John S. Rogers Program

# 2021
# Summer Science Research Poster Conference

Wednesday, September 15
4:30 – 6:00 pm
Stamm Combo

# John S. Rogers Science Research Program

This program prepares outstanding students for careers in the sciences by supporting collaborative scientific research between students and faculty. In addition, the program aims to attract and retain outstanding students and faculty in the mathematical and natural sciences. Rogers fellows are trained not only as scientists, but as scientists who have a responsibility *to communicate the purpose and results of their work to a general audience*.

The following pages contain summaries of the research projects conducted during the summer of 2021. In these abstracts and in the conference posters, the names of the student researchers are followed by their expected year of graduation; the project director's name is listed last. To get the most out of the conference, ask the student presenters to explain to you the essence and significance of their research projects.

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**The Role of Thrombospondin in Neuromuscular Junction Structure and Function.** Grace Woods '22, Norma A. Velázquez Ulloa, *Department of Biology, Lewis & Clark College*.

Thrombospondin (TSP) proteins are involved in the formation of synapses (synaptogenesis) in the mammalian central nervous system. There is one TSP gene in the model organism *Drosophila melanogaster* (fruit flies), but it is not known if TSP in *D. melanogaster* has a similar function in synaptogenesis. We study the role of TSP in the development and function of the *D. melanogaster* neuromuscular junction (NMJ), which is a specialized synapse between a neuron and a muscle. We crossed certain strains of flies to produce progeny that have decreased expression of TSP in neurons, and then performed experiments on these larvae to determine if decreased TSP alters their NMJ structure and their locomotor behavior. To study NMJ structure, we dissected, stained, and imaged larvae, and then analyzed the images of their NMJs for anatomical features. To study locomotor behavior, we took videos of larvae moving freely in an arena and analyzed the videos for velocity, distance, angle of turns, and zones (a measure of how far larvae moved from their point of origin). Using these data, we compared the NMJ structure and behavior of larvae with normal TSP expression to larvae with decreased TSP in neurons. Our preliminary anatomical data suggest a change in NMJ structure in larvae with lowered expression of neuronal TSP. Locomotor data show that larvae with decreased TSP in neurons do not exhibit altered locomotor behaviors. These findings indicate that TSP may be involved in NMJ synaptogenesis in *D. melanogaster*, but that reducing TSP expression in neurons may not change the function of the NMJ in locomotion. We are currently working on validating the decreased expression of TSP, and next we plan on measuring new anatomical features and quantifying additional movement patterns, such as head-turning and body contractions.

**Investigating radial glial clones in the developing brain.** Gila Winefeld '23, Nicole Brockway, Tamily Weissman, *Department of Biology, Lewis & Clark College*.

During early vertebrate brain development, a major class of neural stem cells called radial glia give rise to neurons, or nerve cells, that populate the expanding brain. The radial glial cell, along with its daughter neurons, is termed a "clone." Previous data from our lab and others has shown that a multicolor labeling technique called Brainbow distinguishes among neighboring clones based on color and thus allows the *in vivo* study of multiple cell lineage pathways simultaneously in the developing brain. However, Brainbow does not distinguish between different cell types within a clone, and the spatial and functional relationships between radial glia and their neuron progeny are not fully understood. I have been working toward labeling and visualizing radial glia in living zebrafish using a specific promoter for the glial fibrillary acidic protein (GFAP) driving expression of green fluorescent protein (GFP)-tagged histone H2B. My project will involve a four-color fluorescence analysis in order to identify radial glia (via nuclear localized GFP) in the context of three separate Brainbow fluorescent proteins. I aim to perform *in vivo* experiments assessing gap junction coupling among radial glia and their daughter neurons. Studying clonal dynamics in this way can provide further insight into the significance of neuron lineage and the control of cell proliferation in the embryonic brain.

**Poster title: Hunting for the W Chromosome: Optimizing Sex Determination Research Pipelines for Artemia Sinica**

**Authors' names:** Luca Sax '22, Marwan Elkrewi, Prof. Beatriz Vicoso

**Authors' affiliation:** Lewis & Clark College, Institute of Science & Technology Austria

*Artemia sinica* is a crustacean species living in lakes with high salt concentrations. Its production of long-lasting cysts as offspring makes it ideal fish feed. It hence contributes heavily to the economic success of the aquarium industry. Popularity of *Artemia* has also grown in ecotoxicology where the species is employed to study the effects of nanoplastics on marine environments. Understanding the evolution of its sex chromosomes hence has both industrial and research implications.

It has been known since the 1960s that *A. Sinica* possess a ZW sex chromosome system in which females have ZW gametes while males possess WW sex chromosomes. Even though groups have previously researched *A. sinica's* Z-chromosome, much less is known about the W chromosome. In this project, we optimize the computational approach developed by our group to characterize the gene content of this chromosome. DNA and RNA reads are separated into shorter sequences (k-mers). Comparing the k-mers of male and female individuals allows us to identify which sequences are solely in female DNA/RNA but not in male. We recover RNA-seq reads which contain female specific k-mers, and assemble them into transcripts. Transcripts longer than 200 base pairs comprise our putative W-derived transcripts. We test the influence of sensitivity and specificity of various parameters, including k-mer size, k-mer depth, as well as the fraction of reads to overall k-mer number. We evaluate the quality of the data obtained with various combinations of these parameters, and optimize the pipeline accordingly- facilitating its use in other organisms in the future.

