family in order to counteract the influx of toxic fluoride. These ion channels allow membrane-impermeable fluoride to passively diffuse down an electrochemical gradient. Recently Fluc proteins have been determined to hold an antiparallel homodimer assembly in lipid bilayer membranes. Interestingly, both of the ion channel pores in Fluc are comprised of highly conserved polar residues that align straight along the pore. Mutagenesis applied to this conserved polarity within the pore showed moderate decrease in both Fluc’s efflux functionality and folding capability within the membrane, suggesting a vital role of these polar pore lining residues.

Support HHMI
Characterization of novel regulators of neuronal morphogenesis

Neurons are highly polarized cells with structurally and functionally distinct subcellular compartments – an axon and dendrite(s). Axon-dendrite compartmentalization underlies directional flow of information in the nervous system and is critical for its development and function. Although many extracellular cues and cell-intrinsic factors, including actin and microtubules, that contribute to neuronal polarization have been identified, the molecular mechanisms governing polarization in vivo are not fully understood. The nematode C. elegans provides an excellent model system to study neuronal morphogenesis in vivo. An adult hermaphrodite has a nervous system comprised of 302 neurons with highly stereotyped positions and morphologies. Sixty of these neurons are ciliated and mediate transduction of diverse chemo-, thermo-, and mechanosensory cues. Primary cilia are ubiquitous microtubule-based signaling organelles that play critical roles in development and tissue homeostasis. Defects in cilia structure and function give rise to devastating human disorders collectively termed ciliopathies. Previous work in Sengupta lab identified two candidate regulators of sensory neuron morphology – a conserved signaling and structural scaffolding protein GIRDIN-1 and a junctional component of axonemal doublet microtubules (DMTs) flagellar-associated protein (FAP) FAP20. FAP20 has been previously demonstrated to regulate axonemal stability and motility of Chlamydomonas flagella. Although the cilia in C. elegans sensory neurons are non-motile, the ciliary core organization is highly conserved. Specifically, cilia and flagella in all species are composed of 9 DMTs anchored by the modified mother centriole (basal body) and surrounded by specialized membrane. I generated a putative loss-of-function allele of C. elegans FAP20 homolog using CRISPR/Cas9 and am continuing to characterize the role this gene may play in regulating cilia formation and/or function in sensory neurons. As part of my second project, I began characterizing the role of grdn-1 in regulating morphology of gas-sensing sensory neurons AQR and PQR. Work by others in the lab implicated grdn-1 in controlling early steps of ciliogenesis in head sensory neurons. Along with cilia defects, I uncovered grdn-1-dependent phenotypes suggestive of aberrant polarity in AQR and PQR. To understand the mechanism by which GIRDIN-1 supports neuronal polarity, I will further characterize GIRDIN-1 localization and function in AQR and PQR.

Support Division of Science Summer Research Fellowship (Life Science Scholars)
**Poster # 2015.432**

**Presenter** Pranava Keerthi S (Indian Institute of Science / Chemistry)

**Title** Reticular Synthesis of 2-D Covalent Organic Frameworks Analogous to Graphene

**Authors** Pranava Keerthi S, Casey R. Wade

**Abstract** Reticular synthesis can be described as process of making extended frameworks with predetermined structures using well defined molecular building blocks held together by strong bonds. These solid frameworks are highly crystalline with long range order. Since the molecular building blocks maintain their structural integrity throughout the reaction, we can predict the framework structure and various properties. When the building blocks are held together by strong covalent bonds, covalent organic frameworks (COFs) are generated. In this project, we are exploring the synthesis of novel COFs constructed from a repeating network of triarylborane units arranged in a two dimensional hexagonal lattice analogous to graphene. We have attempted to synthesize a new COF by reaction of 1,4-bis(tributylstannyl)benzene with boron tribromide. The COF products are expected to have high thermal stability due to the formation of B—C covalent bonds. These materials may also have interesting optical or electrical properties due to the conjugated π-electron system connected via the electron-deficient boron atom.

**Support** Brandeis-India Science Scholars Program

**Poster # 2015.433**

**Presenter** Wendy (Ruoxi) Wang (Brandeis / Biochemistry, Biology)

**Title** The Role of Chromosome Territory for DSB-Mediated Gene Conversion Frequency in *Saccharomyces cerevisiae*

**Authors** Wendy (Ruoxi) Wang, Cheng-Sheng Lee, and James E. Haber

**Abstract** DNA double-strand–breaks (DSBs) are one of the most detrimental lesions that disrupts chromosome integrity. Failures in DSB repair are often associated with the development of cancer and other diseases. In eukaryotes, DSBs can be efficiently repaired via homologous recombination (HR), specifically by a process known as gene conversion (GC). We use *Saccharomyces cerevisiae* to study the role of chromosome territory in DSB-mediated gene conversion frequency. First, we assayed viabilities for single-donor strains in which a DSB is induced at the can1 locus by the site-specific HO endonuclease. The DSB could be repaired through gene conversion using an ectopic donor that was placed at different loci. The donor loci were chosen to reflect a range of distance from the DSB, as derived from contact frequency measured by genome-wide chromosome conformation capture. Second, we inserted a reference donor into the single-donor strains and assessed the relative usage of the testing donors using a two-donor competition assay. We found that strains with a donor lying closer to the DSB have higher viabilities and relative usages than strains with a donor located further away, suggesting DSB-mediated GC frequency is highly subjected to chromosome territory. In addition, placing a Recombination Enhancer sequence (RE) adjacent to a donor, deleting the chromosome remodeler FUN30, and increasing donor length have an additive effect to promote viability. Furthermore, when inducing a DSB on Chr.2 and measuring repair via intra-chromosomal donors using the one-donor viability assay, we found that the intra-chromosomal GC frequency is also well correlated with contact frequency and consistent with the Rabl configuration of chromosomes. Our findings provide insight into the role of spatial organization of chromosomes in regulating chromosome dynamics in yeast.

**Support** MRSEC Summer Research Fellowship
Poster # 2015.434
Presenter Leonard Grazian (Brandeis / Mathematics, Physics)
Title The Clustering Behavior of Self-Propelled Polydisperse Dipoles
Authors Leonard Grazian, Yaouen Fily, Michael Hagan, Aparna Baskaran

Abstract Active matter refers to systems in which each constituent member consumes energy to move or exert forces. We study an active matter system composed of dipolar particles that propel themselves through a fluid. We consider particles which have isotropic excluded volume, are probably dispersed in size, and are confined to two dimensions. This system is characterized by two dimensionless parameters: the Peclet number (Pe) that scales the magnitude of the particle’s self-propulsion, and lambda (λ) that scales the strength of the dipolar interaction. We use Brownian dynamics simulations to explore the emergent spatiotemporal patterns of this system. We find that, when the energetic dipolar interactions dominate propulsion, the active dipoles phase separate and form system-spanning partial or complete motile rings. For other parameter values, the particles form motile swarms or mix isotropically.

Support MRSEC Summer Research Fellowship

Poster # 2015.435
Presenter Jessie Ang (Brandeis / Biology)
Title The role of the phosphatase Pph3 in genotoxin-induced targeted autophagy
Authors Jessie Ang, David P. Waterman, Vinay Eapen, and James E. Haber

Abstract DNA damage in eukaryotic cells triggers the DNA damage checkpoint-mediated cell cycle arrest to allow the cells time to repair DNA before damaged chromosomes segregate, which can result in translocations and aneuploidy. If damage remains, budding yeast cells adapt after 9-12 hours by switching off the DNA damage checkpoint and proceeding through the cell cycle despite catastrophic genotoxic stress. This models a cancerous state in which cells continue to perform mitosis even with irreparable chromosomal damage. A novel pathway of autophagy, one of the ways by which protein are degraded, was discovered recently by our lab. This pathway known as GTA (Genotoxin-induced Targeted Autophagy) is induced as a response to DNA damage. To identify genes involved in regulating this pathway, a screen was performed by Haber lab, and the gene PPH3 and its potential target, HOG1, were identified. Deleting PPH3 resulted in heightened autophagy as a result of DNA damage, but the levels of autophagy were not sufficient to cause an adaptation defect. Our data suggests a model in which Pph3 directly inhibits the kinase activity of Hog1 thereby negatively regulating GTA.

