Brandeis Summer SciFest

Undergraduate Research Poster Session

Thursday, August 2, 2012
1:00 – 3:00 pm

Shapiro Science Center Atrium
Brandeis University
Waltham, Massachusetts
**Poster # 2012.101**

**Presenter** John Shen (Brandeis / Chemistry)

**Title** Progress towards surface immobilized cobalt/zirconium bimetallic complex

**Authors** John Shen, Seth Marquard, and Christine Thomas

**Abstract** Ambidentate phosphinoamide ligands have been utilized to support metal-metal interactions in early/late heterobimetallic complexes. Upon two-electron reduction, heterobimetallic Co/Zr complexes featuring unusual coordinatively unsaturated geometries and metal-metal multiple bonds are afforded. These reduced Co/Zr heterobimetallic complexes are capable of performing small molecule activation as well as catalytic cross-coupling reactions. The synthesis of chemically modified Co/Zr heterobimetallic complexes and the functionalization of an electrode surface with the heterobimetallic complex will be discussed.

**Support** Division of Science Summer Research Fellowship

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**Poster # 2012.102**

**Presenter** Kevin Monk (Brandeis / Neuroscience)

**Title** A Tongue's Tale: Licking Microstructure Reveals Rapid Attenuation of Neophobia in Rats

**Authors** Kevin J Monk, Benjamin D Rubin, Jennifer Keene, and Donald B Katz

**Abstract** Neophobia, a term that refers to the initial hesitation that many animals show to a novel food, normally attenuates over the first two encounters of the food. Traditionally measured by comparing the overall consumption of the tastant to the consumption of water, the attenuation of neophobia (AN) has received attention in the past as a model of learning and memory. As neural processes that underlie long-term memory require hours to complete, an assumption of this work is that little AN occurs within minutes of first exposure of the tastant – an assumption that has been largely borne out in data. Unfortunately, this failure to observe may reflect the fact that traditional methods of measuring AN allow only relatively gross and noisy accounts of real-time behavior. With this in mind we compared AN data from a traditional two-bottle test – in which the animal is given the option of a novel tastant (e.g. saccharin) or water and the difference in overall consumption is recorded – with analogous data from a two-bottle brief access test – in which a rat is given 15 seconds access to either water or a novel tastant. At the level of overall consumption, data from both tasks were in good accord; additionally, however, analysis of licking microstructure (that is, analysis of real-time licking behavior) revealed an initial hesitation to consume saccharin that decreased significantly over the course of a twenty-minute tasting session – a more subtle measure of neophobia and its attenuation. Thus, our data suggest not only that the brief-access task is an adequate paradigm to measure conventional AN, but also that, contrary to general consensus, aspects of AN begin within minutes of the very first taste. Future studies will be undertaken to determine if these two similar-yet-distinct behaviors are mediated by similar or distinct mechanisms.

**Support** World of Work Fellowship
**Poster # 2012.103**

**Presenter** Yasmin Marrero (Brandeis / Neuroscience, Psychology)

**Title** Can sucrose cause a truly ‘wanting’ phenomenon (*i.e.* addiction) or is it just yummy?

**Authors** Yasmin Marrero, Madelyn A. Baez-Santiago and Donald B. Katz (Department of Psychology, Volen Center for Complex Systems, Brandeis University)

**Abstract** Addiction is defined as the compulsive use of a substance that interferes with the performance of normal life activities. Research has suggested that addiction represents stimulus “wanting,” which is the function of mesolimbic dopamine and opioid circuits (including those in the basolateral amygdala or BLA) that can be distinguished from those underlying stimulus palatability (*i.e.*, “liking,” which appears to involve other circuits in BLA). Others have suggested that certain schedules of consumption can lead to compulsive sugar seeking behavior that fits the behavioral criteria of addiction, such as bingeing, craving escalation, and withdrawal. Consumption of sucrose activates the same pathways known to be important for cocaine addiction (the aforementioned ‘wanting’ system). It could therefore be predicted that, under certain circumstances, responses to sucrose could be dissociated into those that are addictive behaviors and those that occur because sucrose is highly palatable—and that these behaviors should be differentially affected by specific pharmacological manipulations. We will test this prediction by inhibiting specific BLA subsystems in rats supposedly addicted to sucrose while these rats engage in tasks thought to be driven by either liking or wanting. We predict that antagonism of opioid synapses in BLA will block addiction-related increases in craving for and bingeing on sucrose, without affecting taste preferences for sucrose. This work will provide a strong test of the hypothesis that sucrose is an addictive substance, and of the distinction between systems devoted to liking and wanting.

**Support** Division of Science Summer Research Fellowship

**Poster # 2012.104**

**Presenter** Brendan Hasz (Brandeis / Neuroscience)

**Title** Synaptic Conditions for Phase-Difference Bi-Stability in PING Networks

**Authors** Brendan Hasz and Paul Miller

**Abstract** Gamma-frequency synchronization is found in many cortical regions of the brain. It is thought to play a role in sensory processing, binding different sensory representations through synchronization. However, for this binding to occur, the groups of neurons involved have to be able to bind at a certain phase difference, without reverting to some set phase-relationship regardless of the input. We examine the effect of inter-group synaptic strength on phase-relationships in PING (Pyramidal-InterNeuron Gamma) networks. First, for the single-group case, we find that there are distinct synaptic strength parameter subspaces for generating either slow (20-40 Hz) or fast (60-100 Hz) gamma rhythms. We then connect two such PING networks and determine the phase difference between the two groups as a function of both inter-group synaptic conditions and initial phase difference. We find that while coupling high-frequency PING networks do not result in stable gamma oscillations, certain inter-group synaptic parameter subspaces of coupled slow-PING oscillators do. Some of these parameter subspaces always result in in-phase oscillations, others out-of-phase, and we find some bistable regions where the steady-state phase difference is determined by the initial phase difference. However, these bistable parameter areas are characterised by poor oscillation stability, and are not conserved across trials with slightly randomized network connectivity.

**Support** Computational Neuroscience Traineeship
**Poster # 2012.105**

**Presenter**  Sophie Travis  (Brandeis / Biochemistry, Chemistry)  
**Title**  Structural Study of a Cinchona Alkaloid Catalyst  
**Authors**  Sophie Travis, Yongwei Wu, Li Deng, Dagmar Ringe, and Greg Petsko  
**Abstract**  Cinchona alkaloid catalysts are responsible for highly enantioselective catalysis of over one hundred classes of reaction. It is believed that the key to understanding this enantioselectivity lies in determining the solution conformation of the catalyst. In this study, I have co-crystallized one of these catalysts with tetragonal hen egg white lysozyme. Not only have I obtained an atomic-resolution structure of the catalyst, but also I have developed a new system that may allow visualization of the catalyst-reactant complex and possibly reaction intermediates.  
**Support**  Lerman-Neubauer Fellowship

**Poster # 2012.106**

**Presenter**  Michael Kosowsky  (Brandeis / Mathematics, Physics)  
**Title**  Jansky VLA Observations of the Microquasar SS433 in Different Epochs  
**Authors**  Michael S. Kosowsky, John F.C. Wardle, and David H. Roberts  
**Abstract**  We observed the microquasar SS433 in six different epochs between June 24, 2011, and August 8, 2011, in order to investigate the polarization structure of SS433 in better detail than was possible before. We succeeded in making polarization images to the theoretical limit of the Very Large Array. We found that there are subtle changes in polarization from epoch to epoch as the source expands throughout time. We also found that there was very little differential Faraday Effect across the source as a function of position.  
**Support**  Division of Science Summer Research Fellowship

