John S. Rogers Program

2023
Summer Science Research Poster Conference

Tuesday, September 19
4:30 – 6:00 pm
Stamm Combo, Fowler Center

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 2023. 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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Functional diversity of phospholipases in the *SicTox* gene family. Sammy Kutsch ‘24, Madeline Jones ‘25, Lindy Gewin and Greta Binford, *Department of Biology, Lewis & Clark College.*

Venom is composed of a wide diversity of molecules, with various functions and activities. The *SicTox* gene family includes a variety of Phospholipase D (PLD) proteins found in the venoms of sicariid spiders, including the brown recluse. PLD’s act by cleaving the heads from phospholipids, the molecules that make up the outer membrane of cells. This disrupts the cell membrane leading to biological consequences in the target organism, including dermonecrosis in mammals and neurotoxicity in insect prey. Cell membrane phospholipids themselves are diverse, and the range of *SicTox* substrate specificities target different phospholipid head groups. One hypothesis for the selective factors driving the evolution of different PLD head group specificities is to more effectively target diverse prey types that differ in the phospholipid compositions in their cell membranes. In this project we investigated the functional relevance of different *SicTox* specificities using two different bioassays to compare toxicity in whole organisms and cells as models representing natural prey. Injection assays were performed on *Gryllodes sigillatus* (crickets) and *Drosophila melanogaster* (fruit flies) to compare physiological effects of the individual *SicTox* variants on different prey types. For cytotoxicity assays, we used propidium iodide assays to quantify cellular damage in sf9 cells after treatment with different PLD’s from the *SicTox* gene family. Both of these assays produced inconsistent results, and much of our summer was devoted to refining these assays to increase consistency and allow us to detect more nuanced differences in the effects of these toxin variants. We continue to work to refine these assays.

Elucidating the constraints for ESRRB and STAT3 binding sites in the enhancers for the pluripotency gene *Klf4*

Sarah J Swanson, Torrey M Lind, Sharon E Torigoe
Lewis & Clark College, Department of Biology, Portland, OR

Understanding the transcriptional program for pluripotency is critical for advancing the field of regenerative medicine, particularly for the generation and use of induced pluripotent stem cells. To this end, we have investigated the regulation of the gene *Klf4*, which is best known as one of the four Yamanaka transcription factors for inducing pluripotency. *Klf4* also is expressed specifically in naïve-state pluripotent stem cells (PSCs) but is suppressed in primed-state PSCs. Thus, studying how *Klf4* is transcriptionally regulated can lead to new insight into the naïve-state of pluripotency. Previous work demonstrated that the transcription factors OCT4, SOX, ESRRB and STAT3 assemble at the enhancers to activate *Klf4* expression. Whereas OCT4 and SOX2 are expressed in naïve and primed-states of pluripotency, ESRRB and STAT3 are naïve-specific. Furthermore, ESRRB and STAT3 are recruited by the lead factors OCT4 and SOX2, so we have asked whether there are "grammatical" constraints for the ESRRB and STAT3 binding sites. These include affinity, multiplicity, order, orientation, and spacing, and it has been proposed that these characteristics impact the binding of transcription factors at enhancers and, subsequently, enhancer function. Here, we present work to elucidate constraints for the ESRRB and STAT3 binding sites in the *Klf4* enhancers and how those affect enhancer function.

Investigating the function of a lysosome-related organelle biogenesis factor in *C. elegans* embryos. Madeline Daniel ‘24, Dr. Greg Hermann, *Department of Biology, Lewis & Clark College*.

Lysosome-related organelles (LROs) are cell-type specific organelles that are similar to, but distinct from, lysosomes and endosomes. LROs are derived from the highly-regulated endolysosomal system and are known to have a wide variety of morphologies, contents, and functions depending on the role of the host cell. The proper formation of these compartments is biologically significant, as LROs perform multiple important physiological functions. In order to study LRO biogenesis, the Hermann lab uses the nematode *C. elegans* as a model system. The intestinal cells of *C. elegans* have prominent LROs called gut granules, which contain optically active material and are easy to visualize using standard microscopy techniques.

In a genetic screen for factors that mediate gut granule formation, the Hermann lab determined that the gene *acdh-11*, which encodes an acyl CoA dehydrogenase enzyme, is implicated in regulating gut granule size. However, little is known about the function of ACDH-11 and it is unclear how this protein is involved in promoting normal gut granule formation. I further characterized the function of ACDH-11 in fatty acid metabolism by using GFP-tagged lipid droplets and vital lipid dyes to analyze changes in embryonic lipid levels in response to *acdh-11*(-). I found that *acdh-11*(-) mutant embryos treated with C1-BODIPY-C12 500/510 display higher levels of normalized fluorescence than wild type embryos. Similarly, post-fixed Nile Red staining in *acdh-11*(-) mutant embryos was significantly increased as compared to wild type. These observations are consistent with defects in the cellular β oxidation pathway, and lay the foundations for investigating how ACDH-11 regulates gut granule size.