Support Division of Science Summer Research Fellowship
The Fluc family of proteins function to reduce the toxic effects of fluoride in microorganisms. Fluoride, at toxic levels ~100µM, inhibits key metabolic enzymes such as enolase and pyrophosphatase, which are essential to ATP formation and DNA synthesis. Fluc proteins are fluoride selective channels that exhibit a dual-topology, antiparallel architecture. Two homologous proteins of the Fluc family from *Bordetella pertussis* (BPe) and *Escherichia coli* (EC2) are studied here. Currently, we have a high resolution (2.1Å) structure of BPe, but lack a high resolution structure of EC2. EC2 and BPe have a Histidine tag on the C-terminus with a conserved amino acid linker. Cleavage of this Histidine tag is beneficial in receiving high resolution crystal structures. Histidine tag cleavage of BPe has been shown, while EC2 lacks an optimized way of removing the tag. The amino acid sequence preceding the linker region of amino acids differ between EC2 and BPe. This sequence in BPe has hydrophilic residues, while the analogous sequence in EC2 has hydrophobic residues. It is believed that by changing the amino acid sequence of EC2 in this region to mimic the unconserved region of BPe that the protease cut site will be exposed, and allow for efficient removal of the Histidine tag. In order to cleave this Histidine tag Lys-C, a proteolytic enzyme, is used. Lys-C cleaves on the C-terminal side of Lysine residues. This is especially effective for EC2 given the position of a Lysine residue within the linker before the Histidine tag. Lys-C digestion of this mutated EC2 protein confirms that removal of the hydrophobic residues results in cleavage of the Histidine tag. This allows for a less flexible protein to be crystallized. In order to crystallize protein structures, Monobody chaperones are affixed to the protein to provide different conformations of the protein. Monobody M12 is currently used to chaperone the protein during crystallization. Future goals are to obtain high resolution structures with use of different versions of the Monobody.
The amoeboflagellate genus Naegleria contains a diverse group of species. Although the majority have only been observed to behave asexually, hints of sexuality have been reported for several species. The Fulton lab has studied the organism, Naegleria gruberi, strain NEG, for the past half century without finding a hint of sexual mating. This organism, when starved, undergoes a process of cell differentiation, taking it from an amorphous amoeba to a swimming flagellate. Although only asexual behavior of NEG has been observed in the lab, evidence from the genome suggests that the organism arose from mating and has the ability to undergo mating. Studying NEG has lead us to look at the life cycles of two other divergent species of Naegleria: N. indonesiensis, strain PNG2, and N. minor, strain WT043. These two species behave similarly in the lab and both appear to have a sexual cycle. They undergo a process of differentiation over the course of 8 hours (longer than NEG) under microaerobic conditions in an aquatic environment, requiring low oxygen and high carbon dioxide. The cells undergo two or three unequal divisions during differentiation, producing small flagellates, usually with two flagella, by the end of the process. Interestingly, during the process the cells lose their culturability. Some of these small flagellates can be induced to regain their culturability when incubated with bacteria. We interpret this process to be a sexual cycle in which the initial amoeba cells undergo meiosis, lose culturability, and produce gametes, which are the small flagellates. The phenomenon of regaining culturability we interpret as rare mating of these gametes. These findings provide substantial evidence for the existence of sexuality in these two Naegleria species.
Degenerative diseases such as Alzheimer’s, Diabetes, and Parkinson’s have been associated with mitochondrial dysfunction, a possible mechanism for which could be the gradual accumulation of mutations in mtDNA (Mitochondrial DNA), called Mutational Load, that damage the Electron Transport Chain and promote the formation of harmful Reactive Oxidative Species (ROS). 3’-Azido-3’-Deoxythymadine (AZT), a common Nucleoside Reverse Transcriptase Inhibitor (NRTI) used to treat HIV, has been shown to cause an increase in the number of these mutations. However, previous studies have shown that culturing cells in the presence of Palm Fruit Juice (PFJ), the antioxidant and phenolic-rich byproduct of the extraction of palm oil from Elaeis guineensis, can mitigate the genotoxic effects of AZT. In this study, Isoniazid (INH), an antibacterial drug widely used to treat Tuberculosis (TB) and often in conjunction with AZT treatment was tested for both genotoxic and cytotoxic effects as well as PFJ’s ability to mitigate these effects. Sets of HepG2 (Liver Carcinoma) Cells were cultured in the presence and absence of 88µM INH treatment as well as 75 and 100ug Gallic Acid Equivalents (GAE)/mL of PFJ for 14 or 30 days. These samples were analyzed using a LATE-PCR Lights-On/Lights-Off Assay that interrogates the HV2 region, as well as the CO2 and ND1 genes of the mitochondrial genome. It was found that INH treatment did significantly increase the number of mutations in the HV2 region and ND1 gene, but PFJ concentrations below 75µg GAE/mL did not significantly mitigate the mutations, and concentrations above 75µg GAE/mL had cytostatic and even cytotoxic effects on the cells after 6 days of culture. Previous sequencing data of the AZT induced mutations seem to mimic mutations caused by DNA Polymerase error. To test if PFJ could be preventing these mutations by bolstering impaired DNA Polymerase accuracy, PFJ will be added to an assay that detects Polymerase error by amplifying only Polymerase-mutated strands. Preliminary results show that the blockers have been preventing wild type amplification at 10 DNA copies per well, and PFJ at dilutions below concentrations of 1.36 ppm did not produce interfering fluorescence in the reaction or inhibit amplification. Studying the mitigating effects and mechanisms of PFJ in respect to AZT and INH – induced mutation will help to eventually develop a way to ease the degenerative effects that these drugs have on the mitochondria.
**Poster # 2015.439**

**Presenter** Spencer Jaquet (Greenville College / Biochemistry)

**Title** Applying Immunohistochemistry Techniques to Visualize Viral Injections In Long Evans Rats

**Authors** Spencer Jaquet, Pooja Gupta, Tom Rossetti, Somdeb Banerjee, Marni Gross, Dr. John Lisman

**Abstract** Our knowledge on long-term memory formation is far from complete. While research is being performed to better understand the mechanism behind LTP induction and its relation with memory formation, it is still not decisively determined what is the mechanism for memory storage. We hypothesize that this mechanism lies in the formation of CaMKII/NMDAR complexes within the CA1 region of the hippocampus. To test this hypothesis, we introduce K42M, a transient virus acting as a dominant negative form of CaMKII, to rats and examine the animal’s ability to recall a previously instilled memory (conditioned place avoidance). Within this experiment, I performed immunohistochemistry and analysis techniques on the hippocampal regions of tested rats. With this analysis, it can be confirmed whether or not viral injection occurred at the CA1 region as well as provide evidence that the virus introduced had interacted in a way we expect (temporary expression). Current results on brains injected with fluorescent microspheres are able to clearly display the injected area in the hippocampus. In the future we hope to continue to use this technique to visualize the injection site and confirm injections into the desired CA1 region.

**Support** Cell and Molecular Visualization REU

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**Poster # 2015.440**

**Presenter** Anna Yeh (Brandeis / Biology)

**Title** Split-GFP reporter constructs to visualize exosomal trafficking in *Drosophila*

**Abstract** Exosomes are small secretory vesicles (50-200 nm) of endocytic origin and carry select cargo that impact the recipient cell’s metabolism and signaling pathways. In *Drosophila* at the larval neuromuscular junction (NMJ), we know that exosomes are released at the synapse but we do not know their trafficking route or fate in the recipient cells. We hypothesize that a split-GFP system is a viable tool to study the trafficking patterns of neuronal exosomes. Individually, the two spGFP fragments are non-fluorescent but fluorescence is reconstituted when they interact. We tagged Sunglasses (Sun), an exosomal cargo protein with spGFP11 and Lamp1, a lysosomal marker protein, with spGFP1-10. Depending on where the exosomal cargo is trafficked, we expect to see GFP reconstitution. We transfected S2 cells with the spGFP constructs to visualize the localization of the fusion proteins using confocal light microscopy. Also, we overexpressed the Lamp1 fusion protein with a muscle driver at the larval NMJ. Based on our results, we established that the split-GFP system is a viable tool for studying the trafficking of exosomes. We are in the process of creating *Drosophila* stocks that express both parts of spGFP to establish whether neuronal exosomes are targeted to muscle lysosomes. We will also create early and late endosomal reporter constructs to further track the trafficking and fate of exosomes in different genetic backgrounds. Investigating these exosomal trafficking pathways would aid in developing the exosomal system for targeted therapies in many human diseases.

**Support** Cell and Molecular Visualization REU
Title: Investigating Cross-Talk Between the Signaling and Cytoskeletal Activities of Glia Maturation Factor

Authors: Anindita Chanda, Meredith O. Sweeney, Bruce L. Goode

Abstract: Glia Maturation Factor (GMF), which has two mammalian isoforms called GMFβ and GMFγ, is a 17 kDa protein with many cellular functions. GMFβ was originally identified as a nerve growth factor which promoted the differentiation and survival of neurons. It was also proposed that GMFβ, when phosphorylated on Ser83 by PKA, could enhance signaling through the p38 MAP kinase pathway, leading to the production of cytokines, nerve growth factors, and reactive oxygen species. However, more recently GMFβ and GMFγ have been shown to play crucial roles in cytoskeletal activities; they inhibit actin filament branch nucleation by the Arp2/3 complex and stimulate de-branching of actin filaments. So far, GMF’s cytoskeletal and signaling abilities have been studied in isolation, but we would like to understand how these two activities are coordinated in cells. Thus, we attempted to validate GMF’s effects on p38 MAP kinase signaling. Similarly, we wanted to see whether the PKA-phosphorylated GMF could partake in debranching since Ser83 is located in a region of GMF, called “Site 2”, a region important for its debranching activity. The results from the Western blots suggest that, with the use of forskolin, GMFβ (S83E), but not WT GMFβ, enhances the p38 MAP kinase signaling pathway. These results are inconsistent with the hypothesis that forskolin enhances phosphorylation of GMFβ to promote signaling. TIRF analysis indicated that phosphomimetic GMFβ (S83E) did not interfere with GMFβ’s ability to cause actin filaments to debranch, indicating that this site likely does not regulate debranching. Future directions will use TIRF analysis to understand whether phosphoregulation of other Site 2 residues alters the potency of GMF’s debranching activity.
**Poster # 2015.443**

**Presenter** Pooja Gupta (Brandeis / Biology, Neuroscience)

**Title** Inhibition of the CaMKII/NMDAR Complex Disrupts Maintenance of Spatial Memory

**Authors** Pooja Gupta, Spencer Jaquet, Thomas Rosetti, Somdeb Banjeree, Marni Gross, and John Lisman

**Abstract** Long term potentiation (LTP) has been extensively studied as an underlying mechanism of memory storage. Memories are stored as information at specific synapses and can remain there despite protein turnover. CaMKII is an enzyme that is known to autophosphorylate and is therefore able to keep itself activated to avoid protein turnover. CaMKII with the NMDA receptor forms a complex leading to LTP. However, the complex’s role in the maintenance of actual memory is unknown. The purpose of this research is to determine whether the CaMKII/NMDAR complex is the biochemical entity that maintains memory. We hypothesize that interference with this complex through the use of a transient virus will lead to erasure of memory in rats. Rats form a memory through a spatial avoidance task; catalytically dead CaMKII (HSV-K42M) is then bilaterally injected into CA1 of the dorsal hippocampus. A retention test is conducted ten days later to determine whether the memory has been erased. Preliminary results indicate that rats injected with K42M have little to no retention of the spatial memory when compared to control rats (injected with HSV-GFP). Erasure suggests the necessity of CaMKII in the maintenance of long term memory and hints at the possibility of CaMKII being the memory molecule. Future tests will continue to explore different methods of disrupting the CaMKII/NMDAR complex. These mutants include: CN19- a peptide shown to erase LTP decrease CaMKII/NMDAR complex functionality, and NR2B- a mutant NMDAR that prevents CaMKII from effective binding.

**Support** Cell and Molecular Visualization REU

**Poster # 2015.444**

**Presenter** Sarah A. Pizzano (Brandeis / Biology)

**Title** Neuronal sexual dimorphism in the BNST and mPOA of the mouse brain

**Authors** Sarah Pizzano, Yasuyuki Shima, Sacha Nelson

**Abstract** The brain contains sexually dimorphic neuronal structures suspected of contributing to distinct behaviors in males and females. The functions of these structures have yet to be elucidated. In the expression screening of transgenic mouse lines by the Nelson lab, we found that the mouse line P170 had male-specific expression of a fluorescent reporter gene in the medial preoptic area (mPOA), an established sexually dimorphic nucleus, and the bed nuclei of the stria terminalis (BNST). To discern the functions of these neurons, we tested what social contexts which activated the male specific neurons. Male mice from this line were subjected to social behavioral trials either with male intruders for aggression or hormone-primed females for sexual behaviors. Aggressive behavior was tested with the introduction of a male intruder mouse into the home cage of a P170 male mouse. The interaction was recorded and later scored for frequency and type of aggressive behavior (including chasing and physical attack). Sexual behavior was tested with the introduction of an ovariectomized, hormonally-primed female mouse into the home cage of a P170 male mouse. The interaction was recorded and later scored for frequency and type of sexual behavior (including mounting, intromission, and ejaculation). Immunohistochemical staining for c-fos protein was performed subsequent to each interaction and neuronal activities by the behaviors were mapped by the immediate early gene c-fos expression. After fluorescent imaging, both BNST and mPOA neurons also showed a lack of c-fos expression, indicating c-fos was not activated significantly by the behavioral trials.