**Poster # 2012.107**

**Presenter**  Brendan Reardon  (Brandeis / Physics)  
**Title**  Investigating the Proper Motions of the Blazar 1055+018  
**Authors**  Brendan Reardon, David H. Roberts, and John F.C. Wardle  
**Abstract**  In the sixth paper of the MOJAVE VLBA series, the authors claim proper motion in the third component of 1055+018 by using a mathematical model of the visibility data created by the software DIFMAP. We worked to examine this in detail using an alternative method of analysis. Using the same images within the paper, we calculated the positions and peak flux for each component of the blazar. This was conducted using the software AIPS. MATLAB was used to compare and plot data; all sets agreed and we verified the paper's assertion that the core and 4th component are stationary, while the third has proper motion. We discovered that for the purpose of position analysis, both AIPS and DIFMAP agreed. Additional plots of the intensities of the components were used to compare the two analysis techniques again, and they agreed in general.  
**Support**  Brandeis University Physics
Poster # 2012.108
Presenter Julie Miller (Brandeis / Neuroscience)
Title Immunohistochemistry of Interneurons in Ferret Primary Visual Cortex
Authors Julie Miller, Arani Roy, and Stephen Van Hooser
Abstract Inhibitory interneurons play a critical role in development and functioning of the primary visual cortex. Cortical interneurons display a wide diversity in morphology, electrophysiological activity and immunohistochemical signature, and are accordingly divided into multiple subtypes. To understand better the role of this complex inhibitory circuitry in cortical development, we intend to express genes such as GFP (for structural studies) and channelrhodopsin (ChR2; for functional studies) selectively in subtypes of cortical interneurons. For example, using viral transfection and interneuron-specific promoters, we intend to express ChR2 in specific subtypes of interneurons to optogenetically manipulate their activity in developing ferrets. While interneuron-specific promoters are in development, we are using immunohistochemical staining to visualize populations of interneuron subtypes and chart their developmental profile in ferret visual cortex. In preliminary antibody stainings, we have found distinct developmental trends for interneuron populations that express Parvalbumin (Pv), Calretinin (CR), and Neuropeptide-Y (NPY). These data will help us determine the suitable developmental time windows over which to use certain subtype-specific promoters, and also allow us to validate the efficiency and specificity of the promoter-driven expression.

Poster # 2012.109
Presenter Aaron Chevalier (Brandeis / Physics)
Title Search For Twisted Polarization in Arcsecond Scale Quasar Jets
Authors Aaron Chevalier, Michael Kosowsky, Daniel Koenigsberg, David H. Roberts, and John F.C. Wardle
Abstract The quasar 3C345 displays a seemingly singular 35 degree twist in the polarization of its kiloparsec-scale jet. Because of the vast number of quasars known, it seems unlikely that 3C345 is alone in this respect. By imaging quasars identified as having structure by Murphy, Brown & Perley (1993), and by Perley (1982), and by sampling sources from the VLA calibrator manual, we hope to find additional examples of this property that could help us to better understand the nature of quasar jets.

Poster # 2012.110
Presenter Kyle Van Gorkom (Brandeis / Physics)
Title A New Angle on Quasars: Images from the Young Universe
Authors Kyle Van Gorkom, John F.C. Wardle, and Andreas P. Rauch
Abstract This project studies 129 high redshift quasars—some of the most luminous, distant and youngest objects in the universe. In particular, we are investigating indicators of the orientation of the quasar to the line of sight. The appearance of the quasar changes dramatically with orientation, and we need to determine the orientation to determine the intrinsic properties of the quasar. We make use of radio images from the Very Large Array and optical data from the Sloan Digital Sky Survey.
**Poster # 2012.111**

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

**Title** Site-directed mutagenesis of DNA replication initiator, DnaA

**Authors** Daniel Boyle, Vincent Anthony Sutera, and Susan Lovett

**Abstract** Understanding bacterial replication and how it affects both DNA damage and its repair provides core information of replication in general and is one key towards the development of new antibiotics. Our study focuses on the *E. coli* gene DnaA because it is initiator of replication and is highly conserved among bacteria and many archaea. The goal of this project was to create expression plasmids encoding three separate mutant alleles of 6-Histidine-N-terminal-tagged DnaA and then to purify the resulting recombinant proteins for biochemical analyses. Two mutants, W6A and A154D were produced by site-directed mutagenic PCR and assayed for complementation with a chromosomal DnaA mutant under a variety of conditions that would induce damage. Pilot purifications of the wildtype and A154D mutant by nickel ion column were successful, but due to time constraints we have not yet been able to scale up the purification. The third mutation, R334A, has not been generated due to a rise of suppressor mutations. To reduce the selective pressure against the mutation, I will attempt further constructions in an *E. coli* strain that replicates independent of DnaA. After R334A is generated, purifications can be scaled up and substrate binding and activity assays can be performed. The binding affinity of DnaA for its main substrates, single- and double-stranded DNA, can be studied by gel shift assays, while other properties like oligomerization and nucleotide binding can be observed via crosslinking or binding assays using labelled nucleotides.

**Support** Division of Science Summer Research Fellowship

**Poster # 2012.112**

**Presenter** Nate Shammay (Brandeis / Biochemistry)

**Title** Directly Observing Single Molecules of Lac repressor in Action

**Authors** Nate Shammay and Jeff Gelles

**Abstract** The *E. coli* Lac repressor transcriptional regulation pathway involves binding of Lac repressor to a portion of DNA called the operator. While there is a consensus that in a Lac-bound state, the DNA cannot be transcribed by RNA polymerase (RNAP), it is unknown whether the regulation mechanism involves the prevention of open RNAP-DNA complexes or prevention of closed RNAP-DNA complexes. In order to address this question, a single-cysteine construct of Lac repressor has been purified and labeled with the small fluorescent dye Tetramethyl Rhodamine (TAMRA). We have found this labeled mutant Lac repressor to be functional in both bulk and single molecule experiments and will utilize this new Lac construct to directly observe whether Lac repressor prevents open or closed RNAP-DNA complexes.

**Support** Cell and Molecular Visualization REU
**Title** Synthesis of Multivalent Ligands Designed to Disrupt HIV-1 Tat-peptide/TAR-RNA Interactions

**Authors** Shakara L. Scott, Josh Allgaier and Jason Pontrello

**Abstract** Multiple targets exist in the development of HIV-1 anti-viral drugs, one of which includes the interaction between the transcriptional activator protein (Tat) and the Trans Activation Response (TAR) element of RNA. During transcription, TAR RNA, a 59-base stem-loop structure, located at the 5’ end of all HIV-1 mRNAs recruits Tat. Prior research has shown that the arginine rich motif (ARM) of Tat is integral to the binding interaction between the Tat-peptide and TAR-RNA. In the absence of Tat, the transcription machinery is rendered inept and the polymerase releases the DNA prematurely. We sought to design synthetic polymers that would disrupt the necessary interaction between Tat and TAR-RNA, hindering HIV replication. To target the TAR-RNA, we sought to replicate the basic ARM of Tat by functionalizing the polymer scaffolds derived from the Ring-Opening Metathesis Polymerization (ROMP) with arginine molecules. A Fluorescence Resonance Energy Transfer (FRET) assay is being developed to evaluate the inhibitory effects of these multivalent arginine displays. Continued work includes optimization of the assay and synthesis of other multivalent ligands designed to target TAR-RNA.

**Support** Division of Science Summer Research Fellowship

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**Title** Synthesis of Multivalent Ligands Designed to Control Huntingtin Protein Aggregation

**Authors** Amy Dai¹*, Kaitlin Hulce²*, Charlene Liao³* and Jason K. Pontrello, (*) Authors contributed equally to the work

**Abstract** Protein aggregation in the neurodegenerative disorder Huntington’s disease is linked to excess glutamine repeats (of 36 to over 100) in the mutant huntingtin protein (mHtt) polyglutamine (polyQ) tract. Synthetic polymers displaying amino acid sidechains of various lengths are being developed to interact with the polyQ tract and an adjacent polyproline helix (polyP) such that they may control mHtt aggregation. It is hypothesized that “short” polymers will bind one protein and inhibit aggregation, while “long” polymers will bind multiple proteins and induce aggregation. Theories on glutamine’s ability to bind polyQ and tryptophan’s possible intercalating capabilities with polyP have guided us to display these protein-binding ligands on the synthetic polymer scaffolds. Preliminary data suggests longer polymers of 100mer length induce aggregation of a model polyQ15 peptide as determined by right angle light scattering. Thus far, homopolymers of 10, 25, 50, and 100 units long displaying either glutamine or tryptophan amino acid ligands were prepared using N-hydroxysuccinimide ester-substituted polymers generated from a norbornene monomer. Methods were also pursued to synthesize block displays of multivalent ligands utilizing an amine reaction with isothiocyanate to generate a thiourea linkage of ligand to polymer scaffold. Work is ongoing to further optimize yield of the isothiocyanate product from tryptamine, a derivative of tryptophan. In future experiments, the polyQ15P6 peptide model will be utilized in the light scattering assay to test the ability of tryptophan ligands to aggregate peptides displaying the polyP sequence. In addition, the block vs random copolymer displays of targeting ligands will be evaluated in the assay utilizing the polyQ15P6 peptide.

