

# 2014 Women in Science

Clark Science Center's  
Summer Research Fellows Program





# INTRODUCTION

**“We cultivate the scientist in the next generation of women leaders so they can meet the challenges of our world.”**

In summer 2014 while the students represented in *Women in Science 2014* did their research, a group of Clark Science Center faculty and staff researched, debated, and designed a strategic plan for the sciences at Smith College. After public meetings with students, faculty, and staff, the Summer Strategic Planning Committee’s *Vision for the Future, 2015* was finalized in November 2014. Our strategic plan builds on Smith College’s strength as a national leader in science research and education among liberal arts colleges and responds to the increasing numbers of Smith students studying sciences. Forty percent of our current students have declared a science major, a rate at least double the national average for women. In disciplines in which women are most under-represented (e.g., computer science), our students major at rates up to three times the national average. Strategic planning is also a timely response to societal matters – lower female participation in STEM higher education and later in work, as well as academic, economic, and political leaders’ advocacy of full representation of women in all STEM fields as a matter of equity and good policy based on the benefits that flow from diversity.

*Our Vision for the Future*

**The sciences at Smith will provide transformative opportunities for all students to engage with real problems while empowering them to generate innovative solutions that benefit our world. Our graduates will change the face of science while purposefully building fulfilling lives.**

**Ensuring access for all.** As its first principle of excellence, the Association of American Colleges and Universities’ Liberal Education and America’s Promise (LEAP; 2011) initiative tells us to “aim high—and make excellence inclusive” (p6). For the sciences at Smith, we are guided by understanding that persistence and the best scientific thinking emerge from healthy climates that promote and value a diversity of perspectives. As our strategic direction, we work to address disparities in gender, racial, and socioeconomic representation in the sciences by pairing rigorous learning expectations with robust support and community-building for our students.

**Engaging with the world.** Another essential principle of excellence in undergraduate education is to provide opportunities for students to engage with big questions and tackle real-world problems that connect their

## Strategic Directions

|   |                                 |
|---|---------------------------------|
|  | Ensuring access for all         |
|  | Engaging with the world         |
|  | Developing knowledge and skills |
|  | Fortifying agency and identity  |

knowledge to solutions and action (AAC&U, 2011). At Smith, we are guided by the belief that interactions with bona fide scientific problems connecting our students to the larger world facilitate the best learning. As our strategic direction, the sciences at Smith will strive to engage our students with complex, real-world problems, ranging from local to global, that are often best understood through the multiple disciplinary lenses of the liberal arts.

**Developing knowledge and skills.** Research is a core practice of scientific education at Smith College. We are guided by a shared understanding that best-practices pedagogies and faculty-student research collaborations will result in optimal learning and future success for our students. As we move forward, we build on evidence that through rigorous coursework and undergraduate research opportunities that connect the work of students with cutting-edge faculty scholarship, we develop student mastery of the key concepts and competencies of our disciplines.

**Fortifying agency and identify.** Persistence and success in STEM rest not only on access, opportunity, and knowledge, but also on the actions taken by individual women in particular environments using specific social understandings.

Smith faculty adopt a guiding principle that students' mind sets, metacognition, and identity development are essential to learning as well as professional and personal fulfillment. We understand that through our cultivation of students' agency, confidence, and resourcefulness in learning, we will foster their sense of identity as scientists.

**Undergraduate research is a high-impact educational practice in which the sciences at Smith have considerable expertise.** Research experiences and collaborative projects that occur in research labs, the Science Center's five multidisciplinary research centers, and our field research sites demand the applied and integrative learning that deepens student engagement and learning (AAC&U, 2011). The sciences at Smith have a strong history of providing meaningful research opportunities to students, with a thriving honors program, active faculty research labs in which students participate as collaborators, and almost 50 years of a vibrant Summer Undergraduate Research Fellowship (SURF) Program. Our students present their research in many venues, including at the annual campus-wide *Celebrating Collaborations* exhibition and *Smith in the World Conference*, in public honors thesis presentations, and at regional and national professional meetings in their disciplines. With at least one undergraduate student co-author on a third of science faculty members' peer-reviewed scholarship and SURF participation doubling the likelihood of our students pursuing a graduate degree (data provided, Smith College Institutional Research, 2014), we believe that challenging our students to work at the cutting edge of knowledge helps prepare them for their lives beyond Smith.

***Women in Science 2014* summarizes research done by Smith College's Summer Research Fellowship (SURF) Program participants.** Ever since its 1967 start, SURF has been a cornerstone of Smith's science education. In 2014, 150 students participated in SURF (141 hosted on campus and nearby field sites), supervised by 61 faculty mentor-advisors drawn from the Clark Science Center and connected to its eighteen science, mathematics, and engineering departments and programs and associated centers and units. At summer's end, SURF participants were asked to summarize their research experiences for this publication.

**We have many reasons to be proud of our 2014 SURF researchers.**

- SURF researchers worked on some of the biggest research challenges of our times, including eradicating human disease, reexamining human life and the earth around us at the nano-scale, documenting climate change and its impact on the living world, testing and improving sustainable energy technologies, and developing materials and testing methods not just for earth but also for use in space.
- SURF research took place not just on the Smith campus, but in a wide variety of research settings in the wider world: including, locally (study of local forests, wild life, and water courses at the Ada and Archibald MacLeish Field Station in West Whately, MA and development of new systematics beds in the Botanic Garden), nationally (projects on the Atlantic, Pacific, and Great Lakes shores with NOAA scientists), and internationally (examination of coral reefs in Belize and energy research in Spain).
- Technical know-how, quantitative literacy, and presentation skills grew as students used state-of-the-art instrumentation, analyzed data with specialized software, and presented their results in lab meetings, posters, and conference presentations.
- SURF students learned how to work with mentors and peers and, for some, across the boundaries of academic disciplines with other research teams. More experienced undergraduate researchers learned how to mentor others and take on research leadership roles.

## We are excited about what SURF participants say they learned from SURF<sup>1</sup>

- SURF research deepens students' understanding of research. They discover that scientific research requires active problem-solving, patience, and persistence.
- *"I...feel this summer has vastly helped my ability to approach a scientific problem myself, and be more assertive in my decisions and analyses without total reliance on my research advisor."*  
*"I have found that knowing the instrumentation - how to troubleshoot in case of an issue, learning how to use it correctly and most efficiently - is just as important as reading the scientific literature on my research topic, understanding why it is important to do that research, what we can learn from it and how to proceed to get the results."*
- Each student commits substantial time to SURF research: typically, 30-40 hours per week for 8-10 weeks of the summer.  
*"Summer research is definitely more intensive than academic year research but because of this intensity, I learnt more and faster through the research than I did during the academic year."*
- Many students make a distinct contribution to a collaborative research project with a faculty member and often a joint publication is prepared.  
*"I learned how to write and prepare a manuscript for publication."*
- In many cases, SURF research contributes to the particular student's honors thesis or continues as a special studies project.  
*"[T]his experience confirmed my aspiration to pursue an honors thesis."*
- Students discover the social aspects and interpersonal skills involved in research teamwork.  
*"I also learned how to work in a team, which was especially necessary if we wanted to get anything done. It was amazing to learn from each other and boost our strengths while helping [with] each others' weaknesses."*  
*"I now know so many people that I feel I can comfortably go to and ask questions about plants or jobs or just how to be a scientist!"*  
*"I learned ... self-discipline and autonomy"*
- SURF is where students find direction for graduate school and future careers.  
*"I came to Smith without any specific plans of pursuing a career in chemistry, and today I have no doubts that I would like to attend a graduate school in this field."*  
*"After this research, I am certain that physics will be where my future career lies"*

<sup>1</sup>The comments below are drawn from two surveys SURF participants completed in August 2014. First was a Smith survey (unpublished) in which students were asked what they had learned and how they expected to build on their SURF experience. Second was the SURF III national survey administered by Grinnell College (Smith student comments extracted from an anonymous summary provided by the survey administrators). See the following reports of the SURF survey data across a large number of colleges and universities: Lopatto, D. (2004). Survey of Undergraduate Research Experiences (SURF): First Findings. *Cell Biology Education*, 3, 270-277 and Lopatto, D. (2007). Undergraduate research experiences support science career decisions and active learning. *CBE - Life Sciences Education*, 6, 297-306.

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#### **Smith College offices and units:**

Center for the Environment, Ecological Design &  
Sustainability (CEEDS)  
Online Learning Task Force

Committee on Faculty Compensation and  
Development (CFCD)  
Provost's Office

Some undergraduates were able to participate in summer research internships away from campus with support from the PRAXIS Internship Program.

We wish to recognize and express gratitude to the faculty members and staff who provided supervision, guidance, encouragement and support to SURF participants in the lab, doing field research, on-campus, and away from campus. SURF would not be possible without your devoted and generous contributions.

**Thank you, Smith College students, faculty, staff, friends, and benefactors.** It truly takes a diverse and dedicated community to sustain a program like SURF.

Margaret Lamb, Ph.D,  
Administrative Director, Clark Science Center





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# 1D and 2D $^1\text{H}$ NMR Spectroscopy of the Sp Lesion in DNA Duplexes

Sophia Carroll/2015 and Lindsay Roth/2015

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Exposure to transition metals can damage DNA by oxidizing its nucleobases. Guanine, which has the lowest reduction potential of the DNA bases, is particularly vulnerable to the formation of lesions. The Spiroiminodihydantoin (Sp) lesion, a hyper-oxidized form of guanine, is especially mutagenic, and it can lead to cancer when left unrepaired.<sup>1</sup> Our research investigated the specific structural effects of the Sp lesion on an 11-mer DNA duplex using  $^1\text{H}$  Nuclear Magnetic Resonance (NMR) spectroscopy experiments. Two-dimensional Nuclear Overhauser Effect Spectroscopy (2D-NOESY) experiments show the through-space interactions between protons that are five angstroms or fewer apart as cross-peaks. These cross-peaks form predictable patterns in undamaged DNA duplexes; deviations from this pattern indicate structural damage (e.g. puckering, twisting).<sup>2</sup> Last summer, the cross-peaks in the 2D-NOESY spectrum of the control (non-oxidized) DNA duplex were unequivocally assigned to specific protons and confirmed by the literature.<sup>1</sup> Using an iridium complex as the oxidizing agent, the oligomer containing the Sp lesion was prepared, and the two diastereomers, Sp-R and Sp-S, formed were subsequently separated using High-Performance Liquid Chromatography (HPLC). Each oligomer was then annealed to a complementary bottom strand to form the duplexes. A 2D-NOESY spectrum of the oligomer containing one of the diastereomers was confidently assigned. Based on the literature, this spectrum has been tentatively assigned to the oligomer with the Sp-R confirmation.<sup>1</sup>

This summer, work assigning the 2D-NOESY spectrum of the other diastereomer continued. Additionally, work on base-pair-opening experiments began. These experiments use integrations of one-dimensional (1D)  $^1\text{H}$  NMR spectra to measure the solvent-exchange rates of the imino protons in the duplexes. These rates are lower in stable duplexes, because the conservation of Watson-Crick base-pairing minimizes the imino protons' exposure to the solvent.<sup>3</sup> This summer, the pulse programs for these experiments were designed, and preliminary spectra of the control duplex were acquired (Figure 1). Future work will continue these experiments on the Sp-containing duplexes and place the yet-unmeasured rates in context with those reported for analogous lesions in the literature.<sup>3,4</sup> With the valuable structural insights we will gain from these 1D and 2D  $^1\text{H}$  NMR experiments, we hope to sharpen the picture of the Sp lesion, as well as to suggest how its deleterious effects might be better prevented or repaired.

*(Supported by the Howard Hughes Medical Institute, Carroll and the Schultz Foundation, Roth)*

Advisors: Elizabeth Jamieson, Biochemistry and Chemistry, Cristina Suarez, Chemistry

<sup>1</sup>Khutsishvili, I.; Zhang, N.; Marky, L.A.; Crean, C.; Patel, D.J.; Geacintov, N.E.; Shafirovich, V.; "Thermodynamic Profiles and Nuclear Magnetic Resonance Studies of Oligonucleotide Duplexes Containing Single Diastereomeric Spiroiminodihydantoin Lesions." *Biochemistry*, 2013, 52, 1354-1363.

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<sup>4</sup>Wenke, B. B.; Huiting, L. N.; Frankel, E. B.; Lane, B. F.; Núñez, M. E. "Base Pair Opening in a Deoxynucleotide Duplex Containing a cis-syn Thymine Cyclobutane Dimer Lesion." *Biochemistry*, 2013, 52, 9275-9285.

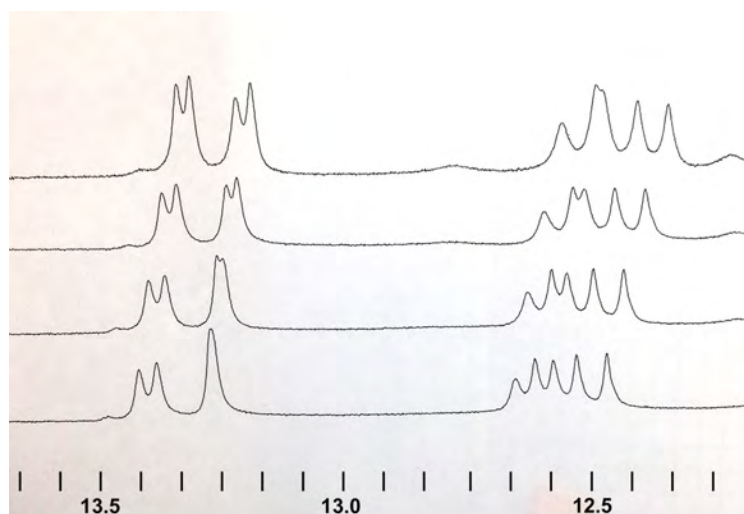


Figure 1. Imino  $^1\text{H}$  NMR spectra from base-pair-opening experiments (stacked) on the control duplex.

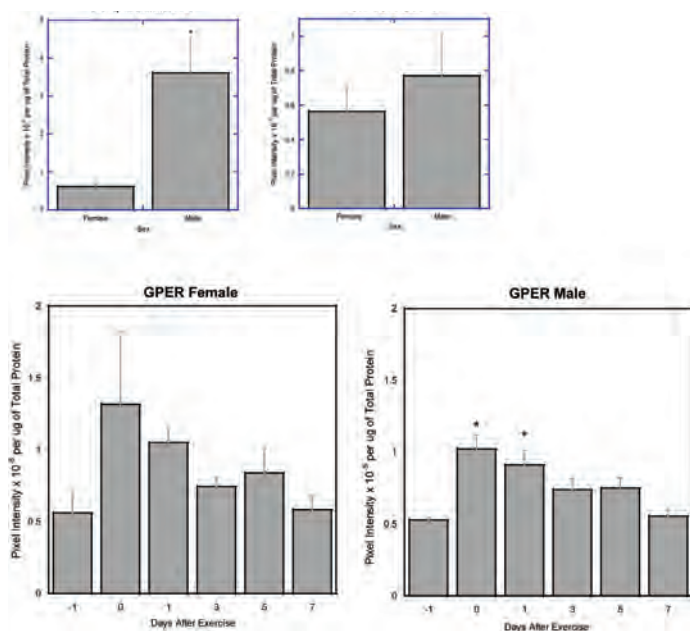


# Estrogen Receptor Analysis After a Single Bout of Exercise in Murine Biceps Brachii

Justine Gelzinis/2014

Everyday our muscles are exercised and when this happens the normal process of muscle damage and repair begins. Recently the estrogen receptors (ERs), ER $\alpha$ , ER $\beta$ , and G-protein coupled estrogen receptor (GPER) were detected in skeletal muscle<sup>1,2</sup>. Estrogen may have an effect on skeletal muscle repair after exercise<sup>3</sup>. My research focuses on ER $\alpha$  and GPER in the biceps brachii of the common house mouse, *Mus musculus* to determine if gender dimorphism exists in expression and if their expression is affected by eccentrically-biased exercise (downhill running).

Murine biceps were removed under protocols approved by the Smith College IACUC. A high ionic strength extraction buffer was used to solubilize the muscle samples. Total protein estimation was accomplished by the Lowry assay. Separation by molecular weight on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was followed by quantitative immunoblot analysis on PVDF membranes using a GPER antibody (Santa Cruz Biotech, sc-48524-R) and an ER $\alpha$  antibody (Cell Signaling, #8644). Skeletal muscle samples were taken at several different time points after exercise. An unexercised adult female muscle extract was used as a standard. Unfortunately, despite many attempts a reliable antibody for ER $\beta$  was not available.



In the unexercised control mice no difference was found in the baseline levels of GPER, however males showed a significantly higher amount of ER $\alpha$  (upper panels of the figure). In a linear model with interaction between time and sex from immediately after a 15 min downhill run (-15°) on a treadmill (Day 0) through Day 7 (lower panels of the figure) there is no gender dimorphism; however, the decrease in levels as a function of time is highly significant for both sexes ( $p = 0.0052$ ), both returning to the unexercised control levels by Day 7. Despite the significant difference in the control levels of ER $\alpha$ , no differences were uncovered for either sex or as a function of time following the downhill exercise in the same linear model for GPER.

We thank Professor Ben Baumer for the statistical analyses.

*(Supported by the Blakeslee Fund in the Biological Sciences)*

Advisor: Stylianos Scordilis, Biochemistry and Biological Sciences

<sup>1</sup>Bjornstrom, L. and M. Sjoberg. 2005. Mechanisms of Estrogen Receptor Signaling: Convergence of Genomic and Nongenomic Actions on Target Genes. *Molecular Endocrinology*, 19(4): 833-842.

<sup>2</sup>Maggiolini, M., and D. Picard. 2010. The Unfolding Stories of GPR30, A New Membrane-bound Estrogen Receptor. *Journal of Endocrinology*, 204: 105-114.

<sup>3</sup>Tiidus, P. M., Holden, D., Bombardier, E., Zajchowski, S., Enns, D., and A. Belcastro. 2001. Estrogen Effect on Post-Exercise Skeletal Muscle Neutrophil Infiltration and Calpain Activity. *Can. J. Physiol. Pharmacol.*, 79: 400-406.

# Myogenic Carbohydrate Transcriptome in Murine C2C12 Cells

Anagha Inguva/2014

Murine C2C12 cells, derived from adult dystrophic mouse thigh muscle simulate in vivo myogenesis (Yaffe and Saxel, 1977). They undergo three distinct stages of C2C12 myogenesis: proliferative myoblasts (day 0); cell cycle withdrawal and fusion to become early myotubes (day 4); and, finally spontaneously contracting late myotubes (day 9). The present study analyzed the changes in the transcriptome of the enzymes involved in carbohydrate metabolism during the three different stages of myogenic development of C2C12 cells.

A carbohydrate metabolism qRT-PCR array was used to examine the mRNA levels of 84 different enzymes involved in carbohydrate metabolism (n=4 per stage). A total of 64 genes showed changes in mRNA levels with time that were statistically significant (ANOVA,  $p < 0.05$ ) from the control group (day 0). Of these 64 genes, 29 genes increased or decreased by two (2.0) fold or higher and were considered to be biologically significant changes. Eleven genes were involved in the TCA cycle, 5 genes were involved in glycolysis, 7 genes were involved in glycogen metabolism, 4 genes were involved in the pentose phosphate pathway and two genes were involved in gluconeogenesis. Isoenzyme mRNA level shifts were also present during the three stages of myogenesis. An example of these shifts occurred in enolase isoforms and isocitrate dehydrogenase-NADP<sup>+</sup> dependent isoforms (Figure 1).

In addition, the enzymatic activities of the rate regulating steps involved in glycolysis, the TCA cycle and glycogen metabolism were measured. The activities of pyruvate kinase, phosphofructokinase, citrate synthase, isocitrate dehydrogenase-NADP<sup>+</sup> dependent and isocitrate dehydrogenase-NAD<sup>+</sup> dependent increased significantly (ANOVA,  $p < 0.05$ ) from the control group (day 0).

These data indicate that the TCA cycle, glycolysis and glycogen metabolism pathways were significantly altered during myogenesis to meet the energetic and synthetic needs of the developing muscle cells. The cell adapts to these needs by increasing mitochondrial biogenesis, greatly enhancing aerobic respiration and increasing the synthesis of contractile proteins. These data also support studies that indicate disparate changes in mRNA transcript levels and protein expression levels, that is, they are not necessarily coupled processes.

*(Supported by the Blakeslee Fund in the Biological Sciences)*

Advisor: Stylianos Scordilis, Biochemistry and Biological Sciences

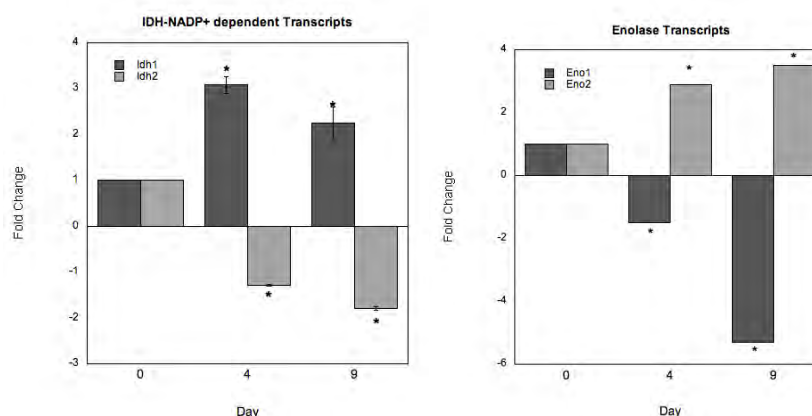


Figure 1: Isoform shifts in IDH-NADP<sup>+</sup> dependent and enolase isoenzyme transcripts during the course of myogenesis. Asterisks indicate changes that were statistically significant ( $p < 0.05$ ).



# Forming Sp-lesions and Digesting Lesion-containing Oligonucleotides for Detection by HPLC

Emma Martin/2016

Oxidative damage to DNA is caused by environmental exposure to high valent metals such as Ir(IV)<sup>1</sup>. Spiroiminodihydantoin (Sp) lesions form by the oxidation of a guanine nucleotide base to form an oxidized 7,8-dihydro-8-oxo-2'-deoxyguanosine intermediate, which is susceptible to further oxidation by Ir(IV)<sup>2</sup>. These lesions are highly mutagenic and cause DNA base pair transversion mutations of G→T and G→C during replication, and arrest the activity of DNA polymerase. Additionally, Sp lesions disrupt hydrogen bonding between DNA base pairs, which causes destabilization of the helix<sup>3</sup>. In this study, 15-mer oligo strands with an 8-oxo-guanine nucleotide were exposed to Ir(IV)<sup>1</sup>, enzymatically digested, and analyzed by HPLC and MS to determine the presence and quantity of Sp lesion synthesized.

Oligonucleotide samples (5'-ACTGATA-8-oxo-GACGCACT-3') were reacted with 100μM Na<sub>2</sub>IrCl<sub>6</sub>·6H<sub>2</sub>O in 0.010M sodium phosphate and 0.10M sodium chloride at 65°C for 30 minutes. The reaction was quenched with 0.5M EDTA, and the desalted by using Amicon Ultra concentrators (MW 3000). The sample was then dried down in an Eppendorf tube and added to a solution containing 10mM Tris-HCL (pH 7.8), 4mM magnesium chloride, (5U/μL) Snake venom phosphodiesterase (SVPD), and (1U/μL) shrimp alkaline phosphatase (SAP). The solutions were incubated at 37°C for 20 hours. Zinc acetate (1.1mM), (1U/μL) nuclease P1, and SAP were added to the sample and incubated at 37°C for 20 hours. The samples were quenched by addition of 20mM EDTA, and the protein was concentrated and analyzed by using HPLC and MS.

Data from HPLC using a Hypercarb column and monitoring the UV absorbance at 260nm in the control oligonucleotide (without an Sp lesion) show four peaks after 13 minutes that represent the four standard DNA nucleosides, while the digestion of the Sp-lesion-containing oligonucleotide shows an additional two Sp-lesion diastereomer peaks between 12 and 13 minutes. This suggests successful formation of the Sp-lesion and effective digestion of the oligonucleotide into individual nucleosides. Analysis by MS shows small non-distinct peaks with the same mass as the Sp-lesion, but further research, including refining the digestion and HPLC procedure, is required to obtain more definitive results. Once Sp-lesion formation and the digestion process are validated by HPLC and MS, it may be applied to more complex strands of DNA.

4 peaks after 13 minutes → A, T, C, G

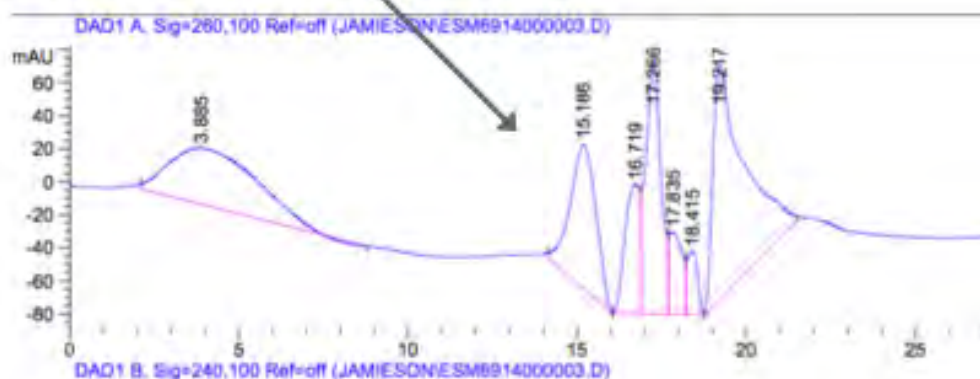


Figure 1: HPLC for a 15-mer oligonucleotide control showing four peaks between 16-20 minutes representing the four standard DNA nucleosides and no presence of the Sp lesion at a UV absorbance of 260nm.



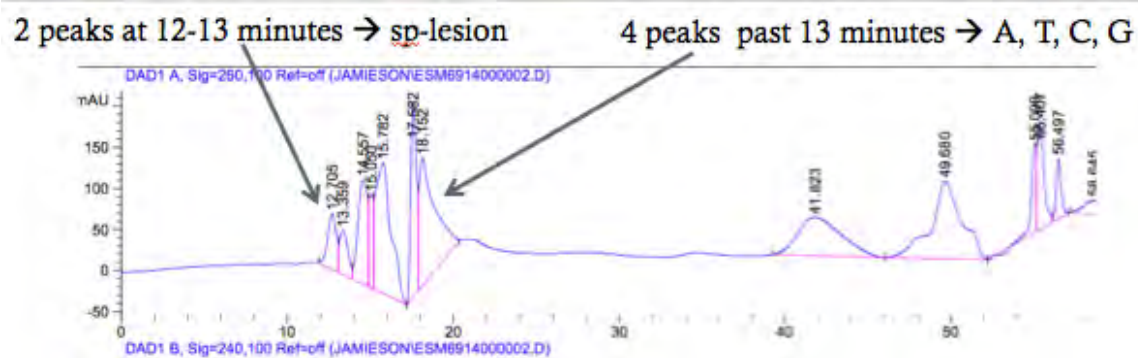


Figure 2: HPLC for a 15-mer oligonucleotide treated with Ir(IV) show two peaks at 12-13 minutes representing the two diastereomers of the Sp lesion and four peaks between 16-20 minutes representing the four standard DNA nucleosides at a UV absorbance of 260nm.

*(Supported by funds from the Committee on Faculty Compensation & Development, Smith College)*

Advisor: Elizabeth Jamieson, Biochemistry and Chemistry

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# The Localization of ER $\alpha$ , ER $\beta$ and GPER Estrogen Receptors in Murine Biceps Brachii

Rebecca A. Ratusnik/2016

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Estrogen is a steroid hormone capable of diffusion through the plasmalemma of muscle cells, where its binding to cytoplasmic, membrane-associated, or nuclear estrogen receptors elicits a response within the skeletal muscle<sup>1</sup>. The three estrogen receptors, alpha (ER $\alpha$ ), beta (ER $\beta$ ), and G-protein linked (GPER), have been implicated in muscle quality maintenance<sup>2</sup>, insulin sensitivity<sup>3</sup>, muscle performance<sup>2</sup>, and exercise recovery<sup>4</sup>. Adult skeletal muscle, is comprised of contractile muscle fibers (cells) of three physiological types that are reflected in their myosin isoforms; Type I (slow), Type IIa (fast oxidative), and Type IIb (fast glycolytic); however the specificity of the three estrogen receptors to fiber type is as yet unknown.

Excised murine biceps brachii were flash frozen and cryosectioned. Immunofluorescence was used to fiber type the specific muscle cells as Type I (slow) or Type II (fast). Protocols were developed for double and triple immunofluorescence of fiber type within the same cryosection, using two or three primary antibodies against Type I muscle-myosin and Type II muscle-myosin, in addition to DAPI, nuclear stain. Epi-fluorescence and laser scanning confocal microscopy (LCSM) techniques were established to image the stained muscle sections (Figure 1). The successful optimization of the triple staining technique as well as imaging using the confocal microscope occupied the majority of the summer research.

Future research will localize the three skeletal muscle estrogen receptors, ER $\alpha$ , ER $\beta$  and GPER in female and male murine biceps brachii. Further, the distinction between the localization in Type IIa and IIb fibers will be investigated. Once all of these baseline results are known, the variable of exercise (downhill running) will be added to determine the time course of changes in the estrogen receptor populations. From these data we will be able to better understand the gender specificity of estrogen receptors expressions in adult skeletal muscle.

*(Supported by the Howard Hughes Medical Institute)*

Advisor: Stylianos Scordilis, Biochemistry and Biological Sciences

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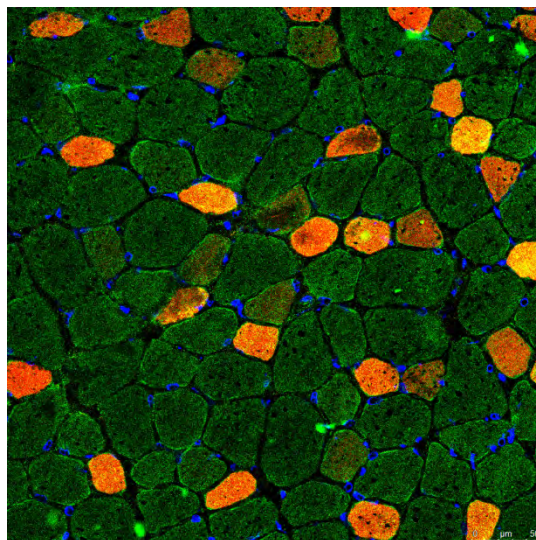


Figure 1. LCSM image of murine biceps brachii cross section (10um) at 40X magnification. Type I muscle myosin (red), Type II muscle myosin (green), and nuclei (blue) are labelled.

# The Effect of Enemy-Free Space on Red-Backed Salamander and Ground Beetle Distributions at MacLeish Field Station

Emily Anderson/2014 and Taylor Jones/2017

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Competition between animals often occurs directly in aggressive interactions or indirectly through competition for resources; however, animals can also compete with each other to avoid being eaten by predators. This can also be thought of as competition for “enemy-free space.”<sup>1</sup> This study used a set of cover board plots to examine distribution and co-occurrence patterns of red-backed salamanders and carabid ground beetles. Based on prior studies, we hypothesized that the salamanders and beetles would interact antagonistically and avoid each other because they are direct competitors for resources.<sup>2</sup> At the same time, we also thought that salamanders and beetles would each avoid areas with mammal tunnels because of the threat of small mammal predators, such as shrews. We sought to answer the following question: do distribution patterns of salamanders and beetles more strongly reflect competitive interactions, or mutual avoidance of predation via co-occurrence in enemy-free space?

To test the spatial relationships between salamanders, beetles, and shrews, we surveyed for the presence of salamanders, carabid ground beetles, and small mammal tunnels under cover boards in study plots at the MacLeish Field Station and at a similar site at Doug Frasier’s property in Chesterfield. The MacLeish site consisted of nine plots, each with seventy small (~1’x1’) wooden boards evenly spaced on the forest floor. The Chesterfield site consisted of six plots of seventy boards each. Since their installation in 2012-13, the boards have been rained on and started to rot, which provides the perfect habitat for these forest floor organisms to live under. For our survey, each board in each plot was lifted, and the presence of salamanders, beetles, and/or mammal tunnels was recorded. The data was analyzed through a series of chi-squared tests in R Studio, comparing salamander vs. beetle presence, salamander vs. tunnel presence, and beetle vs. tunnel presence.

Our results show that predation risk may be a more substantial driver of distribution patterns for salamanders and beetles than direct competition with each other. In particular, we found evidence of salamanders and beetles co-occurring more frequently than expected by chance, and both animals were less likely to occur under boards with small mammal tunnels. Notably, the plots at the MacLeish Field Station had a higher frequency of tunnels than those at Chesterfield, and it was these areas where patterns were strongest, suggesting the animals in plots with more tunnels may be more accustomed to avoiding predators by occupying enemy free space. Therefore, we predicted that the chi-square statistic would be positively correlated with the frequency of tunnels in plots. We found that the chi-square statistic for beetle and salamander avoidance of tunnels is more significant as the frequency of tunnels increases at MacLeish (Fig. 1, 2). This confirms our hypothesis that the salamanders and beetles seem to be avoiding the mammal tunnels to find enemy-free space. This leads to the two potential competitors occurring together more frequently than expected by chance in plots where the mammal tunnels are more frequent (Fig. 3). This result is contrary to what was expected based on competitive interaction, since beetles and salamanders should compete directly with each other for food and habitat, it was predicted that they would avoid each other. However, our results make clear that when there are potential predators present, the two organisms seem to avoid these predators by living under boards with no mammal tunnels (enemy-free space), and are therefore often forced to live together. This pattern suggests that the threat of predation is an overriding force that influences salamander and beetle distribution patterns more than competition.

*(Supported by the B. Elizabeth Horner Fund in the Biological Sciences, Anderson and the Schultz Foundation, Jones)*

Advisor: Jesse Bellemare, Biological Sciences

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<sup>1</sup>Gratton, C., & Welter, S. C. (1999). Does “enemy-free space” exist? Experimental host shifts of an herbivorous fly. *Ecology*, 80(3), 773-785.

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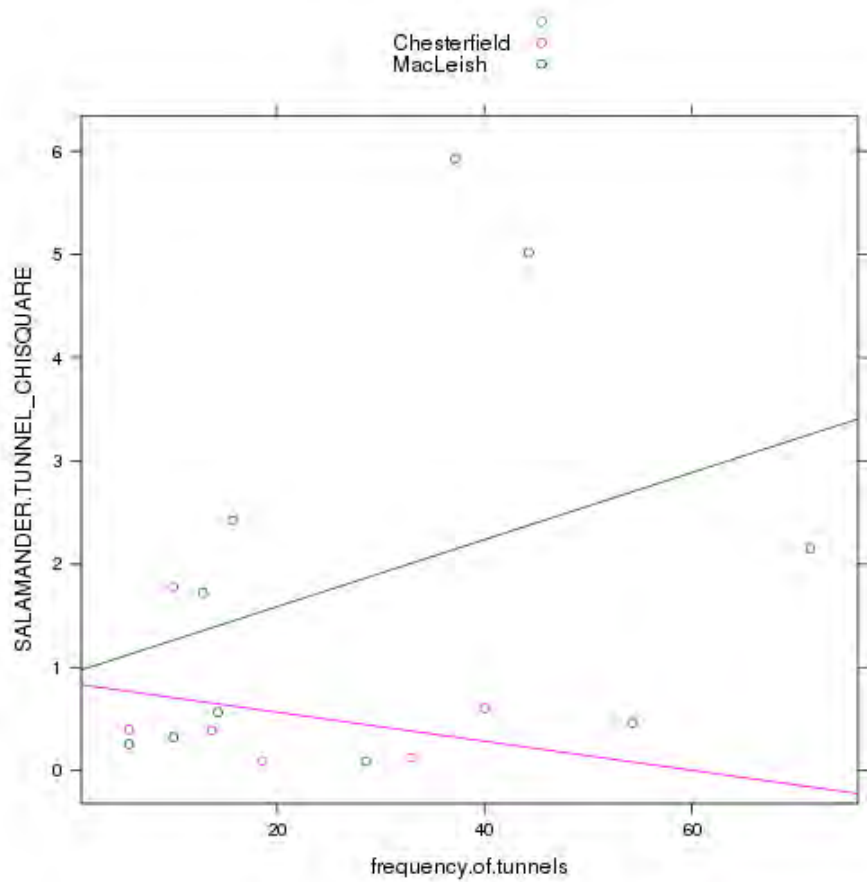


Figure 1: Frequency of Small Mammal Tunnels vs. Chi Square Test Statistic of Salamanders/Tunnels. In these results, higher test statistic values indicated greater avoidance of tunnels by salamanders.