**Support** Provost’s Undergraduate Research Fund
Title  Progress towards single-molecule investigation of TBP-DNA(TATA) remodeling by Mot1

Authors  Ciyue Shen, Stephen Buratowski, Jeff Gelles

Abstract  Transcription is a biological process through which a particular piece of DNA is transcribed into RNA by the RNA polymerase. TATA-binding protein (TBP) is necessary in eukaryotic pre-initiation complex formation in that it specifically binds to an A-T-rich sequence known as TATA-box, and further recruits other transcription factors. Therefore, to better understand the mechanism of TBP regulation is of importance to study gene transcription and protein expression. It has been known that the Snf2/Swi2 ATPase Modifier of Transcription1 (Mot1) is capable of displacing TBP from DNA(TATA) using ATP hydrolysis in purified systems. We want to study if Mot1 functions the same in both purified systems and cell extracts using single-molecule fluorescence microscopy. Saccharomyces cerevisiae TBP encoded by SPT15 gene was purified and labelled with Cy5 (red) dye using sortase labelling method. The labelling efficiency was almost 100% and little free dye was left. Cy5-TBP under the TIRF microscope appeared as small round spot with uniform brightness, suggesting that there is little protein aggregation. Future experiments will focus on comparing how the TBP-DNA(TATA) complex will be remodelled when Mot1 is present in purified systems and yeast nuclear extracts. A blue-dye-labelled (Alex488) dsDNA bearing TATA-box will be tethered onto the glass slide and the colocalization of blue and red spots will be observed using TIRF microscopy as an indication of TBP-DNA(TATA) complex.

Support  Division of Science Summer Research Fellowship

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Title  Effects of caffeine on Rad53 phosphorylation after DNA damage

Authors  Andrea Demos, Gonen Memisoglu and James E. Haber

Abstract  When cells suffer DNA damage, the DNA Damage Checkpoint (DDC) is activated in order to protect the cell from going through mitosis with persisting damage. The DDC cause cell cycle arrest, giving the cell time to repair the break. In budding yeast, S. cerevisiae, a single double strand break initiates the Mec1-dependent checkpoint prior to anaphase. The PI(3) kinase-like kinase (PIKK), Mec1, is responsible for relaying the signal to downstream targets, including an effector kinase called Rad53, which ensure proper cell cycle arrest. A readout of checkpoint activity is the phosphorylation status of Rad53. Pph3, Ptc2, Ptc3 and Glc7 are phosphatases key to extinguishing checkpoint signaling by dephosphorylating targets which include Rad53. Caffeine is a PIKK inhibitor. Previous data from our lab shows that Mec1-dependent Rad53 hyperphosphorylation rapidly disappears after caffeine treatment. In this project, we focus on why this rapid Rad53 dephosphorylation occurs after caffeine treatment. Using a galactose inducible HO endonuclease to cause a double stranded DNA break at a specific locus, we activate the Mec1-dependent DDC. When the checkpoint is activated, we observe hyperphosphorylation of Rad53 by 6 hours. Caffeine causes immediate dephosphorylation of Rad53. However, when using other DNA damaging reagents, like zeocin, MMS and HU where the damage occurs in different forms and at various points in the cell cycle, we do not consistently see this caffeine-dependent Rad53 dephosphorylation. Through this work, we have been able to conclude that caffeine-dependent Rad53 phosphorylation is partly dependent on Pph3 and Glc7. Caffeine treatment does not have any effects on Rad53 abundance. We have also found that the effects of caffeine on Rad53 are cell cycle dependent. In conclusion, these results indicate that Mec1 activity is constantly required to maintain DDC, and phosphatases are active throughout checkpoint arrest.

Support  MRSEC REU
Title Networks of Compartmentalized Belousov-Zhabotinsky (BZ) Reaction in Polydimethylsiloxane (PDMS)

Authors Xiaotong Geng, Thomas Litschel, Seth Fraden

Abstract The non-equilibrium chemistry of the Belousov-Zhabotinsky (BZ) reaction makes it a model experimental system for investigating the synchronization of coupled chemical oscillators. In previous experiments, distinct oscillators were fabricated as surfactant stabilized water-in-oil emulsions in which aqueous droplets of BZ solution were separated by gaps of oil. This emulsion based system allowed us to study the dynamics of diffusively coupled chemical oscillators in linear arrays as well as in two-dimensional networks. Our current project focuses on a new approach—using PDMS to construct microfluidic chips which contain two-dimensional arrays of nanoliter-scale cells. When loaded with BZ solution, the thin PDMS devices form a network of compartmentalized and diffusively interacting BZ reactions. By varying geometrical parameters of the chips' designs, we are able to alter the strength and quality of the chemical coupling between the oscillatory cells, therefore to tune between excitatory and inhibitory coupling. The results will lead us to the future projects which include bioinspired designs with the long-term goal of creating autonomous soft robots.

Support MRSEC Summer Research Fellowship
Rescuing motility defects associated with TDP-43 misexpression in a *Drosophila* model of Amyotrophic Lateral Sclerosis

**Authors** Amanda Shilton, Mugdha Deshpande, Zachary Feiger, Avital Rodal

**Abstract**

Amyotrophic Lateral Sclerosis is a neurodegenerative disease that leads to selective death of motor neurons. It has no cure or definitive treatment. Mutations in the TAR DNA binding protein (TDP 43) have been implicated in familial cases of ALS. We created an ALS model in *Drosophila* by using both overexpression of TDP-43, and TDP-43 loss of function. We wanted to investigate how loss of function or overexpression of TDP-43 affected crawling in third instar larvae compared to the wild-type control. This was done by tracking the movements of the larvae over a period of time and calculating their varying speeds. The results showed that the larvae with a misexpression of TDP 43 had a significant decrease in motility. We are investigating downstream pathways that are involved in this TDP-43 mediated defect, and went on to test possible rescues for these motility defects. The first manipulation we tried was to restore the levels of one of the known targets of TDP-43, the voltage-gated calcium channel cacophony. TDP 43 associates with the cacophony transcript and regulates its splicing. In misexpression of TDP-43, this splicing is affected, which leads to reduced levels of cacophony at the neuro-muscular junction. Re-expression of cacophony rescues axonal transport defects in TDP-43 models (Z. Feiger). It has been shown in previous papers that expressing cacophony rescues crawling in the loss of function model (Morton, 2013). We attempted to rescue larval motility by expressing cacophony cDNA in both overexpression, and loss of function. The loss of function cacophony rescue showed a significant increase in motility compared to the original loss of function genotype. However the rescue for TDP-43 overexpression did not show a significant difference from TDP-43 overexpression alone. The other rescue was aimed at rerouting the growth receptors to early endosome compartments. Variations in TDP 43 levels have been shown to alter the amount of the growth factor receptor thickveins (Tkv) in internal compartments (M. Deshpande), leading to a reduction in growth factor signaling. In TDP-43 misexpression, more Tkv is present in the Rab 11 positive recycling compartments instead of the Rab 5 early endosome. Our manipulation introduced a dominant negative Rab11 (Rab11 DN) in an attempt to restore motility. The Rab11DN rescue for loss of function showed a significant increase in motility compared to the loss of function alone. However larval motility was not restored to wild type levels. Expressing Rab11DN did not rescue motility in TDP-43 overexpression. Overexpression of TDP-43 did not show a rescue in either experiment. The version of TDP-43 being overexpressed is the human
**Title** Using ancestral proteins to unravel the molecular mechanism and the druggability of a modern kinase

**Authors** Yuejiao Zheng, Adelajda Zorba, Christopher Wilson, Janice Villali, Nadja Kern and Dorothee Kern

**Abstract** Proper activity of Aurora A kinase is crucial for cell division. Indeed mutations leading to aberrant Aurora activity underlie many human cancers. Key to Aurora A activity levels in the cell is allosteric regulation by TPX2 binding to the PIF pocket. In addition, TPX2 plays a key role in localizing Aurora to spindle microtubules for subsequent interactions. Despite more than a decade of studies centering on allosteric regulation of Aurora, the molecular mechanism of TPX2 binding and activation of Aurora are still poorly understood. Here, we approach the problem in two ways: first, we use Ancestral Sequence Reconstruction to generate ancestral Aurora proteins and study their ability to be activated by TPX2 in the context of their unique structural identities. Second, we screen for specific monobodies - small fibronectin-domain proteins - that inhibit Aurora through PIF-pocket binding and compare structural basis of inhibition with that of TPX2 activation. We find that incremental changes in Aurora structure throughout the course of evolution serve to accommodate TPX2 binding and mediate allosteric activation. Further, we find through the inhibitory interaction of monobody Mb60 with Aurora's PIF pocket, that the αC-helix that is crucial in Aurora's activation, is now locked in place by Mb60. These results paint a nuanced picture of allosteric activation in which key structural motifs in Aurora develop over time to converge on a specific structural mechanism capable of transferring maximum activation upon TPX2 binding. Understanding of allosteric regulation of Aurora through PIF pocket interactions represents a novel mechanism by which to control Aurora activity and possibly alter cancer pathology.

**Support** Division of Science Summer Research Fellowship

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**Poster # 2015.450**

**Presenter** Keishla Márquez González (Universidad Metropolitana / Cellular Molecular Biology)

**Title** A new role for thermosensors in *Drosophila melanogaster*

**Authors** Keishla Márquez González, Belinda Barbagallo and Paul Garrity

**Abstract** Temperature is a universal parameter experienced by all animals that can have dramatic effects on the biochemical functions of cells. In *Drosophila melanogaster*, neuronal receptors of temperature are well known to control behavioral avoidance of thermal extremes. However, whether they have additional roles in helping animals cope with sub-optimal temperatures is unknown. Preliminary work from the Garrity Lab has shown that mutant animals lacking two neuronally-expressed warmth receptors, Gr28b and TrpA1, become immobilized at normally innocuously warm temperatures (~34°C), suggesting that these thermoreceptors may also play a role in heat tolerance in *Drosophila*. Based on this model, I focused on identifying the neural circuit that sends thermosensory information to the brain to mediate heat tolerance. Using genetic techniques, I have silenced downstream projection neurons and neuropeptide-expressing cells and assessed heat tolerance in the resulting animals. The results of this study will lend insight into the cellular and molecular basis of heat tolerance.

**Support** Cell and Molecular Visualization REU
Title Improving Black Hole Mass Estimates In Quasars With Orientation Information

Authors Jose Miguel G. Bautista and John Wardle

Abstract Quasars are some of the most energetic and distant objects in the observed universe. Their energy comes from the accretion of gas onto a supermassive black hole, and the mass of the black hole can be estimated from the velocity of this gas. We show how to incorporate information about the orientation of the quasar to improve the estimation of the mass of the central black hole.

