Product Labeling and Geographic Origins of Colorado Honeys

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Mentor: Dr. Christy Briles, DC - College of Liberal Arts and Sciences

Honey characterization is based on the determination of its chemical, physical and/or biological properties, including the pollen it contains. The field of melissopalynology, or the study of pollen in honey, is used in the quality control of honey through an analysis of the abundance and concentration of individual pollen types found in it. Honey's floral source and the processing methods used by beekeepers determine the price of and demand for a particular honey. Honeys that are raw and unfiltered with a specified floral source are more expensive than those that have undergone filtration and have no specified source. Clover, wildflower, and alfalfa are the primary honey varieties produced in Colorado, with clover honey bringing the highest price ($2.25/lb wholesale). I analyzed fifteen samples of raw and unfiltered Colorado honeys in order to determine and characterize floral sources, the general location of where the pollen was sourced, and whether the samples were indeed raw and unfiltered. The results of the research suggest that five of the fifteen samples had below average pollen concentrations, three were mislabeled with regard to dominant floral source, and five samples had pollen from outside Colorado (e.g.,. Mexico). Three of the suspect samples were from the same company. In all, eight out of the fifteen honey samples examined were determined to be different than what they claimed on their label. The results highlight the need for more stringent labeling protocols for Colorado honeys, and better regulations to protect abiding honey companies in Colorado and for consumer health.

Synthesis of an Injectable Biomaterial for Bone Regeneration

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Mentor: Dr. Daewon Park, DC - College of Engineering and Applied Science

Over 4 million procedures involving bone grafts are performed annually around the world, and many of these treatments display delayed healing or non-union.

Additionally, the bone graft implantation procedure is highly invasive and is accompanied by an increased risk for many post-operative complications. The focus of this project is to synthesize a novel injectable biomaterial that stimulates bone regeneration, in order to effectively treat bone defects in a minimally invasive manner. The bone-regenerating properties of this material are drawn from the use of hydroxyapatite, the main mineral component of bone and a commonly used synthetic bone graft material. Hydroxyapatite nanoparticles are then chemically conjugated to polymeric biomaterials to improve biocompatibility and to develop reverse thermal gel (RTG) characteristics, so that the material is a liquid a room temperature and becomes a solid gel at elevated temperatures such as body temperature.

The Future As Seen From the Present

Kara Brown, Fine Arts, Sculpture, DC - College of Arts and Media

Mentor: Maria Buszek, DC – College of Arts and Media

The summer of 2015 marked the occurrence of the 56th Biennale de Venezia held in Venice, Italy. This event is one of the most prestigious and widely recognized of the art world do to its international scope. Today it is difficult to distinguish common themes that unite artists in the way that cubism or fauvism did during the twentieth century. My research focuses on determining whether there are themes that have recently begun to develop as a response to the current global state of affairs. In order to do this, I used funding from the UROP grant to attend the Biennale de Venezia for one week in June of 2015. I took extensive notes and documentation of my findings which are presented as part of a media exhibition alongside sculptures and art inspired by work I saw at the Biennale. Since the scope of my project covers artistic subject matter, the research I conducted has informed a formal line of inquiry as well as inspired my own artistic practice. Ultimately I have found that the two are more closely linked than they initially appear.

Pharmacotherapeutic Potential of Disrupting Neuromodulation of Hyper-dopaminergic Neural Activity in

the Co-morbid Expression of Schizophrenia and Drug Addiction

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Other collaborators: Dr. Raibatak Das, Dr. Erik Oleson, DC – College of Liberal Arts and Sciences