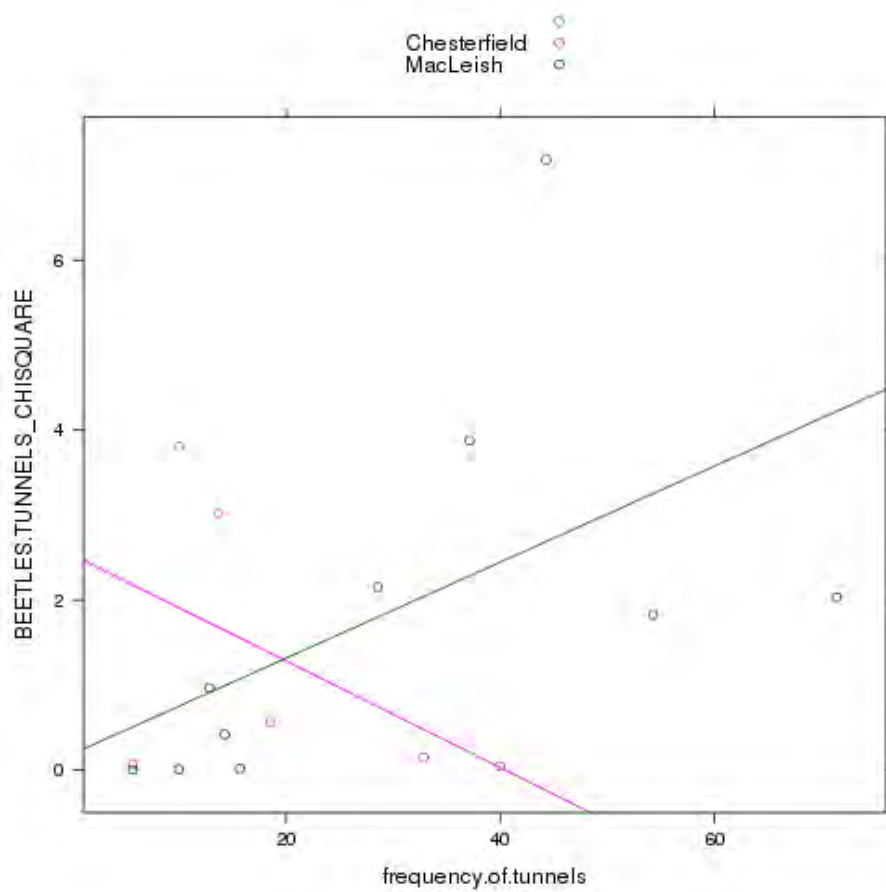


Figure 2: Frequency of Tunnels vs. Chi Square Test Statistic of Beetles/Tunnels. In these results, higher test statistic values indicated greater avoidance of tunnels by beetles.

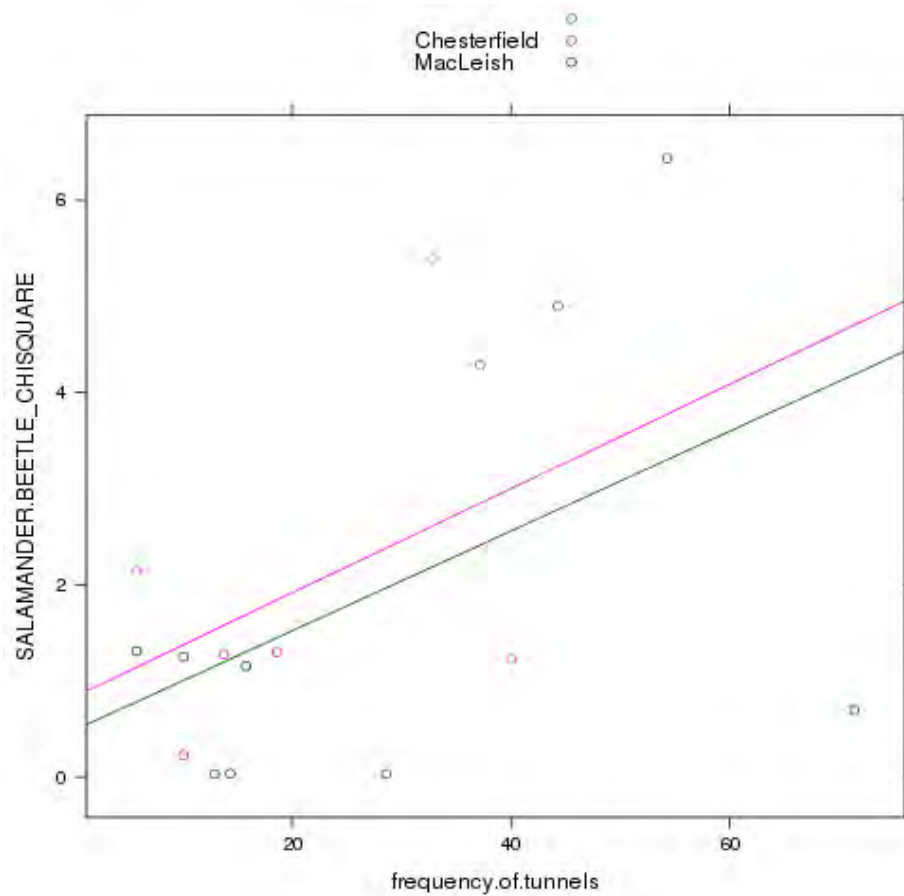


Figure 3: Frequency of Tunnels vs. Chi Square Test Statistic of Salamanders/Beetles showing higher than expected co-occurrence between beetles and salamanders in plots with a higher frequency of small mammal tunnels. This suggests the two potential competitors are co-occurring in enemy free space.

# Slit-Roundabout Signaling in the Development of the Post-optic Commissure

Abigail Antoine/2015

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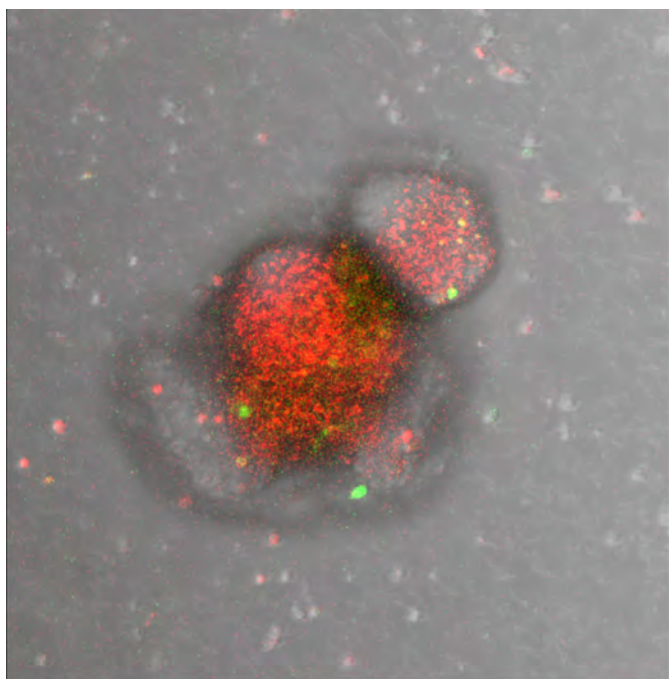
Brain development is a complex process involving multiple cell signaling mechanisms to guide neurons into making proper connections. The development of specific brain structures involves multiple crucial signaling mechanisms for proper formation. Bundles of axons cross the midline and form commissures to connect the two hemispheres of the brain. The Slit-Roundabout protein signaling mechanism is crucial in the formation of the Post-optic Commissure. Slit-Roundabout signaling is understood to act as repellent signaling cues for path-finding axons; however, we propose a new function to this guidance system, in which Slit1a may attract path-finding axons onto the glial bridge to facilitate Post-optic Commissure formation in the zebrafish diencephalon.

This summer was spent researching, learning and employing two new experimental techniques to be used to support my Honors thesis this year. In one such experiment, I researched and learned to make cell and tissue cultures from primary neural tissue. This ex vivo study will allow me to observe the reaction of individual cultured neurons to an isolated Slit signal that is secreted from neural explant tissues. The isolated environment will clarify the neurons' response, allowing us to have a better understanding to the roles of these Slit signals in vivo. I was also able to learn a cell transplantation technique; gastrula-stage cells are transplanted into the diencephalic fate-mapped area to produce a forebrain with mosaic cell expression that will locally misexpress Slit signals. This local misexpression assay will support the ex vivo studies, confirming that isolated areas of Slit1a signaling will ectopically attract path-finding axons away from the permissive substrates of the glial bridge.

Preliminary results suggest that Slit1a does act uniquely from Slit2, attracting axons in a isolated culture environment as well as during the local misexpression assays. Future research will involve further repetitions of the work above as well as applying unbiased statistical analysis to all results. When confirmed, the understanding that Slit1a acts uniquely from the other Slit ligands will add to the understanding of neural developmental as well as promoting further research into the reception of this signal.

*(Supported by the National Science Foundation)*

Advisor: Michael Barresi, Biological Sciences









Sampling from Tidepools at Acadia National Park

# Astroglia are Required for Proper Neurogenesis and Contribute to the Integrity of the Axonal Architecture during Spinal Cord Development in Zebrafish

Jessica Barragan/2015

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Proper development of the nervous system relies upon the elaborate interplay and regulation of neural networks and neural stem cell proliferation. In the developing zebrafish, radial glia (RG) serve as the principle neural stem cell population and thus contribute to the integrity and maintenance of the central nervous system (CNS). Within the neural tube, RG give rise to neuronal and glial progeny, provide signaling cues for pathfinding axons, and facilitate neural regeneration. Though their involvement in constructing the neuronal and glial architecture of the CNS is supported, their direct requirement remains unknown. To determine whether RG are necessary for the generation of cell specific neurogenesis and axon guidance, we generated a genetic cell ablation system using the regulatory elements of the radial glial gfap gene to drive expression of an *E. coli* nitroreductase gene (nfsb) with the fluorescent reporter mCherry specifically within RG. Upon exposing tg(gfap:nfsb-mCherry) embryos to the prodrug Metranidazole (Mtz), the nitroreductase enzyme NTR will convert Mtz into a cytotoxin and cause death of RG cells. To test the requirement of RG in the neural tube, we treated tg(gfap:nfsb-mCherry) embryos with Mtz at 8hpf and labeled for the apoptotic marker anti-activated Caspase 3. In a previous study, we observed cell death in the nfsbSC059 allele at 42hpf; however, recently we began characterizing the SC129 allele and observed cell death throughout the neural tube at 30 hpf. Furthermore, to assay the requirement of RG in maintaining axonal anatomy, we ablated radial glia to characterize the perturbation of axon morphology. Using the SC059 line, we immunolabeled MET treated tg(gfap:nfsb-mCherry) embryos with anti-Acetylated Tubulin ( $\alpha$ AT) antibodies to delineate all axons. By 36hpf, the characteristic longitudinal axons that progress along the DV axis of the spinal cord were disorganized and disrupted. To ensure that this disruption was not caused by neurotoxic effects from MTZ, tg(gfap:GFP) embryos were drug treated and assessed for cell death. By 60hpf, cell death was not present thereby supporting the phenomenon that RG do in fact play a role in mediating and maintaining axonal patterning. These studies as well as our future experiments aim to delineate the contributions that radial glial have in the construction and regeneration of the central nervous system.

*(Supported by the National Science Foundation)*

Advisor: Michael Barresi, Biological Sciences

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# The Effects of Hemlock Woolly Adelgid (*Adelges tsugae*) Invasion on Eastern Hemlock (*Tsuga canadensis*) Leaf Litter Mesofauna Density

Elizabeth Besozzi/2016

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The spread of the invasive Hemlock Woolly Adelgid (*Adelges tsugae*) (HWA) to the Northeastern United States may irreversibly alter the structure of Eastern Hemlock (*Tsuga canadensis*) ecosystems.<sup>1</sup> As evergreen Hemlock stands are replaced by deciduous Black Birch (*Betula lenta*), important habitat characteristics, including soil moisture, organic layer depth, and soil acidity also change. In this study, a survey of leaf litter invertebrates (mesofauna) was conducted across adjacent Hemlock and Birch stands to assess the likely impacts of Hemlock decline on mesofauna in the forest floor. I hypothesized that Hemlock forest mesofauna densities would be higher due to deeper organic layers and more stable environmental conditions.

Six pairs of adjacent Hemlock and Birch plots were surveyed at Smith College's MacLeish Field Station and a location in Chesterfield, MA (n = 12 plots total). In each plot, eight 25 x 25 cm samples of the organic layer were collected in early June 2014. Berlese funnel traps containing 30 g subsamples of the homogenized organic layer were dried for one week to collect mesofauna.

Two broad categories of mesofauna were distinguished which accounted for the majority of specimens: springtails (Collembola) and mites (Acari). Standard t-tests and paired t-tests were employed to test for significant differences in mesofauna densities across sites and between adjacent plots, respectively. Two pairs of Hemlock and Birch plots exhibited statistically significant differences: mite density was significantly higher in Birch plot 2 than Hemlock plot 1 at the MacLeish Field Station ( $P < 0.01$ ), while springtail density was significantly higher in Hemlock plot 2 than in nearby Birch plot 9 at the Chesterfield site ( $P < 0.01$ ). Overall differences in average springtail and mite densities in Hemlock vs. Birch plots across both sites were not statistically significant.

The results of this survey did not clearly support my initial hypothesis, as mesofauna densities did not differ consistently between Birch and Hemlock plots. However, total mesofauna abundances are likely greater in Hemlock forests due to the greater depth of organic layers in this habitat.<sup>1</sup> Zuskwert et al. (2014) did find greater densities of mesofauna in Hemlock plots in a mid-July 2012 survey, a difference they attributed to shallower organic layers and drier conditions in the Birch plots. It is possible that densities did not differ in 2014 because the samples were collected in early summer, while temperatures were relatively low, moisture high, and Birch litter still intact. The similar densities of mesofauna in Hemlock and Birch plots in 2014 may indicate that mesofauna take advantage of high nutrient availability in Birch litter in early summer, and retreat lower into the Birch soil later in summer, as temperatures rise and litter deteriorates. These results suggest that the effect of Hemlock, and its potential loss, on mesofauna will be complex, likely influenced by climatic conditions, seasonal fluctuations in weather, and the effects of these factors on litter temperature and deterioration.

*(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)*

Advisor: Jesse Bellemare, Biological Sciences

<sup>1</sup>Zuskwert, Jenna M., Bellemare, Jesse, Rhodes, Amy L., Sweezy, Theo, Gallogly, Meredith, Acevedo, Stephanie, Taylor, Rebecca S. 2014. Forest Community Structure Differs, but Not Ecosystem Processes, 25 Years after Eastern Hemlock Removal in an Accidental Experiment. *Southeastern Naturalist*, 13 (Special Issue 6): 61-87.

# Assessing Seasonal Photosynthetic Punction of Pominant Species Post-clearcut

Kyle Boyd/2015

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Large-scale forest disturbance affects the exchange of carbon, water and energy between the ecosystem and the atmosphere. Clearcutting is one of the most dramatic disturbances due to the impacts on ecosystem processes and vegetation structure. Due to the complexity in these processes, it is not well understood how the physiology and vegetation structure will change post-clearcut. In the fall of 2008 Christopher Williams' lab commercially clearcut an eight-hectare area in Harvard Forest to study the forest recovery. We studied photosynthetic function and nutrient content in six of the most common species located at the clearcut study site. During June and July a portable photosynthesis system (LI-6400xt) was used to measure leaf-level CO<sub>2</sub>-response curves and light-response curves of *Prunus pensylvanica*, *Prunus serotina*, *Acer rubrum*, *Quercus rubra*, *Rubus idaeus* and *Dennstaedtia punctilobula*. Additionally, leaf tissue samples were analyzed for carbon and nitrogen content. Previous work from the Williams lab during the summer of 2012 provided the basis for comparing changes in photosynthetic capacity. Taken together, this allowed us to measure the photosynthetic capacity, understand potential limitations to photosynthetic capacity, and determine how these change over time. The data suggested similar photosynthetic rates for most species over the past two years. However, preliminary results showed that *Prunus pensylvanica* appeared to be the most efficient in both years, while *Acer rubrum* had the highest C:N ratios. Results from this work will allow us to understand changes in photosynthetic capacity of the most dominant species and how they play a role in ecosystem productivity.

*(Supported by B. Elizabeth Horner Fund in the Biological Sciences)*

Advisor: Danielle Ignace, Biological Sciences



# The Effect of Varying Cargo Rigidity on the Movement of Dynein Ensembles

Karen Chau/2016 and Alyssa Moskites/2015

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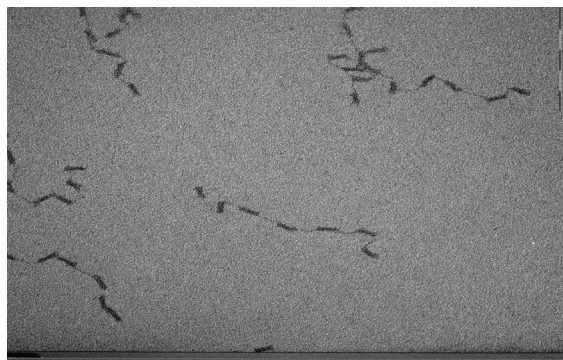


Figure 1. Segmented Chassis 2.0 taken at 200K magnification and 100 kV using transmission electron microscopy.

In eukaryotic cells, intracellular transport depends on motor proteins like dyneins. Dynein is a homodimer with a tail that binds to cargo and two heads that bind ATP to move along microtubules. It is responsible for transporting cargos, which include organelles and vesicles. Since the mechanism that dynein moves is stereospecific, it can only transport cargo towards the minus end of the microtubule or cell center. Ensembles of dynein are often needed to transport cargo, however the ensemble mechanics are not well understood. The overall goal of the experiment is to understand the interaction between dynein ensembles and the biophysics behind their movement as a group.

To establish a control for the experiment, the cargo was synthesized through DNA origami, a technique that offers high precision for making dynein attachment sites. The segmented cargo was designed using Cadnano to have twelve helix bundles arranged in a honeycomb shape. CanDo<sup>1</sup> was used to visually predict the flexibility of the design. After the design was finalized and secondary structures were minimized in the scaffold sequence,<sup>2</sup> the chassis was folded and purified through a glycerol gradient.<sup>3</sup> It was imaged using transmission electron microscopy with uranyl acetate staining at various accelerating voltages and magnification.<sup>4</sup>

Images from TEM confirmed that the cargo folded successfully and according to the design as a segmented chassis. With seven segments and six linker regions, the chassis is more flexible than the previous one.<sup>3</sup> Its flexibility was confirmed by CanDo's visualizations and showed greater fluctuations. This improves the scenario of cargo transport that is recreated in vitro because organelles and vesicles are not rigid. To maximize the cargo's flexibility, staples from the linker regions were not added in one of the foldings, and this produced chassis that resembled clumps. Upon adding the staples that were withheld back into the linker regions, the resulting chassis showed proper segmentation. This confirms the specificity of DNA origami folding and offer options for varying the flexibility of the chassis.

Since the chassis's folding resembles the design, the next step is to attach dynein motors to specific locations of the cargo and conduct a motility assay experiment. The results will generate data to compare the effects of varying the number of attached dynein motors and the rigidity of the cargo on the speed and run length. Ultimately the understanding of how dynein motor ensembles behave can lead to the development of controllable molecular shuttles that can branch into numerous interdisciplinary fields, such as molecular medicine.

*(Supported by National Science Foundation (4CBC), Chau and the Howard Hughes Medical Institute, Moskites)*

Advisor: Nathan Derr, Biological Sciences

<sup>1</sup>Bathe, Mark. "CanDo - Computer-aided Engineering for DNA Origami." CanDo - Computer-aided Engineering for DNA Origami. MIT.

<sup>2</sup>Zucker, Mark, and Nick Markham. "The Mfold Web Server." The Mfold Web Server. Rensselaer Polytechnic Institute.

<sup>3</sup>Derr et al. "Tug-of-War in Motor Protein Ensembles Revealed with a Programmable DNA Origami Scaffold." Science 338.6107 (2012): 662-65.

<sup>4</sup>Douglas et al. "Self-assembly of DNA into Nanoscale Three-dimensional Shapes." Nature 459.7245 (2009): 414-18.



# PAH-Induced Activation of Aryl Hydrocarbon Receptor Signaling and its Effects on Neural Crest Development in Zebrafish

Diane Chen/2014

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On April 20th 2010, the Deepwater Horizon (DWH) oil platform sank, triggering the release of 4.93 million barrels of oil into the Gulf of Mexico from 5000ft below. Concerns have been raised about the effects of crude oil on marine flora and fauna in the Gulf especially those exposed to such toxin during embryonic stages. Examination of native Gulf species is challenging, but the zebrafish provides a tractable model system to directly test the teratogenic effects of crude oil toxins. We investigated the effects of the water accommodated fraction (WAF) of crude oil on zebrafish embryogenesis, and observed cardiovascular and craniofacial malformation that we postulate could be the result of impaired cranial neural crest cell development. Analysis of the DWH oil showed the presence of polycyclic aromatic hydrocarbons (PAHs) and BTEX compounds (benzene, toluene, ethyl benzene, and xylene). We hypothesized that PAHs could also cause similar defects observed in crude-oil mediated teratogenesis. Using a candidate approach, we exposed zebrafish to the 16 PAHs designated priority pollutants by the EPA, and all yielded a similar disappearance of the 6th pharyngeal arch. From our experiments, pharyngeal arch malformation is most likely due to a defect in cranial neural crest migration and not cell survival. Previous studies have shown that PAHs activate the aryl hydrocarbon receptor (AhR) signaling pathway. In my study we investigated whether cross talk between the AhR signaling pathway and canonical Wnt signaling functions as the molecular mechanism to mediate potential neural crest malformations following exposure to PAHs. We found that naphthalene was able to induce AhR signaling activation and the noncanonical Wnt signaling pathway as a promising component in the molecular mechanisms mediating PAH- teratogenesis. Understanding the molecular mechanisms that mediate interactions between the environment and embryo will provide insight into both the regulation of developmental plasticity as well as the risks present in our own environment.

*(Supported by the Nancy Kay Holmes Fund)*

Advisor: Michael Barresi, Biological Sciences

# Exposure to Polycyclic Aromatic Hydrocarbons Cause Defects in Neural Crest Cell Migration Leading to Craniofacial Malformations During Zebrafish Embryogenesis.

Gina Cho/2017

Considered the largest oil spill in US history and greatly impacting a diversity of ecosystems, the Deepwater Horizon oil rig explosion released 200 million gallons of crude oil into the Gulf of Mexico from its occurrence on April 22<sup>nd</sup> 2010 to its capping on July 15<sup>th</sup> 2010. By using zebrafish as a model organism in a controlled laboratory setting, this ongoing project entails studying the developmental defects that crude oil and its components, specifically polycyclic aromatic hydrocarbons (PAH's), may have on vertebrate systems. PAH's are found both naturally and in many industry products that are increasingly contaminating the environment. These studies can provide a stronger awareness of the degree to which PAH's impact not only marine native species, but also human health and the greater environment.<sup>1</sup> Our objective is to discover how PAH's impact life and specifically the developing embryo's most vulnerable stage of life.

We sought to characterize the phenotypic defects caused by exposure to specific PAH's. Zebrafish embryos treated with PAH's cause reductions in the cartilage elements that build the developing skull. We attempted to identify and analyze the cell types and molecular pathways that mediate this teratogenic response. Specifically, we hypothesized that PAH's were somehow impeding the proper development of cranial neural crest cells (NCC), which are migratory multipotent stem cells that give rise to craniofacial structures. This summer, our primary focus was to observe pharyngeal arches: transient structures made up of migratory streams of NCC that form many craniofacial and cardiac tissues of the developing embryo. We treated embryos at 4, 10, 20, and 25 hours post fertilization (hpf) with naphthalene, fluorene, and benzo[a]pyrene, three of the 16 PAH's the U.S. Environmental Protection Agency (EPA) published on their priority pollutant list. We also treated embryos at these time-points with a combined solution consisting of 20% Naphthalene, 10% Fluorene, and 20% Benzo[a]pyrene, and 3:1 and 1:1 dilutions of this solution. Using tg(fli1a:EGFP) transgenic fish to fluorescently label cranial NCC, we were able to assay pharyngeal arch formation following treatment with these different PAH's. At 32 hpf, untreated embryos have six pharyngeal arches: (in order) the mandibular, hyoid, and four branchial arches (Figure 1). The summer's cumulative results showed that many, if not all, of the embryos exposed to these PAH solutions lack one of the four branchial arches when treated at any of the four time-points. However, later treatments yielded a lower percentage of embryos with the branchial arch reduction.

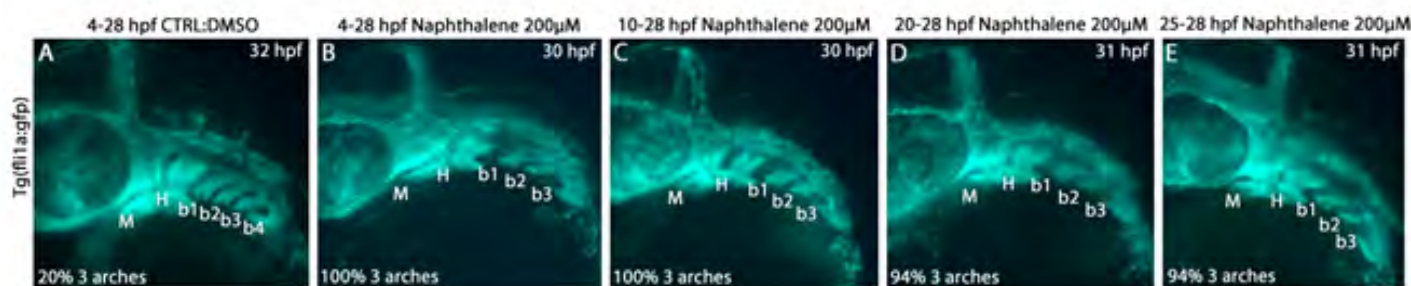


Figure 1 Pharyngeal arch defects induced by naphthalene exposure at different time-points (B-E) (A-E) Fli driven expression of GFP transgenic zebrafish shows cranial NCC forming pharyngeal arches at 30-32 hpf. (A) DMSO control zebrafish show six visible pharyngeal arches: mandibular (M), hyoid (H), and four branchial arches (b1-4). (B-E) Embryos lack a branchial arch (b1-3) at 30 hpf (B,C) and 31 hpf (D,E) when exposed to 200 µM Naphthalene during 4-28 hpf (B), 10-28 hpf (C), 20-28 hpf (D), and 25-28 hpf (E).

Because these arch defects are still observed when embryos are treated only two hours prior to arch assessment during active NCC migration, we hypothesize that PAH's are impacting the ability of NCC to migrate either directly or through modulation of the environmental guidance cues. Future directions of this project include in-situ hybridization assays of NCC populations to spatially visualize and determine if specific genes that are up or downregulated by these PAH's. Our ultimate goal is to understand the molecular pathways involved in mediating PAH-associated teratogenesis.

*(Supported by the Provost's Office, Smith College)*

Advisor: Michael Barresi, Biological Sciences

<sup>1</sup>de Soysa TY, Barresi MJ, Ulrich A, Friedrich T, Pite D, Compton SL, Ok D et al (2012) Macondo crude oil from the deepwater horizon oil spill disrupts specific developmental processes during zebrafish embryogenesis. BMC Biol 10:40

# Inter- and Intra- Specific Competition Between Desert Annual Seed and Seedlings

Samantha Danguilan/2015

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Figure 1 (Left). Petri dish depicting intraspecific *A. nuttallianus* seedling competition on a target *ASNU* seed in the center. Figure 2 (right). Photo of a fully-grown *ASNU* plant growing in the desert. Photo credits to the Western New Mexico University Department of Natural Sciences.

When looking at external environmental factors that influence seed germination and overall plant growth and fitness, two of the main drivers in this process include the environment's overall resource availability and the competition for those resources between and within species. My summer's research focused on how inter- and intra- specific density factors play a role in changing competition patterns between a native and an invasive plant species. Interspecific competition refers to resource rivalry between organisms from different species, while intraspecific competition occurs between organisms from the same species. In one density project, two desert annuals, *A. nuttallianus* (*ASNU*) (Figure 2) and *E. cicutarium* (*ERCI*), were grown in various densities and population combinations to test for differences in germination rates. The results from this experiment showed that germination rates for the native annual, *ASNU*, tended to stay consistent when planted in an intra-specific environment, regardless of density. However, when exposed to even a single *ERCI* in an inter-specific community, the germination rates of *ASNU* seeds declined significantly. Additionally, there seems to be a negative correlation between population density and germination rates for *ERCI* in both inter- and intra-specific communities.

A similar experiment conducted this summer sought to further assess the effects of inter- vs. intra-specific community populations on seed germination rates and timing-- specifically, does "competition sensing" occur more frequently during the earlier seed stages or post-germination during the seedling stages in *ERCI* and *ASNU*? To test for the effect of seedling presence on a target seed's germination, a target seed planted amongst eight seedlings of similar (intraspecific) or differing (interspecific) species was monitored for germination rate (Figure 1). The same procedure was used to test for the effect of seed presence on a target seed's germination rate. In this experiment, germination rates declined and tended to occur later for both *ERCI* and *ASNU* seeds in the presence of *ERCI* seedlings.

The results from both experiments lead us to hypothesize that *ERCI* seedlings may be exhibiting intra- and inter- specific allelopathic effects on surrounding seeds. As a continuation of this project, soil-based experiments will be conducted during the academic year to test for remnant allelopathic chemicals in the soil.

*(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)*

Advisor: Danielle Ignace, Biological Sciences

# Determining the Cause of Varying Endemic Plant Species Richness in the Eastern United States

Claudia Deeg/2017

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The eastern United States is home to a large number of endemic and small-ranged plant species. Endemic species are confined to a particular area, and small-ranged species are found in 70 or fewer counties.<sup>1</sup> I examined 262 small-ranged woody and herbaceous plant species endemic to temperate deciduous forests of the eastern US to investigate the causes of their current distributions relative to environmental factors and biogeographic history.

I used ArcGIS software to gather and analyze data including endemic species richness (ESR) in eastern US counties, mean annual precipitation, mean temperature, elevation range, and distance from the last glacial maximum (LGM). I extracted the number of endemic species per county from raster-based data and used Microsoft Excel to create graphs that explore the relationship between ESR and the various factors. Furthermore, I used ArcMap to create maps of the eastern United States' ESR to illustrate regional patterns and hotspots.

The results showed a linear trend suggesting a positive correlation between mean precipitation and the ESR. Conversely, the results for temperature followed a bell curve, with the number of endemic species increasing up to ~13°C, then decreasing, likely as the climate becomes too arid.

During the LGM (~18,000 years ago), an ice sheet covered a significant portion of the upper Midwest and northeastern United States, rendering the area unsuitable for plant life, and species retreated south. I hypothesized that the ESR per county would increase with greater distance from the LGM. Very few endemic species were found near or within the LGM (Fig. 1), and the total ESR increases exponentially up to 400 km south of the LGM. Beyond 400 km, the richness gradually declines. I hypothesize that once species migrated to suitable climates, they established enduring populations.

The final factor we examined was the elevation range in a county. Areas with greater elevation ranges can buffer native plants against rapid change by allowing species to shift elevation rather than migrating north or south.<sup>2</sup> There is a positive correlation between elevation range and total ESR (Fig. 2). The coefficient of determination indicates that the elevation range in a county is substantially correlated with the ESR ( $R^2=0.2541$ ).

The distribution and richness of endemic species in the eastern United States (Fig. 3) appears to be affected by the four factors explored – mean temperature, mean annual precipitation, distance from the LGM and elevation range. With this data it is possible to better understand how the richness of endemic species throughout the eastern U.S. has been influenced by climactic, physical, and historical factors.

*(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)*

Advisor: Jesse Bellemare, Biological Sciences

1. Bellemare, J. and D. Moeller. 2014. Climate change and forest herbs of temperate deciduous forests. Pp. 460-493 in, F. Gilliam (ed.) The Herbaceous Layer in Forests of Eastern North America, 2<sup>nd</sup> ed. Oxford University Press, Oxford, UK.

2. Sandel, B., L. Arge, B. Dalsgaard, R.G. Davies, K.J. Gaston, W.J. Sutherland, and J.-C. Svenning. 2011. The influence of late quaternary climate-change velocity on species endemism. Science 334: 660-664.



Figure 1. Effect of distance from last glacial maximum on total endemic speices richness

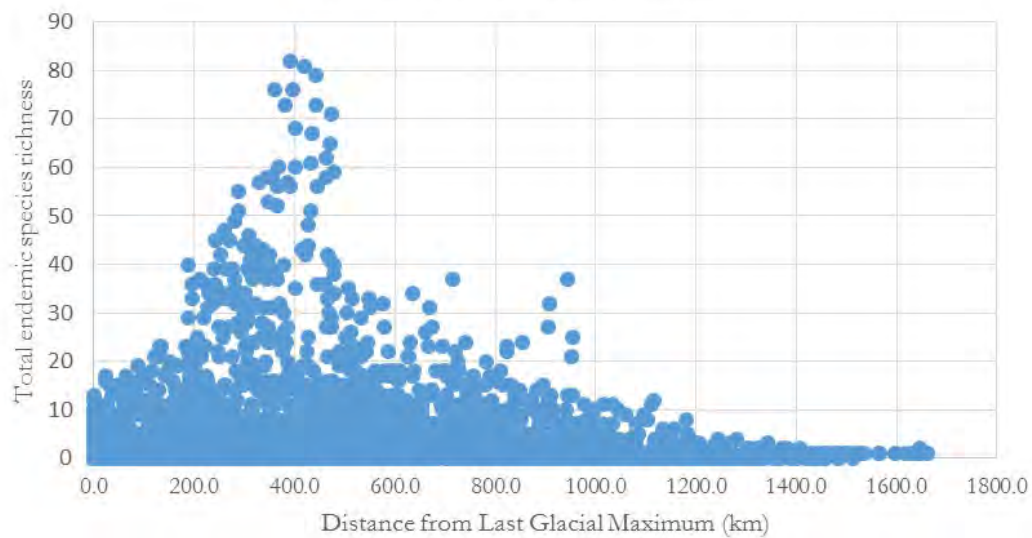
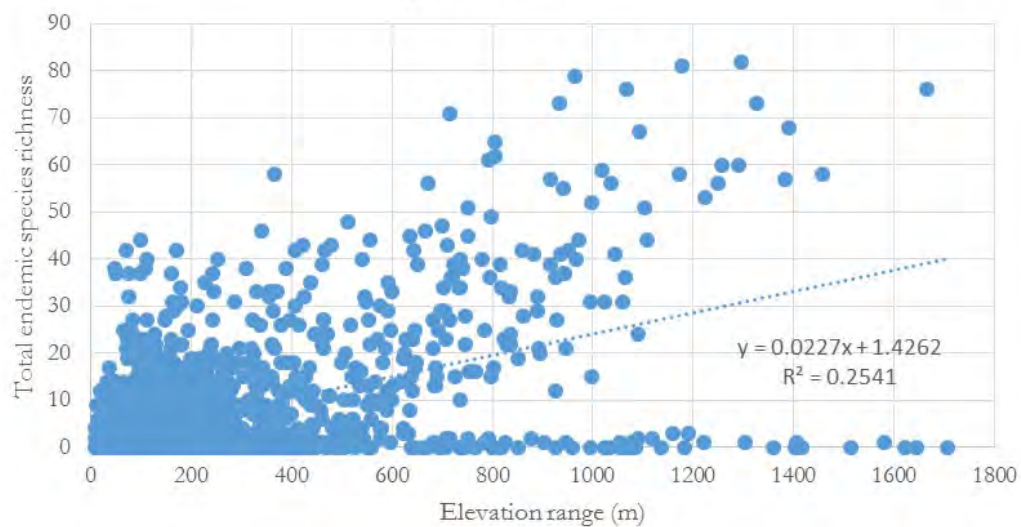
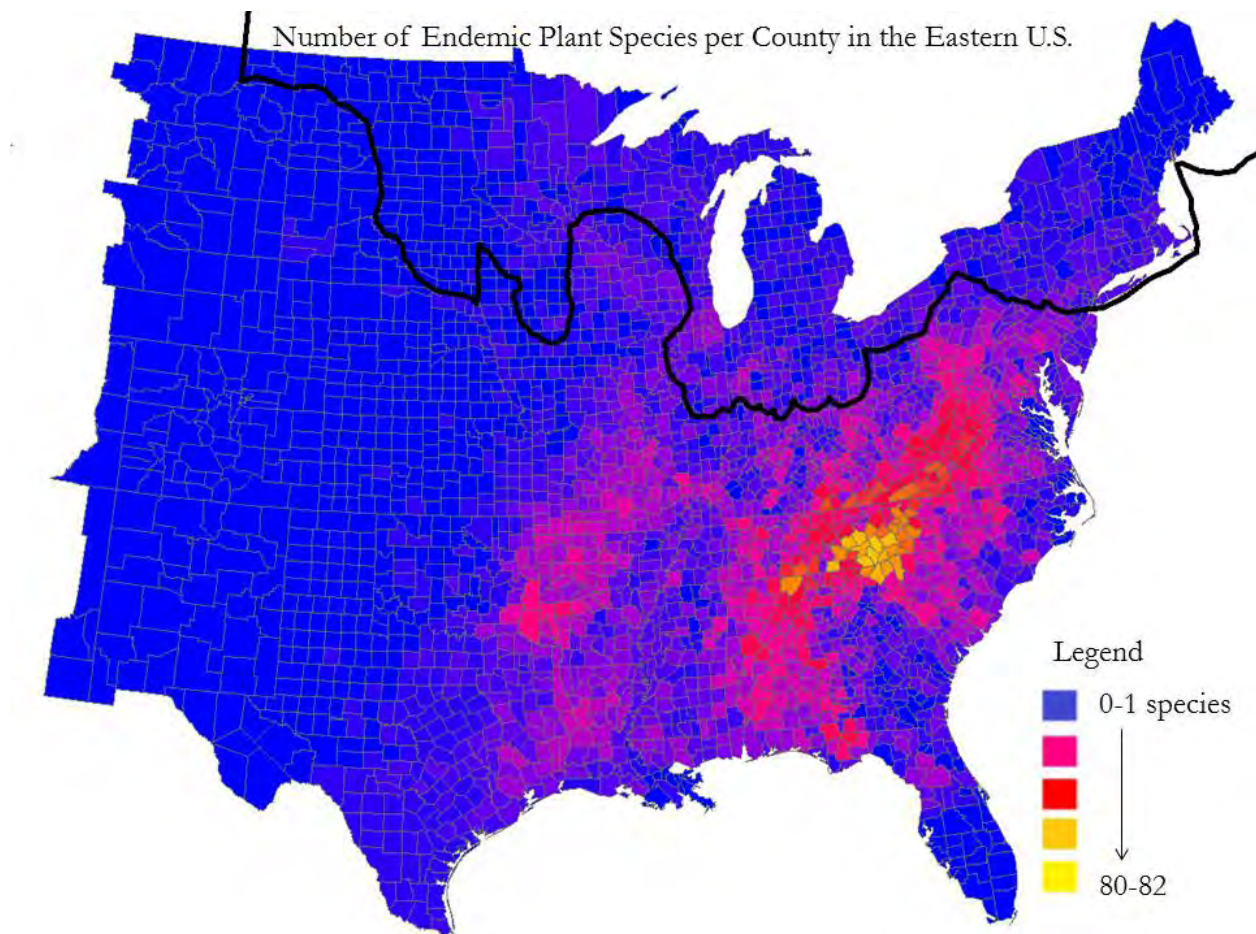


Figure 2. Effect of elevation range of total endemic species richness







## Studying Bacterial Diversity and Sulfate Reducing Bacteria (SRB) Communities in Beaver Ponds of Western MA

Ghida El-Banna/2017

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In this study, we extracted Genomic DNA from water and soil samples to characterize communities of Sulfur Reducing Bacteria (SRB) in beaver ponds in the Avery Brook stream system located in Conway, MA. We hypothesize that SRB's growth varies between outlet and inlet water and between spring and summer because of the role of abiotic inputs and temperature. This study is important because SRB methylate mercury ends up in the water supplies harming the health of many people.

