1

Synthesis of Full Length R521G FUS Protein and Binding Analysis of a G-Quadruplex Structure in MECP2 mRNA

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are neurodegenerative diseases that share common inheritance pathways; however, the pathogenic mechanism by which the symptoms of these disorders develop is not well understood. One mutation leading to the development of ALS and FTD occurs in the fused in sarcoma/translocated in liposarcoma (*FUS/TLS*) gene, encoding for the protein FUS that plays an important role in regulating synaptic function. The FUS protein contains arginine-glycine-glycine (RGG) boxes, an RNA-binding domain that has been shown to specifically bind G-quadruplex secondary structures in messenger RNA (mRNA). One of the ALS FUS mutants, R521G has been shown to form cytoplasmic aggregates with MECP2 mRNA, a neuronal mRNA, however, the RNA recognition motifs involved in this interaction are not known. We postulate that a G quadruplex structure we previously characterized in MECP2 mRNA is recognized specifically by the RGG box region of the FUS protein.

2

Characterization of the Serotonin Transporter in the Lipid Bilayer

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Serotonin (5-HT) is a neurotransmitter; altered serotonin levels contribute to multiple disease states, including but not limited to depression and neuropathic pain. Levels of synaptic serotonin is partially regulated by the serotonin transporter (SERT), making it a potential therapeutic target. Our laboratory seeks to study the structure and function of SERT. Human SERT was expressed by Tetracycline-Regulated Expression (T-REx) Human embryonic kidney cell lines (HEK-293); the product was then purified. Solubilized SERT samples were purified by affinity chromatography and reconstituted. The lipid-accessible structure of the reconstituted transporter in membranes was studied via photo-crosslinking to derivatized cholesterol followed by mass spectrometry (MS). This initial study determined the lipid-facing portions of SERT in the absence of any ligands, as well as identified potential cholesterol binding sites. These studies may be extended to examine state-dependent changes in the transporter.

3

Regulatory Control of Steroid Sulfatase in the NIH-3T3 Fibroblast Cell Line

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The enzyme steroid sulfatase (STS) acts locally to convert inactive, sulfated steroid hormones into their active forms. STS has recently been found to be under the control of the inflammatory NFκB pathway and the anti-inflammatory glucocorticoids. The role of STS in relation to inflammation has only been studied in a few cell types (liver and bone), where STS appears to provide estrogen in order to ameliorate inflammation. We studied the relationship between inflammation and STS in the NIH-3T3 mouse fibroblast cell line, using ^{3}H-E_1S conversion assays in whole cells and homogenates. We confirmed that glucocorticoids were inhibitory of STS and that this relationship was dose-responsive for cortisol. We also found that the LPS treatment stimulated STS, suggesting NFκB involvement. Unexpectedly, the NFκB inhibitor BAY 11-7082 also increased STS activity. These results confirm and extend the model for an inflammatory role of STS in the widely distributed fibroblast cell type.

4

Quantitative Validation of Color Normalization in Histological Images

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Digital pathology is increasingly used as a diagnostic tool for segmenting and classifying histological structures in stained tissue slides. A preliminary step is standardizing slide appearance across a range of variable scanners, stain manufacturers, and differences in stain protocol and preparation. Our research offers a new method of matching color statistics for hematoxylin and eosin (H&E)-stained breast tissue images based on opponent colors, which gives maximum separation between stains. Source images were modified using an algorithm to produce the same color appearance as a different target image. We quantitatively verified the accuracy of our method using several metrics, including chi-square difference test, Quaternion Structural Similarity Index, Earth Mover's Distance, and Kullback–Leibler divergence, and compared our findings with results of other literature methods. To disregard differences in composition between distinct source and target images, we "renormalized" the modified source image back to the original and used the same comparison metrics.

5

Single and Mixed Monolayers of Nonadecanoic Acid and Octacosanoic Acid Self-Organized on Sapphire Surface and their Frictional Properties

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Self-assembled Monolayers (SAMs) are molecular arrays that spontaneously form by adsorption of organic molecules onto a surface. Each molecule is composed of a head group that binds to the substrate, an alkyl chain that provides order and stability, and a tail group that determines surface properties. This project is focused on single and mixed monolayers of nonadecanoic acid (C19) and octacosanoic acid (C28), formed on sapphire. Single monolayers of C19 and C28 and mixed monolayers with ratios of 75%-25%, 50%-50%, and 25%-75% were prepared using solution deposition methods. The samples were analyzed via diffuse reflectance infrared Fourier transform spectroscopy (DRIFT), contact angle measurements, and atomic force microscopy (AFM). DRIFT spectra confirmed the formation of highly ordered monolayers with mono/bidentate binding. Water contact angles indicated a hydrophobic surface, and the degree of hydrophobicity increased with an increase in C28 composition. AFM was used to determine surface topography and analyze interfacial friction.

6

Investigation of the Transfer of Gunshot Residue through a comparison of Gloved, and Non-Gloved Handshakes

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Gunshot residue (GSR) is used in many court cases to determine the shooter, and any witnesses in the vicinity of a gun being fired. GSR is deposited when a gun is fired due to the high pressure reaction which propels the bullet from the barrel. Lead (Pb), Barium (Ba), and Antimony (Sb) must be present in a single spherical particle for GSR conformation. Using a Scanning Electron Microscope/Energy Dispersive Spectroscopy (SEM/EDS), the presence of gunshot residue on suspects or witnesses can be determined by analyzing samples for the characteristics listed above.Gunshot residue transfer has become increasingly researched, though there is still very little know about the quantities that transfer. A comparison of handshake after firing gloved hands, or without gloves was preformed to determine the effects on the quantity of GSR transferred.

7

Detection of Ras-Raf inhibitor binding sites by molecular dynamics simulations

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Ras/Raf, a protein complex implicated in 30% of human tumors, initiates signaling that can contribute to the development of cancer tumors. Several Monocyte Chemoattractant Proteins (MCP) have been detected as inhibitors of this complex by binding to Ras and thus deactivating Raf gene expression. This work is focused on determining the binding site of the MCP compounds in Ras using molecular docking and molecular dynamics (MD) simulations. MCP compounds were docked to the Ras crystal structure available in the Protein Data Bank (PDB) database (PDB ID: 4G0N) using SMINA. MD simulations were performed using Amber between the predicted pose of the MCP compounds and Ras, both constrained but leaving the modeled C-terminal tail unrestrained. The results indicate that the C-terminal complexes with the MCP compound via several hydrophobic interactions. These results can lead to the design of more potent Ras inhibitors which could prevent the development of human cancer tumors.

8

Surface Modification of Titanium and Titanium Aluminum Vanadium using Octadecylphosphonic Acid and Stearic Acid

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Self-assembled monolayers (SAMs) have the capability of altering the physical and chemical properties of a given substrate. SAMs may layer on a substrate in a variety of conformations, including island aggregates or complete monolayer coverage to optimize the altered properties of the substrate. Titanium and titanium aluminum vanadium (Ti-6Al-4V) were modified using mixed SAMs composed of two varying head groups, octadecylphophonic acid (ODPA) and stearic acid (ODCA). ODPA easily attaches to substrates while ODCA is available in numerous reactive functionalities. In order to determine the attachment to the surface, diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) was utilized to analyze the binding of both head groups to the surface. Atomic force microscopy (AFM) was used to determine the homogeneity of the surface by comparing the modified and unmodified surface roughness values. ODPA was found to promote the binding of ODCA to the surface of both the substrates.

9

_N_-acetyl cysteine provides transient neuroprotection against oxidative stress _in vivo_

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The objective of this study was to explore the therapeutic potential of the thiol N-acetyl cysteine (NAC) in an animal model of Parkinson's disease. To accomplish this goal, saline or the free-radical generator 6-hydroxydopamine (6-OHDA) was injected into the mouse striatum to induce degeneration of dopaminergic neurons. The animals were then treated intraperitoneally with saline or NAC and dopamine neurons were examined ten days or three weeks later. NAC prevented dopaminergic loss at ten days post-infusion, but that this effect waned within three weeks. These results were confirmed with multiple independent histological techniques and imaging methods, ranging from 1) tyrosine hydroxylase immunohistochemistry to retrograde tract-tracing with FluoroGold, 2) high resolution epifluorescent microscopy to low resolution infrared imaging, and 3) measurements of both axon terminals and somata. We conclude that NAC provides only transient neuroprotection against oxidative stress and that studies modeling chronic disorders need to include longer time points to ensure sustained neuroprotection.

10

The Effects of Macromolecular Crowding on the Kinetics of Dihydrofolate Reductase

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Biochemical studies have shown that 20-30% of a cell's interior volume is occupied by macromolecules, creating a "crowded" environment that shows thermodynamic and kinetic deviation from dilute solutions. *In vitro* studies have been conducted to simulate crowding effects on bovine dihydrofolate reductase (DHFR) using bovine serum albumin (BSA) and ficoll-70 as model crowding agents. DHFR catalyzes the reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) through a hydride transfer from NADPH in all dividing cells. Tetrahydrofolate is required for the synthesis of thymidylate, essential for DNA synthesis. DHFR's importance in *in vitro* screening of antifolates as potential therapeutics emphasizes the importance of better defining its sensitivity to crowding. Enzyme activity was assayed by measuring depletion of NADPH at 340 nm in the presence of DHF. Both model crowders resulted in a decrease of DHFR activity. Inhibition of DHFR by trimethoprim and methotrexate was also studied to determine their sensitivity to crowding.

11

Determination of Gunshot Residue Settling Velocity

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Gunshot residue (GSR) is a valuable type of trace evidence that is found at crime scenes where a firearm was discharged. GSR can provide investigators information about the individuals present at the scene. Once a firearm is discharged GSR particles are released from various openings of the firearm; this research will study how fast the GSR particles settle to the ground once released. This will be completed by using air suction filters placed at different heights (1,3, and 5 feet). At each height the three filters will be turned on at various time intervals to collect the GSR from the air. Currently, a method was developed to detect GSR using a multi-sample holder to increase efficiency of analyzation using Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDS). The optimized method to determine the settling velocity of GSR will be accomplished by the automated analysis Bruker EDS Esprit Software.

12

Characterization of p250 GAP – miR-132p interactions in the presence of FMRP

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Fragile X syndrome is the most common form of inherited mental impairment, and it is caused by the loss of expression of the fragile X mental retardation protein (FMRP). FMRP interacts with secondary structures of mRNA molecules such as G-quadruplexes and with poly U regions. In this study, we investigated p250GAP mRNA which contains a poly U stretch near the binding site of miR132p, a microRNA that regulates its translation. Two stretches of the p250GAP mRNA were studied: the first, p250GAP poly U short, features an open FMRP poly U binding site, whereas the second, p250GAP Poly U long, potentially has a hairpin structure at the FMRP binding site. We used native polyacrylamide gel electrophoresis (PAGE) to determine how FMRP full length isoform 1 (ISO 1) p affects the binding of p250 GAP mRNA and miR-132p.

13

Development and implementation of a bioreactor to assess spatial and temporal drug delivery profiles used in bone regeneration protocols

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Bone disease and injury affect millions of people around the world, resulting in weakened, degraded, and broken bones. Mobility and limb function are often limited even after treatment or surgery, primarily due to lost bone that fails to heal back. Regeneration of this lost bone can be achieved through implantation of a bone scaffold followed by targeted delivery of angiogenic and osteogenic growth factors. The spatial and temporal delivery profiles of these growth factors play a key role in the extent of vascularized bone formation. Our newly developed bioreactor facilitates large scale testing of many different delivery profiles; the reactor is modular and can be arranged in parallel with other reactors to create large sample size studies. Further exploration of delivery profiles, scaffold materials, and biological factors can lead to discovery of more successful bone regeneration protocols and subsequently, increased recovery rates for those with damaged bones.

14

Drosha mRNA G-Quadruplex Binding with FMRP RGG Box Domain

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Fragile X Syndrome is an inherited intellectual disease caused by an expansion repeat of cytosine-guanine-guanine (CGG) in the 5' untranslated region on the *FMR1* gene which codes for the fragile X mental retardation protein (FMRP). Methylation of the cytosines within this expansion mutation on *FMR1* inhibits the production of FMRP, an RNA-binding protein involved in translation regulation. FMRP binds to specific mRNA secondary structures, including the G-quadruplex. Among other targets FMRP has been shown to regulate the Drosha mRNA, which encodes for an RNAse III enzyme responsible for miRNA processing in the nucleus. We hypothesize that Drosha mRNA forms a G-quadruplex secondary structure in its 5'-untranslated region, which is recognized specifically by FMRP. Various biophysical methods such as NMR, CD spectroscopy, and native PAGE were employed to investigate G-quadruplex formation in the 5' untranslated region of Drosha mRNA and to analyze its interaction with the FMRP arginine-glycine-glycine domain (RGG box).

15

Studying Epidermal Growth Factor Receptor Expression in Manduca sexta

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Although flies and moths have significantly diverged over time, many studies done on *Drosophila melanogaster*, the fruit fly, can also be applied to *Manduca sexta*, the tobacco hornworm moth.
In *Drosophila melanogaster*, the Epidermal Growth Factor Receptor protein, DER, is necessary for the development of a subset of dorsal embryonic muscles. Specifically, activation of DER in the dorsal mesoderm generates two equivalence groups from which muscles will develop. DER may also be necessary for adult muscle development by regulating muscle precursor proliferation. By utilizing a database of expressed sequence tags from *M. sexta*, we have identified and sequenced the *M. sexta DER* homolog (*MsER*). We will characterize the expression patterns of *MsER* by performing in situ hybridizations on day-old embryos. We expect to find gene expression in dorsal mesodermal regions of *M. sexta* embryos, as has been identified in *D. melanogaster*.

16

Macromolecular Crowding Effects on Malate Dehydrogenase

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Traditionally steady state enzyme kinetics have been measured in dilute solutions. Intrinsically, however, the interior of a cell has a crowded macromolecular matrix. Therefore, macromolecular crowding must be studied to better understand its effect on enzymatic reactions as they occur in a simulated intracellular milieu. The model system that we are studying is mammalian malate dehydrogenase. Citrate is an allosteric inhibitor of the enzymatic activity of malate dehydrogenase in the oxidation of NADH by oxaloacetate. In these experiments bovine serum albumin (BSA) was used as a model macromolecular crowding agent. Varying concentrations of BSA significantly change the IC_{50} for citrate inhibition, where IC_{50} represents the concentration of citrate necessary for 50% inhibition of the enzyme rate. The IC_{50} results were extended to experimentally determine K_I values for citrate. These studies represent the first reported observation of enzyme allosteric inhibition showing sensitivity to macromolecular crowding.

17

Bacteria retardant implant surface

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Titanium aluminum vanadium is a common implant material due to its biocompatibility. Bacterial colonization may occur upon or shortly after implantation, ultimately forming a biofilm. Biofilms are communities of sessile bacteria that adhere to surfaces and are difficult to eradicate. Low concentrations of nitric oxide (NO) have been shown to disperse bacteria from biofilms so that systemic antibiotics can be used effectively. As a result, direct delivery of NO to the implant site has the potential to decrease infections. *N*-diaziomdiolates (NONOates), such as diethylene triamine N-diaziomidiolate (DETA NONOate), release two moles of nitric oxide per parent molecule at physiological conditions. Self-assembled monolayers with a carboxylic acid tail group were used as linkers to immobilize DETA, a model for DETA NONOate. Diffuse reflectance infrared Fourier transform spectroscopy was used to characterize the monolayer and to confirm attachment. Attachment was confirmed by the presence of amide I and amide II stretches.

18

Heterologous Expression of *Campylobacter jejuni* NapL Protein in *Escherichia coli*

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NapL is a predicted chaperone protein in the periplasmic nitrate reductase (Nap) in the common pathogen *Campylobacter jejuni (Cje)*, however the physiological function of NapL is not known. It has been determined that NapL may be required during the maturation of the catalytic subunit, NapA, to achieve a fully functioning enzyme. This investigation demonstrates the first time NapL has been heterologously overexpressed and isolated using a poly-histidine tag. Here, a pRSF-Duet 1 (kan) *napL* gene construct synthesized by Genewiz was obtained and overexpressed in competent *Escherichia coli* strain BL21(DE3) cells. Overexpression trials have been conducted to optimize NapL production. Once the system has been optimized, NapL will be purified using affinity purification. To confirm the identity of NapL, LC-MS/MS will be used to identify tryptic peptides.