Support Provost's Undergraduate Research Fund

Poster # 2015.452

Presenter James Lee (Brandeis / Biochemistry)

Title Cutting to the Chase: Mer1, A Critical Splicing Regulator of Meiosis

Authors James Lee, M. Trieu, C. van der Feltz, and D. Pomeranz Krummel

Abstract In human cells, most protein coding genes contain coding segments called exons separated by non-coding segments called introns. Splicing is the process by which the gene's nascent pre-mRNA is converted into a mature mRNA by removal of introns and joining together of exons. Splicing is catalyzed by a mega-dalton assembly called the spliceosome. Despite the vital role that splicing and the spliceosome have in the development of a human cell, little is known about how it is regulated. To study the regulation of splicing, we are pursuing structure-function studies of a protein called Mer1, which has a critical role in regulating splicing of pre-mRNA transcripts in budding yeast during meiosis. Mer1 is composed of two distinct domains: an N-terminal domain, which we hypothesize interacts with the U1 snRNP to nucleate rapid assembly of the spliceosome, and a KH domain, which recognizes a specific intronic sequence. Having previously tagged and expressed the Mer1 KH domain, we are now working to establish the purification of successfully expressed full-length Mer1 protein as a Maltose Binding Protein (MBP) tagged fusion construct.

Support Division of Science Summer Research Fellowship (Life Science Scholars)

Poster # 2015.453

Presenter Ashley Klein (Brandeis / Chemistry)

Title Exploration of Boron Carboxylates For Their Viability to Catalyze Homoallylboration of Aldehydes by Cyclopropylcarbinyl Boronates

Authors Ashley Klein, Stasik Popov, Dr. Isaac Krauss

Abstract Allyl- and crotylboration of aldehydes is a useful and interesting transformation, due to predictable stereochemical outcomes which can be understood through cyclohexane chair-like transition state models. We have recently found that cyclopropanated allyl- and crotylboronate reagents react through transition states analogous to allylation; in an equally predictable manner, they stereoselectively deliver structural motifs which were difficult to access through previous methods. Although this reaction was originally promoted by stoichiometric amounts of PhBCl₂, we have recently found that boron carboxylates can catalyze homoallylation by a ligand exchange mechanism. Herein, we report further explorations in this mode of catalysis, using chiral and achiral ligands including carboxylates and related structures.

Support Provost's Undergraduate Research Fund
Abstract Type 2 Diabetes is a debilitating and highly prevalent disease affecting more than 29 million people in the U.S., thus creating an urgent need for early and effective diagnosis. One postulated mechanism of diabetes is that mitochondrial mutational load builds up over time resulting in mitochondrial dysfunction and onset of disease. Additionally mutational load may serve as an effective biomarker for diabetes progress. Studies using mitochondrial DNA (mtDNA) from liver show that there is a correlation between mutation and disease. However, studies to determine this relationship using liver for analysis make longitudinal studies impossible, so mtDNA in blood plasma was investigated to determine if it was a suitable replacement for liver mtDNA. A significant positive correlation was found between liver mitochondrial mutational load and diabetic state. Due to the complex mixture of mtDNA, such a correlation has not yet been determined in the blood plasma. There does appear to be a correlation between disease state and complexity of the mtDNA population in the blood. This change in population could be due to disease progression or severity. More in depth study of these changes as well as the actual mutational load of the blood plasma will be the focus of our future work.

Support Provost's Undergraduate Research Fund, Malaysian Palm Oil Board
Abstract Prenatal cardiac growth in rats is characterized by proliferation of cardiac myocytes. However, shortly after birth, cardiac myocytes rapidly withdraw from the cell cycle and continue to grow via hypertrophy. This developmental transition takes place concomitantly with the innervation of the heart by sympathetic fibers. Previous work in the Birren Lab has shown that early postnatal chemical sympathectomy with 6-hydroxydopamine in vivo results in rats with smaller hearts. This suggests that the sympathetic nervous system may modulate the transition from proliferation to hypertrophy in cardiac myocytes, leading to changes in heart size and potentially function. I assayed rates of proliferation in cardiac myocytes, cultured with and without neurons, with the proliferative marker Ki67, in order to see whether sympathetic innervation affects the proliferative window of these cells. These experiments showed that cardiac myocytes cultured with sympathetic neurons had an increased proliferative capacity as compared to cardiac myocytes cultured without sympathetic neurons, consistent with previous experiments. I then investigated the molecular mechanisms underlying this regulation. C-myc, Meis1, and ALMS1 have been identified as regulators of the cell cycle in cardiac myocytes. To test whether sympathetic signaling modulated the expression of these genes, expression levels were compared between control and lesioned hearts. C-myc, a gene whose overexpression in mouse hearts results in increased cardiac myocyte number (Jackson et al., 1990), was down-regulated in lesioned hearts at P2. In contrast, Meis1, a gene whose overexpression in mouse hearts results in decreased cardiac myocyte number (Mahmoud et al., 2013), was up-regulated in lesioned hearts at P2 and P7. This suggests that sympathetic signaling may interact with both C-myc and Meis1 in order to prolong the proliferative window and suppress cell cycle withdrawal in cardiac myocytes. Finally ALMS1, a gene whose deficiency results in increased cardiac myocyte number (Shenje et al., 2004), is up-regulated at 2 months in the lesioned hearts, suggesting that it may be involved in maintaining cell cycle withdrawal in the mature cardiac myocyte. These experiments demonstrate that sympathetic innervation regulates the timing of cell cycle withdrawal, potentially through the regulation of genes that are linked to the cell cycle in cardiac myocytes.

Support Provost's Undergraduate Research Fund
Unique Prokaryotic IMP Dehydrogenase Residues Help Determine Inhibitor Selectivity

Alex Cuadros, Masha Rosenberg, Lizbeth Hedstrom

The alarming rise in antibiotic resistant bacteria worldwide demands the need for new antibiotic drug development. The bacterial inosine-5'-monophosphate dehydrogenase (IMPDH) enzyme proves to be a promising target for such drugs due to its structural similarity across bacterial species but exquisite inhibitor binding specificity. The long term goal of the Hedstrom laboratory is to combat antibacterial resistance by developing inhibitors of the IMPDH enzyme to use as new antibiotics. Our experiments aimed to identify and test which bacterial IMPDH inhibitor binding site residues determine selectivity for various inhibitors. Five species of bacteria have IMPDH enzymes that contain a pair of residues near the catalytic active site not present on any other IMPDH, indicating that these two residues could contribute to inhibitor binding specificity. We decided to test whether these two residues are significant by introducing a mutation that deleted Gly346 and Ala347 on the IMPDH of *Acinetobacter baumannii* ATCC 17978. After inducing expression of the mutant enzyme in BL21 ΔguaB competent cells, we purified the protein through a nickel affinity column. By comparing the enzyme kinetics of wild type *A. baumannii* IMPDH versus the kinetics of the mutant we observed no significant changes in $K_m$ or $k_{cat}$. By conducting structure-activity relationship assays, we found that the deletion of Gly346 and Ala347 in the mutant enzyme most profoundly impacted the P-series of inhibitors, causing a 5-fold and 10-fold increase in potency compared to wild type in some cases. Our preliminary results suggest that the absence of the Gly346 and Ala347 residues allows P-series inhibitors to access previously unavailable residues of the active site for which they have a better affinity. Future directions include structure-activity analysis of the IMPDH's of *Legionella pneumophila*, *Corynebacterium diphtheriae*, and *Coxiella burnetti* which contain the same pair of amino acid residues corresponding to those in wild type *A. baumannii* IMPDH.

Support Cell and Molecular Visualization REU

A search for the next cryogenic photoactivatable fluorophore

Conor Lanahan, Marc Nahmani and Gina Turrigiano

Photoactivated localization microscopy (PALM), a technique proposed in the past decade, is a form of super-resolution microscopy that allows molecular distinction of up to 10nm. Recently a new method has been proposed in which conventional PALM resolution is greatly enhanced by performing the imaging at cryogenic temperatures. As a result of the cold temperature many established photoactivatable fluorescent proteins (PAFP) cannot be used due to the nature of the fluorophore activity in a cryogenic state. In this experiment, known PAFP photoactivatable green fluorescent protein (PAGFP), photoactivatable mKate (PAmKate), and photoswitchable mOrange (PSmOrange) were purified from *Escherichia coli* and evaluated for their cryogenic fluorescent characteristics. PAGFP has repeatedly shown consistent activation and fluorescence in the green range while imaging at cryogenic temperatures. Far-red shifted cryo-fluorescent proteins, PAmKate and PSmOrange, are being examined in comparison to PAGFP for their cryogenic state characteristics. Once complete, we are hoping to use two PAFP's in, to the best of our knowledge, the first dual-color cryogenic PALM image.

Support Provost's Undergraduate Research Fund
Title Phase Separation of Oppositely Chiral Rods in Colloidal Membranes

Authors Chunlong Huang, Jerome Fung, Joia Miller, Andrew Balchunas, Zvonimir Dogic

Abstract A membrane is a pliable sheet-like structure acting as a boundary that can separate the interior of a cell from its external environment. Instead of studying biological membranes, we study rod-like viruses with tunable attractive interactions that form monolayer colloidal membranes. Our colloidal membranes are a model system for understanding biological membranes. We recently have observed a phase separation in these colloidal membranes, but the question is what drives them to phase separate. This model system consists of two equal length but oppositely chiral rod-like viruses (1200nm) and non-adsorbing polymer (dextran). This resultant suspension is injected into an observation chamber. Since one type of the rods is labeled with Dylight 550, we can observe the phase separation by using fluorescent microscopy. This experimental study demonstrated that there are bulk phase separation inside the membranes at high dextran concentrations and incomplete phase separation inside the membranes at intermediate dextran concentrations. However, at low dextran concentrations, both types of viruses will mix uniformly. In a near future, we will understand the physical properties in this phase separation state. Consequently, we can understand more biological membranes and potentially help curing cancer.