**Support** (1) Physical Science Scholars, (2) Division of Science Summer Research Fellowship, (3) Jordan-Dreyer Summer Research Assistantship
Characterizing the effect of ATP concentration on the \textit{in vitro} dynamics and self-assembly of microtubules

**Abstract** \textit{In vivo}, microtubules are the fundamental components of the eukaryotic cytoskeleton, providing cells with structural support and playing vital roles in both intracellular transport and cellular division. Much less is understood, however, about the behavior of microtubules outside of their natural cellular environments and their potential value as an amenable biomaterial. We are researching the effect of ATP concentration on \textit{in vitro} samples of microtubules that have been embedded with tracer particles, used to track the mixing and flow of the microtubule network. We are using quantitative analysis of the particles' trajectories and displacements, based on the prominent theories in physics of diffusion and Brownian motion, to allow us to explain qualitative observations of the microtubule samples and draw conclusions about their behavior; our analysis provides insight on the change in microtubule dynamics before and after a critical ATP concentration and the effect on network arrangement. In addition, we employed a simulation program to examine the very fundamentals of microtubule self-assembly. Developing an understanding of these \textit{in vitro} dynamics may eventually lead to the applications of microtubule biomaterials in medicine, for instance for drug delivery, and in science. Understanding the mechanisms of cell behavior, beginning with basic components like microtubules, will help scientists explain more complex functions that are less understood.

**Support** MRSEC
Analysis of candidate ciliary genes in *C. elegans* sensory neurons

**Title** Analysis of candidate ciliary genes in *C. elegans* sensory neurons

**Authors** Stephen Caron, Inna Nechipurenko, and Piali Sengupta

**Abstract** Primary (non-motile) cilia are microtubule-based organelles that project from the surface of most mammalian cells. They have recently emerged as complex signaling centers that sense and transduce diverse developmental and environmental stimuli. Defects in cilia structure and function result in aberrant cellular sensitivity to environmental cues and are associated with many human diseases, collectively known as ciliopathies (Waters and Beales, 2011). Here we examined several candidate ciliary genes for roles in regulating two aspects of ciliogenesis – dye-filling and cilia morphology – in *C. elegans* sensory neurons. We found that mutations in two genes – *khc-1* and *rab-28* – encoding the kinesin-1 motor domain and a small GTPase, respectively, disrupt cilia morphology of the AWB sensory neuron. We will present our ongoing efforts to characterize the function of these genes in sensory cilia development in *C. elegans*.

**Support** Cell and Molecular Visualization REU

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Exploring the Phase Spaces of Active Microtubule Bundles

**Title** Exploring the Phase Spaces of Active Microtubule Bundles

**Authors** Gilead Henkin, Stephen J. DeCamp, Timothy Sanchez, and Zvonimir Dogic

**Abstract** Self-propelled particles that assemble into organized groups are examples of active matter. Active matter can include flocks of birds, schools of fish, or in this case, microtubules moved by kinesin motors. Previous experiments have shown that mixtures of microtubules and kinesin motors have shown overall mixing behavior. Kinesin motors provide sliding forces that drives the extensile behavior of the microtubules, and polyethylene glycol (PEG) provides a depletion force that bundles microtubules together. The exact effect that PEG and kinesin have on the resulting sliding dynamics is unknown and it is the goal of this work to map the phase space of these two ingredients.
**Poster # 2012.119**

**Presenter** Andrew Schultz (University of Massachusetts at Amherst / Biochemistry)

**Title** Investigating Secretion of the Amyloid Precursor Protein (APP) in Exosomes Using Cultured *Drosophila melanogaster* S2 Cells

**Authors** Andrew Schultz, Agata Becalska, and Avital Rodal

**Abstract** The transmembrane Amyloid Precursor Protein (APP) was initially discovered by its amyloid-β peptide (Aβ) form, a component of the plaques found in those suffering from Alzheimer’s disease (AD). Recent studies have focused on the secretion and trafficking of APP throughout the brain during AD pathogenesis. APP has been observed in endosome derived vesicles called exosomes but the mechanism of their secretion or their relevance to disease are poorly understood. Our primary question was to determine if *Drosophila melanogaster* S2 cells could be used as a model system in studying APP exosome secretion. S2 cells were transfected with human APP tagged at its C-terminal end with a green fluorescent protein (GFP) as a means of studying exosomal biogenesis and transfer using fluorescence microscopy. Green puncta were observed in mCherry expressing cells incubated with conditioned medium from Human APP-GFP-expressing S2 cells. Similar results were obtained from co-culturing APP-GFP and mCherry expressing S2 cells. Furthermore, with differential centrifugation we showed that APP cosediments with the predicted exosome fraction. Surprisingly this fraction contained APP recognized by a C-terminal antibody suggesting the packaging of specific fragments of APP into exosomes. Future directions will include additional differential centrifugation experiments with optimized antibodies against APP and exosome markers as well as gradient fractionation experiments to confirm the identity of APP-containing vesicles.

**Support** Cell and Molecular Visualization REU

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**Poster # 2012.120**

**Presenter** Olivia Levine (Brandeis / Biology, Neuroscience)

**Title** Insight into the Role of KCNQ1 and KCNE1 Coupling on Voltage Sensor Movement of the Iks Channel

**Authors** Olivia Levine, Hui Dai, Dahzi Xiong, Leigh Plant, and Steven Goldstein

**Abstract** The Iks potassium channel is a delayed rectifier channel that is essential for normal cardiac function. The channel consists of the α-subunit (KCNQ1), a homotetramer with a central pore domain, and an accessory β-subunit (KCNE1). Mutations in the gene encoding for KCNQ1 have been implicated in human diseases such as Long QT syndrome (LQTS) and Short QT syndrome (SQTS). The interaction between the α- and β-subunits forms the characteristic Iks current, but the mechanism by which this occurs is poorly understood. It has been shown that KCNE1 alters the voltage sensor movement of KCNQ1, significantly slowing its activation kinetics. To further understand the coupling mechanism between KCNQ1 and KCNE1, we will evaluate Iks currents in Wild-Type (WT) and mutant KCNQ1 using two-electrode voltage clamp (TEVC). This will provide insight into the mechanism by which Iks channel kinetics contribute to human heart disease.

**Support** Cell and Molecular Visualization REU
Title Bud6 coordinates polarized actin cable assembly and maintenance of a mother/bud membrane diffusion barrier through its interactions with formins

Authors Jessica Pullen, Brian Graziano, and Bruce Goode.

Abstract Cell polarity in *S. cerevisiae* depends on formin-mediated nucleation of actin cables required for transport of secretory vesicles to the bud tip, and on septin structures at the bud neck, which are required to maintain membrane diffusion barriers between the mother and bud. Our lab recently reported that the cell polarity protein Bud6 serves as an actin nucleation-promoting factor (NPF) for one of the two yeast formins, Bni1, which is located at the bud tip (Graziano *et al.*, 2011; Mol Biol Cell). Other groups have shown that Bud6 is essential for maintenance of an endoplasmic reticulum (ER) membrane diffusion barrier located at the bud neck (Luedeke *et al.*, 2005; J Cell Biol), but whether there is any connection between these two seemingly distinct functions of Bud6 has remained unknown.

Here, we used a genetic approach to explore the potential role of Bud6 in regulating the bud neck formin, Bnr1, and to determine whether there might be a functional connection between its roles in actin cable assembly and maintenance of the ER membrane diffusion barrier. Genetic analyses using bud6 alleles defective in NPF activities in a bni1Δ background showed that Bud6 indeed serves as an NPF in vivo for the bud neck formin Bnr1. Further, these results are supported by strong biochemical evidence obtained outside of this summer project (B. Graziano, unpublished data). Together, these findings show that Bud6 is required in vivo for normal levels of actin cable assembly by the bud neck formin Bnr1.

To test whether Bud6 NPF activities on Bnr1 might also have a role in maintaining the diffusion barrier at the neck, I used FLIP (fluorescence loss in photobleaching) to compare diffusion rates of Sec63-GFP, an integral ER membrane protein, in wild-type and bud6 mutant cells. My analysis revealed that integrated point mutants that specifically impair Bud6 NPF activity are as defective in maintaining the mother/bud ER diffusion barrier as bud6Δ.