To collect sediment samples, we cored vertically into the sediment and then divided the core into pieces, each about 3 cm long. Water samples were collected from the inlet and outlet streams of two ponds and were filtered using a 0.22 µm filter. Genomic DNA was extracted from sediment and soil samples using the PowerSoil and PowerWater DNA Isolation Kit from MoBio Laboratories, Inc. Bacterial *dsrAB* gene and 16S rRNA was amplified using PCR. Some PCR products were gel isolated, cloned and sequenced. Sequences were blasted on GenBank to determine their identities. PCRs to detect specifically each of the six groups of SRB were used as well.

Results show the sequences belonged to diverse bacteria such as *Janthinobacterium*, *Acidobacteria*, *Limnohabitans* sp.Rim47 and *Beta-proteobacterium*. The *dsrAB* PCR succeeded showing that SRB live in some water and soil samples. PCR to amplify part of the SSU operon specific to *Desulfovibrio* (DSV) failed; whereas, primers targeting *Desulfobacter* and *Desulfonema-Desulfo Sarcia-Desulfococcus* Bacteria succeeded suggesting that these two groups are dominant.

A gel of DNA extracted from water and soil later during the summer showed brighter and thicker bands suggesting that the bacterial activity and growth may be higher later in summer. Further research will be conducted over the fall to understand more the behavior of the SRBs in these ponds.

*(Supported by the Howard Hughes Medical Institute)*

Advisors: Laura Katz and Robert Merritt, Biological Sciences

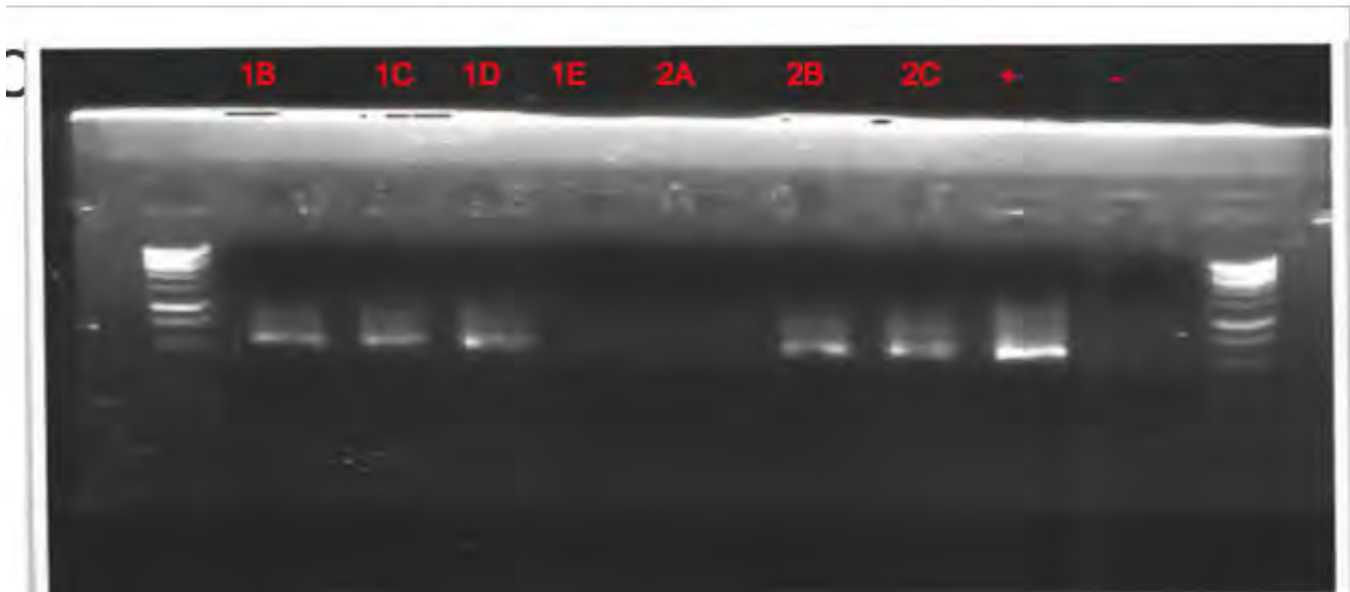
Elizabeth. A. Karr/ 2005

Diversity and Distribution of Sulfate-Reducing Bacteria in Permanently Frozen Lake Fryxell, McMurdo Dry Valleys, Antarctica

Jing Guan/ 2012

Diversity and distribution of sulfate-reducing bacteria in four petroleum reservoirs detected by using 16S rRNA and *dsrAB* genes

A gel for 16S rRNA PCR products for sediment samples; A being the deepest in the core and E being the least deep in the core. 1 and 2 refer to two different sampling locations. + is a positive control and - is PCR blank



# Dengue PCR Test Design

Ridwana Fairuz/2015

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Dengue is a viral disease that occurs in many parts of the world but is especially prominent in tropical and subtropical countries. In recent years it has become an international public health concern. The dengue virus (DEN) is in the genus *Flavivirus* and in the family of *Flaviviridae*. It has four distinct serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). The current method to detect dengue is using serological tests that are not always sensitive and therefore result in a delay in diagnosing the illness. The development and implementation of the more sensitive and lower cost multiplex DNA assay for early detection of the four dengue virus serotypes will enable healthcare professionals, especially in resource-limited environments, to accurately diagnose the virus and its serotype to reduce the chance and incidence of death. The goal of the current project is to create primers and develop a multiplex primer assay for Dengue detection.

Synthetic dengue viral DNA was cloned into *E. Coli* and pure plasmid was extracted from it. The diagnostic primers designed specifically for each Dengue serotype were used to amplify the cloned plasmid DNA in a PCR reaction. The ideal annealing temperature for each serotype was 58 °C for DEN-1, 49 °C for DEN-2, 56 °C for DEN-3, 54.5 °C for DEN-4.

During this fellowship, I learned primer design, cloning techniques and running PCR. Further work needs to be done on this project to probe more precise ideal annealing temperatures.

*(Supported by the Howard Hughes Medical Institute)*

Advisor: Steven Williams, Biological Sciences

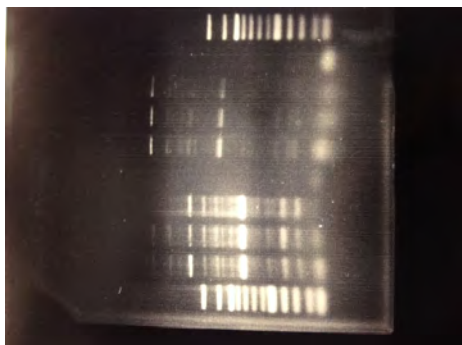


Fig: DEN 3 & 4 PCR product 1% gel

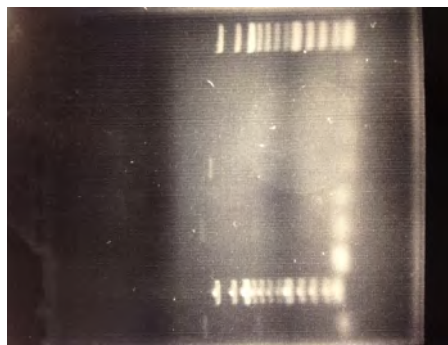


Fig: DEN 1 & 2 PCR product on 1% gel

# Macrofungi Species Richness in Hemlock (*Tsuga canadensis*) vs. Birch (*Betula lenta*) Forest Plots at the Ada and Archibald MacLeish Field Station in Whately, MA. and Chesterfield, MA.

Aliza Fassler/2017

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In New England two exotic insect pests are currently threatening the Eastern Hemlock (*Tsuga canadensis*), the Hemlock Woolly Adelgid (*Adelges tsugae*) and the Elongate Hemlock Scale (*Fiorinia externa*). After Hemlock die-off, the reclaiming tree species is typically Black Birch (*Betula lenta*), a deciduous tree species with very different effects on the forest ecosystem than evergreen Hemlock.<sup>1</sup> Such forest declines have been shown to decrease macrofungal diversity and abundance in other forest types, such as jarrah forests affected by *Phytophthora cinnamomi*.<sup>2</sup> The goal of this research was to determine whether there is a significant difference in macrofungi species richness between Hemlock vs. Birch plots at two sites in Western Massachusetts.

Surveys were conducted on a roughly bi-weekly basis (from 6/6/14 -7/29/14) in 10 x 15m forest plots in Chesterfield, MA and at the Ada and Archibald MacLeish Field Station in Whately, MA. The Hemlock Woolly Adelgid and Hemlock Scale insects have begun infesting hemlocks at the MacLeish site, but not at the Chesterfield site. Nine plots were surveyed at MacLeish (3 Birch, 6 Hemlock) and 6 plots were surveyed at Chesterfield (3 Hemlock, 3 Birch). All mature fruiting bodies within plots were collected. Characteristics of the fruiting bodies were recorded in field notes, scanned images and spore prints. This information was used to determine to genus or species as many of the fruiting bodies as possible, primarily using *Mushrooms and Other Fungi of North America* by Roger Phillips. For each plot the number of distinct morphospecies was recorded.

Data on morphospecies richness from spatially paired Hemlock and Birch plots were analyzed with a series of paired T-tests for each survey date. None of the survey dates at Chesterfield or MacLeish yielded significantly different numbers of morphospecies in Hemlock vs. Birch plots (P-values for these tests ranged from 0.225-0.689). The mean number of morphospecies in Birch plots was consistently higher, although not significantly, at the Chesterfield site then the Hemlock number (Figure 1). The mean number of morphospecies in Birch plots was lower (not significantly) than the mean number of morphospecies in Hemlock for three survey dates (Figure 2). Total abundance of morphospecies in surveys ranged from 10-74 species.

The lack of significantly different morphospecies numbers in Hemlock vs. Birch plots may be due to a lack of host specificity, or the two forest types may have different, but equally species rich macrofungal communities. Research on the Western Hemlock (*Tsuga heterophylla*) has shown that most mycorrhizal species that occur with Western Hemlock are not host specific possibly because they often grow in mixed stands.<sup>3</sup> However, saprobic brown rot species are often found exclusively on conifers.<sup>4,5</sup> This would suggest that brown rot fungal species would be negatively impacted with a shift from Hemlock-dominated forest to Birch. Further research could look at whether individual fungal species occur more frequently in Hemlock vs. Birch, and whether brown rot species are more abundant in Hemlock plots.

*(Supported by the Margaret A. Walsh Grantham Fund)*

Advisor: Jesse Bellemare, Biological Sciences

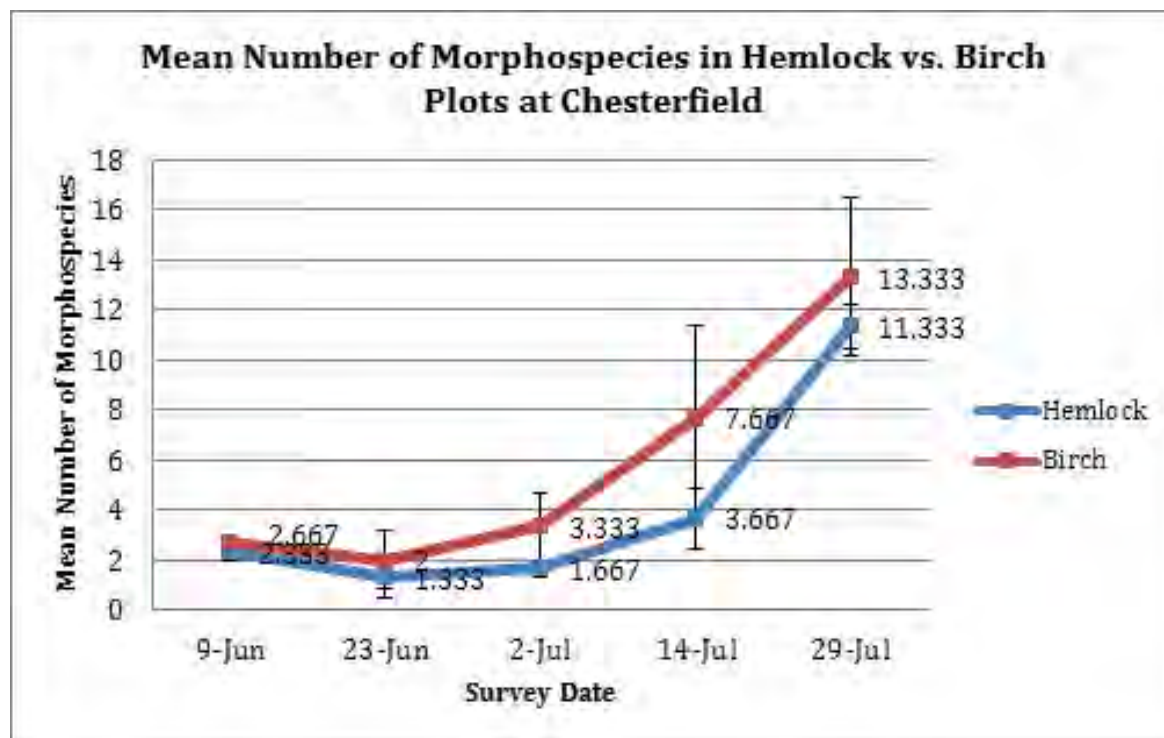
<sup>1</sup> Lovett G. M. et al. (2006). Forest Ecosystem Responses to Exotic Pest and Pathogens in Eastern North America. *BioScience*. Vol. 56 (No.5), 395-405.

<sup>2</sup> Anderson P. et al. (2010). Impact of severe forest dieback caused by *Phytophthora cinnamomi* on macrofungal diversity in northern jarrah forest of Western Australia. *Forest Ecology and Management*. Vol. 259 (5), 1033-1040. <http://www.sciencedirect.com/science/article/pii/S0378112709008949?np=y>

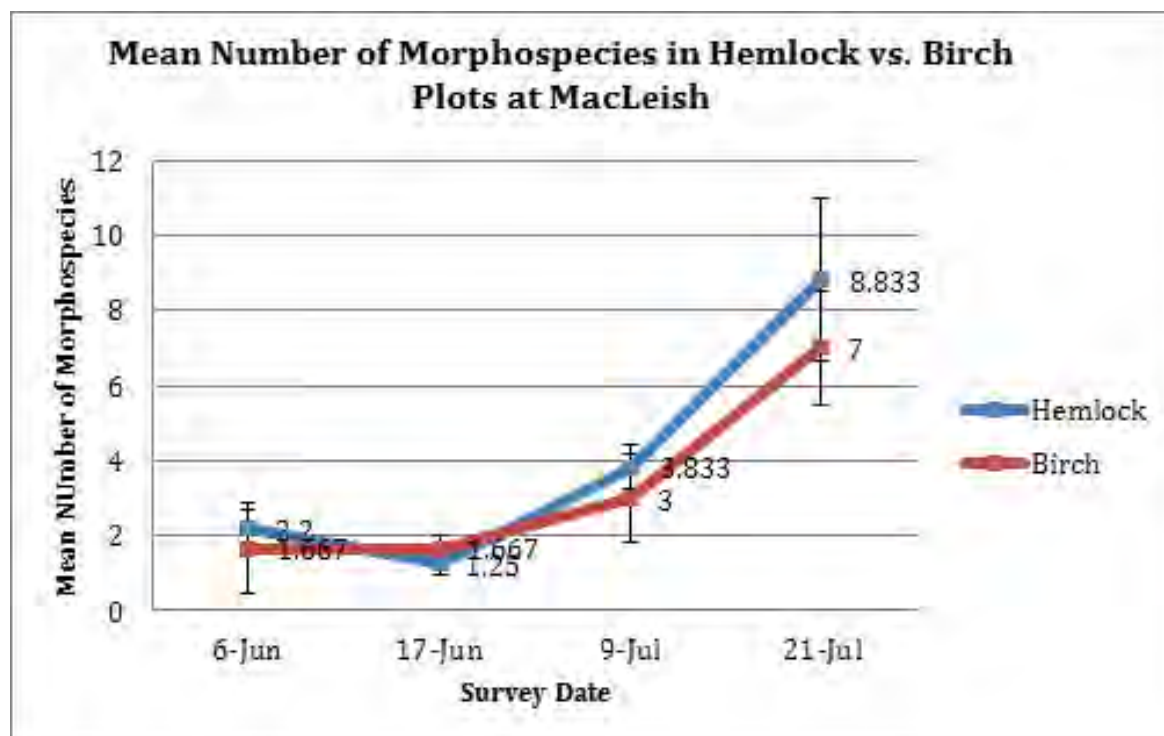
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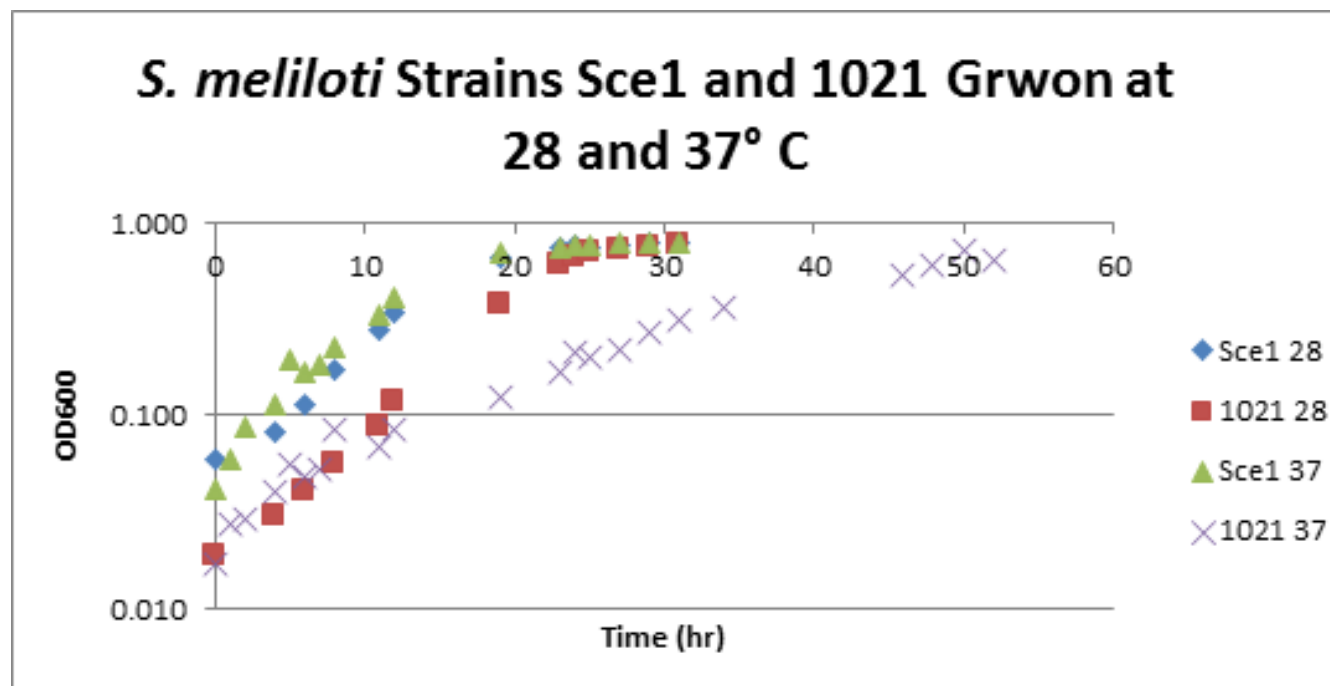
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# Cell Wall and Membrane Properties in *Sinorhizobium meliloti* Sce1 as a Response to NaCl and Temperature Stress

Caroline Finn/2015

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*S. meliloti* is a bacterium endogenous to soil. This bacterium is of crucial importance to plant growth on nitrogen limited soils since it can fix atmospheric nitrogen and supply this biological available nitrogen to the plant in so called root-nodules. *S. meliloti* strain 1021 is a model strain to study root nodulation as well as soil survival. Soil is a heterogeneous environment in which bacteria are exposed to many different stresses. Two such stresses are salinity and drought. In a paper published in 2013, Vriezen et al. reported on the identification of *S. meliloti* mutants carrying tn5LuxAB transcriptional fusions responsive in NaCl stress. Some characterizations on one such mutant (Sce1), including the ability to survive drought, will be presented in Vriezen et al. (2014, in press). Observations of the mutant strain lead to the hypothesis that the tn5luxAB mutation affects cell wall and membrane properties. Thus, tests were run to compare strain 1021 (WT) and mutant Sce1 to identify the function of locus tn5LuxAB. Such tests include creating an ex-conjugate of Sce1 to compare any differential findings between the wild type and mutant. As well, numerous growth curves were run comparing strain 1021 and Sce1 under conditions of desiccative stress such as increased temperature and salinity. Results of these growth curves indicate the possibility of a slight difference between 1021 and Sce1 when grown under temperature stress. Initial results direct that grown at 36°C 1021 grows at a slightly lower rate than Sce1 (Graph 1). Strain 1021 and Sce1 initially grow at the same rate at 37° however, at a point during exponential phase growth of 1021 diverges from the typical growth pattern. Most significantly tests preformed in this study have initiated further and novel research directions to evaluate tn5LuxAB in *S. meliloti*.



Graph 1

Growth curve of strain 1021 and Sce1 grown at 28°C and 37°C on a logarithmic scale.

(Supported by the Schultz Foundation)

Advisors: Jan Chris Vriezen and Christine White-Ziegler, Biological Sciences





# Thermoregulation of Virulence Factors in Uropathogenic *Escherichia coli* using QRT-PCR

Arielle Gomes-Williams/2015 and Addison Reine/2015

## Introduction:

As Uropathogenic *Escherichia coli* (UPEC) infects a host, temperature regulates important virulence factors such as fimbriae, biofilm formation, and toxins (1,7,8). This study focused on six necessary virulence factors: Hemolysin (hly) has the ability to lyse host cells for more efficient iron acquisition to infection, secreted autotransporter toxin (sat), utilizes its toxicity to break down the bladder and kidney cells, flu assists with biofilm formation, ivy defends the infection from the host's lysosome immune response (4,5). PapB and csgA are both operons that encode for adhesions (3,6).

## Methods:

To retrieve samples, cultures were grown under aerobic conditions (shaking at 225 RPM) in M9 minimal media containing glycerol as a carbon source at 23°C. This starter culture was split at the start of the experiment (t=0). After, half of the flasks were shifted to 34°C and 37°C while the control half remained at 23°C. The bacteria were harvested at various time points after the shift in mid-log phase ( $OD_{600} \approx 0.3-0.4$ ), in order to negate growth phase-associated expression changes.

RNA isolation was performed for QRT-PCR to quantify samples.

## Results:

The results determined that papB and csgA increased in expression at 28°C and decreased in expression at 30°C at 4 hours in comparison to the control at 23°C (See Figures 1-4).

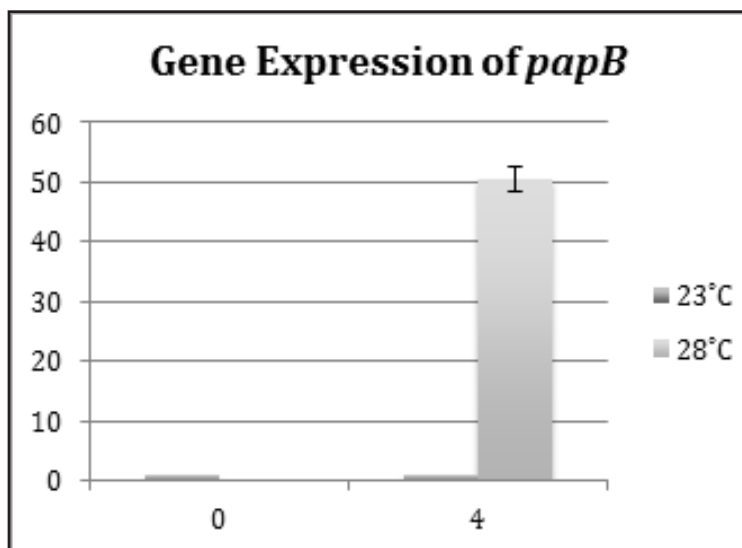


Figure 1. QRT-PCR results of the gene papB at time points 0 hours and 4 hours at 23°C and 28°C

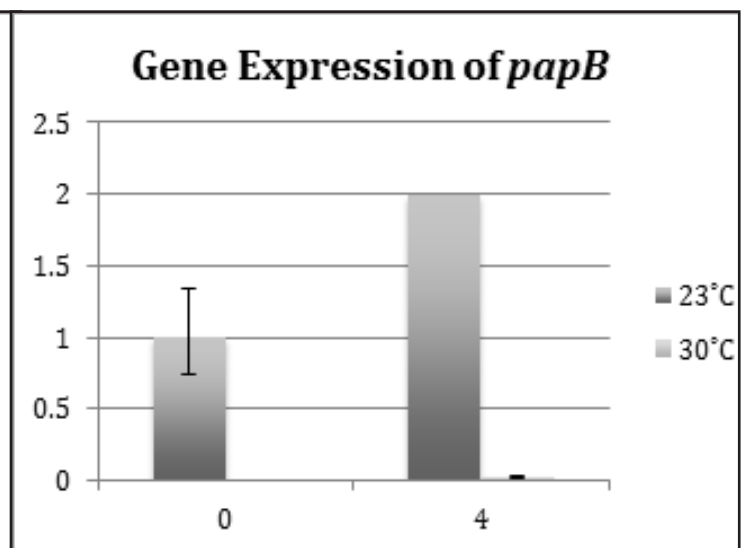


Figure 2. QRT-PCR results of gene papB at time points 0 hours and 4 hours at 23°C and 30°C

The results determined the genes *flu*, *hly*, and *sat* increased at 34°C at 4 hours in comparison to 23°C (See Figure 5-7). The results determined that gene *ivy* decreased at 34°C at 4 hours in comparison to 23°C (See Figure 8).

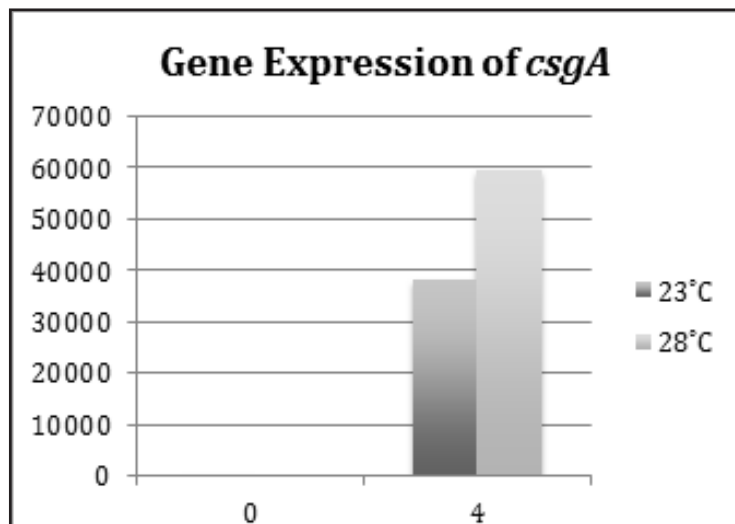


Figure 3. QRT-PCR results of the gene *csgA* at time points 0 hours and 4 hours at 23°C and 28°C

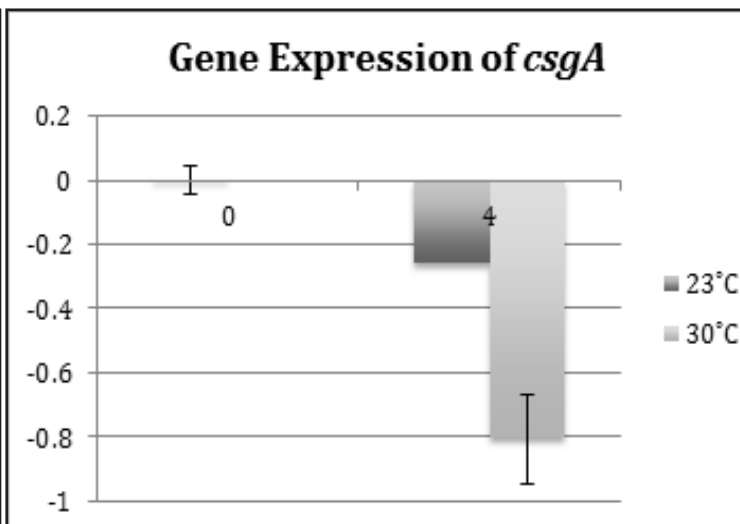


Figure 4. QRT-PCR results of the gene *csgA* at time points 0 hours and 4 hours at 23°C and 30°C

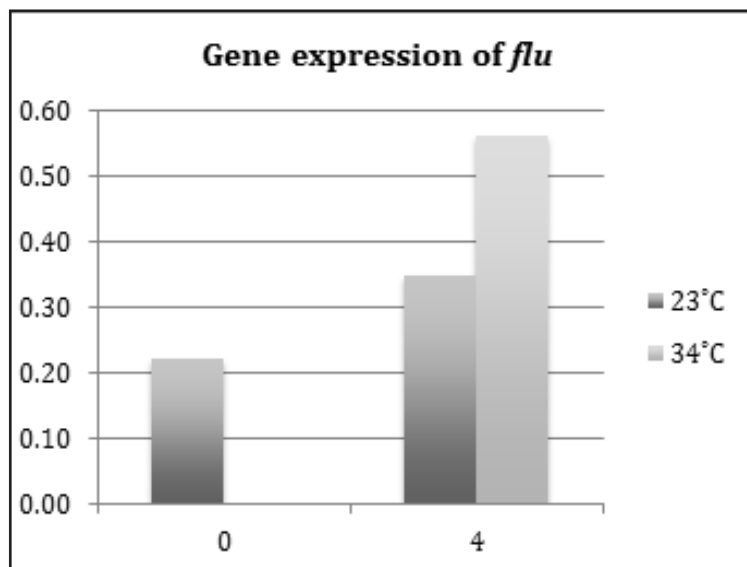


Figure 5. QRT-PCR results of the gene *flu* at time points 0 hours and 4 hours at 23°C and 34°C

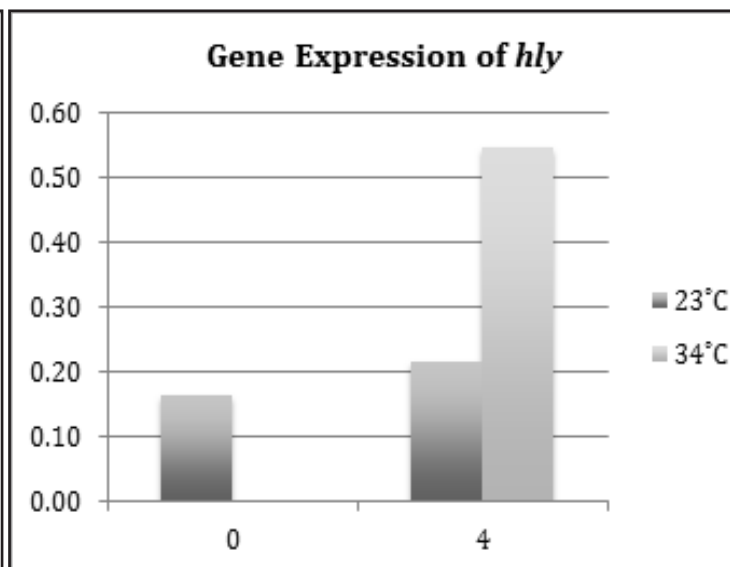


Figure 6. QRT-PCR results of the gene *hly* at time points 0 hours and 4 hours at 23°C and 34°C

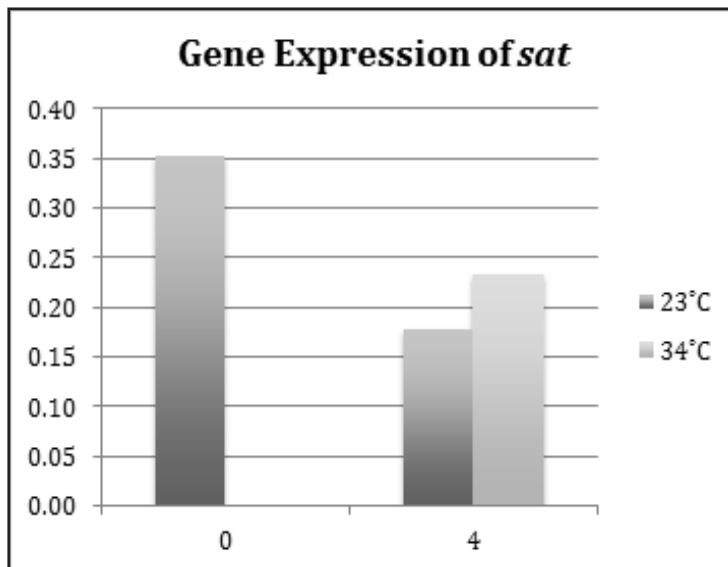


Figure 7. QRT-PCR results of the gene sat at time points 0 hours and 4 hours at 23°C and 34°C

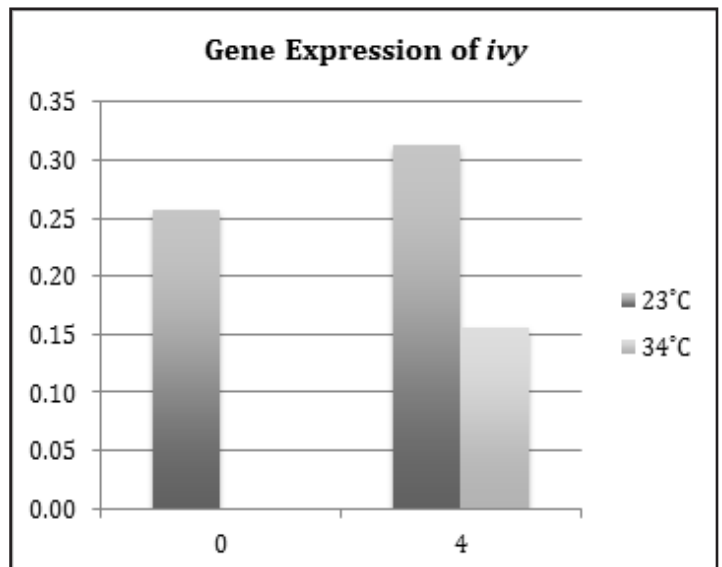


Figure 8. QRT-PCR results of the gene ivy at time points 0 hours and 4 hours at 23°C and 34°C

## Discussion

The expression of each virulence factor changes at different temperatures because the transition from 23 °C to 37 °C is a gradual shift influenced. As the infection gradually increases in temperature, different virulence factor are programed to turn on, while others turn off. For instance, flu, hly, and sat increased at 34 °C, while ivy decreased showing that while the infection does not utilize ivy's lysosome inhibitor against the host immune response at this point in the temperature shift.

