Poster # 2016.605

Miriam Hood* (Brandeis / Biochemistry)

Investigating Active Site Dynamics of Apicomplexans Malate Dehydrogenase by Nuclear Magnetic Resonance

Miriam Hood*, Jacob Wirth, Michelle Fry, Jeffrey Boucher, Brian Beckett, and Douglas L. Theobald

A variety of human diseases including Malaria are caused by unicellular eukaryotes of the genus Apicomplexa, which have evolved highly specific lactate dehydrogenase (LDH) from malate dehydrogenase (MDH). The importance of LDH to the parasitic life cycles of modern Apicomplexans makes it a major drug target. The presence of a promiscuous intermediate in the evolutionary path means the Apicomplexan LDHs and MDHs are an excellent model for studying the role of promiscuous intermediates in the evolution of specificity. The two enzymes share structural and mechanistic similarities but differ in substrate specificity. A six amino acid insert is responsible for the development of pyruvate activity in LDHs. There are two hypothesized conformations for the active site of an ancestor with the six amino acid insert. An LDH conformation of the ancestor in the presence of pyruvate is seen in X-ray crystallography, but an MDH conformation is not seen in the presence of oxaloacetate. Heteronuclear single quantum coherence nuclear magnetic resonance (HSQC-NMR) has been used to visualize the enzyme in the presence of oxaloacetate but was unsuccessful due to the size of the enzyme. Disruptive mutations have been used to create a stable dimer, small enough to produce a well resolved HSQC NMR spectrum. The dimer has been successfully expressed in rich, minimal, and N^{15} labeled media. Purification and steady state kinetic assays of the dimer with both pyruvate and oxaloacetate confirm the bifunctionality of the enzyme and HSQC NMR of the dimer with oxaloacetate is well resolved.

Support: Division of Science Summer Research Fellowship

Poster # 2016.606

Tamar Parmet* (Brandeis / Psychology)

The Neurobiology of the Impact of Innocuous Experience on Later Learning

Tamar Parmet*, Veronica L. Flores, Donald B. Katz

Conditioned taste aversion (CTA) is a simple adaptive form of animal learning whereby a food that causes gastric distress is rendered aversive. Previous research done in the Katz Lab has suggested that experience with salty and sour tastes can alter a rat’s ability to learn an aversion to sweetness; that is to say that experience with multiple flavors strengthens the rat’s conditioned taste aversion to sucrose.

The present study begins our inquiry into the neurobiological underpinnings of this phenomenon. Using c-FOS, a protein byproduct expressed by recently active neurons, I have focused on the gustatory cortex (GC)-a region known to be involved in taste learning. Based on previous studies showing an increase in c-FOS labeling with learning, we hypothesized a difference in the increase in c-FOS expression in GC for rats with prior taste experience. It was found that rats who had prior experience demonstrated less c-FOS. Further investigation showed this result differed for different sub-regions of GC. Specifically, learning in rats that were not pre-exposed to a taste array involved larger increases in c-Fos towards the posterior section of GC, whereas rats that were pre-exposed showed equal amounts of c-FOS across GC. This led us to conclude that taste experience changes the way that the gustatory cortex processes novel tastes.

Support: Bauer Fellowship

Asteisks in author list indicate student or students presenting the poster.
**Poster # 2016.607**

**Nicholas G. Martinez** (University of Kansas / Biochemistry)

**Generation of Catalytically Inactive Molecular Chaperone Hsp90 for Conformational Studies**

Nicholas G. Martinez*, Bin Huang, Timothy O. Street

Heat shock protein 90 (Hsp90) is a ubiquitous dimeric molecular chaperone that is crucial for helping other proteins fold properly. Four different Hsp90 proteins are expressed in mammalian cells: Hsp90a and Hsp90b in the cytosol; Trap-1 in mitochondria; and Grp94 in the endoplasmic reticulum. All Hsp90 proteins require ATP to perform their biological function. ATP hydrolysis drives an essential open-to-closed conformational cycle of the chaperone, however for Grp94, the closure conformational change has been challenging to observe in solution. The Street lab has developed a FRET assay to monitor the rate of ATP-driven closure of Grp94. Intriguingly, Grp94 closure rate is faster in the presence of ATP relative to the rate measured with a non-hydrolysable analog, AMPPNP. This discrepancy could be due to an acceleration of closure from ATP hydrolysis, however we have found that commercial AMPPNP stocks are contaminated with a hydrolyzed product that could be slowing the closure rate. To discriminate between these possibilities, I have introduced a catalytically inactivating substitution mutation in Grp94 (N48CE33A) that allows for ATP binding but eliminates hydrolysis. The objective of my project is to purify this inactive mutant, label it with donor and acceptor fluorophores and study its ATP-driven closure rate by FRET.

**Support:** Cell and Molecular Visualization REU

**Poster # 2016.608**

**Avinoam Singer** (Brandeis / Biochemistry)

**Elucidating the structural basis by which substrate proteins activate Hsp90**

Avinoam Singer*, Timothy Street

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. Mutants with higher basal ATPase rates, and therefore a higher population of the closed state, exhibited a stronger affinity for L2, indicating a preferential binding to the closed state of the chaperone. Furthermore, the mutant R74A exhibited increased affinity for L2 and higher fold activation, while the mutants W191A, W224A, and D217A exhibited decreased affinity and lower fold activation. Together, these four residues appear to compose a patch along the N-domain that may constitute the secondary binding site on HtpG.
Come to Cobalt: Synthesis and Characterization of Novel Cobalt-SNS Complexes for H2 Fuel Catalysis

Jeremy Koob*, Christine M. Thomas§, Cassandra Hayes¥, Uttam Das†, R. T. Baker
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Catalyst development for acceptorless dehydrogenation of ammonia borane (NH3BH3) looks toward a future using hydrogen gas as fuel, upon catalytic release from hydrogen-dense solid molecules. Initial results from the synthesis of a novel family of catalysts indicate several prospective CoI complexes using different sulfur-nitrogen-sulfur (SNS) ligands. Synthesis of a thiolate SNS heterocyclic ligand is shown, and its subsequent ring-opening via coordination to lithium has been achieved with a crystallographic structure shown here of the lithium complex. In addition, cobalt complexes using amido and thiolato SNS ligands are proposed, with initial characterization via NMR spectroscopy. These complexes are useful because the SNS ligands can act cooperatively with the metal center as an inner sphere base, and furthermore the ligands are hemilabile, with the potential of promoting 2e⁻ transitions in catalytic cycles. Synthesis and characterization of this compound in catalytic cycles will provide one of the first examples of a Cobalt-SNS catalyst for NH3BH3 dehydrogenation, and will also yield mechanistic insight into dehydrogenation catalysis.

Support: Division of Science Summer Research Fellowship

Activity of c-Src and Sortase-ligated models

Sara Gelles-Watnick, Chris Wilson, Yizhi Sun, Dorothee Kern

Tyrosine kinase c-Src is a ubiquitously expressed proto-oncogene, implicated in many cancers. The Kern Lab uses NMR to study c-Src's regulation and catalysis mechanisms. A partially labeled version of Full-length Src was needed for NMR studies. Two potential models (Back-ligated Src 1 and Back-ligated Src 2) were developed using a Sortase ligation reaction. By an ATP-NADH Coupled Activity Assay, BL Src 1 was determined to have activity most similar to Full-length Src. Therefore, BL Src 1 will be used in future NMR studies.

Support: Provost's Undergraduate Research Fund
Spatio-temporal receptive field structure and modeling the development of cortical direction selectivity

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

Direction-selective neurons in ferret primary visual cortex (V1) receive synaptic inputs from different spatial locations that vary systematically in response latency, thus leading to spatio-temporal receptive fields (STRF) that are oriented in space-time. Direction selectivity (DS), and this slanted STRF structure, require visual experience to develop. The shape of the STRFs in visually naïve ferrets and the plasticity mechanisms that transform these premature STRFs into the slanted structure remain unknown. We performed intracellular recordings from V1 simple cells in younger and older ferrets, and using white-noise reverse correlation analysis computed their STRFs. The STRFs of DS V1 neurons in older ferrets exhibited slanted structure as expected, while the non-DS cells in younger ferrets revealed strong localized inputs that were not staggered in time. Using this information regarding input patterns in younger ferrets, a feedforward neural network model of V1 inputs was constructed, and the effect of visual experience on the network was simulated by activating it with sweeps of a white bar moving either in one direction (uni-directional training) or two opposing directions (bi-directional training). A network that started with one strong input, and no restrictions on growth of synaptic inputs, did not increase DS following uni- or bi-directional training. However, when we constrained the inputs to grow along one diagonal only, thus assuming an internal structural bias in the network, both uni- and bi-directional training led to increases in DS that are consistent with experimental data. In the future, we intend to expand the size and complexity of the network to better understand the mechanisms involved in DS development.

Support: Computational Neuroscience Traineeship

Elucidating an Inhibitor Binding Mode in Acinetobacter baumannii IMP Dehydrogenase

Alex Cuadros*, Masha Rosenberg, Lizbeth Hedstrom

One of the most pressing public health issues in society today is the alarming rise in antibiotic resistance. Acinetobacter baumannii is a bacterial pathogen prone to high rates of resistance and is responsible for widespread nosocomial infections. To combat such infections, we study the inosine-5’-monophosphate dehydrogenase (IMPDH) enzyme as a promising target for novel antibiotics. IMPDH is the enzyme responsible for catalyzing the oxidation of inosine-5’-monophosphate (IMP) to xanthosine-5’-monophosphate (XMP), the most critical step in synthesizing guanine nucleotides. Cell proliferation is heavily reliant on the guanine nucleotide pool so inhibiting IMPDH activity could provide antibacterial and cancer therapeutics. It is important to note that even though IMPDHs have very similar structures, the potencies of our inhibitors vary greatly for different enzyme orthologs. To probe the mechanism for inhibitor selectivity, we created a mutant form of A. baumannii ATCC 17978 IMPDH that contained a deletion on the mobile loop motif near the enzyme active site. This mutation removed two residues not present in most other IMPDH orthologs, despite them being contained in a highly conserved area of the active site. Since this unique motif is critical for hydrolysis of the product, XMP, we predicted that the mutated residues were interacting with our inhibitors in some capacity. The mutation resulted in a 5-fold increase in inhibitor potency compared to wild type for inhibitor P19. This confirmed a structural determinant for inhibitor selectivity. Interestingly, the mutant form showed no significant difference in potency with respect to inhibitor P24, which only differs from P19 by containing a carbonyl group rather than a vinyl. To characterize this difference in inhibitor potency, we performed a series of steady-state kinetic experiments on the wild type and mutant enzymes with inhibitors P19 and P24. By measuring the rate of NADH coproduct formation, we hope to characterize which enzyme intermediate each inhibitor has a higher affinity for. Future directions for this project include performing stopped-flow kinetic assays on the mutant enzyme to extrapolate the mechanism of inhibitor binding. We would also like to further characterize the difference in potency between a carbonyl carbon on inhibitor P24 versus the vinyl carbon of P19.

Support: Cell and Molecular Visualization REU
**Poster # 2016.615**

**Sabrina McDonnell** (Brandeis / Biochemistry)

**Exploring Interactions Between IMPDH, RNA Polymerase, and Ribosome in *Escherichia coli***

Sabrina McDonnell, Deviprasad Gollapalli, Vincent Sutera, Lizbeth Hedstrom

Inosine 5’-monophosphate dehydrogenase (IMPDH) converts inosine 5’-monophosphate (IMP) to xanthosine 5’-monophosphate (XMP) as a part of the de novo purine biosynthesis pathway. Overexpression of the protein is linked to the proliferation of diseased cells, and inhibitors of IMPDH have been used as anticancer, antiviral, and immunosuppression treatments. Previous research in the Hedstrom Lab suggests that IMPDH interacts with both RNA Polymerase Beta (RNAPB) and ribosome implying that IMPDH may be involved in the regulation of both transcription and translation. Chromosomal mutations and affinity purification were used test for the presence of these interactions. Following affinity purification of IMPDH from wild type *E. coli* and a mock purification from IMPDH null (*ΔguaB*), lysates were compared by mass spectrometry. Both samples contained RNAPB and Ribosome indicating that both proteins bind to the column used for purification. In subsequent experiments, immunoprecipitation with RNAPB antibodies and ribosome antibodies, as well as a polysome analysis, will be used to probe for possible interactions with IMPDH. Tagged chromosomal mutants will also be created to determine if RNAPB and ribosome will come down with IMPDH on different affinity columns.

**Support:** Division of Science Summer Research Fellowship

**Poster # 2016.616**

**Erin Evonne Jean** (Hampton University / Cellular Molecular Biology)

**Understanding the Role of Coactosin in Actin Dynamics**

Erin Evonne Jean, Adam Johnston, Izarys Rivera, Bruce Goode

A fundamental feature of the actin cytoskeleton is its ability to be rapidly assembled and disassembled in cells by the action of a variety of actin-interacting proteins. One family of proteins that are particularly central to the regulation of actin disassembly are the ADF-H (actin disassembly factor homology) domain proteins, which includes Cofilin, an actin-severing factor, and Twinfilin, which accelerates filament end depolymerization. Coactosin, a small protein composed of a single ADF-H domain, is the least well-studied member of the ADF-H protein family. Although it shares close structural homology with Cofilin and other ADF-H domain proteins, its role in actin dynamics is poorly understood. Here, I test the effects of Coactosin on its own and in combination with other disassembly factors in-vitro. A fluorescently-labeled Coactosin will be generated as a tool to help us to understand how Coactosin contributes to actin turnover. In order to label Coactosin in a way that is least likely to affect the function of the protein, the endogenous cysteines will be mutated to serines. Based on previously identified binding sites on Cofilin, a cysteine will be introduced into Coactosin where it is least likely to disrupt these key surfaces. These mutations will result in a single cysteine on the Coactosin protein where the Cy3 dye will bind. TIRF microscopy will be used to visualize how Coactosin interacts with actin and other ADF-H domain proteins in order to better understand the mechanisms by which Coactosin affect actin dynamics.

**Support:** MRSEC REU
Guillermo Narváez-Paliza*, W. Benjamin Rogers

DNA provides a useful tool to control self-assembly of microparticles; not only does it induce self-assembly but it allows the user to specify the way particles interact and bind to each other. Here we study a DNA-coated colloidal system where two non-complementary sequences (A and B) are grafted to polystyrene spheres. A single stranded DNA (ssDNA) linker in solution is used to induce self-assembly. We measure the melting temperatures for a variety of linker lengths (17, 19, 21, and 23 bases in total length) and a range of linker concentrations (1nM–0.8mM). We show that the melting temperature of the material depends on both the length of the linker and the concentration within the sample. A mathematical model is built, assuming local chemical equilibrium and using principles of mass-action, that predicts the yield of aggregation of the system, allowing us to calculate the melting temperature. The use of linkers brings along multiple advantages that direct-hybridization based micromaterials lack, and new characteristics such as a fluid phase re-entry when increasing the concentration of linkers past a threshold concentration. Linker-mediated systems provide a useful tool for the research of biological and synthetic self-assembly processes, as well as having important potential engineering applications.

Support: SMURF (Summer MRSEC Undergrad Research Fellowship)

Adrianna Shy*, Jie Li, Bing Xu

The chemical interactions in a protein is key to understanding its secondary structure. Hydrogen interactions between amino acids in adjacent chains form beta-pleated sheets. 1-pyrenebutyric acid promotes the self-assembly of these small molecules. In this study, focus is placed on the interactions between small peptide chains isolated from the hormone, irisin. We hypothesize that the morphology of a peptide mixture will yield more interactions than a single peptide alone. We also explored recreating the β-sheet structure displayed by irisin. Solid-Phase Peptide Synthesis was used to synthesize the peptides: IQEVN and RMLRF alone, as well as RMLRF, IQEVN and VIGFA with 1-pyrenebutyric acid (Py) attached. Hydrogels were formed, and various techniques such as NMR, TEM, and circular dichroism were used to confirm self-assembly and analyze their β-sheet structure. Results reveal that Py-IQEVN and Py-RMLRF self-assemble on their own. A mixture of these two also show that a β-sheet-like structure was created. The hydrophobicity of Py-VIGFA does not allow hydrogelation, therefore it was not analyzed. However, MTT assays show Py-VIGFA along with Py-RMLRF and Py-IQEVN are toxic to cancer cells at high concentrations. Overall, more interactions happen between RMLRF and IQEVN when 1-pyrenebutyric acid is attached, while single peptides without this molecule show no type of self-assembly. In the future, the morphology of D-peptide molecules will be explored to mimic the L-peptide interactions.