Collectively, my data show that Bud6 stimulates Bnr1-mediated actin cable assembly at the bud neck, and that this function is closely integrated with its previously described role in maintaining the mother/bud ER membrane diffusion barrier. How these functions might be tied together is discussed, and will be experimentally addressed in the remainder of my senior honors thesis project in 2012-2013.

Support Cell and Molecular Visualization REU
Poster # 2012.123

Presenter Michal Price (NAU-Yuma / Biology)

Title Constructing an infectious clone of CrPV and A novel method to eliminate persistent infections from Drosophila cell culture

Authors Michal Price, Michael Spellberg, and Michael T. Marr II

Abstract Cricket paralysis virus (CrPV) is assigned to the order of Picornaviridae. CrPV broadly infects insects and could be used as a safe ecological friendly insecticide for crops. For this reason, understanding their biology is necessary. The goal of this project was to measure the levels of infectious viral particles of CrPV in Drosophila S2 cells upon transfection with a vector expressing the viral genome. Several segments of the viral genome have been amplified from cDNA for cloning. As the method of viral replication and lethality of CrPV is better understood, it may become useful as a biosafe insecticide.

As organisms evolve defenses against viruses, viruses evolve mechanisms to evade those defenses. Viruses have developed specific inhibitors of the RNAi pathway used by invertebrates to fight viral propagation. We utilized the Drosophila S2 cell line which has a low, but persistent viral infection. Persistent infections may confound studies of viral immunity in cell culture. In order to remove the virus we attempted to eliminate the protein that inhibits the RNAi pathway, the Flock House Virus B2 protein, and subjecting cells to 4 hour starvation. By doing this assay it was found that the levels of the viral genome were not reduced. Although this assay did not reduce the viral particles in cell cultures we will next attempt to over express Dcr-2 or otherwise augment its activity to enhance its known anti-viral effects

Support Cell and Molecular Visualization REU

Poster # 2012.124

Presenter Justin Roncaioli (University of Rochester / Biochemistry)

Title Structural Determination of the Yeast U1 snRNP

Authors Justin Roncaioli, Clarisse van der Feltz, and Daniel Pomeranz Krummel

Abstract The yeast S. cerevisiae U1 small nuclear ribonucleoprotein (U1 snRNP) binds to the 5’ splice site of pre-mRNA and initiates assembly of the spliceosome for excision of noncoding intronic sequences. Yeast U1 snRNP (~800 kD) consists of 1 snRNA molecule and 17 proteins. We lack detailed knowledge of its three dimensional structure. Creating a model of its structure is challenging, in part due to its large size large. We are fusing a 10x Histidine tag to the N or C terminus of various yeast U1 snRNP proteins. These tags bind conjugated gold nanoparticles that are easily visible by electron microscopy (EM). Using EM images of these labeled proteins within the complex and structural knowledge of its smaller human counterpart, we hope to create a 3D model of the yeast U1 snRNP. Such a structural model will provide insight into yeast U1 snRNP structure and function, spliceosomal assembly, and the nature of alternative splicing factors in higher eukaryotes.

Support Cell and Molecular Visualization REU
Title  Towards "One-Pot" Manufacture of COC Microfluidic Devices  

Authors  Ariel S. Hyre, Dongshin Kim, and Seth Fraden  

Abstract  Cyclic olefin copolymers (COC) have been considered as novel materials for the creation of optically superior and chemically inert microfluidic devices. A simple and low-cost manufacturing procedure for COC-based chips has been explored, focusing on three key steps: creation of appropriate polymer sheets; hot embossing; and lidding. A heated press assembly was constructed to allow for the mechanical equivalent of a "one-pot reaction" for the creation of COC-based microfluidic devices.

Support  MRSEC

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Title  Establishing Paramecium as a Cilia Model Organism: The Beginning  

Authors  Ellie Kendl, Meaghan Molloy, Thomas Heuser and Daniela Nicastro  

Abstract  Cilia and flagella (cilia from here on) control a multitude of different functions in the human body, and the malfunctioning of these cilia can cause various diseases. In order to treat the causes of these diseases, we must understand the structure and function of cilia. Targeted gene modifications are difficult in currently used model organisms. Paramecium tetraurelia can be easily genetically modified through use of RNAi, and we propose to use cryo-electron tomography (cryo-ET) to study the 3D structure and function of the cilia in this model organism. An efficient deciliating technique is a necessary first step, and we have analyzed various techniques that have been used in the literature. We found that the deciliating agents BaC\textsubscript{12} and CaCl\textsubscript{2}, from preliminary analysis, are better than the others. We also established ways to monitor the efficiency of these techniques, including stress response, cell viability and swimming patterns.

Support  Cell and Molecular Visualization REU
**Poster # 2012.127**

**Presenter** Raul A Ramos (Texas A&M International University / Biology)

**Title** Locomotor Tracking and Sleep Analysis of Micro RNA Library

**Authors** N.C. Donelson, R.A. Ramos, J.B. Slawson, E.Z. Kim, and L.C. Griffith

**Abstract** MicroRNAs (miRNAs) function as translational repressors by pairing with partially complimentary 3′ untranslated regions (3′ UTRs) of target messenger RNAs (mRNAs). In doing so, miRNAs down regulate gene expression and have been proven to effect physiological responses, developmental processes and pathological conditions. The best method to analyze the effects of a family of miRNAs is to create a loss of function. Currently, three methods exist for creating a loss of function. These 3 methods include antisense oligonucleotide inhibitors, genetic knockouts, and the microRNA sponges. MicroRNA sponges function as a decoy target for a miRNA seed family, effectively depressing miRNA targets for an entire family. This enables them to be used in loss-of-function assays. This study examines the effects of miRNAs on locomotion using the microRNA sponges in *Drosophila melanogaster* and the Gal4/UAS system for targeted gene expression. A UAS library of miR lines was created and expressed using Tubulin-Gal4. We conducted a screen of all of the available miR sponge lines using a computer program to track locomotion and analyze data for abnormalities in the movement of flies. Our analysis isolated 15 locomotor deficient lines for further investigation. Future implications of this research can lead to a better understanding of the underlying mechanisms for locomotion in *Drosophila melanogaster*, and potentially humans.

**Support** Division of Science

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**Poster # 2012.128**

**Presenter** Elizabeth Allen (Brandeis / Neuroscience, Classical Studies)

**Title** Investigating the importance of Nek2 and Nek6 in synapse formation

**Authors** Elizabeth Allen, Marissa Kuzirian, Katelyn Kenny, and Suzanne Paradis

**Abstract** The molecular mechanisms that underlie the formation and development of connections between neurons in the brain, called synapses, are not well understood. Proper synapse formation is essential for perception, motor processing, memory, and cognition (McAllister 2007). In order to start to comprehend the biological pathways associated with synapse formation, our lab sought to identify the specific protein kinases involved in this development by conducting an RNAi screen. From this screen, a number of protein kinases were identified as necessary for synapse formation. Interestingly, many NIMA (Never in Mitosis A)-related kinases, specifically the NEK family, are essential. It is my goal to verify that the synaptic phenotypes that were observed in the initial RNAi screen are in fact due to the loss of Nek gene expression. I will focus on two of the NEK family members, Nek2 and Nek6. I used previously-designed hairpins to knockdown Nek2 gene expression and rescue constructs to restore Nek2 gene expression. All experiments were performed in cultured rat hippocampal neurons, then confocal images taken to visualize synapses and synapse density measured. Concurrently, I am designing hairpins and rescue constructs to knockdown and rescue Nek6 gene expression. It is my goal to verify that Nek2 and Nek6 are necessary for synapse formation in cultured hippocampal neurons.