**Preparing for CRISPR-Cas9 Genomic Editing of Mouse Embryonic Stem Cells (mESCs).**
Jack B. Waite '23, Dr. Sharon E. Torigoe, *Department of Biology, Lewis & Clark College*.

Understanding how the sequences of non-coding DNA regulates transcription of cell-type specific genes is critical for gaining insight into cellular differentiation and the advancement of regenerative medicine. In mouse embryonic stem cells (mESCs), transcription of the pluripotency factor *Klf4* is regulated by distal, non-coding enhancers. To study these enhancers, the Torigoe lab has previously used luciferase assays, which can report on the level of transcription when we provide cells with a plasmid containing a regulatory sequence of interest and a reporter gene. The *Klf4* enhancers can thus be "decoded" by mutating key functional nucleotides and measuring for changes in transcriptional output. However, interpretations of luciferase assays are limited, as the enhancers are taken out of their native genomic contexts in plasmids.

Thus, to test the enhancer in its native context, I aim to use Cas9-mediated genomic editing to alter the *Klf4* enhancers *in vivo*. In order to accomplish this, additional mutations, beyond the ones to be tested, must be made to the enhancer. I thus needed to confirm that these additional changes had little effect on enhancer function. Using luciferase assays, it was found that many of these necessary mutations significantly reduced enhancer function. This has led me to investigate an alternative strategy for genomic editing.

**A Model for Integrin-mediated Activation of Rac1 Signaling in Olfactory Epithelium Resident Stem Cells During Injury-induced Regeneration**

Benjamin H. Bromberg[1,2], Jonathan D. Louie[2,3,4], and James E. Schwob[4]
(1) Lewis & Clark College, Portland, OR, USA (2) Medical Scientist Training Program, Tufts University School of Medicine and Graduate School of Biomedical Sciences, Boston, MA, USA (3) Program in Neuroscience, Tufts University Graduate School of Biomedical Sciences, Boston, MA, USA (4) Department of Developmental, Molecular & Chemical Biology, Tufts University School of Medicine, Boston, MA, USA

Horizontal basal cells (HBCs) comprise the reserve population of dormant stem cells in the olfactory epithelium (OE) that activate after severe tissue injury. Understanding the signals that regulate activated HBC (aHBC) cell fate and trigger them to proliferate, differentiate and repopulate all cell types of the OE during tissue repair is a critical step towards building a more comprehensive model of tissue regeneration in the OE. At 24 hours post-injury (24hpi), we found that aHBCs increase apical expression of integrin β1 & β4 (Itgb1 and Itgb4, respectively) and a downstream signaling partner, Rac1. Concomitantly, we found enhanced nuclear localization of phospho-cJun (pcJun), a downstream signaling partner of Rac1 and component of the AP-1 transcription factor that can functionally mediate cellular growth, differentiation and survival. Indeed, inhibition of active Rac1 following activation of primary HBCs *in vitro* results in Rac1 signaling pathway attenuation as demonstrated by diminished pcJun expression. However, it remains unknown whether Rac1 signaling pathway activation occurs as a result of an upstream signaling cascade initiated by integrins at focal adhesions (FAs) in a mode similar to the signaling patterns seen during fibrosis. In addition to increased apical Itgb1 and Itgb4 at 24hpi, we have recently demonstrated that aHBCs also increase apical expression of focal adhesion kinase phosphorylated at tyrosine 925 (pFAKY925), a key component of FAs whose phosphorylation is in part integrin-dependent and can activate Rac1. In total, our data demonstrate that aHBCs spatiotemporally synchronize components of the FA complex at 24hpi, thereby suggesting that FAs may play an integral role in aHBC Rac1 signaling activation and cell fate. Furthermore, blockade of Rac1 activation following HBC activation *in vitro* suggests that Rac1-mediated signaling plays a critical role in regulating aHBC cell fate during OE regeneration. Future experiments will interrogate whether integrin-mediated FAK phosphorylation at FAs facilitates Rac1 signaling pathway activation and the functional impact that Rac1 has on aHBCs both *in vitro* and *in vivo*.

**Deciphering the Cis-Regulatory Code Governing *Klf4* Expression in Pluripotent Stem Cells**
Torrey Lind ('22), Dr. Sharon E. Torigoe. Department of Biology, Lewis & Clark College.

**Abstract**:
Cellular development, morphology, and function are controlled by precise patterns of gene expression. These properties are established by the concerted action of non-coding genomic regulatory elements, or *cis*-regulatory elements (CREs), and their interactions with proteins called transcription factors. Despite years of research, our understanding of how these elements underlie transcriptional organization and regulation remains unclear. To gain further insight into the mechanisms of transcriptional control, the Torigoe lab has focused on deciphering the encoded features of long-distance acting CREs, or enhancers, and how those govern gene expression. Specifically, my work aims to elucidate how specific properties of enhancer sequences regulate the unique expression pattern of *Klf4*, a key gene involved in the maintenance of pluripotency in embryonic stem cells. Exploring these rules will allow us to read the genomic instructions for development and pinpoint enhancer variants underlying disease or evolutionary change.