Understanding the virulence factors that are upregulated at specific temperatures allows us to know which parts of the urethra are the most vulnerable to colonization as well as how quickly UPEC becomes virulent in relation to the thermoregulatory response.

*(Supported by the Howard Hughes Medical Institute)*

Advisor: Christine White-Ziegler, Biological Sciences

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# Using Synthetic Multi-Fluorescence Techniques in Yeast for Single Molecule Microscopy

Malayna Hocker/2015

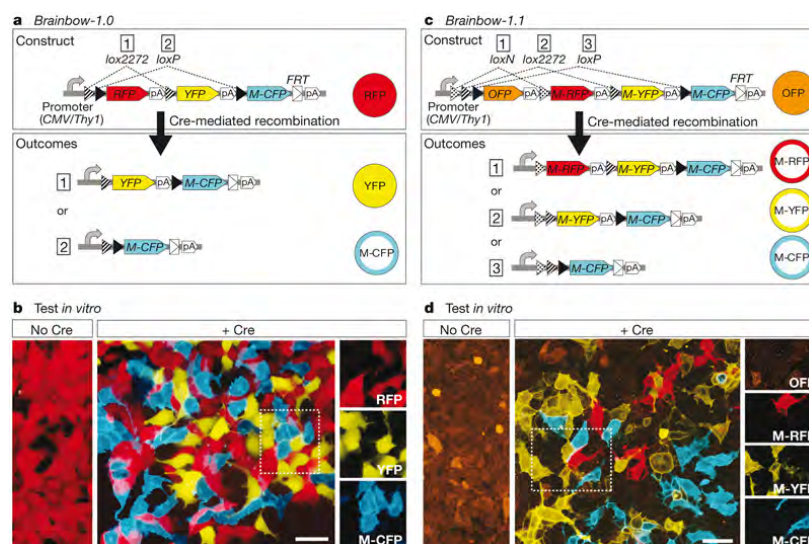


Figure 1: Brainbow 1.0 and 1.1 cassette design.

[http://www.nature.com/nature/journal/v450/n7166/fig\\_tab/nature06293\\_F1.html](http://www.nature.com/nature/journal/v450/n7166/fig_tab/nature06293_F1.html)

Nathan Derr's Lab focuses on using synthetic biology in budding yeast and various microscopy techniques as means of gaining a better understanding of molecular functional processes, primarily Dynein.

In synthetic biology, researchers use genetic modification as an aid in understanding cellular and molecular processes and in order to manipulate and employ said processes for other purposes. One technique used is Brainbow, in which color cassettes are inserted into neurons in order to stochastically produce colors in order to visualize neural pathways more clearly. In this same experiment, the use of a site-specific, inducible recombinase gene, Gal-Cre, allowed for a Brainbow gene cassette to be recombined, offering a larger array of colors (Livet 2007).

In this project, the aim was to modify these techniques to specifically pertain to Dynein in budding yeast. By placing a precisely engineered cassette at the C-terminus of Dynein and using the inducible Gal-Cre recombinase, we can track generations of Dynein based on exposure to galactose in order to learn more about the molecules roles in the cell.

This summer, we worked towards transforming the budding yeast with the Gal-Cre gene, which will allow the cell to produce and utilize the recombinase under the presence of galactose. This occurred in two different transformation steps. First, adenine and uracil deficient yeast with a non-functional adenine gene were transformed, replacing the malformed adenine gene with a functional uracil. Before moving forward, properly transformed cells were selected for and tested using a series of plating and PCR. Plating these cells onto uracil negative plates ensured us that any cells growing, contained uracil, and were therefore transformed with uracil. PCR checked size and placement. The second transformation replaced the previously implanted uracil gene with the Gal-Cre recombinase gene. Although it has not been confirmed through sequencing, PCR indicates that this transformation has been completed successfully.

In further research, we will verify that the transformation was completed correctly, design an experiment unique cassette, and transform it into the Dynein gene. After a complete genetic yeast strain is acquired, microscopy will be used to continue research and observations on mechanisms and roles of Dynein.

*(Supported by the Howard Hughes Medical Institute)*



Advisor: Nathan Derr, Biological Sciences

References:

Livet, Jean et al. (2007). Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature*, Vol 450 (56-63).

# Controlling Fluorescence in *S. Cerevisiae* by Insertion of Cre Recombinase

Naima Javed/2015 and Beck Worrell/2016

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The main goal of our project was to recreate Brainbow in yeast. Brainbow is a new technique which programs cells to express different color fluorescent proteins using Cre-lox recombination depending on the orientation of small genetic recognition sites called lox sites. DNA segments between the lox sites are inverted if the lox are situated in the opposite direction (figure A), or the segments are cut out if the loxP sites are situated in the same direction (figure B).<sup>1</sup>

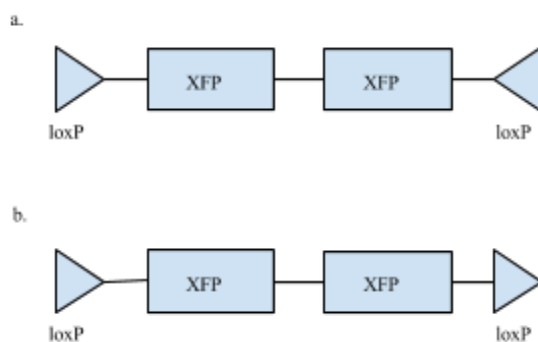


figure A: LoxP sites situated in opposite direction. Results in inversion.

figure B: LoxP sites situated in same direction. Results in excision.

Yeast are particularly easy and useful to work with because they are single-celled, eukaryotic organisms with a short generation time in defined media with the ability to double their population every 90 minutes. They have similar cellular organization to some animals, and their genome can be easily manipulated to add or delete genes.<sup>2</sup>

To begin our research, we grew up the plasmid (RPB20) which contained the uracil (URA) gene and isolated it using a Quigen Mini Prep kit. We then transformed the isolated URA gene into DY1, our starting yeast strain, by targeting a non-functional adenine gene. DY1 was originally deficient of the URA gene so it was possible to select only the transformed colonies by growing them on -URA plates so only cells that made URA survived. We then performed PCR using different colonies and used the colony with the most accurate PCR product as our second strain (DY2).

For our next transformation we isolated the Gal/Cre gene from plasmid (pBF3060) and targeted the now functional URA gene in yeast strain DY2. After the transformation was complete we plated these colonies out on 5FOA plates which selected against cells that produced their own URA, preserving only those cells that have the Gal/Cre gene in the correct place.

Unfortunately, checking for Gal/Cre using Sanger sequencing did not reveal Gal/Cre to be transformed into DY2 which forced us to backtrack, checking to make sure URA did in fact get transformed into DY1. Since experimentation to check for URA in DY2 produced positive results, our current efforts have been focused on repeating our initial transformation of Gal/Cre.

The research completed this summer is only the beginning of this project, which we will continue in the academic year 2014-2015 through special studies. After successfully inserting Gal/Cre into yeast we will insert the brainbow cassette containing lox sites and XFPs. Accomplishing this will allow us to develop future experiments regarding biological sensors and circuits.

*(Supported by the Blakeslee Fund in the Biological Sciences)*

Advisor: Nathan Derr, Biological Sciences

<sup>1</sup> Livet, Jean, Tamily A. Weissman, Hyuno Kang, Ryan W. Draft, Ju Lu, Robyn A. Bennis, Joshua R. Sanes, and Jeff W. Lichtman. "Transgenic Strategies for Combinatorial Expression of Fluorescent Proteins in the Nervous System." *Nature* 450.7166 (2007): 56-62. Web.

<sup>2</sup> Botstein, D. "GENETICS: Yeast as a Model Organism." *Science* 277.5330 (1997): 1259-260. Web.

# Generating a Simple Genetic Bar-code for Molecular Identification of Marine Mammal Parasitic Nematodes: Groundwork for Future Diagnostics

Caroline Keroack/2014

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Marine mammal parasitology is a relatively understudied subject. Very little is known about the nematode parasites that infect these organisms, save those parasites that have a direct impact on human health. Thus, developing an understanding of the basic biology and species diversity of these parasites is of great importance. Controlling parasitic infections, especially those of endangered species, is of utmost importance for conservation. Currently, very little molecular sequence data exists for these organisms. This work focuses on generating molecular data on several marine mammal parasites including morphologically identified specimens (*Acanthocheilonema spirocauda*, *Dipetalonema odendhali*, *Otostrongylus circumlitis*, *Halocercus delphini*, *Anisakidae* spp, *Pseudoterranova* spp, *Crassicauda* spp, *Crassicauda boopis*, *Stenurus minor*), as well as over 100 unidentified parasites from various stranded marine mammals. Work was done on optimizing a simple combination 'bar-code' sequence for species identification. Genes tested to be used in the bar-code include the 12s ribosomal subunit, the 5s ribosomal subunit, the small ribosomal subunit (SSU), cytochrome oxidase c subunit I (COI), and internal transcribed spacer region 2 (ITS2). COI and ITS2 proved useful as a barcode for species identification. When COI and ITS2 failed, SSU was found to be the best substitute. Unidentified gastric parasite of the harbor porpoise (*Phocoena phocoena*) were identified as *Anisakis simplex*, a common and plausible parasite for the aforementioned host, using the COI/ITS2 sequence combination, proving the utility of this gene combination in parasitic nematode species identification. Furthermore these genes will be used as 'anchor' genes in designing long PCR primers to amplify the entire mitochondrial genomes of these organisms. Entire mitochondrial genomes can then be used as potential diagnostic PCR assays (figure I).

*(Supported by the Nancy Kay Holmes Fund)*

Advisor: Steven Williams, Biological Sciences

Acknowledgements: I would like to thank Sea Rogers Williams VMD of the National Marine Life Center, Frederick Wenzel of NOAA, Northeast Fisheries Science Center, Woods Hole, Ma., and Katie Pugliare of the New England Aquarium for providing parasite specimens.

# Xq28 Inversion Polymorphism Assay: Another Approach

Cait Kirby/2015

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For four years I have been researching questions relating to an inversion polymorphism on the long arm of the X chromosome: A) is this inversion polymorphism common to all mammals?, B) can I prove the history and frequency of this inversion?, and C) can I make an accessible teaching lab for students to explore this inversion polymorphism?

This inversion polymorphism involves the emerin and filamin genes, which are bordered by two inverted repeats.<sup>1,2</sup> These inverted repeats are more than 99% identical to one another in humans, and homologous repeats are found in all mammals studied.<sup>3</sup> Most interestingly, the order of the emerin and filamin genes between these repeats is variable in the population - some individuals have their emerin genes closest to their telomeres, in others their filamin genes are telomeric, and some have one X chromosome with one orientation and one with the other.<sup>1,2</sup> There are no phenotypic effects related to one orientation, but the exploration of this inversion polymorphism is fascinating and can act as a guide to teaching genetics.

During my first two SURF summers I was able to design the assay to prove the variable orientations in the population, and we have begun to use that lab in our introductory genetics course at Smith College. During my third SURF summer I focused on sampling populations to determine if there is variability between different groups according to ancestry, as well as to do analysis on SNPs (which is variation at the nucleotide level) and determined that there is a relationship between orientation and SNPs at certain positions. This summer I began to expand my research into other mammals by focusing on questions (A) and (B) above and followed up by experimenting with a new protocol to prove orientation in a new way in a teaching lab setting (C). These SURF experiences have taught me resilience and thoroughness in problem-solving, as well as deepened my desire to do research.

A & B) I used bioinformatics to determine the conserved regions of genes flanking the inverted repeats. I then designed primers in the conserved regions as well as PCR protocols to amplify large regions of DNA, which will ultimately be sequenced. I will design similar assays to the one we currently use in humans to determine orientation in each species. The PCR primers work in humans, and theoretically work in other mammals, but until the receipt of mammalian samples, I am unable to confirm the specificity of this protocol. During the summer months I secured agreements with various zoos in the region to obtain DNA samples from numerous species and am awaiting approval from IACUC to move forward with this experiment.

C) I have begun optimizing a protocol outlined in a recent publication called iPCR.<sup>4</sup> iPCR involves enzyme digestion of genomic DNA with BglII, ligation of the digested fragments with T4 DNA ligase, and PCR amplification of the ligated template to determine orientation. Following the outlined procedure in the paper, I was unable to replicate their results. In my subsequent experiments, I have taken the protocol as a guideline and have received slightly promising results, though the results indicate an issue with our reagents. I will continue these experiments until the protocol is optimized and feasible in a teaching lab setting.

*(Supported by the Blakeslee Fund in the Biological Sciences)*

Advisor: Robert Merritt, Biological Sciences

<sup>1</sup>Small, Kersten, Jane Iber & Stephen T. Warren. Emerin deletion reveals a common X-chromosome inversion mediated by inverted repeats. *Nature Genetics*, 16: 96-99.

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<sup>4</sup>Aguado, Cristina, Magdalena Gaya-Vidal, Sergi Villatoro, Meritxell Oliva, David Izquierdo, Carla Giner-Delgado, Victor Montalvo, Judit Garcia-Gonzalez, Alexander Martinez-Fundichely, Laia Capilla, Aurora Ruiz-Herrera, Xavier Estivill, Marta Puig & Mario Caceres. Validation and Genotyping of Multiple Human Polymorphic Inversion Mediated by Inverted Repeats Reveals a High Degree of Recurrence. *PLOS Genetics* 10: 1-16.

## “Figuring Out” the Xq28 Inversion

Natalie Kolber/2017

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The Xq28 region on the long arm of the X chromosome in humans contains a region characterized by two inverted repeats 11.3 kb in length that are >99% identical. In between these non-coding repeats are the genes that code for filamin (FLNA) and emerin (EMD). Partial deletion of EMD leads to a condition known as Emery-Dreifuss muscular dystrophy, while deletions in FLNA are not survivable. However, the order in which these two genes occur on the chromosome varies between individuals with no apparent phenotypic affect. Chromosomes with FLNA occurring centromeric to EMD are said to carry the plus (+) arrangement, while chromosomes with EMD occurring centromeric to FLNA are said to carry the minus (–) arrangement,<sup>1</sup> progressive muscle weakness and cardiomyopathy. The emerin gene, located in human Xq28, is approximately 2 kb in length, is composed of 6 exons and falls within a 219-kb region that has been completely sequenced. Immediately centromeric to emerin is the 26-kb filamin gene (FLN1). Females, who typically have two X chromosomes, can be either plus homozygotes, minus homozygotes, or heterozygotes. Males, who typically have only one X chromosome, can be either plus or minus hemizygotes.

An investigation in which students use PCR and gel electrophoresis to determine their own gene orientation at this region has shown to be an enlightening lab exercise in Smith classrooms. Not only do students have the opportunity to learn something about their own genomes, but the exercise also helps teach some challenging topics, including changes in chromosome structure and the process of crossing over/gene conversion.

My main task for my five weeks of SURF was to prepare figures for the Merritt lab’s upcoming publication on the topic. I set out to convey concepts including crossing over, gene conversion, and chromosomal inversion loops with the understanding that my audience included students just learning about these ideas. Comprehensible figures would be exceptionally important, given the paper’s pedagogical goals. I created five images for the paper, two of which are below. Additionally, I helped double-check and clean up sequence data that was needed for the paper.

Ongoing investigations by the Xq28 group involve examining whether one orientation is more prevalent in certain racial groups and expanding the research into other eutherians. While I will not be continuing with the Merritt lab, I will continue to contribute figures to the paper in the fall while doing research in another Smith lab.

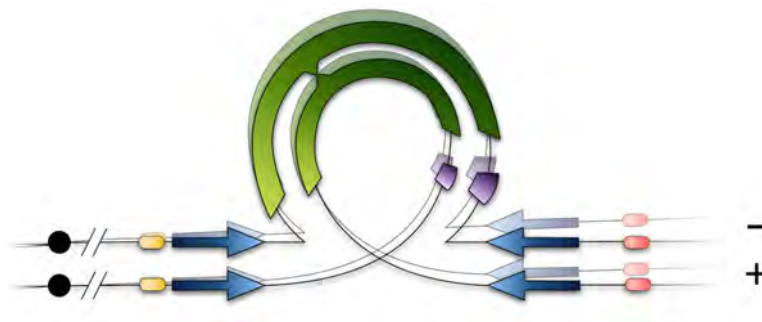
*(Supported by the Blakeslee Fund in the Biological Sciences)*

Advisor: Robert Merritt, Biological Sciences

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1. Small, K., Iber, J. & Warren, S. T. Emerin deletion reveals a common X-chromosome inversion mediated by inverted repeats. *Nat. Genet.* **16**, 96–99 (1997).

a)



B



b)



Figure 1a. A +/- heterozygote in prophase with a paracentric inversion loop and a crossover in the FLMN gene. 1b. This crossover results in a duplicatoin/deletion anaphase bridge and an acentric fragment. Such a chromatid would not be able to separate to produce viable offspring. Together, these figures explain why we tend not to see crossing over in inversion heterozygotes.

# Propagation of Rare and Endangered Species at the Botanic Garden of Smith College

Jacqueline A. Maasch/2015

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The objective of this project was 1) to expand the collection of rare and endangered species at the Smith College Botanic Garden, 2) to propagate these species in order to build populations from which germplasm can be distributed to other botanical institutions, and 3) to test the efficacy of various vegetative propagation methods. Species were selected based on inclusion in the International Union for Conservation of Nature's Red List of Threatened Species or similar criteria, and included 24 members of the following genera: *Acer*, *Amentotaxus*, *Cupressus*, *Cycas*, *Euphorbia*, *Ilex*, *Magnolia*, and *Osa*. Unless an optimal propagation method for a species was previously determined, propagules were subjected to multiple treatments (e.g. a quick basal dip of 20,000 ppm K-IBA hormone, a 24-hour basal soak in 100 ppm K-IBA hormone, and no treatment for control). The results of this project were diverse in nature, and included unexpected success rooting the endangered *Acer skutchii*<sup>1</sup> from cuttings after years of failed efforts; two collecting trips to the Arnold Arboretum, which introduced 9 threatened *Magnolia* species to the Botanic Garden's collection; and compilation of the country's most genetically diverse stock of the endangered *Acer griseum*.<sup>2</sup> As a secondary goal of this project was inter-institutional collaboration and exchange, the distribution of 137 propagules of three endangered *Cupressus*<sup>3</sup> species to 12 institutions in California, Arizona, New Mexico, and Florida was particularly successful. Additionally, I am collaborating with my SURF advisor on a paper that proposes a new nomenclatural category for cultivated plants that would facilitate ex situ plant conservation and germplasm exchange. I will be continuing my work with rare and endangered plants throughout the 2014–2015 school year as a work-study employee.

*(Supported by the Margaret A. Walsh Grantham Fund)*

Advisor: Rob Nicholson, Biological Sciences and Botanic Garden

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<sup>1</sup>Gibbs, D., and C. Yousheng. 2009. The Red List of Maples. Botanic Gardens Conservation International, Richmond, UK.

<sup>2</sup>Ibid.

<sup>3</sup>The IUCN Red List of Threatened Species. Version 2014.2. <[www.iucnredlist.org](http://www.iucnredlist.org)>. Downloaded on 25 August 2014.

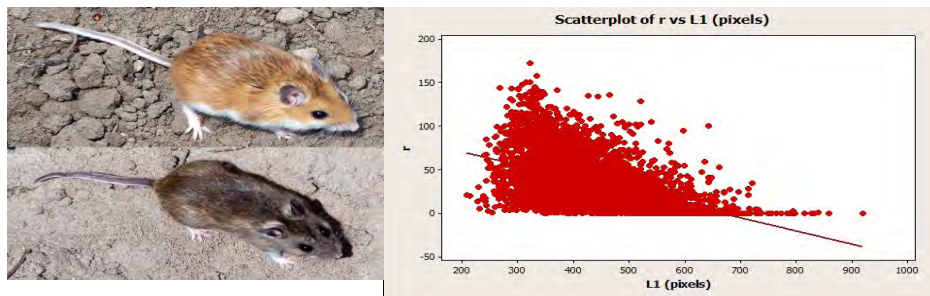
<sup>4</sup>Ibid.



Left: Taking cuttings from *Euphorbia mayurnathanii*, a succulent that is extinct in the wild.<sup>4</sup>  
Right: *Cupressus atlantica* seedlings grown from wild-collected seed.

# An Analysis of Pleiotropic Effects of the Agouti Gene in the Genus *Peromyscus*

Jessica Magri/2017



Is the Agouti gene pleiotropic in *Peromyscus*? A mutation in the Agouti gene may not only determine a deer mouse's coat color but may also have an effect on its body size. *Peromyscus maniculatus gracilis* deer mice that possess non-agouti alleles tend to have a darker coat color and an increased tendency towards obesity than those deer mice that are homozygous for the agouti allele.<sup>1</sup> I collected data on coat color from 45 other species of deer mice and analyzed the data for a relationship with head-body length. If the non-agouti allele influences melanism across species then darker animals should also be heavier.

Over 1500 photographs were taken of deer mice specimens at the United States National Museum. These pictures were standardized for black and white and then, using pixel-color sampling in Adobe Photoshop CS6, red, green, and blue (RGB) values were taken for individual deer mice at the mid-dorsal region. Note that low RGB values represent darker coat colors. Head-body length was also measured for each specimen directly from the photographs. Up to ten mice were sampled from each photograph excluding juveniles. If a subspecies had greater than 20 pictures, pictures were chosen based on location. In total, over 6000 specimens were sampled.

The scatterplot of color vs. head-body length illustrates that the RGB values decreased (got darker) as the specimen's head-body length increased, as predicted. Regression analysis using Minitab software confirms that this trend is significant ( $p < 0.0005$ ), indicating that the Agouti gene may indeed have pleiotropic effects on deer mice.

My research will continue next year when I resume my STRIDE scholarship in the fall. I intend to see if body size and/or coat color is connected to location or gender and if subspecies within species have the same trends.

*(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)*

Advisor: Virginia Hayssen, Biological Sciences

## References:

Hayssen, V. (2001). Body and organ mass in Agouti and non-agouti deer mice. *Comp. Biochem. Physiol., A: Comp. Physiol.* 130, 311-321.

# The Presence and Consequences of Lateral Gene Transfer in Fungi

Jiaqi Mei/2016

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The objective of this independent student research is the classification of opisthokonta (fungi, animals, and their microbial relatives) along with tracing the possible presence of lateral gene transfer in fungi. Since the hypotheses of Aristotle and Plato, biologists have endeavored to create numerous classification models of organisms.<sup>2</sup> Yet, these evolving models of classification corroborate with the idea that the field of phylogenetic taxonomy is in constant flux due to ongoing research, most recently of genomes. Consequently, the classification of organisms and their corresponding biological diversity is often interpreted in terms of morphological, structural, ecological, behavioral, metabolic, and genetic aspects—all relative to the field of microbiology.<sup>2</sup> Yet, recent advances in molecular phylogeny have postulated the notation of quantitative models of phylogenetic “trees.”<sup>22</sup> One complication in these phylogenetic “trees,” however, is the existence of lateral gene transfer (LGT). LGT refers to the transmission of genetic material between species, and contrasts with the traditional view of vertical inheritance from parent to offspring.<sup>2</sup> Although LGT has been extensively studied in bacteria, the presence and consequences of LGT in opisthokonta (and indeed eukaryotes in general) remains relatively unknown.<sup>2</sup>

My research project largely relies on bioinformatics-based methodologies in investigating those LGT events in fungal/opisthokonta genomes. For the purposes of my independent research, I relied on primarily on Python, NCBI, BLAST, Seaview, along with various references to the encyclopedic research sites dedicated to opisthokonta. Initially, I began with analyzing phylogenetic gene trees produced by a phylogenomic pipeline that is available in the Katzlab. The strongest evidence for LGT are examples of interdomain transfers (e.g. from bacteria to fungi), which a considerable quantity of these examples can/are supported by absence/presence trees.<sup>1</sup> The term absence/presence tree refers simply to those phylogenetic gene trees that contain (in this case) bacterial, archaea, and fungal species. By noting bootstrap values, I was then able to assess the individual gene trees in which fungi species were nested within bacteria and/or archaea clades. Afterwards, we ran a python script through the targeted presence/absence trees, noting the number of fungal occurrences (for each individual fungal species) in each of the gene trees.

In the initial run of the phylogenomic pipeline, there were 1550 genes found in at least four of the five major clades (although it was usually vertical transfers). There were only 241 genes that qualified for the criteria set for absence/presence trees, and only 205 genes were judged to show “strong” LGT. After the 241 trees were run through guidance with more stringent parameters, only of the 153 absence/presence trees were considered suitable. From those 153 absence/presence trees, a python script screened and noted the trees for the number and taxa of the specific fungal occurrences in each gene tree. The preliminary results from the python script showed a significant number of LGTs occurring in the Ascomycota and Basidiomycota.

Although, there are significant barrier in prokaryote to eukaryote LGT such as the presence of introns, lack of potential donors or interactions, or the viability of a transfer of genetic material.<sup>1</sup> Recent research studies, however, suggest traces of a feasible if complex relationship between LGT and eukaryotes.<sup>3</sup> Genetic evidence even suggests that the consequences of LGT can influence vital niche specification, metabolic capabilities, and even pathogenicity in eukaryotic organisms.<sup>3</sup> Therefore, in the following academic semester, I plan to continue my research as a special study by examining the timing of the LGT events, or even potential trends and patterns among the genes transferred in the LGTs. Yet, in order to trace and evaluate further consequences of LGT, continuation of bioinformatics research into eukaryotic genomes is crucial. With the extensive sampling of fungal genomes, further examination of LGT in fungi will afford us the capability to extensively analyze these eukaryotic evolutionary mechanisms

*(Supported by the Schultz Foundation)*

Advisor: Laura Katz, Biological Sciences

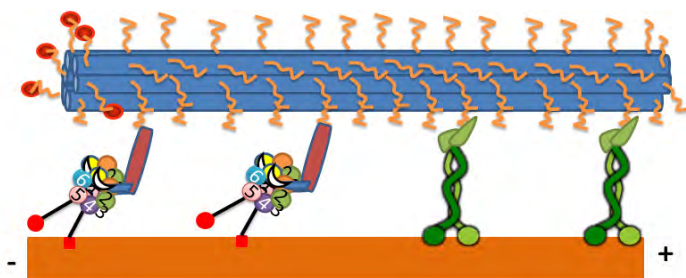
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2. Keeling, P., & Palmer, J. (2008). Horizontal Gene Transfer In Eukaryotic Evolution. *Nature Reviews Genetics*, 605-618.

3. Marcet-Houben, M., & Gabaldón, T. (2010). Acquisition of prokaryotic genes by fungal genomes. *Trends in Genetics*, 5-8.

# ATPase Activity in a Tug-of-War Between Motor Proteins

Jessica Morgan/2017 and Amelia Yeoh/2017



A cell is like a city filled with streets (microtubules and actin) and buildings (organelles).<sup>1</sup> Moving along these streets are cars (motor proteins) with loads of cargo using ATP as fuel. Multiple types of motor proteins (kinesin and dynein) can be used to transport cargo in a cell; however, these motors move in opposite directions and become motionless in a tug-of-war.<sup>1</sup> In this project, we sought to determine the amount of energy required for one motor type to win the tug-of-war and the amount of energy lost when the motor proteins are in an immobile state.

In order to determine the energy consumption of dynein and kinesin motor proteins, we attached dynein and kinesin at different ratios on a synthetic cargo from (Derr et al, 2012). Using an ATP assay, we sought to look at the ATP hydrolysis in each system and measure the conversion of ATP to ADP and inorganic Phosphate using a fluorescence technique. For our control, we synthesized a mutated dynein incapable of hydrolyzing ATP. To create the synthetic cargo, single-stranded DNA was folded into 12-helix bundles and short oligo strands known as staples were used to hold the shape of the DNA structure. Dynein and kinesin motors were purified from yeast cells. Motor proteins were then attached to cargos via complimentary base pairing of handles and antihandles (short oligo strands stemming off of the cargo and motor proteins). To measure the ATPase activity we sought to use an Enzcheck phosphatase kit, which would allow us to detect the optical density of inorganic phosphates as they were released in ATP hydrolysis in a microplate reader. Our results showed that dynein can be successfully purified from yeast cells.

With the time spent on the project this summer, we learned the skills and techniques required to perform our experiment and other experimental work in the Derr Lab. Our plans are to continue with this project in a conjoint special studies, in which we shall work to determine the energy consumption of motor proteins when they are in a tug-of-war.

*(Supported by the Howard Hughes Medical Institute)*

Advisor: Nathan Derr, Biological Sciences

<sup>1</sup>Council-Garcia, C.L. (2002). Cell Structure and Function. Retrieved from [http://biology.unm.edu/ccouncil/Biology\\_124/Summaries/Cell.html](http://biology.unm.edu/ccouncil/Biology_124/Summaries/Cell.html)

<sup>2</sup>Derr, N., Goodman, B.S., Jungmann, R., Leschziner, A.E., Shih, W.M., & Reck-Peterson, S.L. (2012). Tug-of-War in Motor Protein Ensembles Revealed with a Programmable DNA Origami Scaffold. *Science*, 338, 662-665.



# Identifying Specific Zebrafish Radial Fibers (Zrf) 2, 3, and 4 Protein Targets in the Developing Zebrafish Brain: *Zrfs Define the Diencephalic Glial Bridge as a Heterogenous Population of Astroglial Cells.*

Maggie Murgo/2016

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Proper commissure formation, a grouping of nerve fibers that connect the two sides of the central nervous system, in the forebrain is essential for a functional organism. The developing nervous system has helpers known as attractants and repellants that guide axons through the midline, which leads to commissure formation.<sup>1</sup> The Barresi lab uses zebrafish, because their central nervous system is very similar to the mammalian central nervous system. Zebrafish embryos are transparent, which allows us to observe them at various stages in embryonic development. This allows us to study the formation of commissures at cellular and molecular levels.

Barresi and colleagues have shown astroglial cells to be nurturing and supportive to axonal growth.<sup>2</sup> There are four Zrf proteins, which were specifically engineered to help in the understanding of the hindbrain structure. The Zrf-1 antibody has been identified as glial fibrillary acidic protein (Gfap). Gfap is also expressed in astroglial cells, which means astroglial cells can be recognized by the Zrf-1 antibody. The identity of Zrf 2-4 is still unknown.

Identifying the remaining Zrfs is important for understanding more in depth glial development. Using biochemical techniques, including the western blot and immunoprecipitation, the identity of Zrf 2-4 proteins. This summer we were able to confirm the Zrf-1 antibody is Gfap. We are still on the search for the protein identities of Zrf 2-4.

*(Supported by the National Science Foundation)*

Advisor: Michael Barresi, Biological Sciences

<sup>1</sup> Kaprelian Z, Runko E, Imondi R (2001). Axon guidance at the midline choice point. *Developmental Dynamics* 221: 154-181.

<sup>2</sup> Barresi MJ, Huston LD, Chien CB, Karlstrom RO (2005). Hedgehog regulated Slit expression determines commissure and glial cell position in the zebrafish forebrain. *Development*. 132(16): 3643-3656.

# Metagenomic and Bioinformatic Study of the Murine Gastrointestinal Tract Before and After Treatment with the Anthelmintic Medication, *Ivermectin* and *Mebendazole*.

Hangyi Pan/2015

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Infections caused by *Trichuris trichiura* has known to be affecting approximately 800 million people worldwide, with most victims being children. Children suffering from *Trichuris trichiura* infections exhibit signs of malnutrition, stunted growth and intellectual retardation. Despite that the commonly used anthelmintic medication *Ivermectin* and *Mebendazole* are very effective in combating nematodes, both would also work against bacteria and possibly also archaea inside gastrointestinal tract, resulting in changes in microbiome. Infection by *Trichuris muris* in mice has been contributing greatly in understanding *T. trichiura* infections in humans.<sup>1</sup>

This project intends to understand the changes in microbiome after *Trichuris* infection as well as possible disruptions due to anthelmintic treatments such as *Ivermectin* and *Mebendazole* by using mice model. A total of 50 mice were included in this controlled experiment (grouping criteria see Table 1). All mice are genetically the same and were kept at University of Wisconsin where they were infected, treated and cared for with controlled living environment including diet. Fecal samples were collected usually on a weekly schedule and sent to our lab. Microbial DNA was extracted from the received fecal samples. The extracted DNA was later amplified and were barcoded using PCR. The DNA samples would later be sequenced using NextGen sequencer in order to reveal the composition of the microbiome. Bioinformatics would be applied in analyzing the results.

This summer, I have finished extracting DNA from several important time points of collection: the date before infection, the date after successful infection and the date after treatment. Blue group consistently yield good quality DNA (comparing to other groups) (concentration  $\geq 20\text{ng}/\mu\text{L}$ , 260/280 value  $\in [1.80, 2.00]$ , with distinct peak at 260 in nanodrop spectrometer results) and was thus the first group used for PCR. Four sets of primers recommended by Klindworth et al. (2013) were tested for best performance in amplifying V4 region of 16s rRNA gene for both archaea and bacteria, and A519F, 802R were proven to work best in our experiment, even comparing to previous primer set used in our experiment<sup>2</sup> New primers were barcoded and attached with adaptors for later sequencing on Illumina MiSeq Sequencer following instructions in Caporaso et al. (2011).<sup>3</sup>

To understand how *Trichuris* infection and its treatment would disrupt the equilibrium in microbiome, I will sequence the V4 region of 16s rRNA gene when I continue this project in fall. Together with a statistics student, we will be working together using bioinformatics and statistics to analyze and understand the significance of data generated by the sequencer.

(Supported by the Howard Hughes Medical Institute)

Advisor: Steven Williams, Biological Sciences

Table 1. Group set up for animal experiment

| Group Color (Tube labels)            | Details                                  |
|--------------------------------------|--|
| Red (1-10)                           | Not exposed and not treated              |
| Black (1-10)                         | Not exposed and treated                  |
| Green (2, 5, 9)                      | Successful infection and not treated     |
| Blue (1, 2, 3, 4, 5, 6, 7)           | Successful infection and treated         |
| Purple (Blue 8, 9, 10 Green 1, 6, 8) | Exposed but no infection and not treated |
| Yellow (Green 3, 4, 7, 10)           | Cleared the infection and not treated    |

Reference:

J.E. Klementowicz, M.A. Travis and R.K. Grencis.(2012) *Trichuris muris*: a model of gastrointestinal parasite infection. *Seminars in Immunopathology*, Nov;34(6):815-28.