**Support** Division of Science Summer Research Fellowship
Selective Spin Labeling of *E. coli* GMP Reductase for Field-Cycling NMR

**Abstract**

Guanosine monophosphate reductase (GMPR) plays an important role in the conversion of nucleoside and nucleotide derivatives of guanine to adenine nucleotides. It is proposed that this reaction follows a two-step mechanism, of which the first step is a deamination reaction and the second step is a hydride transfer. Inosine monophosphate dehydrogenase (IMPDH) performs a similar reaction to convert IMP to XMP utilizing analogous residues and an identical covalent intermediate, E-XMP*. Both reactions involve a conformation change: a mobile protein flap is mobile in the IMPDH reaction and the cofactor, NADPH, moves in the GMPR reaction. Our goal is to study the dynamics of the NADPH molecule in each of the steps of the GMPR reaction. High resolution field-cycling $^{31}$P NMR will be used in conjunction with spin labeling to learn more about the role of NADPH. However, *Escherichia coli* GMPR contains seven cysteine residues, and so isolating a single cysteine residue for spin labeling while protecting the active site cysteine proves difficult. Here, we use cysteine modification methods to discover which cysteine residues are exposed and buried the *E. coli* GMPR in order to selectively spin label one cysteine residue. By reacting GMPR with Ellman’s reagent to modify the reduced cysteine residues and monitoring its subsequent activity, it was shown that two cysteine residues are exposed in the protein’s natural fold. It proved to be difficult to determine which cysteine residues were labeled through modification with Ellman’s reagent and 4-vinylpyridine. For more precise determination of which residues were exposed, modification with N-ethylmaleimide followed by iodoacetamide was used. The samples were analyzed using mass spectroscopy and results are forthcoming.

**Support**

Hedstrom Laboratory

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Micro-phase Separation in Bi-Disperse Colloidal Membranes

**Abstract**

We self-assemble membrane-like structures using a mixture of rod-like viruses and a depletion agent. We show that colloidal membranes composed of bi-disperse viruses micro phase-separate into domains rich or poor in longer viruses. The phase diagram of this system was explored at various stoichiometric ratios of the two kinds of viruses and at various depletant concentrations. At lower ratios of long to short rods, phase separation occurred in the form of domains similar to lipid rafts, while at higher concentrations the colloidal membranes were homogenous. Future studies will explore the effects of chirality and charge density on this phase behavior.

**Support**

MRSEC
**Poster # 2012.131**

**Presenter** Meaghan Molloy (University of Massachusetts Amherst / Chemistry)

**Title** Cryo-Electron Tomography of *Paramecium tetraurelia* Axoneme

**Authors** Meaghan Molloy, Ellie Kendl, Thomas Heuser, and Daniela Nicastro

**Abstract** Cilia are highly conserved biological nano-machines found across many species from algae to humans. Cilia are present on almost every cell in the human body and perform a variety of crucial functions ranging from the sense of smell to the filtration of blood in the kidneys. Defects in cilia cause a broad spectrum of human diseases. Contrary to currently used model organisms which have poor genetic tools for targeting specific cilia genes, *Paramecium tetraurelia* allows straightforward gene modification utilizing RNAi. Thus we are introducing them as a model organism in our laboratory to study the structure and function of cilia. We successfully isolated cilia and axonemes (demembranated cilia) using a combination of a cold and calcium shock. We then visualized the sample with two different microscopy techniques. Chemical fixation with uranyl acetate allowed for a quick assessment of the sample by traditional electron microscopy. However to visualize the three dimensional ultrastructure as close to the native state as possible, we prepared samples for cryo-electron tomography. As these tools have been established, future studies can focus on the important relationship between structure and function of specific cilia proteins by utilizing RNAi and cryo-electron tomography in tandem.

**Support** MRSEC

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**Poster # 2012.132**

**Presenter** Allison Matthews (Mount Holyoke College / Biochemistry)

**Title** How does CDC5 overexpression affect the DNA Damage Checkpoint?

**Authors** Allison Matthews, Vinay Eapen and James Haber

**Abstract** DNA is essential to life, but it is subject to damage from interaction with various chemicals and environmental stressors. The induction of DNA damage activates the DNA damage checkpoint. The purpose of this G2/M checkpoint is to allow the cell time to repair the DNA, in order to preserve genome stability and ensure the stable transmission of genetic information. Loss of this checkpoint leads to an increased likelihood of carcinogenesis due to decreased genome fidelity.

The DNA damage checkpoint was studied in budding yeast by inducing an irreparable double stranded break in the DNA with HO endonuclease. Wild type cells adapt to this break within 24 hours, while mutation of several proteins involved in checkpoint de-activation and signaling cause the cells to be adaptation deficient, or unable to turn off the checkpoint. Previous studies have shown that the polo kinase Cdc5 promotes adaptation to the checkpoint and decreased Rad53 hyperphosphorylation. We have found that overexpressing CDC5 rescues some adaptation defective cell lines, and shortens the checkpoint arrest, showing that CDC5 may be involved in termination of the DNA damage checkpoint.

**Support** Cell and Molecular Visualization REU
Poster # 2012.133

Presenter John Taylor Chavis III (University of Maryland, Baltimore County / Chemistry)

Title Dynamical Behavior of Pulse-Coupled Chemical Oscillators: Asymmetric Coupling Strengths

Authors John Taylor Chavis III, Viktor Horvath, and Irving Epstein

Abstract Studies of coupled chemical oscillators typically employ systems in which coupling is realized by continuous mass exchange. In many biological systems (e.g., neural networks, fireflies), however, oscillators are coupled by short pulses, often with a time delay. A system of two pulse-coupled Belousov-Zhabotinsky oscillators with matching initial conditions and coupling strengths has been previously studied. However, the occurrence of matching conditions in nature is not likely, therefore we investigated a system where the coupling strengths differ significantly. Here we demonstrate our latest results which confirm that activatory coupling gives rise to synchronization even if the coupling strengths are unequal. In addition to this, we found that asymmetric inhibitory coupling results in out of phase and resonant oscillations in this system if moderate coupling strengths are used.

Support MRSEC, HHMI EXROP, MARC U*STAR at UMBC

Poster # 2012.134

Presenter Helen Stolyar (Brandeis / Chemistry)

Title Cycloaddition of Nitrile Oxides and Bishomoallylic Alcohols

Authors Helen Stolyar and Isaac J. Krauss

Abstract The Krauss lab has recently developed methods of homoallylation. As part of this ongoing project, we have explored a potential use for the bishomoallylic alcohols produced by those methods. Research conducted by Carreira and Lohse-Fraefel, in which they developed a method for hydroxyl-directed, diastereoselective cycloaddition of nitrile oxides and homoallylic alcohols, indicated that 3+2 cycloadditions could potentially work for bishomoallylic alcohols under optimal conditions. Expanding Carreira’s method to cycloadditions with bishomoallylic alcohols broadens the scope of polyketide building blocks that can be created using our homoallylation method. Several Lewis acids were tried, and DiBAl-H seemed to work best, producing only one diastereomer and no byproducts. We hope that this method will eventually be effective for a variety of nitrile oxides and bishomoallylic alcohols.


Support Jordan-Dreyer Summer Research Assistantship
The Role of H-NS Proteins in DNA condensation and Transcriptional Regulation of *Escherichia coli*

Laura Swanson, Christopher S. Weitzel, and Susan T. Lovett

When studying bacterial responses to the environment, one important element to investigate is the cell’s nucleoid structure. It has been found that these structures often vary in response to the levels of available nutrients. The stringent response in bacteria is one such example. The stringent response is activated when the bacteria are deprived of nutrients and is signaled by the accumulation of the small nucleotide ppGpp. Bacterial chromosome structure can also be dictated by nucleoid binding proteins, for example H-NS. H-NS is an abundant DNA binding protein that has recently been discovered to be both necessary and sufficient for the compaction of DNA into a toroid shape. To gain insight into the mechanism of H-NS mediated nucleoid organization, several plasmid borne mutants were constructed. Upon overexpression, these proteins were found to affect the compaction and localization of the *E.coli* chromosome. The assembly of an H-NS protein with a non-aggregating fluorescent protein tag was also attempted in order to better determine what role these proteins play and whether or not they specifically localize during compaction.