**Investigation of OCT4/SOX2 Binding Sequences from the Enhancers for the *Klf4* Gene.**
Savannah C. Myers '23, Alexis R. Traeger '21, Dr. Sharon E. Torigoe, *Department of Biology, Lewis & Clark College*.

Transcription is the first step in the process of gene expression and activity, and understanding the mechanisms for transcription regulation provides valuable insight into the function of genes in organisms. An important step in gene regulation is the binding of transcription factors to their binding sites to modulate the activities of RNA polymerase. To study this process, we use the model gene *Klf4,* which is a pluripotency factor. *Klf4* contains three enhancers, called E1, E2, and E3, which are non-coding regulatory sequences that facilitate gene expression but are not necessarily right next to the coding portion of the gene. For *Klf4,* the binding of transcription factors OCT4 and SOX2 is necessary for expression of the gene. To our surprise, the binding sites located in the three enhancer regions of the gene are relatively low affinity compared to the consensus sequence. To confirm the binding affinities of the OCT4-SOX2 composite site from all three *Klf4* enhancers, we conducted biochemical experiments to see the impact on individual protein binding, synergistic protein binding, and binding when the sequence has been mutated. These results underscore the impact of the specific DNA sequence in directing transcription factor binding and, subsequently, gene expression.

**Functional Diversity of Brown Recluse Spider Venom Toxins.** Abby Prager '22, Jemma Montgomery '23, Greta Binford & Pamela Zobel-Thropp, *Department of Biology, Lewis & Clark College*.

Different proteins found within the largest protein family in Sicariid venom, SicTox, target different phospholipids found within cell membranes. These proteins catalyze the cleavage of the phospholipid headgroup which leads to cell death due to compromised membrane integrity. Previous research has found that some SixTox proteins isolated from the venom of Sicariids have specificity for either sphingomyelin (SM) or ceramide phosphoethanolamine (CPE). We tested four different proteins from the venom of *Loxosceles arizonica* and *Sicarius terrosus:* a CPE specific protein from *S. terrosus,* a non-specific protein from *L. arizonica,* and a SM specific protein from *L. arizonica*, along with a protein variant with a single point mutation of the SM specific protein that wipes out function. We tested the effects of these proteins on two different cell lines, Sf9 from *Spodoptera frugiperda* which has equal concentrations of SM and CPE, and S2R+ cells from *Drosophila melanogaster,* which is CPE-rich. We found that each enzyme affected the morphology of both of these different cell types, but there are anecdotal differences in the magnitude of the effect. Future work will pursue improved quantification of those effects with the goal of standardized comparisons. We predict that proteins with different phospholipid specificity will have different morphological effects within and across cells with different phospholipid compositions.

**Development of AXL-Degrading Proteolysis Targeting Chimeras (PROTACs) for Treatment of Pancreatic Cancer**

Cassidy Floyd-Driscoll '24, *Lewis & Clark College* Ashley Jensen, Ruben Muñoz, Haiyong Han, *Molecular Medicine Division, Translational Genomics Research Institute, Phoenix, AZ*

We tested the activity of 12 novel proteolysis targeting chimeras (PROTACs) on pancreatic cancer cell lines. Due to their common structure, these compounds induce the degradation of AXL proteins, thus causing cancer cell death. We observed the survival rate of treated MIA PaCa-2 and PANC-1 cells using Sulforhodamine B (SRB) staining, and we performed Western Blotting to determine AXL levels in treated cells.

The XW-3-ELA4 and XW-3-ELA5 compounds had the greatest effect on MIA PaCa-2 cell survival, and the TAM-977 and TAM-6C compounds had the greatest effect on PANC-1 cell survival. Of these, the XW-3-ELA4 and XW-3-ELA5 compounds were shown to be the most active in degrading AXL protein. The success of these compounds provides guidance for future development of targeted pancreatic cancer therapies.

**Screening the Million Mutation collection for defects in gut granule biogenesis in *Caenorhabditis elegans*.** David Nhek '22, Frances Courtemanche '24, Caitlin P. Morris '16, Jared L. Delahaye '12, Greg Hermann, *Department of Biology, Lewis & Clark College*.

Lysosome-Related Organelles (LROs) are a diverse group of cell type-specific organelles, each with its own distinct functions, morphologies, and contents. LROs and lysosomes are both derived from the endocytic trafficking pathways. *Caenorhabditis elegans* have LROs called gut granules in their intestinal cells. Gut granules contain optically active birefringent and autofluorescent materials that can act as gut granule markers. Currently, there are 33 known genes that function in gut granules biogenesis. Many of which encode for protein complexes that function in mammalian LRO formation such as AP-3, BLOC-1, HOPS complex, and Rab32/38. At least 150 genes are involved in lysosome formation in *Saccharomyces cerevisiae*. Given the similarities between lysosomes and LROs, it is likely that there are many undiscovered factors that function in LRO biogenesis. To identify new factors, we have been screening *C. elegans* strains that belong to the Million Mutation Collection for a loss and/or reduction of birefringence and autofluorescence compartments or a change in their morphologies. We identified 129 strains that have altered gut granule number and/or morphology. Many do not appear to contain mutations in any genes previously implicated in LRO biogenesis. Following identification, we then began the process of genetic characterization of 6 strains with altered gut granule size or number. Genetic characterization of new strains is necessary to identify novel genes that impact LRO biogenesis and maintenance when mutated. Through identifying and studying the function of this new gene, we can gain more insight into the LRO biogenesis and maintenance in *C. elegans* and other organisms.