Mentor: Dr. Erik Oleson, DC - College of Liberal Arts and Sciences

Schizophrenia is a debilitating psychopathology that is exacerbated by patients showing a predilection for addictive behavior. The high co-morbidity between schizophrenia and drug addiction theoretically arises from a hyperdopaminergic state in schizophrenia, pre-sensitizing the neural mechanisms that invigorate drug seeking. Our research attempts to establish the causality of DA in eliciting a pro-psychotic response in a conditioned avoidance task, which is a classical screen with high predictive validity for determining the efficacy of anti-psychotic drugs. We then attempt to counteract that response pharmacologically. Historically, both typical and atypical antipsychotics target the dopamine D2 receptor, but a number of issues exist with these pharmacotherapies that result in poor compliance. We propose an alternative method of treatment that targets upstream modulators of DAergic neurons in the mesocorticolimbic pathway that will potentially ameliorate both the schizophrenic symptoms and drug-seeking behavior. To achieve this, our group artificially induces a hyperdopaminergic state in transgenic rats by utilizing Gq-coupled DREADD virus technology. We then systemically administer antagonists of the cannabinoid CB1 receptor and orexin OX1 receptor, which we have previously demonstrated to modulate DA neural activity. Preliminary results show a DREADD-induced hyper-dopaminergic state elicits a pro-psychotic response in a classic pharmacological screen, as well as increases locomotor activity and motivation for cocaine; whereas, an anti-psychotic response and reduced motivation for cocaine is observed when either the CB1 or OX1 antagonist drug is administered. These results

show promise for targeting upstream modulators of DA function in the treatment of co-morbid diagnoses of schizophrenia and drug addiction.

The Impact of Nutrient Pollution on Ammonia-Oxidizing Microbial Communities Residing in Freshwater Ecosystems

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Mentor: Dr. Annika C. Moiser, DC - College of Liberal Arts and Sciences

Microbial nitrification (the oxidation of ammonia into nitrite and nitrate) is thought to be a critical, rate-limiting step in the removal of nitrogen pollution from freshwater systems. Here, we enriched ammonia-oxidizing bacteria (AOB, which oxidize ammonia to nitrite) and nitrite-oxidizing bacteria (NOB, which oxidize nitrite to nitrate) in cultures derived from freshwater streams in the Denver metropolitan area. Functional gene PCR and Illumina MiSeq sequence analyses showed that cultures contain NOB belonging to the Nitrobacter genus and AOB belonging to the betaproteobacteria phylum. Nitrite production and consumption in the enrichment cultures have been monitored for more than one year. We investigated the impact of nutrient pollution on the growth and survival of freshwater AOB and NOB by exposing the enrichment cultures to elevated nitrite environments (up to 100mM nitrite). Growth was monitored by measuring changes in nitrite and nitrate concentrations. Understanding the physiology of these organisms will shed light onto how well these organisms may adapt to changing concentrations of nitrite that could be observed in the case of nutrient pollution, and how these changes affect the global nitrogen cycle in freshwater ecosystems. This research will help to preserve the resources these freshwater ecosystems provide for the Denver metropolitan area.

Protein-membrane Binding: Detection Using Single Molecule TIRF Microscopy

Marissa DeLima, Biology, DC - College of Liberal Arts and Sciences

Mentor: Dr. Jefferson Knight, DC - College of Liberal Arts and Sciences

This exhibit demonstrates biomolecular interactions such as the cumulative effect of multivalent attractions on interfacial protein-membrane binding. We have shown the stoichiometry of peripheral protein-membrane interactions can be measured based on single-molecule diffusion using supported lipid bilayers. Here we apply this technique to granuphilin, a synaptotagmin-like protein containing tandem membrane-targeting C2 domains, C2A and C2B. Granuphilin C2A binds simple lipid membranes containing anionic lipids such as phosphatidylserine (PS), but C2B affinity for PS is undetectable using standard approaches. Here, we set out to determine the PS affinity of C2B based on a comparison of the diffusion rates of the C2A domain and the C2AB tandem on supported lipid bilayers. Total internal reflection florescence (TIRF) microscopy with single particle tracking was used to identify diffusion constants of each individual or tandem C2 domain. Granuphilin C2A displays a lateral diffusion constant comparable to other C2 domains. However, the diffusion of the granuphilin C2AB tandem on the same membrane appears to be slower; suggesting substantial PS contacts for the C2B domain within the C2AB tandem. This effect represents a weak but potentially physiologically relevant interaction that influences the membrane-bound state of this strong membrane binding protein. This research shows an exciting new approach to looking at protein-membrane interactions using single molecule techniques.

The MAGL Inhibitor MJN110 Alters Social Behavior and Differentially Impacts mTOR Phosphorylation in Astrocytes and Neurons in the Medial Prefrontal Cortex of Adolescent Rats.