Investigating The Proximal Promoter In Klf4 Regulation. Johnny Pang ‘26, Dr. Sharon E. Torigoe, *Department of Biology, Lewis & Clark College.*

The expression of a gene is controlled by non-coding regulatory elements in the genome. Some examples include the promoter and enhancers, and they contain binding sites for transcription factors, which subsequently control gene transcription.

OCT4, SOX2, and KLF4 are some of the essential factors to establish and maintain pluripotent stem cells (PSCs), which can self-renew and differentiate into all functional cell types. By investigating how regulatory elements govern transcription of *Klf4*, we can gain a better understanding on how other pluripotency genes may be regulated.

The proximal promoter is a regulatory element found upstream of the transcription start site. For *Klf4*, the proximal promoter contains transcription factor binding sites, which are grouped into seven clusters. Previous work in the Torigoe lab has shown that each cluster has a different impact on transcription. To understand why each cluster has different activity in the proximal promoter, I investigated the role of cluster orientation. My initial results suggest orientation has a minimal role in the activities of the most and least active clusters. Future projects will focus on testing the binding affinity and location on cluster function.

Exploring how C-terminal domain phosphorylation affects α-synuclein aggregation in Parkinson's disease. Brendan J. Creeks '24, Nicole L. Brockway, Tamily A. Weissman. *Department of Biology, BCMB Program, Lewis & Clark College.*

Parkinson's disease (PD) is a neurodegenerative disease for which there is no cure. Lewy bodies, protein aggregations found in neurons, are the hallmark indicator of PD. Lewy bodies are composed primarily of α-synuclein, a 140 amino acid protein. The causes of α-synuclein aggregation are largely unknown. Phosphorylation is associated with aggregation, specifically at serine-129, which is in the inherently disordered C-terminal domain of α-synuclein. Research suggests that alterations to α-synuclein's C-terminal domain could play a critical role in its aggregation. Due to their proximity to each other in the C-terminal domain, I hypothesize that α-synuclein phosphorylation at serine-129 along with tyrosines 125, 133, and 136 together promote aggregation. I will test this by expressing different forms of green fluorescent protein-tagged human α-synuclein in zebrafish embryos via microinjection. I will then perform fluorescence recovery after photobleaching (FRAP) to measure the mobility of α-synuclein *in vivo*, indirectly measuring protein aggregation. This summer, I successfully performed FRAP on zebrafish expressing WT-α-synuclein. In addition, I generated the DNA plasmids that I will be testing for my thesis: one that mimics phosphorylation and one that prevents phosphorylation at the four C-terminal domain sites. I predict that the phosphomimetic α-synuclein will be more aggregated, have slower movement, and thus less fluorescence recovery than the WT-α-synuclein and the phosphorylation-inhibited α-synuclein.

Using a Phosphomimetic Approach to Determine the Effects of Phosphorylation at S129 and Y136 on Alpha-Synuclein Aggregation, Kiki Zawadzki '26, Nicole Brockway, Tamily Weissman-Unni,. *Department of Biology, Lewis & Clark College*

Parkinson's disease is a lethal and progressive neurodegenerative disease, currently diagnosable with certainty only after a patient's death by identifying Lewy Bodies in Substantia Nigra neurons. Lewy Bodies are an accumulation of various protein aggregates, predominantly made up of alpha-synuclein (α-syn). The phosphorylation of α-syn has been considered the key to understanding the mechanism of its aggregation and its relationship with PD pathology. One site of particular interest is serine 129 (S129) in the C-terminal of α-syn. In healthy brains, only 4 percent of α-syn is phosphorylated at S129, while in brains with PD, over 90 percent of α-syn is phosphorylated at S129 (Fujiwara et al., 2002). Though phosphorylation of S129 may be critical for PD pathogenesis, a previous study concluded that the phosphorylation of this site alone does not seem to promote aggregation (Weston et al., 2021). Using a zebrafish model, we aim to study the effects of neighboring phosphorylation sites on α-syn aggregation. The Weissman lab is studying sites tyrosine 125, 133, and 136, while this study only addresses results pertaining to serine site 129 and tyrosine 136. To determine whether phosphorylating different combinations of S129, Y125, Y133, and Y136 changes aggregation behavior, we use FRAP (fluorescence recovery after photobleaching) to measure the mobility of different forms of the protein (α-syn). For this experiment, we have constructed plasmids containing point mutations at S129 and our selected sites that are either all phosphomimetic (aspartic acid - D) or all phospho-inhibitory (alanine - A). Data collection is still ongoing and no conclusions have been made.