A. Klindworth, E. Pruesse, T. Schweer *et al.* (2013) Evaluation of general 16s ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acid Research*, 41(1). Doi:10.1093/nar/gks808

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## Developing the REP-detective

Marina I. Papaïakovou

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The high-prevalence neglected tropical diseases (NTDs) exhibit a global disease burden that exceeds malaria, STHs, tuberculosis and other better known health conditions; they also represent a potent force in trapping the world's poorest people in poverty and will still remain a worldwide health-threat for as long as poverty and disadvantage persists in the developing world. The term of "soil-transmitted diseases" applies to a group of parasites whose life cycle usually depends on a period of development outside the human host, typically in moist, warm soil. The main soil-transmitted helminth infections, ascariasis (*Ascaris Lumbricoides*), trichuriasis (*Trichuris Trichiura*), strongyloidiasis (*Strongyloides Stercoralis*) and hookworm (*Ancylostoma Duodenale*, *Necator Americanus*), are common clinical disorders in humankind. That's almost 4 billion infections around the globe making these the most common infections of humanity. This project is part of my research (Master's thesis); we attempted to find, after applying high-throughput sequencing to the genomes of all these parasites, the most highly-repetitive sequences in their genomes in order to design primer/probe sets for these on a qPCR-based diagnostic assay; DNA-based diagnostic is more accurate, sensitive and species specific than any other technique currently being used on-or out-of-the field. After designing with precision the primers/probes we evaluated their specificity, optimal concentrations and limitations; next step would be to test the individual assays for each parasite with infectious and/or blind samples from endemic countries. At this point we have tested 176 samples from Ethiopia (endemic country and prevalent in all of the above mentioned parasites); from those 176 samples 88 were received from NIH after processing the stool samples, and the other half from an accredited university in Netherlands. So not only we were able to estimate/determine the infection burden but also it was a good approach to evaluate the different stool DNA extraction method used in these two places (NIH and Netherlands) (stool preservation is a big issue in the endemic countries, since most of them lack the infrastructure to use high-analysis extraction kits or even centrifuges). Currently while waiting for comparison with what it's been considered as state of the art (golden standard Kato Katz), we are processing the data, evaluating the Ct cutoffs for false positive/negative/nonspecific signals and we are planning on publishing and presenting our work this fall since our results seem promising.

*(Supported by the Blakeslee Fund in the Biological Sciences)*

Advisor: Steven Williams, Biological Sciences

Acknowledgements: I would like to thank Nils Pilotte and Steven Williams for support for this project and the Bill and Melinda Gates Foundation for its support of this and related research in the Williams lab.

# Quantitative Analysis of Commissure Formation Using ArcMap (GIS)

Jin Sook Park/2015

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Axon-glia interactions in the developing brain are caused by Roundabout (Robo) receptors detecting Slit proteins, which are known to act as repulsive cues. These interactions guide bundles of axons, known as commissures, along a glial cell “bridge” in order to form the necessary connections within the nervous system.<sup>1</sup> Among the Slit proteins, Slit1a appears to play a distinct role from Slit2 and Slit3 as an attractive cue in axon guidance.<sup>2</sup> One of the major questions that we are investigating is how commissure formation changes when one or more components of the Slit/Robo signal-receptor pathway are disrupted. Another equally important question that I am focusing on is: How can we objectively analyze the resulting different phenotypes? What types of data can we obtain from quantifying the commissure phenotypes?

Using a zebrafish model system, we studied embryonic forebrains to determine the role of the Slit/Robo pathway and to see if certain mutations resulted in specific phenotypes, especially in the formation of the post-optic commissure (POC). We needed to quantify the POC to compare and contrast different commissures, and possibly measure the amount of deviation a specific mutation may produce. We utilized geographic programs (GIS), known as ArcMap and GeoDa, to develop a method to quantify the POC using an unbiased perspective.

With help from the Spatial Analysis lab, we quantified the POC based on the intensity of the pixels of the forebrain images. Using ArcMap, we were able to establish four potential measurements that we would be able to extract from the POCs. The first type of measurement was a cluster analysis which informed us in a numerical and visual way how the POC formed. The patterning of the pixel intensities of the POC is examined and a Moran's I was calculated, which showed the extent of either a clustered or dispersed pattern in the POC. The second type of measurement was a map of statistical significance where ArcMap was able to determine whether or not the cluster patterns of the POC were due to chance. The third type of measurement was average axon movement where we were able to calculate how far an individual axon had wandered away from an ideal POC formation. The last type is a measurement of the dimensions of the zebrafish forebrain, where the distance between forebrain structures or size of the forebrain itself would be measured for comparison between different groups of embryos. Currently, we are attempting to automate the cluster analysis in order to provide an efficient and rapid method of quantifying the POC. We are using a built-in tool in ArcMap known as ModelBuilder where different tools can be inserted in a specified order. Once the model is complete, it would have the potential ability to go through all of the steps of a cluster analysis on a POC automatically. Eventually, we would want to automate as much of the analysis process as possible by also including it in the ModelBuilder interface.

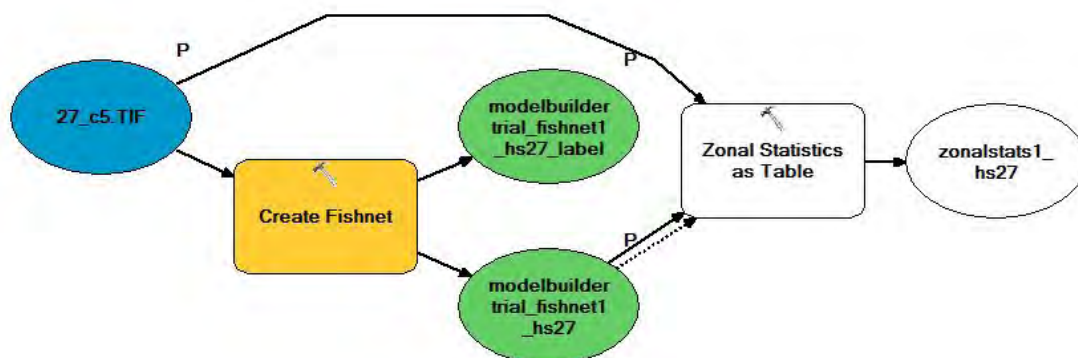
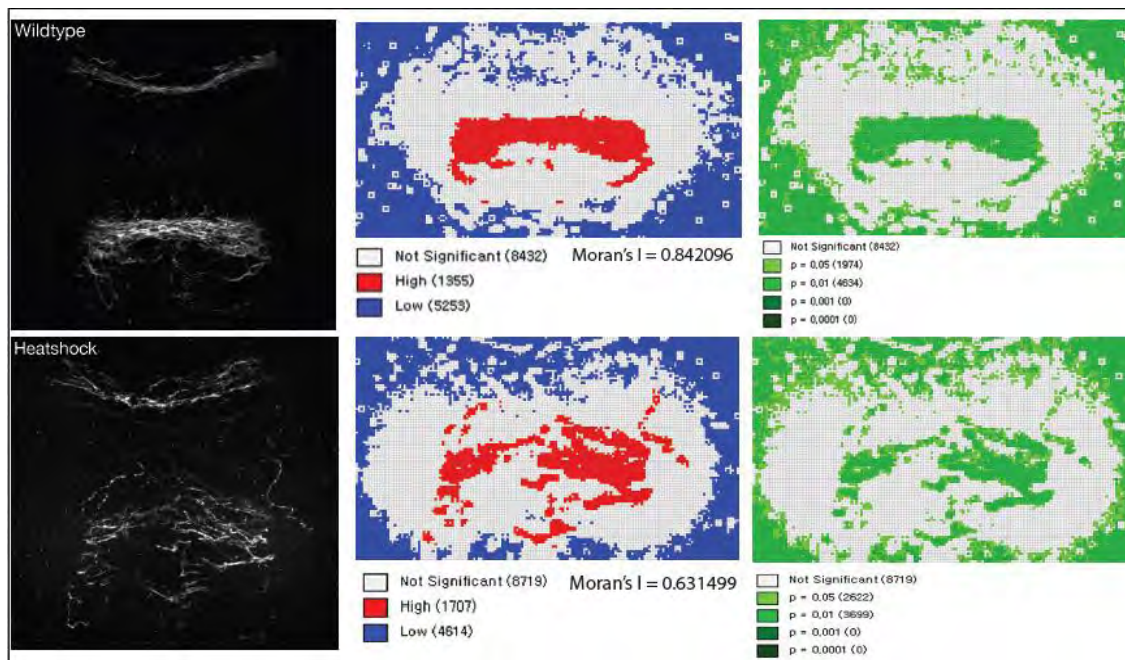
The quantitative analysis using ArcMap provides an objective method to examine phenotypes and to compare one commissure to another. We can establish what a standard wild type commissure looks like, in addition to creating standards for the different transgenic and mutant phenotypes. This would make comparisons of phenotypes more meaningful and easier to visualize.

*(Supported by the Schultz Foundation)*

Advisor: Michael Barresi, Biological Sciences

<sup>1</sup>Rasband, K., Hardy, M. and Chien, C. B. 2003. Generating X: formation of the optic chiasm. *Neuron*, 39: 885-8.

<sup>2</sup>Barresi, M. J., Hutson, L. D., Chien, C. B. and Karlstrom, R. O. 2005. Hedgehog regulated Slit expression determines commissure and glial cell position in the zebrafish forebrain. *Development*, 132: 3643-56.





# Temperature Regulation of the Stress-Response Regulator RpoS in Commensal and Pathogenic *Escherichia coli*

Fei Peng/2016

Temperature is an important signaling cue for *Escherichia coli* (*E. coli*) to distinguish the human host from external environments. Understanding how *E. coli* responds to temperature changes will help identify potential targets for anti-infective therapies. Three different strains of *E. coli*, commensal K-12, uropathogenic *E. coli* (UPEC), and enteropathogenic *E. coli* (EPEC) are studied in the White-Ziegler lab. My research project focused on investigating the effect of temperature change on the expression of the protein RpoS. RpoS is a sigma factor that directs transcription of the stress-response genes in *E. coli* at low temperature.<sup>1</sup> These genes help bacteria survive a variety of stresses including low pH, high osmolality, and oxidative stress.<sup>1</sup>

Growth experiments with the three strains of *E. coli* (K-12, UPEC, and EPEC) were done to obtain cell pellets under two different temperatures (23 °C and 37 °C) at various time points (0, 0.5, 1, and 4 hours). Proteins were isolated from the cell pellets and the protein levels were quantitated using the bicinchoninic acid assay. Equivalent amounts of total protein were analyzed by Western blot to determine the level of RpoS under different temperatures and in varying strains of *E. coli*.

Based on the Western blot results for *E. coli* K-12, the levels of RpoS stayed the same at the various time points at 23 °C, confirming that low temperature growth leads to RpoS expression (Fig. 1). After the temperature was shifted up to 37 °C, RpoS started to decrease at the t=1 hour time point and was virtually absent by t=4 hours (Fig. 1). Similarly, for the UPEC strain, Western blot results demonstrated constant RpoS levels at 23 °C (Fig. 2). However, in contrast to K-12, RpoS levels remained relatively high at 37 °C throughout the time course (Fig. 2).

Together, these results indicate that commensal and uropathogenic bacteria regulate RpoS expression differently based on temperature. However, both strains retain high RpoS expression during the first few hours upon a shift to human body temperature that may help them survive the environment stresses of the host environment. Future experiments will be needed to determine how the levels of RpoS change as temperature shifts up in the EPEC strain. I will continue this research as part of a Special Studies in the upcoming semester.

*(Supported by the Committee on Faculty Compensation and Development, Smith College and the Schultz Foundation)*

Advisor: Christine White-Ziegler, Biological Sciences

<sup>1</sup>White-Ziegler, C.A., Um, S., Pérez, N.M., Berns, A.L., Malhowski, A.J., & Young, S. (2008). Low temperature (23 °C) increases expression of biofilm-, cold-shock- and RpoS-dependent genes in *Escherichia coli* K-12. *Microbiology*. 154(Pt 1):148–66.

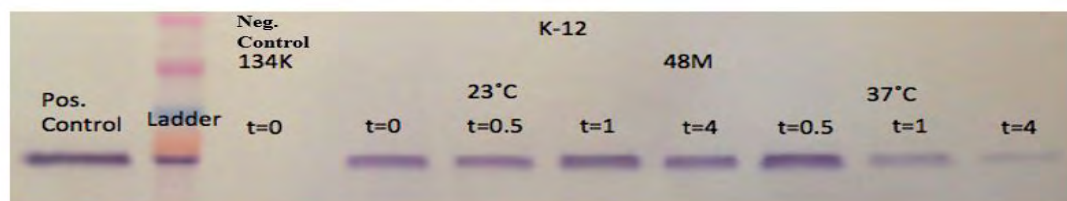


Figure 1. Western blot membrane result for K-12. The band intensity indicates the level of RpoS at the specific time point and temperature.

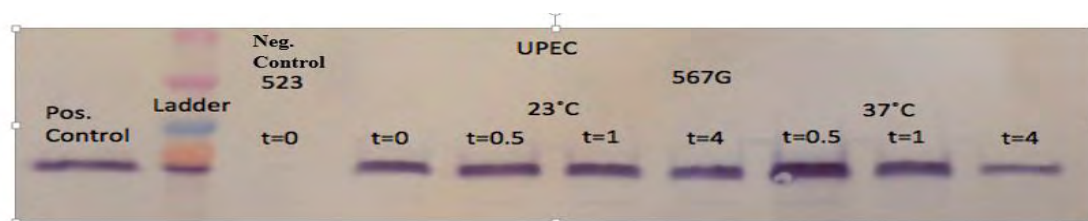


Figure 2. Western blot membrane result for UPEC. The band intensity indicates the level of RpoS at the specific time point and temperature.

# The Effect of Habitat on Patterns in Canidae Body Size Measurements

Kate Pielmeier/2015

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We think of bodies as developing proportionately; larger animals should have proportionately larger features. However, is that always the case? To address this question, I compiled data on Canidae species. The family Canidae is comprised of 13 genera and 35 extant species, including wolves, foxes, jackals, and wild dogs.<sup>1</sup> Canid species are incredibly well distributed; representatives of the family can be found on every continent with the exception of Antarctica.<sup>1</sup> Thus, canids are found in a variety of different climates and habitats; as a result, some species may have differing proportions.

Standard allometric measurements, body mass, head-body length, tail length, hind foot length, and ear length, were compiled for each canid species from over 200 papers from the primary literature as well as from preserved specimens in three museums. Averages for each measurement were weighted by sample size. Finally, regression and analyses of covariance were conducted using Minitab in order to assess trends.

The data exhibit a strong correlation between head-body length and body mass as well as between head-body length and hind foot length. However, strong correlations between head-body length and tail length or ear length were not apparent. The regressions for tail length and ear length reveal several outliers: *Speothos venaticus*, *Atelocynus microtis*, *Otocyon megalotis*, and *Vulpes zerda*. Both inhabiting dense tropical rainforests, *S. venaticus* and *A. microtis* have much shorter ears with respect to head-body length.<sup>2,3</sup> *S. venaticus* also has a much shorter tail with respect to its head-body length. In a heavily vegetated rainforest, shorter extremities may be beneficial as they are less likely to become caught on vegetation. On the other hand, both *O. megalotis* and *V. zerda* inhabit sparse, arid climates with higher temperatures. *O. megalotis* and *V. zerda* probably have much larger ears in order to dispel excess heat for thermoregulation.<sup>1</sup>

In sum, while the features of most canids appear to be proportional, not all canids conform to the overarching trends. Non-conforming species inhabit climates in which a particular adaptation, such as disproportionately larger ears, may be beneficial. Further research would include incorporating reproductive data for each species in order to assess correlations between reproductive and allometric patterns. Finally, I plan to expand upon this study by completing an honors thesis with Professor Hayssen, my research and academic advisor, and look forward to furthering my research.

*(Supported by the B. Elizabeth Horner Fund in the Biological Sciences)*

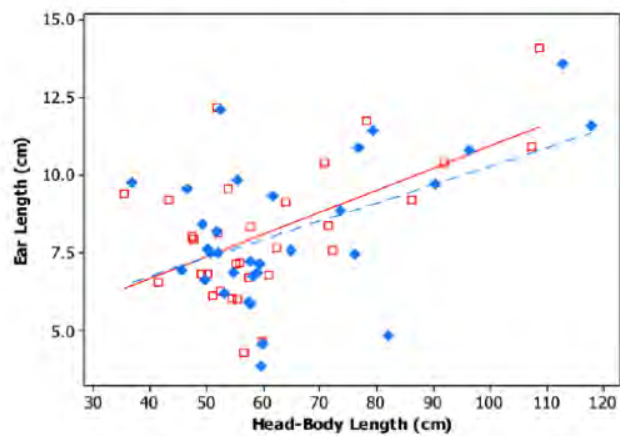
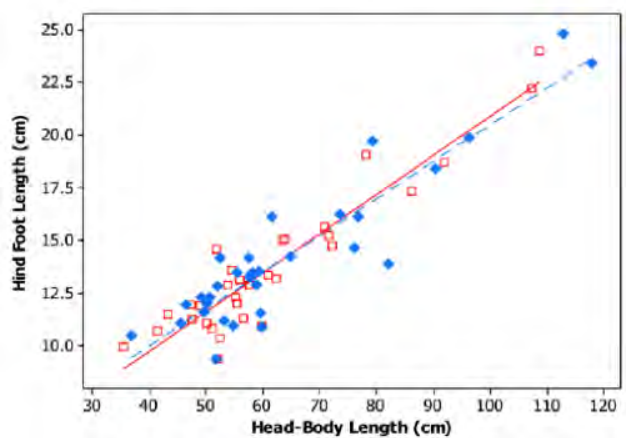
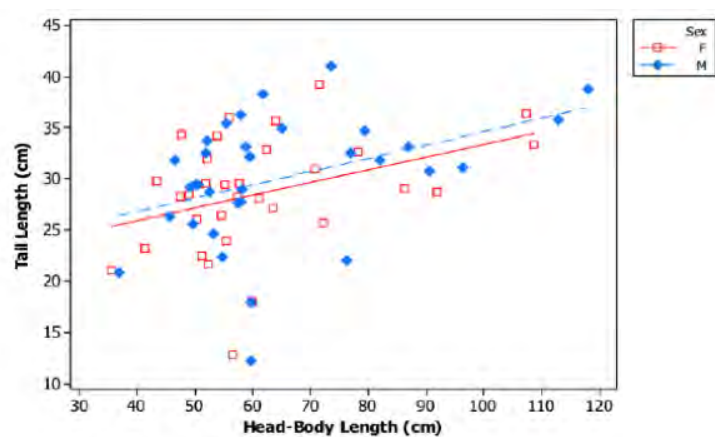
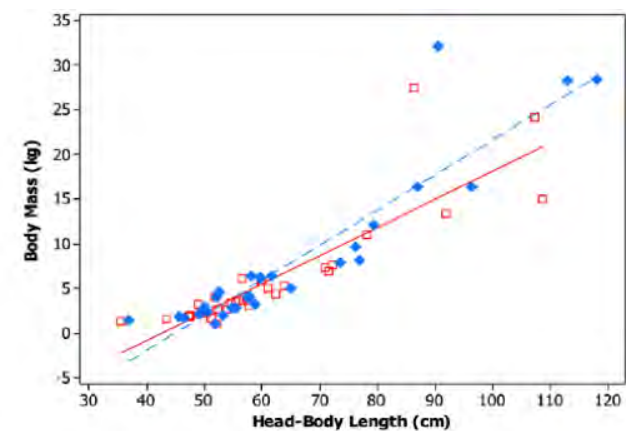
Advisor: Virginia Hayssen, Biological Sciences

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<sup>1</sup> Wilson, D.E. and Mittermeier, R.A. (2009). Family Canidae. In Handbook of the Mammals of the World. (Vol. 1, pp. 352-446). Barcelona, Spain: Lynx Edicions.

<sup>2</sup> Berta, A. (1986). *Atelocynus microtis*. Mammalian Species 256: 1-3.

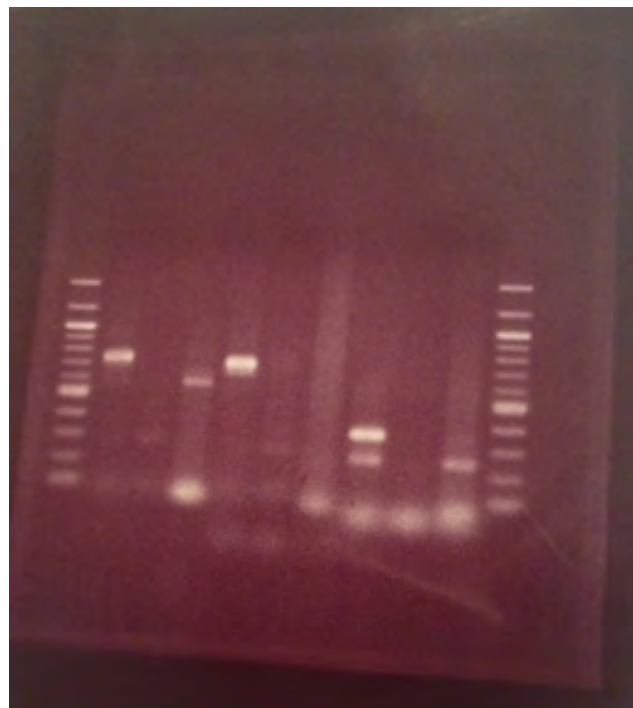
<sup>3</sup> Lima, E.S. et al. (2009). Habitat use and diet of bush dogs, *Speothos venaticus*, in the Northern Pantanal, Mato Grosso, Brazil. Mammalia 73: 13-19.



# Identification of *Centella asiatica*, *Neuroleena lobota*, and *Quisqualis indica* as Medicinal Herbs Containing Antifilarial Activity to Target Lymphatic Filariasis Parasites

Meghna Purkayastha/2016

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Gel electrophoresis capture of a DNA extraction of *Quisqualis indica* that was used to confirm the identification of the plant.<sup>1</sup>

Lymphatic filariasis (LF), a globally neglected tropical disease, is a parasitic illness that is known to affect over 120 million people in Southeast Asia and Africa, according to the Center for Disease Control.<sup>1</sup> LF is the second leading cause of long-term disability worldwide, and it is caused by the human parasites: *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*, and *Brugia pahangi*. The Steven Williams Lab (SAW Lab) is using *Centella asiatica*, *Neuroleena lobota*, and *Quisqualis indica* as medicinal herbs native to East Asia, Guatemala, and China respectively to develop a drug that exhibits antifilarial activity on the LF parasites. Continued research must emphasize the legitimacy of the sources from where the plants were obtained. In order to proceed with animal testing and further drug development, DNA extractions of all three plants were performed and sequenced.

The Phire Plant Direct PCR Kit<sup>3</sup> protocol revealed DNA for the *Neuroleena lobota* leaves and *Quisqualis indica* seeds. To extract DNA from *Centella asiatica* bark, a bark specific, more intensive protocol was carried out.<sup>4</sup> All three DNA extractions were prepared for a Polymerase Chain Reaction (PCR) using three different primer combinations that amplify the plants in the ITS (Internal Transcribed Spacer) region. The ITS region of the 18S-5.8S-26S nuclear ribosomal citron is commonly used for phylogenetic reference, or identification in plants. Each three PCR products were sequenced using Sanger Sequencing by following the Thermal Cycle Sequencing protocol.<sup>5</sup> The sequencing results were analyzed using the FinchTV and the NCBI Basic Local Alignment Search Tool (BLAST) programs to compare the sequence results in a plant specific database. There was a positive identification of *Quisqualis indica* as *Quisqualis indica*, with a 94% match and 0 E value score. However, there was a 75% identification match and 4e-17 E value score for what was intended to be *Neuroleena lobota* as *Wellemia sebi* fungal strain. *Centella asiatica* remains unidentified because the Sanger sequencing method was unable to recognize the sample, and the sequence was messy.

The *Quisqualis indica* identification was the most contested result because the plant was purchased from an unknown seller on eBay. The seeds were sowed in the Smith College Plant House so that the plant identification will always be known when performing crude extractions. For the *Neuroleena lobota* sequence, the *Wellemia sebi* fungus DNA was higher concentration than the *Neuroleena lobota* DNA, so the sequence did not identify *Neuroleena lobota*. This may be due to the fact that the *Neuroleena lobota* used in the lab was not fresh. In order to correctly identify the plant, a new *Neuroleena lobota* plant must be ordered and/or grown. To properly sequence *Centella asiatica* bark, new primer combinations can be researched to best optimize the PCR reaction.

Bark in particular is a difficult sample to process because it contains many sugars due to the presence of the phloem. Thus, adjustments can also be made to best optimize the Thermal Sequencing Reaction protocol by increasing the amount of primer to sure it is not diluted, and increasing the amount of PCR product. Research will continue throughout the academic school year for accurate identifications of the plants so that animal testing can ensue. Eventually, with the collaboration of the Shea Lab synthesizing the *Neurolaena lobata* compounds, the SAW lab hopes to synthesize each of the three plants as potential drug candidates to fight LF.

*(Supported by the Howard Hughes Medical Institute)*

Advisor: Steven Williams, Biological Sciences

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Purkayastha, Meghna. Gel electrophoresis capture of a DNA extraction of *Quisqualis*

indica that was used to confirm the identification of the plant following the Thermal Cycle Sequencing protocol and the Sanger Sequencing method, July 2014.

<sup>2</sup> “Parasites-Lymphatic Filariasis” Center for Disease Control and Prevention. <http://www.cdc.gov/parasites/lymphaticfilariasis/biology.html>

<sup>3</sup>“Thermo Scientific Phire Plant Direct PCR Product Information” Thermo Scientific. <http://www.thermoscientificbio.com/uploadedFiles/Resources/tech-manual-f-130-phire-plant-direct-pcr-kit.pdf>

<sup>4</sup> R.M.L. Novaes, J.G. Rodrigues and M.B. Lovato, “An efficient protocol for tissue sampling and DNA isolation from the stem bark of Leguminosae trees” February 2009.

<sup>5</sup>“Big Dye Terminator v3.1 Cycle Sequencing Kit” Applied Biosystems. [http://mvz.berkeley.edu/egl/inserts/Big\\_Dye\\_v3.1\\_Protocol\\_Manual.pdf](http://mvz.berkeley.edu/egl/inserts/Big_Dye_v3.1_Protocol_Manual.pdf)





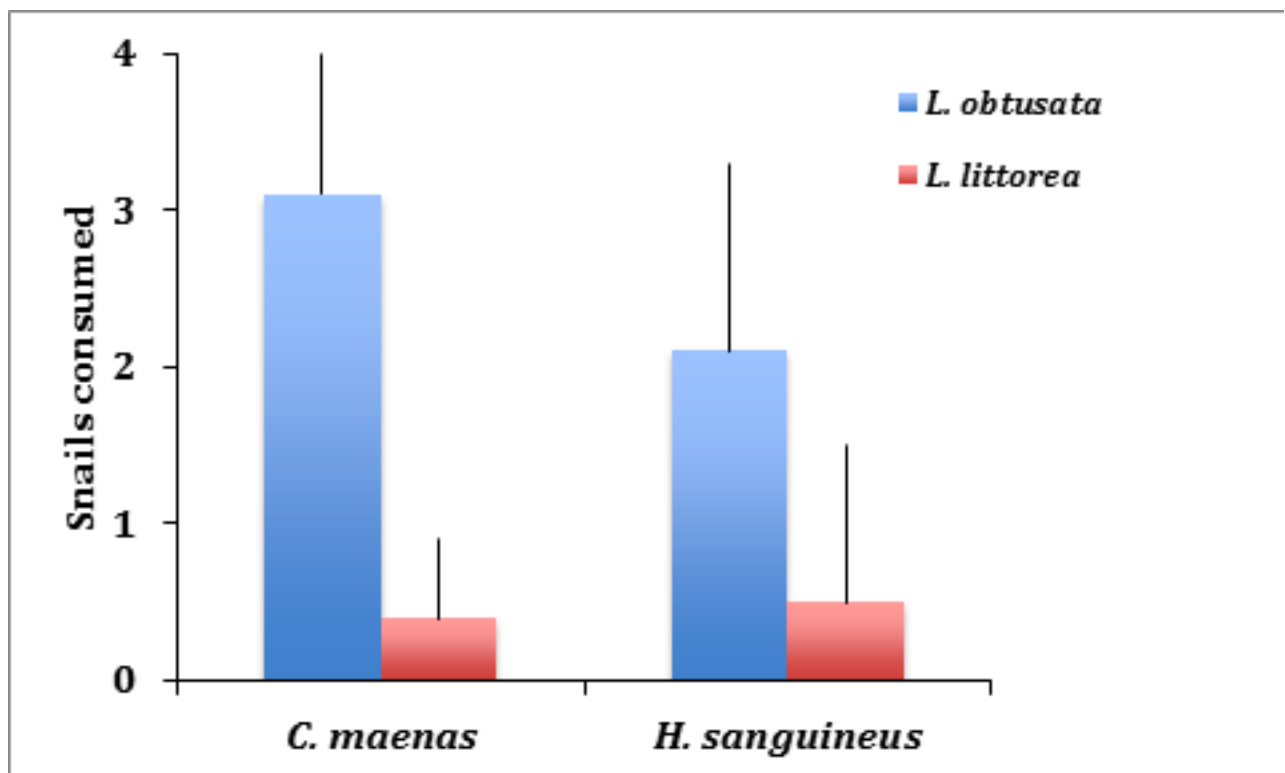


Figure 2. Mean (+SD) number of snails (*Littorina obtusata* and *L. littorea*) consumed by *Carcinus maenas* and *Hemigrapsus sanguineus* (n=10).



Figure 3. Example of broken and chipped snail shells of *L. littorea* and *L. obtusata* in a prey preference experiment with green crab, *C. maenas*.

# The Role of Wnt5b During Neurogenesis in the Zebrafish Spinal Cord

Catalina Sakai/2016

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In vertebrate organisms, stem cells regulate development through proliferation, self-renewal, and giving rise to different cell types within the organism. Correct and proper mechanisms behind this system are vital, and an increased understanding of the processes provides insight into developmental diseases. Radial glial cells are a type of neuronal stem cell, and we have shown that when functioning correctly, will proliferate and give rise to other neuronal cells including interneurons, motor neurons and oligodendrocyte precursor cells.<sup>1</sup> This process is regulated by a variety of factors that we have yet to fully understand. In a previous screen for genes required for radial glial development in zebrafish, a mutation in the *wnt5b* gene produced an increase in mitotic radial glial cells within the zebrafish spinal cord.<sup>2</sup> This project focuses on ascertaining how Wnt5b, a secreted signaling protein, regulates proper proliferation of radial glial cells in the spinal cord using zebrafish as a vertebrate model.

This summer we began our characterization of the *wnt5b*<sup>hi1780</sup> mutant identified in the screen. To do this, we developed a new genotyping protocol using PCR to distinguish between wild type and mutant siblings. To determine whether Wnt5b regulates radial glial proliferation we collected and fixed *wnt5b*<sup>hi1780</sup> mutant embryos at specific time points corresponding with relevant stages of neurogenesis and performed immunocytochemistry to label radial glial cells (GFAP) and cells in mitosis (PH3). We will continue this investigation in the school year by using fluorescent microscopy to observe the number of radial glial cells in mitosis at these specific time points throughout neural development. Since these neuronal stem cells give rise to other cell types in the spinal cord, we hypothesize that an increase in mitotic radial glia could potentially affect the number of downstream neuronal cells.

Secondly, we also set out to establish several transgenic lines to assess whether *wnt5b* functions to modulate  $\beta$ -catenin signaling in order to influence neurogenesis. Wnt5b is known to be a non-canonical signaling protein, but examination of regeneration has suggested interplay with  $\beta$ -catenin-dependent signaling.<sup>3</sup> We believe that Wnt5b antagonizes canonical Wnt/ $\beta$ -catenin-dependent signaling to regulate radial glial division. Following loss of *wnt5b*,  $\beta$ -catenin signaling is hyperactive and leads to increased proliferation of radial glial cells. We will investigate this question by manipulating Wnt5b activity in Wnt/ $\beta$ -catenin reporter lines to determine if the two pathways are interacting to affect radial glial division. Together these experiments will be helpful for further understanding radial glial division.

*(Supported by the Provost Office, Smith College)*

Advisor: Michael Barresi, Biological Sciences

## References:

<sup>1</sup> Johnson, K., et al., Kif11 dependent cell cycle progression in radial glial cells is required for proper neurogenesis in the zebrafish neural tube. *Dev. Biol.* (2014).

<sup>2</sup> Barresi, M., et al., Essential genes for astroglial development and axon pathfinding during zebrafish embryogenesis. *Developmental dynamics* (2010).

<sup>3</sup> Chien, A., et al., A Wnt Survival Guide: From Flies to Human Disease. *J Invest Dermatol.* (2009).





# The Zebrafish Diencephalic Glial Bridge is Made Up of a Heterogeneous Population of Astroglial Cells During and After Forebrain Commissure Formation

Caitlin Schneider/2015

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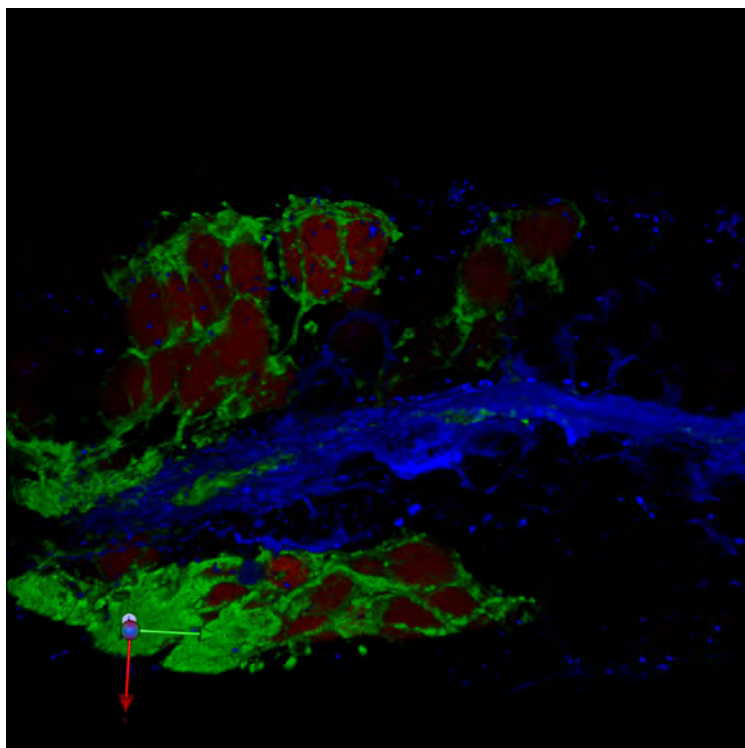
In the embryonic nervous system of bilaterally symmetric, neuronal axons are guided toward and away from the midline by attractant and repellent cues. Those axons that cross the midline form bundles of nerve fibers, called commissures, that connect the two sides of the brain and enable communication of information. The Barresi lab uses zebrafish to elucidate the developmental mechanisms of these processes. Of particular interest is the post-optic commissure (POC) of the zebrafish forebrain, as it is the first of three major commissures to form. Specifically, we aim to characterize the population of non-neuronal cells, astroglia, that are present at the midline before, during, and after POC formation. We hypothesize that these cells provide a growth substrate that guides axons across the midline. Moreover, we believe that different subtypes of astroglia present at the midline are molecularly distinct and may serve different roles.

Using a combination of molecular and biochemical techniques, we have begun defining the morphological and molecular identities of astroglia present at the midline of the POC. Firstly, we used a method of cellular transplantation to generate transgenic and fluorescent clonal cells in a wild-type embryo. By generating small populations of transgenic cells in the region of the POC, we were able to identify four types of astroglia at the midline: long-process or “classic” radial glia; short-process radial glia; mesenchymal glia; and olig2+ (a marker of oligodendrocyte precursor cells) bridge glia. Each of these cell types are found in specific regions of the POC.

Next, we used Western blot analysis to determine the identity of three proteins that differentially label the above mentioned cell types. These proteins, zebrafish radial fibers (Zrf) 2, 3, and 4, may molecularly define the differences between these groups of cells. Preliminary Western blot results are inconclusive, and identifying these proteins will be an area of further study.

*(Supported by the Schulz Foundation)*

Advisor: Michael Barresi, Biological Sciences



3-dimensional view of confocal microscopy image of the post-optic commissure. Axons are labeled in blue and transplanted astroglia clonal cells are in green (cell membranes) and red (cell nuclei).