Support Cell and Molecular Visualization REU

Homomethyallylation of Aldehydes and Homoallylboration of Imines

Stephanie Chun and Isaac Krauss

While there are several known methods for stereoselectively allylating carbonyls and related compounds, the number of homoallylation reactions, with one more carbon in the chain, is significantly smaller. Thebishomoallyl products are more complex than their allylation counterparts, and can potentially add up to three stereocenters. Our group previously developed cyclopropylcarbinyl boron reagents to homoallylate and homocrotylate aldehydes, and here, we modify the cyclopropylcarbinyl boronate to add a methyl delta to the hydroxyl in the product alcohol. We have obtained some of the desired homoallylated alcohols, but also the undesired methylcyclopropylcarbinyl transfer product. In a related research area, we are attempting to expand the reaction’s substrate scope to imines, producing bishomoallyl amines. As of this writing, the aluminum imines used have not reacted with the boronate. We are continuing to test a wider variety of reaction conditions to investigate whether we can favor the correct homoallylation reaction and easily synthesize bishomoallyl amines.

Support Jordan-Dreyer Summer Research Assistantship
**Poster # 2012.137**

**Presenter** Brian Williams (Brandeis / Chemistry)

**Title** Discovery of small molecules capable of stabilizing DJ-1, a protein involved in Parkinson disease

**Authors** Brian A. Williams, Joseph P. Salisbury, Jared R. Auclair, Jeffrey N. Agar

**Abstract** Recessive mutations in the gene PARK7, which encodes for the homodimeric protein DJ-1, are associated with Parkinson disease (PD). A number of these mutations, as well as oxidative damage, interfere with the protein’s stability. We hypothesized that chemical warheads targeting a specific DJ-1 residue would stabilize the protein (chemical names and target are being withheld until a provisional patent is filed). In a mass spectrometry-based screen, we identified two compounds that bound this target specifically, and that they also increased the thermostability of wild-type DJ-1. We have undertaken expression and purification of variants of DJ-1 in order to determine whether the identified chemicals can restore stability and confirm the site of their reaction. Chemicals capable of stabilizing DJ-1 may provide a potential strategy for developing therapeutic agents against PD.

**Support** Jordan-Dreyer Summer Research Assistantship

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**Poster # 2012.138**

**Presenter** Matthew J. Zunitch (Brandeis / Biology, Neuroscience)

**Title** Cellular Mechanisms for Packaging the Amyloid Precursor Protein into Exosomes

**Authors** Matthew J. Zunitch, Christina Luo, and Avital Rodal

**Abstract** Exosomes are small (50-100 nm in diameter) membrane-bound compartments that are released from cells containing cargo relevant for cell signaling and survival. We have observed that the Amyloid Precursor Protein (APP) is secreted from *Drosophila* motor neurons in exosome-like compartments. These exosomes are hypothesized to be diffusible carriers of the toxic proteolytic product of APP in Alzheimer’s Disease (AD), but this has yet to be demonstrated in intact animal nervous systems. Using the *Drosophila* model we have manipulated membrane traffic via mutations in Rab11, Shibire/dynamin, and the ESCRT and Retromer complexes. We have also tested the role of BACE1, a protease involved in toxic APP processing in AD, in APP exosome formation. We examined the effects of these mutants on APP localization and APP-containing exosome secretion in the larval neuromuscular junction (NMJ) by imaging NMJs using confocal light microscopy and subsequently quantifying fluorescence. Preliminary results indicate that both exosome-related membrane trafficking machinery and APP processing proteases regulate APP exosome formation and/or contents. In the immediate future more NMJs will be imaged and processed in order to elucidate whether other membrane trafficking proteins act as regulators along the APP exosome secretion pathway and could be potential points of therapeutic intervention in Alzheimer’s disease.

**Support** Division of Science Summer Research Fellowship
Adenylate Kinases (ADKs) play an important role in the maintenance of cellular energy homeostasis by catalyzing the reversible phosphoryl-transfer reaction that interconverts ATP and AMP to ADPs. Our studies of two ADK isoforms (thermophilic and mesophilic) showed that they have significantly different catalytic activities despite having similar active sites. This difference can be explained by the effect of second shell residues located outside of the active site. To test this hypothesis, we replaced the residue 138 in thermoADK (aADK) with corresponding residue from mesoADK (eADK). Through kinetic assays, we found that the Y138F mutant of aADK is 20% faster than wild-type aADK. Although the effect of the mutation on the catalytic efficiency of ADK was in the direction we predicted, its magnitude was less than expected. Consequently, Y138 is unlikely to be the only significant second shell residue in the enzyme. The overall difference in activity may also be due to the evolutionary factor of thermo-stability of each enzyme since ADK function is well buffered for the entire range of temperatures its respective organism may experience in nature. We determined the enthalpic and entropic contributions to the energy for phosphoryl transfer for the different isoforms by measuring the temperature dependence of the catalysis. Our preliminary data indicate that ADKs decrease their enthalpic and increase their entropic contributions in the catalysis as they evolve with the Earth in time. To further advance our understanding of the relationship between efficiency of catalysis and evolution of ADKs, we constructed a phylogenetic tree from known ADKs in different species based on sequences and structural information. As ADK are important in maintaining cellular homeostasis, it is no surprise that they are present in humans as well. Although seven human ADK isoforms are found to date, very little mechanistic studies have been done on them, especially with the more recently discovered AK 7 and AK 8. We began to isolate and purify human ADK isoform 7 in hope to do mechanistic studies on it in the future. Understanding the phosphoryl-transfer mechanism in human ADK can further the evolution studies of how this ubiquitous protein evolves throughout the Earth’s history.
Beat Frequency in *Chlamydomonas reinhardtii* Under Red and Green Light

**Title**: Beat Frequency in *Chlamydomonas reinhardtii* Under Red and Green Light  

**Authors**: Shani Aharon, Rebecca Cohn, Arvind Gopinath, and Azadeh Samadani  

**Abstract**: The motion of the green alga *Chlamydomonas reinhardtii* is driven by two cilia, cis (c) and trans (t), distinguishable based on their proximity to the eyespot. In the absence of an external light gradient, *Chlamydomonas* exhibits a helical motion, which consists of periods of long runs, where the two cilia beat in synchrony and short bursts of asynchronous tumbles. *Chlamydomonas* displays phototactic behavior, maximally toward green light of wavelength 500 nm. The goal of this project is to understand how the beating pattern of the two cilia, and therefore their run-and-tumble behavior, is modified during phototaxis. To this end, we have immobilized the cells in a glass channel and visualized their cilia using high speed and high resolution, microscopy. We have analyzed the beating frequencies of the c and t cilia in 18 individual cells using an in-house image processing code. In this poster, I present our quantitative measurement of the individual frequencies, relative frequencies and synchronicity between cilia under red or green light. Our preliminary results indicate that the beat frequency of a single cilium changes slightly as a function of time. This inherent temporal variability in the beat frequency leads to periods of synchrony followed by asynchronous beat pattern. Under green light the beat frequency of the c cilium is consistently lower than the t cilium. There is no such clear pattern under the red light. The variability in the mean beat frequency of c vs. t cilia is much greater among different cells, than the temporal variation of the same variable in one cell. Further analysis will reveal the frequency and duration of runs (synchronous beat pattern) vs. tumbling (asynchronous beat pattern) under red vs. green light.

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Identifying substrate specifying residues in Lactate Dehydrogenase of *Plasmodium falciparum*

**Title**: Identifying substrate specifying residues in Lactate Dehydrogenase of *Plasmodium falciparum*  

**Authors**: Joseph R. Jacobowitz, Jeffrey I. Boucher, and Douglas L. Theobald  

**Abstract**: Following gene duplication events, enzymes are capable of evolving novel functions. One example of this is the evolution Lactate Dehydrogenase(LDH) after the duplication of the Malate Dehydrogenase(MDH) gene in the Apicomplexa phylum. Despite their common ancestor, it has been shown that both modern LDH and MDH in *Plasmodium falciparum* maintain high levels of specificity towards their respective substrates. Investigations have suggested that the mutation of an Arginine to a Lysine in position 102 and a six residue insertion are largely responsible for the shift in specificity over time. Our study focuses on the identification of the amino acids within the six residue insertion that have the most influence over LDH’s function by individually mutating residues 89-93 to Alanines. While the $k_{cat}$ values for mutants S89A, D90A, and K91A do not deviate remarkably from the wild-type LDH, E92A shows a 74% increase and W93A shows over a 99% decrease in turnover number. When examining the $k_{cat}/k_m$ it is notable that W93A also showed a 99% decrease in substrate specificity. The profound effect of mutation on Tryptophan 93 appears to be consistent with the residue’s high level of conservation throughout modern Apicomplexan LDH. As our investigation progresses, we plan to mutate the insertions flanking residues so as to characterize their effect on the LDH’s function and specificity.
**Poster # 2012.142**

**Presenter** Alexandra De Denko (Brandeis / Chemistry)

**Title** Synthesis and Investigation of Heterobimetallic Zr/Co Complexes with mixed Phosphinoamide Donor Sets

**Authors** Alexandra De Denko, Subramaniam Kuppuswamy and Christine M. Thomas

**Abstract** Recently, our group has investigated a series of heterobimetallic Co/Zr complexes and the effect of dative metal-metal interactions in these complexes on redox potentials and reactivity toward small molecule activation. These previously studied complexes are comprised of three identical phosphinoamide linkers to provide a three-fold symmetric scaffold. Different phosphine and amide substituents have been found to lead to dramatically different electronic properties and reactivity. We have now turned our attention to mixed donor sets and will discuss the effects of such substituent variation on metal-metal interactions and reactivity.