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Other collaborator: Esteban Loetz

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The mammalian target of rapamycin (mTOR) is a protein kinase expressed in neurons and glial cells with an important role in plasticity through regulation of protein synthesis. Activation of mTOR through phosphorylation (p-mTOR) can be mediated by numerous extracellular signals, but the effects of the endocannabinoid system on mTOR phosphorylation are unknown. 2-arachidonoylglycerol (2AG) is one of the primary endocannabinoids present in the brain, and is broken down largely by the enzyme MAGL. The novel compound MJN110 is a potent MAGL inhibitor shown to increase central 2AG levels. Here, two doses (1 and 5 mg/kg) of MJN110 or vehicle were administered systemically to adolescent rats prior to a single social encounter with a novel adolescent rat. The lower dose of MJN110 increased play behaviors, while the higher dose decreased social interaction, including play behaviors. p-mTOR expression was assessed using immunohistochemistry (IHC) in the prelimbic (PL) and infralimbic (IL) regions of the medial prefrontal cortex (mPFC). Cells were identified as neurons or glia based on morphology. In vehicle treated rats, a novel social encounter increased glial p-mTOR expression in PL and IL. The higher dose of MJN110 produced a robust decrease in glial p-mTOR expression, and an increase in neuronal p-mTOR expression. Double-label fluorescent IHC revealed that p-mTOR was expressed in astrocytes but not in microglia. These results suggest that 2AG has opposite and dose-dependent effects on social behavior as well as on mTOR phosphorylation in neurons and astrocytes. In a separate experiment, astroglial p-mTOR expression was greater in the mPFC in adolescent rats than in adults. These results suggest that astroglial mTOR signaling in the mPFC may be involved in adolescent social behavior, and is modulated by the endocannabinoid 2AG.

A Small Puppet Theater Company for Spanish-Speakers

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Mentor: Dr. Andres Lema-Hincapie, DC - College of Liberal Arts and Sciences

The Denver Children's Affairs reported that 39% of the students in Denver public schools are Spanish speakers, including non-English language learners. It has been our experience that in Denver there are very few sources of live educational entertainment available for this demographic group. By taking advantage of the whimsical and theatrical components of puppet theater, we have been determined to bring Spanish speaking families together and stimulate an exciting acquisition of knowledge for Hispanic children (as well as their parents) on crucial aspects of healthy childhood development. With a storyline based on Walt Disney's Alice in Wonderland, we have incorporated information regarding healthy diets, oral hygiene, and immunizations into the adventures of our protagonist Alicia and her friend Andi the Cheshire Cat as they save the inhabitants of the enchanted forest from the evil King Viruso and his bacteria henchmen. Our goal is that after watching these performances, the Hispanic parents and children will have acquired basic fundamental information about these three topics. The parents will then be able to encourage their children to consistently implement this newly acquired knowledge into their lives so that they may be more prepared for the next upcoming stage in their children's physical and mental development.

Combined QM/MM Dynamics Simulations of Proton Transfer in E. coli CLC Chloride Ion Transport Protein

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Adam Duster, Chemistry, DC - College of Liberal Arts and Sciences

Mentor: Dr. Hai Lin, DC - College of Liberal Arts and Sciences

The CLC family of transmembrane proteins include both Cl– channels and Cl–/H+ antiporters, which play critical roles in many cellular processes, such as extreme acid response in E. coli and acidification of membranes in humans. The E. coli CLC (EcCLC) antiporter has been extensively characterized; however, it remains a mystery how the proton is shuttled through a largely hydrophobic gap of ~15 Å between the two gating glutamic acid residues. Previous molecular dynamics studies have suggested transient formation of a water wire in this gap which could provide a pathway for proton transport. Here, we aim to elucidate the detailed process of proton

transport through EcCLC by performing combined QM/MM dynamics simulations, in which the proton is treated explicitly and the reorganization of the covalent and hydrogen bonds during proton relay is described quantum mechanically. Acknowledgments: This project is supported by the NSF(CHE-09523337 and CHE-1564349), XSEDE (CHE-140070), Camille and Henry Dreyfus Foundation (TH-14-028), and the Undergraduate Research Opportunity Program of the University of Colorado Denver. We thank Prof. E. Tajkhorshid for the geometries from MM simulations.

The Lakewood Gulch: A Waterway in Peril

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Mentor: Mr. Drew Bixby, DC - College of Liberal Arts and Sciences

The Lakewood Gulch is an artery that flows in the South Platte River basin. Significantly smaller than the heavily utilized Clear Creek, the Lakewood Gulch bubbles from the water table beneath the base of North Green Mountain, gathers water from other gulches and the run-off from nearby neighborhoods, and flows into the South Platte River. Even though it is a naturally flowing stream, the Lakewood Gulch has been converted into a floodway to prevent property damage to the encroaching housing developments. In addition to pollution from storm drainage, trash from camping and surrounding residences and natural erosion have added to its destruction. This is a journalistic project that, through my field observations, photography, and research, tells the story of a waterway in peril.