Support MRSEC REU
Title Changes in neuronal morphology and connectivity upon TDP-43 misregulation

Authors Gabriela A. Rodríguez, Josiah Herzog, Mugdha Deshpande, Suzanna Paradis & Avital Rodal

Abstract Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that affects the lower motor neurons of the brain stem and spinal cord as well as the upper motor neurons of the cortex. In the disease state, the lower motor neurons detach from the muscle ultimately resulting in neuronal atrophy and death. Frontotemporal Lobar Degeneration (FTLD), another neurodegenerative disease characterized by atrophy of the frontal and temporal cortices, is related to ALS in that they both share a similar cellular pathology. A small but significant percentage of ALS cases are caused by genetic mutations in the gene TAR DNA Binding protein (TDP-43). TDP-43 contains two RNA-recognition motifs (RRM), a glycine rich region, a nuclear localization signal (NLS) and a nuclear export signal (NES). Interestingly, the majority of ALS and FTLD patients present with neuronal aggregations of TDP-43, suggesting that the misexpression of this gene could be one of the main causes of these neurodegenerative diseases. TDP-43 plays several roles throughout the cell and one of its main features is its ability to bind RNA. Due to this multifunctionality and to the ubiquitous expression of TDP-43, it has been difficult to elucidate which specific cellular processes go wrong in disease states. Unpublished studies from the Paradis and Rodal labs demonstrate that overexpression of wild-type TPD-43 in cultured cortical neurons causes decreased dendritic growth. Further, this phenotype requires a functional RNA binding domain, as overexpression of TDP-43 containing mutations in the RRM1 region (TDP-43 F147_149L) fail to suppress dendritic growth. Taking this information into consideration I hypothesized that synapse formation in these dendrites might also be affected by TDP-43 misregulation. To answer this question I performed synapse assays by quantifying synapse density on 14DIV cortical neurons in which I overexpressed wild-type or mutant TDP-43. My initial experiments indicate that the overexpression of the wild-type TDP-43 and RNA Binding Mutation (F147_149L) has no effect on the synapse formation in cortical neurons.

Support Cell and Molecular Visualization REU
Title: The role of Bik1 and formin interactions in coordinating the actin cytoskeleton
Authors: Julia L. Schiantarelli, Jessica Henty-Ridilla, Bruce L. Goode

Abstract: It is well known that the coordination of the actin and microtubule (MT) cytoskeletons is required for diverse cellular processes, morphologies, and motility. These active biological polymers consume energy in order to assemble into different types of highly cellular structures, i.e., filaments, bundles, or branched arrays. However, there is relatively little known about how the dynamics of the two systems are coordinated. Recent observations in the Goode lab show that the microtubule plus end-binding protein CLIP-170 directly regulates formin-mediated actin polymerization through a conserved sequence motif, discovered in yeast Smy1, known as Formin Elongation Effector Domain (FEED). Thus, providing a new link between actin and microtubule dynamics. My project asks whether similar formin regulation extends to the budding yeast homolog of CLIP-170, Bik1. In yeast, Bik1 has been shown to regulate microtubule dynamics, and to localize to microtubules. I will ask whether Bik1 additionally regulates the actin assembly activities of the two yeast formins, Bnr1 and Bni1. Thus far, I have performed sequence alignments with the FEED motif from yeast Smy1 with Bik1. I have also used biochemical approaches to transform, express and purify both yeast formins and assessed their activity on actin filaments in bulk fluorescence assays as well as Total Internal Reflection (TIRF) microscopy assays. I hope to learn whether the CLIP-170 function is conserved across evolution, and to take advantage of this genetically powerful system for future in vivo analyses.

Support: MRSEC Summer Research Fellowship

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Title: Incorporation of Pd-PCP Metallolinkers into Metal-Organic Frameworks for Heterogeneous Catalysis
Authors: Samuel M. Bhutto, Casey R. Wade

Abstract: Metal-organic frameworks, or MOFs, are porous coordination networks consisting of metal ions or clusters linked by organic molecules bearing donor functional groups. Owing to their porous nature and tunability, these materials have the potential to act as supports for heterogeneous catalysts. Although framework metal ions may exhibit catalytic activity, their involvement in catalytic processes is limited by metal identity and the availability of coordination sites and redox states. The use of organic linkers decorated with catalytically active functional groups or non-framework metals preserves the crystallinity of the MOF while also introducing an active site for catalysis. My project focuses on the synthesis of palladium diphosphine pincer metallolinkers (Pd-PCP) decorated with carboxylic acids for MOF assembly. Diphosphine pincer ligands form highly stable complexes with late transition metals, and the resulting complexes have been shown to catalyze a wide variety of chemical transformations. Pd-PCP metallolinker derivatives bearing benzoic acid and biphenyl carboxylic acid phosphine substituents have been successfully synthesized. The benzoic acid derivative has also successfully been incorporated into a MOF with Cu²⁺.

Support: Jordan-Dreyer Summer Research Assistantship
Incorporation of Pt and Pd Pincer Complexes into Metal-Organic Frameworks for Heterogeneous Catalysis

Rebecca E. Sternberg, Casey R. Wade

Abstract

Metal-organic frameworks, or MOFs, are porous coordination polymers assembled from metal ions or clusters and functionalized organic linkers. These materials have the potential to act as supports for heterogeneous catalysis. Although framework metal ions—which fulfill the role of the structural component—may exhibit catalytic activity, their involvement in catalytic processes is limited by metal identity and the availability of coordination sites and redox states. However, the use of organic linkers with additional functional groups can allow for the incorporation of catalytically active, non-framework metals. Diphosphine pincer ligands form highly stable complexes with late transition metals which have been shown to catalyze a wide variety of chemical transformations. This project seeks to incorporate platinum and palladium “PCP” pincer metallolinkers decorated with carboxylic acid groups into three dimensional MOFs for use in heterogeneous catalysis. These metallolinkers have been synthesized, and initial screening of MOF assembly reactions has shown that they produce isostructural MOFs with Cu$^{2+}$ as characterized by powder X-ray diffraction.

Support
MRSEC REU

Characterizing the domains of a soluble Guanylyl Cyclase protein for use in optogenetics

Josiane Fofana, Benjamin Morehouse, Erin Devine, Daniel D. Oprian

Abstract

A soluble Guanylyl Cyclase protein (GCwCC) was recently discovered and determined to be involved in a fungal phototaxis. However, the signaling pathway still remains unknown. Guanylyl Cyclase is composed of a coiled-coil domain (CC) and a catalytic domain (GC) similar to the one found in vertebrates. Our goal in this project is to characterize each domain and determine their role in the light response of the fungus. In order to do so, we obtained GCwCC and a Guanylyl Cyclase without the coiled-coil domain (GCnoCC) through PCR mutagenesis. So far, only GCwCC could be successfully expressed. This confirms the need to further investigate the role of the coiled-coil domain in the catalytic activity of this soluble Guanylyl Cyclase protein.

Support
Adar Family and Friends Fellowship
Title Fronto-central theta oscillations during multisensory divided attention

Authors Arielle S. Keller, Lisa Payne & Robert Sekuler

Abstract Although it is known that alpha oscillations play a role in suppressing distractions when just one sensory modality must be attended, are the same neural mechanisms involved when attention must be paid to multiple sensory modalities? For an answer, we investigated how divided attention impacted processing of auditory and visual sequences. In Experiment 1, subjects performed an oddball task with auditory, visual, or simultaneous audiovisual sequences in separate blocks, while the electroencephalogram was recorded using high-density scalp electrodes. During a divided-attention audiovisual condition, an oddball (a rare and unusual stimulus) could occur in either the auditory or the visual domain, requiring that attention be divided between modalities. Fronto-central theta band (4-7 Hz) activity source-localized to the anterior cingulate was strongest in this audiovisual condition. Given that the anterior cingulate plays a role in divided attention (Corbetta et al., 1991) and given that theta-band activity has been associated with both multisensory processing (Sakowitz et al., 2000) and working memory (Gevins et al., 1997), Experiment 2 sought to differentiate these possible roles of fronto-central theta activity during multisensory divided attention. Using a modified version of the oddball task from Experiment 1, the results of Experiment 2 showed that theta power was present in single-modality conditions and was independent of working memory load. Ruling out theta’s association with multisensory processing and working memory, we concluded that fronto-central theta activity is likely a marker of divided attention.

Support Computational Neuroscience Traineeship

Poster # 2015.465

Presenter Steven Wasserman (Brandeis / Biochemistry, Biological Physics)

Title SH3 domains as a means for protein binding and autoinhibition

Authors Steven Wasserman, Emily Messelaar, Avital Rodal

Abstract Nervous Wreck (Nwk) is a member of the F-BAR/SH3 family of membrane remodeling proteins, able to bind to membranes and generate curvature. Mutations in nwk are associated with synaptic overgrowth at the neuromuscular junction of Drosophila larvae. SH3 domains, found in F-BAR proteins, bind proline-rich domain (PRD) in binding partners, and also autoinhibit F-BAR domains via electrostatic interactions. Two proteins are known to bind to the two SH3 domains of Nwk: Wiskott-Aldrich Syndrome protein (WASP), which activates Arp2/3 and actin nucleation, binds to the first, SH3a, while Dynamin-associating protein 160kD (Dap160), involved in the localization of Nwk in larval NMJs, binds to the second of these domains, SH3b. To better understand these interactions, we used GST pull down assays to test the interactions. The results of these assays show that the hydrophobic N-terminus of the SH3b domain may be important for Dap160 interaction, and that the SH3b domain may inhibit WASP/SH3a interactions. Future experiments will use FRET to understand the autoinhibition of Nwk and how Dap160 activates its binding activity.

Support QBReC Program
Abstract  Protein knockdown is an invaluable method to study the protein function. With previous work done in Hedstrom’s lab, a target protein can be degraded, induced by a recognition enzyme inhibitor linked to an N,N,N-triboc-protected arginine (Boc3Arg), such as trimethoprim-Boc3Arg can degrade e.coli DHFR. For my project, methotrexate (MTX) was used as the known inhibitor linked on Boc3-Arginine to target mammalian DHFR. The inhibitor MTX-Boc3Arg, as well as its derivatives carrying a methyl ester or benzyloxy carbonyl protection group, was synthesized with a 6-step method. The products were confirmed by H-NMR spectra. Both in vitro and in vivo assays were done to test their effect on mediating degradation. The results of both assays showed that Boc3Arg-MTX and its derivatives can induce degradation of mammalian DHFR, and the efficiency of degradation varies depending on the protection group. Boc3Arg-MTX and its derivatives provide a strategy to directly degrade endogenous protein. The attachment of MTX moiety broadens the application of Boc3-Arg to degrade a new protein found in all organisms.

Poster # 2015.466
Presenter Zhongrui Zhang (Brandeis / Biochemistry)
Title Small Molecule Inhibitor Methotrexate-Boc3Arginine Targets Dihydrofolate Reductase for Degradation
Authors Zhongrui Zhang, Yuntao Shi, Lizbeth Hedstrom

Abstract  Hsp90 is a highly conserved, dimeric molecular chaperone composed of 3 domains, N-, M-, and C- per monomer. Bacterial Hsp90, HtpG, has a very slow rate of ATP consumption, though introduction of a substrate protein to HtpG significantly increases its ATPase rate. The primary binding site for substrates has been shown to be located between the M- and C- domains with secondary cross-monomer contacts that are responsible for activation occurring along the N- or M-domains. In order to determine the location of these cross-monomer contacts, 16 residues located along the N- and M- domains were mutated to an alanine. I then titrated in L2, a known substrate of HtpG, and measured the ATPase rate of each mutant using an enzyme-linked assay. The mutant W191A exhibited a decreased Km, the mutants K103A and R74A exhibited increased Vmax, and the mutants I338A and R87A exhibited decreased Vmax. These results indicate strongly electrostatic dependent effects as well as a possible location of the secondary binding site on HtpG.