**Support** Jordan-Dreyer Summer Research Assistantship

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**Poster # 2012.144**

**Presenter** Abigail Zadina (Brandeis / Neuroscience)

**Title** Transcriptional profiling of neurons specific to circadian rhythms in Drosophila

**Authors** Abigail Zadina, Kate Abruzzi, and Michael Rosbash

**Abstract** Circadian rhythms are free-running oscillations that occur every 24 hours and serve to synchronize physiological and behavioral processes to external changes in the environment. In fly brains, the ventral lateral neurons (LNvs) and dorsal lateral neurons (LNds) are specific to circadian function and help to maintain behavioral activity patterns. To better characterize the involvement of these cells in circadian function, we manually sorted these cells from dissociated fly brains and made cDNA libraries from cell-specific RNA collected at two time-points. Preliminary data confirms that the sorted LNvs are enriched for transcripts specific to this cell type. Future experiments, involving next-generation sequencing, will help elucidate what processes may be under circadian control in these cells.

**Support** Computational Neuroscience Traineeship

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**Poster # 2012.145**

**Presenter** Adam Drake (Brandeis / Biochemistry)

**Title** Estimation of an Alpha-Helical Transmembrane Protein Replacement Matrix that Improves The Inference of Transmembrane Protein Phylogenies

**Authors** Adam Drake, Marcus Kelly, and Douglas Theobald

**Abstract** We present an amino acid replacement matrix that better models the evolution of alpha-helical transmembrane proteins than previously published matrices. Our matrix shows that the incorporation of structural information into the data improves the fit of transmembrane protein phylogenies to the data that was used to estimate them. Further, large differences in our inferred tree topologies implies that our replacement model reveals that the evolutionary process for transmembrane proteins is distinct from that of soluble proteins. Our method incorporated a hierarchical Bayesian method for model inference that differs from the conventional methods of rate matrix estimation. However, our method estimates a rate matrix with significant improvements over rate matrices inferred with the same data but using previously published methods. We measured the performance of our amino acid matrix by comparing phylogenies estimated with it to phylogenies estimated with other replacement matrices.
Title Insights into the activation mechanism of Aurora A Kinase

Authors Nadja Kern¹, Vanessa Buosi¹, Chris Wilson¹, Adelajda Zorba¹, Angela M. Gronenborn², and Dorothee Kern¹ (1) Dept. of Biochemistry, Howard Hughes Medical Institute, Brandeis University, Waltham, MA. (2) Department of Structural Biology, University of Pittsburgh, Pittsburgh

Abstract Aurora A, one of the three variants of the family of Aurora kinases, has elicited much interest in the past decade as a powerful pharmacological target for cancer treatment. Previous research has shown that the phosphorylation of at least one of two key residues in the activation segment of Aurora A is necessary for its activation. The phosphorylation rates of Aurora A kinase using three different mutants of the activation peptide as substrates, T288E, T287E, and T287ET288E, were measured in order to determine which residue on Aurora A gets phosphorylated during the autoactivation mechanism. The results confirmed that only residue T288 must be phosphorylated in order for the Aurora A kinase to be active. Due to the conclusive results on the mutants of the Aurora peptide, kinase assays were done using mutants of the Aurora A kinase itself. Results revealed that the mutant T287ET288E has a small activity (80 times slower than the wild type). This residual activity is most likely due to the fact that the glutamates mimic the electronegativity of phosphates. Although a member of the Ser/Thr family of protein kinases, the mechanism of activation of Aurora A kinase appears to differ in several aspects from the prototype of this family, cAMP-dependent protein kinase A (PKA). It is thus very intriguing to analyze how Aurora A kinase is self-sufficient in carrying autophosphorylation compared to how it phosphorylates several substrates like histone H3, Aurora A peptide, kemptide and TPX2. We used kinase assays measuring both nucleotides (ATP/ADP/AMP) and peptides (phosphorylated and non phosphorylated) concentration with both Aurora A and PKA on the different substrates. Results indicate that the nature and/or the length of the peptide largely affect the rate of the phosphoryl transfer. In an attempt to study Aurora A kinase activity in a more biological context, we then focused on its natural substrate TPX2. Literature as well as our own data prove that TPX2 locks Aurora A into its active conformation because it prevents T288 from being dephosphorylated by phosphatases. Our data shows that when no phosphatase is present, the phosphorylation rate of Aurora A peptide is much slower without TPX2 present, indicating that the affinity for Aurora A kinase is higher for TPX2 compared to Aurora A peptide. Moreover, our western blot data using an antibody that recognizes specifically the phosphorylated T288 show that despite the presence of phosphatase, TPX2 binding keeps T288 phosphorylated.

Poster # 2012.147

Presenter Adam Ollanik (Brandeis / Physics)

Title Droplet Microfluidics: Creation, Interrogation, Manipulation

Abstract Droplet microfluidics facilitates high throughput, low volume experiments for biochemical assays in an individual environment. By using a fluorescent readout the droplets are controlled and manipulated. The goal of this project is to refine, tune, and operate a single machine, created this past year by an Olin Scope team working in conjunction with the Fraden Lab, capable of achieving all of these processes. The desired capabilities are droplet creation, interrogation, and on-demand merging and sorting.

Support MRSEC
**Poster # 2012.148**

**Presenter** Padraig Niall Murphy (Brandeis / Biochemistry, Biology)

**Title** Probing the Mechanism of Phosphoryl Transfer in Adenylate Kinase

**Authors** Padraig Niall Murphy, Roman Agafonov, Lien Phung, Young-Jin Cho, and Dorothee Kern

**Abstract** Phosphoryl-transfer reactions are ubiquitous in almost all life processes including the storage of genetic information, energy transfer, and signaling, because of their ability to form high-energy, stable bonds with an enormous array of different substrates. Much research has been done to examine the mechanism in which these essential bonds form in solution, but surprisingly very little is known about how the chemical step occurs in an enzyme. Using a technique historically used to study phosphoryl transfer in solution, the manipulation of leaving-group or nucleophile activation, the character of the transition state of the phosphoryl transfer of adenylate kinase was examined. By previously establishing that mutations of arginines to lysines in the active site do not affect substrate binding, three arginine residues were mutated to lysines to discover the affect upon turnover rates. Each individual mutation alters the process of nucleophile and leaving-group activation in opposite trends (depending upon the mechanism), contingent upon the direction of the reaction. The change in the ratio between the forward and reverse reaction rates suggests that the transition state is more loose or dissociative in character, indicating a metaphosphate-like bonding scheme. On-enzyme equilibrium measurements using equilibrium quench experiments were performed to establish the validity of the turnover rates and to ensure that the mutations did not affect other aspects of the protein. With this information as well as our previous results, we have gained new insight into how adenylate kinase performs the chemical step of the phosphoryl transfer.