Binding of Granuphilin C2A Domain to Membrane by Molecular Dynamic Simulations

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Mentor: Dr. Hai Lin, DC - College of Liberal Arts and Sciences

Granuphilin is a membrane binding protein found in insulin secreting pancreatic ß-cells, where it assists in docking vesicles to the plasma membrane prior to exocytosis.1 Investigating the process by which granuphilin assists in the exocytosis of insulin can lead to the development of therapeutic compounds to aid in insulin secretion for diabetes. Previous studies have shown that the granuphilin C2A domain binds with a micromolar affinity to lipid membranes containing phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) and to its soluble analog inositol triphosphate (IP3); however, the domain binds much stronger, with a nanomolar affinity, to lipid membranes containing both PI(4,5)P2 and negativity charged lipids such as palmitoyl-oleoyl phosphatidylserine (POPS).2,3 We hypothesize that the increased affinity arises from concerted binding at multiple sites on the granuphilin C2A domain. Through docking calculations of IP3 to the C2A domain, we identify the principle binding site for the PI(4,5)P2 head group on the granuphilin C2A domain. By employing molecular dynamic simulations of the C2A domain in the presence of membrane models, we show that multiple sites on the C2A domain bind concertedly at the surface of the lipid membrane. The results of our computational analysis are in line with the experimental findings. [1] Torii, S. et al. Mol Cell Biol. 2002, 22, 5518–5526. [2] Wan, C. et al. Chem Phys Lipids. 2015, 186, 61-67. [3] Lyakhova, T.A. et al. Chem Phys Lipids. 2014, 182, 29–37.

Activation of the Nigrostriatal Dopamine Pathway Strengthens Fear Extinction

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Extinction of aversive memories is critical for the treatment of anxiety disorders, but extinction memories are fragile and depend on the context in which they were learned. Novel means to strengthen fear extinction and reduce the return of fear in contexts outside of the extinction context are needed. Prior work suggests that dopamine (DA) can strengthen fear extinction learning, but the specific DA circuits involved are unknown. We used viral-mediated transfer to express a designer receptor exclusively

activated by a designer drug (DREADD) into DA neurons of the substantia nigra compacta, a midbrain region containing DA projections to the dorsal striatum, to begin to investigate whether activation of the nigrostriatal DA pathway can facilitate fear extinction and reduce the return of fear. Male wild type or TH-Cre rats received injections of a CRE-recombinase-dependent DREADD bilaterally into the substantia nigra pars compacta. After 3 weeks to allow for viral gene expression, rats were exposed to auditory fear conditioning. The next 2 days, rats received either vehicle or the designer drug CNO (1 mg/kg i.p.) 30 minutes before exposure to auditory fear extinction. The next day rats were placed drug-free into either the familiar extinction context or a novel context and exposed to the auditory stimulus. Results indicate that activation of nigrostriatal DA neurons with DREADD can enhance fear extinction in such a way as to reduce the return of fear in novel contexts. These data suggest that the nigrostriatal DA pathway is a novel target for the augmentation of fear extinction.

Effects of Social Isolation on Aggressive Behaviors in Stalk-eyed Flies

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Aggression is a vital behavior in many animal species as a means of obtaining resources, mating, and overall survival. Therefore, the presence of aggressive behaviors in a species can also be an indicator of evolutionary fitness. Stalk-eyed flies, Teleopsis dalmanni, have elongated eyestalks that protrude laterally out of their heads with eye bulbs residing on each end. Males and females of this species and other related species in the Diopsidae family are often sexually dimorphic – meaning that males and females are morphologically distinct. As stalk-eyed flies exhibit aggressive behavior in competition over access to food and mates, they are a useful model species for studying aggressive behaviors in invertebrates. This study examines the effects that social isolation has on aggressive behavior in stalk-eyed flies. Social isolation implies the removal of a sexually mature fly from a normal population cage into separate housing for the period of 7 days. We hypothesized that socially isolated stalk-eyed flies would exhibit an increased quantity and intensity of aggressive

behaviors. This was tested by pairing a size-matched isolate with a control fly in a partitioned fighting coliseum. After a period of 24 hours, the flies were provided with corn medium to encourage fighting and the number and intensity of each behavior was recorded and analyzed. Preliminary data suggests a trend toward isolates exhibiting a larger number high intensity aggressive behaviors and overall wins. We will discuss the results in the context of a larger sample of fights.