Investigating whether disease-associated alpha-synuclein phosphorylation drives aggregation. Brandon Apresa ‘24, Nicole L. Brockway, Tamily A. Weissman-Unni, *Department of Biology, Lewis & Clark College*

Parkinson’s disease (PD) is a common neurodegenerative disease affecting 1% of the population above the age of 60 (Tysnes & Storstein, 2017). Currently there is no known cure, only treatments that can lessen symptoms temporarily. Lewy bodies, which are found in the neurons of patients, are the hallmark indicator of PD. These abnormalities are cytoplasmic aggregates that are predominantly composed of the 140-amino acid protein alpha-synuclein. It is unclear why alpha-synuclein aggregates, but previous research has demonstrated that phosphorylation at specific residues plays an important role in its aggregation. To further test whether phosphorylation drives aggregation, I made genetic manipulations to specific sites (serine-129 and tyrosine-133) in the alpha-synuclein protein and then expressed it in zebrafish along with a green fluorescent protein tag. I performed fluorescence recovery after photobleaching (FRAP) on the axon terminals of neurons within different zebrafish constructs (wild-type synuclein, phosphorylated (phosphomimetic), and non-phosphorylatable) *in vivo* in order to measure the protein’s aggregation. Data collected from FRAP will help us to learn whether phosphorylation at these disease-associated sites drives alpha-synuclein aggregation.

Joint phosphorylation of Y125 and S129 sites may cause alpha-synuclein aggregation. Lily Schainker ‘25, Nicole Brockway, Tamily Weissman, and the *Department of Biology & Psychology, Lewis & Clark College.*

Despite Parkinson’s Disease (PD) being the second most common neurodegenerative disease in the world, there are limited treatments available. Current treatments target symptoms, but are ineffective in preventing the underlying progression of the disease. The presence of Lewy Bodies, an intracellular aggregate protein located within the cytoplasm of neurons, is a key pathological indicator of PD. Abnormal protein aggregation is predominantly made up of a 140-amino acid protein called alpha-synuclein. The relationship between neuron degeneration and Lewy Bodies is unclear, thus the factors that lead to alpha-synuclein protein aggregation and cause the formation of these Lewy Bodies must first be better understood.

Phosphorylation is a significant post-translational modification of alpha-synuclein that may play a role in the protein’s function. We use genetic methods to mimic or inhibit phosphorylation via point mutations at S129 and Y125 sites in human alpha-synuclein, changing this site to alanine (A) as well as phosphomimetic aspartate (D). Our research attempts to quantify the aggregation of different forms of alpha-synuclein by measuring protein mobility *in vivo.* I generated Y125A/S129A and Y125D/S129D alpha-synuclein DNA expression clones and then injected forms of alpha-synuclein DNA into zebrafish embryos post-fertilization. Fast-developing zebrafish are a powerful model organism for this *in vivo* analysis. Zebrafish have no endogenous alpha-synuclein, allowing for exogenous human alpha-synuclein DNA to be the only form of alpha-synuclein present. We then measure this protein’s mobility using photobleaching methods and test whether certain phosphorylation sites, such as S129 and Y125, drive aggregation of alpha-synuclein when compared to wild-type alpha-synuclein.

Developmental Nicotine Exposure and Mutants. Maddie Dopp ‘24, Cassidy Floyd-Driscoll ‘24, Avi Strok ‘25, Renee Pieri ‘25, Karis Huh ‘26, Norma Velazquez Ulloa, *Department of biology and biochemistry and molecular biology, Lewis and Clark College. Neuroscience program, Santa Clara University.*

It is well established that tobacco is harmful for people's health and development. The main addictive component of tobacco is nicotine. We are using flies to study how nicotine and addiction to nicotine affects the behavior and lifespan of fruit flies.

We have been testing mutant strains to identify different responses to nicotine compared to the control strain. We have identified three mutant strains with interesting characteristics. We compared the developmental progression of the *2-10* mutant to the *white* Berlin (*wB)* strain of *Drosophila melanogaster* as the control. We found that compared to the control, the *2-10* mutant produced a significantly higher pupae population but less than a quarter survived into adulthood. We exposed the *2-34* mutant and control fly to developmental nicotine exposure. We compared the number of larvae and pupae and adult flies over time in these two flies in response to nicotine or control food. We found that the *2-34* mutant fly is significantly resistant to nicotine throughout all stages of development. After this developmental characterization we crossed these mutants with another fly to allow us to visualize the tissues by causing the locations where the mutated gene is expressed to glow green. We used a confocal microscope to take images of these tissues. In this poster we show the representative images for each of the previously mentioned mutant strains as well as another mutant called *8-86.* We checked expression in the brain and the gut as well as some other tissues and found consistent gut expression in all strains but brain expression varied. Lastly, we used molecular biology to determine where in the genome these mutations were located. We made progress and mapped the *8-86* fly to the gene *frizzled* and we will continue experiments to map the other two mutants in the fall.