Support: MRSEC REU
Emergent Structures in Dense Active Fluids
Jianshuo Qiu*, Elias Putzig, Aparna Baskaran

Conventional fluid materials are composed of inanimate building blocks. Much current research deals with active fluids, which are composed of self-propelled particles and carry significantly different properties, such as energy consumption and collective motility. In this research, we study the emergent structures in active fluids at high densities in two dimensions. We hypothesize that at high densities, the emergent structures are composed of multiple asters with no disordered region between any two asters. The research is implemented through both numerical and analytical methods. In the numerical method, a FTCS (Forward-Time Central-Space) scheme is used to write the code and to numerically solve the dynamical equations that describe the density and the polarization density of the self-propelled particles. Codes are run on the High Performance Computing Cluster, with different values of parameters called the particle propulsion speed and the inter-particle interaction parameter. The results are used to make plots of the density and the polarization density. The emergent structures are then identified from the plots. In the analytic method, called linear stability analysis, the nonlinear dynamical equations are approximated as linear equations around the homogeneous steady state solution to the dynamical equations. The conditions when this state becomes unstable are obtained and used to explain the emergent structures found. Finally, we find the structure composed of multiple asters with some disordered region between them, and the empirical relations between aster number, aster radius and parameters are obtained. Other new structures, such as streams, and extended single aster are also found. An empirical phase diagram of the distribution of different structure in the parameter space is plotted. In future research, the reason why disordered region appears between multiple asters needs to be explained and other new emergent structures will be further studied.

Support: SMURF (Summer MRSEC Undergrad Research Fellowship)

Elastic Buckling of Rigid Bio-polymers
Margaret Morris*, Walter Schwenger, Feodor Hilitski, Zvonimir Dogic

Bio-polymers play an important role in many critical processes in organisms ranging from providing structural rigidity to motility. In this experiment we examine the buckling properties of rigid rod-like flagella. Experiments with rod-like bio-polymers like flagella and microtubules tend to assume these filaments behave as Eulerian rigid rods. Euler's theory predicts that as a rod is buckled, the force required to buckle it further increases as buckling becomes more extreme. In previous experiments, our lab has found that this is not the case with microtubules. Microtubules become easier to buckle when they are extremely bent. We now explore the buckling properties of flagella, which are made of microtubules, to see if they behave as microtubules or as Eulerian rods. We use laser tweezers to buckle flagella. We attach beads to either end of a straight flagellum taken from Salmonella, Then using optical tweezers, we compress the filament until it buckles. For small forces, bead displacement from a laser trap scales linearly with force, allowing us to find a relationship between applied force and degree of buckling. Our initial results suggest that force required to bend flagella increases with increased buckling. So, unlike microtubules, flagella follow the trend predicted by Euler's theory. We will continue testing this for different lengths of flagella. Because flagella behave as classical rods, our experiments will also allow us to determine persistence length of flagella through mechanical means.

Support: SMURF (Summer MRSEC Undergrad Research Fellowship)
Identifying Key Players in Growth Factor Signaling Defects in Amyotrophic Lateral Sclerosis

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

Amyotrophic Lateral Sclerosis is a neurodegenerative disease that leads to selective death of motor neurons. Mutations in the TAR DNA binding protein (TDP-43), which has thousands of targets in the cell, have been implicated in ALS. The current model of TDP-43 pathology shows that there is dysfunction both when endogenous TDP-43 is lost, as well as when there is a toxic gain of function. We created an ALS model in *Drosophila melanogaster* using both loss and gain of function. We found that TDP-43 misexpression results in premature death, larval motility defects, and decreased synaptic growth at the neuromuscular junction (NMJ). TDP-43 misexpression also causes reduced Bone Morphogenic Protein (BMP) signaling, which is important for synapse growth. We observed decreased levels of synaptic pMAD (the end product of the BMP pathway responsible for inducing transcription of the genes responsible for growth) although, surprisingly, nuclear pMAD was not affected. There was a shift in the BMP receptor thickveins (Tkv) from the early endosome which is signal permissive to the recycling endosome which is signal non-permissive. We then found that rerouting of BMP receptors by introducing a dominant negative Rab11 partially suppresses the synaptic growth, signaling, and crawling defects associated with TDP-43 misregulation. It however still remains unclear where in the pathway TDP-43 is acting, and which downstream targets are leading to neurodegeneration. Importins are nuclear import/export proteins that allow substrate entry into the nucleus and also may have synaptic functions. It has been previously shown that importin beta-11 mutants exhibit a similar loss of NMJ but not nuclear BMP signaling. Our current focus is whether or not importin beta-11 acts in the same pathway as TDP-43. We will also look at whether disabling retrograde traffic helps to rescue levels of synaptic pMAD in larvae that are misexpressing TDP-43.

**Support:** SMURF (Summer MRSEC Undergrad Research Fellowship)

Initially, we tried to use DNA-mediated transformation to test gene functions. Instead, we discovered an efficient gene-silencing mechanism. We used the \textit{ump} gene, which is involved in the synthesis of uracil and converts 5-fluoroorotic acid [FOA] into a toxin. When the \textit{ump} gene is silenced, the cells can grow in the presence of FOA, allowing for selection of “transformed” cells. Introduction of a \textit{ump} gene with engineered deletions causes highly efficient FOA-resistance, absence of \textit{ump} RNA, and lack of UMP synthase enzyme activity. Surprisingly, the wildtype \textit{ump} gene sequence remained unchanged in the FOA-resistant cells. Introducing the wildtype \textit{ump} gene also induced FOA-resistance. Preliminary experiments suggested that invading homologous DNA causes silencing through DNA methylation. Bisulfite sequencing revealed extensive methylation of the \textit{ump} gene in FOA-resistant cells, and none in control cells that had not been exposed to the \textit{ump} DNA. Bisulfite sequencing results revealed an unusual type of methylation, non-CpG, in which bases other than guanine follow methylated cytosines. In mammals, non-CpG methylation is associated with stem cells and adult neural cells, but its function remains unknown. We repeated silencing using the wildtype \textit{ump} gene, and found significantly more methylation in the treated cells than in control cells that had been electroporated without DNA. The majority of the methylation was again non-CpG. To test if the invading DNA silences only homologous DNA, we looked at a gene involved in flagellar motility, \textit{cam1} [calmodulin-1], and found no methylation. We also tested if 500-nt PCR segments of the \textit{ump} gene will silence the entire gene. We found that the PCR segment near the beginning of the gene caused silencing, while the segment near the end did not. In addition, we grew FOA-resistant “transformants” in 5-azacytidine [AzaC], which demethylates cytosine. The AzaC treatment caused the cells to lose their FOA-resistance. DNA samples from all these trials have been analyzed using a new method of sequencing being developed at NEB, Apobec sequencing, which offers a more rigorous analysis than bisulfite sequencing. Future experiments include testing if another invading \textit{Naegleria} gene will silence its endogenous homologous gene. 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

Angelina Gallego*, Gael Prado, W. Benjamin Rogers

Every cell in the human body contains a membrane that separates inside from outside. The cellular membrane also executes a wide variety of other notable functions, from transport of nutrients to sensing and responding to the outside environment. These functions are carried out by membrane proteins, which cannot act alone and need to organize into intricate structures to function. However, the process by which membrane proteins self-assemble remains unknown. We have made progress developing a model system to study self-assembly of membrane bound objects. We use an emulsion of water and oil stabilized by phospholipids as our model ‘cell,’ and polystyrene colloids—micron sized plastic beads—as our model ‘proteins.’ Unlike real proteins, the colloids are large enough to be imaged with an optical microscope, yet small enough to still exhibit Brownian motion. Lastly, we use DNA hybridization to induce attractive interactions between the particles and the membrane: DNA strands grafted to the particles can bind to complementary DNA strands inserted in the membrane. This results in an adhesive interaction that is chemically specific and tunable: by changing the temperature only a few degrees, we can switch the interaction from very weak to effectively irreversible. Preliminary experiments suggest that we can organize particles on a model membrane and we anticipate that our unique approach might ultimately help shed light on how objects organize on cell surfaces.

Support: MRSEC REU
Poster # 2016.624

**Remi Boros** (Brandeis / Physics)

**Driving Chaos Off the Grid - Analyzing Networks in Microfluidic Lattices of Chemical Oscillators**

Rémi Boros*, Thomas Litschel, Mike Norton, Ian Hunter, Seth Fraden

In nature, living organisms often synchronize to form large, coherent structures. While this synchronous coupling is essential to life as we know it, it remains exceedingly difficult to control. Controlling synchronizations requires the direct manipulation of individual organisms, something that is currently unfeasible on a large scale. To better study large-scale synchronizations, our lab constructs lattices of nanoliter-scale PDMS wells filled with the chemically oscillating Belousov-Zhabotinsky reaction. Depending on their architectures, these lattices can induce different oscillatory patterns between individual wells and their neighbors. With the addition of a photosensitive catalyst, the BZ reaction becomes light sensitive, making it possible to inhibit wells individually when exposed to blue light. Using this technique, one can influence large scale synchronous behaviors on these lattices with relative ease.

**Support:** Bauer Fellowship

Poster # 2016.625

**Sarah Lipitz** (Brandeis / Neuroscience, Psychology)

**Effects of Age on Recruiting the Medial Prefrontal Cortex During Self-Referential Encoding**

Sarah Lipitz*, Angela Gutchess

The medial prefrontal cortex (mPFC) is recruited during self-referencing, a cognitive process in which information is related to oneself. In one reported fMRI experiment, younger and older adults were asked to rate adjectives for how well they described themselves; after a 30 minute delay, participants completed a surprise recognition task probing for those adjectives. The results of this study suggest that mPFC recruitment is associated with successful encoding of self-referential memories in both age groups. However, focal differences in neural activation seem to arise between younger and older adults, where younger adults recruit regions involved in emotional processing while older adults recruit regions involved in cognitive control. The present study aims to further understand the effect of age on neural activation during successful self-referential encoding by using more nuanced analyses such as functional connectivity and multivoxel pattern analysis, or MVPA. Preliminary analyses reveal that both younger and older adults recruit mPFC when encoding adjectives later successfully recognized, yet older adults recruit regions associated with cognitive control such as middle frontal cortex and superior prefrontal cortex. Future connectivity analyses will aim to detect regions functionally linked to mPFC recruitment for younger versus older adults, while MVPA analyses will aim to predict age group and remembered versus forgotten trials based on voxel-specific patterns of neural activation.

**Support:** Bauer Fellowship
**Poster # 2016.626**

**Niya Wilkins** (Hampton University / Environmental Science)

**Making Double Emulsions in Thermoplastic Microfluidic Devices**

Niya Wilkins*, Achini Opathalage

Microfluidics or Lab-On-Chip, is a technology that uses nanoliter to picoliter volumes to manipulate fluids. In this study, our focus is to make a robust double emulsion; water/oil/water and oil/water/oil using thermoplastic microfluidic devices. Double emulsion systems are mainly used in pharmaceutical industry for drug delivery and also for encapsulating cells and proteins. Our approach is to use Cyclic Olefin Copolymer (COC) as the thermoplastic material to make the microfluidics device because of its lower manufacturing costs, improved solvent resistance and ease of surface treatments. We report a method of making masters, creating replicas, sealing devices and modifying the surface of COC microfluidic devices. We melt the COC pallets on to a PDMS master to replicate the drop making design, perform a solvent based sealing and bind thick PDMS sheets using silane chemical coupling for the interconnects. We have made water drops in oil and oil drops in water in hydrogenated and fluorinated oil/surfactant based systems, with and without surface modifications to COC. This study continues in developing a robust double emulsion system in fluorinated oil based systems.

**Support:** MRSEC REU, Nano HU Program

**Poster # 2016.627**

**Angela Berry** (Hampton University / Pre-Pharmacy)

**The Nanometer-Scale Stepping Behaviors of Kinesin 401 and Kinesin 365**

Angela Berry*, Kun-Ta Wu, Zvonimir Dogic

Kinesins are molecular motor proteins that move along microtubules in eukaryotic cells. Kinesin 401 is a processive molecular motor that has two legs walking on a microtubule, whereas Kinesin 365 is a non-processive motor that has one leg, therefore hopping on a microtubule. When assembled into kinesin clusters, these motors convert ATP into ADP, driving microtubule network. Kinesins promote intracellular activity, but the role of their nano-scale stepping behaviors in micron-scale network activity remains unclear. Here, we synthesize an active gel, a network comprised of crosslinked microtubules driven by molecular motor clusters. The clusters are comprised of kinesin motors crosslinked by streptavidin. We measured the network activity driven by kinesins 401 and 365. Kinesin 365 reduces the molecular crowding of kinesin motors and microtubules. We found that kinesin 365 enhanced network activity, implying that non-processive motors drive the gel network more efficiently. Our finding not only paves the way to outlining fundamental stepping behaviors of non-processive motors, but also sheds light on designing molecular motors that promote intracellular activity.

**Support:** MRSEC REU
The Role of PlexinB Receptors in Synapse Formation

Dena Goldblatt*, Jacqueline E. McDermott, Suzanne Paradis

Proper cell-to-cell communication and circuit function in the brain depends on the establishment of neuronal connections, or synapses. Since the brain is composed of both excitatory and inhibitory synaptic connections, a specific excitation/inhibition (E/I) balance is required for proper nervous system function. Disruptions to this E/I balance are associated with diseases such as epilepsy, autism spectrum disorders, and schizophrenia. Previously, our lab has shown that the molecule Semaphorin4D (Sema4D) is required for inhibitory synapse formation and signals through the PlexinB1 receptor in hippocampal rat neurons. However, the role of the PlexinB1 and PlexinB2 receptors in excitatory synapse formation is unknown, as well as the identity of the Semaphorin ligand(s) which signal through PlexinB2 to mediate synapse formation. Here, we show that PlexinB2, but not PlexinB1, is required for proper inhibitory synapse formation, while neither receptor is required for excitatory synapse formation. Next, we tested possible PlexinB2 ligands in Cos7 cell binding and collapse assays. The binding intensity of the putative PlexinB2 ligands Sema4C, Sema4D, and Sema4G is significantly increased compared to control cells. Furthermore, PlexinB2 mediated cell collapse increased with treatments of Sema4C, Sema4D, or Sema4G ligands compared to a control treatment. Taken together, we show that PlexinB1 is not required for either inhibitory or excitatory synapse formation, while PlexinB2 is uniquely required for inhibitory synapse development. Additionally, Sema4C, Sema4D, and Sema4G bind and signal through the PlexinB2 receptor to promote cos7 cell collapse, and are potential ligands for PlexinB2 in neurons. Future experiments will aim to determine the role of Sema4C and Sema4G in synapse development, and whether they signal through PlexinB2 to do so.

Support: Alt Fellow

Poster # 2016.629

Senmiao Sun (Brandeis / Biochemistry, Neuroscience)

Essential phenylalanine box in a unique fluoride ion channel (Fluc)

Senmiao Sun*, Nicholas B. Last and Christopher Miller

The Fluc family of F\textsuperscript{−} ion channels, built as dual-topology antiparallel dimers, provides an efflux pathway for microorganisms to deal with the toxicity of environmental F\textsuperscript{−}. Recent studies of their structures have discovered two conserved phenylalanine residues forming a central “Phe-box”, an aromatic-halide coordination motif never before seen in proteins. Understanding the chemical mechanism of this aromatic-anion coordination geometry is essential for understanding the F\textsuperscript{−} pathway and the channels high selectivity for F\textsuperscript{−} over Cl\textsuperscript{−}. Here several mutations of each phenylalanine residue have been made and the function of those mutants has been tested. A wide variety of amino acids, including polar, nonpolar and aromatic side chains have been tried. Almost all of them cause complete loss of function of the Fluc, even the structurally similar tyrosine mutant. However, surprisingly, the methionine mutant retains the ability to transport F\textsuperscript{−}. Crystallization and single channel measurements will be carried out to gain further insights into the effect of both the functional and non-functional constructs.

Support: HHMI
Micah Margolis*, David Roberts

From past work, the radio galaxy 3C 219 was hypothesized to contain traces of star formation, due to its characterization as a restarting galaxy. Data cubes (matrices with a third dimension), which are comprised of a series of images of the galaxy at different wavelengths, were created by Weil (2014) from data originally taken by the Gemini North 8-Meter Telescope in March 2005. Each image contained 68 x 49 square spatial pixels over a wavelength range of either 2142 or 1911 spectral pixels depending on the observation, forming a data cube of over 7 million pixels. The Active Galactic Nucleus (AGN) was subtracted away from each original cube by modeling the AGN with a Moffat distribution (a variation of the Normal distribution), creating residual data cubes. Principal Component Analysis (PCA) was then performed on the original and residual data cubes, producing tomograms and eigenspectra that would then be analyzed for signs of star formation. Test cases, or synthetic data cubes, were created to try these methods, with success. However, the results of these methods on the real data from the six observations of 3C 219 have yet to show signs of star formation in the galaxy.

Support: Physics Department
Neural circuit control of physiological homeostasis
Abby Daniels*, Belinda Barbagallo, Paul Garrity

Thermotolerance is essential for maintaining the homeostasis of an organism by allowing it to withstand a range of temperatures. This is especially important for insects due to their inability to regulate body temperature. Work from the Garrity lab has shown that a neural circuit mediates physiology in response to cold exposure in *D. melanogaster*. One target tissue of this pathway is the heart, which slows down as temperature decreases. To explore this connection further, we investigated whether all components of the circuit were necessary to maintain heart rate and explored the effects of the dopamine inhibiting drug 3-iodotyrosine (3-IY) on cold tolerance. We found that when a part of the circuit was eliminated the animals' heart rates were lower than those of the control animals. However, the 3-IY trials did not share this phenotype although treated animals' chill coma percentages did not differ from the controls. When mutants were shifted from 18°C to 25°C for an hour, a high chill coma phenotype and low heart rates relative to the controls were observed and the opposite was true for 25°C to 18°C. These observations support the proposed cold processing circuit and suggest that functions acutely to maintain physiological function during cold exposure.