Lipid Coated Gold Nanoparticles For Ultra-Sensitive Label Free Quantitation of Protein Adsorption Kinetics

Desmond Hamilton, Chemistry, DC - College of Liberal Arts and Sciences

Mentor: Dr. Scott Reed, DC - College of Liberal Arts and Sciences

Synaptotagmin (syt) family proteins are involved in exocytosis, a process in certain cells that allows the release of neurotransmitters and/or hormones such as insulin. Syt proteins are part of the biological machinery that promotes the fusion of secretory vesicles with the cell's membrane, the final step in exocytosis. Here, we investigate the syt7 C2A domain, a part of the syt7 protein that is responsible for docking and inserting into the membrane. In order to study the curvature dependence of its membrane binding, we use three different sized lipid-coated spherical gold nanoparticles (LCAuNP). LCAuNPs allow for ultra-sensitive, label-free detection of protein adsorption. The LCAuNPs were constructed from the ground up through a multistage process. Octahedral gold nanoparticles were synthesized and transformed into spherical gold nanoparticles. The hybrid-membrane was constructed upon the spherical nanoparticles with the addition of synthetic lipid vesicles. Propane thiol adhered the hybrid-membrane to the surface of the nanoparticles and the LCAuNP solution was purified with centrifugation. Syt adsorption studies were accomplished by monitoring small changes in the wavelengths of light that the nanoparticles interact with. The LCAuNPs limit of detection was found to be 9 nM, and the three sizes were able to show size-dependent slow adsorption kinetics that have not previously been observed.

New Tool Reveals Site-Specific Differences in the Size of Osteocyte Lacunae

Adam Rauff, Bioengineering, DC - College of Engineering and Applied Science

Mentor: Dr. Dana Carpenter, DC - College of Engineering and Applied Science

New Tool Reveals Site-Specific Differences in the Size of Osteocyte Lacunae Rauff A1, Heveran CM2, Ferguson VL2, Carpenter RD3 1Department of Bioegineering, University of Colorado Denver; 2Department of Mechanical Engineering, University of Colorado Boulder; 3Department of Mechanical Engineering, University of Colorado Denver Osteocytes, or bone maintenance cells, reside within mineralized bone tissue in voids called lacunae. The osteocyte network responds to mechanical stimuli and actively directs the remodeling of bone structure. Because lacunae comprise an abundant component of bone microstructure, they may play significant roles in both mechano-sensation and the mechanical integrity of bone. The purpose of this study was to quantify three dimensional characteristics of lacunae. Samples were acquired from bones of humans, mice, and cows. The samples were stained, embedded, and polished in preparation for microscopy. A confocal microscope was used to acquire 3D “stacks” of 2D images. The images were reconstructed into 3D objects and processed using new software developed as part of the study. The new software was used to measure lacunar volume, density, directionality, radius, and anisotropy. One novel finding of the study was a site-specific difference in lacunar size: lacunar volumes in the femur ($n = 48$) and tibia ($n = 86$) of mice were 410 ± 90 and 296 ± 126, respectively ($p<0.001$). This larger size was also reflected in the lacunar surface area, which was 366 ± 55 in the femur and 268 ± 80 in the tibia ($p<0.001$). These site-specific differences in lacunar morphology could reflect differences in the mechanical function of the two bones. However, further study is needed to address this question. Additionally, the software developed in this study can be used to investigate changes in lacunar morphology that may occur due to obesity, diabetes, chronic kidney disease, and exposure to microgravity.