19

Optimizing PbS Quantum Dot Solar Cells Synthesis

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Using PbS quantum dots (QDs) of approximately 950 nm in diameter p—n type semiconductor solar cells were fabricated to achieve a high current density. The solar cells were made from patterned FTO on glass substrates, spin coated with TiO2 as an electron blocking layer, treated with TiCl4 for thirty minutes and finally PbS QDs were deposited onto the substrates. In an attempt to increase the fill factor and ultimately the efficiency, ZnO nanoparticles and TiO2 solutions were tested and compared as blocking layer methods. Problems with the ZnO nanoparticles occurred because of the extreme conditions that ZnO nanoparticles require and their tendency to grow without ideal conditions. ZnO nanoparticles were the lesser of the two methods, but many papers claim that they are actually the better of the two.

20

Salamander Skin Peptides Inhibit the Proliferation of *Batrachochytrium dendrobatidis*

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Batrachochytrium dendrobatidis (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*) are fungal pathogens that infect amphibian skin and restrict osmoregulation which can cause mortality. In recent decades, these fungi have been a contributor to the major population declines of amphibians throughout the world. Some amphibian species are highly susceptible to the fungus, while others resist or tolerate infection. Peptides secreted from the skin act as a primary defense mechanism, and that differences in susceptibility are due to the variations in natural peptide concentrations among species. The hypothesis tested was that skin peptides would inhibit the growth of *Bd* in red-legged salamanders (*Plethodon shermani),* a species that is resistant to *Bd* infection*.* It was found that skin peptides from *P. Shermani* successfully inhibited the growth of *Bd* at high concentrations. Thus, skin peptides found in the secretions of *P. shermani* salamanders do confer resistance to the growth of the *Bd* fungus.

21

Search for $B_s \rightarrow \eta \eta'$ and $B_s \rightarrow \pi^0 \eta'$ in Belle Data

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We search for the decays $B_s \rightarrow \eta \eta'$ and $B_s \rightarrow \pi^0 \eta'$ using 121.4 fb^{-1} of data collected at the Y(5S) resonance with the Belle detector at the KEKB asymmetric-energy electron-positron collider. These decay modes are suppressed in the Standard Model of particle physics and proceed through $b \rightarrow u$ transitions, or $b \rightarrow s$ transitions, which are sensitive to new physics. The expected branching fractions in the Standard Model are 33.5×10^{-6} for $B_s \rightarrow \eta \eta'$ and 0.12×10^{-6} for $B_s \rightarrow \pi^0 \eta'$. Neither decay mode has been observed yet. We use Monte Carlo simulation to optimize our selection criteria for signal events and a Neural Network to separate signal from background events in order to make an accurate measurement of these branching fractions.

22

Removing Electron Beam Lithography Resist Residue from the Surface of MoS_2 using UV/Ozone

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In order to fabricate electronic devices using two-dimensional materials, lithography is required which leaves 1-2 nm of resist residues on the sample surface. Here, UV/Ozone is used to remove the resist residues from the surface of MoS_2, and the effectiveness of the treatment is quantified via atomic force microscopy (AFM). UV/Ozone exposure times ranged from 2 to 25 minutes and the effect of exposure time was examined. Results show that UV/Ozone exposure alone makes the resist residues more soluble in polar solvents (in this study, acetone and isopropyl alcohol) and reduces the total amount of residues found on the MoS_2 surface. However, there exists a tradeoff between removing the resist residues and damaging the MoS_2. Specifically, at longer exposure times (>10 minutes), the MoS_2 surface shows visible etching and is oxidized by the UV/Ozone treatment.

23

Insights into Single-Site Catalysts: Density Functional Theory Study of Iron Atoms on Amorphous Silica Surfaces

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Recent experimental work has shown that single-site catalysts involving isolated iron atoms on amorphous silica have unexpected and remarkable properties for activating C—H bonds in methane for reactions such as upgrading methane to ethylene and aromatics. We use density functional theory to study the energetic properties of iron atoms embedded on the surface of amorphous silica. Our goal is to shed light on the mechanism of the experimentally-observed activation of iron clusters on amorphous silica. We have identified different embedding sites on model surfaces of amorphous silica and have computed the optimized geometries, electronic properties, and energies of iron substitution.

24

Automatization for Preprocessing Laboratory Data

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Utilizing laboratory data in machine learning algorithms often requires extensive manual preprocessing. We aimed to develop an automated, efficient algorithm to preprocess laboratory results. Our algorithm focused on automating five preprocessing steps: 1) removing invalid test results, 2) classifying tests as categorical or continuous, 3) standardizing test units and categorical results, 4) flagging results as normal/abnormal, and 5) identifying results for manual review. Currently, the algorithm can automatically remove invalid test results with acceptable error, distinguish categorical/continuous tests, flag continuous tests, standardize test units and identify results for manual review. Standardization and flagging of categorical test results has proven to be a challenging task and is still in development. Once completed, our algorithm will reduce time spent manually preprocessing data and thus facilitate efficient, secondary use of laboratory data for machine learning algorithms.

25

Using Genomics to Study the Diversity of Red Foxes in Central Asia

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Red foxes (*Vulpes vulpes)* are omnivores found in a wide range of habitats, including forests, mountains, deserts, and urban areas. Their ability to adapt to a wide range of different habitats has allowed them to migrate throughout the world. Presently, the red fox has the largest range of any extant mammal. The phylogenetics of red foxes in Central Asia has not been well studied. The main goal of
this study was to compare genetic variation among different populations of red fox in Central Asia. The populations included in this study were from India, China, Pakistan, Mongolia, and Kyrgyzstan. 1,459 scat samples were collected from these areas.

26

Formation and Dissolution dynamics of silver filaments through an ion-conducting polymer film

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Using Conductive Atomic Force Microscopy (C-AFM) the formation and dissolution dynamics of an ion-conducting polymer film was characterized. This is part of the overall project which is creating polymer cubes with imbedded silver nanoparticles that can have its electrical and optical properties reconfigured with an applied external field. In our research we spin coated a polymer solution consisting of various concentrations of Polyethylene Glycol Diacrylate (PEGDA), 1-butyl-3-methylimidazilum tetrafluroborate (Ionic Liquid), and Silver Tetrafluroborate (AgBF4), onto a SiO2/Si substrate with 100nm of silver thermally evaporated on the SiO2. We measured the film thickness as a function of spin-speed using spectroscopic ellipsometry. To vary the film thickness, the spin speed, PEGDA concentration, and PEGDA molecular weight were varied. C-AFM was used to quantify the formation and dissolution times of conductive filaments between the silver underneath the film and the AFM tip. The formation and dissolution rates were calculated by dividing the measured times by the film thickness.

27

Restored Catalytic Activity of [Cu(Me_6TREN)Cl][Cl] in ATRA Using Ascorbic Acid as a Reducing Agent to Activate Less Active Monohalogenated Substrates

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Copper-catalyzed atom transfer radical addition (ATRA) is used for C-C bond formation through the addition of alkyl halides across various alkenes. Copper complexes with Me_6TREN (tris(2-dimethylaminoethyl)amine ligand are approximately ten times more active than with TPMA (tris(2-pyridylmethyl)amine) due to higher reported K_{ATRA} values. The side product of the oxidation of ascorbic acid is the generation of H-X, (X=Cl, Br), which protonates the Me_6TREN ligand. Previous UV/Vis studies of [Cu^{II}(Me_6TREN)Cl][Cl] in the presence of HCl confirmed decomposition of the copper complex under such conditions. ATRA studies of the addition of CCl_4 across alkenes catalyzed by [Cu(Me_6TREN)Cl][Cl] illustrated that nitrogen containing bases can inhibit the protonation of the ligand. We have reported values as high as 82% of much more synthetically attractive compounds originating from less reactive monohalogenated substrates. Column chromatography was utilized to isolate monoadducts which were also characterized via ^{1}H NMR and QTOF-MS.

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28

Designing a Translator to Facilitate Biochemical Network Reconstruction from Multiple Language Processing Formalisms

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Biochemical network reconstructions facilitate no-cost, in silico drug screening and toxicological experimentation. To assemble these models, tedious curation of reaction databases has been automated by algorithms that employ natural language processing, systematically extracting information about thousands of reactions from scientific journals. However, the output of these data mining algorithms differs in content and format depending on the needs of their creators. By studying the output of three reading algorithms, we designed a translator to accurately convert between several output formats. The translator successfully converts between the FRIES, MITRE, and TRIPS/DRUM formats and a custom formalism. In testing, the translator accurately converted 99.96 percent of 10,107 relevant fields. In addition, the translator was optimized for ease of use and distribution as command-line Python, Windows desktop, and Windows store applications. A translator between output conventions bridges a technical “language barrier”, allowing researchers to work interdependently toward the goal of functional network reconstructions.

29

Generation of Baculovirus for the Expression of Mutant Human Glycine Receptors in Sf9 Insect Cells

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Human alpha 1 glycine receptors (hGlyR) are inhibitory, post-synaptic ion channels and a member of the Cys-loop pentameric ligand gated ion channel family. Despite recent crystallographic data, limited structural information was available concerning allosteric states. Previously, protein crosslinking reagents were coupled with mass spectroscopy analysis to gain insight into the structure of the receptor in various allosteric states. Moreover, additional single cysteine point mutations were used to bind the crosslinker, MTS-benzophenone, and conduct structural studies. Baculovirus containing mutant hGlyR were tranfected in Sf9 cells to generate viral stocks. Viral titers were then performed to determine the concentration of viral plaque forming units (pfu) for several mutant hGlyRs and ranged from $1.02x10^4$ to $1.46x10^7$ pfu/mL. These results indicated that the viral stocks must be amplified before they can be used to infect large quantities of Sf9 cultures with a high multiplicity of infection (MOI) and these studies are ongoing.

30

Alpha-arrestin regulation of potassium channel Kir 2.1 trafficking

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Kir2.1 is an inward rectifying potassium channel of the KCNJ family found in mammalian cardiomyocytes. Though KCNJ proteins are critical for maintaining potassium homeostasis, regulation of their trafficking is poorly understood. We recently found that a family of trafficking adaptors, the α-arrestins, regulates Kir2.1 localization using a yeast model system. We have shown that three α-arrestins (Ldb19, Aly1, Aly2) control intracellular sorting of Kir2.1 and have assessed Kir2.1 localization and protein abundance using fluorescence microscopy, immunoblot analyses, and growth curve assays in the yeast model system. To next define the possible contributions of mammalian α-arrestins on KCNJ trafficking we have initiated studies to determine the effect of mammalian α-arrestin overexpression on 1) KCNJ-regulated potassium currents using two-electrode voltage electrophysiology in *Xenopus* oocytes, and 2) KCNJ abundance in mammalian cell culture. The combination of these studies helps to convey the similarities and differences in α-arrestin function from yeast to vertebrate model systems.

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Synthesis of a Fluorescent Turn-on Lead Sensor

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Low levels of lead have detrimental effects on the environment and human health. A turn-on chemo sensor for the lead (II) ion was developed. Initially, the synthetic pathway consisted of numerous steps with low yields. Currently, a smaller, six-step process with greater yields is used. Upon coordination to a lead ion (Pb(II)), the chemically synthetic molecule exhibits an intense fluorescence. The fascinating aspect of Leadglow is its ability to fluoresce with low concentrations of lead in solution, down to 10 ppb levels. The refined synthetic scheme of Leadglow is presented. Future work on the molecule consists of studying its fluorescence for real world applications.

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Evidence for Cross-Hemispheric Preconditioning in Experimental Parkinson's Disease

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Parkinson's disease (PD) usually emerges unilaterally, with deficits eventually appearing on the contralateral side only after a delay. It is well established the exposure to mild stress can precondition cells against subsequent toxic challenges. Consistent with the principles of preconditioning, we report that unilateral infusions of the oxidative toxicant 6-hydroxydopamine (6-OHDA) abolish contralateral loss of dopaminergic neurons (*i.e.* cell bodies and terminals) in response to a second 6-OHDA infusion in the opposite hemisphere. Motor asymmetries in turning behavior and forelimb use were consistent with these histological observations. 6-OHDA infusions increased activation of the phosphokinase ERK2 and expression levels of the antioxidant enzyme CuZn superoxide dismutase (SOD1) in both striata. These findings are the first to support the existence of cross-hemispheric preconditioning in experimental PD. If these results generalize to humans, Parkinson's pathology may progress more slowly on the side opposite to the initial deficits because the contralateral hemisphere is better prepared to combat future insults.

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Finding A Low-cost, Reliable Internal Standard Reference for Quantitative Analysis using X-ray Powder Diffraction

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Internal standards for X-Ray powder diffraction are used to calibrate and align the instrumentation. Additionally, they can allow for quantitative analysis of the crystalline components of a mixture. The National Institute of Standards and Technology (NIST) sell several standard reference materials, but due to the high cost they become inaccessible for use in an undergraduate laboratory course. This project focuses on finding an alternative, low-cost, but reliable internal standard for quantitative analysis. In this project, two candidate internal standards, cerium oxide and zinc oxide, obtained from a commercial supplier are being explored as standards using a Lite Salt mixture of varying amounts of sodium chloride and potassium chloride. The results will be evaluated in terms of both precision and accuracy. Once identified the standard reference material will be used in the advanced integrated laboratory course at Duquesne University.

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Association of Low-Frequency Variants in Regulatory Regions with Non-Syndromic Orofacial Clefts

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Orofacial clefts (OFCs) are a category of birth defects occurring in 1 per 700 births. To determine genetic factors contributing to risk of non-syndromic OFCs, genome-wide association studies have been performed, detecting a multitude of associations between genetic variants and OFCs. However, OFC heritability is not entirely explained by these findings. Therefore, this study tested associations between low-frequency variants (minor allele frequency <1%) in regulatory craniofacial enhancers and non-syndromic OFCs using two statistical tests: Sequence Kernel Association Test (SKAT) and Combined Multivariate and Collapsing Method (CMC). This study revealed an association between cleft palate and an enhancer on chromosome 3, known to regulate the branchial arch during development (SKAT p-value:4.95×10^{-5}). There was also a suggestive association between cleft lip with or without cleft palate and an enhancer on chromosome 9 (SKAT p-value:5.0×10^{-3}, CMC p-value:1.4×10^{-3}). These findings demonstrate that low-frequency variants in regulatory regions contribute to the complex etiology of OFCs.

35

Generation of the ScUbI and KinDel yeast strain libraries as tools for phenotypic analyses with Aly1 and Aly2 mutants

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Cells respond to cues in their extracellular environment by selectively redistributing proteins. This reorganization is imperative for cell survival and is regulated, in part, by α-arrestins. How is α-arrestin-mediated trafficking regulated? We know that modification by ubiquitination and phosphorylation both control α-arrestin function. To help us identify specific α-arrestin regulators, we are building two unique yeast gene deletion libraries: 1) the *Saccharomyces cerevisiae* Ubiquitin Interactome (ScUbI) library and 2) the Kinase and phosphatase Deletion library (KinDel). These libraries will contain all the non-essential genes annotated as important for ubiquitination/ubiquitin interaction or phospho-regulation, respectively. We will use these libraries to screen for gene deletions that result in loss of phenotypes associated with α-arrestin function, using α-arrestins Aly1 and Aly2 as an initial paradigm. To accomplish our goal, several new and important high-throughput screening tools have been established, and these new protocols will be described here.

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Introducing Cysteine Mutations in Human α1 Glycine Receptors *via* Site-Directed Mutagenesis

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Human α1 glycine receptors (GlyR), a member of the pentameric ligand-gated ion channel superfamily is transiently permeant to chloride ions upon glycine binding. Creating single site-specific cysteine (Cys) point mutations is an initial step in examining GlyR allostery. Two types of cysteine mutations were made—a single Cys substitution in a wild type *Cys null* background, and the identical substitution in a non-desensitizing double GlyR variant with sensitivity to ivermectin. Mutations in a wild type background allow the desensitized state to be studied in the presence of excess glycine, and closed state to be studied in the absence of glycine. Cysteine mutations in ivermectin sensitive GlyR can be used to study the open state in the presence of excess ivermectin. Subsequent structural studies will allow a more complete understanding of the protein's allostery, prompting better drug design for chronic pain and hyperekplexia.