Examining the physiological changes of pupil dilation with increasing complexity of auditory stimuli
Austin Luor*, Eriko Atagi, Arthur Wingfield

Changes to the autonomic nervous system are an inevitable part of aging. One such change is that the older adults' pupillary response is more limited in range of dilation (i.e the reduction of the size of the pupils of the eyes to ambient light) than those of young adults, known as senile miosis (Piquado et al., 2010; Van Gerven et al., 2004). Young and older adults also differ in the velocity of the eye to reach maximum contraction and to return to baseline after offset of a light stimulus (Bitsios et al., 1996). Interestingly, changes in pupil size also correlate with perceptual and cognitive effort—the greater the effort expended, the larger the pupil size. Thus pupillometry has been used as a measure of cognitive effort (e.g. Kuchinsky et al., 2013). However, the impact of aging on the physiological pupillary response and cognitive effort has received little attention. This study aims to investigate the relationship between the pupillary reaction to auditory stimuli; ranging from tones, to words, to more effortful resolution of full sentences, to the velocity to peak and peak-size as pupillary changes to those elicited by changes in light intensity. In sum, this study examines differences in pupil measurement parameters elicited by a range of complexity differences of auditory stimuli as well as any differences between younger and older adult listeners.

Support: Provost's Undergraduate Research Fund
Screening for genes involved in repairing QP mutations by template switching

Kati Vu*, Julie Klaric, Stephen Gross, Susan Lovett

Genome stability is critical for life since DNA mutations can result in disease or cell death. A quasi-palindrome (QP) is an imperfect, inverted repeat and a mutational hotspot found within many organisms, including *E. coli*. QP mutations are known to occur by nascent strand misalignment or template switching. To further expand our knowledge about template-switch events within QP’s, we used the QP5 and QP6 reporters to conduct a forward genetic screen in *E. coli*. Since QP5 and QP6 reporters only report template switch events, our screen identifies genes involved in template-switch events within QPs. Thus far, I have identified the following two genes in the QP5 strain: *potE* and a glucosyltransferase gene. In the QP6 strain, I have identified *cyaA* gene, *yedZ*, and an unknown gene involved in sugar transport. Unfortunately, the genes I have classified in my screen recently are not relevant to template-switch events, but I am currently screening more genes. When the summer is over, the Lovett lab will continue to screen thousands of mutants to identify more genes involved in stimulating and repressing template-switch events within QPs. To increase our understanding of the mechanism of template-switch events at QP sites, we hope to find more proteins involved in these mutational events.

Support: Cell and Molecular Visualization REU

Enzyme-Regulated Self Assembly of Cholesterol Conjugates Against Drug-Resistant Ovarian Cancer Cells

Huaimin Wang, Zhaoqianqi Feng, Dongdong Wu, Mike Rigney, Jie Zhou, Yujie Jiang*, Bing Xu

Ovarian cancer treatment is challenging because tumors are usually found late and a high proportion develops resistance to platinum-based chemotherapy.1 Here we report the use of cholesterol conjugates to selectively kill cancer cells, including platinum-resistant human ovarian carcinoma cell lines (A2780cis). The cholesterol conjugates exhibit greater potency and selectivity than cisplatin against A2780cis in cell assay. D-tyrosine cholesterol conjugate (1b) is generated through enzymatic dephosphorylation of D-phosphotyrosine cholesterol precursor (1a). Nanoscale self-assembly of 1a in-situ on the surface and inside the cancer cell triggers extrinsic and intrinsic cell death signaling, inducing cell death. In addition to interacting with actin filaments and microtubules during cell death, the assembly of 1b poses microheterogeneity in cell membrane. Our work demonstrates a promising approach to overcome the drug-resistance barrier in chemotherapy by adopting EISA to control the nanoscale self-assembly of the cholesterol conjugates.

Homeostatic changes in gene expression and axonal morphology in response to network activity deprivation

Robin Schectman*, Vera Valakh, Sacha Nelson

Epilepsy is a neurological disorder correlated with an overall increase in network excitability, but its underlying mechanisms are not well-understood. We are using a mouse cortical slice culture model to explore underlying causes of hyperexcitability that potentially contribute to epileptogenesis. By creating a prolonged activity blockade with voltage-gated sodium channel blocker tetrodotoxin (TTX) we can induce seizure-like activity in the network. One of the biophysical changes that occurs is a decrease in excitatory synaptic input onto inhibitory interneurons. We investigated whether the ErbB4 pathway is affected by prolonged activity withdrawal. We see that although there is no change in mRNA levels of ErbB4, there is a significant decrease at the protein level in TTX-treated slices. In the future, we will investigate whether this is the only pathway responsible for the excitatory synaptic changes onto inhibitory neurons that we see and further study these and other potential mechanisms that contribute to epileptogenesis.
Predominant Algorithm for Computationally Modeling Single-Neuron Morphology Shown Insufficient in the Stomatogastric Ganglion

The 26 neurons that compose the stomatogastric ganglion (STG) of the crab, Cancer borealis, are among the most deeply studied and best understood models for neuronal circuitry. When trying to decipher the vastness of such circuits, it is essential to understand the physical organization of each cell; however, there has hitherto been little success in objectively capturing the optimal course of development for any single neuron. In a 2010 paper entitled "One Rule to Grow Them All: A General Theory of Neuronal Branching and Its Practical Application," building upon the morphological doctrine of famed neuroanatomist Ramón y Cajal, a method was proposed to calculate a so-called “balancing factor”—a measure of morphologic favorability based on two things: conservation of resources, or cytoplasmic investment, and electrotonic efficiency, or conduction time (Cuntz et al., 2010). To investigate this method, the cell bodies of individually-extracted STG neurons were injected with Lucifer Yellow fluorescent dye and then treated with paraformaldehyde before confocal microscopy generated several hundred cross-sections of each cell. These cross-sections were then consolidated using KNOSSOS 3D imaging, which enabled for manual tracing of individual cells’ dendrites and deep computational analysis of their morphological properties using a combination of independently developed Python applications and the in-house "Quantifying Morphology" software suite developed by Alexander Sutton and Ted Brookings. As the title of the aforementioned paper suggests, the concept of balancing factor was incorporated into a greater computational algorithm for simulating the optimal growth of all neurons, and this algorithm lie at the core of Hermann Cuntz’s TREES toolbox, a MATLAB software suite for generating what are supposed to be realistic and accurate models of single neurons. Using data from a deeply analyzed gastric mill neuron (GM) and a similarly analyzed lateral gastric neuron (LG), several artificial neurons were generated using the TREES toolbox, capturing the entire range of balancing factors. Despite the claims made by Hermann Cuntz and his co-authors, striking dissimilarities exist between the real neurons of the STG and their artificial counterparts at all balancing factors. This apparent disparity suggests that perhaps current methods for simulating neuronal morphology remain inadequate in the characterization of all neurons, particularly those in the STG of Cancer borealis.

Support: QBReC
Let-7 is required for development, survival, and fertility in *Drosophila*

Katherine Dorfman *, Patricia R. Goodwin, Leslie C. Griffith

The microRNA let-7 is highly conserved and is known to play important roles in development, cell proliferation, differentiation, motility, survival, fertility, and cancer. However, previous experiments aimed at testing the role of let-7 have deleted the whole let-7 complex, including let-7, mir-100, and mir-125. The mutation that we used, *let-7*2, on the other hand, is a 23 base pair deletion specifically in the *let-7* locus created by CRISPR (and verified by DNA sequencing), which gives us a much better sense of the role of let-7 in particular. For our experiments, we tested the role of let-7 in development, survival, fertility, and locomotion. To test for development, crosses of *let-7*2/CyO and +/CyO using female virgins and males were set up at 18°C, 25°C, and 29°C, and emerging flies were genotyped based on their wings. We found that significantly fewer homozygote *let-7*2 mutants emerged, which implies that let-7 is required for development. For the survival experiment, *let-7*2 homozygotes and control *w*CS flies were collected from crosses set up at 25°C and placed in vials with up to 10 other flies into incubators set to 18°C, 25°C, and 29°C, and surviving flies were counted at least 6 days a week. We found that *let-7*2 homozygotes died sooner than controls, showing that let-7 is important for survival. We also found that the *let-7* deletion had a larger effect on male survival, especially at 29°C, showing that it may affect the two sexes differently. To test for fertility, crosses were set up at 25°C with virgin females and males, and were monitored to see whether new flies hatched. Crosses with *let-7*2 homozygotes had fewer or no progeny, which shows that let-7 is important for fertility. For locomotion testing, *let-7*2 and *w*CS control males and females were monitored in a Drosophila Activity Monitor (Trikentics), and the number of beam breaks per minute awake was recorded. We found that there was no significant difference in the number of beam breaks, showing that let-7 does not play a role in locomotion. Lastly, we tested to see whether enriched food (Nutri-Fly GF) would improve survival and development. We found that the enriched food did not improve the survivorship and the eclosion rate of flies in *let-7*2 and control homozygous flies. In conclusion, we found that let-7 is important for development, adult survival, and fertility, but not for locomotion.

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**Poster # 2016.639**

**Megan Leubner** (Brandeis / Neuroscience)

**The Molecular Basis of Memory: Calcium-calmodulin-dependent protein kinase II (CaMKII) is necessary for the maintenance of long-term potentiation and behavioral memory**

Megan Leubner*, Tom Rossetti, John Lisman

Long-term potentiation (LTP) is a leading hypothesis for the mechanism of memory and involves induction, maintenance and expression processes. The aim of this research project was to reveal the role of Calcium/Calmodulin-dependent protein kinase II (CaMKII) in the maintenance mechanisms of LTP. This aim was addressed through the use of an erasure test in vivo by applying a transient dominant-negative form of CaMKII (K42M) to the CA1 region of the rat hippocampus. K42M was applied after the rat was trained using conditioned place aversion, a hippocampal-dependent form of memory. Memory retention was then tested a week later. The rats injected with K42M entered the shock zone during retention testing more quickly and frequently as compared with the rats injected with GFP, indicating that this form of behavioral memory can be erased by this procedure. Rats from both groups were capable of relearning. This is the first demonstration of the reversal of memory.

**Support:** Bauer Fellowship
Semaphorin 4D as a Possible Treatment for Epilepsy

Keaton Unroe*, Daniel Acker, Suzanne Paradis

Epilepsy is a common neurological disorder characterized by seizures. The Paradis Lab was able to ameliorate seizures in mice in vivo by treating with the protein Semaphorin4D (Sema4D). We hypothesize that the effect of Sema4D on seizures is mediated by Sema4D’s activity as a fast-acting positive regulator of inhibitory, GABAergic synapse formation. However, questions remain about the long-term effects of modulating inhibitory synapse formation. These include the persistence of newly formed synapses. To address this question, we treated hippocampal cell cultures with Sema4D and assessed synapse density after zero or 48 hours. The results of this assay are so far inconclusive. Another question is whether Sema4D treatment negative behavioral side effects. To address this question, we treated mice with Sema4D by intracerebral infusion. We then tested the mice for increased anxiety using a common behavioral assay, the light-dark box test, at zero and 48 hours after treatment. Our preliminary results from this assay suggest Sema4D treatment does not alter anxiety behavior in mice.

Support: Cell and Molecular Visualization REU

Characterizing the role of grdn-1 in regulating neuronal morphology

Sofia A. Lavrentyeva*, Inna V. Nechipurenko, Piali Sengupta

Neurons are highly specialized cells with structurally and functionally distinct axon and dendrite(s). Axon-dendrite polarity dictates the directional flow of information in the nervous system. Although many extracellular and cell-intrinsic factors contributing to cell polarity have been identified, the molecular mechanisms governing polarization in vivo are not fully understood. Previous work in the Sengupta lab has identified the conserved signaling and structural scaffolding protein GRDN-1 as a regulator of neuron morphology in C. elegans. Work by others has implicated grdn-1 in controlling early steps of ciliogenesis in amphid sensory neurons. In addition, it appears that grdn-1 acts in a molecular signaling pathway to establish and/or maintain polarity in the gas-sensing neuron AQR. We find that in grdn-1 animals, AQR neurons exhibit defects in migration and dendrite outgrowth. Analysis of conserved GRDN-1 domains shows the presence of a Dishevelled binding GCV motif at its C-terminus. GRDN-1 mutant protein lacking the GCV motif fails to rescue AQR defects when expressed in grdn-1 mutant background. Disheveled proteins are key mediators of Wnt signaling, suggesting GRDN-1 may function in the Wnt pathway to regulate AQR morphogenesis. Here, I present my ongoing efforts to characterize the role of GRDN-1 in regulating AQR morphology.

Support: Cell and Molecular Visualization REU

Bitter Sweet: Drosophila Interaction with Denatonium through Ionotropic Receptor 25a

Josefine Striepen*, Gonzalo Budelli, Paul Garrity

Drosophila Ionotropic Receptors (IRs) play regulatory roles in the gustatory, olfactory and chemosensory systems. Cold sensing IR25a is involved in fly larvae’s aversive behavior towards Denatonium Benzoate. The relationship between IR25a and denatonium in adult flies is still unknown and to understand the receptor’s vast importance it is crucial to study it in all of its functions. Using CAFE taste tests and labellar sensilla electrophysiological tip recordings we studied the denatonium aversion behavior. We found that IR25a doesn’t appear to play a crucial role in this behavior in adult flies. We also found no significant difference in neuron activity between genotypes in sensillum S5, which detects denatonium. Looking forward, we want to focus on the possibility of more than one receptor controlling this behavior.

Support: Cell and Molecular Visualization REU
Behavioral State Switching in Star Graph Networks

Jesse Held*, Matthew Carl Cambria, Nate Tompkins, Seth Fraden

Biological networks are vast and complex, which make them very hard to study. We in the Fraden lab at Brandeis University attempt to understand some of the simplest forms of behaviors which can arise from communicative networks by creating networks, which can more easily be observed and studied. We do this by fabricating the geometries of networks called star graphs and creating nodes consisting of the oscillatory Belousov-Zhabotinsky (BZ) reactions. In this geometry we have previously observed 3 states, the unlocked, locked, and center-silent states. We are now using a photo catalytic additive and a programmable illumination microscope to inhibit certain droplets at certain times, in order to modify the network geometry by changing how many drops are communicating and how they communicate, to try to cycle through the different states allowed by the geometry in the hopes of later building more complex networks with a wider variety of behaviors we can study and control. So far we have been able to use this method to transition a network from a locked to an unlocked state, and we are currently working on experiments that transition a network from a center silent-state to a locked state.

Support: QBReC

Exploring the Phase Transition of a filamentous virus

Billy Chau*, Joia Miller, Marc Ridilla, Zvonimir Dogic

Vesicles in cells are used to distribute nutrients throughout the organelles and allow for particles to enter the cell (endocytosis) and leave the cell (exocytosis). We are mimicking the structure of a vesicle using rod shape viruses. We use these micro scale membranes made out of nano-viruses in order to measure their mechanical properties.

Phase-Dependent Response to Excitatory Activity in the Belousov-Zhabotinsky Reaction

Alexander Mitchell*, Michael Norton, Seth Fraden

The Belousov-Zhabotinsky reaction (BZ) is a chemical oscillator known for its colorful “spikes” of oxidized metal catalyst. This oscillatory behavior results from pairing a fast-acting autocatalytic activator with a slow-acting inhibitor. Influx of activator or inhibitor affects the spiking behavior of wells; influx of activator can cause premature spiking, while influx of inhibitor can delay the spike. We sought to determine a theoretical region of synchronization between a discrete periodic pulse of activator and a single well. A theoretical well was constructed using the dimensional Oregonator model in Matlab. We first generated a phase response curve (PRC) using a single discrete pulse, applied at different phase points. We call maximum of the PRC \( \phi_m \), \( \Delta \phi_m \). Pulses had an amplitude of 1mM, about 0.12% of the maximum value of the activator (800mM). Simulations ran at amplitudes within an order of magnitude of 1mM showed similar trends in phase response, which scaled linearly with pulse amplitude. Using the PRC, we constructed a Poincaré Map for several different periodic pulses of 1mM activator to see which maps contained stable fixed points, indicative of synchronization between the pulse and the spiking of the well. We call the natural period of the oscillator \( T_0 \) and the period of the pulse \( T_s \). We found synchronization occurred in the region \( |T_0-T_s| \leq \Delta \phi_m \). We also found that in cases where \( |T_0-T_s| = \Delta \phi_m \), the fixed point was at phase \( \phi_m \), and when \( |T_0-T_s| > \Delta \phi_m \), a ghost of the attractor was still clearly visible at \( \phi_m \), suggesting the region around \( \phi_m \) is critical for establishing synchronization. We plan to compare these findings to simulations of continuous periodic pulsing and experimental results of coupled wells, with the goal of creating a general model to describe synchronization in activator-coupled regimes of different well geometries.