Support Division of Science Summer Research Fellowship
**Poster # 2015.468**

**Presenter** Matthew Davis (Brandeis / Chemistry)

**Title** Synthesis of Fmoc-Homopropargylglycine for Large-Scale Glycopeptide Synthesis

**Authors** Matthew N. Davis, Jennifer K. Bailey, Satoru Horiya, J. Sebastian Temme, Dan N. Nguyen, and Isaac J. Krauss

**Abstract** Much work on treatment and vaccination against HIV in recent years has focused on broadly neutralizing antibodies (bnAbs). One bnAb of particular interest to our group is 2G12, which neutralizes the largely conserved HIV surface protein gp120. Gp120 is a trimeric protein covered with high-mannose glycans, and 2G12 neutralizes it by binding to these glycans. For our synthetic approach, we used an alkyne-containing amino acid, homopropargylglycine (HPG), in place of Methionine, as well as the click reaction with Man9-azide to form glycopeptides. My specific research has been to improve the overall synthesis of HPG. HPG is a very expensive compound to buy, and we discovered that the method we had been using to synthesize it lead to partial racemization of the HPG. My goal was to modify the method to produce non-racemic HPG while still having a good yield. Once I have synthesized a significant amount of HPG, my work will focus on synthesizing the winners from our selection of the best 2G12 binders and mutating or truncating them to determine which regions have the most important effects on binding.

**Support** Jordan-Dreyer Summer Research Assistantship

**Poster # 2015.469**

**Presenter** Jonathan Spyreas (Brandeis / Biological physics)

**Title** Carbon Fiber Electrode Bundles for Dense Recordings of Neural Circuits

**Authors** David Landsman*, Jonathan Spyreas*, Nora Anderson, Neil Ritter, Steve Van Hooser

*Contributed equally

**Abstract** The total number of cells that can be simultaneously recorded is limited by current electrode technology. Here, we describe a method for building low cost, customizable ultradense-electrode arrays for in vivo extracellular recording at arbitrary depths. This solution to solve these issues is the utilization of carbon fibers and 3D printing. 3D printing allows for rapid customizable structure of the electrodes, originally based on the 16 channel design used for superficial recordings in HVC of the Zebra Finch in the Gardner lab at BU. With channel densities that would not be possible with other materials and methods of manufacturing, carbon fibers have the benefit of having lower impedance than most other materials currently used, making them excellent for capturing signals from the brain. The fibers are able to be coated so that their signals do not interfere with the other channels of the electrode bundle. The use of these technologies will enable further understanding of the connections among brain structures, such as those between thalamus and visual cortex. These recordings will lead to a deeper understanding of fine-scale circuitry that underlies system-level responses in the regions. Continuous iterations of the coating material’s design and the building process will further improve these electrodes. These improvements will lead to future electrodes that will number in the hundreds of channels, by use of the tools pioneered in this relatively small scale 16 channel electrode.
Title: Self-Assembly of Multidimensional Colloidal Structures by Tuning Osmotic Pressure and Constituent Particle Ratios

Authors: Tarik Phillips, Andrew Balchunas, Joia Miller, Jerome Fung, Zvonimir Dogic

Abstract: M13K07* is a rod-like virus strain that exhibits hard core repulsions in a colloidal system. When a depletant is also added, it lends the rods an additional attractive force leading to the rods’ self-assembly into ordered structures. These micron-sized rods can come together to form various shapes: single-layered membranes, stacks of membranes, and even 3D structures such as tactoids and catenoids. These systems serve as an important model for study as they can form structures very similar to biological membranes but on a larger scale. M13K07* is of great interest as it has a feature contrary to the current model of physical characteristics of colloidal membranes. M13K07* resists twisting under optical tweezing. It also forms catenoids which have negative curvature, a physical trait not often seen in biology. Catenoid formation is a phenomenon that occurs under specific levels of depletant concentration and when mixing M13K07* with a different strain, thereby doping the pure sample. However, the exact ranges for these values that cause formation have yet to be mapped. We have tested varied ratios of M13K07* and M13 mixtures under different concentrations of Dextran (depletant). Using Differential Interference Contrast (DIC) Microscopy, we have characterized the stabilized states that exist at these points. This has led to a Phase Space that indicates which structures exist at which conditions and some of the requirements for catenoid formation. This formation begins around 92% M13K07* to 8% M13 in a range from 39 to 45 mg/mL Dextran concentration. As the doping levels increase, the catenoid forming region also widens running from 39 to 55 mg/mL Dextran with a mixture of 80% M13K07* to 20% M13. Future studies will take place in order to more precisely isolate where the transition from membrane to catenoid takes place and explore the effects of even further doping.

Support: MRSEC REU

Poster # 2015.471

Presenter: Zoe Brown (Brandeis / Neuroscience, Psychology)

Title: Examining the Behavioral and Physiological Effects of Prosody on Sentence Processing

Abstract: Speech prosody encompasses word stress, pitch contour, and pauses, which can help indicate clause boundaries to the listener. I investigated the effect of prosody on speech recognition as measured by recall accuracy and cognitive effort. Young adults were asked to listen to and recall sentences, half of which contained prosody congruent with the syntactic structure, and the other half had been computer-edited to place the prosody in conflict with syntax. Recall accuracy was significantly lower for the incongruent condition than that of the congruent condition. For the incongruent sentences, participants often shifted their responses to match the prosodic marking, which indicated that prosody often had a strong lure over the intended syntactic parse. To measure effort, pupil dilation was continuously recorded using an eye tracker, and was time-locked to the sentences via MATLAB. Pupillometry data are currently being analyzed, and I expect that pupillometry will be able to be used as a sensitive measure of cognitive effort. If so, as the incongruent condition produced more errors, there should be an overall larger pupil dilation, reflecting an increase in effort, for this condition.

Support: Bauer Summer Undergraduate Research Fellowship
Abstract Patterns formation in animals is an interesting topic to explore, since it may disclose the mechanism underlying the self-organized structures in living organisms. Some Turing patterns show striking resemblance to patterns on animal skin and shells and may shed light on a possible origin of morphogenesis in living organisms. Belousov-Zhabotinsky Aerosol OT system has been shown to exhibit rich variety of patterns under a wide range of concentrations and temperatures. In our experiment, we investigate the sensitivity of Turing patterns in a BZ reverse microemulsion system to illumination by different light intensities. We showed that BZ reaction in two dimensional space is a good system to study pattern formation, especially we have mapped the spatiotemporal patterns formed at various concentrations of sulfuric acid, sodium bromate, malonic acid together with various droplet fractions. In addition to this, we have verified that illumination can transform pattern from labyrinth to spots.

Support Jordan-Dreyer Summer Research Assistantship
Title
Polymorphic transitions in helical flagella

Authors
Henry Snow, Walter Schwenger, Sevim Yardimci, Thomas Gibaud, Zvonimir Dogic

Abstract
Biological polymers have a critical role in many cellular processes. Here, we present bacterial flagella as rigid rods, but demonstrate that flagellar geometry gives rise to unique new qualities. Flagella are purified from Salmonella typhimurium with a helical shape; by modifying the self-assembly of flagellin monomers in vitro, we create a variety of helical polymorphs, as well as a straight polymorph, and, through controlled polymerization, block copolymer filaments which consist of a helical and a straight component. Through observing controlled transitions in shape of these flagella, as well as studying dynamics and rheology, we demonstrate the unique nature of bacterial flagella and their utility as an interesting model colloid in the field of soft condensed matter.

Support
QBReC Program

Poster # 2015.475

Presenter Ethan Chan (Brandeis / Chemistry)

Title
Indefinitely Oscillating Chemical Cells: Initial Design and Future Direction

Authors
Ethan Chan, Thomas Litschel, Camille Girabawe, Nathan Tompkins, Seth Fraden

Abstract
Synchronized oscillators are very common in nature, from flashing fireflies to spiking neurons, and even the steps of walking humans. The study of synchronization is important to the development of synthetic assemblies of chemical cells that will produce chemo-mechanical oscillations mimicking the behavior of biological soft matter such as a beating heart. An experimental system commonly used to study synchronization is the oscillatory Belousov-Zhabotinsky (BZ) reaction where the oxidation and reduction of a metal species in solution results in oscillatory color changes corresponding to its oxidation state. This project’s goal is to design an open system in which the reagents are continuously supplied to the chemical cells so that they can oscillate indefinitely. Microfluidic channels and wells in polydimethylsiloxane (PDMS) were designed to allow for continuous flow of chemicals to the wells, which would function as chemical oscillators. Experiments performed on the initial design did not result in oscillations due to inadequate mixing of chemicals and bubble formation. Modifications to the design are currently in progress in order to address these issues, but have not yet been tested.
**Title**  
Epigenetic Gene Silencing Induced by Invading Homologous DNA in *Naegleria gruberi*

**Authors**  

**Abstract**  
Our lab chose *Naegleria gruberi* as a model organism to study epigenetics because of its ability to undergo a dramatic phenotypic change from amoeba to flagellate. Initially, we tried to transform genes to test their functions. Instead, we discovered an efficient gene-silencing mechanism. We tried to transform the ump gene, which is involved in the synthesis of uracil and converts 5-fluoroorotic acid [FOA] into a toxin. When the ump gene is silenced, the cells can grow in FOA, allowing for selection of “transformed” cells. Introduction of a ump gene with engineered deletions causes highly efficient FOA-resistance, absence of ump RNA, and lack of UMP synthase enzyme activity. Surprisingly, the wild type ump gene sequence remained unchanged in the FOA-resistant cells. Introducing the wild type ump gene also induces FOA-resistance. Preliminary experiments suggested that invading homologous DNA cause silencing through DNA methylation. Bisulfite sequencing revealed extensive methylation of the ump gene in FOA-resistant cells, and none in control cells that had not been exposed to the ump DNA. The sequencing showed an unusual type of methylation, non-CpG, in which bases other than guanine follow methylated cytosines. To test if the invading DNA silences only homologous DNA, we looked at a gene involved in flagellar motility, *cam1*, and found no methylation. We are repeating silencing using the wild type ump gene to see if the methylation occurs on the same bases again. We are also determining if 500-nt PCR segments of the ump gene will silence the entire gene. Future experiments include testing if another invading Naegleria gene will silence its endogenous homologous gene. Furthermore, we will test if the loss of FOA-resistance is accompanied by loss of non-CpG methylation by growing FOA-resistant cells in 5-azacytidine, which demethylates cytosine. The high efficiency of silencing suggests that an intermediate amplifies the effect of the invading homologous DNA. Perhaps this system could be manipulated to efficiently silence the genes of other organisms.