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Conversion of Phosphatidylcholine to Phosphatidic Acid using Phospholipase D for Human α1 Glycine Receptor Studies

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Phosphatidic acid (PA), a diacyl-glycerolphospholipid, is an important membrane signaling lipid which influences disease and transmembrane Cys-loop receptor functionality and conformation. To determine the unique protein-lipid interactions of PA, comparative crosslinking studies with PA and its precursor lipid, phosphatidylcholine (PC) will be conducted. Using a biological enzyme, phospholipase D (PLD), 95% plant PC was reacted with PLD in a 9:1 ratio for two hours. PA was, then, extracted using an altered Bligh and Dyer method. Lipid content was confirmed by two dimensional thin layer chromatography and quadrupole time of flight mass spectrometry. Then, the protocol was repeated using pacFA-18:1 PC contains a photoactivatable diazirine (This compound is unavailable as a photoactivatable PA). After extraction and dilution, pacFA-18:1 PA interactions can be studied and compared to PC labeling of human α1 glycine receptor, a Cys-loop receptor, gleaning new information about specific interactions of anionic lipids and glycine receptor function.

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Serotonin transporter

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Serotonin transporters are integral transmembrane proteins responsible for the reuptake of serotonin from the synapse to the presynaptic neuron. The issue investigated in this study is the allostery of the protein when it reuptakes serotonin back into the cell. To study this issue, a cysteine was introduced on the loops of the protein. One cysteine was introduced at a time and after expressing the protein, a MTS-benzophenone crosslinker was used to bind to form a disulfide bond to the cysteine mutation. The protein was stabilized in different states, and the results were compared with each other to determine protein movement. This research will increase our knowledge of the structure of serotonin transporters and can potentially be used to develop better medications for people who have depression and anxiety.
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Evaluating Multiple Classifier Methods for Patient Specific Result Prediction in Learning Electronic Medical Records (LEMR)

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The Learning Electronic Medical Records (LEMR) attempts to help avoid Information Overload (IO) for clinicians by highlighting results of predicted interest for specific patients. We tested multiple Machine Learning (ML) algorithms against curated sets of records to determine an optimal classification method. We compared Random Forest (RF) ML to Lasso and Ridge Logistic Regressions (LR) as well as to Support Vector Machines (SVM) to see which one better predicted relevant results of specific patient's clinical state. We found that RF showed a better overall Area Under the Receiver Operating Characteristic (AUROC) of 0.63 than either 0.60 in Lasso LR or 0.42 in SVM. The difference in performance of ML algorithms on predicting results of relevance in individual patients suggest that ensemble methods combining multiple models could lead to further improvement on classification methods in LEMR.

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Adsorption of Arsenic by Activated Carbon

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Arsenic is a toxic element that has the potential to enter ground water sources either by erosion or from runoff of agricultural or industrial sites which can contaminate drinking water. Chronic exposure to arsenic has been linked to non-carcinogenic effects such as nausea, vomiting, partial paralysis, blindness, and cancers of the bladder, lung, skin, kidney, and liver. The project focuses on the removal of sodium arsenate from aqueous media using an activated carbon filter that has been doped with Fe(lll) hydroxide. The filtrate will be analyzed using ion chromatography to test for removal of the sodium arsenate from the initial aqueous solution.

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Endogenous defenses against severe oxidative stress in astrocytes

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Astrocytes are the most abundant glial cell type in the brain and are highly stress-resistant. Here we show that primary cortical astrocytes surviving high concentrations of the oxidative toxin paraquat do not die in response to subsequent oxidative challenges. This phenomenon of glial stress tolerance was so robust that inhibition of multiple defensive proteins, such as heat shock proteins 32 and 70, the antioxidant glutathione, and the kinases ERK1/2, Akt, and JNK completely failed to attenuate the protective effect. These results demonstrate that astrocytes are not weakened by exposure to severe stress, unlike neurons. Rather, they fail to respond with any additional cell loss in a manner that is independent of multiple endogenous defenses. We speculate that astrocytes evolved to tolerate severe stress in order to continue to fulfill their neurosupportive roles in highly damaged brain regions.

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Determining the Critical Mixing Speed of Fluidization in an Agitated Reactor

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For processes involving Liquid-Solid and Gas-Liquid-Solid stirred tank reactors, it is critical to identify the operating and geometrical parameters for optimum catalyst fluidization. Improper understanding of the effect of mixing on catalyst fluidization during reactor operation will inevitably have a negative effect on the reaction performance and product quality, and may unnecessarily increase mixing power consumption and costs. Thus the aim of this work is to experimentally determine the critical mixing speed of fluidization, which is the minimum speed at which all particles in the vessel achieves suspension, for multiple Liquid-Solid systems in an agitated reactor. An empirical correlation for determining the mixing performance will be developed. Previous works investigating the critical mixing speed of fluidization had significant discrepancies in their predictions, and there are still a few different parameters that, between all the investigations, have not been tested, such as the effect of baffles, which this work will address.

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Synthesis and Characterization of Dithione Ligands and Mixed Ligand Molybdenum Complexes

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Coordination complexes of 1,2-dithiolenes are fascinating molecules that exhibit unique structural and spectroscopic properties. 1,2-dithiolenes have uses in various material science applications and are also important for the development of mimics for biological metal centers. This project will focus on the synthesis of such ligands and these ligands will used to synthesize mixed ligand molybdenum complexes that may act as a mimic for certain biological metal centers. Both anaerobic and aerobic reaction conditions will be employed and all complexes will be characterized using various spectroscopic techniques such as ^{1}H NMR, IR, and UV-Vis spectroscopy.

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Evidence for formyl and α-hydrogen bonding in asymmetric organic reactions from single crystal X-ray diffraction and density functional theory

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Koga reported that alkoxyaluminium dichloride, a Lewis acid catalyst, worked effectively to form highly stereoselective products but did not offer a molecular interpretation. In attempt to develop a model for stereoselectivity, Corey and coworkers introduced the formyl hydrogen bond, but did not explain Koga's entire stereochemical data set. We hypothesize that the α-hydrogen is a novel catalytic element along with Corey's formyl hydrogen bond to explain the stability of the Lewis acid and predict the stereoselectivity of adducts in significant organic reactions. To test our hypothesis, we modeled BF_3 with N,N-dimethylformamide, N,N-dimethylacetamide and N,N-dimethylacrylamide computationally using M062X/jul-cc-pvDz combined with single crystal X-ray diffraction to determine the structure and conformation of the complexes. Our preliminary results show that the BF_3-DMF computational model is consistent with the crystallographic structure. The influence of formyl and α-hydrogen bonding upon the dienophile-Lewis acid complex conformation will be presented and discussed.

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Optimizing Electrospun Gel and Composite Polymer Electrolytes for Improved Li – S Battery Performance

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Sulfur is a promising candidate as cathode material for lithium batteries because of its high theoretical specific energy of 2,567 Wh/kg and capacity of 1,672 mAhr/g, outperforming current $LiCoO_2$ or $LiMnO_2$ cathode capabilities. However, sulfur electrodes suffer from poor cycling stability due to the low electronic conductivity of sulfur and highly soluble nature of lithium polysulfides which undergo crossover and coat onto the anode, leading to cell failure. Replacing organic electrolytes with a gel/composite polymer electrolyte (GPEs) has shown promising results in mitigating this, albeit still suffering from poor ionic conductivity. This work focuses on creating an ionically conducting, flexible GPE capable of preventing polysulfide crossover by electrospinning various polymers along with organic lithium containing salts and mesoporous filler nanoparticles such as SiO_2 and TiO_2. The results of testing these electrospun fiber mats as a separator – electrolyte complex against commercial sulfur cathodes are reported.

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Correlation of PFC activity with Social Development in Children using Non-Invasive Optical Imaging

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The Pre Frontal Cortex is involved in executive function and control and plays a large part in the social development of young children. It has been revealed that signs of irritability in children could parallel neural defects within this specific brain region. As a child grows, the pre frontal cortex is crucial in developing a child's social behavior and their ability to regulate emotion. In order to track the progress of this region, fNIRS technology was used to measure blood flow and make explicit inferences concerning irritable and non-irritable children. Subjects played computer games that allowed them to experience an array of emotions, which enabled us to measure all possible activity in the PFC. It was revealed that children with mental disability or irritable behavior showed less activation of this brain area compared to normally developing children, providing a potential basis for clinical diagnosis in the future.

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Design of a Packed Bead Column for Extracorporeal CO_2 Removal through Respiratory Hemodialysis

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Extracorporeal carbon dioxide removal ($ECCO_2R$) is a promising treatment for patients with acute lung failure, including acute respiratory distress syndrome and acute exacerbations of COPD. It removes excess CO_2 from patients' blood. In the human body, over 90% of carbon dioxide is transported in blood as bicarbonate. Based on this fact, respiratory hemodialysis, one form of $ECCO_2R$, is developed to remove the bicarbonate through traditional hemodialysis techniques. To prevent micronutrient loss during the treatment, the dialysate needs to be recycled. In this study, a packed bead column was designed to remove bicarbonate from the dialysate during its recycling. Carbonic anhydrase, an enzyme catalyzing the conversion of bicarbonate to dissolved CO_2, was immobilized on to the polystyrene beads. The dissolved CO_2 was bubbled out of the dialysate by using pure oxygen gas. The immobilized enzyme activity was determined and the column's CO_2 removal rate was tested in an assembled device.

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Correlation of PFC activity with Social Development in Children using Non-Invasive Optical Imaging

Kolar, Brian; Seese, Brett; Perlman, Susan; Clark, Russell J.
Department of Psychiatry
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The Pre Frontal Cortex is involved in executive function and control and plays a large part in the social development of young children. It has been revealed that signs of irritability in children could parallel neural defects within this specific brain region. As a child grows, the pre frontal cortex is crucial in developing a child's social behavior and their ability to regulate emotion. In order to track the progress of this region, fNIRS technology was used to measure blood flow and make explicit inferences concerning irritable and non-irritable children. Subjects played computer games that allowed them to experience an array of emotions, which enabled us to measure all possible activity in the PFC. It was revealed that children with mental disability or irritable behavior showed less activation of this brain area compared to normally developing children, providing a potential basis for clinical diagnosis in the future.

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The Synthesis, Structure, and Characterization of Quaternary Diamond-Like Semiconductor Selenides with Applications in Infrared Non-Linear Optics

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Quaternary diamond-like semiconductors (DLSs) of the formula I_2-II-IV-VI_4, and its structural variation as I_4-II-IV_2-VI_7, were synthesized and studied to determine their viability in infrared (IR) non-linear optical (NLO) devices. Currently, the commercially available options for IR NLO devices are ternary DLSs, such as $ZnGeP_2$, $AgGaS_2$, and $AgGaSe_2$. These ternary compounds have serious drawbacks such as difficulty of crystal growth and low laser damage thresholds (LDTs). Quaternary DLSs are promising candidates for IR NLO devices as a result of increased LDTs and compositional flexibility that can be exploited to tune properties, for example, the bandgap and the NLO susceptibility. Pure-phase compounds are necessary for optical property measurements. Toward this goal, the quaternary compounds were synthesized via high-temperature solid state synthesis, and analyzed through X-ray powder diffraction and diffuse reflectance UV-Vis-NIR spectroscopy. The crystal products were imaged using scanning electron microscopy, and semi-quantitative composition was obtained using energy dispersive spectroscopy.

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Development of novel Surface-mediated gene transfer (SMGT) system for gene transfection in bone implants.

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Surface mediated gene transfer (SMGT) is a promising method for regenerative medicine. SMGT typically employs controlled release of plasmid DNA (pDNA) from a multilayer structure comprised of alternating bilayers of polycationic and polyanionic, synthetic or natural polymers. However, SMGT currently has limited clinical translation due to poor gene transfection efficiency. We have accordingly developed a novel SMGT system comprised of synthetic polycationic and polyanionic polymers which incorporate calcium phosphate nanoparticles complexed to pDNA (NanoCaPs-pDNA). The current work was developed on non-biodegradable, titanium, and biodegradable, magnesium substrates. Surface characterization via Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), ellipsometry, and dynamic contact angle (CA) measurement have confirmed the successful fabrication of our SGMT system. Preliminary *in vitro* studies with human embryonic kidney (HEK-293) cells have demonstrated our SMGT as a superior gene transfection system.

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Optimizing Exome Cancer Workflows on the Bridges Supercomputer.

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Workflows for cancer data utilize a variety of bioinformatics tools with different performance characteristics. These characteristics may vary substantially depending on the physical hardware in which the workflow is run. In this project we used publically available exome sequencing data to examine the performance of a University of Pittsburgh developed variant-calling workflow on the Pittsburgh Supercomputing Center's newest supercomputer, "Bridges", and how various file systems can be used to improve the throughput of these workflows. This exome workflow has been benchmarked to characterize the four available file systems on Bridges to provide guidance to users of the system. In addition we looked at the tools in the pipeline individually to examine the parallel performance of the tools. We found that optimal filesystem selection is tool-dependent and is highly variable depending on the way in which the workflow is structured. Likewise, the parallel performance of the pipeline is also highly tool-dependent.

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Modeling the Evolution of *Pseudomonas aeruginosa* in Biofilms

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Cystic fibrosis patients have a buildup of mucus in their respiratory tracts that promotes chronic infections due to bacteria accumulation. *Pseudomonas aeruginosa* is a common pathogen in cystic fibrosis patients that establishes infections in part by creating biofilms to help it resist clearing agents. In addition to the biofilm's inherent durability, biofilm formation allows rapid adaptation of the bacterial population, leading to high genetic and physiological diversity. A model of biofilm evolution is needed to help predict the progression of chronic *P. aeruginosa* infections in cystic fibrosis patients. Experimental evolution of *P. aeruginosa* for nearly 600 generations under biofilm conditions produced metagenomic data detailing the population's mutational composition at 6 time points throughout the experiment, as well as clonal mutation data at the end of the experiment. The data was analyzed and models summarizing the pathogen's evolution were created by determining the prevalence of key mutations throughout the experiment.

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The Effect of Resveratrol & Resveratrol Nano emulsions on Breast Cancer Cell Proliferation

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Breast cancer is a disease that affects more than 1.7 million women each year. Breast cancer cells are mutated so that they gain the ability to keep dividing without control to form tumors. In this study, we are investigating the effect of the natural product, resveratrol, on cancer cell growth. Natural products are attractive therapeutic option as they leave heathy cells undamaged. Resveratrol was added to breast cancer cells to examine the effect on cancer cell proliferation using an MTT assay. As the bioavailability of resveratrol is low, we used resveratrol nanoemulsions made in Dr. Janjic's lab for comparison to free reseveratrol. Nanoemulsions are phagocytosed by macrophages to deliver the reseveratrol to the cells and thereby increase the bioavailability of resveratrol. The completion of these studies will increase our understanding of how natural products effect breast cancer cell proliferation.

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The role of α-arrestin ubiquitination on protein stability

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Proteins are removed from the cell surface in response to environmental changes. The α-arrestins are a critical class of protein trafficking adaptor needed for this regulated protein trafficking. Specifically, -arrestins bind to the ubiquitin ligase, Rsp5, and recruit it to transmembrane cargo proteins. Ubiquitination of cargo serves as a mark for their internalization. However, the α-arrestin itself is also ubiquitinated in and the functional consequence of this modification is unclear. Our goal is to define how ubiquitination impacts the function for α-arrestins Aly1 and Aly2. We make use of two Aly1 and Aly2 mutants: 1) PPxG mutants that no longer bind Rsp5, and therefore fail to ubiquitinate both α-arrestins and cargos, and 2) K-to-R mutants in which the ubiquitination site in Aly1 and Aly2 is mutated to arginine to prevent α-arrestin ubiquitination but leave Rsp5-binding intact. We are assessing the impact these mutations have on Aly1 and Aly2 protein stability.

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Quaternary Diamond-Like Semiconductors for Infrared Nonlinear Optical Devices: Synthesis and Characterization

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Commercially available crystals for infrared (IR) nonlinear optical (NLO) devices are ternary diamond-like semiconductors (DLSs), such as $AgGaS_2$. These NLO crystals shift the coherent, monochromatic light of lasers to other desirable wavelengths for military and medical applications. Unfortunately, these materials exhibit drawbacks including low laser damage thresholds (LDTs) and difficult crystal growth. To overcome this issue, quaternary DLSs, which offer compositional flexibility that allows for the tuning of optical properties, are being pursued. This project targets DLSs, Cu_2MnGeS_4, Ag_2FeSiS_4, and Ag_2ZnSiS_4, for which no NLO studies have been performed. In order to properly assess the viability of these DLSs in infrared NLO devices, pure-phase materials are needed. The compounds were prepared via high-temperature, solid-state synthesis and characterized using X-ray powder diffraction. Scanning electron microscopy was utilized to study the morphology of the synthesized materials and energy dispersive spectroscopy was used to assess the homogeneity and composition of the samples.