Support: Computational Neuroscience Traineeship
Analysis of Sexually Dimorphic Neurons in Mice

Felicia Lee*, Yasuyuki Shima, Sarah Pizzano, Sacha Nelson

Behavioral differences between males and females are supposed to arise from structural differences between the two sexes. The medial preoptic area (MPOA) and bed nucleus of the stria terminalis (BST) are well-studied sexually dimorphic nuclei that lie downstream of the vomeronasal organ (VNO), suggesting that olfaction plays an important role in controlling sex-typical behaviors. We noticed that only males from the transgenic mouse line, P170, showed reporter gene expression in the MPOA and BST, so we hypothesized that P170 neurons were involved in male-specific behaviors, such as inter-male aggression, mating, infanticide, and parenting. Preliminary results suggest that P170 neurons are not activated by inter-male aggression or mating. However, we observed that fathers had fewer reporter expressing neurons in the MPOA and BST, suggesting that the neurons may be involved in infanticide, a behavior mainly exhibited by virgins but not fathers. Initial results indicate expression of the neural activity marker, c-Fos protein, in P170 neurons after infanticide, but more mice will need to be tested to further support this observation. Furthermore, we found sexually dimorphic expression in the medial amygdala (MeA) of P170 mice, suggesting that the MeA, which receives information from the VNO, is also crucial for male-specific behaviors.

Support: Cell and Molecular Visualization REU

How Does a Crustacean Neuronal Circuit Respond to Changes in Extracellular [K+]?

Lily He*, Manaswini Kar, Daniel Powell, Eve Marder

Over the lifetime of a crab, the pyloric neuronal circuit faithfully maintains rhythmic motor output despite various environmental disturbances. In this study, we wanted to investigate short-term and long-term responses to superfusions of saline with elevated or depressed potassium ion concentrations across animals. In the first set of experiments, we superfused modified saline for ten minutes at a time. We found that the burst frequency of the pyloric circuit pacemaker decreased moderately in saline with half the normal potassium ion concentration and that the burst frequency decreased significantly (even falling to zero in some animals) in saline with twice the normal concentration. All preparations recovered activity comparable to baseline levels after removal of modified saline. In the second set of experiments, we saw that the pacemaker burst frequency first increased slightly, then decreased significantly or even dropped to zero within the first few minutes of the hour-long superfusion of saline with twice the normal potassium ion concentration. All preparations recovered within the hour-long superfusion, indicating the existence of a homeostatic mechanism that allows the circuit to reach a particular activity level despite altered environmental conditions. Furthermore, the degree of decrease in burst frequency varied across animals exposed to virtually the same environmental conditions, thus revealing the wide animal-to-animal variability in response to a perturbation.

Support: Provost's Undergraduate Research Fund
Ancient protein points to life's origin: Knock-in of ancestral adenylate kinase alters growth profile of a modern mesophile

Nina Feinberg*, Christopher Wilson, Dorothee Kern

Much evidence points to life originating in a warm environment (>70˚C); however, the molecular evolution that accompanied the earth’s cooling is still an emerging field of study. As the planet cooled, microorganisms necessarily adapted to falling temperatures (<30˚C). Enzymes have evolved along with bacteria to function at mesophilic temperatures (15-40˚C). Modern ancestral protein reconstruction allowed the recreation of ancestral enzymes from the Adenylate kinase (ADK) family, dating back to nearly 3 billion years. In order to study the temperature dependency of ADK’s enzymatic rate on organismal fitness, we replaced the endogenous ADK of modern Escherichia coli with an ancestral counterpart, a modern thermophilic ADK, and wildtype E. coli ADK. After growing the strains at different temperatures, it became evident that bacteria expressing ancestral ADK do not grow well at low temperatures and grow better than wildtype at high temperatures. This demonstrates that an increase in activity of ancient enzymes at lower temperatures was vital to organismal fitness and subsequent evolution.

Support: HHMI

Ligand-Specific Interactions in the Hsp90 N-Terminal Domain

Brian Gzemski*, Timothy Street

Hsp90 is a molecular chaperone which has a homolog found in many different organisms, ranging from E. coli to humans. The Hsp90 family plays an important role in many different processes and has recently become a target for cancer. Hsp90 has an ATPase that causes the structure of the protein to change depending on whether or not it has hydrolyzed ATP. The ATPase is what drives the protein’s function. This led to many prospective drugs being developed to inhibit the ATPase ability of the chaperone. Some of the drugs have different basic structures, so they should have different contacts with the ATP binding pocket. From x-ray crystallography structures of the N-terminal domain (the part of Hsp90 that binds to ATP) with separate drugs the contacts of each drug can be determined. A script was created to analyze the residues that contact the ligand of several crystal structures in order to deduce specific residue-drug contacts. There are unique contacts of different drugs, and these should allow for ligand specific mutations in the protein to help better understand ligand binding specificity.

Spectral Tuning of an Opsin-Guanylyl Cyclase Fusion Protein for Optogenetic Use

Aaron E. Ammerman*, Daniel D. Oprian

Rho-GC (bacteriorhodopsin-guanylyl cyclase) is a fusion protein responsible for phototaxis in the fungus Blastocladiella emersonii. We are researching Rho-GC for use as an optogenetic tool. We wish to move expression into adult flies to show function as an optogenetic tool. Wild type Rho-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 Rho-GC from a green absorption maximum to red to make the protein sensitive to deeper penetrating light. The binding pocket of bacteriorhodopsins is comprised of 3 key residues: Lys 216, Asp 85, and Asp 212. Lys 216 binds covalently to retinal by means of a protonated Schiff base linkage, while Asp 85 and Asp 212 serve as counterions to the positively charged nitrogen. Past studies with microbial rhodopsins have found that changing these counterion residues, as well as other residues involved in retinal binding, can result in color shifts. With this in mind, mutations were designed at analog residues in Rho-GC, yielding three spectral tuning mutants: E254D, D380N (both blue shifted mutants), and D380E (a red shifted mutant). Further experiments are necessary to determine new residues to mutate as well as to test the activity of all spectral tuning mutants.

Support: Bauer Fellowship
**Poster # 2016.652**

**Bethany Rennich (Brandeis / Biochemistry, Neuroscience)**

**Investigating Surf2 in the context of conditioned taste aversion**

Bethany Rennich*, David Levitan, Sacha Nelson

The ability to form associations between stimuli and consequences in the environment and turn them into long term memories is an important survival mechanism. Conditioned taste aversion, a robust, single trial learning paradigm offers a behavioral model to investigate changes in gene expression that contribute to memory formation. Here we study Surf2, a gene downregulated in excitatory cells of the basolateral amygdala following conditioned taste aversion. Cell culture methods were used to overexpress an HA tagged Surf2 construct. Immunohistochemistry and biochemical fractionation were used to analyze the cellular localization of Surf2 protein. The immunohistochemistry results suggest that Surf2 localizes primarily to the nucleus. Several attempts at biochemical fractionation failed to effectively separate the nucleus from the cytoplasm, leaving us unable to confirm the nuclear localization of Surf2. Further study will use in vivo viral overexpression to repeat the fractionation and immunohistochemistry experiments. In addition, behavioral experiments will determine the effect Surf2 overexpression has on memory formation.

**Support:** Bauer Fellowship

**Poster # 2016.653**

**Susan Okrah (Hampton University / Chemical Engineering)**

**Observations of Three Ringed Networks of BZ Droplets**

Susan Okrah*, Jesse Held, Nate Tompkins, Seth Fraden

Synchronization is a critical of the cardiovascular system. When a person has a cardiac arrest or a heart murmur, the rhythm of their heart is changed. Because the heart is a complex network to study, we use a simpler network with similar qualities to study these patterns. The Belousov-Zhabotinsky reaction or BZ reaction is a nonlinear chemical oscillator. Just like the heart, BZ has a specific synchronization pattern. Over a period of time, this coupled oscillator continually changes from a red reduced state to a blue oxidized state. For this research, we wanted to observe a ring network of BZ. As a wave traveled in one direction in this network, we wanted to find a way to switch the direction of the wave by inhibiting a single drop. Using a microfluidic drop maker, droplets of a consistent size were then observed in one of two environments: capillaries or silicon chips. For capillaries, precut capillaries filled with these droplets were attached to a glass slide using an epoxy mixture. For silicon chips, the BZ droplets were carefully squeezed onto the desired etching, sealed with a glass slide and placed in a special clamping device. For both methods, observations were recorded using the Programmable Illumination Microscope, or PIM. In capillaries, we observed two isolated rings spontaneously switch direction after two droplets in the ring simultaneously oscillated. In silicon chips, a new sloshing state was observed. Instead of completing a full cycle through all three droplets, the ring continually switches direction. As a future direction, we want to continue engineer a method to control the direction of these rings. We also want to find and examine the parameters that cause the sloshing state to occur.

**Support:** MRSEC REU
**Poster # 2016.654**

**Kimberly Montano** (Brandeis / Biology, Sociology)

**Human Papilloma Virus and Cervical Cancer: Detecting High-Risk mixed infections using single-closed tube LATE-PCR**

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

Cervical cancer is linked to chronic infection by one or more of thirteen High-Risk Human Papillomavirus (HR-HPV) types. Each infecting HR-HPV type contributes separately to cancer risk. In the case of mixed infections, total cancer risk corresponds to the sum of the contributions of individual infecting HR-HPV types. The FDA-approved HR-HPV PCR test does not provide an estimate of cancer risk. This test detects all HR-HPV types but only identifies HPV 16 & HPV18.

Using synthetic targets, I showed that a LATE-PCR test constructed by the Wangh laboratory detects and identifies each of the thirteen HR-HPV types in a single-tube assay in the presence of 5000 copies human genomic DNA. The assay also identifies the 15 most common dual HR-HPV mixtures reported in the US. Control and demonstrates co-detection of two targets where one HR-HPV type is in 100-fold in excess to a second HR-HPV type.

**Support:** Provost's Undergraduate Research Fund, SSSP Experiential Learning Fund

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**Poster # 2016.655**

**SoJin Chon** (Brandeis / Biology)

**Insights from a bacterial diet: How studying terpene metabolizing bacteria plays a part in forest blight prevention**

Chon, SoJin*; Wong, Nathan; Cooper, Deani; Ferrazzoli, Alex; Futcher, Jeffery; Pochapsky, Thomas; Lovett, Susan

Terpenes, a large class of organic compounds, are ubiquitous in plants, perfumes, and cleaning products. Although naturally occurring in pine trees as a chemical defense mechanism, terpenes can be used by certain microbes as an energy resource; this immunity is the cause of the current forest blight in North America. We isolated different genera of terpene metabolizing bacteria to find the genes responsible. After we do whole genome sequencing on purified genomic DNA, we will be able to scrutinize the characteristics of these genes.

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**Poster # 2016.656**

**Victor Suarez** (Kean University / Biology, Chemistry)

**Quantitative Comparison of Nuclear and Somatic CaMKIV Expression in Pyramidal Visual Cortex Neurons**

Victor M. Suarez*, Anne Joseph, Nathaniel Miska, Gina Turrigiano

Homeostatic plasticity is vital for neuron networks to maintain equilibrium in the face of perturbations. This is accomplished through a series of mechanisms that allow neurons to regulate their firing rates around a stable firing rate set point (FRSP). While this phenomenon is observed both in vivo and in vitro, the process of maintaining a FRSP is not very well understood. Calcium-calmodulin kinase IV (CaMKIV) is suspected to play a critical role in the structure of FRSPs. We hypothesize that a drastic shift in activity beyond the current FRSP results in CaMKIV activation leading to translocation to the nucleus to regulate transcription. To test this, immunocytochemistry was utilized to assess nuclear versus somatic CaMKIV expression and obtain a ratio of the two in vitro in visual cortical pyramidal neurons treated with tetrodoxin (TTX) or bicuculline (BCC). Preliminary data indicates that TTX treated cells exhibit significantly higher nuclear/somatic CaMKIV localization when compared to control cells; however, BCC data indicates no significant difference. Results suggest an inverse relationship between CaMKIV and activity to moderate neuron homeostasis.

**Support:** Cell and Molecular Visualization REU
HMLα and HMRα, two crucial loci on chromosome III, are in a heterochromatin region and hence inaccessible for transcription or cleavage by HO endonuclease. However, upon double-strand break (DSB) at the MAT locus, both HMLα and HMRα become available for strand invasion. The mechanism behind this remains elusive. Of particular interest are post-translational modifications within the globular domains of histones that may facilitate chromatin rearrangement for mating type switching to occur. To this end, three sites, two on H3 and one on H4, were analyzed: H3K56 and H3S57, which are located at the DNA entry/exit site, and H4K91, located at a histone-histone interface. Acetylation of H3K56 has been shown to increase DNA unwrapping, and implicated in chromatin assembly and DNA repair, and phosphorylation of H3S57 has been shown to interplay with H3K56 in recovery from S-phase stress. H4K91 is associated with telomeric silencing and its acetylation appears to regulate nucleosome assembly/disassembly. Strains of H3K56 and H4K91 that both mimicked constitutively acetylated and unacetylated states were employed, as well as strains that mimicked constitutively phosphorylated and unphosphorylated H3S57. These were transformed with a plasmid containing the gene for HO endonuclease under control of the GAL promoter, and a viability assay was performed by comparing survival on YEP-Gal and YEPD. Only H3S57A, the constitutively unphosphorylated mimic, exhibited significantly (four-fold) reduced survival upon YEP-Gal induction of DSB, indicating the strain was relatively unsuccessful in DSB repair. In the mating switch assay, the colonies surviving induction of Ho endonuclease were predominantly ‘a’ mating type, suggesting only one Ho endonuclease cut. H4K91Q mutant reduced the frequency of spontaneous mutations significantly as observed from scoring of Ura- phenotype on 5-FoA plates. This strain will be further tested for LYS2 and CAN1 mutation suppression. All strains will undergo further mating type switching assays, and an altered viability assay will be performed, in which Gal induction will be limited to one hour.

Support: Cell and Molecular Visualization REU

**Poster # 2016.658**

**Zhongrui Zhang** (Brandeis / Biochemistry)

mTORC1 Signal Inhibitor Cbz-Boc3-Arginine Interacts with Ubiquilin

Zhongrui Zhang*, Lizbeth Hedstrom

The mammalian target of rapamycin complex 1 (mTORC1) is a regulator of protein translation and autophagy. mTORC1 has been implicated in many diseases, such as cancer and type 2 diabetes. Previous studies in our laboratory found a new small molecule that can inhibit mTORC1 signaling, Cbz-Arginine-Boc3 (Cbz-B3A). Instead of binding directly to mTORC1, the inhibition mechanism involves ubiquilin. We hypothesized that ubiquilins bind directly to Cbz-B3A and the complex of ubiquilin-Cbz-B3A might inhibit mTORC1 signaling pathway. The protein pulldown assay between Boc3Arg-amine and ubiquilin1, 2 was done to show that interaction between Boc3-Arg and ubiquilin exists. We also tested which part of ubiquilin interacts with B3A, because ubiquilin has three major parts, ubiquitin-domain end, ubiquitin-associated end and a middle truncate. As the result, the middle truncate is most likely the part that binds to B3A.
Coupling Behaviors of Spatially Arranged Oscillating Gels

Yongwoon Kim*, Zulma Jiménez, Irving Epstein

The Belousov-Zhabotinsky reaction is a chemical oscillator, in which the oxidation state of its catalyst oscillates from Mn+ to M(n+1)+. This property can be exploited to elicit swelling-deswelling behaviors in hydrogel networks crosslinked with the catalyst, a conversion of chemical energy to mechanical energy. When multiples of these gels are placed close to one another, the oscillations of one can affect the others because of the diffusion of intermediate species; this is known as coupling. We arrange a number of hydrogels of similar shape and size in several arrangements to examine their coupling behaviors. Understanding these phenomena can lead to future designs in soft robotics, with said gels serving as chemomechanical actuators.

Support: Jordan-Dreyer Summer Research Assistantship

Rhythmic release of an anti-depressant by an enzyme system

Zhiheng Wang*, Zulma Jiménez, Viktor Horváth, Irving Epstein

Sarcosine is a derivative of glycine, and has previously shown to help patients with obsessive-compulsive disorder (OCD). The antidepressant activity is a result of enhancing the activity of NMDA receptors in the brain, which help the brain adapt and involve in memory. Since sarcosine is a product of enzymatic action of creatinase on creatine, we use this enzymatic system as the source of sarcosine. Here, we aim to deliver sarcosine in a self-sustained and oscillatory manner; for this purpose, we couple a pH oscillator with the enzymatic reaction mentioned above. Our initial simulations show the existence of bistability which depends on the concentration of creatine.