**The genetic mechanisms of nicotine and ethanol cross-tolerance in *Drosophila melanogaster*.** Sonya Lee '24, Luke Rotello '22, Norma Velazquez Ulloa, *Department of Biology, Lewis & Clark College*

Nicotine and ethanol are prevalent drugs of abuse across the world. Epidemiological studies demonstrate a correlation in the use of these drugs; however, many studies about drugs of abuse focus on a single drug. We use *Drosophila melanogaster*, the common fruit fly, as a model for elucidating mechanisms of cross-tolerance between nicotine and ethanol. Our experiments tested mutant genetic lines that have been identified as either sensitive (2-3) or resistant (2-21, 2-62, 8-86) to nicotine based on previous survival data. To validate the sensitivity of the selected mutants on nicotine and alcohol, we crossed mutant flies on control food (5% water), alcohol food (5% ethanol), and nicotine food (1.8 mM nicotine) and studied the progeny's development and behavior. To study development, we recorded the number of larvae, pupa, and empty pupa from days 7 to 14 and measured dry weight of adult progeny. To study behavior, we performed an ethanol sedation assay on adult progeny. Using these data, we compared the mutant strains to our control, finding that 8-86 prevents developmental delay in alcohol but has no effect on dry weight. For behavior, our data suggest that 8-86 decreases the effect of alcohol. We are working to perform nicotine sensitivity testing to further examine nicotine and ethanol cross-tolerance in *Drosophila melanogaster*.

**Salma Bashir**

**Title: Sequence determinants of substrate specificity in brown recluse venom toxins**

**Abstract:** Sicariidae toxins (*SicTox*) are abundant components in the venom of brown recluse spiders. They are neurotoxic on insects and can cause dermonecrosis in mammals. *SicTox* variants differ in phospholipid substrate preference with enzymes in the α clade acting primarily on the common sphingolipid sphingomyelin (SM), and enzymes of the β clade showing variable preference towards both sphingomyelin (SM) and ceramide phosphoethanolamine (CPE). We are investigating the amino acid changes that evolved to cause this switch in substrate specificity, and we hypothesize the role of a conserved aromatic cage in *SicTox* from the α clade. To test the influence of the aromatic cage, we used site-directed mutagenesis to eliminate the cage in the α clade protein L1-αIII1i from the 9hilean brown recluse spider *Loxosceles laeta*. Activity assays demonstrate that mutant L1-αIII1i has decreased activity on SM relative to wild-type L1-αIII1i, while neither the wild-type nor the mutant enzyme have significant activity on CPE. Further research is in process to determine if the mutant remains able to bind membranes. This research aims to provide insight into the sequence determinants of substrate specificity in the highly diverse *SicTox* family.

**Clonal Coordination in Interkinetic Nuclear Migration.** Julia Litz ‘22, Nicole Brockway, Tamily Weissman-Unni, *Department of Biology, Lewis and Clark College*

In early embryonic development, radial glia cells play a crucial role in producing the majority of neurons in the brain. When radial glia divide asymmetrically, they produce daughter neurons that then use the radial fiber as a scaffold before migrating into the growing brain. The unit containing a radial glial cell and its daughter neurons is called a clone. During the cell cycle, radial glial cell bodies move along their fibers towards and away from the ventricle in a process called interkinetic nuclear migration (IKNM). Although this process was first identified almost 100 years ago, little is understood about why cells undergo interkinetic nuclear migration and what regulates the process. It is even possible that there is coordination across clones during IKNM. To study IKNM, Brainbow fluorescence can be used to visualize clones in embryonic zebrafish on a confocal microscope. My goal is to develop new methods for quantifying clonal coordination and dynamics within clones during IKNM. One such tool will involve in vivo time-lapse microscopy with 10-minute intervals to fully capture the relatively rapid stages of mitosis. Another tool will involve creating a graphical approach to quantify IKNM across multiple clones. If coordination is found among neighboring clones, it could suggest a role for an extracellular factor or communication among clones. Studying clonal coordination is important because it allows us to better understand how clones may contribute to overall brain development and function.