# Next Generation Sequencing Analysis Comparing *A. viteae* and *B. malayi*

Marie Jacques M. Seignon/2015J

*A. viteae* and *B. malayi* are two nematodes known to be responsible for the infectious disease filariasis in animals and humans respectively.<sup>1</sup> *B. malayi* has a bacterial intracellular symbiont, Wolbachia, found in a majority of nematodes, believed to help with development, fertility and survival of the worm within its host; while, *A. viteae* is one of a few that lacks this symbiont.<sup>1, 2, 3</sup>

In this continuing independent research project, we tackled the next-generation sequencing for the transcriptome of these two nematodes in order to assess differential gene expression and possibly isolate genes that are similar in function from this assessment and would explain why *A. viteae* is able to survive within its host without the help of Wolbachia. After creating the cDNA library of both nematodes and sequencing with a MiSeq, we made use of the web-based platform for biomedical research data analysis, Galaxy (Mason instance, Indiana University). Following the Tuxedo protocol for RNAseq differential gene expression analysis, using the latest tool versions available (Mason as of July 2014), we were able to visualize the expression pattern of possible genes present in these worms using CummeRbund.<sup>4, 5</sup> A parallel visualization of the Cuffdiff output from Galaxy was also done using CummeRbund in Rstudio and obtained identical graph outputs.<sup>6, fig1</sup>

Since we were unable to locate a proper annotated version of the genomic reference sequence of *A. viteae*, an incomplete, but recent annotation of the *B. malayi*'s reference sequence was used on both nematodes' Miseq output for mapping and alignment.<sup>7</sup> This makes any assessment of gene expression from our *A. viteae* outputs suspicious since they are a different species of worm. We are currently working on the de novo assembly of our *A. viteae* Miseq output in order to create an annotated reference sequence that will be used for reanalysis of our data through Tuxedo on Mason Galaxy as well as a parallel analysis through command line following a set of protocols using several analysis tools in the Unix terminal interface.<sup>8</sup>

(Supported by the Howard Hughes Medical Institute)

Adviser: Steven Williams, Biological Sciences

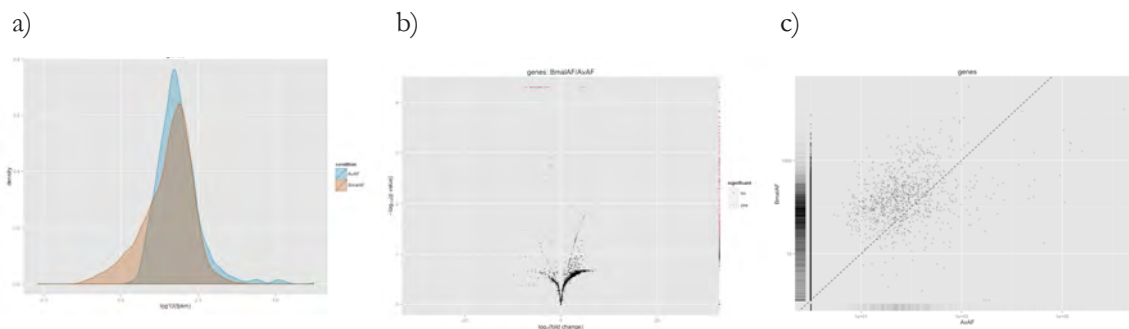


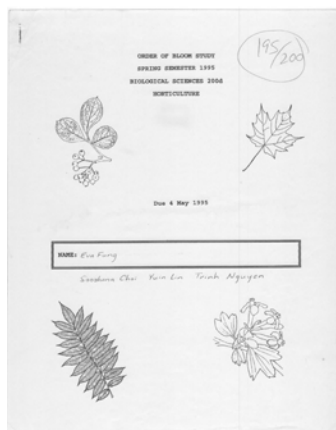
Figure 1: Cuffdiff visualization of possible genes expressed in both nematodes using CummeRbund in Rstudio. a) Density plot representing the distribution of abundance of transcripts between the two samples. b) Volcano plot showing the relationship between the fold-change of genes and the statistical significance of that change, the red dots being the ones identified as significantly differentially expressed (p-value 0.05). c) Scatter plot, which shows the apparent expression level of genes in *A. viteae* against the expression level in *B. malayi* for all possible genes identified.





# Refining and Expanding Smith College's Data Tracking Capacity for Phenologic Study of Local Flora

Tara Stark/2015



| ID | A    | B    | C         | D         | E         | F           | G          | H          | I         | J                      | K        |
|----|------|------|-----------|-----------|-----------|-------------|------------|------------|-----------|------------------------|----------|
| 1  | ID   | YEAR | BudBurst  | FirstLeaf | AllLeaves | FirstFlower | FullFlower | FirstFruit | FullFruit |                        |          |
| 2  | Alba | 1992 | 3/23/1992 | 3/23/1992 | 4/30/1992 | 3/23/1992   | 4/14/2002  |            |           |                        |          |
| 3  |      | 1995 | 3/20/1995 | 3/27/1995 | 4/17/1995 | 3/20/1995   | 3/27/1995  | 5/1/1995   |           |                        |          |
| 4  | Alba | 1995 | 3/20/1995 | 3/27/1995 | 4/10/1995 | 3/20/1995   | 3/27/1995  | 5/1/1995   |           |                        |          |
| 5  |      | 1999 | 3/23/1999 | 4/6/1999  | 4/27/1999 | 3/30/1999   | 4/6/1999   | 4/6/1999   |           |                        |          |
| 6  | Alba | 1999 | 3/30/1999 | 4/6/1999  | 4/27/1999 | 3/30/1999   | 4/6/1999   | 4/6/1999   |           |                        |          |
| 7  |      | 2000 | 3/21/2000 |           |           |             |            |            |           |                        |          |
| 8  | Alba | 2000 | 3/7/2000  | 3/28/2000 | 4/25/2000 | 3/21/2000   | 3/28/2000  |            |           | no full bloom, "dead?" | 4/4/2000 |
| 9  | Alba | 2002 | 4/15/2002 | 4/26/2002 |           | 4/1/2002    | 4/29/2002  |            |           |                        |          |
| 10 | Alba | 2003 | 4/3/2003  | 4/15/2003 | 5/1/2003  | 4/3/2003    | 4/11/2003  |            |           |                        |          |
| 11 | Alba | 2005 | 3/24/2005 | 4/14/2005 |           | 4/7/2005    | 4/14/2005  | 4/28/2005  |           |                        |          |
| 12 | Alba | 2010 | 3/6/2010  | 3/25/2010 | 4/5/2010  | 3/6/2010    | 3/25/2010  | 4/26/2010  |           |                        |          |
| 13 | Alba | 2011 | 4/15/2011 | 4/27/2011 |           | 4/15/2011   | 4/15/2011  |            |           |                        |          |
| 14 | Alba | 2013 | 3/25/2013 | 4/6/2013  |           | 4/1/2013    | 4/6/2013   |            |           |                        |          |
| 15 |      | 2014 | 4/10/2014 | 4/24/2014 | 5/1/2014  | 4/10/2014   | 4/17/2014  |            |           |                        |          |

Since the early 1980s, the spring Horticulture class has been following the return of flowers and leaves to plants on campus through an assignment called Order of Bloom. Observing a list averaging around 50 plants on campus, students make weekly observations of the emergence of buds and subsequent phenological developments through the end of the semester. The purpose of my research was to develop a better method for organizing and storing this data, and to make it accessible and useful to other institutions or organizations also interested in tracking phenological changes.

In order to reformat the data, I collected primary sources of data in the form of paper copies of Order of Bloom assignments spanning over two decades. I focused on developing data sets for plants which had five or more years of data records and that are still on campus. I set up spreadsheets for each species individually and recorded five key phenological stages (bud burst, first leaf, all leaves, first flower, full flower, first fruit, and fruits mature) that I interpreted from the existing key code. I was able to observe changes that occurred year to year and recognize patterns that emerged in the data sets.

The overwhelming majority of the data I analyzed indicated the potential for significant phenological changes year to year due to weather, but no overwhelming gradual or consistent change in bloom time over the past 20+ years due to climate change. To make this data more useful to the scientific community, I contacted Dr. John O'Keefe, an ecologist at Harvard Forest, who has developed a long-term phenology data set of native woody species in the area. A meeting was arranged at Harvard Forest with Gaby Immerman, Smith's horticulture lab instructor, as well as John O'Keefe, and Emery Boose, a senior investigator at Harvard Forest. We discussed collaborating with Harvard Forest to enhance the research happening at both institutions, and build a more comprehensive data set for native woody species. Beginning spring semester of 2015, the horticulture class will complete a modified assignment that focuses specifically on distinct phenological changes so that more accurate dates can be recorded. Hopefully an ongoing relationship will be formed between Smith and Harvard Forest to make this data more accessible and useful to the scientific community.

*(Supported by the Schultz Foundation)*

Advisors: Michael Marcotrigiano, Biological Sciences and Botanic Garden and Jon Caris, Environmental Science and Policy and Spatial Analysis Lab

# Theoretical and Practical Renovation of the Systematics Beds at the Smith College Botanic Garden

Elizabeth Strohbeck/2017

The renovation of the Systematics Beds at the Smith College Botanic Garden has been taking place over the course of the past three years. This project has brought that work to fruition, combining a new design reflecting the course of evolutionary history, a modernized DNA-based understanding of plant relationships, and an updated family and species plant list.

The primary focus of research over the course of this summer was on refining the list of plant species in conjunction with the director and collections manager, using GIS-based waypoints to establish the planned beds on the ground, and working with the staff to produce materials tailored to maintaining the renovated garden over the long term. On the organizational side, the use of Google Drive™ spreadsheets was crucial in allowing multiple members of the Botanic Garden faculty and staff to simultaneously view and edit the evolving plant list in real time. The logistical establishment of the planned beds proved to be more complex than expected, and beds were re-adjusted and re-aligned to accommodate the needs of both visitors and preexisting landscape trees such as the Ginkgo biloba. Additionally, the stones representing evolutionary relationships between families were embedded, and altered bed corners were re-mapped using ArcGIS software.

Smith's teaching collection is extensive and renowned, and this is the first renovation of the systematics collection since the 1960's. As the plan created over the summer is brought into action over the course of this fall and next spring, our new collection will once again represent the newest botanical and scientific knowledge of plant taxonomy and evolution, displayed in a comprehensible manner for students from Smith as well as nearby colleges and institutions. Highly visible and academically significant, this project brings together the academic and aesthetic, the digital and organismal, and represents a highly successful collaboration between faculty, staff and students, in multiple academic and administrative departments.

This research was presented to fellow Botanic Garden Interns on August 13<sup>th</sup>, and will be utilized this fall by Professor Jesse Bellemare and his class.

*(Supported by the Schultz Foundation)*

Advisor: Michael Marcotrigiano, Biological Sciences and Botanic Garden



| Plant Family Key   |                  |
|--------------------|------------------|
| 1. Solanaceae      | 8. Lamiaceae (1) |
| a. Annonaceae      | a. Boraginaceae  |
| b. Asteraceae      | b. Ranunculaceae |
| c. Caryophyllaceae | c. Agrostaceae   |
| d. Lauraceae       | d. Veliaceae     |
| e. Magnoliaceae    |                  |
| 2. Ranunculaceae   | 9. Rubiaceae     |
| a. Ranunculaceae   | a. Rubiaceae     |
| b. Ranunculaceae   | b. Rubiaceae     |
| c. Ranunculaceae   | c. Rubiaceae     |
| d. Ranunculaceae   | d. Rubiaceae     |
| e. Ranunculaceae   | e. Rubiaceae     |
| 3. Ranunculaceae   | 10. Rubiaceae    |
| a. Ranunculaceae   | a. Rubiaceae     |
| b. Ranunculaceae   | b. Rubiaceae     |
| c. Ranunculaceae   | c. Rubiaceae     |
| d. Ranunculaceae   | d. Rubiaceae     |
| e. Ranunculaceae   | e. Rubiaceae     |
| 4. Ranunculaceae   | 11. Rubiaceae    |
| a. Ranunculaceae   | a. Rubiaceae     |
| b. Ranunculaceae   | b. Rubiaceae     |
| c. Ranunculaceae   | c. Rubiaceae     |
| d. Ranunculaceae   | d. Rubiaceae     |
| e. Ranunculaceae   | e. Rubiaceae     |
| 5. Ranunculaceae   | 12. Rubiaceae    |
| a. Ranunculaceae   | a. Rubiaceae     |
| b. Ranunculaceae   | b. Rubiaceae     |
| c. Ranunculaceae   | c. Rubiaceae     |
| d. Ranunculaceae   | d. Rubiaceae     |
| e. Ranunculaceae   | e. Rubiaceae     |
| 6. Ranunculaceae   | 13. Rubiaceae    |
| a. Ranunculaceae   | a. Rubiaceae     |
| b. Ranunculaceae   | b. Rubiaceae     |
| c. Ranunculaceae   | c. Rubiaceae     |
| d. Ranunculaceae   | d. Rubiaceae     |
| e. Ranunculaceae   | e. Rubiaceae     |
| 7. Ranunculaceae   | 14. Rubiaceae    |
| a. Ranunculaceae   | a. Rubiaceae     |
| b. Ranunculaceae   | b. Rubiaceae     |
| c. Ranunculaceae   | c. Rubiaceae     |
| d. Ranunculaceae   | d. Rubiaceae     |
| e. Ranunculaceae   | e. Rubiaceae     |
| 8. Ranunculaceae   | 15. Rubiaceae    |
| a. Ranunculaceae   | a. Rubiaceae     |
| b. Ranunculaceae   | b. Rubiaceae     |
| c. Ranunculaceae   | c. Rubiaceae     |
| d. Ranunculaceae   | d. Rubiaceae     |
| e. Ranunculaceae   | e. Rubiaceae     |

# Diversity of Sulfur-Reducing Bacteria in the Beaver Ponds of Avery Brook, MA

Eirini Tsekitsidou/2016

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My SURF project focused on the diversity of sulfur-reducing bacteria (SRB) collected from water and sediment core at the Avery Brook beaver ponds, located just northwest of Smith College, in Whatley, MA. Sulfur can be reduced and made available to other organisms by both bacteria and archaea whose metabolism involves the transformation of sulfate ( $\text{SO}_4^{2-}$ ) into sulfide ( $\text{S}^{2-}$ ). SRBs are of increasing importance to us because sulfur reduction influences the biogeochemical cycling of elements like nitrogen and carbon, has beneficial applications in cleaning industrial waste, and also produces methylated mercury, which is then bioaccumulated in organisms (eg. fish) that humans eat. SRBs can be found in most environments, but they prefer anoxic conditions since they are anaerobic. Beaver ponds are a suitable place for SRB collection, because seasonal fluctuations and beaver activities alter the water flow and create the ideal conditions for SRBs to grow.

For this study, I worked with Bob Merritt, Ghida El-Banna and Dail Laughinghouse to collect water and sediment core samples from both the inlet and outlet areas of two beaver ponds. To isolate bacterial genetic material, I first performed water filtration and then DNA extraction from both the water and sediment. I then amplified some genes of interest via Polymerase Chain Reaction (PCR), using a thermocycler. The genes I was initially interested in were universal 16S rRNA, a highly conserved ribosomal gene in bacteria, and DSRAB, a part of dissimilatory sulfite reductase (DSR). DSR is an operon critical for sulfur metabolism and unique to sulfur reducers. Later on, instead of using DSRAB to identify SRB presence I used primers that were specific to amplifying a part of the 16S rRNA sequence of each of the six clades of SRBs. Refining PCRs was a lengthy process, because the cycling conditions needed to be optimized before results were repeatable. PCR success was assessed by running amplicons through a gel within an electric field (gel electrophoresis).

My initial hypothesis was that SRBs would be more abundant at the anoxic outlet part of the pond. Gel electrophoresis results of the initial 16S rRNA PCR were mostly positive, indicating that bacteria were present in most of the samples, both inlet and outlet. However, results from trying to detect DSRAB were disappointing, probably due to a glitch in the PCR process. Furthermore, cloning and sequencing of DSR fragments did not reveal any SRB present. For this reason I shifted my focus to the genera-specific 16S rRNA amplicons and I was able to detect the presence of two SRB genera, namely *Desulfobulbus* and *Desulfonema-desulfo sarcina-desulfococcus*. Right now I am preparing my DNA samples to be sequenced on a large scale through NextGen Sequencing, in order to assess what types of bacterial species are present in my samples, and to map these data according to the depth and pond origin of each sample.

My goal beyond this summer is to learn how to analyze the sequences I obtained using bioinformatics and to then draw conclusions about the diversity of SRB communities when conditions such as depth, water flow, and temperature/oxygen levels vary in the pond.

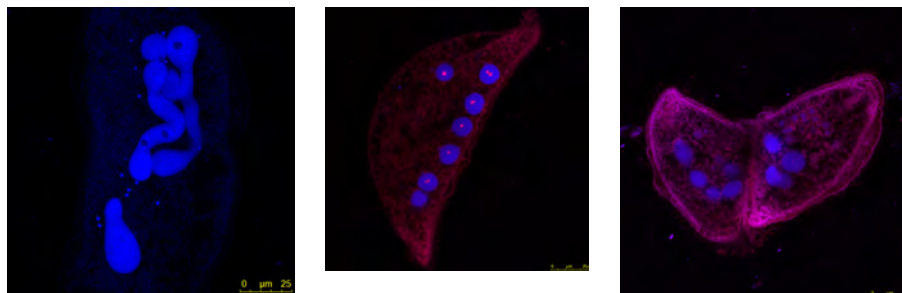
*(Supported by the Blakeslee Fund in the Biological Sciences)*

Advisors: Robert Merritt and Laura Katz, Biological Sciences



# Mapping the Lifecycle of *Blepharisma americanum*

Megan Wancura/2017



Ciliates, microbial eukaryotes characterized by having two distinct genomes, are incredibly diverse. Having both a somatic macronucleus and a germline micronucleus, these organisms are interesting to study due to their unusual genome features, which allow them to evolve quickly. Ciliates can divide asexually or conjugate to share genetic information. Blepharisma, a genus of Ciliates, are particularly interesting because species of Blepharisma are abundantly diverse in the evolution rates of several genes. Life cycles of several Blepharisma species have been proposed in the past<sup>1</sup>, but the life cycle of *Blepharisma americanum* remains unknown. This ciliate is easily grown and maintained in lab cultures, and therefore is an ideal model organism to study complex genome arrangements.

To obtain a general life cycle of Blepharisma, literature over Blepharisma species was consulted<sup>1</sup>. Blepharisma americanum taken from the same lab culture were fixed using PFA and Triton-x100 and stained with DAPI. Upon UV imaging with a Leica confocal microscope, holes were found in the macronuclei of the organisms. To address if this was an artifact of fixation or a real phenomenon, experiments were done altering the fixation protocol. Upon imaging with UV and 633nm lasers, bright red filled the empty holes. Live imaging was attempted, placing Blepharisma in 2% agarose and imaging with UV, but cells exploded too quickly for imaging.

Each experiment yielded some cells with at least one macronucleus with a red dot. Cells in different life cycle stages showed different rates of this phenomenon, but not enough cells were collected to get a significant statistical ratio, although no immediate pattern clearly emerged. The images taken depict clearly how diverse the genome arrangement of Blepharisma is over its life cycle, although the images with the red dot cannot be used until the issue of its identity is resolved. The red dot could possibly be blepharismmin, an auto-fluorescent pigment Blepharisma contain, as the absorbance spectrum of blepharismmin matches up with using the 633nm laser.

More experiments need to be done to identify the red dot. Different microscopy techniques may provide a clearer view inside the macronuclei. However, even if the organisms imaged did not show this strange phenomenon, a full life cycle could not be laid out from the images taken as not every cycle was imaged and most of the cells were vegetative. Next semester I will explore microscopy techniques and image more cells to draw a more definitive conclusion.

*(Supported by the National Science Foundation)*

Advisor: Laura Katz, Biological Sciences

Guise, Arthur C. (1973). *Blepharisma: the biology of a light sensitive protozoan*. Stanford University Press.



# Ciliates and Copepods in the Planktonic Food Web

Cameah Wood/2015

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The research that I conducted this summer examined the relationships within the planktonic food web. The main organisms I focused on are ciliates and copepods. Ciliates are unicellular eukaryotes. These microorganisms feed on nano and pico-sized organisms such as bacteria, cryptophytes, and other heterotrophic flagellates (Sherr and Sherr 2002, Grattenpanche et al. 2011). Copepods are abundant metazoans. They feed on various planktonic organisms that can include diatoms and microzooplankton such as ciliates and dinoflagellates (Saiz and Calbet. 2011). My research looked at the grazing habits of copepods and their effects on ciliates. My hypothesis is copepods display selectivity towards aloricate (naked) ciliates over loricate (shelled). This project is important because it explores the dynamics of the planktonic food web, the key members within it, and the larger impacts it can have on the environment.

For this study, I examined microcosm experiments that were conducted in July of 2013 at the University of Connecticut Avery Point, Groton CT. Here at Smith College, I extracted the DNA using phenol chloroform, and conducted numerous pcr's to amplify the DNA. From here I was able to put the DNA in a DGGE (denaturant gradient gel electrophoresis). A DGGE allows for us to examine and see all the haplotypes present in a community sample. From here, the product present on the DGGE gel was used for sequencing. In the end, I found numerous trends that directed us to the result that copepods are displaying selectivity for aloricate or naked ciliates over loricate or shelled ciliates, and this was consistent across four DGGE's.

After conducting several DGGE's, I was able to see clear trends of copepods selecting to graze on aloricate ciliates over loricate. I was able to note the difference in abundance depending on the amount of copepods present in each sample, and was also able to note that it seems as if the copepods are feeding on loricate ciliates after aloricate abundance dwindled. Even though this is one conclusion for the activity present within the DGGE's, another idea is that copepods are not feeding on the ciliates. It may be possible that the copepods within the sample are feeding on phytoplankton and other nano-sized organisms. With this, the ciliate abundance begins to die due to the reduction in food supply. This research will be continued in the fall of 2015 as well as a publication.

*(Supported by the National Science Foundation)*

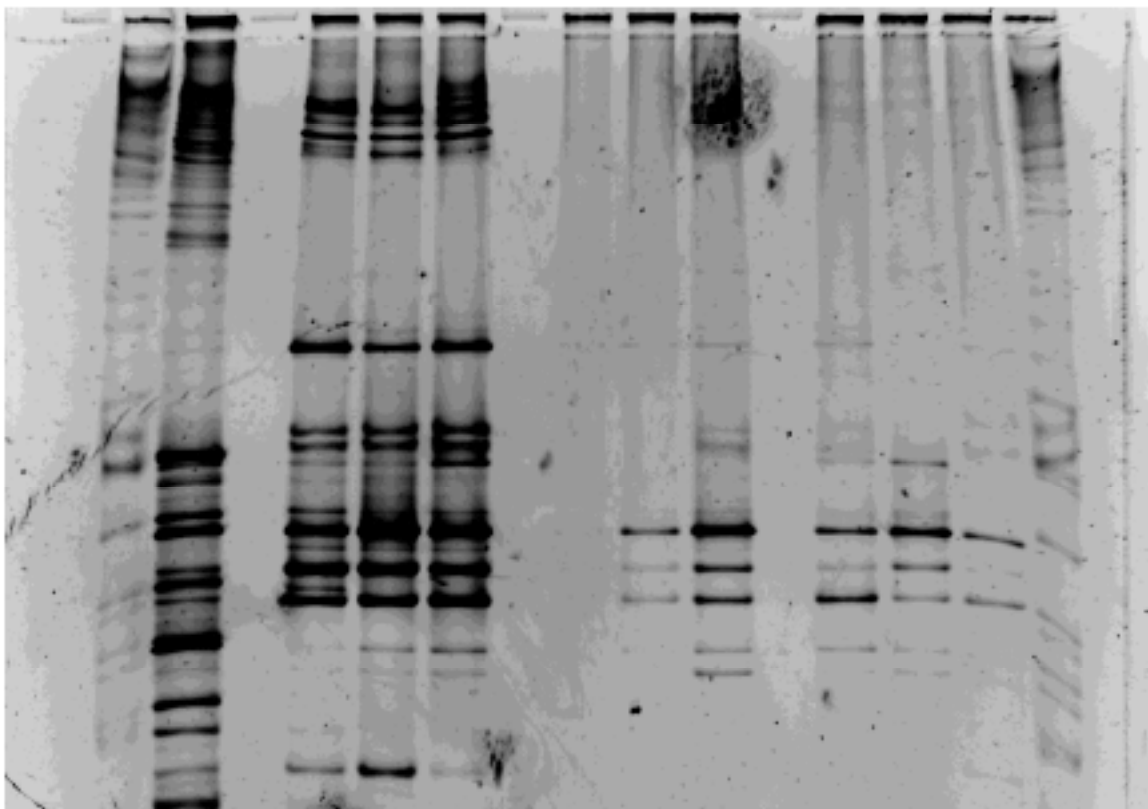
Laura Katz, Biological Sciences

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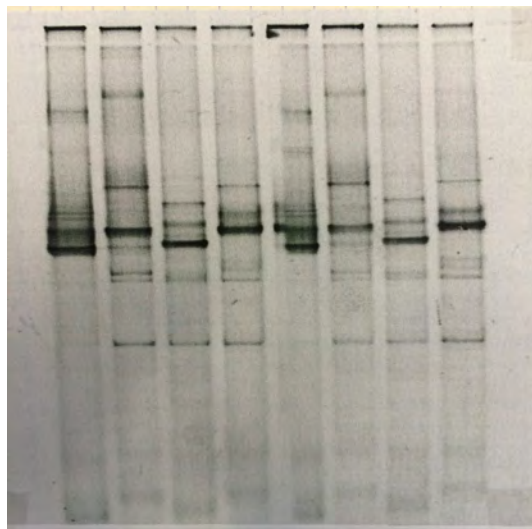
**Image of DGGE Conducted this summer displaying different ciliate haplotypes**





# Using DGGE as a Means of Assessing Biodiversity of Testate Amoebae

Jennifer Yoo/2016



Testate amoebae are a great clade for environmental studies, especially because their sensitivity allows scientists to visualize the effects of different environmental parameters. Testate amoebae also inhabit various habitats, from the soil to the ocean, and are abundantly scattered, which makes them useful to study. More specifically, they dominate peatlands, where hydrology levels are critical. Studies have shown that testate amoebae are bioindicators for hydrology levels and this makes them model organisms to study climate change.<sup>1</sup> Due to this sensitivity, testate amoebae are able to reflect environmental conditions through species density and richness. In the bigger picture, testate amoebae are great bioindicator species for understanding and predicting environmental changes.

The main goal for this summer was to apply and optimize the DGGE (Denaturing Gradient Gel Electrophoresis) technique to a newly sampled community of testate amoebae that was freshly picked from a nearby bog. To do this, testate amoebae were handpicked cell by cell until about 50-70 cells were picked. The DNA was then extracted using phenol-chloroform. Following extraction, multiple rounds of PCR were then executed to make sure the DNA was present. At this point, I faced many challenges. The DNA did not seem to show up and by the end, the lab concluded that the phusion, which is crucial component in a master mix, did not work well with community DNA.

The purpose of this experiment is to revolutionize the way scientists obtain molecular data on these protists as well as apply a new technique to uncover a serious environmental issue. Along the way, I learned that research is not a smooth and straightforward endeavor. Many obstacles were faced, however it was a rewarding experience to be able to learn and absorb new knowledge through a research setting. I hope to further explore this project and gain interesting results with more experiments in the coming year.

*(Supported by the Howard Hughes Medical Institute)*

Advisor: Laura Katz, Biological Sciences

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# Design and Synthesis of DNA-small Molecule Catalyst Conjugates for Site Selective Chemistry

Emilia Argüello/2016

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Attaining site-selectivity in complex chemical contexts, such as living organisms is challenging given the presence of multiple instances of the same functional group and many other potentially competing molecules in the sample. To perform a selective reaction inside a living being, a catalyst must be able to discriminate among other similar or identical functionalities in order to leave the organism's biological workings unharmed. Currently, methods to achieve selectivity and biocompatibility typically rely upon a uniquely functionalized target substrate.<sup>1</sup> However, a method for targeting naturally occurring small molecules is yet to be reported.

My research project aims to achieve this type of chemical selectivity through the use of DNA-small molecule conjugates (DCats), which combine the binding specificity and high affinity of DNA aptamers<sup>2</sup> with the chemical power of a covalently attached catalyst (Figure 1). In analogy to enzymes, we hypothesize the aptamer will bind to its substrate, facilitating a specific chemical reaction by increasing the effective concentration of the DCat-bound molecule and the covalently tethered catalyst.

I focused specifically in the design of a new branch of DCats for ester hydrolysis reactions on porphyrin substrates. Substrate synthesis was explored by combining a commercially available porphyrin precursor, Protoporphyrin IX (PPIX) with a fluorescently active molecule, umbelliferone, under a variety of esterifying conditions. Proton NMR analysis suggests the chlorination of PPIX's carboxylic acid chains with oxalyl chloride followed by treatment with the umbelliferone alcohol is the most successful synthetic route,<sup>3</sup> although PPIX's light- and water-sensitive nature still makes synthesis and analysis a delicate task.

DCats suitable for ester hydrolysis were built by combining a well-studied porphyrin-binding DNA sequence, T30695,<sup>4</sup> with a molecular linker and an imidazole catalyst through amide coupling chemistry explored earlier in this lab. The reaction mixture underwent a preliminary purification using a NAP-5 disposable column, followed by a more rigorous HPLC purification with acetonitrile and triethylammonium acetate to collect the desired DCat adducts. Promising collected fractions were lyophilized and analyzed via ESI-MS to confirm their identity.

Due to unexpected behavior of the PPIX-binding sequence during the reaction and purification, synthesis of DCats for PPIX-umbelliferone ester hydrolysis was not accomplished. Thus, it was not possible to perform a thorough assay to study the effect of the DCat in this reaction. Preceding experiments with different DCats on other ester substrates have, nonetheless, pointed that DCats are more reactive than small-molecule catalysts alone and, hence, are promising tools for selective chemistry. This research will be continued in the spring of 2015 as a special studies project and in 2016 as a possible honors thesis.

*(Supported by the Schultz Foundation)*

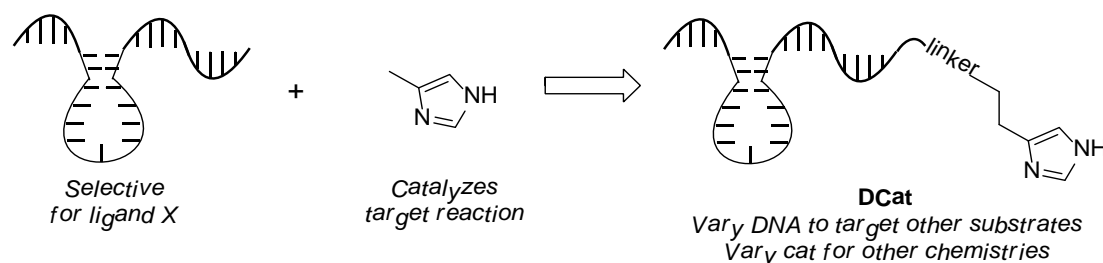


Figure 1: Modular assembly of a DCat through the coupling of a functionally modified DNA aptamer for a defined target and a desired catalyst.

Advisor: David Gorin, Chemistry

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# RAFT Synthesis of Di-block Copolymers for Biomaterial Applications

Anna Carroll/2016

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Modern medicine demands versatile novel biomaterials. Di-block copolymers composed of inert, water-soluble polyethylene glycol (PEG), along with 2-vinyl-4,4'-dimethylazlactone (VDMA), which allows for diverse functionalization with a variety of therapeutics, demonstrate potential in a wide variety of biomedical applications.<sup>1</sup> This summer, we explored creating copolymers capable of self-assembly into micelles for later conjugation to adhesive and anti-bacterial peptides to create antimicrobial coatings for medical implants, which will serve to reduce hospital-acquired infections.<sup>2</sup>

VDMA monomer<sup>3</sup>, along with chain transfer agents (CTA), compounds for carrying out controlled polymerizations, were synthesized. After inexpensively producing a version of a pricy commercially available carboxylic acid-terminated CTA<sup>4</sup>, a Steglich esterification reaction was performed to produce a custom alkyne-terminated CTA<sup>5,6</sup>. The CTAs were purified using column chromatography, and all syntheses were evaluated using proton and carbon NMR spectroscopy. Using the first CTA, numerous controlled RAFT polymerizations were performed. The conversion percentages for these polymerizations were determined using proton NMR, relating the integration of a monomer peak to its corresponding polymer peak, and their molecular weight distributions were examined through gel permeation chromatography (GPC).

After producing pure VDMA in nearly 50% yields, much of the valuable monomer was compiled for future experiments. Additionally, the carboxylic acid-terminated CTA was efficiently synthesized in an over 75% yield and successfully controlled mono and di-block RAFT polymerizations as well as the commercial compound in high conversions. The alkyne-terminated CTA synthesis yielded little, impure product but did effectively control polymerization, as evidenced by low variation molecular weight distributions observed.

With an expanded understanding of how to perform effective, controlled RAFT polymerizations, future steps include synthesizing and purifying additional custom alkyne-terminated CTAs, as well as producing di-block copolymers with sufficient PEG for required water solubility to drive micelle assembly. Additionally, we plan to analyze these micelles with spectrophotometry and light scattering studies and functionalize them using selective “click” chemistry on the alkyne-terminated micelle arms. Finally, we plan to attach the micelles to surfaces and test their antimicrobial properties, as part of a Special Studies this semester.

*(Supported by the Schultz Foundation)*

Advisor: Maren Buck, Chemistry

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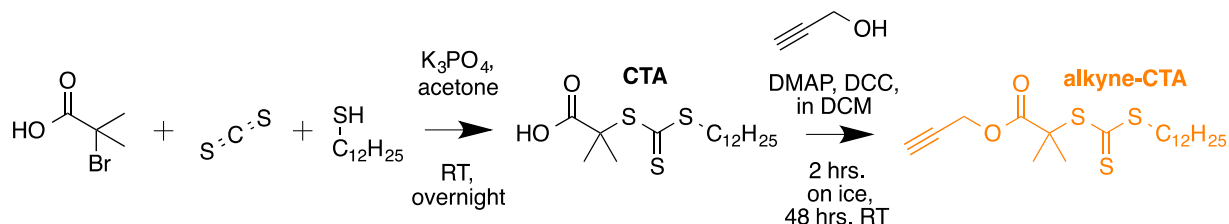
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## CTA Synthesis:





# DNA-Small Molecule Catalyst Conjugates for Site-Selective Chemistry

Drew Colman/2015

In organic chemistry, there is currently no way to transform a small molecule among many with the same functional groups. If such an approach is achieved, this chemistry could be applicable to living systems, which contain complex environments with many molecules of similar structures. Our project goal is to functionalize one target molecule among many potential competitors, through the use of DNA as a macromolecular targeting domain. DNA aptamers can fold into three-dimensional structures, which allows them to target very specific substrates. Our lab has previously linked DNA aptamers to a small-molecule catalyst (imidazole). These structures are DNA-small molecule catalyst conjugates (DCats, figure 1A); we hypothesize that DCats can select for and bind molecules with functional groups that exist in living organisms to the small-molecule catalyst. This summer, I attempted to synthesize an adenosine ester substrate, which would demonstrate that DCats perform ester hydrolysis more effectively than free small-molecule catalysts.

The adenosine ester is composed of two initial compounds: a fluorescently active molecule, umbelliferone, and a molecule for which a DNA sequence has a binding affinity, adenosine (figure 1B). The substrate is fluorogenic—it is only fluorescent after hydrolysis occurs, so its hydrolysis can be monitored by fluorescence signal. To synthesize the ester, first an isopropylidene-protected adenosine was oxidized at the carbon-5 position to form a carboxylic acid (figure 1B). Once oxidized, two steps were necessary: deprotect the adenosine and form the adenosine umbelliferone ester (figure 1B). Either step could be attempted first; both routes were explored.

The protected adenosine was oxidized reproducibly with high yield. The carboxy-adenosine was successfully deprotected, but the ester has not yet been synthesized from either protected or deprotected carboxy-adenosine. TLC evidence suggests that ester may have formed, but then hydrolyzed or otherwise decomposed during purification. The adenosine DCat has been synthesized, so once the adenosine ester has been created its DCat effectiveness will be monitored.

The adenosine ester will hopefully address lack of hydrolysis completion in the fluorescence assay that arose with cholic acid and R-ibuprofen esters, which also have corresponding DNA aptamers. Once DCat-mediated site-selective chemistry is achieved, many biomedical applications are possible, including hydrolysis in bacterial quorum sensing. I helped present a poster at the American Chemical Society, Connecticut Valley Section in the spring of 2013. Additionally, I have presented posters at Smith College's Celebrating Collaborations in 2013 and 2014. I plan to continue this project this year as an honors thesis.