Support: Bauer Fellowship

Effect of Sodium Bromide on Pattern Formation in the Chlorine Dioxide-Iodine-Malonic Acid (CDIMA) Reaction-Diffusion System

Yunqiao (Josephine) Gan*, Delora K. Gaskins, Irving R. Epstein, Milos Dolnik

Patterns are ubiquitous in biology. Stripes and spots found on the skins of animals and waves on the leaves are some examples. Similar patterns can be found in abiotic systems as well. Alan Turing proposed that patterns could arise from morphogens that react and diffuse. These patterns result from small perturbations to the uniform steady state and have a characteristic wavelength. We study the pattern formation in the chlorine dioxide-iodine-malonic acid (CDIMA) system and this system is the prototypical one for the study of Turing patterns. We used numerical simulations to study the effects of iodide concentration and flow rate in the stirred feed solution and observed two different steady states and two different oscillatory phenomena. We also performed experiments increasing the concentration of bromide and observed increases in pattern wavelength. We varied the ramp rate and observed that a uniform steady state could be obtained at higher ramp rates that was bistable with the large wavelength patterned state.

Support: Jordan-Dreyer Summer Research Assistantship
**Poster # 2016.662**  
**Richard Haburcak** (Brandeis / Chemistry, Mathematics)  
**Ligand-Receptor Interaction Modulates Energy Landscape of Enzyme-Instructed Assembly of Small Molecules**  
Richard Haburcak*, Junfeng Shi, Bing Xu  
The concurrence of enzymatic reaction and ligand-receptor interactions is common for proteins, but rare for small molecules and has yet to be explored. Here we show that ligand-receptor interaction modulates the morphology and free energy landscape of molecular assemblies formed by enzyme-instructed assembly (EISA) of small molecules. While the absence of ligand-receptor interaction allows enzymatic dephosphorylation by alkaline phosphatase (ALP) of a small peptide precursor (1P) to generate the hydrogelator (1) that self-assembles to form long nanofibers, the presence of the ligand-receptor interaction between D-Ala-D-Ala and vancomycin (2) biases assembly to form unstructured aggregates. Ligand-receptor modulation of EISA is analogous to the mechanism of action of amyloid aggregate targeting drugs and molecular chaperones in protein folding. As the first example of using ligand-receptor interaction to modulate the kinetics of enzymatic self-assembly, this work offers new insights for understanding the emergent behavior of sophisticated molecular systems having multiple and parallel processes.  
**Support:** Division of Science Summer Research Fellowship

**Poster # 2016.663**  
**Ben Pomerantz** (Brandeis / Biology)  
**Cyclic-di-Nucleotide Hydrolysis by a Diiron Phosphodiesterase from the Vibrio cholerae Pathogen**  
Ben Pomerantz*, Maria-Eirini Pandelia  
*Vibrio cholerae* VCA0681 is a phosphodiesterase featuring two tandem HD motifs, (denoted as HD-[HD-GYP]) each of which can bind two iron atoms. This protein hydrolyzes cyclic dinucleotides, in particular c-di-GMP and the hybrid 3',3'-cGAMP. Though activity has been shown to be Fe-dependent, the chemical nature of the active cofactor and the catalytic mechanism are unknown. A crystal structure for these enzymes is lacking, which would be of paramount importance to resolve substrate positioning and selectivity as well as resolving the role of the second metal site. A homology model of VCA0681 was constructed but crystallization attempts were not successful. The homologous HD-GYP from *Shewanella oneidensis* was chosen as a more stable and suitable target for crystallization experiments, which are currently under way. We have performed activity assays in order to determine the oxidation state of the cofactor that performs the chemistry and our results support that the mixed-valence Fe(II)Fe(III) is more catalytically competent compared to the fully reduced Fe(II)Fe(II) form. Intriguingly, not all PDEs of this superfamily are Fe-dependent (some demonstrate a larger degree of promiscuity) and an initial phylogenetic analysis has revealed that there is a correlation between function and cofactor evolution. This study exemplifies the need of structural and biochemical information to ascertain the functional cofactor in HD enzymes and opens up new frontiers for the discovery of unknown metal clusters performing a variety of hydrolytic reactions.  
**Support:** Division of Science Summer Research Fellowship
**Poster # 2016.664**

**Kristin Diamantides (Brandeis / Biology)**

**Bacterial Genome Constructions of lacZ to Study Homologous Recombination Rates**

Kristin Diamantides*, Vincent Sutera, Susan Lovett

Genetic recombination is the process that allows for the rearrangement of genetic material between sources through a variety of mechanisms that increases genetic diversity. Homologous recombination is one such mechanism that allows DNA to cross over at similar sequences for this exchange to occur. Though genetic recombination’s function is to increase gene diversity between generations, it is particularly useful and necessary in the repair of mutated genes or broken chromosomal sequences. Our objective is to study the repair of a chromosomal frameshift deletion of lacZ+, called the lacZ Active Site Delete (ASD), through homologous recombination between lacZASD and full or partial wild type lacZ+ sequences inserted at various minutes on the *Escherichia coli* (*E. coli*) chromosome. The lacZASD is a deletion of five base pairs in lacZ+ resulting in a frameshift mutation of the glutamic acid at amino acid position 461 resulting in the cells inability to metabolize the sugar lactose. The lacZASD sequence will be inserted into the chromosome at the attTn7 site located at minute 84.1. Full or partial wild type lacZ+ sequences will be inserted at FRT (flippase recognition target) sites utilizing FLP recombinase in various positions around the *E. coli* chromosome depending on the bacterial strain. The goal of this experimental study will be used to investigate the recombination rates in relation to different distances and donor sizes between the wild type donor sequence and the mutated sequence at the attTn7 site.

**Support: Provost's Undergraduate Research Fund**

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**Poster # 2016.665**

**Laura Bonvini (Brandeis / Chemistry)**

**Analysis of Alpha-Synuclein Oligomers in Human Brain Tissue**

Laura Bonvini*, Xinyue Liu, John Sanderson, Thomas C. Pochapsky, Tim Bartels, Dennis J. Selkoe

Aggregates of normally soluble neuronal protein alpha-synuclein represent a causative factor of Parkinson’s Disease (PD) and dementia with Lewy Bodies (DLB). This study aims to identify the toxic αS oligomers that develop aggregation. In collaboration with Dr. Selkoe and Dr. Soto Lab, size exclusion chromatography (SEC), protein misfolding cyclic amplification assay (PMCA) and enzyme-linked immunosorbent assay (ELISA) were the techniques employed. In solution with different amounts of amyloid seeds, soluble alpha-synuclein fractions were observed to be aggregating after approximately 100 hours. The fifth fractions of soluble alpha synuclein extracted from two different control and DLB brains were identified to be the ones mostly developing aggregates.

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**Poster # 2016.666**

**Rohin Biswas (Indian Institute of Science, Bangalore / Chemistry)**

**Synthesis And Characterization of Metal-Organic Frameworks (MOFs) Containing Pd Diphosphine Pincer Complexes**

Rohin Biswas*, Abebu Kassie, Casey R.Wade

A porous Hf metal-organic framework Hf_{6}O_{4}(OH)_{4}(L-PdX)_{3} has been synthesized from linkers based on Pd diphosphinite pincer complexes ([L-PdX]^{+} = [(2,6-(OPAr)_{2}C_{6}H_{3})PdX]^{+}, Ar = p-C_{6}H_{4}CO_{2}^{−}, X = Cl, I). Powder X-ray diffraction (PXRD) measurements indicate that the Hf MOF is isostructural with the Zr analogue Zr_{6}O_{4}(OH)_{4}(L-PdX)_{3} and retains crystallinity after exposure to strong acid. Treatment of Hf_{6}O_{4}(OH)_{4}(L-PdX)_{3} with aqueous NaI results in Cl^{-}/I^{-} ligand exchange to generate Hf_{6}O_{4}(OH)_{4}(L-Pdl)_{3}. Reaction of Hf_{6}O_{4}(OH)_{4}(L-PdX)_{3} and Hf_{6}O_{4}(OH)_{4}(L-Pdl)_{3} with Phl(O_{2}CCF_{3})_{2} results in I^{-}/CF_{3}CO_{2}^{−} ligand exchange. Future studies are aimed at comparing the catalytic activity of the Zr and Hf MOFs toward transfer hydrogenation of organic substrates.

**Support: Brandeis India Science Scholar**
**Poster # 2016.667**

**Anna Rothstein** (Brandeis / Biology, Chemistry)

**Synthesis and characterization of a zirconium metal organic framework containing a platinum pincer complex**

Anna Rothstein*, Neil T. Mucha, Casey R. Wade

Metal organic frameworks (MOFs) are coordination networks composed of organic ligands and metal ions that self-assemble in solution. These highly ordered materials have applications in gas storage, molecular separation, and catalysis. The strategy of immobilizing catalytically active transition metal complexes in MOFs via self-assembly can prevent intermolecular decomposition pathways observed in solution and potentially allow for the study of reactive intermediates. The Wade group recently reported the synthesis and catalytic activity of a zirconium MOF based on Pd–POCOP [POCOP = 2,6-(OPAr$_2$)$_2$C$_6$H$_3$; Ar = p-C$_6$H$_4$CO$_2$H] pincer complexes. As an extension of this project, we have successfully synthesized a neutral diphosphine PNNNP pincer ligand [PNNNP = 2,6-(HNPR)$_2$C$_5$H$_3$N; R = p-C$_6$H$_4$CO$_2$H] containing P–N bonds. This ligand has successfully been used to synthesize both Pd and Pt PNNNP pincer complexes. Solvothermal reaction of these complexes with ZrCl$_4$ in 4/1 mixture of dimethylformamide/acetic acid yield MOFs after 16 hours. Initial characterization of these materials by powder X-ray diffraction has shown that MOFs prepared with this ligand are both crystalline in nature and isostructural to the Pd–POCOP MOF previously established by the Wade group. Furthermore, the Pt–PNNNP MOF is porous and exhibits a BET surface area of 2616 m$^2$mmol$^{-1}$ as determined through nitrogen adsorption experiments. Pt–PNNNP MOF was digested using trifluoroacetic acid then analyzed by $^{31}$P and $^1$H NMR spectroscopy. These spectra demonstrate that the linker remains intact within the MOF though halogen exchange occurs at Pt. This exchange is evidenced by elemental analysis data and by chemical shifts of the MOF ($^{31}$P δ = 65.3, 61.9) closely resembling those observed for the homogenous complex ($^{31}$P δ = 62.9).

Support: Jordan-Dreyer Summer Research Assistantship

**Poster # 2016.668**

**Rachel Voss** (Brandeis / Chemistry)

**Oxidation induced breathing effects in chromium azolate MOFs**

Rachel N. Voss*, Neil T. Mucha, Casey R. Wade

Metal-organic frameworks (MOFs) are three-dimensional coordination polymers comprised of organic and inorganic subunits connected by self-assembled metal-ligand bonds. A chromium-based MOF was synthesized through the reaction of Cr(CO)$_6$ and 1,4-di(2H-tetrazole-5yl)benzene (H$_2$BDT) under a nitrogen atmosphere. This chromium azolate MOF, [Cr(BDT)(DMF)] (DMF), is comprised of chains of chromium atoms bridged with dimethylformamide (DMF) solvent molecules and connected by tetrazolate linker molecules. This material forms rhombus-shaped channels that can exhibit framework flexibility, or breathing effects, when external stimuli are applied. The inclusion or exclusion of solvent molecules or oxidation of the chromium centers are two external parameters that can contribute to framework changes. The effects of oxidation state on the framework flexibility of [Cr(BDT)(DMF)](DMF) were studied by oxidizing the as synthesized form of [Cr(BDT)(DMF)](DMF) (CrBDT-DMF-assynth) with both air and elemental sulfur. These materials were then analyzed via powder X-ray diffraction (PXRD) and nitrogen adsorption. As synthesized materials were determined to be microcrystalline under all reaction conditions tested, as observed via PXRD. To study the solvent effects on framework flexibility, suspensions of CrBDT-DMF-assynth were washed with both DMF or ethanol. Use of either solvent resulted in framework structure changes as determined by PXRD. These changes are also readily observed through a change in the shape of the nitrogen adsorption isotherms for ethanol-rinsed materials and suggest that ethanol-rinsed materials require higher partial pressures to induce microporosity than DMF-rinsed MOFs.

Support: Jordan-Dreyer Summer Research Assistantship
Blurb

Over the last couple years, three-dimensional (3D) cell culture systems have found widespread applications in the biomedical field, due to their capability of mimicking the native microenvironment necessary for cell–cell and cell–matrix interactions. In particular, supramolecular hydrogels that consist of peptidic nanofibrils have attracted considerable attention as a potential substitution of matrigels for 3D cell culture. In previous studies, we identified the self-assembling nature of certain short-sequence peptides dephosphorylated by ectophosphatases overexpressed on the surface of cancer cells (enzyme-instructed self-assembly, or EISA). The hydrogels that form upon EISA can be cytotoxic; however, the biocompatibility of the gels formed is crucial to having a successful 3D cell culture. Our focus was on an immunoreceptor tyrosine-based inhibitory motif (ITIM) with the sequence of LYYYYL. The ITIMs are hypothesized to be cytotoxic due to recent findings from our lab that the more tyrosines there are in a peptide, the more cytotoxic it became; however, this has yet to be demonstrated. Using standard solid phase peptide synthesis (SPPS) to synthesize the three motifs of interest (L, D and retro-inverso versions) in addition to two control peptides (L and D versions), we characterized the resulting hydrogels by optical imaging, transmission electron microscopy (TEM), rheometry and circular dichroism (CD) spectroscopy. We also tested the stability of the protein using a protein kinase digestion test, and quantified the dephosphorylation rate of the precursor peptides using the analytical high-performance liquid-chromatography (HPLC). We examined the cytotoxicity of the peptides on HeLa, Saos-2 and HS-5 cells by using cell viability assays such as MTT and Live/Dead Cell Viability Assays (2D and 3D). During our study of EISA of the ITIM-based peptides, we unexpectedly found that the supramolecular hydrogel of retro-inverso peptide of the ITIM epitope is more cell compatible than the hydrogel of the corresponding L-peptide of the ITIM. As the first example of the work on supramolecular hydrogel of retro-inverso peptides, this work illustrates a new approach to designing biocompatible soft materials from a less explored pool of candidates of peptides.

Support: SMURF (Summer MRSEC Undergrad Research Fellowship)
**Hannah Kilcoyne** (Brandeis / Biology)

**Quantification of Prolonged Sedation in Infants and Correlation to Brain MRI Findings**

Hannah W. Kilcoyne*, Russell W. Jennings, Patricia E. Grant, Dusica Bajic

**Introduction.** Infants routinely undergo prolonged sedation with opioids and benzodiazepines for proper clinical management, as part of the standard of care. Recent evidence suggests that prolonged exposure to pain medication may have negative effects on infant brain development. The clinical impact of such treatment on the youngest of patients is largely unknown.

**Hypothesis.** Prolonged sedation with opioids and benzodiazepines is associated with increased incidence of abnormalities in full-term patients as per brain MRI scan in comparison to healthy full-term controls.

**Methods.** We compared patients with healthy controls at two ages: <6 months (N=4 patients and 3 controls) and 6-12 months (N=3 patients and 3 control) as per IRB approval at Boston Children’s Hospital. We quantified the amounts of drugs used for prolonged sedation management that included muscle relaxants (cisatracurium, rocuronium), opioids (fentanyl, morphine, methadone), and benzodiazepines (midazolam, lorazepam). We analyzed: (1) average daily doses during sedation and weaning per group (mg/kg/day ± SD); (2) total treatment doses per patient (mg/kg/day); and (3) individual daily doses over time (mg/kg) only for morphine and midazolam. The number of anesthesia events and individual neuroradiology reports were also presented. Pearson’s correlation coefficient was used to measure the linear relations between different variables analyzed.

**Results.** Morphine and midazolam were the two drugs used the most frequently for prolonged sedation and were administered at the highest doses. Neuroradiology reports showed abnormalities in extra-axial space, parenchyma, and/or white matter structures that were not present in the controls. Our preliminary data show positive linear relationships for the average daily dose of administered morphine (r=0.934, p=0.0021) and midazolam (r=0.810, p=0.0272) with the number of neuroradiological abnormalities. Possible confounding factors, including the number of anesthesia events (r=0.398, p=0.3772), days of sedation (r=0.397, p=0.3784), and days of weaning (r=0.407, p=0.3655) do not appear to be associated with the number of neuroradiological abnormalities.

**Conclusions.** Prolonged sedation with morphine and midazolam is strongly associated with increased incidence of neuroradiological abnormalities in infants younger than 12 months of age. Given the current standard of care using these drugs, further investigations should investigate how prolonged sedation with opioids and benzodiazepines can affect brain development and give rise to potential functional alterations later in life.