Adult Nicotine Exposure in White Berlin *Drosophila Melanogaster.* Maddie Dopp ‘24, Cassidy Floyd-Driscoll ‘24, Avi Strok ‘25, Karis Huh ‘26, Norma Velazquez Ulloa, *Department of biology and biochemistry and molecular biology, Lewis and Clark College. Neuroscience program, Santa Clara University.*

Tobacco has been well established as being harmful and its main addictive component is nicotine. We are using flies to study how the behavior and lifespan of fruit flies is affected by nicotine exposure of adult flies during several days.

We used different behavioral assays to study the effects of nicotine exposure in adult fruit flies of the *white* Berlin (*wB*) strain of the *Drosophila melanogaster* species. We tested the survival rate of adult fruit flies by separating them by sex and giving different groups various levels of nicotine in their food and compared them against the control flies that received no nicotine. We found that higher levels of nicotine decreased the flies’ lifespan. We also tested the flies' ability to smell after being exposed to nicotine and found that their sense of smell was not affected, unlike the sense of smell of humans who smoke. We used capillary feeder (CaFe) assays to determine if the flies showed increased consumption of nicotine compared to their control counterparts. We have conducted 3 assays, but each one had different results. When combining all assays for analysis, we found very little difference in consumption over time between the control and nicotine-exposed flies. Lastly, we performed a negative geotaxis assay designed to mimic the human symptom of shaky hands to see if the flies also displayed an increased motor function behavior after being exposed to nicotine. Our results show that the female flies had a statistically significant difference in locomotion compared to the control, while male flies did not have a significant difference. Additional experiments with the CaFe assay will determine whether or not there is escalation. Preliminary data suggests that the *2-34* mutant strain responds differently than wB flies to adult nicotine exposure. Future experiments will further test this mutant.

Detangling details of OCT4 and SOX2 binding site affinity. Katya F Schwieterman ‘24, Alexis R Traeger ‘21, Savannah C Myers ‘23, RuthMabel Boytz ‘20, Jack B Waite’23, Sharon E Torigoe. Department of Biology, Lewis & Clark College.

OCT4 and SOX2 are proteins that control characteristics of pluripotent stem cells by attaching themselves to specific DNA sequences. These proteins are also required to transform adult somatic cells into pluripotent stem cells. To advance our understanding of the maintenance and regulation of pluripotency, prior investigations by multiple groups have sought to characterize OCT4 and SOX2 interactions with DNA. This work yielded partial structures of both proteins from x-ray crystallography, specifically the DNA-binding domains in complex with DNA. Additionally, computational analyses of many DNA sequences from many organisms have led to position weight matrices (PWMs). PWMs show the frequencies of DNA nucleotides, the letters of DNA, at each position in the DNA sequence where OCT4 and SOX2 bind. While these previous studies provide valuable insight into DNA binding activity of OCT4 and SOX2, they raise new questions about the influence of each nucleotide on interactions with these cell-programming proteins. To this end, we have performed single nucleotide substitution analysis of the OCT4-SOX2 binding sequence in luciferase reporter assays. Our results are generally consistent with prior work, but some substitution mutations had unexpected outcomes. We analyzed mutations with unexpected results in electrophoretic mobility shift assays to better characterize binding effects of individual nucleotides in the sequence. These assays led us to several sequences that bind OCT4-SOX2 exceptionally well and suggested that several nucleotides may have different effects on binding than predicted. This suggests that there may be more nuance to OCT4 and SOX2 binding interactions than previously understood.

Regeneration Dynamics in a Pacific Northwest Old Growth Forest

Melissa Even ‘24, Madison Johnson ‘25, Kai Larson ‘25, Margaret Metz *Department of Biology, Lewis & Clark College*.

The early seed and seedling life stages are vulnerable periods in a tree’s life with high mortality rates. Seedlings that germinate in the spring must survive the summer drought and later years in an understory with many larger plants competing for sunlight, herbivores, pathogens, and other hazards. A seedling’s environment might fluctuate year to year with changing temperature and precipitation and because of changes to the surrounding plant community. The long-lived trees of Pacific Northwest conifer forests produce potentially millions of seeds in their lifetime, but only a handful of these will germinate in a location with the right conditions to survive and grow to reach the forest canopy. In this project we examined the physical and vegetative conditions that best support seedling germination and growth in an old-growth forest in southwestern Washington.

Within the Wind River Forest Dynamics Plot in Gifford Pinchot National Forest we censused 160 long-term monitoring sites consisting of a seed trap and an adjacent 1-m^2 vegetation plot. We marked, mapped, and identified every seedling <50 cm in height. Those older than one year were tagged, measured, and mapped to be tracked next year. We also censused the herbaceous vegetation in each plot. We returned from 3 weeks of field work to digitize our seedling data to create maps for additional censuses. This work is part of a long-term study, and the data can be used to examine how germination rates, survival rates, and growth rates for individual tree species differ across parts of the forest that differ in moisture and light availability and in the plant competitors in the neighborhood. Our work this summer was abbreviated due to uncertain NSF funding, and we were unable to undertake any statistical analysis of our data during that time. A goal of our research is to provide broadly accessible datasets to the scientific community. Studies using this data have identified how forests react to climate stressors, the carbon storage potential of old growth, and that despite their age, old growth forests still exist in a very dynamic

Evaluating the use of *Sargassum* oil for cosmetic applications. Elizabeth Young ’25, Marielys Torres Díaz, Liz M. Díaz-Vázquez, Lewis & Clark College, University of Puerto Rico—Río Piedras Campus.