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Testing the Limits of Causal Analysis

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This project explores some limits of causal analysis in dynamic systems, integrating perspectives from sources across decades: Levins's moving equilibrium models, the LoopAnalyst package, Dash's explorations of temporality and causality, Pearl's approach to causal inference, and contemporary causal discovery algorithms. The long term and short term effects of an intervention are sometimes paradoxical: manipulating a parameter in order to influence a target variable may initially cause the expected change, but dynamics may then take the variable in the opposite direction. Our R package LevinsLoops graphically explores pre-selected and user-defined dynamic systems. The Dynamics graph shows the trajectory from starting point to equilibrium. The Moving Equilibrium graph shows the equilibrium changing as a selected parameter changes. LevinsLoops demonstrates how the paradoxes occur and why the time frame matters. Future extensions will generate simulated data sets to test causal algorithms, and study apoptosis.

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Evaluation of novel copper complexes as catalysts in atom transfer radical addition (ATRA) reactions

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The effectiveness of ligands N,N-bis((4- methoxy-3,5-dimethylpyridin-2- yl)methyl)-N',N'- dimethlyethylenediamine (T*2M1) and N-(2-(dimethylamino)ethyl)-N-((4- methoxy-3,5-dimethylpyridin-2- yl)methyl)-N',N'- dimethlyethylenediamine (T*1M2) complexed with CuX_2 (X=Cl, Br) as catalysts atom transfer radical addition (ATRA) reactions in comparison to tris(2-pyridylmethyl)amine (TPMA) was investigated. Photo initiated ATRA reactions were conducted with [Cu(L)X][X] (L=TPMA, T*2M1, T*1M2; X=Cl,Br) catalysts with a variety of alkenes and alkyl halides. Yields for the reaction of bromoacetonitrile to methyl acrylate with a catalyst loading of 1 mol % to alkene were 58% for $[Cu^{II}(T*2M1)(Br)][Br]$ compared to 64% for $[Cu^{II}(TPMA)(Br)][Br]$ with $[Cu^{II}(T*1M2)(Br)][Br]$ showing much lower yields. Yields for the addition of 2-bromopropionitrile to methyl acrylate with a catalyst loading of 1 mol % to alkene were 94% for $[Cu^{II}(T*1M2)(Br)][Br]$ compared to 82% for $[Cu^{II}(TPMA)(Br)][Br]$. $[Cu^{II}(T*2M1)(Br)][Br]$ and $[Cu^{II}(T*1M2)(Br)][Br]$ showed comparable yields to $[Cu^{II}(TPMA)(Br)][Br]$ for the ATRA reactions of 2-bromopropionitrile to alkenes.

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Gas Sensing MOF Array Investigation with Information Entropy

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Gas sensing technologies as they exist today are used in a wide range of applications, from environmental monitoring to explosives and drug detection.[1] Beyond the simple detection of a single molecular species, some gas sensors are composed of arrays of polymer or metal oxide substrates that collectively give complex signature responses to multi-component gas mixtures. While existing sensing substrate technologies are restricted in sensitivity by low surface areas, it has been proposed that metal-organic frameworks (MOFs) deposited on detecting substrates can increase sensitivity through their high surface areas, reproducibility, and tunability.[2] The high tunability of MOFs leads to a difficulty in identifying the most effective combinations of MOFs in gas sensing arrays, however. This research investigates the use of information entropy as a measure of the effectiveness of different MOF arrays that can be used for gas sensing applications.

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Edge guided curvature based denoising

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Recent studies suggest that various denoising methods can be improved upon by denoising the curvature information of the noisy image instead of processing the image directly. We have observed that while the object boundaries within an image always benefit from this approach, smooth regions do not always enjoy the same benefits. In this research we are currently exploring mechanisms within this framework for treating smooth areas and edge regions differently to generate an optimal reconstruction across the entire image.

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Characterization of Spore-Associated Protein D for Use in the Development of *Streptomyces coelicolor* Spores for Vaccine Delivery

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One type of potential vaccine production explores the use of exospores from filamentous bacteria, such as *Streptomyces coelicolor*. Previously we showed that, spore-associated protein D (SapD) fused with the β-subunit of the heat labile toxin (LTB) of *E. coli* localizes to the surface of *S. coelicolor* spores. Now, the primary focus of this project is to determine the location of the unknown secretion signal by creating truncations of SapD fused to LTB. The SapD-LTB C-Terminal truncation encoding constructs were inserted into the *S. coelicolor* chromosome in order to express the various C-Terminal truncated protein fusions and determine if they localize to the spore coat. Spore-associated proteins are being extracted and will be characterized using SDS-PAGE and Western blot techniques. Because of the durability and longevity of spores and their associated proteins, antigens expressed on exospores could potentially result in a vaccine with extended shelf life.

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An Image Analysis Tool for Mapping Cilia-Driven Particle Crystallization

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An important aspect of particle-based drug delivery is the degree of crystallinity of the delivered particles *in vivo*. Brownian motion affects particles in a fluid under 6 microns in diameter enable the formation of crystalline structures. Larger particles require that a driving force be applied to them. In the case where particles are inhaled into human lungs, cilia within the lungs may impart such a force on the particles in an effort to clear them from the airways. In this work, images were taken using confocal microscopy of particles on well-differentiated, ciliated human bronchial epithelial cell cultures. Stacked 2D image files of the particles were generated post-assembly. Using an augmented form of the Circular Hough Transform function, each stack of particles was evaluated and particle centers were identified. From the data obtained during each stack evaluation, the overall crystal structure in this 3D system can be determined.

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A Graphical User Interface for Monitoring Glucose-Insulin Dynamics in Critical Care Patients

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Hyperglycemia is often present in critical care patients, and these patients must be closely monitored to regulate their glycemic levels. In order to prevent hyperglycemia, the patients can be treated effectively with insulin based on a mathematical model and control algorithm that monitors glucose-insulin dynamics. To aid clinicians, we synthesized a graphical user interface (GUI) in MATLAB that can interact with the dynamic patient model. This model and GUI contain time-varying parameters that capture patient-specific fluctuations. The GUI also describes glucose levels after subcutaneous or intravenous insulin administration, meal administration and other exogenous glucose inputs. The insulin sensitivity can be updated to accurately represent different patients. Overall, this GUI provides a patient-tailorable display of glucose-insulin dynamics in individuals over a clinician-specified time period with a goal of mitigating hyper- and hypoglycemia.

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QSAR Models: Identifying Substructures Key in Binding and Predicting Ligand Affinities to SERT

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Adverse effects and lack of efficacy of antidepressants can derive from poor selectivity and binding affinity towards their target. Selective serotonin reuptake inhibitors, or SSRIs, such as paroxetine and escitalopram, are commonly prescribed to treat mental disorders derived from serotonin deficiencies. Through an analysis of the structure-activity relationships of SERT binding compounds, substructures' effects on binding affinity can be identified. In this project, we assembled data sets of >1,500 DAT ligands and >2,500 SERT ligands to train QSAR models. The predictive QSAR models can estimate affinity and selectivity of ligands to the serotonin reuptake transporter and highlight moieties important to affinity. To test the estimates of the models we calculated the binding free energy of paroxetine and citalopram through molecular dynamics simulations. Additionally, a derivative of citalopram was generated with SeeSAR; its predicted binding affinity to SERT was greater than that of its parent.

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Probing the Strange Quark in the Proton

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The widespread belief is that the proton and neutron, commonly known as nucleons, are each composed of three elementary particles called quarks. But in the last few decades, experiments showed that the mass, momentum, spin, and electromagnetic properties of the three quarks do not add up to the known proprieties of the nucleon. Theory predicts that a ``sea'' of virtual pairs of quarks and anti-quarks, along with the strong force carrier particles called gluons, should account for the difference. Our interest of study is in the contribution of the third lighter quark, known as the "Strange quark," and its anti-quark counterpart. Kaon particles, formed of strange and up quarks, will allow us to probe the strange quark. Database development and geometry programming for charged particle detector will be used to further examine the data involving these kaons. We will present our work on the hit reconstruction for the experiments.

65

Modeling RGG Box Interactions with RNA G-quadruplex Binding Domains

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Hexanucleotide repeat expansions (HREs), e.g. (G4C2)n, of the C9orf72 gene, are the leading cause of the neurodegenerative diseases Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). One mechanism for pathogenesis is protein sequestration by RNA G-quadruplexes formed from HREs within the nucleus, thus inhibiting the protein's normal function. FMRP, a regulatory RNA-binding protein (RBP), often complexes with RNA G-quadruplexes by an arginine-glycine-rich region or RGG box to control protein translation. Crystal structures were adapted to create a (G4C2)4 RNA G-quadruplex, and PEPFOLD3 was used to identify viable ligand structures from an FMRP RGG box FASTA sequence. Here, we present the binding of FMRP to HRE RNA G-quadruplexes using a variety of molecular dynamics programs such as CHARMM-GUI, NAMD, and ACEMD. The preliminary results further validate RGG box/quadruplex interactions in the absence of a stem region, and the importance of specific peptide residues that are key in RGG box anchoring.

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Monte Carlo Simulations of 2D Mercedes-Benz Water

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The 2D Mercedes-Benz (MB) model of water is a viable computational means of qualitatively representing unusual properties of water that are experimentally observed. A Monte Carlo program written in python was used to perform simulations on systems of *N*=64 MB water molecules. We show that the MB model qualitatively displays the volume and density anomalies, as well as the effect of hydrogen bonding. By changing the reduced temperature (k_BT = .20 and k_BT = .14, respectively), we created a MB water and MB ice system. Upon freezing, the graphs of area vs. steps were plotted and it was shown that the volume of the ice system was greater. Since $d = m\frac{1}{v}$ and mass was constant, this showed that MB ice is less dense than MB water. Also, the potential energy of a nonpolar system was compared to that of MB water, showing that hydrogen bonds significantly reduce overall system potential energy.

~~**CAELIN CELANI**~~

67

Synthesis and Characterization of Quaternary Diamond-Like Semiconductors

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Diamond-like semiconductors are compounds with characteristically wide bandgaps. This makes them useful for electrical and optical applications. The goal of this research is to synthesize quaternary diamond-like semiconductors (DLSs) using high-temperature solid-state synthesis. Focusing specifically on the compound Li_2ZnSnS_4, which has potential in areas such as infrared nonlinear optical devices. This experiment will investigate conditions of high-temperature solid-state reactions including varying the elemental ratios to obtain phase-pure samples of Li_2ZnSiS_4. The phase-purity will be assessed using X-ray powder diffraction (XRPD). Scanning electron microscopy coupled with energy dispersive spectroscopy will be utilized to determine the surface morphology and approximate elemental ratio of the compounds.

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Interactive Interface for Designing Docetaxel Treatment Schedules in Cancer Chemotherapy

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Chemotherapy schedules balance drug efficacy and toxicity for solid tumor cancer patients. Existing models simulate drug responses, but are typically designed for a nominal patient or population, and parameters must be altered to capture individual response. We designed a graphical user interface (GUI) in Python that would allow clinicians to interact with toxicity and response models, thereby allowing them to (re)design chemotherapy schedules and check predicted patient response in real-time. Pharmacokinetic, pharmacodynamic effect, and toxicity models in response to treatment with Docetaxel are used. The GUI simulates tumor size and absolute neutrophil count (ANC) for a specified patient on a clinician-determined schedule. Clinicians can update the model, using measured ANCs or tumor sizes during patient visits, or update the treatment schedule. The result is an interactive GUI that shows individual patient responses to treatment and allows clinicians to quickly evaluate potential outcomes of different treatment schedules.

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Stabilizing an Optical Circuit with Proper Equipment and a Secure Cart Design~~Title~~

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~~The project over the past couple months was to design a functioning~~ We have built a Michelson Interferometer to measure the wavelength of an unknown laser. A crucial part of the interferometer is the layout of the optical circuit. The circuit was designed where the beam would be evenly split along two pathways. A moving cart mounted on an air track to reduce friction holds~~held the~~ retroreflectors in each arm of the interferometer, which return~~ed~~ the beam back along the pathway. The beams ~~would~~ recombine on a ~~beamsplitter~~beam splitter creating interference fringes~~and a "fringing" effect indicative of interference would take place~~. The number of fringes that appear as the cart moves is proportional to the wavelength of the laser. ~~The t~~Two factors in making sure that the Interferometer operate~~d~~ smoothly ~~was~~are the alignment of the optics and the stability of the air track. To ~~counter-~~improve the alignment ~~issue~~, a "cat's eye" retroreflector ~~called a cat's eye was used to gain extreme accuracy~~was installed to replace the existing corner-cube retroreflector. To improve~~solve~~ the stability of the air track~~issue~~, the various~~specific~~ cart design~~s~~ were built using a 3D printer and tested to minimize ~~was considered to enable the optics to float without~~ any disruptive shaking. Taking care of these two points promise~~d~~ a reliable comparison between ~~the~~ two lasers.

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Gas-phase Reactions of Alcohol Solvated Group II Cations

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The chemistry of the group II cations is of great interest because Mg and Ca are essential to life, and Sr and Ba play important roles in materials and industry. Tandem mass spectrometry allows species-specific studies of metal ions and complexes. Here were used electrospray ionization to create gas-phase, doubly charged complexes containing group II cations and simple alcohols, which are the simplest models for solvated cations. Collision-induced dissociation (CID) and ion-molecule reactions were used to determine behavior of the solvated ions. We found that regardless of the cation or alcohol (methanol, ethanol, 1-propanol or 2-propanol), CID of $[M(ROH)_4]^{2+}$ caused elimination of a single alcohol ligand. However, CID of $[M(ROH)_3]^{2+}$ causes ligand elimination and charge reduction reactions. For methanol, the charge reduction occurs by proton transfer and formation of singly-charged metal-methoxides. For the larger alcohols, heterolytic cleavage of the alcohols is observed, leading to formation of singly-charged metal-hydroxide products.

~~**Isolation and Characterization of an Antibiotic Resistant Bacterium from Produced Water**~~

~~Jenna Kuhn and John F Stolz~~
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~~Development of natural gas through unconventional drilling and hydraulic fracking well fluid generates production fluids as well as releases formation water. These fluids are not sterile and microbial activity can result in potentially harmful compounds as well as well deterioration and decreased gas production. Produced water from hydraulically fracked wells contains a variety of microbes able to withstand a high total dissolved salt concentration (TDS) environment. This study focused on isolating a single strain by growing cultures in high TDS liquid media and streak plating, analyzing microbial morphology using light microscopy, and identifying the strain through 16S rRNA gene analysis. Antibiotic resistance testing found the strain to be sensitive to erythromycin, but resistant to oxacillin and nalidixic acid. The microbe grew optimally at 10.0 % NaCl, pH of 6, and 24°C.~~

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Community-Engaged Learning in the Undergraduate Research Program

Ames, C.; Behun, M.; Bizarro, M.; Callipare, A.; Clemenza, P.; Cox, A; DeMarco, E.; Evans, C.; Furgurson, M.; Furnari, H.;Garces, A.; Gartland, N.; Hilton, E.; Magyan, A.; Malik, F.; Pirl, J.; Renk, M.; Resko, Z.; O'Donnell, A; Woodley, S..
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Kinetics of Transport through Nuclear Pore Complexes

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The focus of this research is the transport of large molecules (>40 kD in size) through nuclear pore complexes via interactions between transport proteins and the many

This summer, 17 undergraduates in the Duquesne University Research Program (URP) participated in community-engaged learning at the Center of Life, a community organization in Pittsburgh that offers a free summer camp for children. Working in teams, URP students translated themes from their research into interactive science activities that they shared with middle-school aged children. URP students spent about 2 hours per week over the 10 week program preparing for, engaging with, and reflecting on the experience. By working collaboratively with the Center of Life, Duquesne URP students developed disciplinary and professional skills while engaging with the civic issue of science literacy. At the same time, campers were exposed to topics in cell biology, conservation biology, neuroscience, and pain empathy, thereby contributing to the scientific literacy of the campers and inspiring an interest in science.