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**Ji Eun Bae** (Brandeis / Biology)

**Characterization of Cas9’s Mutagenic Signature in S. cerevisiae**

Ji Eun Bae*, Brenda Lemos, James E. Haber

The CRISPR/Cas9 endonuclease efficiently enables the modification of target genomes—which has long been a challenge for scientists—by creating a double-strand break at a desired locus. Because of its efficiency across all the common model organisms as well as its relative price, speed and accessibility, this novel mechanism emerged as one of the most popular tools utilized in science and technology today. Despite its simplicity over other gene editing tools, the RNA-guided system lacks expansive studies outlining the details of its mechanisms. In this study, we used Cas9 to implement site-directed mutagenesis in the CAN1 locus of Saccharomyces cerevisiae to better understand how Cas9 cuts DNA, and how DNA is subsequently repaired. In our study, we used the CAN1 locus because the site is well-studied and can be conveniently used as a selection marker.
**Poster # 2016.672**

**Ethan Glantz** (Brandeis / Neuroscience)

**Understanding the Functional Connectivity between Hippocampus and Medial Prefrontal Cortex Through Chemogenetic Inactivation**

Ethan H. Glantz*, Dennis M.S. Maharjan , Shantanu P. Jadhav

The Hippocampus has long been identified as a center for creating and retrieving short-term spatial memories. Additionally, the medial prefrontal cortex has critical roles in executive control, storage of long-term memories, working memory, and memory guided decision-making. It is known that both dorsal and ventral areas of CA1 in Hippocampus have direct monosynaptic projections as well as indirect projections to the medial prefrontal cortex. Although the connectivity between the hippocampus and medial prefrontal cortex is implicated in spatial working memory, the exact nature this functional connectivity plays in behavior and cognition is not fully understood.

Using DREADDs as a chemogenetic approach to in vivo inactivation of the hippocampus in rats, we will attempt to further understand the cognitive and behavioral affect of disrupting the connection between the Hippocampus and Medial Prefrontal Cortex.

**Support:** Computational Neuroscience Traineeship

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**Poster # 2016.673**

**Zoe Brown** (Brandeis / Neuroscience, Psychology)

**Effects of Hearing Acuity on Use of Prosody in Speech Comprehension**

Zoe Brown*, Nicole Amichetti, Arthur Wingfield

Speech prosody, which encompasses word stress, pitch contour, and pauses in spoken sentences, is a feature that helps signal syntactic boundaries to the listener and thereby reduces processing effort. An experiment is reported in which this effect on processing effort was examined in the context of reduced hearing acuity. Normal-hearing young adults and older adults with good-hearing and with mild-to-moderate hearing loss listened to and recalled sentences with a prosodic pattern that coincided with the syntactically defined clause boundary, sentences with prosody placed in conflict with syntax, and sentences with no prosodic marking. Pupil dilation was used as a measure of processing effort, with larger dilations reflecting larger amounts of effort. For each participant group, recall accuracy was significantly greater for congruent prosody than for incongruent prosody. Recall accuracy was significantly higher among the young adults compared to the older good-hearing and older poor-hearing participants. Unlike the young adults and the older good-hearing adults, older poor-hearing adults more often shifted their responses to match the prosodic marking than to stay with the intended syntax; this suggests that reduced hearing acuity may require a greater reliance on prosody. The young adults and older adults with good hearing showed a significantly greater peak amplitude of pupil dilation for sentences heard with incongruent prosody compared to those heard with congruent prosody, suggesting increased effort to process sentences with prosody placed in conflict with syntax.

**Support:** Provost's Undergraduate Research Fund
Investigating the effect of self-paced listening on the relationship between hearing acuity and speech recall

Emily Cohen*, Eriko Atagi, Arthur Wingfield

Increased perceptual effort, due to hearing loss or difficult listening conditions, negatively affects listeners’ comprehension and recall performance—even when the words are audible (e.g., McCoy et al., 2005; Rabbitt, 1991; Wingfield et al., 2005). However, Piquado et al. (2012) demonstrated that allowing young adult listeners with mild-to-moderate hearing loss to control the speed of auditory input by self-pacing through passages ameliorates this negative effect of poor hearing acuity on recall. The current study investigates this effect of self-paced listening on poor recall in both young and older adults. Four passages are presented to the young and older adult listeners in self-paced or continuous conditions. In the continuous condition, passages play continuously from beginning to end; in the self-paced condition, passages pause after each sentence and allow the listener to continue at their own pace. Two passages are presented at +10 dB from the listener’s speech reception threshold (hard-to-hear) and two at +25 dB (easy-to-hear). After each passage finishes, listeners are prompted to begin their free recall of the passage with as much detail as possible. Each listener’s recall is analyzed for amount and degree of detail recalled using propositional analysis (Peelle, et al., 2015). While data are still being analyzed, recall of the self-paced listening condition presented at the easy-to-hear level is expected to be more accurate and more detailed than the continuous listening condition at the hard-to-hear level. These results are expected to have a greater effect for older adults than young adults.

Support: Bauer Fellowship

Investigating the Role of the Small GTPase Rem2 in a PTZ Seizure Model

Isabel Smith*, Sarah Richards, Anna Moore, Suzanne Paradis, Stephen Van Hooser

The small GTPase Rem2 is a member of the RGK family of Ras-like GTPases which inhibit voltage gated calcium channels (Correll 2008). Rem2 is the most highly expressed RGK protein in the nervous system and is a regulator of synapse formation and dendritic morphology, which we have seen in vitro in hippocampal neurons (Paradis 2007; Ghiretti 2011). Expression of Rem2 is very high in the hippocampus and specifically concentrated in area CA3, where seizures often originate (Liput et al. 2016; Prince 1978). In mice, Rem2 mRNA and protein expression are highest two weeks after birth, a time when dendritic spines and synapses increase drastically in rodent neurons, representing a role of Rem2 in vivo (Liput et al. 2016; Moore et al. 2016, in revision). With the assumption that spine density decreases with Rem2 knockout in area CA3, an area where seizures often initiate (Prince 1978), it is hypothesized that loss of Rem2 may cause resistance to seizure initiation. Data analysis revealed that the initial hypothesis was incorrect. However, a strong effect was seen upon comparison of seizure susceptibility in Rem2 KO mice and WT mice. It was thought that knockout of Rem2 would cause resistance to chemically-induced seizures, but it appears that Rem2 KO mice are significantly more susceptible to seizures induced by PTZ. We hypothesize that this effect is related to the increased intrinsic excitability of Rem2 KO cells, as was quantified in previous studies of responses from layer 2/3 pyramidal neurons (Moore et al. 2016, in revision).
A memo from Millennial Kinases to The Lost Generation: “We are not so lazy after all!”

Ayantu Temesgen*, Sarita Biswas, Roman V. Agafonov, Dorothee Kern

Src and Abl are two non-receptor Tyrosine kinases that have similar structures but different regulatory mechanisms. Kinases are enzymes that phosphorylate Ser, Thr and Tyr residues by transferring a phosphate group from ATP. Phosphorylation often functions to turn a protein “on” or “off,” and plays a central role in cellular communication. Uncontrolled kinase activity leads to uncontrolled cell growth and ultimately cancer making kinases important drug targets.

In order to gain insight into the evolution of kinase regulation, we resurrected common ancestors of Src and Abl, and then Src, Abl and the Tec and Fer families. We manipulated various regulators, such as myristoylation for Abl and tail phosphorylation for Src, to get quantitative information on the distinct regulatory modes of the modern enzymes. We then used the same methods on the ancestors in order to understand the evolution of regulation.

Support: Bauer Fellowship

Effect of Limited Activation of Fusion Protein on Probability of Viral Membrane Fusion

Frankie Marchan*, Tijana Ivanovic

Enveloped viruses enter cells by fusion of viral and cellular membranes. Trimeric protein hemagglutinin (HA) serves as both the attachment and fusion protein of influenza virus. HA is produced as an inactive precursor, HA0. To activate it for fusion, all three monomers of HA0 must be cleaved into HA1 and HA2 polypeptides. Cleaved and uncleaved HAs bind receptors equivalently via the globular head-domain of HA1. Cleavage enables the low-pH-triggered conformation changes in HA required for fusion. Cleavage also liberates hydrophobic peptide sequences (fusion peptides) at the newly formed N-termini of the HA2 monomers. Hundreds of HAs and thousands of receptor molecules are embedded within a single interface of the fusing membranes. Individual HAs undergo conformational changes at random as a result of proton binding. They reach for the target membrane, but not all HAs succeed in inserting, instead becoming spontaneously and permanently inactivated. Individual HAs that insert remain stretched out between the two membranes as ‘extended intermediates’ attempting to bring the two membranes together. Fusion occurs only when several neighboring HAs insert so as to pull together on the same membrane region. The aim of this project was to produce a reagent to study the probability of HA insertion vs. inactivation as a function of HA-receptor interaction. By limiting proteolytic activation of HAs, we will generate virions with about 50% chance of fusing. Based on computer simulations of fusion, we predict that, in that regime, small changes in the probability of HA insertion vs. inactivation would result in large changes in fusion yield. We have successfully produced virions that incorporate completely intact HAs. Uncleaved virions have greatly reduced infectivity, but their infectious potential is recovered upon cleavage. We have further established conditions that allow us to vary the extent of HA cleavage on virions. This sets the stage for experiments aimed to quantify the fraction of fully cleaved HAs on virions and to measure the effects of limited HA activation on the probability of HA insertion during membrane fusion.

Support: QBReC
Species Identification: Closed-Tube Barcoding of Naegleria Species

John Deng*, Nicky Sirianni, Heather Schiller, Chandler Fulton, Lawrence Wangh

Members of the genus 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. Linear-After-The-Exponential (LATE) PCR and a set of nine Lights on/Lights off probes was utilized in order to rapidly distinguish and identify different Naegleria species. As different species bind to the probes at different temperatures, a unique fluorescent signal can be produced for each species. A fluorescent library of signals was first established with known Naegleria DNA, followed by tests of freshly isolated Naegleria samples. So far, different Naegleria species have each produced different patterns, allowing for their rapid identification and differentiation.

Support: Division of Science Summer Research Fellowship

Poster # 2016.679

Kyra Hamel (Brandeis / Biochemistry, Biophysics)

Look at Those Curves: Measuring Thermodynamic Properties of Multi-Strand DNA

Kyra Hamel*, W. Benjamin Rogers

In the natural world, the basic building blocks of life are capable of self-assembling into complex structures. Is it possible to achieve artificial analogs using micron-sized polystyrene beads as programmable atoms? Beads can be coated with densely packed single-stranded DNA of specific sequences then reversibly bound to a bead with a complementary base pair sequence. The bases release their hold on each other, turning double-stranded DNA into single-stranded as temperature increases, known as DNA melting. Interactions can be predicted to a degree of accuracy, but information is still limited. To further complicate the matter, our system involves three different DNA strands: two short, distinct strands and a third strand complementary to both. The third oligonucleotide is a linker, and when cooled it should connect the other two strands. Further, if the linker is used to link two DNA coated beads, conformational change could occur without changing any other variables. If we can accurately predict the thermodynamics of this kind of system, the linker strands can be designed to a desired affinity and fit for the experiment, such as an evolving, multi-segmented Pokemon! We succeed in using spectrometry to detect the melting temperature of two and three strand oligonucleotide systems and the thermodynamic properties of the former. We find that current prediction methods are not extremely accurate, a linker is a promising variable for manipulation, and there is potential in predicting the thermodynamics of a three strand DNA system from its respective two strand components.

Support: QBReC
Investigation of the Native circadian CLOCK-complex in Drosophila melanogaster

Micael Maya-Peinl*, Weifei Luo, Michael Rosbash

Circadian clocks are defined by molecular reactions that take place throughout the day and regulate almost various aspects of an organism’s routine from digestive metabolism to sleeping patterns. Concentration, accumulation, and steps involved all contribute to the timing mechanism. The biological ‘clocks’ are highly conserved and are present in a wide variety of organisms from mammals to bacteria. The central clock in Drosophila melanogaster is a heterodimer of CLOCK and CYCLE which are transcription factors that activate thousands of genes. There are several uncharacterized proteins synchronizing with CLK/CYC which we hope to determine through Co-IP of the complex at different time points. Learning more about the complex allows for further studies on the downstream effects of these proteins. A native complex allows us to identify which proteins are present based on size. Complementary methods include size-exclusion chromatography. The ability to assay the molecular weight on a native PAGE would help us understand which proteins -as well as how many- are binding to the complex at these different times of day. This knowledge helps refine the understanding of the molecular basis for timekeeping. Development of a protocol to assay the complex in native conformation would be beneficial to the field for future studies of the molecular clock as a complex.

Support: HHMI EXROP

Engineering Caenorhabditis elegans for Phototaxis

Kamsi Odinammadu*, Melissa Trieu, Anna H. Hartmann, Piali Sengupta, Daniel Oprian

The organism Blastocladiella emersonii is an aquatic fungus that was found to use the second messenger cGMP and retinal for phototaxis in the zoospore phase of its life cycle. B. emersonii phototaxis is mediated by a fusion protein, RhoGC, made up of a microbial rhodopsin domain and a eukaryotic guanylyl cyclase catalytic domain. Our lab is interested in this unique protein to elucidate the mechanism by which light and retinal control activity of the guanylyl cyclase domain and to develop the protein as an optogenetic tool, extending its use in different laboratories as a technique to control cGMP levels in cells and organisms with light. Here we begin studies to express RhoGC in the AWB olfactory and AWC chemo/thermosensory neurons of Caenorhabditis elegans to test whether we can engineer this organism for phototaxis. We describe here construction of vectors for targeted expression of a RhoGC gene into C. elegans. We will next insert the finished vectors into C. elegans and test their viability for induced phototaxis.

Support: Biochemistry Department

JVLA Imaging of X-Shaped Radio Galaxies.I

Carly C. KleinStern*, Kevin Wang, David Roberts

The goal of this work is to study the morphologies of X-shaped radio galaxies (XRGs) and learn if they are “true” XRGs. True XRGs are those formed by a flip or drift of the axis of the black hole at the center of the galaxy. Back flow processes do not produce true XRGs. The data from each source are calibrated for instrumental response and self-calibrated, and then images are made. Of the sources discussed in this poster, J0702+5002 is an example of a back flow process, J0845+4031 is an axis drift, and J0914+1715 and J1043+3131 resists classification at this time. The spectral index images show intensity as a function of frequency. In depth data analysis will begin when all data have been collected (Fall 2016).

Support: Provost's Undergraduate Research Fund
**Poster # 2016.683**

**Kevin Wang** (Brandeis / Physics)

**JVLA Imaging of X-Shaped Radio Galaxies. II**

Kevin Wang*, David H. Roberts

We obtained polarization data on a set of X-shaped radio galaxies and automated the calibration process for these data by modifying the pipeline written by the NRAO. These data have to be calibrated separately because of an additional source of error due to the instrumental polarization. Having calibrated the data, we made polarization images of these galaxies, some of which are displayed on our poster. By collecting information about the polarization of these sources, we hope to further elucidate the nature of these interesting galaxies.

**Support:** Physics Department

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**Poster # 2016.684**

**Natalie Harris** (University of Maryland Baltimore County / Biochemistry)

**Role of Tryptophan Residues in a Novel Opsin Fusion Protein**

Natalie Harris*, Lindsey Lamarche, Aaron Ammerman, Daniel Oprian

RhoPDE is a novel UV sensitive rhodopsin-phosphodiesterase fusion protein identified in the genome of chanoflagellate Salpingoeca rosetta, which degrades the phosphodiester bond in the second messenger cGMP. This naturally occurring enzyme has potential for fine-tuned control of cyclic nucleotide signaling pathways, making it an advantageous tool for optogenetics. The microbial transmembrane opsin domain binds retinal making a visible pigment at 490nm, typical of classic opsins. Due to the connection of the phosphodiesterase (PDE) to this opsin domain, it is expected that the activity of PDE would be light-dependent. However, exposure of RhoPDE to visible light has no effect on the PDE, which is constitutively active. RhoPDE exhibits an uncharacteristically high UV absorbance at 280nm which has been localized to the N-terminus of the protein. This phenomenon may be the result of tryptophan residues in this region. This project seeks to determine the resulting functional and spectral properties of RhoPDE upon mutation of all the tryptophans in the UV-sensitive region of the protein. Eight W to F point mutants of RhoPDE were generated via site-directed mutagenesis, expressed in HEK 293 cells, purified, and tested via UV-Vis spectroscopy to determine spectral properties. Absorbance spectroscopy indicated that many of the mutants bound retinal well producing WT spectra, while others did not express as well. One mutant in particular, W63F, showed a double peak in the visible region. All eight mutants retained the abnormally high UV absorbance comparable to that of RhoPDE WT.

**Support:** Cell and Molecular Visualization REU

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**Poster # 2016.685**

**Eduardo da Veiga Beltrame** (Brandeis / Biophysics)

**Implementation of a simple to use Gene Transcription Simulator**

Eduardo da Veiga Beltrame*, Jane Kondev

There is currently no user friendly tool for modeling gene expression at the level of individual polymerases elongating through the gene. There is a large diversity of published models which share many common features, but are generally inaccessible to newcomers. We have implemented a stochastic transcription simulator that is simple to use and customize, available at www.genesim.org. This platform should be useful for individuals who are not interested in developing models from scratch either analytically or computationally, but would still like to investigate how gene expression is affected by the characteristics of a genetic circuit, such as length, binding or elongation rate. It can also yield predictions for systems too complicated to be treated analytically, whose dynamics would not be intuitively apparent.