Progressive increases in the ocean temperature, disruption in sea currents, and pollution are some of the causes of the massive influx of *Sargassum*, a holopalegic species of brown macroalgae, in the Caribbean region. Many efforts are being developed to reduce the ecological and economic impact of these macroalgae accumulated on the beaches. Macroalgae represent a versatile biomass source with many potential applications. The high content of bioactive molecules in marine algae makes them attractive for the sustainable development of cosmeceutical ingredients. In this project, we evaluate different organic solvents for extracting sargasso oil with ASE and identify its component compounds with GC-MS. The project’s main objectives include sargasso oil’s high-yield recovery, characterization of the oil, and application in a cosmetic formulation. We find that sargasso oil could serve as a cosmetic ingredient due to its antioxidant activity, photostability, and fatty acid profile content, including many skin-loving molecules.

Noble Metal Nanoparticle Shape Control. Zane Mullally ‘25, Abigail Ramírez ‘25, Kenzie Stewart ‘25, Anne K. Bentley, Department of Chemistry, Lewis and Clark College.

Gold nanocrystals can be used as catalysts for reactions such as the CO_2 reduction reaction and in fields such as medicine and renewable energy. The effectiveness of these nanoparticles can vary depending on the shape produced, particularly the crystalline surface (or facet) exposed to the surrounding environment by that respective shape. Due to differences in the density of atoms on their surfaces, some facets display better catalytic behavior than others; thus, control over the shape formed can improve reaction outcomes. Our lab used water-based colloidal synthesis methods to create nanoscale Au octahedra, cubes, and rhombic dodecahedra and characterize them with UV-vis spectroscopy and scanning electron microscopy. Initial work began to use these shapes as templates for the constant potential electrochemical growth of bimetallic noble metal nanoparticles.

Making Moves: Investigating the Region of Binding Between Dynein IC-2C and Dynactin p150^{Glued}. AJ Di Nicola ‘24, Henri Danzelaud, Paul Cleary ’24, Nikolaus M. Loening, *Biochemistry and Molecular Biology Program, Lewis & Clark College*.

Dynein is a multi-subunit motor protein integral to the transport of molecular cargo throughout the cell. In mammals, dynein only exhibits movement when interacting with the protein dynactin. This interaction is regulated through the binding of the intrinsically disordered intermediate chain (IC) subunit of dynein with the p150^{Glued} subunit of dynactin. Specifically, the single alpha helix (SAH) region of dynein IC (residues 1-38) interacts with the coiled-coil 1B (CC1B) region of dynactin p150^{Glued} (residues 358-555). Previous research has only narrowed down the CC1B residues that directly interact with IC to residues 382-531. To further narrow down which CC1B residues play a significant role in dynein-dynactin binding, we used nuclear magnetic resonance (NMR) spectroscopy to measure the intermolecular paramagnetic relaxation enhancements (PREs) of ^{15}N-labeled IC-2C$_{1-96}$ when interacting with paramagnetically-labeled p150^{Glued} CC1B$_{382-531}$ single-cysteine mutants. PRE NMR results suggest that the binding site of the SAH region of IC-2C$_{1-96}$ on p150^{Glued} CC1B$_{382-531}$ includes residues 432 to 448. PRE NMR results also suggest that the helix 2 (H2) region of IC-2C$_{1-96}$ (residues 52-66) appears to transiently interact near residue 511 on p150^{Glued} CC1B$_{382-531}$. Additional research is needed to explore whether the transient interaction observed between IC-2C H2 and p150^{Glued} CC1B$_{382-531}$ contributes to binding and whether the single-cysteine mutations and MTSL label affect binding in this system.

Designing Stabilizing Mutations of p150^{Glued} CC1B and Optimizing Salt Concentrations to Aid the Characterization of IC/p150^{Glued} Binding. Paul Cleary '24, Nikolaus Loening, *Biochemistry and Molecular Biology Program, Lewis & Clark College*

In mammals, the motor protein dynein binds to another protein, dynactin, to move cargo inside of cells. Both dynein and dynactin contain multiple subunits, two of which, dynein intermediate chain (IC) and dynactin p150^{Glued}, have been shown to interact directly with one another. The exact specifics of the binding between IC and p150^{Glued} are unknown, so this research project was focused on better elucidating which amino acids in p150^{Glued} are involved in binding IC. In addition, we hoped to make smaller constructs of p150^{Glued} that are more amenable to structural characterization methods. These smaller constructs suffer from reduced structural stability, so we focused on introducing specific mutations to stabilize them.