**Support**  
New England Biolabs

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**Poster # 2015.477**

**Presenter**  
Aaron Ammerman (Brandeis / Biochemistry)

**Title**  
Spectral Tuning of a Guanylyl Cyclase for Optogenetic Use

**Authors**  
Aaron E. Ammerman, Erin L. Devine, Daniel D. Oprian

**Abstract**  
We are researching Guanylyl Cyclase (GC) for use as an optogenetic tool. We wish to move expression into adult flies to control cyclic nucleotide levels. Wild type GC absorbs green light, which would not penetrate the cuticle of an adult fly (meaning the protein would not be activated). We investigated methods to spectrally tune GC from a green absorption maximum to red to make the protein sensitive to deeper penetrating light. The binding pocket is comprised of 3 key residues: Lys 216, Asp 85, and Asp 212. Past studies have found that changing the Asp residues can result in color shifts. With this in mind, three mutants were designed: E73Q, E73T, and D199N. At this time, only the E73Q and E73T mutants have been successfully tested. After purifying the protein with immunoaffinity techniques, it was found that the E73Q and E73T mutants had no effect on the color absorbance of the protein. Further experiments are necessary to test the D199N mutant, as well as to test the effect of other mutated residues on spectral tuning and optimize protein purification.

**Support**  
Schiff Fellows
Using Bimolecular Fluorescence Complementation to Visualize Protein-Protein Interaction

Authors Fanny Zhou, Leigh D. Plant, Steve A. N. Goldstein

Abstract Fluorescent proteins have become a common tool in biomedical science research to study the location, trafficking, and operation of a range of intracellular molecules. Bimolecular Fluorescence Complementation (BiFC) is a fairly recent technique developed to observe the interaction between two proteins. This approach is based on the idea that fluorescence can be recovered between two non-fluorescent fragments that are split from one fluorescent protein. This experiment attempts to discover if subunits from two different potassium channels (K2P1 and K2P2) are in association with each other. These two potassium channels are expressed on the plasma membrane and are responsible for regulation of cell excitability and resting potential in eukaryotic cells. K2P1 and K2P2 were tagged with one non-fluorescent fragment each in various combinations and visualized with an epifluorescence microscope. Using BiFC, it was shown that these two potassium channels formed a heterodimer with each other due to detection of a fluorescence emission. Future experiments include using TIRF microscopy to determine if these two channels are on the plasma membrane, where we expect them to be, and FRET to visualize a three protein complex with SUMO.

Support Schiff Fellows

Rapid Prototyping Thermoplastics

Authors Austin Prince, Ali Aghvami, Seth Fraden

Abstract Cyclic Olefin Copolymer (COC) has emerged as an effective polymer for microfluidic chips in the past decade. In comparison to standard PDMS elastomer based microfluidic chips, COC provides excellent UV transparency and low absorbance, which is very useful for purposes such as protein crystallization. We are seeking to construct a rapid prototyping method for the fabrication of microfluidic COC chips by utilizing hot embossing and solvent bonding. A method has been developed using a flexible PDMS master and a linear press in order to hot emboss COC chips. Lidding the devices proved to be the most difficult part of the fabrication process. Strength of bond, channel integrity, and absence of interstitial air bubbles are the most important factors in bonding. We sought to use a solvent bonding method taking advantage of Case II diffusion phenomena in order to create a shallow plasticized front, which is sharply separated from the glassy, embossed polymer. By using this method the polymer chains of the embossed and lidding piece can interweave creating a strong bond. Our results indicate that COC exhibits Case II diffusion in a 80:20 wt% solution of ethanol and decalin. Further work must be done in order to determine the right soaking time in solution.
**Poster # 2015.480**

**Presenter** Katy Lehmann (Brandeis / Biology)

**Title** The Lowe Syndrome Protein OCRL Regulates PIP2 Levels in Endosomes

**Authors** Katy Lehmann, Steve DelSignore, Sarah Biber, Avital Rodal

**Abstract** Lowe Syndrome is an x-linked disease that causes neurological abnormalities, glaucoma and renal failure, due to a mutation in the OCRL gene. The OCRL protein localizes to endocytic compartments and plays an essential role in trafficking and signaling. However, it is not well understood how mutations in OCRL cause the phenotypes typically associated with Lowe Syndrome. We found the Drosophila model of the disease (dOCRLΔ3 animals) exhibits an innate immune response phenotype even in the absence of an immune challenge. OCRL is a phosphoinositide phosphatase and regulates phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2). Given this, and the observed phenotype, we chose to investigate the role of OCRL in converting PI(4,5)P2 into PI(4)P in innate immune cells. We used immunohistochemistry and confocal imaging to look at PIP2 levels and PIP2 colocalization with Rab5 positive early endosome compartments and Rab11 positive recycling endosome compartments. Results show an increase in overall PIP2 levels in the dOCRLΔ3 background. Initial results show an increase in colocalization of PIP2 and Rab5 compartments and Rab11 compartments in the dOCRLΔ3 model. Our future investigations will determine whether the increase in colocalization is due to the overall increase in PIP2 levels. We will also repeat the same experiment with a dOCRLΔ3 Rab7-GFP model to investigate PIP2 in late endosomes and, we will create Rab5, 7 and 11 constitutively active and dominant negative models to determine defects in which endocytic compartment cause the phenotypes we see in the Drosophila model. This research will help us understand the mechanism of Lowe Syndrome and lead to treatments for patients.

**Support** Lowe Syndrome Association

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**Poster # 2015.481**

**Presenter** Pranav Kantroo (Indian Institute of Science, Bangalore / Mathematics)

**Title** A Study of Homomorphisms from a Genus-2 Surface

**Authors** Pranav Kantroo, Prof. Daniel Ruberman, Dr. Carl Wang Erickson

**Abstract** An important way to characterize the behaviour of a topological space is by studying its fundamental group. The fundamental group captures the topological properties of a space in an algebraic sense by giving paths that start and end at the same fixed base point an algebraic structure. In this study, we attempt to understand how different gluing maps can lead to different resultant spaces by comparing the pushout diagrams for the gluing action and counting the homomorphisms from the resultant spaces to a given group G. We are able to find a condition on when we can have a correspondence between homomorphisms from the resultant spaces to the group G. We then consider a specific case that relates the problem with the mutation action in knot theory.

**Support** Brandeis-India Science Scholars Program
Poster # 2015.482

**Presenter** Patrick Sheehan (Clarion University of Pennsylvania / Biology)

**Title** Visualizing Activity-independent Changes in Inhibitory Synapses with Array Tomography

**Authors** Y. Escobedo Lozoya, B. Isaac, S. Nelson

**Abstract** We are studying the effects of activity deprivation on inhibitory circuitry in relation to epileptic encephalopathies such as Infantile Spasm syndrome. We hypothesize that prolonged activity deprivation during development causes homeostatic plasticity changes that result in these epileptic encephalopathies. By applying Tetrodotoxin (TTX) to our mouse cortex in vitro model, we have found that miniature inhibitory synaptic currents (mIPSC) were significantly decreased in L5 pyramidal neurons, causing greater hyperexcitation. In this study, we wish to measure the structural changes that might be occurring at synapses. Using Array Tomography, which allows uniform antibody penetration and Z axis resolution through ultrathin-sectioning, we will be able to label synapses to detect how inhibitory synapses respond to TTX treatment. Our results so far show that all the steps of Array Tomography can be executed within our current facilities and that the specimens can support antibody multiplexing as well as multiple elutions and restainings.

**Support** Cell and Molecular Visualization REU

Poster # 2015.483

**Presenter** James R. Weiss (Brandeis / Biology, Neuroscience)

**Title** Temperature Sensitivity in the Cardiac Ganglion of *C. borealis*

**Authors** James R. Weiss, Adriane G. Otopalik, Eve Marder

**Abstract** Cold-blooded animals must maintain proper nervous system function at a wide range of temperatures. The crustacean stomatogastric ganglion, a small motor circuit, shows precise temperature compensation despite varying temperature sensitivities among the ion channels and receptors of its constituent neurons (Tang, 2010). In this experiment I recorded from a different crustacean motor circuit, the cardiac ganglion (CG), which acts as the heart’s pacemaker. The CG is comparatively simpler, consisting of four small cells (SCs) and five large cells (LCs), and its activity is still highly temperature-sensitive. The CG’s activity was recorded extracellularly while being subjected to steadily increasing and decreasing temperature ramps from 6-24°C. I found that the cardiac ganglion’s burst frequency adjusts to increasing and decreasing temperature at different rates, while other characteristics of the burst were also temperature-sensitive. With these preliminary data I will next be looking at whether hysteresis plays a role in temperature acclimation in the CG.

**Support** Computational Neuroscience Traineeship
Title: Investigating the Effects of Rem2 on Dendritic Arborisation *in vivo*

Authors: Shikhar Saxena, Jose Garcia, Sarah Richards, Anna Moore, Suzanne Paradis and Stephen D. Van Hooser

Abstract: The nervous system has the amazing capacity to transform sensory experience from the environment into changes in neuronal activity. While we know that sensory experience has a lasting effect on the brain, we have yet to discover the molecules responsible for these alterations. The small Ras-like GTPase Rem2 has been implicated in modulating several aspects of neuronal morphological development. Recent studies have suggested that Rem2 is a negative regulator of dendritic complexity *in vitro* and *in vivo* in *X. laevis* (Ghiretti *et al.* 2014, Ghiretti & Paradis 2011). Furthermore, these differences were amplified when cells were chronically depolarized suggesting that Rem2 is regulated by activity (Ghiretti *et al.* 2014). Here, we investigated the role of Rem2 in the development of V1 dendritic arbors in the mouse both under typical conditions and when visual experience is manipulated by dark rearing during and proximal to the critical period for ocular dominance i.e. between 24 and 30 days after birth. Wild type and Rem2 knockout littermate mice were either typically reared or dark reared until P21 or P30. We analysed their dendritic complexity using 3D Sholl analysis and by measuring branch length, branch order. Our results promise to give an insight into the activity dependence role of Rem2 in this critical period as our preliminary data suggested that knockout of Rem2 modestly increases the dendritic complexity and that Rem2 knockout animals show a reduction in dark reared induced reduction in complexity.