*(Supported by the Research Corporation for Science Advancement)*

Advisor: David Gorin, Chemistry

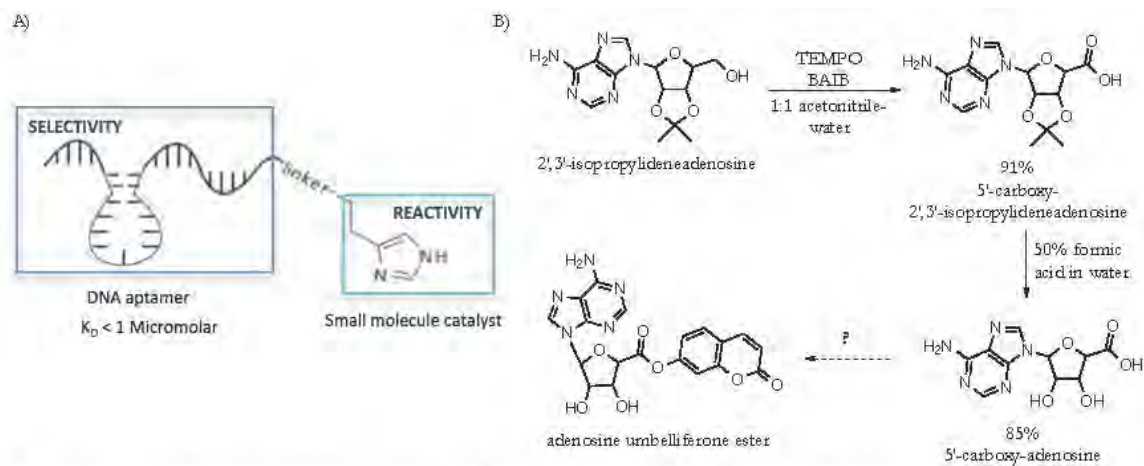


Figure 1. A) DNA-small molecule catalyst conjugate (DCat): both selective and reactive. B) Synthetic route thus far to an adenosine umbelliferone ester.

# Exploration of *Neurolaena Lobata*: Extraction and Experimentation with Active Compounds

Sophia Deady/2016

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The study of naturally occurring chemical compounds is crucial to our understanding of plants' medicinal properties. This summer, our interest in natural products chemistry focused on *Neurolaena Lobata*, a plant used in Central America and the Caribbean to treat a wide range of parasitic ailments.<sup>1</sup> The medicinal properties of *N. Lobata* are due to the presence of sesquiterpene lactones, plant terpenoids known for their antibacterial and pharmaceutical uses.<sup>2</sup> For this research, neurolenin, a type of sesquiterpene lactone active in *N. Lobata*, was extracted and studied. Prior research in the Shea and Williams Labs showed that the extraction of both isomers of neurolenin (A and B) was possible, and that these compounds have shown anti-filarial effects against the parasites responsible for lymphatic filariasis. The research this summer was undertaken to continue the extraction and purification of both neurolenin isomers, to experiment with the acetate functional group on neurolenin B, and to develop the curriculum for a research-based organic chemistry lab course.

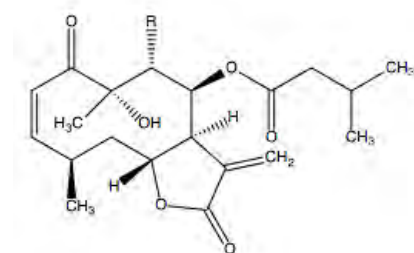


Figure 1: Structure of Neurolenin: Neurolenin B, R= OAc Neurolenin A, R= H

Over the course of five weeks, multiple extractions of neurolenin were successfully completed. The compounds were extracted from dried leaves using a Soxhlet apparatus, the extract was stirred with activated charcoal to remove inactive compounds,<sup>3</sup> and Neurolenins A and B were isolated by column chromatography. One of the goals was to obtain a greater supply of both neurolenin isomers for use in the Williams Lab, as well as a supply for experimentation and possible modification.

Another goal was to find a way to selectively convert the acetate group on Neurolenin B to an alcohol. This would allow for esterification and the creation of novel compounds, which could be assessed for their antifilarial properties in the Williams Lab. Reactions on similar compounds in the literature were identified and attempted. Experimentation with this functional group is still in the early stages, but promising directions have been identified. The research conducted this summer has helped to refine the extraction process of neurolenins and offers an effective road-map for a research-based lab course.

*(Supported by the Schultz Foundation)*

Advisor: Kevin Shea, Chemistry

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# Clinical Analysis of the Correlation Between ASD and GI Dysfunction

Charlotte Dzialo/2016

Autism, a developmental disorder affecting 1 in 88 children in the United States today, is characterized by impaired social interaction and communication and the presence of limited, repetitive and stereotyped interests and behaviors (Johnson and Myers, 2007; Vandereycken, 2003). There is no uniform set of biomarkers specific to ASD, thus the diagnostic of ASD is entirely dependent on behavioral observation according to the DSM-IV criteria. “Elimination” diets, which are typically casein- and gluten-free diets have been reported to reduce negative behavior in children with ASD. This summer I examined the autism literature to determine if there is any credible evidence to support the use of elimination diets, and to determine overall why parents find improvements in their children’s behavior.

Somewhat surprisingly, I found that there is little consistent data available about the true prevalence of GI dysfunction in an average, unselected population of autistic children 3 with, the rates reported ranging from 9% to 91.4%.<sup>1</sup> This wide variability in reported rates is in part due to the impaired communication of individuals with ASD, which makes recognizing and characterizing gastrointestinal dysfunction challenging.

**TABLE 3** Diagnostic Evaluation of Gastrointestinal Symptoms and Disorders in Individuals With ASDs

| Symptom  | Possible Associated Gastrointestinal Disorder  | Definition  | Diagnostic Evaluations to Be Considered  |
|--|--|---|--|
| Sleep disturbance  | GERD   | Parental/provider report  | (1) Diagnostic trial of proton-pump inhibitor; (2) pH probe, EGD   |
| Self-injurious behavior, tantrums, aggression, oppositional behavior   | Constipation, GERD, gastritis, intestinal inflammation   | Parental/provider report  | (1) Abdominal radiograph; (2) diagnostic trial of proton-pump inhibitor or PEG 3350; (3) pH probe, EGD, colonoscopy  |
| Chronic diarrhea   | Malabsorption, maldigestion  | ≥3 loose stools daily for >2 wk   | (1) Stool analysis for occult blood, enteric pathogens, ova/parasites ( <i>Giardia</i> or <i>Cryptosporidium</i> ), <i>Clostridium difficile</i> ; (2) consider PEG 3350 if overflow diarrhea is a possibility; (3) lactose breath test (or measure lactase-specific activity), EGD, colonoscopy |
| Straining to pass stool, hard or infrequent stool  | Constipation   | ≤2 hard stools per week (Bristol stool score)   | (1) Abdominal radiograph to look for fecal impaction; (2) diagnostic trial of PEG 3350   |
| Perceived abdominal discomfort: pressing abdomen, holding abdomen and crying, problem behaviors related to meals | Constipation, GERD, intestinal inflammation, malabsorption, maldigestion                           |   | (1) Diagnostic trial of proton-pump inhibitor or PEG 3350; (2) abdominal radiograph; (3) lactose breath test (or measure lactase-specific activity); (4) pH probe, EGD, colonoscopy  |
| Flatulence and/or bloating   | Constipation, lactose intolerance, enteric infection with <i>Giardia</i> or <i>Cryptosporidium</i> |   | (1) Abdominal radiograph; (2) diagnostic trial of PEG 3350 or lactose restriction; (3) lactose breath test or EGD (measure lactase-specific activity)  |
| Any or all of the above  | FAP, IBS   | FAP: abdominal pain without demonstrable evidence of anatomic, metabolic, infectious, inflammatory, neoplastic, or other pathologic condition<br>IBS: FAP associated with alteration in bowel movements | (1) Behavioral soothing; (2) diet enhancements with fruits, fiber, sufficient fluids; (3) increase in routines for sleep and toilet time   |

EGD indicates esophagogastrroduodenoscopy; PEG, polyethylene glycol.

The current hypothesis explaining this prevalence include: an unregulated mucosal immune response, abnormal microbiota, and IgE mediated food allergies. Figure 1 shown above presents typical negative behavior associated with GI dysfunction. Since Elimination diets can affect an array of mechanisms these diets might reduce negative behaviors associated with common GI symptoms by altering cytokine levels, eliminating allergens and/or balancing microbiota, to name just a few possibilities.

Without a clear pathological mechanism for GI dysfunction in autism there can be no definitive evidence to support the use of Elimination diets. However, there have been many reports of decreased negative behaviors as a result of reduced discomfort and pain associated with GI problems. It is hoped that future research will clarify the role of metabolic disorders, allergic/toxic reactions, and inflammatory changes in the etiology of gastrointestinal disturbances in individuals with ASDs.<sup>1</sup> Yet for now, the use of Elimination diets is a practical and noninvasive treatment that may result in reduced pain, thus improve behavior in children who cannot communicate their discomfort.

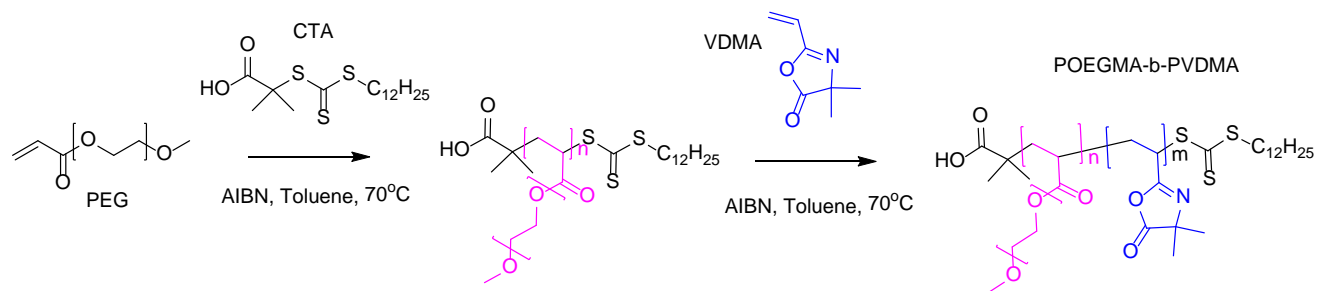
*(Supported by the Chemistry Alumnae Gift Fund)*

Advisor: David Bickar

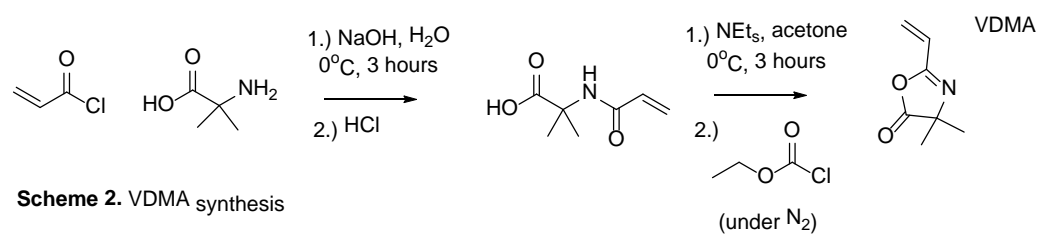
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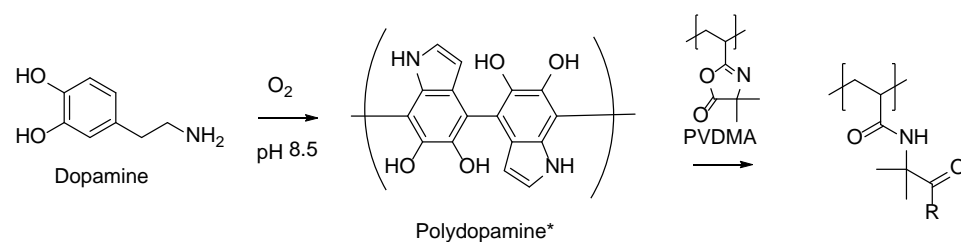




**Scheme 1.** Schematic illustration of the RAFT synthesized block copolymer POEGMA-b-PVDMA (where  $n$  and  $m$  are the number of each monomer subunit).



**Scheme 2.** VDMA synthesis



**Scheme 3.** Schematic illustration of dopamine oxidation and PVDMA grafting.  
\*Proposed structure of polydopamine, R = polydopamine



# Developing a Method for Ancient Ceramic Provenance Studies Using Handheld X-Ray Fluorescence Spectroscopy

Rebecca Gerdes/2015

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The provenance of an ancient ceramic, or where it originated, can give insight into contact between ancient communities, and is determined from many factors, including chemical composition.<sup>1,2</sup> For chemical analysis, X-ray fluorescence spectroscopy (XRF) is often used because it is non-destructive. In XRF, an area of the ceramic is excited by X-rays and releases excess energy in patterns of fluorescence lines unique to each element, which the instrument counts as pulses to determine composition. While guidelines exist for XRF-based provenance studies, each study must be tailored to its instrument and samples. Our work focuses on developing an XRF-based method for investigating the provenance of Corinthian-style ceramics from the Classics Department's Van Buren Antiquities Collection (VBAC).

XRF spectra of areas of ten VBAC ancient Corinthian-style ceramics were taken using a hand-held XRF (HHXRF) spectrometer. The potential, current, filter, collection time, and vacuum settings were varied to focus on elements that distinguish Corinthian ceramics from those of other ancient regions. Element concentrations were calculated from spectra using ARTAX software by approximating peak areas. Figure 1(a) shows the site where a spectrum was taken on an aryballos-shaped ceramic (repository no. 25.30).

Comparison of experimental concentrations with published values indicates that the current HHXRF calibration and parameters do not provide reliable data on the composition of the samples. Ancient ceramics should be about 50% silicon (Si) and less than 10% iron (Fe),<sup>2</sup> but with current settings, Si peaks are dwarfed by Fe peaks. Figure 1(b) shows an XRF spectrum of the site in (a) taken at 40 keV, 1.70  $\mu$ A, no vacuum, and an aluminum/titanium filter for 60 seconds, with distinguishing peaks marked.

Research will be continued in an honors thesis by R. Gerdes in 2014-2015 to correct the calibration and monitor the performance of the instrument via a glass standard. The method will be used to investigate the provenance of the VBAC's Corinthian-style ceramics. Special thanks to Scott Bradbury, Classics, for access to the Van Buren Antiquities Collection

*(Supported by the Schultz Foundation)*

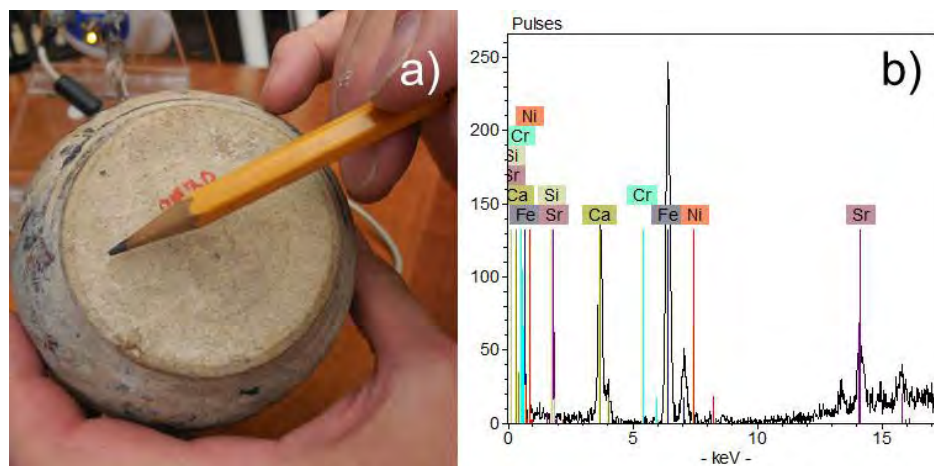


Figure 1. (a) An area of a Corinthian aryballos analyzed by XRF, and (b) the corresponding XRF spectrum.

Advisors: Kate Queeney, Chemistry, and David Dempsey, Smith College Museum of Art

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<sup>2</sup> Jones, R.E. *Greek & Cypriot Pottery: A Review of Scientific Studies*. Athens: British School at Athens, 1986.

# Synthetic Polymer Hydrogels for Tissue Engineering and Regenerative Medicine

Pooja Hindocha/2016

Hydrogels are cross-linked polymers that can closely replicate the nature of the extracellular matrix of a cell for several reasons. They absorb large quantities of water, permit the efficient transport of oxygen, nutrients, and waste, and can be designed to have mechanical and biochemical properties that resemble natural tissues. Moreover, they allow for the study of cellular function in a 3D environment. Lastly, since the materials used to make these hydrogels closely resemble naturally occurring materials, they can be bioactive, non-immunogenic, non-cytotoxic and biocompatible.<sup>1</sup>

This summer, the research I did focused on making the foundation structures required to form the hydrogel. Our first goal was to synthesize our base molecule, an azlactone trimer. This trimer is made from the cyclization of a molecule which is a modified version of the amino acid alanine. We did this using various approaches, such as anionic polymerization and acid-catalyzed polymerization (Figure 1a). The second goal was to functionalize the trimer and make supramolecular building blocks, which in aqueous environments would self-assemble and form hydrogels. We used various methods to create these supramolecular building blocks, which involved having non-covalent interactions between our host molecule (Beta cyclodextrin) and guest molecule (adamantane) (Figure 1b).

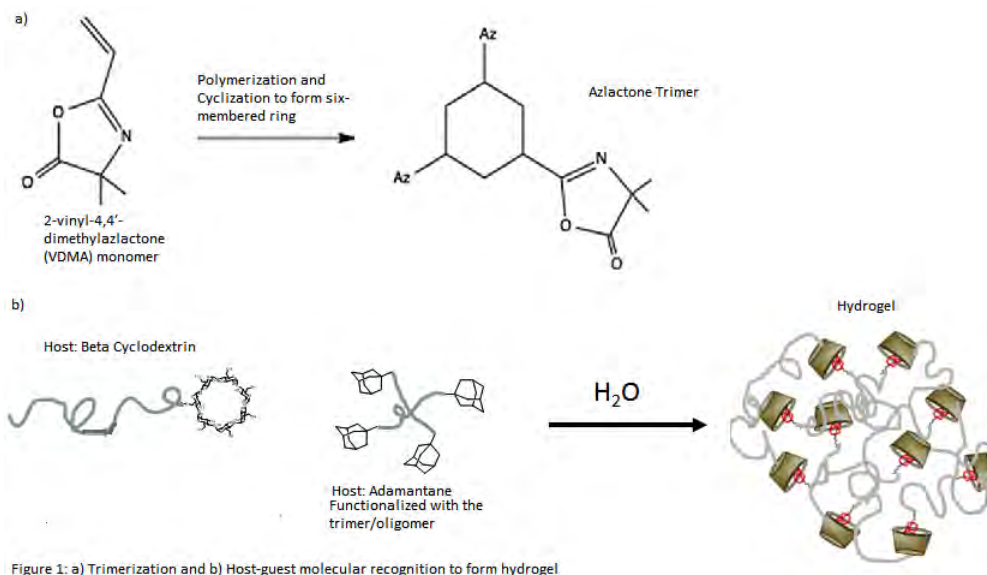
Electrospray-Ionization Mass Spectrometry (ESI-MS) was used to identify the product of our trimerization reaction, and thus far, it seems that acid-catalyzed polymerization is providing more promising results as we continue to try and form the trimer. Furthermore, our preliminary results of the reaction of the functionalized oligomer with adamantane, as shown by ATR-IR, also seems to have gone successfully. More analysis will be carried out as I continue with this project in the fall with special studies. Having had quite a few opportunities during the summer to present our work and gain feedback from different faculty members on how to troubleshoot any problems and look at new approaches, we hope to be able to make our hydrogel soon and study cells in this synthetic yet malleable environment.

*(Supported by the Schiffer Fund)*

Advisor: Maren Buck, Chemistry

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# Synthesis Optimization of Cyclo[-L-Phe-D-N-Me-Ala]4 via Solid Phase Peptide Synthesis

Chloe Jones/2015

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Due to the growing threat of bacterial resistance, there is a strong need for a new form of antibiotics. Cyclic peptides such as Cyclo[-L-Phe-D-Me-Ala]4 have shown to inhibit bacterial growth by disrupting the bacterial cell wall.<sup>1</sup> This particular peptide is composed of rings of alternating L-phenylalanine and MeN-D-Alanine that stack using beta-sheet hydrogen bonding. M. Reza Ghadiri has confirmed cyclic peptide potency against both resistant and nonresistant strains of bacteria, thereby showing promise to end the drug resistance crisis<sup>2</sup>. The hydrophobic outer ring side chains of Cyclo[-L-Phe-D-Me-Ala]4 allow the structure to permeate the lipid bilayer of the bacterial cell wall, while the hydrophilic core allows the tube to possibly be a potassium ion transporter.<sup>3</sup>

This project was mainly focused on the optimization for synthesis and purification of a dimer version of this nanotube, Cyclo[-L-Phe-D-N-Me-Ala]4. The difference being a methyl group on the nitrogen of D-alanine to prevent infinite beta-sheet stacking. This dimer was synthesized via solid phase synthesis starting with Wang-Phe-Fmoc resin and using dichloromethane to swell. For each coupling the resin was washed using dichloromethane and dimethylformaldehyde, and deprotected with 20% piperidine in DMF. A coupling solution containing either Fmoc-Me-N-D- Ala-OH or Fmoc-L-Phe-OH was added. This linear peptide synthesis utilized a new apparatus that semi-automated the washing and draining sequences and overall shortened the synthesis time by approximately 50%.

There was also a challenge left by previous work in the purification of the peptide. Both the cyclopeptide and linear peptide when purified through an HPLC showed 6-8 peaks with similar integration with an absorbance at 350 nm. The remainder of the summer was dedicated to optimizing HPLC protocol and scaling up the runs in order to identify these peaks. We have seen evidence through runs of 10 ml/min of possible conformer activity, or unreacted smaller peptide chains. Once identified, the peptide can be purified and cyclized to test ion-transport activity. I plan to continue this synthesis and optimization during fall 2014.

*(Supported by the Alumnae Gift Fund in Chemistry)*

Advisor(s): David Bickar and Cristina Suarez, Chemistry

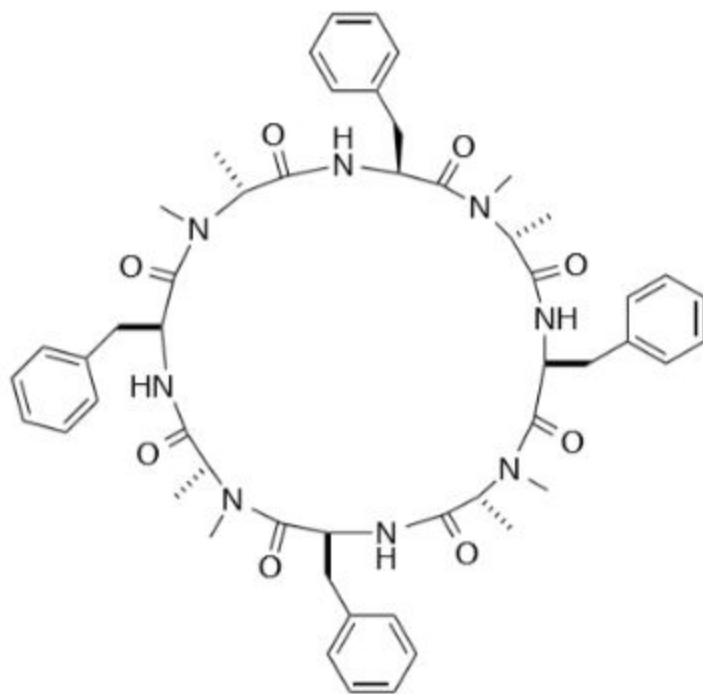


Figure 1: Final cyclo-peptide product

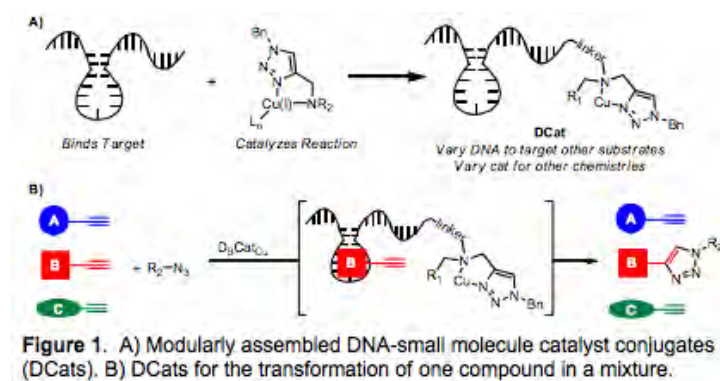
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- <sup>2</sup>Fletcher, J. T., Finlay, J. A., Callow, M. E., Callow, J. A., and Ghadiri, M. R. *Chem.-Eur. J.* 2007, 13, 4008-4013.
- <sup>3</sup>Granja, J. R., and Ghadiri, M. R. *J. Am. Chem. Soc.* 1994, 116, 10785-10786.

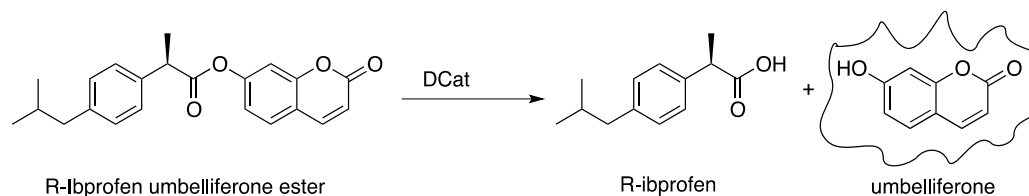
# DNA-Catalyst-Conjugates

Shimu Liu/2015

Chemical reactions in complex biological or environmental systems are gradually attracting more attention. The challenges of doing such chemical transformations are selectivity. Suppose we want molecule A to react with our target molecule, there might be molecule B and C in the biological environment that have the same active functional group as A does and they can also react with our target molecule. In this case, our desired reaction won't be selectively specific to molecule A. In Professor Gorin's lab, we are aiming to provide solutions for these problems by using DNA-small catalyst conjugates (DCats). A DCat is a DNA aptamer attached to a catalyst with small molecular weight (Figure 1A). We expect the aptamer to increase the selectivity since it can bind to a specific target molecule, and after aptamer-target binding, the small molecule catalyst can react with the target molecule. By applying DCat in the previous example, we should be able to selectively perform the transformation of molecule A even with the presence of B and C.



Last summer, I did SURF on this project and my major focus was the proof of concept of DCats. In order to show that DCats are actually effective in catalyzing desired reactions, my partner Drew and I designed and optimized a fluorogenic assay, which can display visible changes of the reactions after catalyst is added. Since the previously synthesized DCats we worked with were designed to target ester hydrolysis reaction, we made the ester substrates containing this fluorescent piece called umbelliferone ((Scheme 1). With the help of a microplate reader, we were able to monitor the change of the fluorescent signal after addition of DCat and learn about the rate and yield of the reaction. The problem we encountered with this assay was the low solubility of the substrate in water, which decreases the interaction between the DCat and the substrate. After a series of experiments, it was found that raising the temperature to 65°C and increasing the percentage of organic solvent (DMSO or DMF) in the assay both helped to get the fluorescent signal higher, which essentially means more umbelliferone was generated. However, since we're hoping to apply DCats in biological context, both high temperature and high concentration of DMSO should be avoided.



Scheme 1. Hydrolysis reaction of one umbelliferone ester substrate with DCat



This summer, I started to investigate a new direction of the DCat project and worked on porphyrin derivatives and G-quadruplex DNA aptamers (figure 1). Porphyrins are highly aromatic compounds and have strong pi-stacking interaction with the G-quad DNA structures. It also gives us more flexibility in syntheses of both the substrates and the DCats. During the substrate synthesis we realized that protoporphyrin IX (figure 2), the specific porphyrin derivative we worked with, is unstable and decomposes easily during the esterification. A more stable derivative, mesoporphyrin IX was tested and will be used for future studies. The DCat synthesis also gave us some hard time during the procedure of HPLC purification. We tried to conquer the difficulty by denaturing the DNA and future analysis is required to see if the synthesis actually generated the DCat products we expected.

*(Supported by the Schiffer Fund)*

Advisor: David Gorin, Chemistry

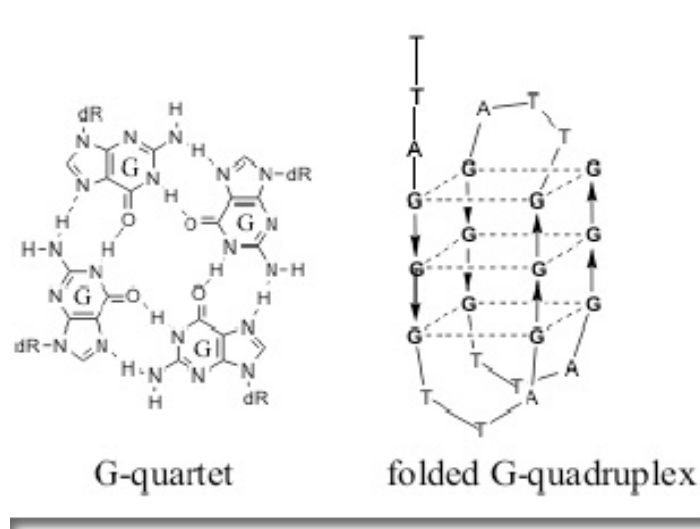
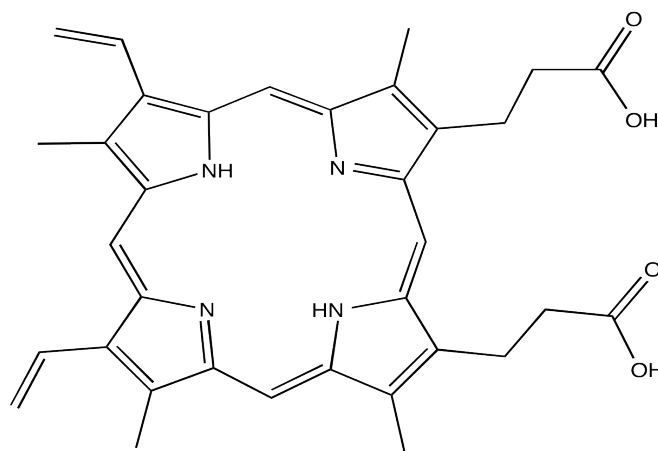


Figure 1. G-quadruplex DNA aptamers



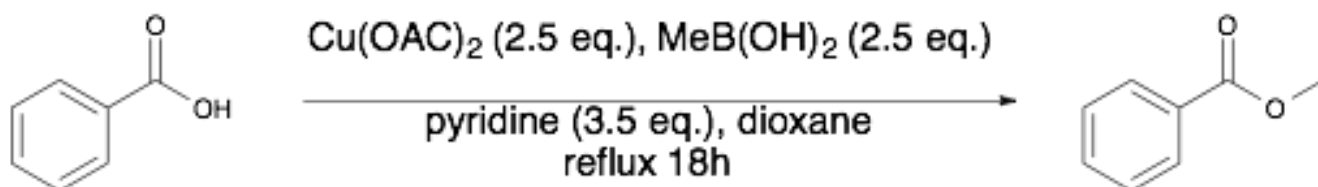
## protoporphyrin IX

Figure 2. Structure of protoporphyrin IX

# Catalytic Methylation of Oxygen Nucleophiles

Noelia Martinez-Muñoz/2016

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Methylation is an important method in organic synthesis. Commonly used methylating agents include diazomethane and trimethylsilyldiazomethane. Diazomethane is an explosive gas, must be made in the lab just before use, and is highly toxic. Trimethylsilyldiazomethane is a “safer” derivative of diazomethane because it is a liquid and therefore more stable; but is also acutely toxic. Our goal is to find milder conditions for methylation. Dimethyl carbonate is being studied as a greener and safer alternative to current methylating agents.

Previous work in our lab optimized the conditions for the methylation of carboxylic acid derivatives using dimethyl carbonate. The conditions for each carboxylic acid nucleophile used were 0.4 equivalents of potassium carbonate, 20 equivalents of dimethyl carbonate in a reaction flask heated to 90 degrees Celsius and run overnight. Part of our goal for this summer was to expand both the nucleophile and electrophile scope. We used nitrogen nucleophiles, such as benzotriazole, to see if they would methylate and got a 90% yield (benzotriazole has two nucleophilic locations that are methylated in a 2:1 ratio). We also used other electrophiles such as ethylmethyl carbonate and ethyl carbonate to see if methylation was preferred over ethylation under our conditions. Results showed that ethylation only occurs at 120 degrees Celsius with ethyl carbonate. Previous mechanistic studies concluded that our reaction undergoes an SN2-like mechanism which would limit the type of substrates we can use. We expanded our substrate pool and realized our reaction is not as limited as we had thought.

Towards the end of the summer our focus shifted to copper mediated methylation. Previously, Gonzalez et al. showed that a nitrogen nucleophile, aniline, could be methylated using methyl boronic acid, copper acetate, pyridine, dioxane and refluxing for 18 hours.<sup>1</sup> We hypothesized that testing their methods with an oxygen nucleophile would also methylate. We started by successfully replicating the aniline methylation and proceeded to substituting oxygen nucleophiles in. An aryl alcohol, an aryl carboxylic acid, and an alkyl behaving alcohol were used. The benzoic acid produced a 34% yield. The remainder of the time was spent optimizing the conditions of the reaction to obtain higher yields. We used Gas Chromatography/Mass Spectroscopy to quantify our yields on 20mg scales. We found that using Cu (II) CO<sub>3</sub> as the catalyst gave the highest yields. We also experimented with solvents, the incubation period, bases, and other substrates and adjusted the amount of material we used. Results showed that lowering the amount of copper to 0.75 equivalents and changing the solvent to dimethyl carbonate gave approximately the same yields as the control (yields varied based on GC/MS). These results are important because using less copper is more efficient and dimethyl carbonate is safer than dioxane.<sup>2</sup> We will continue to test different conditions and scale up the reactions to get isolated yields.

*(Supported by the American Chemical Society and Petroleum Research Fund)*

Advisor: David Gorin, Chemistry

<sup>1</sup> Gonzalez, I.; Mosquera, J.; Guerrero, C.; Rodriguez, R.; Cruces, J. Org. Lett. 2009, 11, 1677.

<sup>2</sup> ACS GCI Pharmaceutical Roundtable. Solvent Selection Guide 2.0. 21 Mar 2011. (Accessed Aug 29, 2014).

# Theoretical Calculation of Oxidative Addition of H<sub>2</sub> to Rhodium Compounds

Irene Marusoi/2015

My summer research was based on kinetic analysis done by Halpern et al.<sup>1</sup> on hydrogenation of [RhCl (PPh<sub>3</sub>)<sub>3</sub>] and [RhCl (PPh<sub>3</sub>)<sub>2</sub>] with an aim of synthesizing [RhCl H<sub>2</sub> (PPh<sub>3</sub>)<sub>3</sub>]. In this case hydrogenation was examined in two ways. The first hydrogenation is summarized as the dissociation of RhCl (PPh<sub>3</sub>)<sub>3</sub> to form RhCl (PPh<sub>3</sub>)<sub>2</sub>. RhCl (PPh<sub>3</sub>)<sub>2</sub> is very reactive with H<sub>2</sub>. PPh<sub>3</sub> then adds to the adduct to form Rhodium octahedral complex as shown. [RhCl (PPh<sub>3</sub>)<sub>3</sub> → (PPh<sub>3</sub>)<sub>2</sub> + PPh<sub>3</sub> (1), RhCl (PPh<sub>3</sub>)<sub>2</sub> + H<sub>2</sub> → RhClH<sub>2</sub> (PPh<sub>3</sub>)<sub>2</sub> (2), RhClH<sub>2</sub> (PPh<sub>3</sub>)<sub>2</sub> + PPh<sub>3</sub> → RhClH<sub>2</sub> (PPh<sub>3</sub>)<sub>3</sub> (3)]. The second pathway is the addition of H<sub>2</sub> to a square planar [RhCl (PPh<sub>3</sub>)<sub>3</sub>]. As established by Halpern's findings, the dissociation pathway had a lower activation energy as compared to the direct addition of H<sub>2</sub> to the square planar. It is also important to note that, in the kinetic study summarized, benzene was not active but it was used as a solvent.

My studies were as summarized in scheme 1 with predicted substitution of benzene for one of the X ligands that would result in lower activation energy required for hydrogenation. My projected reaction pathways were as follows: [RhCl (C<sub>6</sub>H<sub>6</sub>)(X)<sub>2</sub>]<sub>3</sub> → (X<sub>3</sub>)<sub>2</sub> + C<sub>6</sub>H<sub>6</sub> (1), RhCl (X<sub>3</sub>)<sub>2</sub> + H<sub>2</sub> + C<sub>6</sub>H<sub>6</sub> → RhClH<sub>2</sub> (X<sub>3</sub>)<sub>2</sub> + C<sub>6</sub>H<sub>6</sub> (2), RhClH<sub>2</sub> (X<sub>3</sub>)<sub>2</sub> + X<sub>3</sub> + C<sub>6</sub>H<sub>6</sub> → RhClH<sub>2</sub> (X<sub>3</sub>)<sub>3</sub> + C<sub>6</sub>H<sub>6</sub> (3)]. All calculations were optimized using Gaussian 09 program and optimized using LANL2DZ basis set and RMO62X method of calculation.