The binding of all proteins is heavily influenced by the buffer solution that they are in. Consequently, we looked at the effects of salt concentrations on the IC-p150^{Glued} interaction to determine the optimal conditions for studying these proteins.

Studying the Structure and Binding Characteristics of the N-Terminal Domain of the Dynein Intermediate Chain 1 Isoform A. Logan Hausler '25, Nikolaus Loening, *Department of Biochemistry, Lewis and Clark College*

In mammalian cells, alternative splicing of genes IC-1 and IC-2 produces six distinct dynein intermediate chain (IC) isoforms. Of these isoforms, IC-1A is specifically expressed in neural tissues such as the brain and spinal cord, whereas IC-2C is ubiquitously expressed in a wide range of tissues. While there are several differences in the sequences of IC-1A and IC-2C, the primary difference, which presumably leads to the distinct behavior of these isoforms, lies in the presence of 15 additional hydrophobic amino acids in the linker 2 (L2) region of IC-1A. To study IC-1A, we plan to use nuclear magnetic resonance (NMR) spectroscopy to help elucidate the reason for the distinctive behavior between IC-1A and IC-2C. To achieve this, we utilized recombinant protein expression and a series of chromatography steps to obtain purified IC-1A samples, which were then studied by NMR. A major obstacle encountered was the lack of NMR peaks for the 15 additional amino acids in the L2 region of IC-1A. We hypothesized that the lack of peaks was a result of aggregation due to the hydrophobicity of this region of the protein. Initial mitigation strategies, such as the incremental addition of CHAPS detergent or a 50 mM mixture of glutamine and arginine to the sample buffer, proved ineffective. The inclusion of 500 mM guanidinium chloride resulted in NMR spectra in which the peaks for these hydrophobic residues could be observed, but is not an ideal additive as it compromises the native protein structure. To overcome the challenge that these hydrophobic residues introduce for NMR spectroscopy, we engineered a truncated IC-1A construct that only has the L2 region (amino acids 67-112), as opposed to the original 38-112 sequence. This truncated construct has been successfully designed and synthesized, and NMR spectrum acquisition will be underway soon.

Degradation of Glyphosate Under Variable Conditions. Owen Beale ‘24, Dr. Louis Y. Kuo, *Department of Chemistry, Lewis & Clark College.*

Phosphonates used as herbicides are prevalent in the environment, both in soil and water runoff. Phosphorus recovery is extremely important and a mechanistic understanding of how organophosphates degrade is needed in order to predict and control the process, allowing for recycling of phosphorus. The degradation of the phosphonate glyphosate was studied using molybdenum compounds that break it down under mild conditions. This included two molybdenum peroxide compounds, one ligated to picolinic acid and the other to the amino acid glycine. These catalysts break down glyphosate differently from one another and form different intermediates depending on reaction conditions. The results of these reactions were studied using nuclear magnetic resonance spectroscopy (NMR), of both hydrogen and phosphorus nuclei. Degradation of glyphosate with the molybdenum picolinato compound showed some similarities to the abiotic degradation of glyphosate, while results with the molybdenum glycinato compound showed some similarities to biotic degradation. For both catalysts changing reaction conditions such as the ratio of catalyst to glyphosate, the presence of hydrogen peroxide, and temperature influenced the degradation to progress differently.

Catalyst Comparisons: Optimizing Reaction Conditions for Glyphosate Degradation. Katie Caudill ‘25, Dr. Louis Y. Kuo, *Department of Chemistry, Lewis and Clark College.*

This research studied how different catalysts and catalytic conditions affect the degradation rate of glyphosate, the active ingredient in Roundup herbicide. The catalysts investigated were all picolinato peroxo coordination complexes, using the metal ions molybdenum(VI) (Pic-Mo) and tungsten(VI) (Pic-W). By employing ^{1}H and ^{31}P nuclear magnetic resonance (NMR) spectroscopy, the precise rate of reaction for different conditions could be measured. The results showed that with an equimolar amount of Pic-Mo and Pic-W in different vials of equimolar acetic acid buffer, hydrogen peroxide, and glyphosate, the Pic-Mo was the superior catalyst. This suggests that the primary degradation pathway that glyphosate undergoes could involve a free radical electron, as molybdenum(V) is more stable than tungsten(V).

Glyphosate Degradation for Polyphosphate Accumulating Organism Supported Phosphate Reclamation. Mia Bell ‘24, Dr. Louis Y. Kuo, *Department of Chemistry, Lewis & Clark College*.