**Support** Howard Hughes Medical Institute

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**Poster # 2012.149**

**Presenter** Nicholas L. Medina (Brandeis / Biology)

**Title** Defining the mechanism behind sympathetic regulation of cardiomyocyte development in neonatal laboratory Rattus norvegicus

**Authors** Nicholas L. Medina, Rebecca E. Kreipke, and Susan J. Birren

**Abstract** Cardiomyocyte development is regulated by sympathetic neurons, and defining how the sympathetic nervous system regulates heart development has implications in cardiac recovery from tissue damage in the adult organ. Preliminary evidence suggests that sympathetic innervation regulates the transition of cardiomyocytes from hyperplasia (proliferation) to hypertrophy (individual cell growth) by prolonging the period during which these heart cells proliferate. We asked whether β-adrenergic signaling regulated cardiomyocyte withdrawal from the cell cycle. A series of in-vitro experiments were performed using isoproterenol (2 µM) and proprananol (10 µM) (β-adrenergic agonist and antagonist, respectively) that screened for an effect of β-adrenergic signaling on cardiomyocyte proliferation. Inconclusive results were found in cardiomyocytes 3 and 4 days in vitro (DIV) in both myocyte-only and sympathetic neuron co-cultures due to uncontrolled variability. Additionally, to screen for particular cardiomyocyte-derived factors called matrix metalloproteinases (MMP2, MMP9, and Timp1), comparative RT-PCR was used to detect levels of gene expression in cardiomyocytes at 2 and 9 DIV in myocyte-only and sympathetic co-cultures. These results were inconclusive, but hinted at differences in MMP2 expression in cardiomyocytes at 2 DIV in the different culture environments. Future experiments will continue testing levels of MMP expression and screening for other cardiomyocyte-derived factors that are regulated by sympathetic innervation during development.

**Support** Science Posse Summer Funding
Abstract

Gene targeting is a genetic technique that uses homologous recombination to change an endogenous gene. Although many studies identified key genetic requirements for gene targeting and it is being used in experiments every day to delete a gene, add a gene, or replace a gene, many aspects of the mechanism is still unknown. I have been studying one phenomenon that results from gene targeting, referred as "hit-and-run" transformatation, initially described by Kraus, Leung and Haber (2001) for budding yeast. In Kraus's paper, it has been found that some cells make plasmid-like curcular DNA during intended gene targeting. For this to occur, the targeting fragment had to begin to recombine with the target locus but somehow ended up by copying adjacent sequences, including a nearby origin of replication, and then became an autonomously replicating circle. How such circles are formed or what mechanism drives the formation of circular DNA is not well understood. In order to study more about the "hit-and-run" events, I examined gene targeting in Saccharomyces cerevisiae, in which an ade2::URA3 fragment was to delete the wild type ADE2 gene. When I performed regular gene targeting transformation using the ade2::URA3 PCR product, selecting for URA3, I observed three different results: (1) about 29% were stable Ade- Ura+ transformants that represent normal gene targeting; (2) about 67% were Ade+ Ura+ events where the URA3 sequences apparently integrated at another location by non-homologous end joining and (3) about 4% were Ade+ Ura+ transformants in which the URA3 marker could be lost by selection on 5-FOA, which is the result expected for a hit-and run event. I went further and tried to examine the FOA-resistant isolates that resulted from ade2::URA3 gene targeting, and using inverse PCR, I found out that some of these hit-and-run events were indeed circles. The sizes of the circles vary among the isolates. Further DNA sequence analysis should show how much DNA was copied and how the ends of the DNA were ligated together to form a circle.

Poster # 2012.151

Presenter Pengfei Li (U Mass Amherst / Mechanical Engineering)

Title Role of Bend-Splay Anisotropy in Structure Formation in Active Polar Fluids

Authors Pengfei Li, Arvind Gopinath, Michael F. Hagan, and Aparna Baskaran

Abstract We study a continuum model for self-propelled particles with an aligning interaction in two dimensions. This model generalizes earlier work to include anisotropy between bend and splay fluctuations and dynamic feedback between structure and activity. We numerically map out the phenomenology of the system in various regions of parameter space. New results include the existence of an oscillatory state that alternates between a striped structure and a spiral.

Support MRSEC
**Poster # 2012.152**

**Presenter** James Chin (Brandeis / Biochemistry)  
**Title** Computational docking of enzyme inhibitors  
**Authors** James Chin and Lizbeth Hedstrom  
**Abstract** Inosine monophosphate dehydrogenase (IMPDH) catalyzes the oxidation of inosine monophosphate (IMP) to xanthosine monophosphate (XMP), a critical step in guanine nucleotide biosynthesis. Due to the role of IMPDH in cell proliferation, IMPDH is an attractive target for antimicrobial drugs. Compound D73 has promising antibacterial activity, but has poor solubility and insufficient metabolic stability. As crystal structures of the IMPDH•D73 complexes are not available, homology modeling and docking simulations may provide crucial lowest energy ligand binding poses that allow for further drug optimization. Initial rigid docking of ligands into homology models of bacterial IMPDH has been an unreliable predictor of lowest energy ligand binding conformations. In the future, we will use orthogonal random walk (OSRW) free energy simulations to calculate the relative binding affinities of various analogs of D73 with improved solubility and metabolic stability.

**Support** Computational Neuroscience Traineeship

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**Poster # 2012.153**

**Presenter** Alyssa Schwartz (University of Rochester / Chemical Engineering)  
**Title** Belousov-Zhabotinsky mechanical oscillation of poly-NIPAAm network with Ruthenium Catalyst modification  
**Authors** Alyssa Schwartz, Ye Zhang, Seth Fraden, Irving Epstein, and Bing Xu  
**Abstract** The Belousov-Zhabotinsky (BZ) reaction is a self-oscillatory reaction caused by non-equilibrium thermodynamics. A hydrogel backbone was constructed of a NIPAAm monomer, a Ruthenium catalyst, and Bis crosslinker, which was exposed to light at 430nm to form particles about 600um in diameter and 80um high. Catalytic materials undergo periodic oxidation and reduction which transfers chemical energy to a mechanical oscillation. The chemical oscillations affect the mechanical change, where the gel is expanded in the oxidized state, and contracted in the reduced state, caused by changes in hydration. Ability to control the mechanical force of the gel makes it a promising candidate for creating smart materials that can walk, talk and think. These cylindrical particles had a thick layer around the radial edge and were thinner in the middle, which allowed for a volume change that is up to ten times larger than previously seen in the literature. This large volume change indicates there is a higher level of efficiency over a series of periodic shrinking and swelling in a flow cell with BZ conditions.

**Support** MRSEC
The Role Of Mph1 And Sgs1 Helicases In Modulating Gene Targeting Efficiency in *Saccharomyces cerevisiae*

**Title** The Role Of Mph1 And Sgs1 Helicases In Modulating Gene Targeting Efficiency in *Saccharomyces cerevisiae*

**Authors** Rosmel E. Hernandez, Ranjith Anand, and James E. Haber

**Abstract** Gene targeting is a technique for DNA manipulation that replaces the target DNA sequence with the DNA sequence of interest (donor) by making use of shared homologies between them. The process typically includes a method to deliver DNA into the cells. Subsequent to its internalization, the donor is subject to processing by various enzymes present inside the cell nucleus that includes DNA resection, strand invasion/assimilation and integration. Thus, many cellular factors can affect gene-targeting efficiency. Using budding yeast *Saccharomyces cerevisiae* as the model organism, we wish to determine the role of Sgs1 and Mph1 helicases in modulating gene targeting efficiency. Sgs1 is a 3'-5' helicase that is known to be involved in DNA resection. Mph1, also a 3'-5' helicase, has been shown to be involved in the creation and disruption of strand invasion intermediates. These helicases have also been shown to negatively affect a homology-dependent repair pathway called Break Induced Replication (BIR) in which only one end of the broken DNA shares homology with the donor. Gene targeting also involves "one-ended" events at both sides of the targeting fragment. In order to address the roles of the above helicases in gene targeting, a DNA construct coding for the antibiotic marker HPH was made, which was used to integrate at a known location within chromosome V. An autonomously replicating circular plasmid containing the autotrophic marker TRP1 served as transformation control. The efficiencies of HPH marker integration were determined in the Wild Type (WT) yeast cells and in mutants lacking Sgs1 (sgs1Δ) and Mph1 (mph1Δ) helicases by selecting for the cells that gained the ability to grow on media containing the antibiotic hygromycin (HYG+) and lacking the amino acid tryptophan (TRP1+). Our results show that, compared to the WT, in sgs1Δ mutants and in mph1Δ mutants, integration of HPH marker increased by 4 and 11-fold respectively and in the sgs1Δmph1Δ double mutant by 36-fold, suggesting that Sgs1 and Mph1 helicases are negative modulators of gene targeting. The accuracy of gene targeting will be determined by further experiments.

**Support** POSSE
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