**Support:** QBReC
Flap Deletion May Have an Influence on Penicillium brevicompactum IMP Dehydrogenase Resistance to Mycophenolic Acid

Sarah Zainelabdin*, Runhan Yu, Lizbeth Hedstrom

Microbial drug resistance is a growing problem requiring scientists to take a closer look at drug-targets interactions. One such target is Inosine-5’-monophosphate dehydrogenase (IMPDH) which plays an important role in the biosynthesis of guanine nucleotides. Inhibitors of IMPDH have been used to limit the guanine nucleotide pool which will consequently limit cell proliferation. Mycophenolic Acid (MPA) successfully inhibits eukaryotic IMPDH and is used as an immunosuppressant drug to limit B and T cell growth. The MPA producing filamentous fungi, *Penicillium brevicompactum*, contains two genes coding for two isozymes of IMPDH; Pb-IMPDH-A and Pb-IMPDH-B, which are respectively 17- and 103-fold more resistant to MPA in-comparison to other eukaryotic IMPDHs. Interestingly, the resistance of Pb-IMPDH is not due to a mutation in the active site rather, the active sites of Pb-IMPDH-A and Pb-IMPDH-B are conserved when compared to both MPA-sensitive and MPA-resistant eukaryotic strains. By looking at the amino acid sequence and the 3D structure of various IMPDH-types, we found that the flap region (residues 432-487)* which falls over the active site is longer in MPA-resistant strains. We used site-directed mutagenesis to create two mutants: a short deletion mutant (D450-S454) and a long deletion mutant (K443-S454) in the flap region of Pb-IMPDH-A. We will characterize the wild type Pb-A and Pb-B and our mutated enzymes in three ways: 1) measure the kinetic activity on its substrate and cofactor 2) gather MPA IC50 values and 3) determine the inhibition mode of MPA. By doing so, we hope to better understand the interactions of this ubiquitous drug-target.

* Numbering based on Pb-IMPDH-A.

Support: Cell and Molecular Visualization REU

Determining the Relationship Between Nervous Wreck and the Cell Membrane at Different Phosphoinositide Levels

Amber B. Jones *, Steven J. DelSignore, Avital A. Rodal

Nervous Wreck (Nwk), a neuronal F-Bar protein, plays a specific role in cell membrane curvature and deformation. The cell membrane contains these lipid components, Phosphoinositides (PIP), that have the capability of being phosphorylated or dephosphorylated by lipid kinases and phosphatases respectively. When phosphorylated, the cell membrane becomes more negative, and this negative charge recruits an electrostatic interaction between Nwk and the cell membrane. Despite knowing this specific information, there is not much known on how Nwk gets off of the cell membrane. In order to answer this question, PIP2 levels were manipulated to make the cell membrane more or less negative in different experimental assays. One assay deals with purifying the phosphatase domain of OCRL and targeting a purified cell membrane with purified Nwk. The other methods include using Fluorescence Recovery After Photobleaching (FRAP) in larval neurons and S2 cells in order to determine the dynamics of the relationship between Nervous Wreck’s ability to bind to the cell membrane when exposed to different PIP concentrations. When doing the phosphatase purification, the expression of the protein in *E. Coli* resulted in the protein remaining insoluble after trying two different protocols. Supplemental purification attempts are underway, using yeast rather than *E. Coli*, because of yeast’s potential success. In a preliminary FRAP experiment, there was a localization of endogenously tagged Nwk in the synaptic bouton of the larval neuron. Nearly four minutes after Nwk was photobleached, there was hardly any recovery to original fluorescence, which suggests that the Nwk is tightly assembled to the cell membrane, and recovery would be limited. The original S2 cell experiment resulted in Nwk remaining on the cell membrane and the phosphatase remaining cytoplasmic. In order to further continue this project, there is the plan to use the acquired information to develop an optogenetic tool for the lab’s use to alter membrane composition. By understanding the dynamics of this relationship, there is the potential to be able to engineer biological systems in such a way that can control the activation and deactivation of membrane deformation and understand Nwk during in vivo processes like neuron growth.

Support: MRSEC REU
Species, Variation, and Global Distribution of Naegleria

Heather Schiller*, Elaine Lai, John Deng, Lawrence Wangh, Chandler Fulton

The genus Naegleria is comprised of aquatic, single-celled, eukaryotic amoeboflagellates that can be found in freshwater sources around the world. What was first thought to be a genus containing only one species of Naegleria — Naegleria gruberi — turned out to be a highly variable genus with a tremendous amount of diversity. Despite their capacity for sexual reproduction, Naegleria mainly reproduce asexually in both the laboratory and nature. It is within this realm of high variability and remarkable distribution that our interest lies. In this study, we explored the possibility for coexistence among species of Naegleria on a smaller scale: a birdbath. There were at least two species of Naegleria found in successive isolations from birdbath water samples, and in one single sample, analysis is in the process of being performed as to the amount of species present. Future experiments will involve identifying the species of Naegleria found in each of the water samples. Moreover, the mystery of distribution will be further explored by examining the possibility of Naegleria inhabitation, growth, and travel in a unique vehicle: the inside of a migratory dragonfly.

Interplay of Temperature and Neuromodulation on the Activity of the Crustacean Cardiac Pacemaker

James Weiss, Eve Marder

Environmental temperature perturbations pose a constant threat to the stability of neuronal function. The cardiac ganglion (CG), a central pattern generator (CPG), is able to maintain healthy rhythmic activity over a 30°C temperature range, modifying its output with changes as slight as 0.5°C (Jury and Watson, 2000). Recent studies have confirmed the role of neuromodulators as agents of regulation of crustacean CPGs in response to both acute and mild temperature changes (Chen et al., 2014; Staïdele et al., 2015). The goal of the present study is to observe the effects of neuromodulatory substances on the isolated CG across a broad range of biologically-relevant temperatures. I recorded activity from the CG extracellularly from 7°C to 31°C in saline and in the presence of one of five neuromodulatory substances. Q10 temperature coefficients of the CG’s burst frequency in control conditions (n=31) were 1.76±.45, with some CGs nearly temperature invariant (Q10 = 1.1) while others increased 6-fold over the observed range (Q10 = 2.7). The efficacy of each substance in altering the CG’s burst frequency and number of spikes per burst was found to have its own signature temperature dependence. In the presence of excitatory modulators, the change in burst rate over temperature increased, causing previously temperature-invariant CGs to become more temperature dependent. CCAP, typically considered excitatory, elicited a wide range of temperature-dependent responses on an animal-to-animal basis. The inhibitory substances GABA and Allatostatin-3 (AST-3) completely silenced activity of the CG at low temperatures. Interestingly, most but not all preparations overcame silencing at high temperatures, ranging from reduced to elevated frequencies in comparison to temperature-paired controls. These data enrich our knowledge of neuromodulatory dynamics in a temperature-varying environment.

Support: Computational Neuroscience Traineeship
Elon Mathieson (Brandeis / Neuroscience)

Tasty Place Cells; Multimodality in the Hippocampus

Elon R Mathieson*, Linnea Herzog, Donald B Katz, Shantanu P. Jadhav

The hippocampus plays a central role in spatial learning and memory. In fact, the CA1 region of the rat hippocampus can be considered a spatial map since it consists of neurons known as place cells that fire in a specific location of the animal’s environment. These cells have been known to not only respond to specific locations but other stimuli such as odors. However, there is little research in understanding the role of the hippocampus in relation to taste learning. Prior research has shown that inactivation of the hippocampus interestingly enhances conditioned taste aversion (CTA), a type of associative learning. As a result, the aim of the experiment was to characterize how hippocampal CA1 neurons respond to tastes. Preliminary results suggest that there may be place cells in the hippocampus that also respond to tastes. These “tasty place cells” may play a role in taste learning paradigms such as CTA, as well as offer a relationship between location and taste responses.

Support: Bauer Fellowship

Karen Wang (Emory University / Biology)

Title: Evaluating the pH and Salt Dependence of ATP Hydrolysis in Trap1

Karen Wang*, Timothy Street

Heat shock protein 90 (Hsp90) is a specialized molecular chaperone that assists with protein folding and maintains cellular homeostasis for numerous client proteins. Hsp90’s functional cycle relies on ATP hydrolysis, through which binding of ATP at the N-terminal domain causes the protein to go through a conformational change from an open state to a closed state. Using an enzyme linked assay, the ATPase rate of mitochondrial Hsp90 (Trap1) was measured at various ATP concentrations in different pH and salt conditions. As pH and salt concentration increased, Trap1 exhibited increased $K_{cat}$, and a smaller range of $K_m$ values at high pH than at low pH. These results indicate that higher pH and salt concentrations result in an increased rate of ATP consumption in Trap1.

David Landesman (Brandeis / Biology, Neuroscience)

Carbon Fiber Electrode Bundles for Dense Recordings of Neural Circuits

David Landesman*, Andrea Stacy, Neil Ritter, Stephen Van Hooser

The total number of cells that can be recorded simultaneously is limited by current electrode technology. We describe a method for building low-cost, customizable dense electrode arrays for in vivo neural recording using carbon fiber. The progression of the electrode occurred in multiple stages resulting in the current model which has grips, a guide tube, and sturdier construction.
Gaining a Toehold in the Thermodynamics of DNA Strand Displacement

Olivia Zou*, W. Benjamin Rogers

DNA strands are versatile molecules that have a wide variety of applications. We can easily control how and which strands bind and unbind based on Watson-Crick base pairing. This ease of complementarity and specific base pairing allows us to use DNA as a structural material. From programming DNA circuits to constructing three-dimensional structures, researchers can use DNA as more than just biological carriers of information. The thermodynamics of DNA hybridization also contributes a great deal to how DNA structures hybridize. For this research, we look at the behavior of competing DNA strands. We aim to understand how displacement strands affect the equilibrium concentrations of the various species. Our specific scenario involves two strands, A and B, which are partially complementary to each other. Two other strands, D1 and D2, are the displacement strands. We find that our data agrees with nearest-neighbor model predictions for reactions with zero or one displacement strand; however, our data for two displacement strand reactions do not agree. This leads to some questions about the thermodynamics of strand displacement.

Support: QBReC

Generation of Methylene-coupled Products via Reductive Elimination from an Oxidied Gold(III-III) A-Frame

David Barnes*, Benjamin Reiner, Casey Wade

Multinuclear gold complexes have received increased attention in organometallic chemistry in the past four decades. These complexes have been used to explore structure and bonding as well as the role of multinuclearity on catalytic and stoichiometric transformations. We have focused our efforts on binuclear gold complexes bearing phosphorus diylid ligands. These complexes are nucleophilic at gold which allows facile activation of small molecules. Notably treatment of complex 1 with dibromomethane cleanly affords complex 2 in a 2-center, 4-electron oxidation. Schmidbaur and coworkers have reported that alkylation of complex 2 (Ar = phenyl) affords complex 3 which, upon thermolysis in the solid state, liberates propane and regenerates. While the generation of a methylene inserted product is intriguing, the high temperatures required to effect the transformation (>150 °C) would preclude the use of compound 1 as a practical methylene insertion agent. Considering the high barrier to reductive elimination, we hypothesized that increasing the size of the substituents at phosphorus may lower the barrier to reductive elimination by increasing steric congestion around the gold sites. Reducing the barrier to reductive elimination may allow use of these gold complexes for catalytic methylene insertion under mild and safe conditions. Realizing this goal would provide a valuable tool for synthetic chemists. Herein we report our efforts toward the synthesis and reactivity of binuclear gold complexes bearing sterically bulky diylid ligands.

Support: MRSEC REU
Electrochemical Preparation of Polyaniline/Gold film

Johnson Agyapong*, Antony Epps*, Kenton Meronard, Amir Saheb

Polyaniline (PANI) is a conductive polymer produced by polymerizing aniline. Owing to favorable properties like easy synthesis and ability to change oxidation state with simple oxidation/reduction (Surwade, 2010), there have been numerous research projects based on the potential applications of PANI. Aniline can be polymerized chemically or electrochemically; however, the latter was utilized in this experiment because the final product did not require extraction from any solvent, oxidant or monomer (Chemistry of polyaniline). Our short-term goal was to deposit gold clusters on the PANI film and potentially create a DNA hybridization biosensor. We successfully electropolymerized polyaniline on ITO substrates and platinum (Pt) electrodes but we were unable to achieve our short-term goal. We went through up to 8 gold treatments but our methods of characterization could not detect any gold present on the PANI film. We believe that gold clusters were present but too tiny to be detected by our methods of characterization. Saheb et al, illustrate that detection of low Au clusters was unsuccessful and the only way gold clusters has been detected is by using XPS (2008).

Support: Hampton University PREM REU

Ruth-Love Damoah (Brandeis / Biology)

One in a Million: Sensitive Molecular Assay for TB Point of Care Testing.

Ruth-Love Damoah*, John Rice, Aquiles Sanchez, Lawrence Wangh

Tuberculosis (TB), though curable, infects about a third of the world’s population and claims over a million lives annually. In addition, there is a continuous rise in the percentage of drug resistant strains. A point of care (POC) test that is sensitive, inexpensive and provides drug susceptibility information could greatly reduce the number of annual TB victims, and curb the development of drug resistance. Our laboratory is developing a LATE PCR assay which targets the beta subunit of RNA polymerase (rpoB) of Mycobacterium tuberculosis (Mtb). Mutations in the 81bp rifampicin resistance-determining region (RRDR) of rpoB are indicative of drug resistance to rifampicin, an important first-line drug. My assay can detect a single copy of Mtb in a background of 58,000 human genomes. Additionally, the assay can distinguish the drug-sensitive wildtype (WT) rpoB from at least 21 different drug-resistant mutational types using a novel probe technology called Lights-Off Only. Once completed, this assay will be useful for detecting Mtb DNA in oral samples. Oral samples serve as more useful and less invasive screening for TB in children and HIV-infected patients than sputum.
**Poster # 2016.697**

**Scott MacDonald** (Brandeis / Biology, Chemistry)

**Direct Evolution of glycoDNAs Antigenic for a Carbohydrate Epitope on HIV**

Scott W. MacDonald*, Jennifer K. Bailey, J. Sebastian Temme, Isaac J. Krauss

Monoclonal antibody 2G12 neutralizes a broad range of HIV isolates by binding a cluster of high-mannose glycans on HIV envelope gp120. Structures that mimic the 2G12 epitope are of interest in HIV vaccine design as they could elicit antibodies that neutralize the virus in a broad manner, similarly to 2G12, through vaccination. As an approach to generate such immunogens, SELMA (SELection with Modified Aptamers) was developed. SELMA is a directed evolution approach used to develop DNA scaffolds that cluster glycans (glycoDNAs) in the optimal spacing and orientation for recognition by a carbohydrate-binding target. These glycoDNA libraries are generated by attachment of azide sugars to an alkyne-modified base via “click” chemistry. To select for the most antigenic binders the library is incubated with a carbohydrate-binding target. The library fraction bound to the target is amplified and then reglycosylated, affording the 2nd-generation library for the next round of selection. Previously, this protocol was applied to the discovery of glycoDNAs that mimic the 2G12 epitope on HIV and bind to the 2G12 antibody. The winners from that selection are tightly recognized by 2G12 with an affinity comparable to the natural interaction between 2G12 and its epitope on gp120. Herein, we use SELMA to select for 2G12-binders from a new library in order to test whether selection with this library produces constructs similar to those from the previous selection and to validate the published protocol.

**Support:** Jordan-Dreyer Summer Research Assistantship

**Poster # 2016.698**

**Jair Flores** (University of Maryland, Baltimore County / Biochemistry)

**Understanding the Evolution of Circadian Rhythm in Cyanobacteria by ABC Protein Complexes**

Jair Flores*, Warintra Pitsawong, Marc Hoemberger, Dorothee Kern

*Synechococcus elongatus*, a cyanobacterium, has a circadian clock whose mechanism relies on three proteins: KaiA, KaiB, and KaiC. Of the three, KaiC, a hexameric protein, auto-phosphorylates and dephosphorylates throughout the day; this is the core protein in the complex. KaiA and KaiB are regulatory proteins that activate and suppress phosphorylation causing KaiC to oscillate through different phosphorylation states. As of now, not much is known about the functions of the circadian clock mechanism, its connection to the environment, or how it compares to other bacterium that lack one or both regulatory proteins. In this study, *T. vulcanus* will be compared to *S. elongatus*. We will run an assay composed of purified proteins with a set amount of ATP in a constant temperature. Samples will be collected at specified time points which will be analyzed via HPLC and SDS-PAGE. HPLC will be utilized to analyze the rate ADP production while the SDS-PAGE will allow us to analyze the phosphorylation states of the protein complex. Results of both analysis will be compared to those of *S. elongatus*. We see similar results from the SDS-PAGE, however we require more consistent results to come to a conclusion. *T. vulcanus* is five times faster in terms of ADP hydrolysis than *S. elongatus*. In the future we plan on running a similar assay on two other bacteria: one that lacks KaiA, *Rhodobact. sphaeroides*, and one that lacks both KaiA and KaiB, *Pyrococcus horikoshii*. Through analysis of these bacterium, we hope to have a better understanding of this timing mechanism.