**Exploring Unexpected Substrates of a Brown Recluse Venom Toxin**

Keeley Alexander, Dr. Greta Binford, Dr. Pamela Zobel-Thropp, Dr. Matthew Cordes

There are a variety of biological advantages for venomous organisms to have antifungal proteins in their venom and this could be true for brown recluse (sicariid) spiders. There are hundreds of *SicTox* protein homologs which constitute over 50% of proteins in sicariid venom, but many of them have active sites with slight differences that lead to diverse lipid substrate specificity. Out of those hundreds, it is possible that one could have a preference for lipid substrates found in fungus. Previous attempts at categorizing *SicTox* venom protein, Lox_βII, have shown that this protein in particular does not have a high preference for the same lipid substrates as some of its other well-studied homologs. The βII protein has a slightly larger active site and may cleave lipids with larger headgroups. In an attempt to further characterize the substrate preference of the βII protein, I added purified βII to *Saccharomyces cerevisiae,* or budding yeast, a common species of fungus that can be easily grown and monitored in a lab setting. I performed and imaged results from serial dilution and disk diffusion assays, two different methods to quantify any effect the βII protein had on the yeast's growth rate. Yeast is a type of fungus with a membrane lipid called phosphatidylinositol, which has a larger headgroup than other common *SicTox* substrates found more commonly in non-fungal organisms. I performed and imaged both of these preliminary tests multiple times with varying concentrations of added βII protein. Additions of higher concentrations—from anywhere above 30μg of purified protein— showed stunted yeast growth within 30 minutes of βII exposure, indicating a possibility of βII activity on fungal lipid membranes. However, stronger evidence of antifungal activity by the protein is needed in order to verify the added protein as the sole cause of the stunted yeast growth. Additionally, while antifungal activity displayed by this enzyme could potentially inform antifungal action on a more broad scale, the concentration of venom needed to display activity is not realistic when considering the biological relevance of a potential antifungal protein in a spider's venom.

**Does alpha-synuclein modulate DNA repair?**

Saheli Singh'22, Zoe Cook'18, Nicole Brockway, Tamily Weissman-Unni, *Department of Biology, Lewis & Clark College*

Parkinson's disease (PD) is a progressive neurodegenerative disease in which a protein called alpha-synclein (α-syn) aggregates in dopaminergic neurons. While alpha-synuclein's role in disease pathology is unknown, research has shown α-syn localization in the nucleus and presynaptic terminals. Currently, it is understood that α-syn functions as a vesicle recycling agent in presynpatic terminals; however, its role in the nucleus remains elusive. Recent research in cell culture suggests that alpha-synuclein functions in the nucleus as a DNA-repair protein. We aim to test whether alpha-synuclein serves a role in repairing DNA using *in vivo* imaging techniques in zebrafish. By exploiting the visual advantages of transparency during zebrafish development, we are able to measure DNA repair levels using a non-homologous end-joining (NHEJ) reporter that drives green fluorescent protein (GFP) expression. Currently, we have successfully expressed the neurod:NHEJ reporter-GFP construct in living zebrafish that appears to track DNA repair. As α-syn is typically a free-floating monomer that can easily enter the nucleus, it is possible that when asyn is aggregated in PD, it is no longer able to enter the nucleus and repair DNA, potentially providing an explanation for neuronal death in PD. Identifying alpha-synuclein's function in the nucleus will allow for a deeper understanding of alpha-synuclein's role in disease and may lead to therapeutic advances.

**Regeneration and Diversity in an Old Growth Pacific Northwest Forest.**
Olivia Brackin ‘22, Kaitlin Emmett ‘22, Mila Pruiett ‘22, Dr. Margaret Metz
*Department of Biology, Lewis & Clark College.*

Plant pathogens can cause large outbreaks of disease that have detrimental effects on the agricultural and natural world. Two examples include the Irish Potato Famine in the 19th century and the continued destruction of California’s forests by sudden oak death. Both of these epidemics, among many others, were caused by oomycetes, a pathogenic relative of algae. While these history-altering outbreaks highlight the impact of oomycetes, there is little known about the role these pathogens play in their endemic environments.

Oomycetes are native to Pacific Northwest forests, and they are potentially regulating forest diversity by preferentially attacking some species over others. The Metz lab aims to study oomycetes' role in shaping diversity at the old-growth Wind River Experimental Forest in southwestern Washington. Annually, a network of plots is treated to kill specific pathogens, oomycetes and fungi. Treated and untreated plots are then censused, tracking which individual seedling conifers survive each year. Seed rain is also quantified to calculate germination rate per treatment. These two annual censuses provide long-term data on survival and germination rates for all conifer species and treatments. The Metz lab hopes to utilize this data to understand if oomycetes are preferentially attacking certain species or affecting overall conifer survival rates, ultimately influencing forest diversity.

**Degradation of Glyphosate with a Molybdenum(VI) Polystyrene Bead Catalyst**. Morgan Bashore ’22, Dr. Louis Y. Kuo, *Department of Chemistry, Lewis & Clark College, Portland, OR 97219, United States*

A polystyrene-supported molybdenum(VI) bead catalyst (called Mo-Y beads) has been developed in an effort to degrade glyphosate, an herbicide commonly known as Roundup, into safer products, as well as recover phosphate as a phosphorus commodity. The Mo-Y beads are activated with hydrogen peroxide in a buffer solution around pH 6 to oxidize the glyphosate. This research is working towards determining all products in the degradation and the pathways followed in degradation. Both nuclear magnetic resonance (NMR) and gas chromatography/mass spectroscopy (GC/MS) have been utilized to characterize the reaction products. Through these techniques, we have been able to determine glycine, formate, carbon dioxide, and phosphate as some of the products; 70% of the initial glyphosate was converted to glycine and formate, while the other 30% as gaseous products such as carbon dioxide. To better account for all carbons in the products, $^{13}$C-labeled glyphosate has been synthesized to degrade and track through $^{1}$H, $^{13}$C, and $^{31}$P NMR as well as GC/MS. Ultimately, the phosphate will be recovered to then be recycled as a calcium salt fertilizer. This project is a part of the effort to combat the rapidly decreasing phosphorus resource.