A Study of the Morphological and Genomic Effect of Melatonin and/or 17 □-Estradiol (E_2)/Progesterone (P_4) Hormone Therapy (HT) in HER2/*neu* Mouse Mammary Tissue

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Prempro,™ which contains conjugated equine estrogens (CEE) and medroxy progesterone acetate (MPA), but not Premarin (contains CEE only) increased breast cancer incidence and mortality; however, the underlying mechanisms of this are not clear, but may involve MPA effects on mammary ductal differentiation and tumorigenesis. Ten different treatment groups containing E_2 and varying doses of P_4, CEE/MPA, alone or in combination with melatonin, were tested in 2-3 month old female HER2/neu mice, a transgenic mammary cancer mouse model. Our data demonstrate that mammary ductal differentiation that is associated with cancer protection (decrease in ductal elongation and increase in tertiary branching) was paralleled by temporal changes in pERK 1/2, pERK5, and Runx2 expression. Although the overexpression of any of these proteins may lead to tumorigenesis, the protein signature exhibited in the presence of melatonin induced changes that are theorized to be the most protective against cancer, offering menopausal women a safe HT alternative.

disordered FG nucleoporin proteins that line the inside of nuclear pore complexes (NPCs). In this work, a bead-spring polymer chain represents individual nucleoporins, and spherical nanoparticles model transport proteins. Molecular dynamics simulations provide insight into the kinetics of transport, i.e., the time evolution of cargos and transport proteins infiltrating the NPC and crossing the nuclear envelope. Diffusion across the NPC is observed in the presence of nanoparticle concentration imbalance, as expected. Additionally, as nanoparticle size increases traversal becomes confined to a central aqueous channel region whose size is determined by the degree of extension of the polymers. This supports another application of the polymer brush model (aside from the study of NPCs), namely, as a possible biomimetic valve.

Genetic Association of PKD2 with Dental Caries

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Dental caries (decay), the most common disease worldwide, costs over $50 billion annually in the US. Though heritable, few genes for dental caries have been identified and replicated. A genome-wide association study of dental caries implicated the genomic region containing the dentin/bone extracellular matrix genes and two adjacent genes (PKD2, ABCG2). As a follow-up study, we analyzed the effects of 49 genetic polymorphisms chosen to tag the genetic variation in this region, while adjusting for age, sex, and ancestry using samples from 6 studies (n=4000). Age- and race-stratified analyses were performed and results were combined using meta-analysis. In black adults, a polymorphism in PKD2 was significantly associated with dental caries ($p<0.003$). Variants in PKD2, ABCG2, and MEPE were significantly associated with dental caries in individual studies. These results lend additional support to the hypothesis that variation in this genomic region influences risk of dental caries.

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Computational Alanine Scanning of Vitamin D Receptor bound to Vitamin D Ligands

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Identifying the importance of residues at protein-protein interfaces and within a substrate binding pocket is a challenge experimentally and computationally. Alanine scanning has been used to analyze protein-protein and protein-small molecule interactions experimentally. The method works by mutating selected wild-type residues to alanine and then determining the corresponding change in free energy. The interactions between Vitamin D ligands and the Vitamin D receptor (VDR) have been experimentally studied utilizing this method. In this study, a thermodynamic cycle – perturbation method was used

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Using the Oculus Rift for Molecular Visualization

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Molecular virtualization software such as VMD and Pymol, have had a significant amount of popularity among the computational science community. This has pushed members of the community to approach new methods to enhance the experience such as virtual reality (VR). Using the Oculus Rift, a VR headset, we have created new features within a program known as MolecularRift (Norrby et al. JCM 2015), a molecular structure viewer for Windows, to enhance the features of MolecularRift. We have added audio and identification of additional intermolecular

to simulate alanine scanning and to computationally determine the important residues in the VDR binding pocket. The results from these calculations are compared to the experimental values in order to validate the computational alanine scanning method for protein-small molecule studies, as well as to quantitatively identify the significant pocket residues necessary for the binding of Vitamin D and Vitamin D agonists.

forces.

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Combining mass spectrometry and vibrational spectroscopy to characterize gas-phase nickel and manganese oxo-anions.

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Nickel and manganese oxo species catalyze a wide range of chemical transformations. The mechanisms by which metal-oxo species catalyze reactions, and the influence of local chemical environment on activity, can be difficult to study in condensed phase systems. Gas-phase investigations using tandem mass spectrometry can provide important information about reactivity for specific species. Here we used electrospray ionization and collision-induced dissociation to make metal-oxo ions in the gas phase from metal-nitrate anions. Ion structures were determined using wavelength-selective infrared multiple-photon photodissociation (IRMPD) spectroscopy and density functional theory calculations. Using comparisons between experimental and predicted infrared spectra, assignments of nitrate N-O and O-N-O stretches are possible, as is the determination of the structure of species such as $[M(NO_3)_3]^-$, $[MO(NO_3)_2]^-$ and $[MO_2(NO_3)]^-$. In addition, the comparison of experimental to predicted spectra allow the spin states of the respective species to be determined.

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Optimizing High Performance Big Data Cancer Workflows

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Appropriate optimization of bioinformatic workflows is vital to improve the timely discovery of variants implicated in cancer genomics. Sequenced human brain tumor data was assembled to optimize tool implementations and run various components of RNAseq workflows. The measurable information produced by these tools account for the success rate and overall efficiency of a standardized and simultaneous analysis. We used the NCBI SRA database to retrieve 16 transcriptomic datasets containing over 850 million reads to be used as input data. We used these datasets to benchmark various file systems on the Bridges supercomputer to improve overall workflow throughput. We report critical recommendations on selections of appropriate file systems and node types to efficiently execute these workflows.

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A new paradigm of protein allostery: Insight through the Gaussian network model

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Allostery in proteins regulates many medically relevant biological phenomena: Biochemical feedback loops, disease mechanisms, and signaling pathways are just some examples. Prediction of allosterically linked protein sites is critical to the discovery of novel drug targeting sites on proteins. Traditionally, experiments have been the key players in allosteric site discovery. Experimental methods however are costly and time consuming. A simple computational method for discovery built into an already well-developed theory is needed. Here we present a novel way to determine if two sites within a protein are allosterically linked. Gaussian network model analysis shows that experimentally linked allosteric sites in three proteins (HIV-1 RT, Tryptophan synthase, DnaK) show strong cross-correlation and fast signaling times between one another. These findings suggest that prediction of allosteric hotspots within proteins is possible through the use of an elastic network model.

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Design and Analysis of Multiplexed Photoacoustic Flow Cytometry System for Early Detection of Metastatic Melanoma

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Photoacoustic flow cytometry (PAFC) is a process which allows for earlier in vitro detection of metastatic melanoma cells than other current methods. This is achieved when a pulsed laser irradiates a small sample of blood passing through a flow chamber, enabling pigmented melanoma cells to absorb energy and produce an acoustic wave. The current PAFC system analyzes a 1 mL sample in 33 minutes, proving inefficient for testing larger volumes of blood. To solve this problem, we multiplexed the system by splitting the laser beam and the flow into two chambers, each with its own acoustic sensor. We programmed software to control the apparatus and collect data. We performed t-tests on acoustic signals for various samples to show similarity between the multiplexed system and the original device.

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Using Multitask Learning to Predict Signaling and Regulatory Pathways in Cancer

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Computational methods have been developed to reconstruct cellular response networks from perturbation studies. However, when modeling response networks for specific perturbation, researchers often only use a limited set of experimental data which can lead to overfitting. To address these issues we developed multitask learning framework to reconstruct molecular response networks. We combine related perturbation experiments (for example, the same drug applied to different types of cancer cells) to constrain the learning of network parameters across all cells while at the same time a unique network is still learned for each cell type. We applied our method to reconstruct drug response networks for different cancer types using expression experiments. The reconstructed networks identified several of the key proteins and pathways involved in cancer drug response as shared between different cancer types. In addition, these data can help us predict the rate at which the drugs are efficient in treating these cancers.

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Macromolecular Crowding Effects on Lactate Dehydrogenase: Evidence of Enzymatic Cooperativity

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This project continues its focus on steady-state enzyme kinetics of lactate dehydrogenase (LDH) within highly crowded environments. LDH is a cytosolic enzyme that catalyzes the conversion of pyruvate to lactate and the oxidation of NADH to NAD^+ as the final step in lactic acid fermentation. Bovine serum albumin (BSA), a soluble globular protein, was the primary model crowder studied due to its ability to significantly inhibit LDH activity, and because proteins exist in higher concentration by dry mass within most cells. Through experimentation with BSA and by pushing the limits of kinetic measurements to low NADH concentrations, we have found unprecedented evidence of mammalian LDH cooperativity. Previously, only bacterial LDH had shown such behavior. Our experiments have demonstrated LDH to exhibit positive cooperativity with NADH as the variable substrate, with a Hill coefficient of up to 2.5 in a non-crowded environment and decreasing cooperativity as the concentration of BSA increased.

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Convolutional Neural Networks for Ligand-Protein Scoring

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Computational approaches to drug discovery reduce the time and cost associated with experimental assays, which screen thousands of compounds for ligands that can bind with a target protein. Computationally testing a ligand on a protein target is done by using a scoring function to predict binding affinity. The scoring function is constrained by our knowledge of ligand binding since features must be chosen based on our prior knowledge. A convolutional neural network (CNN) eliminates feature selection and makes use of all structural and cheminformatic data. Using a dataset of known bindable ligands consisting of correct and incorrect binding poses, we have been able to optimize a CNN to successfully score poses with a higher accuracy than the scoring function used by Autodock Vina. Expanding the dataset with compounds that can and cannot bind, we show that CNNs have the potential to score whether a ligand can bind a protein target.

82

Synthesis of Small Functionalized Molecules Using Photo Induced Copper-Catalyzed Atom Transfer Radical Addition (ATRA)

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Copper-catalyzed atom transfer radical addition (ATRA) has shown to be an effective method for the synthesis of small functionalized molecules. The bifunctional alkyl halide initiator 1,2-bis(bromoisobutyryloxy)ethane (BBiBE) was used for the ATRA of various acrylates (methyl, ethyl, propargyl, ethylene glycol methyl ether, butyl). The reactions were performed at ambient temperature and catalyzed by $[Cu^{II}(Me_6TREN)Br][Br]$ (Me_6TREN=Tris[2-(dimethylamino)ethyl]amine) mediated by UV light. The reactions were initially performed in dimethyl sulfoxide (DMSO) due to the high K_{ATRA}; however, methanol (MeOH) was evaluated as a possible alternative. The ATRA of ethyl acrylate to BBiBE in DMSO afforded quantitative conversion and a yield of 28% using 0.4 mol-% catalyst, relative to initiator. When performed under identical conditions in MeOH, an increased yield of 67% was observed. Moving forward with optimization, varying alkene concentrations will be employed. This methodology can eventually be extended to other multifunctional initiators.

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Development and Application of a 3D Scientific Visualization Tool

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Annotation of biological datasets precedes important applications including disease detection, machine learning, and teaching. However, it is often difficult or even impossible to confidently identify biological structures in the arbitrary plane produced by the image capture process. Here, we describe our expansion of a three-dimensional volume browsing software, which we used to annotate Zebrafish neuromast hair cells. These cells are distinguishable by a teardrop shape and hair extension, without which no conclusive identification can be made. With our volume browser, electron micrographs of cells could be viewed in any plane in three-dimensional space, allowing “rotation” of images to produce discernible cell shapes. By allowing the user to view a volumetric dataset from any angle, the volume browser can transform inconclusive data into useful context for annotation. Future implementations will include tools for viewing 2D traces in a 3D context and methods for orienting the user while navigating a volume.

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Biophysical Characterization of G-Quadruplex Secondary Structure in pre-miR-125b-2

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Small, noncoding miRNA has been shown to fulfill a significant role in mRNA translational regulation. Specifically, G-quadruplex secondary structure formation in the 5’ and 3’ UTRs of certain miRNAs has been demonstrated to both affect mRNA translation and act as a binding motif for certain organic molecules. Furthermore, quadruplex formation can occur during miRNA nuclear processing itself to self-regulate such activity as the sequence is processed from its pri-miRNA to pre-miRNA to mature miRNA forms. Pre-miR-125b-2, an immature form of microRNA 125b, has been hypothesized to form such a G4 structure which could regulate the amount of mature miRNA formed from DICER processing, providing indirect means for translation regulation. Pre-miR-125b-2 mRNA secondary structure was quantitatively characterized using biophysical methods including NMR, CD spectroscopy, and UV-Vis spectroscopy. Once characterized, native PAGE was conducted in order to qualitatively demonstrate KCl-dependent conformational changes as well as intermolecular vs intramolecular formation of the quadruplex.

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STR Forensic Typing and Development of a Tetranucleotide Multiplex Assay for Snow Leopards

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Current methods of identification of individual snow leopards using a dinucleotide multiplex can be improved by implementing a tetranucleotide multiplex assay. By using tetranucleotide repeats, the assay will become more robust due to the larger differences in allele size. This robustness enables more consistent genotyping by avoiding "+A stutter." The avoidance makes it possible to obtain resolution down to specific base pairs. Base pair calling discrepancies are reduced when using tetranucleotide assays. This reduction makes data sharing across laboratories more consistent. Using scat samples, a previously developed tetranucleotide assay for domestic cats was optimized to obtain the individual identification of snow leopards. The tetranucleotide assay was coupled with an amelogenin assay for sex identification. Traditional applications improved by the use of tetranucleotide assays include population monitoring and determining relatedness. Forensic applications include region of origin determination and identification in poaching cases for a more effective approach to law enforcement.

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Composite Tissue Engineering: Toward the Development of a Bioengineered Vascularized Soft Tissue Flap

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Millions of Americans require reconstructive surgery after severe tissue loss or damage due to traumatic injury or tumor resection, often times requiring surgical reconstruction using autologous soft tissue flaps. We hypothesized that we could make the first steps towards generating an off-the-shelf free flap by developing a protocol to effectively decellularize a soft tissue flap (using a rodent model). Specifically, abdominal wall flaps were harvested from Sprague Dawley outbred rats and were cannulated (via the femoral artery) with a 24G cannula. To determine the optimal protocol for complete decellularization, flaps were perfused with multiple combinations of chemical/biological reagents, with or without mechanical agitation. The degree of decelluarization was determined by the presence of nuclei visible on a hematoxylin and eosin (H&E) stain and by the overall residual DNA content and base pair size. This work represents the first step (to our knowledge) in developing the first off-the-shelf vascularized free flap.

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Autonomous Cell Collection for Photoacoustic Flow Cytometry System

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Biomedical Engineering
Duquesne University

An independent data collection method has been created for gathering melanoma cells that were detected by way of the Photoacoustic Flow Cytometry System (PFCS). The previous process of collecting data was manually moving a 96 well plate underneath the falling droplets to collect them. Automating the collection process allows the person running the flow system to focus on other tasks, such as running other parts of the system. The collection system itself is a 96 well plate that moves by means of an X and Y gantry system. The system works in correlation with a LABVIEW program that runs the PFCS. As the PFCS runs it determines if cells moving through it are melanoma. If nothing is detected then the general waste is dropped into the outer wells. Once a melanoma cell has been identified the gantry moves a center well underneath the droplet.

88

Planning Optimal and Personalized Surgical Myectomy Treatment for Hypertrophic Cardiomyopathy

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Continuing off work to create a virtual representation of a heart with hypertrophic cardiomyopathy or other outflow tract obstructions, the goal was to create a patient specific cardiac geometry. The apical, mid-ventricular region was realistic in terms of mechanical properties and shape. Beginning with the 3D rendering that was used for the virtual surgery, five molds of patients' hearts were created. Dermosol, a compound with similar properties to human muscle, was used to fill the molds and create physical copies of the hearts. This technique, combined with the virtual tool, shows precise locations of hypertrophic cardiomyopathy and creates a better understanding of each patient's heart. The final models were compared to the original by using an MRI to rescan the created copy. A surgery was then performed on the model which was rescanned and put through the cycle again. This allowed for a visualization of the accuracy of the surgery.

89

Wetting Behaviour of Silica Nanoparticles coated with a perfluorinated self-assembled monolayer (SAM)

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Using a nanometer-thick film of hydrophobic polymer coated on a hydrophilic substrate, it is possible to achieve simultaneous hydrophilic/oleophobic effect that may be useful in a variety of applications including oil-water separation and self-cleaning surfaces. Applying a self-assembled monolayer (SAM) of hydrophobic perfluorinated polymer onto a hydrophilic silica nanoparticle, it is hypothesized that water will penetrate the polymer due to the small water molecule size, thus the SAM/nanoparticle will display hydrophilic properties. Wettability of these nanoparticles was studied using various nanoparticle concentrations from 5 to 50 g/L using dip-coating and drop casting techniques. Initial tests indicate that dip-coating is not an effective method for applying a uniform nanoparticle film onto a flat substrate. Drop casting was used effectively to demonstrate that particles did not exhibit simultaneous hydrophilic/oleophobic properties but instead showed simultaneous superhydrophobic/ superoleophillic properties. This behaviour is rationalized based on surface topography and nanoparticle wettability.