**Support:** Biochemistry Department
Poster # 2016.699

**Michael Perlow** (Brandeis / Biological Physics)

**Powering Active Colloids with DNA polymerization**

Michael Perlow*, Olivia Zou, W. Benjamin Rogers

Colloids are mixtures of different materials which do not dissolve, nor quickly separate. A subset of colloids, called active colloids, consume chemical energy to move, including self-catalyzed autonomous propulsion. DNA isothermal polymerase chain reaction (PCR) was used as a chemical energy source, being reactive without the need of thermocycling. Polystyrene microbeads (PS) mixed in water, a type of colloid, was mixed in a PCR solution, which were then recorded to observe any changes in diffusivity and movement. Mixing PCR reagents with PS beads shows possible ballistic motion in the PS caused by the PCR, compared to Brownian motion seen in PS alone. Further experiments are needed to fully explore and describe this observed behavior.

**Support:** QBReC

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Poster # 2016.700

**Danielle Robbins** (Brandeis / Neuroscience)

**Gene Silencing with Age in the Mouse CNS**

Danielle Robbins*, Erin Clark, Sacha Nelson

Changes in gene expression that either regulate or are regulated by aging in the brain are poorly understood. Previous work has shown that at the transcriptional level, brain ageing in mice displays parallels with human neurodegenerative disorders. These types of studies look at the transcriptional profile in aging brains have the potential to shed new light on age related illnesses involving cognitive decline such as dementia, Parkinson, and Alzheimers. These are all hugely important diseases with increasing diagnoses every year. Thus, understanding the mechanisms behind transcriptional changes with age can ultimately add to the body of research on mechanism behind cognitive decline.

**Support:** Provost's Undergraduate Research Fund

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Poster # 2016.701

**Mark Sherer** (Brandeis / Neuroscience)

**The development of a molecular toolkit to probe in vivo CaMKII-subunit exchange**

Sherer, M.*, Flyer-Adams, J.G., Griffith, L.C.

The majority of organisms rely on the ability to store and retrieve memories for basic survival skills. Long-term memory (LTM) can last decades, yet protein half-life is much shorter, making us to wonder how memory is maintained at the molecular level. The serine/threonine Ca\(^{2+}\)/calmodulin -dependent kinase II (CaMKII) is required for LTM and is a plausible candidate in the search for molecular memory (Herring and Nicoll 2016). A mechanism detailing how CaMKII could maintain LTM in spite of protein turnover was recently demonstrate *in vitro* but has yet to be proven *in vivo* (Stratton et al., 2014). Elucidating this mechanism *in vivo* is crucial for understanding CaMKII’s role in memory, and key to finding a molecular basis for memory. CaMKII’s structural and biochemical properties qualify it as an important molecule in memory processing. CaMKII activates in the presence of Ca\(^{2+}\)/calmodulin but can self-modify to extend its enzymatic activity beyond the window of neuronal activity. Individual CaMKII molecules assemble into a twelve-subunit CaMKII holoenzyme in which cooperative activation occurs such that inactive subunits can be co-activated by active subunits within the holoenzyme (Stratton et al., 2013). In the presence of Ca\(^{2+}\)/calmodulin, these holoenzymes undergo *in vitro* subunit exchange, and spread their state of activation to newly inserted subunits (Stratton et al., 2014). This subunit exchange process could function *in vivo* to maintain CaMKII holoenzyme activity despite protein turnover, highlighting CaMKII as a molecular mediator of LTM.

To investigate *in vivo* CaMKII subunit exchange, we will use the fruit fly *Drosophila melanogaster* to express two tagged CaMKII proteins in a temporally distinct manner and observe their co-integration within the same holoenzyme both biochemically and using FRET microscopy. Here, we have cloned the first reporter constructs for generation of fly lines to enable validation of in-vivo CaMKII subunit exchange, and thus identification of a molecular mechanism for LTM maintenance.

**Support:** Computational Neuroscience Traineeship
Tiny Tumblebeads: Making Spinning Colloids Roll with DNA-Mediated Friction

Zachariah Trotz*, W. Benjamin Rogers

Living organisms exhibit many behaviors that inanimate materials do not: They move, organize, and replicate, and in the field of active matter, there is a growing interest in engineering materials that share some of these remarkable features. Here we aim to develop a new model experimental system combining colloids and DNA to study artificial motion at the microscale. We use paramagnetic particles which rotate when subjected to an applied, rotating magnetic field. DNA grafted to the particles can hybridize with complementary DNA grafted to coverslip, coupling this rotation to translation. In this poster, I will describe the progress we have made in building the rotating magnetic field setup, as well as in developing protocols for labeling glass and colloids with DNA. In the future, we hope that this model system will help us explore how ‘friction’ due to ligand-receptor interactions affects motion at the microscale.

Support: Provost's Undergraduate Research Fund

Investigating the PER and TIM protein profiles in Drosophila melanogaster circadian clock mutants

Michael Wang*, Maisa Araujo, Weifei Luo, Michael Rosbash

A circadian rhythm is known to be any set of biological changes that oscillate within an organism over a period of twenty-four hours due to the natural day/night cycle stemming from the earth's rotation. One such circadian rhythm is the cycling of two circadian proteins, PERIOD (PER) and TIMELESS (TIM), in Drosophila melanogaster. These proteins are involved in a transcriptional feedback loop that begins with a heterodimer composed of two other non-cycling circadian proteins – CLOCK (CLK) and CYCLE (CYC) –, which serves as a transcription factor for a great number of fly genes. Among these genes are per and tim, whose protein products, the aforementioned PER and TIM, form yet another heterodimer that acts as a transcriptional repressor against CLK/CYC. In the presence of light, TIM degrades, leaving PER vulnerable to phosphorylation by a protein called DOUBLETIME (DBT) and its own subsequent degradation, which allows CLK/CYC to resume their function in transcriptional promotion. However, despite our current understanding of molecular CLK and its transcriptional feedback loop, the subtler details regarding interactions between PER, TIM, and DBT are still ambiguous.

Using western blot and immunohistochemistry techniques, we aim to provide a preliminary investigation of the interconnected workings of PER, TIM, and DBT by considering the effects on PER and TIM expression that may arise due to a number of mutations affecting the tim and dbt genes in drosophila over the course of six circadian time-points over the course of a twenty-four hour, light-dark cycle “day”; these mutations specifically being the deletion of specific E-box sequences found on the tim gene and two separate single base-pair deletion mutations on the dbt gene that lengthen or shorten the daily circadian period in their hosts, respectively. Should results be consistent with prior literature, levels of PER and TIM ought to correspondingly rise and fall in adherence to the dbtL mutant’s shorter period and persist for longer in the dbtS mutant, while there’ll be an overall lower concentration of PER and TIM in the flies characterized by the tim E-box deletions.
Shubham Singh (Indian Institute of Science, Bangalore / Physics)

Modeling fluctuation driven interaction between colloidal rafts: Casimir Effect

Shubham Singh*, Bulbul Chakraborty

A mixture of opposite chirality rods, instead of just bulk phase separating out, can form interesting structures under the suitable depletant concentration. Experiments have shown that when a membrane of right handed short rods is coalesced into bigger membrane made of opposite chirality long rods, it breaks into small circular rafts that are stable in time and repel each other. However, the rafts form a ordered structure if the background is taken to be a mixture of left and right long rods instead of only left ones. The interaction energy between the rafts has a minima suggesting the presence of some attractive component besides the usual repulsive force as in former case.

We think that since the attractive component is due to Casimir like forces arising because of the large fluctuations and long range correlations of the background mixture of long rods.

In the brief study, we have simulated the Casimir interaction using the simple nearest neighbour 2D Ising model to understand the interaction between the colloidal rafts.

Support: Brandeis India Science Scholar

Radhika Jangi (Brandeis / Biochemistry)

Examining Rare Codon Clusters in Hsp90

Radhika Jangi*, Timothy Street

Most amino acids are encoded by multiple codons. These synonymous codons are used with differing frequencies during translation. Previous work has shown that, rather than being randomly dispersed throughout genes, rare codons typically appear in clusters. The %MinMax algorithm calculates the relative rarity of a nucleotide sequence that codes for a protein (Clarke IV and Clark, 2008). HtpG is the prokaryotic analog of Hsp90, which is a chaperone protein. By applying the algorithm to various bacterial HtpG sequences, we were able to distinguish a pattern in which rare codons clustered around amino acids that are close in proximity to the end of the N-terminal domain and the end of the middle domain for HtpG. These rare codon clusters may be beneficial in translation and folding in Hsp90.

Julia Schiantarelli (Brandeis / Biology)

The role of Bik1 and formin interactions in coordinating the actin cytoskeleton

Julia Schiantarelli*, Julian Eskin, Bruce Goode

Tight coordination of the actin and microtubule (MT) cytoskeletons is required for diverse cellular processes, such as maintaining cellular morphology and motility. These active biological polymers consume energy in order to assemble into different types of 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 results 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, providing a new link between actin and microtubule dynamics. In my project, I address the question of whether similar formin regulation extends to Bik1, the budding yeast homolog of CLIP-170. In yeast, Bik1 has been shown to regulate microtubule dynamics, and to localize to microtubules. I have tested whether purified Bik1 can regulate the in vitro actin assembly activities of the two yeast formins, Bnr1 and Bni1, and found that it specifically inhibits just one formin: Bnr1. I have also imaged bik1Δ cells to identify actin cable defects or related phenotypes, and found that they are enlarged and have more cables than wild type cells. Finally, I have observed the dynamic localization and behavior of 3GFP-Bik1 in live cells using spinning disc confocal microscopy. Future in vivo and in vitro analyses will help elucidate to what extent formin regulation by CLIP-170 is conserved across evolution.

Support: Division of Science Summer Research Fellowship
Determining Selectivity in Bacterial IMPDH Inhibitor Sensitivity

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Antibiotic resistance is becoming an increasing problem in the treatment of bacterial pathogens. A deeper understanding of IMPDH inhibition at a biochemical level may implicate a route to the development of novel antimicrobial drugs. Inosine 5’-monophosphate dehydrogenase (IMPDH) is a tetrameric enzyme found in almost all organisms that catalyzes the oxidation of IMP to XMP, a key step in the biosynthesis of guanine nucleotides. As the guanine nucleotide pool controls proliferation and other cellular processes, inhibition of IMPDH activity results in cell death. Several inhibitors of bacterial IMPDH have already been developed. While all bacterial IMPDHs with Tyr358 and Ala165 are sensitive to these inhibitors there is still quite a bit of variation in inhibitor sensitivity between the IMPDHs of various bacterial species. Our goal is to determine which residues within the IMPDH protein affect sensitivity to inhibitors. Specifically, we hypothesized that the N22D mutation in the IMPDHs of the bacteria Francisella tularensis would alter its sensitivity to inhibitors. The bacterial species Bm and Sa, which demonstrate the most similar inhibitor sensitivity despite relatively different sequences, both have aspartic acid in this position. We believe that the N22D mutation in FtIMPDH will cause its inhibitor sensitivity to be more similar to that of Bm and Sa. The N22D mutation was incorporated into FtIMPDH through site-directed mutagenesis (SDM). The mutant will be analyzed with both kinetic and IC50 studies, and compared to wild type FtIMPDH. It was found that the $K_M$ of NAD binding in the N22D mutant (155uM) is half of what it is with the wild type enzyme (380uM). Because the inhibitors bind in the NAD binding site, a change in NAD binding affinity could indicate a change in inhibitor sensitivity, however experiments that specifically test inhibitor binding have not yet been conducted.

Motilal Uttarkabat (Indian Institute of Science / Biology)

Purification of IMPDH from Mycobacterium tuberculosis

Motilal Uttarkabat*, Devi Gollapalli, Sabrina McDonnell, Lizbeth Hedstrom

Tuberculosis (TB) remains one of the rapidly spreading infectious diseases with increased incidence of multi drug resistant (MDR) strains. The enzyme inosine-5’-monophosphate dehydrogenase (IMPDH) catalyses NAD dependent oxidation of IMP into xanthosine monophosphate (XMP). Inhibition of IMPDH enzyme depletes the in vivo guanine nucleotide concentration making it a prime drug target. Mycobacterium tuberculosis has 3 probable IMPDHs as compared to one found in most bacteria. They are designated guaB1, guaB2, and guaB3. The active and essential enzyme IMPDH-2 was purified from M. tuberculosis H37Rv cells by using DEAE-sepharose followed by IMP-sepharose column chromatography. The purpose is to study the enzyme in natural form and to find if any complex is formed with other IMPDHs.

Support: Brandeis India Science Scholar
Dahlia Kushinsky, Eve Marder

Central pattern generators (CPGs) including the stomatogastric ganglion (STG) and cardiac ganglion (CG) of Cancer borealis are circuits responsible for consistent and rhythmic muscle movements and are regulated by neuromodulation. Extensive work has been done to determine the effects of these neuromodulators upon the circuits in vitro, but little work has been done to see the effects of their application in vivo. Additionally, little is known about the interaction between pyloric and cardiac rhythms in vivo throughout the animal’s life. This research sought to a) determine the effects of exogenous application of neuromodulator on the heart and b) investigate whether there exists a correlation between the cardiac and pyloric rhythms in vivo. This was achieved by recording muscle movement of the heart and/or dorsal dilator muscle while also injecting neuromodulator into the animal to determine its effects. From this investigation, it was concluded that GABA was consistently inhibitory, proctolin produced short bouts of inhibition, and serotonin produced different results in all animals. There also appears to be no correlation between cardiac and pyloric rhythms in the animal, although additional experiments must be done to verify this finding. Overall, this experiment demonstrated that the application of neuromodulator in vivo may cause different effects upon the heart of C. borealis than found with the isolated circuit, indicating that there is much more to be learned about the use of neuromodulators throughout the animal’s life to regulate these CPGs.

Support: Computational Neuroscience Traineeship

Phillix Esquea, Yihao Zhuang

Isolating P450 gene in wild bacterial genome

Cytochrome P450s are hemoproteins that catalyze reactions in electron transfer chains in human and bacteria. The focus of this experiment was to isolate wild-type P450 gene from a bacterial genomic DNA found in the wild by PCR. The gene was further expressed to obtain the wild-type P450 protein. Future experiments will focus on how various point mutations in P450 gene alter the functionality of P450 protein.

Everett Weber

Time Dependence of Variance in the Limiting Pool Mechanism

The limiting pool mechanism has been proposed as a way to control the size of structures within cells. However, it has been shown through both simulation and theory that this mechanism alone cannot control the sizes of multiple structures competing for the same pool of subunits, instead having the size of each structure entering a random walk. The variance in each of these lengths has been described previously in the first, linear, domain, but was previously not described in detail in the asymptotic domain that follows. In the case of two competing filamentous structures, stochastic simulations were run and compared to a calculation of variance over time for diffusion inside a one dimensional box. The calculation closely matched both the linear and asymptotic domains of the variance. In future experiments, the case of three or more structures should be investigated.
Building Logic Gate based on Thermodynamic Model

Ziwei Huang*, Lishibanya Mohapatra, Gabriel Bronk, Jane Kondev

When working on the problem of gene expression, we are interesting in the effect of transcriptional factors in modulating the rate of transcription during gene expression. Specifically, we study how the cooperative binding of transcriptional factors affect the rate and use this property to build the ANG logic gate. This gate consists of two types of activators (A) and (B) respectively as the inputs and the output is the transcription rate (Tx). We use a simple thermodynamic model and the Boltzmann distribution to find the optimal value of the input (A) and (B) respectively so that the input-output relation is closest to an AND gate.

Support: HHMI

Crystallographic Comparison of Electron Difference Densities in Fluc

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Fluc membrane proteins are functional dimers that expel fluoride ions from bacteria and lower eukaryotic organisms when they encounter weak hydrofluoric acid challenges. Fluc architecture is unique in that each subunit of the functional dimer is oriented opposite to its monomer mate. X-ray crystallography structures from homologous proteins of the Fluc family, Bordetella pertussis (Bpe) and Escherichia coli (Ec2), solved in the presence of “monobody” chaperones, illuminate Fluc’s antiparallel architecture by hinting at its two antiparallel pores which run along the dimer interface. In Bpe, electron difference densities at four positions along the two proposed pores are coordinated by the polar side chains of asparagine, two serines, and a novel edge on quadrupole interaction with a phenylalanine ring. Ec2 however only has two electron difference densities, one in each pore in a two-fold symmetric position. The goal of this study is to determine if the electron difference densities found in Bpe and Ec2 are in fact fluoride ions. To accomplish this goal we quantitatively measured all solutions pertinent to the purification and crystallization of Bpe and Ec2 through a fluoride ion selective electrode and standard addition of fluoride. Our results indicate a lower limit of fluoride contamination in our solutions at 1.0 uM, far below the expected KD of a fluoride ion binding to Bpe or Ec2. Ec2 purified in fluoride free conditions does in fact crystallize with a “monobody” chaperone and we are in the process of determining the structure. Comparison of a fluoride containing structure and fluoride free structure, in particular the presence or absence of electron difference densities within the proposed pores, will be completed.

Support: HHMI