**Electrochemical Interactions between Common Groundwater Ions and Iron.** Marie Solis '22, Barbara A. Balko, *Department of Chemistry, Lewis & Clark College*.

In this research, the open circuit potential (OCP) and oxidation-reduction potential (ORP) of iron were measured as a function of time. The OCP is a measure of how reactive or corrosive the iron surface is. A high OCP (near -0.3 V) indicates that the iron is passivated. A low OCP (near -0.8 V) suggests that the passive film is removed. In contrast, the ORP is a measure of the oxidizing or reducing tendencies of the solution; a high ORP indicates the presence of an oxidizing agent (such as $O_2$) and a low ORP indicates the presence of a reducing agent (such as Fe); depassivated Fe will lower the ORP while passivated Fe and $O_2$ will raise the ORP. The effect of the iron particle size, the groundwater ions present in solutions, and the presence of $O_2$ on the formation and dissolution of the passive film were studied. The different iron sizes that were studied were bulk iron, Fluka iron (large particles), Fischer electrolytic iron (small particles), and freshly prepared nano-iron. The different solutions used were 10 mM ferrous sulfate ($FeSO_4$) and 10 mM sodium chloride (NaCl), both under anaerobic and aerobic conditions.

Our results showed that for all iron particles except the nano-sized, $FeSO_4$ removed the passive film and kept it off the iron surface, even in aerobic conditions. The NaCl did remove the passive film as well, but in the presence of $O_2$, some of the iron particles grew a passive film. The freshly prepared nano-iron did not have a passive film. However, in aerobic conditions (even in the presence of $FeSO_4$), a passive film formed immediately. This suggests that the freshly prepared nano-iron is extremely reactive, likely because of the absence of an initial passive film and the large surface area to volume ratio. ORP measurements suggested that in anaerobic solutions, the nano-size iron slowly grew a passive film. These findings can be used to improve the performance of iron permeable barriers used for the remediation of groundwater.

**Investigation of Sulfide Oxidation using Novel Molybdenum (VI) Catalysts.**
Drew Blauth '23, Louis Y. Kuo, *Department of Chemistry, Lewis & Clark College*.

Oxidative desulfurization is a process by which sulfides are removed from petroleum such that less pollutants are released when the petroleum is burned. An important part of this process is oxidation of the sulfides, which is performed by combining petroleum, peroxide, and a catalyst. The impact of the size of a catalyst on its effectiveness was explored by analyzing the ability of two such compounds to catalyze a specific oxidation reaction involving a model sulfide. Both novel catalysts contained a molybdenum (VI) complex and nearly identical ligands in an attempt to control other variables that impact their ability to catalyze sulfide oxidation. The only difference was the size of four identical groups attached to one of the catalyst's ligands.

Kinetics runs were performed using an NMR spectrometer to determine how quickly the oxidation reaction took place at different temperatures using one of the two catalysts, hydrogen peroxide, and the model sulfide. Analysis of this and of other work performed using Excel revealed that there was a significantly larger activation energy associated with the larger catalyst, meaning that it was less effective than the smaller catalyst. This suggests that the size of a catalyst and its ability to catalyze sulfide oxidation are inversely related. Future research will focus on smaller catalysts in order to further investigate this relationship while also attempting to identify more effective catalysts

**Protein-Capped Gold Nanoparticles Under Solar Illumination**
By Rachael Rice

Nanoparticles are miniscule pieces of matter that can be used for a wide variety of applications such as disease screening, food sensors, and in hair and cosmetic products. Due to popularity, their properties are important to study and investigate. Our lab focused on gold nanoparticles (AuNPs). New studies have sought to assess the effects of changes in environmental factors, such as amount of sunlight, temperature, and/or pH on both the structure and function of AuNPs. We studied the effect of UV and solar light on the stability of the capping agent BSA and/or BSA-capped gold nanoparticles.The stability of BSA as a capping agent was first assessed, with the BSA suspended in a phosphate buffer solution, under both UV light and the solar simulator. Gold nanoparticles were made by reducing chloroauric acid with trisodium citrate in water, and UV-vis spectroscopy confirmed their stability in solution. Gold nanoparticles capped with BSA were then tested under both UV light and the solar simulator. BSA's structure has a large percentage of alpha helices which can be used to look at changes in structure. Results were assessed by comparing percent alpha helicity before and after light exposure. Calculations were done using the program DICHROWEB. Stable AuNPs were synthesized and suspended in solution. Under both the UV light and solar simulator, BSA showed a decrease in percent alpha helicity indicating a change in structure. Under UV light, the localized surface plasmon resonance peak of BSA-capped AuNPs showed a right shift to a lower energy. This is consistent with the nanoparticles beginning to agglomerate. These light sources have the effect of destabilizing the overall structure of the capped nanoparticle. Light exposure appears to be changing the conformation of the capping agent BSA and causing the AuNPs to agglomerate; causing them to be in the early stages of crashing out. This could have implications in both wastewater treatment and aquatic environments. Sun and UV light are known wastewater treatment methods, but it is unknown what effects destabilization and agglomeration of nanoparticles will have in the treatment process. If nanoparticles are being released into sunny environments, this could result in problems for aquatic ecosystems as well. Further research should be done to determine the effects.