Glyphosate is associated with numerous environmental and health risks, necessitating its degradation into non-toxic chemical components. Abiotic degradation of the compound, induced by a polystyrene-supported molybdenum(VI) catalyst, can yield valuable free phosphate. This phosphate is a limited resource that can be converted into a high value fertilizer. Thus, the importance of glyphosate degradation is reinforced by extending glyphosate dephosphorylation towards phosphate recovery. In theory, the recovered phosphate could undergo bioaccumulation with polyphosphate accumulating organisms (PAOs) to increase the concentration of the compound and produce viable metal phosphate salt (fertilizer) upon chemical precipitation. This research focused on adapting an enhanced biological phosphorus removal (EBPR) system to the benchtop. After finding ways to replicate/adapt equipment from local wastewater treatment plants, PAOs were subjected to anaerobic and aerobic conditions to induce the uptake and release of free phosphate. Trials were performed under mildly acidic conditions at room temperature. Results were tracked using a malachite green phosphate assay (Sigma Aldrich MAK308) and monitored via UV-vis spectroscopy. Preliminary data suggest that the benchtop adaptation of the commercial EBPR system is effective.

Glyphosate Synthesis, Recrystallization, and Degradation. Isabel Willis ‘23, Dr. Louis Kuo
Department of Chemistry, Lewis and Clark College.

This research examined the mechanism for the degradation of the herbicide glyphosate. C3-deuterated glyphosate was synthesized using deuterated paraformaldehyde. The resulting glyphosate with deuteriums on carbon 3 was recrystallized and a crystal structure was obtained through x-ray crystallography. Visualization of olefinic signals on proton nuclear magnetic resonance (NMR) allowed the tracking of an isomerization within the mechanism. The initial rates of the appearance of these signals were plotted against temperature to obtain a value for the enthalpy. The rate of degradation for the deuterated glyphosate was compared to that of protonated glyphosate on phosphorus NMR. This showed secondary deuterium isotope effects and confirmed that there is no carbon-hydrogen bond breakage at carbon three.

Rehearsing Disaster: Understanding Earthquake Preparedness in an Interactive Environment. Kat Berge ‘25, Nora Cesareo-Dense ‘25, Jake Darnell ‘24, Evan Eldridge ‘24, Sam Flores ‘23, Ismael Jaramillo ‘24, Elizabeth Safran, *Department of Environmental Studies,* Erik Nilsen, *Department of Psychology,* Peter Drake, *Department of Mathematical Sciences,* Bryan Sebok, *Department of Rhetoric and Media Studies, Lewis & Clark College.*

Located inland of the Cascadia Subduction Zone, the Pacific Northwest is poised to experience an earthquake of 8-9 magnitude. Given the state of regional infrastructure and disaster education, this event is expected to result in numerous casualties and economic turmoil. Nonetheless, many Pacific Northwest residents, particularly those aged 18-29, are unprepared and uninformed. Our research uses video games to explore young adults’ motivation to prepare for disaster. The current study focuses on the effect that in-game identification with location and residence type has on individual preparedness. The game is set in Portland and features iconic landmarks. Half the participants will be from the Portland Metro Area and half from the Seattle/Tacoma area. We hypothesize that individuals residing in Portland will be more impacted by the game than those in the Seattle area. Participants will be randomly assigned to one of two in-game scenarios: house or apartment, with different, condition-based tasks. We hypothesize that participants benefit most by playing the version that mirrors their actual living situation.

Upon securing clean water and the means to manage bodily waste, players can finish the game or earn additional points by aiding non-player characters through item exchanges. Participants will complete a questionnaire pre- and post-game with follow-up questions three months later. The questionnaire measures learning, self-efficacy, outcome expectations, and intent to act relative to a series of preparedness and coping actions. Finally, participants will be asked about level of anxiety, sense of responsibility, and risk perception around the Cascadia earthquake.

Trust in Data: How Consistent are Low-Cost Air Quality Sensors? Karl Peterson ‘23, Isaac Heintze ‘25, Dr. Jessica Kleiss, *Department of Environmental Studies, Lewis & Clark College.*

This study investigates potential use cases (eg. classroom use, citizen science) of low cost personal sensors to investigate air quality. Researchers also considered environmental justice oriented applications with community partners, Parkrose-Argay Opportunity Coalition and Argos Scientific. 24 Atmotube sensors were tested for precision in different scenarios across different metrics such as Particulate Matter, Total Volatile Organic Compounds, and GPS coordinates.

The Atmotube sensor communicates via bluetooth, and it was discovered that upon connecting to the sensor, data quality diminished; leaving brief periods with no data. (Fig.1). Researchers additionally found that sensors read rather inconsistently over a period of time, showing up in an oscillation when plotted (Fig. 2); this inconsistency was later fixed by averaging data over a 15 minute period.

Due to variable data quality the applications are limited to educational and community engagement use and are optimized through techniques such as time averaging. The sensors, and the framework and analysis surrounding them, can be applied in the classroom in order to give students hands-on experience of air quality field observations, data analysis, and spatial mapping.