90

Differentiation of Counterfeit Medicines via X-Ray Powder Diffraction

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Counterfeit medication is fake medication. It is a dosage form that contains no active ingredient, or an adulterated active ingredient. The purpose of this project is to see if X-Ray Powder Diffraction (XRPD) is a viable option for distinguishing between the presence or absence of an active ingredient. Diffractograms of ingredients in different formulations of OTC medicines and placebos were compared to analyze distinctions between the medicines containing the active ingredient, and those that do not. These experiments were performed to see whether a qualitative (XRPD) difference between functioning medicines and their adulterated placebos could be identified and used as a method of counterfeit medicine detection. This data and information can potentially be used to create a lab to teach pharmacy students about XRPD and counterfeit medicines.

91

A Mechanistic Model for Dual-Stimulation of the JAK-STAT Pathway

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The janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway translates extracellular signals into altered gene expression in the nucleus. Abnormal activity of this pathway is associated with cancer since STAT1 regulates cell death, and STAT3 regulates cell survival. Possible cancer treatments involve inhibiting STAT3 while leaving STAT1 unaffected. To study how this could be achieved, we built a mechanistic model of the pathway to predict the cellular response to stimulation from multiple cytokines. This model predicts the activation of STAT1 and STAT3 from stimulation of interleukin-6 (IL6), interferon gamma (IFNγ), and oncostatin-M (OSM). Pathway reactions were generated using rule-based modeling (RBM), where rules were based on the interactions between pathway components. Markov Chain Monte Carlo sampling was used to fit the rate constant parameters. Our model shows that competition for JAK strongly influences the kinetics of cellular response to stimulation with combinations of cytokines.

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Development of a web-based visualization tool for exploration of protein topic model

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Assessing the similarity of proteins in cellular location, structure and function is crucial for understanding proteins' roles in disease. Substantial efforts have been made to improve the understanding by considering location and function independently, in this work, we aimed to consider both protein location and function using a topic model. The location of the protein within the topic model space can be inferred from the topic model. We designed an interface to show the protein images, names and associated Gene Ontology terms (GO). The information about the various proteins were gathered from the Human Protein Atlas (HPA), which consist of fluorescence stained images. It allows the user to show and hide two separate channels. This visualization tool also allows users to quickly identify and reorder neighboring proteins in terms of their model proximity. The interface also has a zoom in display function to expose the details of each depicted protein images.

93

Identification of novel developmental genes in *Streptomyces coelicolor* via random transposon mutagenesis

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Streptomyces coelicolor is a soil-dwelling sporulating bacterium. Random transposon mutagenesis was employed to identify functions encoded by novel developmental genes involved in its complex life cycle through visual screens and phase-contrast microscopy. The insertion sites were discovered by chromosomal DNA isolation, restriction enzyme digestion, ligation, and then an inverse PCR reaction followed by sequencing. In mutant NBS96, the transposon insertion site was found in an intergenic region upstream of divergently transcribed genes SCO2255 and SCO2256, genes encoding a putative membrane spanning protein and a transferase, respectively. Once each insertion site is identified for additional mutants, gene(s) will be deleted by means of a λ Red recombination. A second set of analysis will be performed to determine if there is linkage between the mutation and the phenotype of the mutant strains. This research provides multiple developmental genes of interest for further study.

94

Synthesis and Analysis of Li_2SnS_3 for Potential Applications in Solid-State Lithium Ion Batteries

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Organic liquid electrolytes pose serious safety issues; however, solid-state electrolytes used in solid-state lithium ion batteries are safer due to their environmental stability. The Li^+ ion conductivity of Li_2SnS_3 makes it a promising solid-state electrolyte. Li_2SnS_3 is a wide-gap semiconductor with a sodium chloride-like structure. Initial Li^+ ion conductivity data collected by impedance spectroscopy were obtained from pellets of 56% of the theoretical density; however, much higher conductivities are expected for pellets closer to the theoretical density. Therefore, it is necessary to develop a method for producing denser pellets, which first requires a phase-pure compound to be prepared. This summer, the preparation of Li_2SnS_3 was carried out via high-temperature solid-state synthesis. Phase purity was assessed using X-ray powder diffraction. Scanning electron microscopy was used to study the morphology of the crystals, and energy dispersive spectroscopy was used to determine a semi-quantitative elemental composition. UV/Vis/NIR spectroscopy was also carried out.

95

Electrochemically stable Sulfate – based conducting MOFs as hosts for Sulfur in Li – S batteries

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Sulfur, with a theoretical capacity of 1675 mAh/g, could potentially improve the energy and power density of current lithium ion batteries[1, 2]. However lithium-sulfur batteries are plagued with many problems stemming from polysulfide dissolution which result in a fade in capacity leading to eventual cell failure[3, 4]. Infiltrating sulfur into porous carbonaceous matrices has shown improvements in the performance by reducing polysulfide dissolution[5, 6]. However, these approaches did not show complete elimination of polysulfide dissolution[7-9]. We build this project upon our group's previous work using carbonate – based MOFs as hosts for Li – S battery. An electrochemically stable MOF was prepared using sulfate precursors and organic linkers followed by solution infiltration of sulfur. The sulfur infiltrated MOFs prevented polysulfide dissolution resulting in improved columbic efficiency. In current cycling tests, the sulfate based MOF cathodes achieved an initial discharge capacity of 1365 mAh/g. Results of these studies will be presented and discussed.

96

Provision of Glycerophosphoinositol Causes Cell Growth Inhibition in aly1Δaly2Δ Mutants Overexpressing *GIT1*

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The Git1 permease is responsible for the uptake of the phospholipid metabolites, glycerophosphoinositol (GPI) and glycerophosphocholine (GPC) in *Saccharomyces cerevisiae*. Unpublished data suggests that the alpha-arrestins Aly1 and Aly2 regulate the endocytosis of the Git1 permease, as Git1 is retained at the cell surface in an aly1Δaly2Δ mutant. Importantly, provision of the Git1 substrate, GPI, to aly1Δaly2Δ cells constitutively expressing *GIT1* results in growth inhibition. This inhibition is not observed when other Git1 substrates, GPC and glycerol-3-phosphate, are provided. To identify proteins that are involved in the growth inhibition by GPI, tools are being developed to employ the Yeast Genomic Tiling Library (a previously constructed library containing >95% of the functional yeast genome in 1,588 plasmids) to screen for genes that restore cell growth upon overexpression.

97

Exploration of Quinazoline-based inhibitors of MEK5

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According to the National Cancer Institute over 180,000 women and 2,000 men in the US will be diagnosed with breast cancer this year. The most aggressive breast cancer is triple-negative breast cancer (TNBC). TNBC displays aggressive growth and invasiveness. Targeting the unique metabolism of TNBC may be reasonable therapeutic strategy. One enzyme that is upregulated in TNBC is MEK5. A previously synthesized compound by Craig Thomas (2011, BOMCL, 3152) in an SAR study of CK1 was a 47 nM inhibitor at MEK5. From this lead compound, structural modification was conducted to increase potency and selectivity for MEK5. We have used computational analysis and analog design to devise novel compounds. From these preliminary studies, we identified two regions for making changes to the lead molecule with the goal of increasing potency and selectivity. The design and synthesis of these compounds will be presented.

98

Characterizing the Fusion of Spore-Associated Protein A to an Antigenic Marker in Bacterial Exospores as a method of Vaccine Delivery

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Endospores from unicellular bacteria that express recombinant antigenic markers fused to spore coat proteins (Cot) have been proven an effective vaccine mechanism. Exospores produced by non-pathogenic, filamentous bacteria such as *Streptomyces coelicolor* may also be used. *S. coelicolor* encodes multiple secreted spore associated proteins. The aim was to characterize the secretion and localization of spore associated protein A (SapA) when fused with an antigenic protein at the C-terminus. In *E. coli*, the gene for the *E. coli* ß-subunit heat labile toxin (*ltb*) was fused to the 3' end of *sapA* by recombineering and introduced into *S. coelicolor* by conjugation. Proteins from strains expressing SapA-Ltb were extracted and Western Blot analysis demonstrated that the fusion protein is secreted and localized to the spore coat. Now 3' truncations are being made to find the minimum amount of SapA necessary for secretion and localization. This may be a viable vaccine mechanism.

99

Interactions of the Fragile X Mental Retardation Protein with a G-quadruplex Structure in the BACE1 mRNA 3' Untranslated Region

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Fragile X Syndrome is an inherited genetic disease caused by the hypermethylation of a CGG expansion repeat in the *FMR1* gene, which leads to the loss of translation of fragile X mental retardation protein (FMRP). FMRP binds to messenger RNA (mRNA) G-quadruplexes by using its arginine-glycine-glycine (RGG box) binding domain. We hypothesize that beta-secretase 1 (BACE1) messenger RNA sequence forms a G-quadruplex structure that will be bound with high affinity and high specificity by the FMRP RGG box. In order to characterize the RNA G-quadruplex structure, we used biophysical methods such as circular dichroism (CD) spectroscopy, UV/Vis spectroscopy, ^{1}H-nuclear magnetic resonance (^{1}H NMR) spectroscopy, and native polyacrylamide gel electrophoresis (PAGE). We then investigated the interactions of FMRP RGG box to the G-quadruplex structure using electrophoretic mobility shift assay (EMSA).

100

Baculovirus Expression of Recombinant Rat Serotonin Transporter Containing a Single Point-Mutation for Crosslinking Studies

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Initial studies were conducted in preparation for future structural studies of the rat serotonin reuptake transporter (rSERT), a sodium-chloride-dependent membrane protein that reuptakes serotonin back into the presynaptic neurons after delivering its chemical message. To investigate allosteric states of this transporter, two samples of rSERT containing single reactive cysteines were expressed utilizing baculovirus expression systems for further crosslinking studies. Point mutations, L406 and S252 were introduced at specific locations on the extracellular loops of rSERT where crystal structures have poor resolution. These point-mutations were transformed into DH10Bac cells and selected using blue/white screening to produce the recombinant baculovirus shuttle vector (bacmid). The bacmid was harvested from small cell cultures and amplified to higher concentrations that are necessary to infect cultures of Sf9 cells that can produce the milligram quantities of rSERT protein required for future studies.

101

Detection and Identification of Radioactive Elements using a homemade Gamma Ray Spectrometer

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Gamma Ray Spectroscopy is a technique used to detect and identify radioactive isotope, such as ^{223}Ra, ^{226}Ra, ^{228}Ra from the radioactive decay of ^{235}U, ^{238}U, and ^{232}Th, respectively. We present a device that will be able to detect and identify these elements. The sample is placed near a Cerium Lutetium-Yttrium Oxyortho Silicate crystal, which absorbs gammas rays and re-emits the energy as blue light. The light then strikes a silicon avalanche photodiode array that converts the light energy into electric current. The current pulse is sent into an LC circuit causing the circuit produce an exponentially decaying sinusoidal wave. The energy of the incident gamma ray is logarithmically proportionally to the number of maxima found in the wave. The maxima are counted digitally and are used to identify the radioactive element. The device will be installed in a robotic boat that is designed to autonomously take water samples.

102

Characterization of a Spore-Associated Protein to Study Assembly of *Streptomyces* Spores

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Streptomyces coelicolor is a model non-pathogenic filamentous bacterium that produces chains of spores from aerial hyphae. Linear plasmid SCP1 contains *sapC, sapE, and sapD,* which are genes that code for spore-associated proteins. Spore-associated protein SapC does not contain a normal secretion signal nor is the mechanism of secretion known. Our strategy to study secretion and spore coat assembly was to use recombineering to fuse the β subunit of the *E. coli* heat labile toxin (LTB) to the C-terminus of SapC. Results characterizing this fusion suggest that SapC-LTB was secreted and assembled on the spore surface. The goal of this project was to further characterize SapC-LTB through LTB fusions to truncations of the C-terminus (SapC'-LTB) and construct a fusion at the N-terminus (LTB-SapC). The use of Sap protein fusions to passenger proteins might lead to an additional method for vaccine delivery by displaying epitopes on recombinant *Streptomyces* spore surfaces.

103

Evaluating protein-protein interaction prediction methods by using machine learning evaluation metrics

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[5] Internship for Biomedical Research, Informatics and Computer Science

Identifying protein-protein interactions is key for mapping an organism's protein interactome. A complete interactome provides useful topological information for understanding biochemical pathways, predicting protein function, identifying disease-related proteins, and designing target-specific drugs. While experimental methods for detecting protein-protein interactions exist, these methods are time-consuming and costly. Computational methods can aid in guiding experiments by identifying novel interactions. For this reason, in the past decade several protein interaction prediction methods have emerged. In order compare their performance, this study evaluates three PPI prediction methods by using machine learning evaluation metrics. A novel aspect implemented is the evaluation of predicted PPIs on hub proteins. The evaluated methods are: FpClass, hPRINT, and PIPs. Among the evaluated methods, the best performing method was FpClass.

104

Characterization of Thin-Film Copper and Oxidation Using in situ Environmental Transmission Electron Microscopy

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Corrosion costs the U.S.A. a few percent of its GNP annually. In situ atomic scale characterization offers detailed dynamics of corrosion for fundamental understanding. We prepared thin-films (~70 nm) of copper, a model metal system, on single crystal NaCl substrates using e-beam evaporation. We performed in situ reduction/oxidation of Cu within a Hitachi H9500 environmental transmission electron microscope. The Cu films were annealed in hydrogen gas to remove the native oxide and to create clean copper facets, and then exposed to oxygen gas at 250°C. We noticed facet-dependent differences at the early stages of Cu-oxide growth in situ, where the relative oxidation rates by facet varied such that (100) < (110) < (111). These results can be used to guide future work on how to control corrosion by manipulating the crystal planes and surface defects used in corrosion-protection applications including municipal water transport, turbine blades, and integrated circuits.

105

Site-Directed Mutagenesis in the Rat Serotonin Transporter (rSERT)

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The serotonin transporter (SERT), a neurotransmitter sodium symporter (NSS), is responsible for the reuptake of serotonin (5-HT) to terminate the neurotransmitter action at the receptor sites. Dysregulation of serotonin concentration in the synapse is related to depression and anxiety, as well as other neurological conditions. Selective serotonin reuptake inhibitors (SSRI) such as fluoxetine inhibit SERT so that 5-HT synaptic concentrations remain elevated. By making single point cysteine mutations at various locations on the transporter, ultimately photocrosslinking studies can be carried out to determine state-dependent crosslinks to provide data for more accurate modeling of SERT. Three single point mutations: S252C, Y232C, and S522C, were successfully inserted into rSERT in pFastBac vector. This vector contained rSERT DNA plasmid with eight of its cysteines removed. The recombinant vector was Dpn1 digested and grown in XL10-Gold ultracompetent cells. DNA is then extracted from the cells and sequenced to verify the presence of the mutation.

106

Design and Implementation of Optoelectronic Sensors in a High-Resolution Wavelength Meter

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Optoelectronic systems constitute an important interface between experiments in AMO physics and modern data collection devices. Proper design and construction of photo-detectors is key in experimental optics; our current experiment requires an interferometer-based laser wavelength meter that implements purpose-built detection and data acquisition electronics. Observation of the output of these detectors can inform further modification of the optical circuit, and with proper calibration the device should produce highly accurate wavelength measurements at sub-picometer resolutions. Here we discuss the design of the detection equipment and analysis of output data in relation to the performance of the optical circuit.

107

Using RNA sequencing data to detect simultaneous mRNA transcription and degradation in Zebrafish embryogenesis

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Gene expression during the first stages of embryogenesis is dependent on both maternally deposited mRNAs, which are actively degraded at a later stage, and on *d e novo* zygotic transcription after zygotic genome activation (ZGA). Depending on the timepoint at which a gene's expression is required, it can be controlled by either or both of these mechanisms. The dynamic interaction of these processes, however, is poorly understood. We aim to use RNA seq data sets from Zebrafish embryos at timepoints both before and after zygotic genome activation to identify genes regulated by both mechanisms. We will use exon reads to measure expression levels, with an increase in reads postZGA representing *d e novo*zygotic transcription, while a decrease represents maternal decay. Simultaneously, intron signal will also be used to detect *d e novo* transcription, as only newly transcribed zygotic products will contain introns.Together, this data will aid further understanding of embryogenesis.