**Novel Phosphorus Chemistry by Molybdenum(VI) Complexes.** Liz LeJeune '22, Louis Y. Kuo, *Department of Chemistry, Lewis & Clark College*.

This research investigated the interactions between various organophosphates and two molybdenum(VI) complexes: Mo-diperoxo and bihyat-$MoO_2$. With nuclear magnetic resonance (NMR) spectroscopy, degraded phosphonate and possible chelate products have been tracked.

The Mo-diperoxo degrades glyphosate, the primary component of Roundup, into little AMPA and primarily free phosphate, which can be used in products like fertilizers and detergents. When both the Mo-diperoxo and bihyat-$MoO_2$ interact with select phosphonic acids, they likely form chelates in which the molybdenum metal binds to the phosphorus compounds.

**Exploring the Structural Basis of Dynein Regulation**
Ashley Phan ‘24, Meghan Sheldrake ‘23, Claire Yung ‘22, Nikolaus M. Loening
*Department of Chemistry, Lewis & Clark College*

We have conducted an experimental study to help elucidate how the motor protein dynein and one of its binding partners, dynactin, interact. Dyneins are responsible for transporting a variety of cargos inside of cells. The binding interaction between dynein and dynactin is one mechanism that determines the type of cargo that is transported as well as the destination and timing. Our research focused on an isoform of the intermediate chain (IC) of cytoplasmic dynein, IC-2C, and the $p150^{Glued}$ subunit of dynactin. Using various biophysical techniques, we aim to better characterize the region of $p150^{Glued}$ that interacts with IC-2C. Additionally, we investigated the activity and efficacy of TEV protease, an enzyme that we used in the process of purifying IC-2C and $p150^{Glued}$ proteins. Our final focus aimed to design mutations that stabilize the structure of the region of $p150^{Glued}$ that interacts with IC-2C.

In order to study these proteins, we genetically engineered bacteria (*Escherichia coli*) to produce wild type (WT) and various mutant versions of the IC-2C and $p150^{Glued}$ proteins. After growing cultures of *E. coli* that expressed the mutant strains in an incubator, we lysed the cells using sonication and purified our target proteins using three chromatographic steps: immobilized metal affinity chromatography (IMAC), reverse IMAC (after cleavage of the target protein by TEV protease), and then size exclusion chromatography. These purified samples will be used to carry out isothermal titration calorimetry, circular dichroism measurements, and nuclear magnetic resonance spectroscopy measurements.

This summer we produced and purified many useful mutants of the dynein IC subunit and dynactin $p150^{Glued}$ subunit that will be used for further binding interaction characterization experiments. Furthermore, we determined the ideal protease to substrate ratio for TEV cleavage which will make protein purification more efficient.

**Rehearsing Disaster: Understanding Earthquake Preparedness Behavior in an Interactive Environment** Altaf Barelvi ‘23, Shohrukh Jalolov ‘21, Sylvia Kunz ‘23, Jensen Kraus ‘21, Ross Miyabuchi ‘21, Isaiah Nduku ‘22, Skye Russ ‘23, Sarah Wood ‘22, Peter Drake, *Department of Mathematical Sciences, Lewis & Clark College,* Erik Nilsen, *Department of Psychology, Lewis & Clark College,* Bryan Sebok, *Department of Rhetoric and Media Studies, Lewis & Clark College,* Elizabeth Safran, *Department of Environmental Studies, Lewis & Clark College*

Our project seeks to use educational video games to help users better understand earthquake preparedness.

Our first experiment is nearing completion and we have gotten a good baseline of results for the rest of our experiments. We sought to compare our video game with a free internet search, and their impact on participants intent to act, outcome expectation, and self efficacy for earthquake preparedness actions.

At the moment, we are working on the game for our second experiment, which will help us see how the location and living circumstances in our game, plus the living circumstances of the participants affect the amount of information each participant retains.

The answers to these questions are still in progress. But so far, the results from our first experiment seem to indicate that participants had a better understanding of earthquake preparedness after playing our game compared to conducting an online search.