Password Cracking as a Medium for Introducing Cybersecurity Skills and Student Autonomy. Emily Tanabe '24, Cole McCorkendale '25, Matt Chio '25, Jens Mache, Aurelio Puente, Richard Weiss, *Department of Mathematical Sciences, Lewis & Clark College, Evergreen State College.*

We've designed an introductory password-cracking exercise that gives students the opportunity to develop foundational cybersecurity skills while increasing their confidence and agency. This exercise aims to educate students about the brittle nature of passwords while increasing students' cybersecurity soft skills, such as collaboration, autonomy, and problem-solving. To do so, the exercise uses popular educational methods such as the Gradual Release of Responsibility model and tools like guiding questions.The exercise is holistic, hands-on, and consists of three scaffolded levels:

- Password guessing, intelligence-gathering, and spear phishing.
- Manually attempting a "credential stuffing" attack on a simple password.
- Scripting an automated password-cracking tool.

This exercise will educate students about passwords, how to attack them, and how to choose secure passwords while building foundational cybersecurity skills and keeping less experienced students interested, engaged, and motivated.

Dependable Computing. Caitlyn Wilde '25, Wyeth Greenlaw Rollins '24, Andrew Fowler '24, Alain Kägi, Jens Mache, Jorge Martinez, *Department of Mathematical Sciences, Lewis and Clark College.*

Cybersecurity incidents are daily occurrences. One approach to this problem is to detect and to respond to such occurrences. Another approach is to build systems that are impervious to such attacks. As a proof of concept, we are building a networked temperature sensor that we can mathematically prove to be resistant to certain classes of attacks. The applications for such sensors include incubators in biological laboratories.

We are developing this sensor on top of a single-board Odroid C2 computer. Its software is written in the C programming language and the plan is to prove the correctness of this program's implementation against a formal specification with the help of the proof assistant Isabelle/HOL. At this time, we have completed most components of the networking stack and we have started the proof of the fragment reassembly process.

Moral Judgements of Racial Passing Shaped by Ideology. Mary Prentice ‘23, Dr. Diana Leonard, *Department of Psychology, Lewis & Clark College.*

The purpose of this study was to investigate how moral judgements of *racial passing* are shaped by endorsement of *ideology*. This study aimed to replicate previous research and expand upon it by adding in an ideology manipulation to see if a colorblind, multicultural, or a control would affect judgments of racial passers. We found that ideology scores were correlated with behavior judgments and character judgments in response to racial passing, as well as social distancing from the target. Additionally, the multicultural manipulation affected acted as a mediator for harsh judgements of racial passing. Further research to investigate these findings is necessary as well as a redesign of our study.

Physicochemical Characterization of Magnetic Erythrocyte-Based Micromotors *Makena Andersen[1,3], Qi Wang[2,3], Jamel Ali[2,3] 1.Lewis & Clark College, 2.FAMU College of Engineering, 3.Florida State University*

Magnetic micromotors are small scale devices which convert magnetic forces and torques into rotational and translational motion. Micromotors of various designs and compositions have been investigated for their capacity to be used in biomedical applications, such as active targeted drug delivery, nano surgery, biopsy, and localized diagnosis. As mature red blood cells (RBCs) have lost their nuclei and most of their organelles, they become naturally versatile cargo-carriers. Combined with their immunosuppressive properties, preventing them from being removed by immune cells during their circulation in the bloodstream, makes them ideal drug carriers. By implanting magnetic nanoparticles into the RBCs, their movements can be controlled using a single magnetic field through various mediums. The physical properties of the RBC motors (critical frequency and magnetic moment), along with the osmolarities of the preparation suspension, were determined to create guidelines for magnetically guiding the micromotors with accuracy.

The Dynamics of Microswimmers: optical trapping as a tool to explore the physics of swimming *C. reinhardtii*, Lily Johnson ’24, Hugh Pettitt-Kenney ’25, Albert J. Bae, *Department of Physics, Lewis & Clark College.*

Low Reynolds number hydrodynamics occur in the regime where the drag force of a liquid dominates over the inertia acting on objects in the liquid. In this project, we sought to understand the role of low Reynolds number mechanics in the flagella-driven propulsion of *Chlamydomonas reinhardtii,* which we refer to as microswimmers. We used optical trapping, a method by which we apply highly focused laser light to hold a specimen in place and allow us to take force measurements based on the light’s deflection. We implemented this with a series of lenses and mirrors, a quadrant detector, piezo-electric stage controller, Blackfly high-speed video camera, and two microscope objectives. Using high-speed microscopy in combination with computer simulations, we analyzed the flagellar movements and their relation to the force measurements to identify how the trajectory of the flagella allows the cell to move within this low Reynolds paradigm. This initial data and effort in creating a functional apparatus will be used to further investigate the interactions between microswimmers and their environment.

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