108

Structure and Dynamics of Ligated and Un-Ligated $5HT_{2C}$ Receptor in a Molecular Dynamics Environment

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Dysfunction of the $5HT_{2C}$ receptor has been implicated in schizophrenia and depression and thus has been the target of recent studies attempting to design novel therapies for such disorders. Before novel therapies can be developed, the binding pocket and conformations of binding states must be determined for $5HT_{2C}$. The aim of this project was to create a high accuracy homology model of $5HT_{2C}$ from the D_3 and $beta_2$-adrenergic receptors and to use the homology model to computationally observe the conformational changes occurring in the binding pocket with agonists/antagonists bound. $5HT_{2C}$ homology models were created using both Modeller and the ITASSER server and LowModeMD in MOE was used to find the most favorable intracellular loop conformation. HTMD was used to build, equilibrate, and run production of the homology model-bilayer membrane-water system. The system equilibration and production runs with agonists/antagonists bound were run on a local GPU.

109

Design & Development of a Low-Cost Particle Inflow Gun

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Biolistic particle delivery systems, commonly known as gene guns, employ high-velocity, DNA-coated microparticles (commonly gold or tungsten) to achieve direct gene transfer in both plant and animal cells. Pressurized helium, among other inert gases, is used to accelerate the particles and bombard living cell cultures. In an effort to expand biolistic research, an effective, low-cost particle inflow gun was 3D modeled and subsequently built using mostly off-the-shelf components. The parts were sourced from various retailers and the design incorporated a minimal number of 3D printed components. The device is intuitive, provides users with a basic understanding of pneumatic systems, and can be modified by the end user for specific, dedicated applications. The gene gun will be used to transform both carrot cultures and mushroom cells with green fluorescent proteins (GFPs).

110

Exploration of Quinazolines as TTBK1 inhibitors

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Amyotrophic lateral sclerosis (ALS) is characterized by intraneuronal accumulation of hyper-phosphorylated tau and TDP-43 protein aggregates. TDP-43 forms a component of stress granules, inclusion bodies that sequester and protect RNA from oxidative damage during oxidative stress or viral infection. TDP-43 can be viewed as providing a normal protective role. Over-activation of TDP-43 has been associated with neuronal death by aggressively sequestering RNA and impairing transport, impairing autophagy, and resulting in excitotoxicity in neurons. It has been recently recognized that the enzymes that over-activate TDP-43 are TTBK1 and TTBK2. Both enzymes generate the over-activated pSer409/pSer140 form of TDP-43. There may be undesirable effects from off-target inhibition of TTBK2. Although a screen of commercially available kinase inhibitors has identified moderately active TTBK1 inhibitors, they lack selectivity for TTBK1 versus TTBK2 inhibition. The design and synthesis of substituted quinazolines as TTBK1 selective inhibitors will be presented.

111

Calculating Binding Affinities for SERT to Determine Binding Pockets

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Binding free energies are important in determining binding pocket locations for pharmaceutical drugs to adhere to which controls the function of said protein. Contemporary attempts for calculating the binding free energy of protein complexes such as empirical knowledge based approaches, coarse-grained potentials, and continuum dielectric solvent treatments are insufficient at yielding reliable binding free energies. The serotonin transporter (SERT), which is responsible for transporting serotonin from the synaptic cleft to the pre-synaptic neuron terminating the effects of serotonin and simultaneously enabling its reuse, is of interest because of its implicated role in depression and addiction. The free energy calculations were computed using free energy perturbation molecular dynamic (FEP/MD) simulations using CHARMM-GUI's Ligand Binder. The pockets were identified through the analysis of the free energy calculations.

112

Optimizing the quality and throughput of electrospun poly(glycerol sebacate) vascular grafts

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Electrospun poly(glycerol sebacate) (PGS) is used to fabricate vascular grafts. We optimized the quality and process of fabrication of our PGS grafts by decreasing time electrospinning and residual carrier polymer after curing. We accomplished our aims by improving the wash methods and implementing multiple-needle electrospinning. We showed that a more aggressive wash with a lower mass graft to solvent volume reduced the amount of PVA in the graft from the previous methods of washing of 9.7±2.5% to 5.8±0.47% by weight. Two needles were successfully spun at the same time, and essentially doubled the rate at which PGS/PVA solution was electrospun on to a mandrel when paired with a needle array. Fiber diameter and alignment were monitored with ImageJ. The graft will be implanted into a sheep model to test its regenerative potential.

113

Protein-Protein Interaction Impact Prediction

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Identifying pairs of proteins that are likely to interact is a well-defined problem in machine learning. As a result, thousands of these pairs have been generated by Protein-Protein Interaction (PPI) prediction algorithms. Confirming an interaction takes time, resources, and expertise, so it is not feasible to examine all possible PPIs. To help scientists determine which predicted PPIs to study, we attempt to predict the scientific impact of a PPI. “Impact” is a nebulous term, so we predict the number of citations a PPI discovery paper will receive. Several key centrality features are calculated from the topology of a PPI network, with proteins as nodes and interactions as edges. These features are a replication from scratch of previous work, and do show some predictive ability. Presently, we are investigating functional characteristics of proteins to determine if any may be novel prediction features which increase the performance of our model.

114

Scalable Informatics Tools for Investigating Tumor Heterogeneity in Breast Cancer

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It has been proposed that intratumor heterogeneity, a prominent feature of many malignancies, may have diagnostic and prognostic value. Specifically, tumors represent a complex dynamic ecosystem where heterogeneity acts as the substrate for tumor evolution and as one of the main drivers of disease progression and resistance to therapy. Therefore, it is essential to develop and test spatial intratumor heterogeneity metrics that correlate with various clinical outcomes . In the present study, we propose a high throughput pipeline that will form the basis for a set of opensource informatics tools for integrating and analyzing data obtained from a variety of imaging modalities. In particular, we employ a deep learning approach to identify discriminative cellular distributions or “signatures” that can be used to characterize breast cancer subtypes. Lastly, our distributed implementation currently targets Apache Spark, a powerful cluster computing framework, and should begin to mitigate the difficulties associated with quantitative big imaging.

115

Thermodynamics and Structure of the RNA Sequence $(GGGGCC)_4$

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A mutation in the *C9orf72* gene results in an RNA sequence $(GGGGCC)_4$ that can adopt both G-quadruplex and hairpin conformations and is associated with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). The role of ionic conditions in stabilizing the G-quadruplex and hairpin conformations of this RNA sequence was investigated using molecular dynamics and metadynamics simulations. Each structure was studied in both 150 mM KCl and 5 mM $MgCl_2$ with the NAMD simulation package. The metadynamics algorithm was then used to compute the free energy as a function of the deviation from ideal G-quadruplex, hairpin, and random coil RNA. Free-energy landscapes for all four systems were obtained. Analysis of ion distribution of lowest-energy structures indicated that K^+ binding occurs within the G-tetrad units of the G-quadruplex, while Mg^{2+} interacts with the hairpin's backbone. The resulting structural and thermodynamic data provide insight into the mechanisms by which these structures interconvert.

116

Evaluation of a mini-barcode primer set for use in monitoring ray-finned fish communities from environmental DNA samples.

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The state-adopted method of electrified benthic trawling for the standard survey of fish communities on large river systems (Allegheny, Monongahela, and Ohio) has been labor intensive, expensive, and imposed dangers for both organisms and surveyors. Advancements in Environmental DNA and mini-barcode technology with next-generation sequencing have potential to provide improved detection of aquatic organisms from water samples. A previously designed primer set, which amplified a partial mitochondrial Cytochrome Oxidase (COI) gene, was evaluated for its ability to detect and identify a wide range of ray-finned fishes. COI sequences from GenBank were aligned to test primer selectivity. After PCR optimization, primers were tested on genomic DNAs and amplicon identity was verified through capillary sequencing and BLAST analysis. Candidate primers were not optimally designed to amplify all ray-finned fishes and were sensitive to human contamination. Future work will focus on developing a new universal primer set exclusively for ray-finned fishes.

117

Biophysical Characterization of a G-quadruplex Structure on the FMR1 Gene at Exon 12

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Fragile X syndrome (FXS) is an inherited form of mental impairment, caused by a trinucleotide expansion within the Fmr1 gene, which leads to the loss of expression of the fragile X mental retardation protein (FMRP). FMRP is a RNA-binding protein whose arginine-glycine-glycine (RGG box) domain binds with high affinity and specificity to the G-rich regions of RNA that form G-quadruplex structures. The Fmr1 mRNA can undergo alternative splicing producing several isoforms whose biological functions are not fully elucidated. The mRNA encoding for the longest FMRP isoform 1 has a G rich region at the junction of exons 12 and 13, which we predict could form a G-quadruplex recognized by FMRP. This project investigated a possible connection between the Fmr1 mRNA and the FMRP protein. *In vitro* transcription, native PAGE, circular dichroism (CD) and nuclear magnetic resonance spectroscopy (NMR) were used to characterize the G-quadruplex at the Fmr1 exon 12 junction.

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Fundamental Difference Between Oxalate and Malonate Decarboxylation

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Decarboxylation of oxalate differs from malonate and other β-keto acids under enzymatic and nonenzymatic conditions. However, the difference is not well defined. In our research, the nonenzymatic decarboxylation of oxalate has been studied with explicit water molecules (n=0-7) employed in a hydrogen bonding “buckle” using the PM6 semi-empirical method and Truhlar’s M062X density functional with Pople’s 6-31+G(d) basis set. To address fully solvated systems, Morokuma’s ONIOM method was also implemented, where oxalate was treated by quantum methods and TIP3P water. The stable orthogonal conformation identified for ground state malonate monoanion could not be located for oxalate because it is a transition structure. This implies that oxalate does not adopt a stabilizing orthogonal position in its ground state and instead assumes a planar position. This research will lead to understanding the mechanism of the decarboxylation under enzymatic conditions and lead to a detailed understanding on how oxalate differs from β-keto acids.

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Development and characterization of a pilot gold standard annotation set for clinical cancer genomics

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Clinical natural language processing (NLP) can be used to extract important information from clinical notes such as pathology notes. Data obtained with NLP methods serves a multitude of purposes such as improving patient safety, educating health providers and accruing patients into clinical trials. An important new application of NLP is the correlation of genomic information to patient phenotype and outcome. The aim of my project is to pilot test a gold standard annotation process for molecular diagnostic reports for a variety of cancers. The gold standard annotation set will later be used to develop natural language processing methods that will accurately extract information about the test results from each patient’s report. For this project, I created an annotation guideline, annotation schema, and annotated 150 reports. I measured inter-annotator agreement, and performed a descriptive stat of the frequency and distribution of the annotations across the corpus, using the R- programming language.

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Ground state stabilization of malonate by water molecules

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Decarboxylation reactions are key enzymatic steps vital to biochemical processes. Surprisingly, differences between the enzymatic and nonenzymatic chemical processes are not well defined and are often a result of speculation. Recently, non-catalyzed rate constants and enthalpies of activation for the decarboxylation of malonate have been reported by Wolfenden. In this first phase of our research, the nonenzymatic decarboxylation of malonate has been studied with explicit water molecules employed in a hydrogen bonding “buckle” (n=0-5) using Truhlar’s M062X density functional and Dunning’s jul-cc-pvdz basis set. Our efforts have led to the identification of a conformation previously not considered, which we refer to as “orthogonal.” Our work sets the foundation to assess enzymatic catalysis, where arylmalonate decarboxylases (AMDase) catalyze the decarboxylation of α-aryl-α-methylmalonates. The difference between the enzymatic and nonenzymatic decarboxylation mechanisms has the potential to create a new paradigm in understanding enzymatic catalysis.

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Potential energy surface of five-, six-, and seven-membered ring oxocarbenium ions

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Nucleophilic additions to five-, six-, and seven-membered ring oxocarbenium ions require reliable, predictive models to be synthetically useful. However, ring conformations and barriers are relatively unknown, yet are an important factor in deducing selectivity. As our first step, the PM6 semiempirical method and Truhlar's M062X density functional with Dunning's jul-cc-pv[d,t,q]z correlation consistent basis sets were used to determine the potential energy surface of the oxocarbenium ions. Unsubstituted five- and six-membered ions have shown a pair of enantiomers connected by a single transition structure of less than 1 kcal/mol and 5 kcal/mol, respectively. The energy diagram of seven-membered rings has multiple ground states and transition structures, having a highest energy barrier near 10 kcal/mol. Our results provide the foundation for a general stereochemical model that can be developed to predict accurately the selectivity of the oxocarbenium ion in its reactions with nucleophiles, where current Felkin-Anh and Cram chelate models are limited.

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Novel binding and shuttling catalytic cycle of the aldol reaction by Trost dinuclear zinc semi-crown ligands

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Semi-crown ligands have been a significant advance in the asymmetric Mukaiyama aldol reaction; however, the three-dimensional structure, mechanism, and major factors that control the stereoselectivity have not yet been fully elucidated. We have determined plausible dinuclear zinc-based semi-crown ligand three-dimensional structures using the M06-2X functional paired with Dunning basis sets. Our results reveal a unique mechanistic cycle, where a C2 symmetric ligand works by employing nontraditional hydrogen bonding to predispose aldol reactants into reactive positions and produce high stereoselectivity of the aldol reaction. Specifically, we investigate the structural and energetic findings and consequences of the formal and □-hydrogen bonds that occur separately for the electrophile and the nucleophile with the semi-crown ligand in the Mukaiyama aldol reaction. Our results contribute to the development of a rational strategy for designing catalysts for the Mukaiyama aldol reaction, which serves to be an excellent template to expand to other asymmetric processes.

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Comparison of hydrometra and pyometra in client-owned vs. feral cats brought for routine ovariohysterectomy to a spay-neuter clinic.

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A longitudinal study of feral cats (*Felis catus*) was initiated in 2006. Infectious disease prevalence, bacterial load, body condition and overall health data as well a genetic analysis via STR markers have been collected. In this study, the incidence of feline uterine infection from cats brought to a spay and neuter clinic for routine spay (ovariohysterectomy) is analyzed as well as a comparison in occurrence between client-owned and feral cats. Feline uterine infections are clinically assessed as hydrometra, characterized by the accumulation of serous fluid within the uterus, and pyometra, an infected uterus. Hydrometra occurs secondary to progesterone-induced hyperplasia of the endometrium. Pyometra is considered to be a life-threatening condition that requires quick treatment, usually through ovariohysterectomy. Currently, both hydrometra and pyometra are thought to be uncommon in cats. This analysis is to our knowledge the first of its kind for non-pure breed cats and reveals that pyometra is often symptomless.

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Optimization of Silica DNA Extraction Technique from Compromised Horse Bone

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DNA extraction from skeletal material is burdened by low yields or degraded samples. Ancient DNA has the added complications of contamination and PCR inhibitors. In this study, an environmentally exposed and twenty year old horse femur serves a dual purpose to extract DNA for a horse parentage study and provide a matrix material analogous to Ancient DNA. The horse femur was drilled in several locations, some laterally and others from the head of the femur. The subsequent bone powder was weighed and aliquots of 0.125, 0.150, 0.250, and 0.500 (grams) were incubated at 56^0C for different time periods of three, six, nine (hours) and overnight to determine the optimal weight and digestion time. The extracted DNA was quantified via Nanodrop and amplified with a commercial horse STR kit (StockMarks). Samples were genotyped using GeneMarker. This study found that 0.150 grams and overnight incubation were the best for compromised skeletal material.

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Reactivation of Acetylcholinesterase

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Acetylcholinesterase (AChE) is essential to humans and other species. When AChE is damaged, a build up of acetylcholine occurs in the nerve synapse. This results in the paralysis of surrounding muscles. Acetylcholinesterase has an active site at the bottom of a long and narrow gorge. At the bottom of this gorge is the catalytic triad: catalytic serine(S203), glutamic acid(E334), and histidine(H447). Organophosphates(OPs) covalently inactivate AChE by phosphonylating the catalytic serine residue. Some commonly known OP's are methamidophos, fenamiphos, diisopropylfluorophosphate (DFP), and paraoxon. Possible therapeutics for organophosphate poisoning are oximes. Oximes are used to reactivate AChE by reacting with the OP-conjugate and removing it from the serine residue. However, many difficulties arise when considering these oximes for therapeutics. Firstly, each OP reacts with AChE to form a unique OP-conjugate. The efficiency of the therapeutics depends on the structure of both the oxime and the OP. The depth of the gorge, about twenty angstroms, makes it difficult for entry and exit of possible reactivators. Oximes are also generally positively charged compounds that have difficulty crossing the blood-brain barrier. Lastly, oximes can also act as AChE inhibitors when the AChE has not formed an OP-conjugate. In order to improve our understanding of therapeutic oximes and to develop further therapeutics, we have employed a combination of computational procedures to analyze the efficiency of certain oximes. These said oximes were previously chosen for their reactivation ability potential.