Investigation of Cellular Regulatory Elements

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Sanju Garimella, Biology, DC - College of Liberal Arts and Sciences

Mentor: Dr. Christopher Phiel, DC - College of Liberal Arts and Sciences

Glycogen synthase kinase-3 (GSK-3) is a protein kinase involved in many intracellular regulatory events. A novel role for GSK-3 was recently discovered by our lab – the regulation of mRNA methylation, referred to as m6A (methylation of

adenosine bases at the C6 position). The m6A modification of mRNA is believed to control the stability of mRNA, and thus the persistence of gene expression. We have found evidence that GSK-3 controls m6A levels by regulating the enzyme that demethylates mRNA, FTO. In this study, we quantify m6A mRNA levels in mouse embryonic stem cells using two different techniques. In addition, we have cloned the FTO gene into a lentiviral vector, which will permit us to overexpress FTO in a variety of cell types, including induced pluripotent stem (iPS) cells. Progress on these

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1700 Years of Anthropogenic Influences: An Ecosystem Study of Northern Vietnam's Van Don Island

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Christopher Andersen, History, DC - College of Liberal Arts and Sciences

Mentor: Dr. Christy Briles, DC - College of Liberal Arts and Sciences

Vietnam has a long history of human occupation (>4000 years) making it a significant region for studying anthropogenic factors influencing tropical ecosystems. A sediment core from Van Don Island was analyzed using a multi-proxy approach to examine the degree to which humans impacted the landscape. Charcoal and pollen were used to reconstruct fire and vegetation, coprostanols to approximate population levels, and carbon/nitrogen ratios and loss-on-ignition data to determine wetland productivity. The data suggests three distinct zones in the record. The first zone (1750-1000 cal yr BP) indicates a small population on the island, likely practicing rice agriculture while minimally impacting the environment. The second zone (1000-450 cal yr BP) indicates a significant decrease or movement of people away from the site location and a shift away from local cultivation of rice to other uses of the island. Historical sources suggest the island was used as a port in this time period, trading goods between the capital of Northern Vietnam and other nearby Asian countries. The third zone (450 cal yr BP to present) indicates a shift back to agriculture and away from trade as population increases greatly. We assume this shift is initially due to a movement of the northern capital away from the Red River Delta and, later on, because of a change in ship size, greater regulation, and island isolation from foreigners. Future research involves analyzing a core taken in December 2015 at the Van Don port site to compare the spatial impact of trade and agriculture.

Investigating the Differences in Membrane Binding Cooperativity between the Tandem C2 Domains of Synaptotagmin 1 and Synaptotagmin 7

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Mentor: Dr. Jefferson Knight, DC - College of Liberal Arts and Sciences

Synaptotagmin 1 (Syt1), a Ca2+ sensor involved in exocytosis in pre-synaptic neurons, contains a characteristic tandem C2 sequence (C2AB) which inserts into anionic membranes in the presence of Ca2+. Previous studies have shown that the C2AB fragment of Syt1 inserts into target membranes more deeply than either individual C2A or C2B domain, suggesting cooperative interaction between C2A and C2B in the membrane-docked state. This behavior stands in apparent contrast to that of another family member, Syt7, whose C2A and C2B domains have been shown to bind membranes independently. To compare the cooperative behaviors of the C2 domains in these two isoforms, the dissociation kinetics (off-rates) of protein-liposome complexes were measured upon addition of the Ca2+chelator EDTA using stopped-flow fluorescence spectroscopy. Using liposomes composed of a 1:1 mixture of phosphatidylcholine and phosphatidylserine (PC/PS), the Syt1 C2AB tandem domain exhibits a much slower off-rate than either of the single domains. In contrast, dissociation kinetics for Syt7 C2AB were best fit to a two-step model in which each rate constant matches those from the individual domains. This preliminary result supports the presence of interdomain interactions in the membrane-docked state of Syt1 C2AB but not Syt7 C2AB. These experiments were performed with protein domains purified using affinity chromatography with high-salt washes and verified to be >95% free of nucleic acid contaminants; ongoing work aims to assess effects of polyanions on this apparent cooperative behavior.

Three-Way Mirror

Joey Verbeke, Music, Recording Arts, DC - College of Arts and Media

Mentor: Mr. Jeff Merkel, DC - College of Arts and Media

When you think of a mirror, a reflection of what you look like at that current point in time is probably the only thing that comes to mind. This project took on the challenge of leveraging art and technology to explore the way in which one can interact within the context of a mirror. Using a two-way mirror, a computer screen behind it, face tracking technology, and mixing this all with creative code I was able to create a way in which a participant is able to interact through time with the previous person to look in the mirror. "Three-Way Mirror" is also an expression of our times, in which so much of our interaction is done through technology, and how we abstract our digital self from what's really on the other side of the mirror.