Support: Brandeis-India Science Scholars Program

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Poster # 2015.485

Presenter: Beth He (Brandeis / Biology, HSSP)

Title: Plasmid Cloning to Identify miRNA Targets Using TRIBE and *in vivo* Monitoring of Neuronal Firing

Authors: Beth He, Xiao Chen, Michael Rosbash

Abstract: Plasmid cloning techniques have been used in the *Drosophila* genome to identify miRNA targets and monitoring *in vivo* neuronal firing using mCherry and luciferase tags. Ago 1, an RNA binding protein, has been cloned into PMT, metal-ion induced, plasmid. Similarly, several activity-induced enhancers have been cloned into cre-luc gene. In the future, newly cloned PMT plasmid will be transfected into S2 cells and sequenced to find Ago1 binding targets. Since Cre-luc has both an mCherry and luciferase tag, in-vivo monitoring of the effect of cloned enhancers will be done.
Title Phase response of oscillating BZ droplets to light perturbations with varying Ru(bpy)$_2^{2+}$ concentrations

Authors Anna Kolstad, Nathan Tompkins, Seth Fraden

Abstract Patterns of synchronization are commonly found in nature: for example, in the beating of heart cells, or in the spontaneous synchronization of neurons. Using emulsion droplets containing a light-sensitive variation of the Belousov-Zhabotinsky (BZ) reaction, a nonlinear, oscillating chemical reaction, we previously created 2-D arrays of coupled oscillating droplets which exhibit similar patterns of synchronization. However, the magnitude of our previous system’s response to light was not great enough to allow us to externally control the network’s synchronization patterns through light perturbations. Using a Programmable Illumination Microscope (PIM), we optically isolated single BZ droplets and perturbed the droplets with 3-second light pulses during different phases of the droplets’ periods in order to measure the resulting phase response (a forward or backward shift in the droplet’s phase of oscillation) as a function of phase at which the perturbation occurred. Previously, using 1.2mM light-sensitive catalyst Ru(bpy)$_2^{2+}$Cl$_2$, the maximum phase response observed was ~-0.1 radians. By replacing Ru(bpy)$_2^{2+}$Cl$_2$ with Ru(bpy)$_2^{2+}$SO$_4$ and increasing the concentration of Ru(bpy)$_2^{2+}$ from 1.2mM to 3.5mM and 7.0mM, we observed a maximum phase response of -2/3pi radians using the 3.5mM system and -pi radians using the 7.0mM system. With this 30-fold increase in phase response due to light perturbation, we may now proceed with development of synchronization engineering techniques to manipulate networks of coupled oscillating droplets, beginning with the goal of switching the direction of coupled oscillations within a three-droplet triangle, and ultimately leading in the direction of the development of soft robotics.

Support MRSEC REU
Title Direct visualization and comparison of the three human Cofilins during actin filament severing and disassembly

Authors Samantha M. Chin, Silvia Jansen, and Bruce L. Goode

Abstract The functions of cellular actin filament networks depend on their ability to be dynamically remodeled, and at the heart of regulating actin network turnover is the Actin-Depolymerizing Factor (ADF)/Cofilin family of severing proteins. Mammals express three family members: Cof1, Cof2, and ADF. Cof1 and ADF are ubiquitously expressed, and thus often found in the same cell types, whereas Cof2 appears to be muscle-specific. Genetic studies indicate that Cof1 and ADF have both overlapping and unique functions in non-muscle cells, and biochemical studies have begun to define differences in activities of the three Cofilins. What has been lacking to date however, is a rigorous quantitative comparison of the effects of the three Cofilins on actin filament severing and disassembly. Here, we performed such an analysis using bulk fluorescence and multi-wavelength TIRF microscopy assays. Our results show that all three Cofilins bind to ADP-actin monomers with similar affinities, yet Cof2 and ADF have 8-fold and 4-fold higher efficiencies in severing filaments compared to Cof1. Similar effects were observed using muscle and non-muscle actin. We also directly visualized fluorescently labelled, functional Cof1 and Cof2 molecules on filaments in real time. Cof1 and Cof2 bound to filaments with similar kinetics, but Cof2 exhibited a shorter time interval from binding to severing, explaining its higher severing efficiency. Similarly, Cof2 and ADF were more effective than Cof1 in disassembling filaments in the presence of Coronin-1B and AIP1. Finally, by combining different Cofilins in the same reactions, we observed novel relationships in which specific pairs have additive severing effects while other pairs show neutral or inhibitory effects. Taken together, these data establish key quantitative differences in the activities of the three human Cofilins, alone and in combinations, with important implications for how these proteins are utilized in cells for tuning rates of actin network turnover.

Poster # 2015.489

Presenter Micael Maya-Peinl (UC San Diego / Biochemistry)

Title Investigating Crucial Proteins of the Circadian CLOCK-Complex in Drosophila melanogaster

Authors Maya-Peinl, M., Luo W., Rosbash, M.

Abstract Circadian rhythms are based around molecular clocks which help time the many functions of an organism. They are present in many species, including ourselves and Drosophila melanogaster; in which both clocks are relatively similar. Proteins, mRNA and DNA interactions cycle at different times of the day to generate the complex timing that is a circadian rhythm. While the basic, overlying circadian clock for D. melanogaster has been determined, we hypothesize that many more proteins of the CLOCK complex exist at specific time points during the evening for Drosophila. In D. melanogaster, the CLOCK protein peaks at ZT 14 while PER is peaked at ZT 18. We use this information to track down the CLOCK-complex using FLAG and HBH epitopes and specific antibodies against to both FLAG and PER. Silver stains and protein immunoblots were used to test for the presence of the CLOCK protein and complex at the times ZT 14, ZT 18, and ZT 22. For ZT 18, we've managed to gather enough protein for Mass Spectrometry analysis and we are currently awaiting results. Regardless, we do expect to find more proteins in unknown quantities as part of the circadian rhythm at ZT 18. We expect to send ZT 14 and ZT 22 samples for mass spectrometry in the near future. Further experimentation could be conducted on more time points such as ZT 10 for a better understanding of the evening oscillations of any proteins the spectrometry might detect.

Support HHMI EXROP
Title Developmental changes in spatio-temporal receptive fields of ferret V1 neurons

Authors Benyamin Meschede-Krasa, Arani Roy, Wesley Alford, Stephen Van Hooser

Abstract Direction-selective neurons in mammalian V1 have been observed to have slanted spatio-temporal receptive fields (STRF), indicating that they are sensitive to both space and time in a manner that is unseparable. Direction selectivity (DS), however, requires visual experience and is therefore not observed in visually naïve animals. Intracellular recordings were taken from both young, visually inexperienced ferrets and older ferrets. A cell's STRF was visualized using cross-correlation analysis in order to determine whether visual experience reinforces a pre-existing slant in the visually inexperienced animals' STRFs (pre-existing bias model) or if it organizes the broad and weak array of inputs to V1 into a slant (flexible model). From our analysis we can corroborate that the STRFs of DS V1 neurons in ferrets exhibit a slant as predicted. However, non-DS cells in visually naïve animals seem to exhibit both slanted and non-slanted STRFs, suggesting the existence of an early direction-selective input to cells that have not yet acquired DS.

Support Computational Neuroscience Traineeship

Poster # 2015.491

Presenter James R. Weiss (Brandeis / Biology, Neuroscience)

Title Just Keep Swimming: Dopamine's Neuromodulatory Effects on the Optomotor Response in Larval Zebrafish

Authors James R. Weiss, Urvashi Jha, Vatsala Thirumalai

Abstract In zebrafish, the optomotor response (OMR) allows the fish to maintain its spatial position by orienting toward and swimming in the direction of the perceived source of environmental motion. Reticulospinal neurons (RSNs), a set of descending motor control neurons, provide the necessary drive to turn and propel the fish. Distinct populations of RSNs are active during forward swims, left turns, and right turns (Orger et al, 2008.) Dopamine has previously been implicated as a key neuromodulator of locomotor behavior in larval zebrafish (Thirumalai, 2008). Here, we exposed head-mounted zebrafish to forward- and side-moving gratings to elicit OMR. We tested changes in swim behavior (tailbeat frequency, tail amplitude, and swim bout duration) in response to dopamine, quinpirole a D2 receptor agonist, and L741,626, a D2 agonist, using a high-speed camera. We found that while dopamine does not significantly affect OMR, quinpirole, a D2 receptor agonist, increased the duration of swim bouts and L-741,626, a D2 antagonist, increased the amplitude of turns. These data suggest dopaminergic modulation of swim behavior in response to OMR. Future projects include using calcium imaging to monitor neural activity while eliciting OMR in vivo.

Support Computational Neuroscience Traineeship, Brandeis-India Initiative
**Poster # 2015.492**

**Presenter** Ruth-Love Damoah (Brandeis / Biology)

**Title** Detecting Mycobacterium Tuberculosis Using LEL-PCR

**Authors** Ruth-Love Damoah, John Rice M.S., Dr. Aquiles Sanchez, Dr. Lawrence Wangh

**Abstract** Tuberculosis (TB), though treatable and curable, still claims 1.3 million lives annually. One of the contributing factors to this alarming statistic is the absence of a timely, comprehensive, and cheap diagnostic tool that is useful to all patient groups. A novel molecular assay targeting rpoB, a subunit of the DNA-directed RNA polymerase gene, is being developed to identify Mycobacterium tuberculosis (MTB) and its resistance to rifampicin. This assay utilizes the Linear-Expo-Linear polymerase chain reaction (LEL-PCR). It is sensitive down to single molecules.

**Support** Brandeis Study Abroad

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**Poster # 2015.494**

**Presenter** Jared Swezey-Gleason (Brandeis / Neuroscience)

**Title** The Analysis of Diffusion and Structural MRI in Applications to the Aging Brain

**Authors** Michael Jared Swezey-Gleason, Nina Linde Reislev, Ellen Garde

**Abstract** Within the field of aging brain research, Diffusion and Structural Magnetic Resonance Imaging (MRI) are important tools for revealing the properties of the brain’s microstructure and how they might be related to healthy brain aging. MRI plays a role in determining the effect of exercise on the brain and how brain plasticity might affect the brain’s long term function, questions critical to the study of dementia and Alzheimer’s disease. Here, Diffusion Weighted image processing, using FMRIB Software Library Topup and Eddy, is reviewed (Smith, 2004; Yamada, 2014). Due to image artefacts arising during scanning, images need to be preprocessed prior to any further analyses. First, the diffusion weighted images were preprocessed to correct for image inhomogeneities and subject motion during scanning. Next, the diffusion tensor model is fitted to explore microstructural properties of the brain white matter, mainly focusing on the mean diffusivity and fractional anisotropy and their relationship to neurobiological lesioning (Westlye LT et al, 2009). These microstructural measures were then compared with measures from structural T1-weighted and T2-weighted MRI. We show how the various image modalities can highlight various tissue properties, and we are thereby able to perform a broad characterization of the brain tissue in vivo. Understanding of these modalities is used to explore research on the topics of healthy aging, brain maturation, and white matter tractography are presented and discussed (Watanabe, 2012; Mukherjee, 2006; Nossin-Manor, 2013).