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Identifying new protein trafficking networks regulated by α-arrestins using evolutionary rate covariation (ERC)

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Alpha-arrestins are trafficking adaptors that bind a ubiquitin ligase and recruit it to membrane proteins to regulate protein trafficking. Few α-arrestin-cargo pairs are known, and identification of these pairs is challenging due to the transient nature of these associations and the inherent biochemical difficulties of working with membrane proteins. To identify new α-arrestin-regulated cargos we used Evolutionary Rate Covariation (ERC), a computational approach that uses DNA sequences to identify genes with similar evolutionary histories. Multiple known α-arrestin cargos were among the top hits in ERC when we compared the evolutionary rate of α-arrestins against the genes in yeast. We validate these new α-arrestin cargos by assessing the localization and abundance of GFP-tagged cargo proteins in cells lacking specific α-arrestins. We have confirmed that at least five of our predicted ERC cargos are regulated by α-arrestins and are currently validating more α-arrestin cargo pairs.

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Primer Optimization for Allelic Databasing of *Felis Catus*

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Domestic cats (*Felis catis*) leave ample traces of DNA in and around their territories. These traces are invaluable in forensic science for potential individualization at crime scenes. We aim to add utility to these DNA traces in crime scenes though the creation of an allelic database of STRs in a Western Pennsylvania population of feral cats. The first goal of this project is to use molecular DNA techniques, including PCR and Capillary Electrophoresis, to optimize a set of five established DNA primers for use on extracted DNA samples from ear tips of individual cats to create a multiplex for optimum clarity and consistency for analysis. Secondly, we aim to use those same molecular techniques, along with GeneMarker analysis software, to create a comprehensive database of STRs for use in forensic science. Finally, we aim to use our methods to examine kinship patterns in our colonies of feral cats.

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Exploring the Formation and Intrinsic Reactivity of Gas-phase Carbanions Bound to Group II Cations

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Carbanions, anions with an unshared electron pair and formal negative charge at a carbon center, are widely used in organic synthesis. Carbanions typically coordinate a counter cation, and Grignard reagents are among the most common examples. One of our research goals is to determine and understand the intrinsic reactivity of such species. Here, electrospray ionization and collision induced dissociation (CID) were used to prepare group II metal-ion complexes with carbanions in the gas-phase, where interactions with substrates can be studied in a controlled fashion. We evaluated the CID of Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} coordinated by with either acetate or propionate to form cationic metal-methide $[MCH_3]^+$ and metal-ethide $[MC_2H_5]^+$ complexes respectively by decarboxylation. Isolation of these products to react with gas-phase H_2O and CH_3OH yielded metal-hydroxides $[MOH]^+$ and metal-methoxides $[MOCH_3]^+$ to varying degrees. Differences in apparent reaction energetics and rates are rationalized with the use of density functional theory calculations.

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An Active Laser Polarization Controller for Laser Cooling Experiments

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We present a design for a homemade microcontroller-driven active polarization controller designed for use in atomic laser cooling experiments. The device is a modified Mach-Zehnder interferometer. The optical path length of one arm of the interferometer is adjusted by tilting a glass plate, resulting in a relative phase shift between the two arms of the interferometer. When light from the two arms recombines, interference creates the desired output polarization. The polarization is monitored by a pair of orthogonal Brewster plates, and the measurement is used to generate an error signal for the digital controller which adjusts the position of the tilt plate. The device can generate any linear polarization and can switch polarizations in a fraction of a second, and costs a fraction of commercial devices.

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Quantum modeling and mathematical prediction of surface phenomena

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The computational modeling of metal oxide surfaces using quantum chemistry continues to be plagued with technical issues, demands of great computational resources, and lack of systemic studies that define accuracy and convergence of computed properties. Specifically, we have found that the predicted limit on some properties, such as surface energy, of large rutile 110 slab models may be determined using a set of smaller surface models (as few as 7 layers). A closed-form solution, derived from an exponentially decaying function, allows for algebraic determination of the limit using the values from the first three even depth slabs. In general, we find that the exponential function is able to model closely the limit of surface energy computed by density functional methods with all electron basis sets. Our mathematical predictive method of determining slab properties can significantly reduce the computational resources to achieve a converged and accurate solution.

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A healthcare tool for analyzing and managing genomic data

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The usefulness of a patient's genomic information for personalizing their care relies heavily on the ability of healthcare individuals to effectively analyze it. Currently, there are many different databases used for storing large amounts of valuable information regarding genes and genetic variations as they relate to disease and other health risks. The current state of these databases pose a potential problem for those attempting to use the information for genomic data analysis. Our approach is to use computer algorithms to mine these databases for clinically significant information that healthcare professionals can use to personalize treatment for patients. The envisioned system would compile all the information extracted into a single database that is reliable and up to date with current research efforts. This exploitation of data has the potential to make treating disease more efficient for the benefit of both patients and healthcare providers alike.

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Recording Neuronal Responses to Bladder Pain in the Central Nucleus of the Amygdala

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Interstitial cystitis/bladder pain syndrome (IC/BPS) is a common, poorly treated chronic pain disorder. Many of the symptoms of IC/BPS (e.g. affective abnormalities) suggest a central nervous system component. However, the role for the brain in processing and modulating bladder pain is largely unknown. Without understanding how the brain reacts to the painful stimuli, it is difficult to treat IC/BPS patients. To investigate the hypothesis that dynamic changes in the brain account for symptoms of IC/BPS, in-vivo recordings of the left and right central amygdala using carbon-fiber electrodes were made during painful bladder distention in *Mus musculus* (mouse). Once a neuron was detected during the recording process, urinary bladder distensions (UBD) were used in order to observe how the neuron responded to a noxious stimulus. Based upon the pain neurons recorded thus far, it appears that neurons inhibited by UBD have higher firing rates than those excited by UBD.

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Natural Products Decrease Breast Cancer Cell Proliferation

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Statistically, 1 in 8 women will develop invasive breast cancer within their lifetime and the predicted number of new breast cancer cases in 2016 exceeds 240,000. Recently, natural products such as resveratrol, a natural stilbenoid found in grapes and other food sources, have been shown to decrease breast cancer cell viability and proliferation with less toxicity than traditional therapies. Unfortunately, resveratrol has a low bioavailability due to rapid metabolism and excretion. Therefore, it is necessary to examine the effect of other natural products in breast cancer models. Naturally occurring cyanobacteria from Curaçao were collected by the Tidgewell Lab at Duquesne University. Ten fractions of cyanobacteria were tested on triple negative (MBA-MD-231) and estrogen receptor positive (MCF-7) breast cancer cells. Decreased proliferation was noted following treatment with 5 of the fractions. These data suggest that natural products may be useful treatments for breast cancer.

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Impact of Pro-Inflammatory Cytokine CCL2 on Neural Stem/Progenitor Cells

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Inflammatory responses to viral infection result in the release of several types of proteins, such as C-C motif chemokine ligand 2 (CCL2). During measles virus (MV) infection of the brain, CCL2 is highly expressed in neonatal, but not adult, mice. CCL2 also induces chemotaxis of neural stem/progenitor cells (NSPCs), which show decreased neurogenesis during MV infection in neonatal mice. CCL2 was investigated for alterations to NSPC activity. Primary murine NSPCs were treated with CCL2 (5-300 ng/ml) and examined for changes in proliferation and differentiation. BrdU staining and neurosphere diameter measurements showed a minimal, but significant, effect on NSPC proliferation. In-cell westerns and traditional western blot analyses show no effect on differentiation into either astrocytic or neuronal lineages. These data indicate CCL2 has no significant effect on NSPC growth or differentiation, and thus may have an alternate role in inflammatory response apart from a direct effect on NSPCs.

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Crosslinking Investigation of Human Glycine Receptor using pacFA-18:1 and Tandem Mass Spectrometry

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Glycine is an inhibitory neurotransmitter commonly associated with proper sensory and motor function. The glycine receptor is a pentameric ligand gated ion channel that further hyperpolarizes the post-synaptic neuron when chloride flows in through the channel, thus making it harder for the cell to generate an action potential. The modulation of glycinergic neurotransmission is associated with chronic and inflammatory pain, a disease that affects more patients than heart disease, diabetes and cancer combined. A molecular understanding of the glycine receptor allostery is unknown due to limitations in the crystal structures. Our lab seeks to better resolve this by studying interactions of the receptor with surrounding transmembrane lipids. Lipids are a requirement for proper functioning of the protein. Using a photoactivatable phosphatidylcholine crosslinker, we performed tandem mass spectrometry to identify the specific amino acids on the protein that interact with the lipids. Results to date will be presented.

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Exploring clinically observed sex differences of bladder nociception in a mouse model

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Urologic chronic pelvic pain (UCPP) syndromes affect 4-12 million nationwide, and burden patients with generalized pelvic pain, bladder pain, increased urination, and a constant urge to urinate. While UCPP affects both genders, these disorders predominate in women. Previous studies have shown that female rats display greater bladder sensitivity than male rats in a model of visceral pain. However, bladder sensitivity decreases in ovariectomized female rats. Thus, sex hormones may play an activational role in modulating bladder sensitivity and be involved in facilitating the observed sex differences. In these studies, we are using mice to explore sex differences in bladder nociception and blood estrogen concentrations in response to bladder injury through sensitization and urinary bladder distentions. It is intended that this study will provide basic understandings of gender differences in visceral nociception so that we can uncover the molecular differences between males and females in the future.

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New Delivery Strategies on Nanoscale for Inflammatory Pain Treatment with Resveratrol - Size does matter!

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Resveratrol is a naturally occurring compound found in grapes and peanuts that has been found to inhibit the activation of Na^+ channels in the dorsal root ganglia, preventing pain signals from being transmitted to the brain. Resveratrol inhibits NF-kB in macrophages, thus preventing the transcription of inflammatory chemokines and cytokines. However, resveratrol has poor water solubility, chemical stability, and bioavailability. Encapsulation of resveratrol into nanoemulsions and/or microemulsions can improve upon its clinical usefulness by overcoming these problems. Nanoemulsions (100-200 nm) are phagocytized by monocytes upon i.v. injection, thus inhibiting inflammation, while microemulsions (20-50 nm) avoid monocytes' uptake and can therefore directly target the site of injury. Furthermore, to fully explore future clinical usefulness, nano- and microemulsions were incorporated into thermoresponsive hydrogels for targeted localized injury treatment with resveratrol. To evaluate potential for future use in inflammatory pain select formulations were evaluated using Luminex® Multiplex and ELISA analyses.

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Protein trafficking adaptor recognition of target proteins: Finding putative sequence motifs in α-arrestin cargos

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Alpha-arrestins, an exciting class of trafficking adaptors, control cell-environment interaction by regulating membrane protein trafficking. Given their critical role in the cell and the important clinical implications of this function, it is imperative that we gain insight into how α-arrestins recognize their protein cargos. The method by which α-arrestins interact with their cargos is currently undefined, and is hampered by the small number of defined α-arrestin-cargo pairs. To identify regions important for α-arrestin-cargo interaction, we have taken computational and bioinformatical approaches to search for amino acid motifs present in the group of transmembrane proteins regulated by α-arrestins. To date, no significant sequence motif has been identified among the limited group of established α-arrestin cargos. However, the search is currently being expanded to include a larger number of potential cargos to improve our ability to define sequence motifs. In the future, putative motifs will be tested *in vivo*.

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Examining the Physiological Effects of Anxiety on Exercise in Healthy Human Subjects

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Pain management is critical for those suffering from severe chronic or acute pain. The use of more osteopathic techniques such as exercise have recently risen in popularity due to widely noted therapeutic effects. As with any treatment, the validity of exercise must be evaluated to gain insight about its proper dosage and side effects, as well as how other factors may affect its potency. This study sought to observe whether social observation during exercise changes physiological measures of stress in healthy participants. We hypothesize that physical measures of stress will increase in the presence of the anxiety-inducing confederate, with more dramatic increases in heart rate and levels of the stress hormone cortisol. Given the mutual dependency between the psychological and biological components of the pain experience, examining the relationship between anxiety and exercise is vital in understanding exercise as a modality for treating pain.

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Exploration of Curaçaoan cyanobacterial collections for activity against triple negative breast cancer cells

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Cyanobacteria are photosynthetic prokaryotic organisms found in diverse terrestrial and marine habitats. Marine cyanobacteria are a rich source of complex bioactive secondary metabolites. These secondary metabolites exhibit activity as anti-inflammatory, anti-cancer, anti-fungal, anti-bacteria, trypsin inhibitor, neurotoxic and hepatotoxic compounds. This project is interested in discovering new compounds from cyanobacteria that have activity against the Triple Negative Breast Cancer (TNBC) cell line MDA-MB-231. Two collections of cyanobacteria were obtained by snorkel at Cas Abao and Playa Kalki beaches in Curaçao, Netherlands Antilles. These collections were extracted in 1:2 MeOH:DCM and given extract codes of DUQ0017 and DUQ0018, respectively. Extracts were then fractionated utilizing flash column chromatography into 9 sub-fractions, A-I. Ten fractions, DUQ0017D-H and DUQ0018D-H, were screened against MDA-MB-231 cells. DUQ0017D, DUQ0017H, DUQ0018D and DUQ0018G displayed cytotoxic activity, between 37% to 51%, at a concentration of 1mg/ml. Structure elucidation is underway utilizing NMR, HPLC and LC-MS techniques. Results to date will be presented.

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Construction of a Model for Social Exercise Anxiety in Healthy Human Subjects

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Anxiety is recognized as a debilitating illness that has devastating impacts on quality of life and contributes to millions of dollars of lost productivity and increased health care costs. Anxiety is difficult to treat and pharmacologic interventions are often not effective, expensive, or can have many side effects. Aerobic exercise is a known method of anxiety relief. However, exercising in a public setting can induce anxiety, which lessens the therapeutic and psychological benefits of exercise. The aim of this study was to develop and test a new measure of social stress that models social observation experienced in an exercise setting. Subject participation included an intake session followed by 30 minutes of moderate intensity treadmill walking either alone (control) or closely watched (intruder). Physiologic and psychologic measures of anxiety were examined. If our model induces anxiety, it will be used in future studies investigating the ability of exercise to attenuate anxiety.

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Detection of macrophage expressed MCP-1 in CCI rat model of neuropathic pain

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Neuropathic pain, a type of chronic pain, is modeled in a chronic constriction injury (CCI) of the sciatic nerve in rats. This model elicits hypersensitive behavior as well as an inflammatory response. A theranostic nanoemulsion with anti-inflammatory drug (celecoxib) has been developed by Dr. Jelena Janjic. When injected intravenously, the nanoemulsion droplets are then phagocytosed by monocytes that become macrophages at site of injury.This investigation used immunofluorescence to determine if the number of CD-68 positive macrophages at the site of CCI was reduced in the presence of nanoemulsion loaded with celecoxib. Additionally, we assayed the expression of MCP-1, a chemokine that can differentiate distinct classes of macrophages. A blind study on the presence of celecoxib was conducted, and it was hypothesized that rats that were injected with the drug would have fewer macrophages infiltrating the injured sciatic nerve. Conventional epi-fluorescence, as well as confocal microscopy, was used to observe a trend illustrating a reduction in the number of macrophages in half of the sample, indicative of the influence of the drug-loaded nanoemulsion.

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Isolation of serotonin receptor 2C ligands from a cyanobacterial sample from Curaçao

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Cyanobacteria are photosynthetic prokaryotes found in diverse terrestrial and aquatic habitats. Secondary metabolites produced by marine cyanobacteria exhibit pharmacological activity and resemble endogenous ligands to mammalian receptors. Our lab is interested in discovering novel compounds from cyanobacteria that have activity at targets, such as serotonin receptors, involved in CNS disorders. One particular sample of marine cyanobacterium, field classified as *Symploca*, was collected off the coast of Santa Barbara Beach, Curaçao and was subjected to dichloromethane/methanol extraction and fractionation by column chromatography. Phylogenetic analysis is being carried out using 16S rRNA sequencing to determine the species of cyanobacteria. Screening against G-protein coupled receptors has showed marked affinity for the serotonin receptor 2C (5-HT_{2C}). Structure elucidation was attempted utilizing NMR and LC-MS techniques. Unfortunately, during the isolation process contamination from the plasticizer dioctyl phthalate was found. Due to such contamination, our current focus has shifted towards removing this contaminant from fractions.