**Building Dependable Computing Systems with a Focus on Cybersecurity.** Linus Brogan '22, Natalie Zoz '23, Dylan Angel '23, Charlie Compton '23, Syd Parker '22, Alain Kägi, Jens Mache, *Department of Mathematical Sciences, Lewis & Clark College*

Not a day goes by that we do not hear about a new cybersecurity incident. Writing provably correct code would alleviate this concern, but it is a neglected area of investigation. We draw inspiration from the Resurrecting Duckling paradigm first described by Frank Stajano and Ross Anderson. We focus on improving the security of Internet of Things (IoT) devices by demonstrating that it is practical to develop dependable systems that minimize risk and prioritize security through formal verification. We developed the foundation for a simple autonomous temperature sensor that allows authenticated remote agents to access its data. With this sensor in mind, we have chosen and familiarized ourselves with a verified microkernel to provide a minimal, secure foundation for our application compared to larger operating systems. While the development of our application is still ongoing, we aim to implement our application into this temperature sensor and other IoT devices in the future. At this time, our main focus is deploying our temperature sensor in collecting and protecting data in biological research labs. Because IoT devices are becoming increasingly common, it is our goal to build secure applications that work to protect the security and wellbeing of their users.

**Acoustics of the Mandolin.** Connor Robertson '22, Gretchen Schowalter '22, Stephen L. Tufte, *Department of Physics, Lewis & Clark College*.

The mandolin is an acoustically unique musical instrument with elements similar to guitars and violins. Inspired by a classic guitar study, we test if the mandolin's low frequency response can be modelled as a simple 3-mass coupled oscillator system. We measured the response of the three masses in question (the mandolin's front plate, the back plate, and the air within the body) using microphones, accelerometers, and force probes. From this data we calculate a quantity called "mobility," which describes how much the mandolin moves when it is excited at different frequencies. We used this parameter to compare our experiments to the model. A telltale feature of a system of coupled oscillators is the influence of one oscillator on the rest of the system. For example, when we attach mass to the back plate of the mandolin to lower its resonant frequency, we observe that the vibration frequencies of the front plate also change. From a detailed comparison of our measurements to the theoretical model, we conclude that the low-frequency behavior of the mandolin can be accurately modeled using the 3-oscillator model.

**Deep Learning Phase Transitions in Quantum Chromodynamics.** Stephen Baker, *Department of Astronautical Engineering, University of Southern California;* Christian Ermann '22, *Department of Physics, Lewis & Clark College;* Mohamed Anber, *Centre for Particle Theory, Department of Mathematical Sciences, Durham University*.

We have studied the ability of convolutional neural networks to learn deconfinement phase transitions in quantum chromodynamics (QCD). A metropolis Monte Carlo simulation was used to generate states for different configurations of the X-Y spin model with discrete-symmetry-preserving perturbations, which is mapped to QCD. Convolutional neural networks were trained on states far from the critical region and then evaluated on states within the critical region. The critical temperatures and exponents of the systems were calculated from both the Monte Carlo data and the network predictions. Convolutional neural networks were able to determine critical temperatures near the expected values for some configurations of the X-Y spin model.

**Inclusive Pedagogy in Educational Games.** Ruth Makonnen '22, Sunny Broadhead '22, *Department of Psychology, Lewis & Clark College*.

Edugames, and edularps as a subtype of edugames, are becoming a more popular tool for education as an alternative teaching style that can result in higher memory retention for some learners (Wouters, van Nimwegen, van Oostendorp, & van der Spek, 2013). However, we must consider that participants enter educational settings with different levels of preparation, motivation, interest, and varying cultural perspectives—all of which can affect their in-classroom and in-game experiences (Dewsbury, 2018).

Our research project this summer aims to explore ways in which educational games, more specifically edularp, can incorporate an inclusive pedagogical framework. We want to explore the link between the presence or absence of this framework and the experience participants have during the edugame regarding feelings of inclusion, engagement, and learning outcomes. As this is a brand new project, our poster today mainly describes elements of our background research, our intended methods, and our research hypothesis.

**Exploring the Relationship Between Trait Resilience, Cognitive Control, Eating Habits, and Stress During the COVID-19 Pandemic.** Andrew Z. Steinberg '21, Hanna R. Wright '23, & Todd D. Watson, *Department of Psychology, Lewis & Clark College*

We remotely assessed individual differences in trait resilience, cognitive control in the context of stressors, potentially unhealthy (emotional eating) and healthy (intuitive eating) eating habits, and stress levels during the COVID-19 pandemic in a community-based sample of young adults (N=38). Separately, we used a remote version of the classic Stroop paradigm to explore the relationship between these variables and the behavioral correlates of exerting cognitive control over attentional capture by three types of distracting cues/primes (images of high calorie foods, nature scenes, and neutral gray scale shapes). We found that trait resilience correlated with higher levels of self-reported cognitive control, better performance across categories of cues in the Stroop task, higher levels of intuitive eating, and lower levels of both emotional eating and overall stress. Further, self-reported cognitive control was associated with lower levels of COVID-specific fears and worries during the pandemic. Finally, we found that while food images led to attentional capture (slower response times) to at least some categories of Stroop stimuli across participants, the specific magnitude of this effect was not related to trait resilience or eating habits. Taken together, these data support the hypothesis that trait resilience and cognitive control are related, and predict a constellation of potentially healthy behaviors (e.g. higher levels of intuitive eating) and outcomes (e.g. lower stress levels) in the context of a global pandemic. The data also suggest that the relationship between trait resilience and cognitive control is consistent across distracting stimuli, and is not specifically related to control over food cues.

# Index of Student Researchers