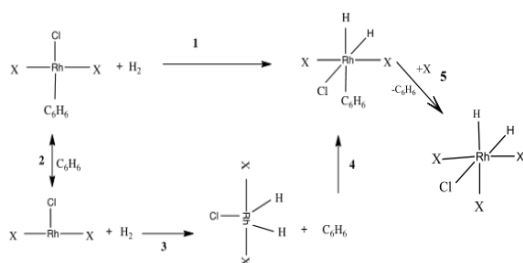
My main goal was to understand transition states for each reaction pathway described in the scheme. I further obtained a substitution transition state for formation of [RhCl (C<sub>6</sub>H<sub>6</sub>) (X)<sub>2</sub>] from a 5-coordinated rhodium compound [RhCl (C<sub>6</sub>H<sub>6</sub>) (X)<sub>3</sub>].

Final results indicated that the substituted reaction path had a lower activation (+14.41kcal/mole) compared the dissociation pathway described by Halpern et al (+31.08kcal/mole). The results agree with the predicted substitution statement stated above; benzene pathway is more favorable than the dissociation pathway. More experiments and literature searching ought to be done in the future in order to support this theory.

*(Supported by the Schultz Foundation)*

Adviser: Robert Linck, Chemistry

<sup>1</sup>Halpern, I.; Wong, C.S.; I.C.S.Chem.Comm.1973, 629-630



Scheme 1: Reaction pathways of Oxidative addition of H<sub>2</sub> to form an octahedral di-hydrogen complex involving benzene substitution .X=CO, PH

# Ligand-Ligand Interactions in $\text{Mn}(\text{CO})_5\text{X}$ Compounds

Alexandra Maryashina/2017

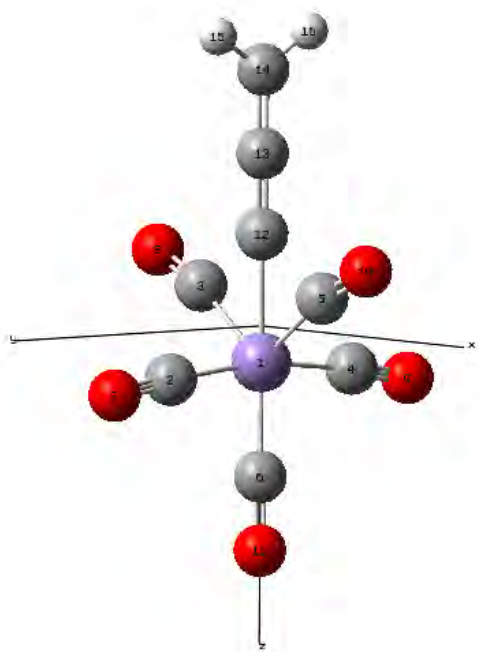
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The compounds we studied in this work,  $\text{Mn}(\text{CO})_5\text{X}$ , are important in understanding insertion reactions. They are observed to distort from octahedral geometry depending on the nature of the ligand X. In compounds of appropriate symmetry, there are two equatorial X-Mn-CO angles. The angle in the xz-plane that we calculated using the Gaussian09 package varies from  $90^\circ$  to  $52^\circ$ , while the angle in the yz-plane is distorted significantly less and is dependent upon the xz-plane angle. A plot of the average distortion angle versus the charge on  $\text{Mn}(\text{CO})_5$  fragment shows that the biggest distortion occurs when the ligand X is the best donor – see the figure.

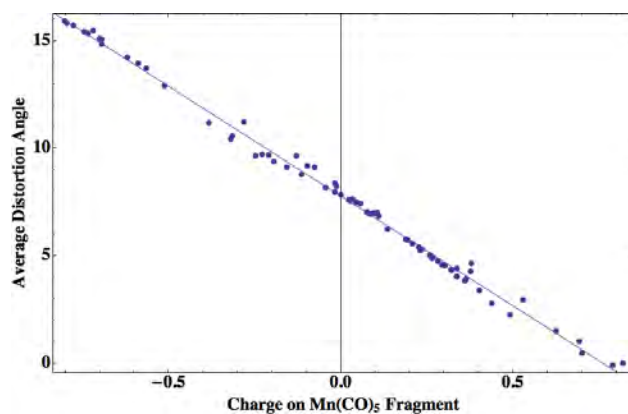
In this research, we discovered the reason two of the equatorial angles behave differently from the other two. We suggest a model of  $b_1$  interaction, a conjugative donation from the filled ligand  $\text{X p}_x$  orbital to the empty  $\pi_z^*$  orbitals on the carbonyls in the xz-plane. As the orbital overlap becomes larger the distortion angle increases. We found that the C-O bond distance of the carbonyls lengthens with distortion angle, which is consistent with donation into the carbonyl antibond. In addition, both a Natural Bonding Orbital and Amsterdam Density Functional analyses show the increase in  $\pi_z^*$  electron population to be expected from this interaction. In the case of the carbonyls in the yz-plane, the filled  $\text{p}_y$  orbital on the ligand X is not stabilized by the conjugation effect, and therefore cannot overlap as well with the carbonyls in the yz-plane. In compounds with a big distortion, such as  $\text{Mn}(\text{CO})_5\text{CCH}_2$  shown below, the yz-plane carbonyls actually bend away from the X ligand. However, even in these highly distorted compounds, known as “post-bifurcation compounds”, the average angle still relates to the charge on  $\text{Mn}(\text{CO})_5$  fragment in the same way. We hope to continue this research on post-bifurcation compounds during the upcoming academic year and ultimately understand how the yz-plane carbonyls are dependent on the carbonyls in the xz-plane.

*(Supported by Committee on Faculty Compensation and Development, Smith College)*

Advisor: Robert Linck, Chemistry



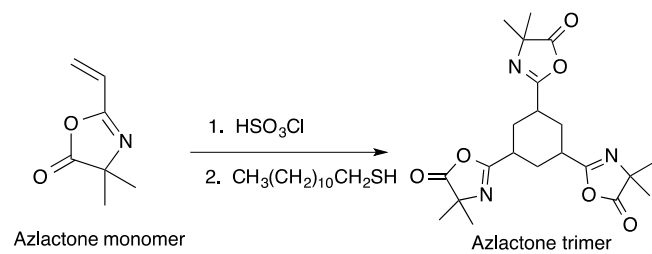
Bifurcation in  $\text{Mn}(\text{CO})_5\text{CCH}_2$



Plot of average distortion angle as a function of charge on  $\text{Mn}(\text{CO})_5$  fragment



Figure 2.



The two-step synthesis of the azlactone trimer through activation of the amine and the Michael addition of the thiol group.



# Clinical Analysis of the Correlation Between the Increasing Prevalence of Regressive Autism and the Incidence of Viral Co-Infection

Mojdeh Mostafavi/2015

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## Background

Autism, a developmental disorder affecting 1 in 68 children in the United States today, has increased dramatically since ~1970.<sup>1</sup> If the prevalence continues to increase at the current rate, 1 in 15 children born in the year 2015 will develop the disorder. Although several explanations for this increase have been proposed, its cause, like that of the disorder, is still unknown. Within the autism spectrum there are multiple types with different symptoms and etiologies. Those diagnosed with classic autism can be further divided into two subtypes. One subtype, sometimes called severe/regressive autism, is characterized by apparently normal development before an abrupt regression occurs between the ages of 12 and 24 months. Individuals with this subtype have been found to have a significantly reduced incidence of seizure disorders and increased intellectual ability, but to also have more associated comorbidities including G.I. disorders.<sup>2</sup>

## Proposal

We propose that some genetically predisposed children have an increased risk of contracting a specific viral infection at ~12 months. This infection causes their already limited immune response to be further suppressed, and permits a second, normally benign virus to multiply and enter the brain, where it eventually results in regressive autism. There are several lines of evidence that support this hypothesis; the abrupt onset of symptoms, starting between 12 and 24 months of age, is at a time when a child's immunity is at its lowest and viral infections are common. The gender ratio for children with severe/regressive autism, about 8 boys to every girl, matches the ratio observed in some common childhood viral infections and may reflect the differences between males and females in their immune response to these viruses. Finally, several of the viruses exhibit neurotropic behavior in immunocompromised individuals.

## Clinical Study

Our objective is to see if specific viral infections are critically associated with regressive autism. Children under the age of four, who have been diagnosed with regressive autism and their age-matched peers, will be tested to identify prior viral infections. Analyses will include serological assays for (1) the presence of viral genetic material, (2) antibody responses to specific viruses of interest and (3) immunosuppression. This work will allow development of better diagnostic tools for autism and may suggest ways to limit the severity of the disease. During this summer we initiated a clinical study to test our hypothesis, first by refining our study design and then applying for funding, and beginning the recruitment of study subjects.

*(Supported by the Schultz Foundation)*

Advisor: David Bickar, Chemistry

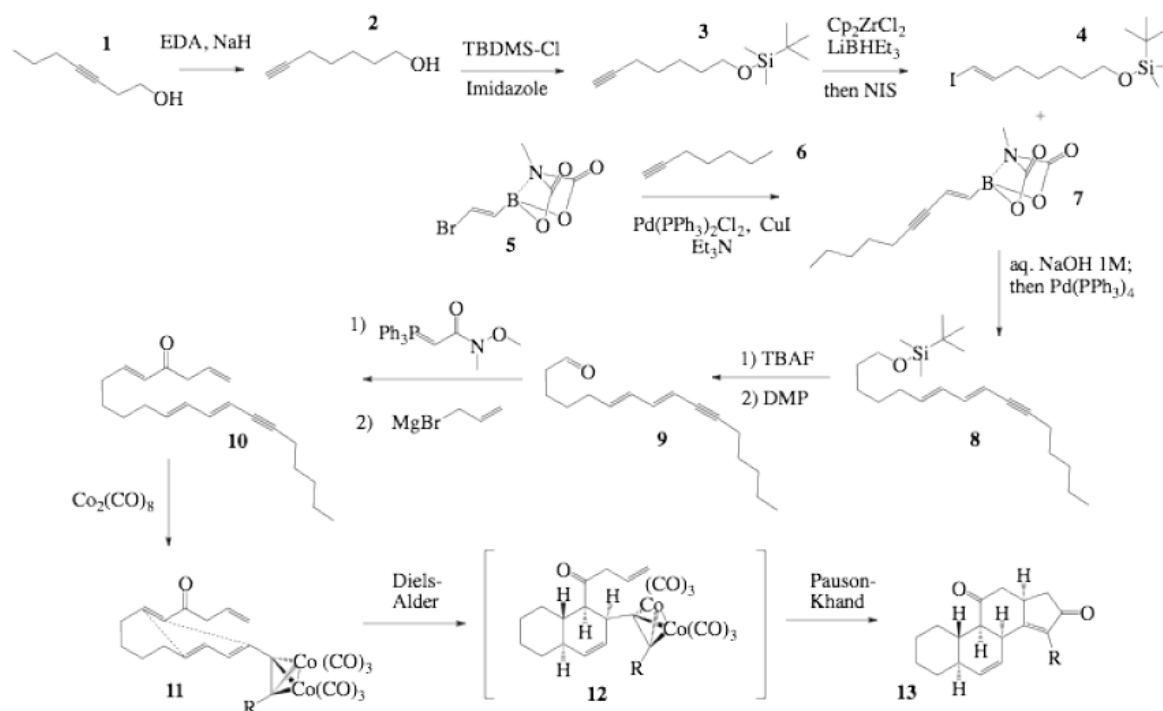
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Doshi-Velez, Finale, Yaorong Ge, and Isaac Kohane. "Comorbidity Clusters in Autism Spectrum Disorders: An Electronic Health Record Time-Series Analysis." Pediatrics 133.1 (2014): e54-e63.

# Development of a Tandem Diels-Alder/Pauson-Khand Reaction for the Formation of Tetracycles

Zulema Peralta/2015



In organic chemistry there is a need to develop new methods for activating dienes. In this project, a cobalt-complex alkyne is used as an electron withdrawing group to activate the diene in intermediate 10 and facilitate the formation of our final product, compound 13. For the past few years the main focus of this project has been to successfully produce enough material of compound 11 so as to run a one-pot tandem Diels-Alder/Pauson-Khand reaction. Although it is believed that Elsa Hinds '13 synthesized compound 10 in the spring of her senior year there is no spectra with which to prove her success because there was not enough material to take a proton NMR.

Last year, Natalie Vaninov '14 was able to modify the hydrozirconation/iodination reaction, as well as the purification method used on the Sonogashira product that leads to compound 7. Vaninov changed the electrophilic source of iodide from Iodine to N-iodosuccinimide leading to a more effective, and somewhat more pure, formation of compound 4. After listening to a presentation by Seiko Fuji, Vaninov decided to purify her Sonogashira product using what Seiko called the "catch and release" method. The catch part of the method entails using a less polar solvent such as diethyl ether and 1.5% methanol to remove the unwanted side products, while the release portion necessitates the use of a more polar substance such as ethyl acetate so as to release compound 7.

This summer I have focused my efforts on successfully producing enough material of compound 4 and compound 7 so as to run a Suzuki reaction. During my five weeks I was able to produce thirteen grams of compound 1, successfully protect the alcohol, and run hydrozirconation/iodination reactions on about half of the product. When trying to purify compound 4, I was faced with problems which Hinds and Vaninov had also experienced; I ran a column with 120 fractions where the product did not successfully separate. After trying various solvents, I determined that using a 1.5% ethyl acetate to hexanes, as opposed to 0.3% ethyl acetate to hexanes, removed the majority of the side product and allowed for the product to be collected in 70 fractions as opposed to 120. Regardless, even though this solvent system seems to be the correct one, 70 fractions are still too many to collect. I have decided to make my column shorter in an attempt to collect my product faster but with the same purity.

I have also synthesized compound 7 and have enough to continue with the following step in my synthetic scheme. This upcoming school year I will continue my research as an Honors Thesis in hopes of finally being able to run a Suzuki reaction effectively, as well as the final tandem Diels-Alder/Pauson-Khand reaction.

*(Supported by Howard Hughes Medical Institute)*

Advisor: Kevin Shea, Chemistry

# Investigating the Relation of Dopamine Level in Saliva and Parkinson's Disease

Jingyi Sui/ 2017

This study is to test dopamine level in saliva, which can help identify people who might develop PD before they are diagnosed. Motor symptoms of PD include tremor, disturbed gait, and rigidity. However, by the time the patients show these symptoms and are diagnosed, they would have lost more than 60% dopaminergic neurons. Many patients have non-motor symptoms before they are diagnosed with PD, which include sleep disorders, diminished color discrimination, and cardiac arrhythmia. These non-motor symptoms might be related to loss of dopaminergic neurons in the peripheral nervous system. Therefore, testing dopamine level of saliva would help identify people who might develop PD.

For this study, HPLC is used to test dopamine concentration. A standard of dopamine concentrations against the peaks shown on HPLC is needed first. Dopamine was dissolved in buffer solution of pH 3, and stabilized by sodium bisulfite with a concentration of 5 mM. The concentrations of dopamine were set from 10 pg/mL to 1 ug/mL, because the dopamine concentration in human saliva is about 30 pg/mL. The internal standard, 3,4-dihydrobenzylamine hydrobromide, was also added in the real saliva test, which, therefore, needed a standard curve of concentration against the signals shown in HPLC as well. The concentrations of the internal standard were on about the same scales as those of dopamine. Real saliva test was run after finding both standards. The dopamine in saliva, mixed with known amount of internal standard, was extracted by EVOLUTE WCX, and tested using HPLC.

The standards of dopamine and the internal standard found in the end were still not satisfying enough. Figure 1 shows the standard of dopamine concentration, which is made by averaging all of the possible data.

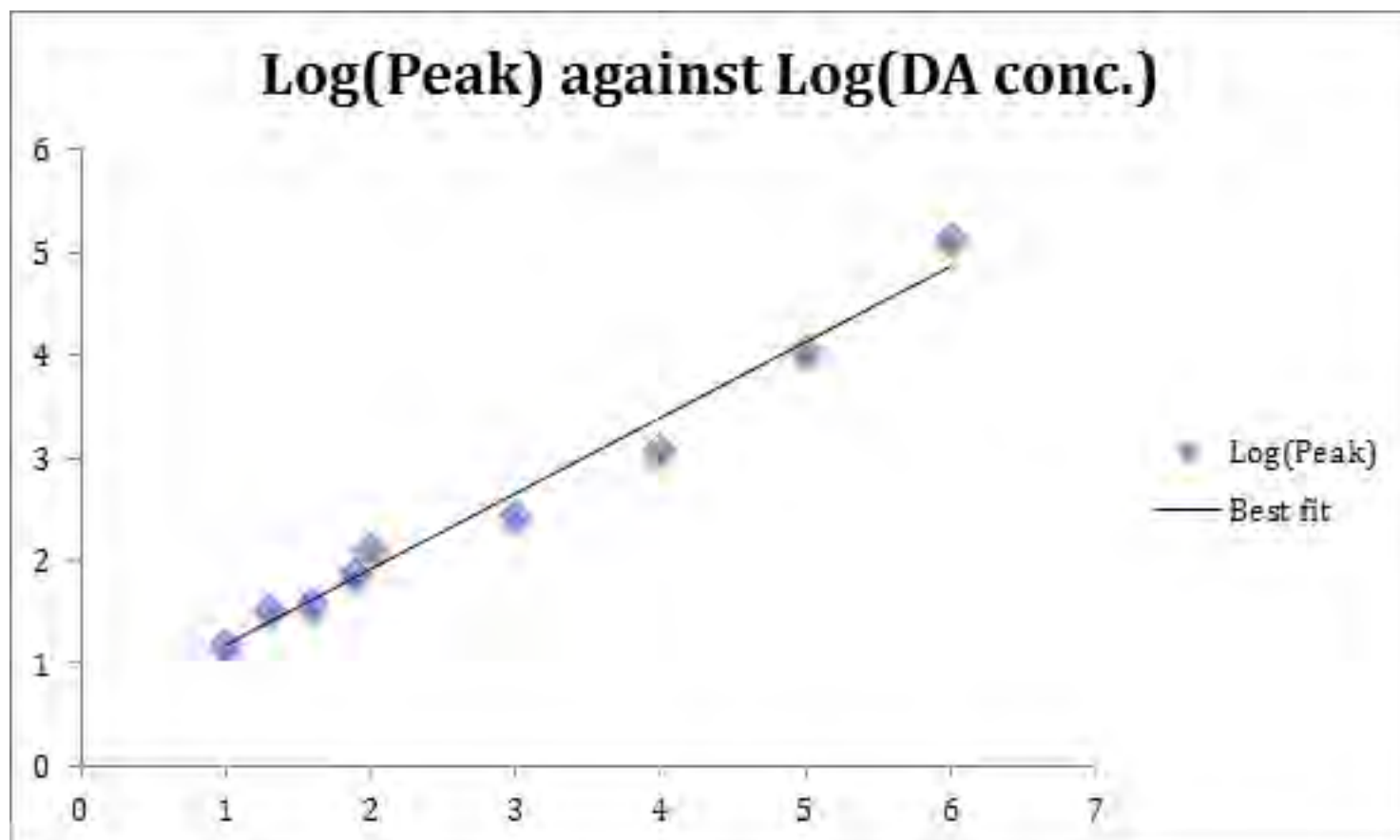


Figure 1

Also, I tested my own saliva although the real concentration of dopamine could not be determined. For future study, more dopamine solutions can be made and tested to get an accurate standard.

*(Supported by Howard Hughes Medical Institute)*

Advisor: David Bickar, Chemistry

Goldenberg, Marvin M. "Medical Management of Parkinson's Disease." *National Center for Biotechnology Information*. U.S. National Library of Medicine, 29 Mar. 0006. Web. 29 Aug. 2014.

# Synthesis of 2,6-Dialkylcyclohexanol Derivatives for Use as Potential Anesthetics

Julia Yun/2016

Propofol (Fig. 1) is a commonly used intravenous anesthetic agent that binds to the GABA<sub>A</sub> receptor in the central nervous system. The structure-activity relationship (SAR) analyses have shown significance of aliphatic groups adjacent to the hydroxyl group in propofol's anesthetic properties.<sup>1</sup> In Adam Hall's Laboratory, numerous isomeric mixtures of cyclohexanol derivatives with aliphatic groups on 2,6-positions were tested and received positive results. In order to test the possibility of GABA<sub>A</sub> receptor being stereoselective, previous works from Kelly Smith and Alexandra Gatsios have focused on separating the diastereomers of 2,6-dimethylcyclohexanol and 2,6-diethylcyclohexanol. However, the inability to identify the cyclohexanols in UV light and their low molecular weight has made the separation a struggle. Therefore, the goal of this project was to place phenyl groups in 4-position of 1,4-cyclohexanedione in order to synthesize UV active and higher molecular weight cyclohexanol derivatives, and to synthesize 2,6-diisopropylcyclohexanol (Fig. 1) for further GABA<sub>A</sub> receptor stereoselective testing.

Two different approaches were made for placing phenyl groups in 4-position of the commercially available 1,4-cyclohexanedione. In the first method, 1,4-cyclohexanedione was reacted with phenol and sulfuric acid in a mixture of water and 1,4-dioxane solvent to synthesize 4,4-bis(4'-hydroxyphenyl) cyclohexanone (Fig. 2).<sup>2</sup> <sup>1</sup>H and <sup>13</sup>C NMR spectra illustrated that no reaction occurred, despite the length of reaction time or concentration of reagents. 1,4-cyclohexanedione was also reacted with benzene and trifluoromethanesulfonic acid (Fig. 3).<sup>3</sup> The spectra for this reaction depicted both ketones on 1,4-cyclohexanedione reacting with benzene.

The synthesis of 2,6-diisopropylcyclohexanol occurs over several reactions, starting with synthesizing 2-cyclohexenyl pivalate by reacting trimethylacetyl chloride with 2-cyclohexen-1-ol, dichloromethane, and pyridine for nucleophilic substitution (Fig. 4).<sup>4</sup> The product was purified via flash column chromatography and run through a Grignard reaction with copper cyanide and isopropyl magnesium bromide to produce 3-(2-propyl)cyclohexene (Fig. 4).<sup>5</sup> This product was purified via vacuum distillation, but was only able to gain small product yield due to technical difficulties in the purification process. Both purified and crude 3-(2-propyl)cyclohexene was reacted with mCPBA and dichloromethane to synthesize 3-isopropyl-1,2-epoxycyclohexane (Fig. 4), which was purified via aqueous workup.<sup>6</sup> The <sup>1</sup>H NMR of pure 3-isopropyl-1,2-epoxycyclohexane matched the peaks reported in Portalier et al. The resulting peaks from using crude 3-(2-propyl) cyclohexene showed majority of 3-isopropyl-1,2-epoxycyclohexane and 3-(2-propyl) cyclohexene peaks, indicating that there may be selective reactivity for mCPBA between the two alkene structures.

Due to unsuccessful results in both reactions producing diphenyl derivatives, I decided to move on and focus on synthesizing 2,6-diisopropylcyclohexanol. The next step of this project is to perform flash column chromatography on the crude 3-isopropyl-1,2-epoxycyclohexane to confirm whether vacuum distillation in the previous reaction is necessary. Also, the synthesis will be continued by performing another Grignard reaction on 3-isopropyl-1,2-epoxycyclohexane, which will be followed by the separation of 2-diisopropylcyclohexanol isomers. These isomers will be tested for their anesthetic potency and will hopefully enhance the understanding of 2,6-disubstituted cyclohexanol molecules' anesthetic properties.

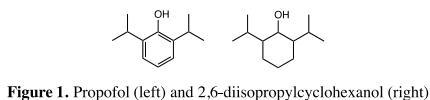


Figure 1. Propofol (left) and 2,6-diisopropylcyclohexanol (right)

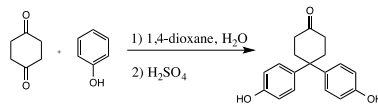


Figure 2. Synthesis of 4,4-bis(4'-hydroxyphenyl)cyclohexanone

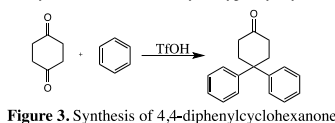


Figure 3. Synthesis of 4,4-diphenylcyclohexanone

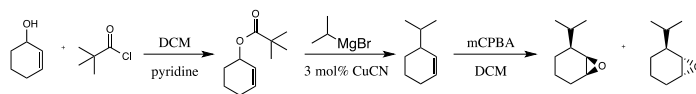


Figure 4. Synthesis of 3-isopropyl-1,2-epoxycyclohexane



*(Supported by the Schultz Foundation)*

Advisor: Kevin Shea, Chemistry

#### References

<sup>1</sup>Hall, A.C.; Griffith, T. N.; Tsikolia, M.; Kotey, F. O.; Gill, N., Humbert, D. J.; Watt, E. E.; Yermolina, Y. A.; Goel, S.; El-Ghendy, B.; Hall, C. D. Eur. J. Pharmacol. 2011, 667, 175-81.

<sup>2</sup>Aitipamula, S.; Nangia, A. Chem. Eur. J. 2005, 11, 6727-6742.

<sup>3</sup>Klump, D. A.; Garza, M.; Jones, A.; Mendoza, S. J. Org. Chem. 1999, 64, 6702-6705.

<sup>4</sup>Bourque, L. E.; Cleary, P.A.; Woerpel, K. A. J. Am. Chem. Soc. 2007, 129, 12602-12603.

<sup>5</sup>Tseng, C. C.; Paisley, S. D.; Goering, H. L. J. Org. Chem. 1986, 51, 2884-2891.

<sup>6</sup>Portaier, F.; Bourdreux, F.; Marrot, J.; Moreau, X.; Coeffard, V.; Greck, C. Org. Lett. 2013, 15, 5642-5645.

# The Global Proverbs Project

Li Chai/2016

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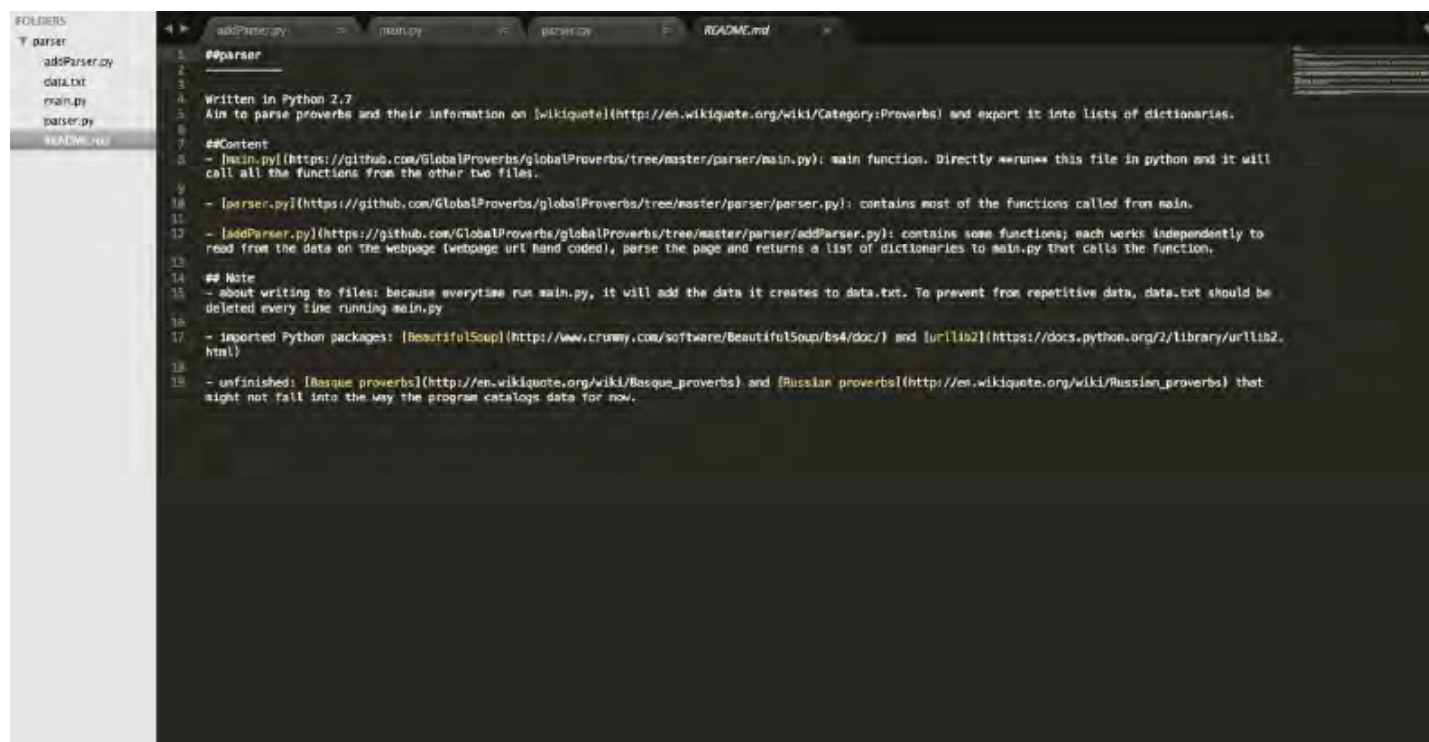
The Global Proverbs Project is a research project that focuses on gathering and creating a multilingual database with which to store and analyze proverbs. The database is built upon Django web framework and MySQL. It allows users to get access to proverbs annotated with cultural, temporal, geographic, and thematic information and to edit proverbs' information. One way to populate such a database is to use other data sources with permissive copyrights such as out of copyright books about proverbs, and other knowledge repositories such as Wikipedia. My primary focus this summer was to parse proverbs from Wikiquote—a free online quote database built collaboratively using Wiki software, a sister project to Wikipedia—into a list that includes the proverbs and their annotations.

I used urllib2 module to open urls, and BeautifulSoup, a Python library that creates a parse tree for parsed pages in HTML, to help locate the part of content on a webpage I needed. By looping through 'li' tags that contain a proverb and its annotation, the function—parser—can obtain the information and convert them into one dictionary. After iterating through all the proverbs within one proverb page on Wikiquote, the parser function eventually returns a list of dictionaries containing all of the necessary content on this page. Some of the difficulties I encountered came from the difference in categorization of how people contribute their knowledge to the webpage. Information on some web-pages was incomplete or not well-organized, especially those in languages not widely spoken. Those pages usually have a emphasized line at the top, "This article needs cleanup," showing their irregular structure. In these cases, the parser needed to be customized for each page, so I wrote more functions that were similar to the original parser function but each was only applicable to one webpage. These additional functions were stored in addParser.py.

Another issue that arose is related to the fact that one proverb may have different versions. Our project is expected to show multilingual versions of one proverb. Therefore, after I completed the parser function, I added a reformat function to be called at the end of parser. It will loop through each dictionary of the complete list again and search if there is a label in the dictionary containing the keyword "equivalent". Eventually when importing the result to our database, program will find all the versions of one proverb under "proverbs" label in a dictionary.

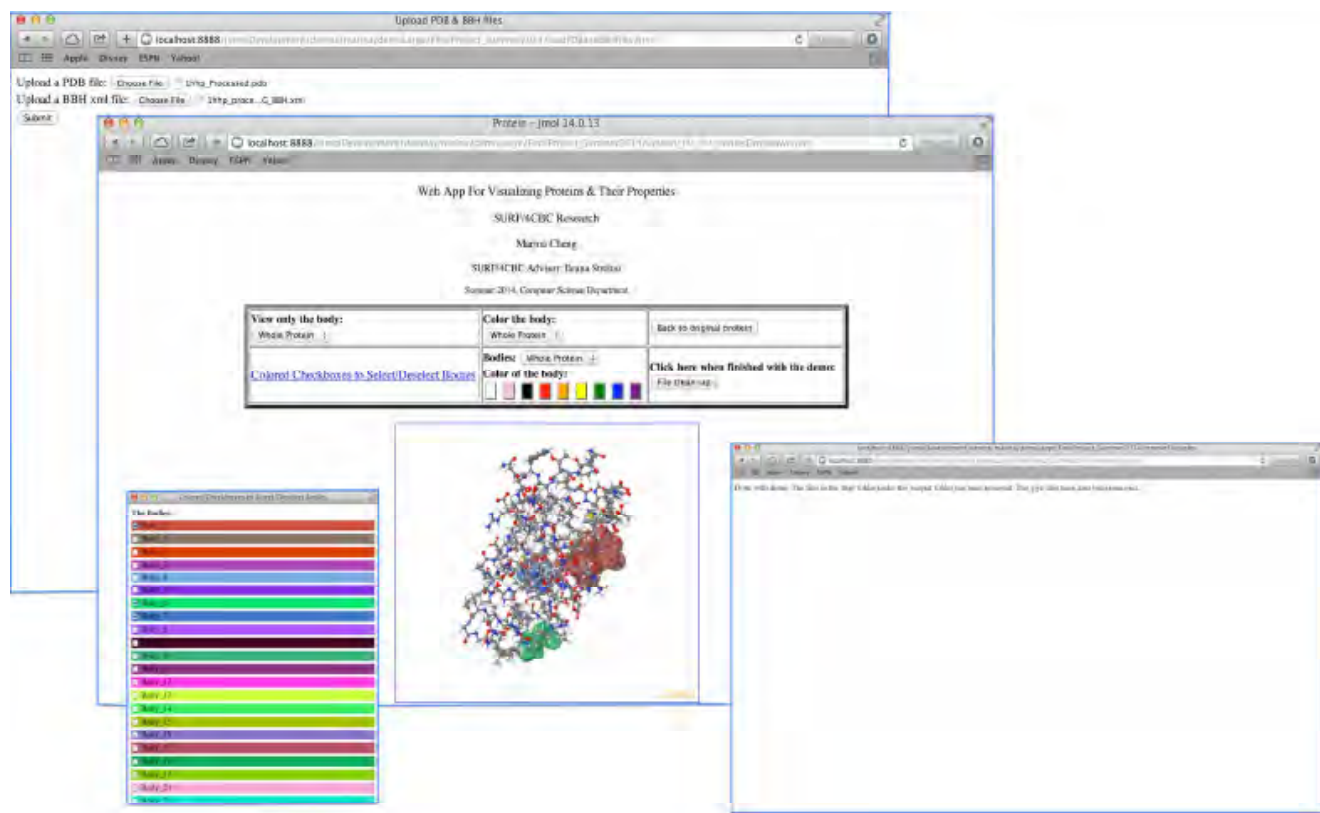
*(Supported by the Schultz Foundation)*

Advisor: Eitan Mendelowitz, Computer Science



# Web App For Visualizing In Silico Proteins and Their Properties

Marina Cheng/2017



Proteins are macromolecules that play a pivotal role in biological processes in living organisms. Their 3D structure and how they are folded determines their chemical properties. To help biologists and chemists determine the functions of proteins, I worked on creating a web app that allows researchers to more quickly and accurately determine the functions of the protein of interest.

Professor Streinu's lab created the KINARI (KINematic And Rigidity analysis of proteins) software, which calculates and analyzes biomolecules' rigidity and flexibility.<sup>1</sup> Currently, KINARI uses Jmol apps, which cause problems when users have different versions of Java installed, so we wanted a more stable method of visualizing the proteins. I thus helped start the transformation of imbedding JsMol apps instead of Jmol apps, and in the process, learned and recorded what kind of JsMol-related functions one could put in web apps.

I learned new web development tools including PHP, HTML, CSS, JavaScript, and JQuery, and along with Python, created a user interface that is easy to use. I used KINARI's output of rigidity analysis (a PDB and BBH file) to visualize the clusters and produced various smaller individual apps in higher order of complexity and combined the most useful ones into one.

I made an interactive web app demonstration in which the user can view and interact with any protein or a particular calcium atom of a 1CLN protein more closely and effectively. The image above shows screen-shots of another web app. Users first load PDB and BBH files, leading them to a different interactive page where the user could open a pop-up window of a list of color-coded checkboxes that when clicked on, puts an isoSurface with that particular color around the body, each of which represents a cluster defined by the bar-body-hinge file. They could also select a body from the drop-down menus, which either isolates or colors the body in the JsMol box and in the drop-down menu. They may put an isoSurface of the color of their choice around a body, and by clicking on the "Back to Original Protein" button, may revert back to the original protein at anytime. The "File clean-up" button cleans out all the protein-specific files outputted by the various Python programs once the user is finished with the demonstration.

My work provides a foundation for future work with updating KINARI and a more efficient method for researchers to visualize and interact with proteins.

*(Supported by the National Science Foundation (4CBC))*

Advisor: Ileana Streinu, Computer Science

<sup>1</sup>Naomi Fox, Filip Jagodzinski, Yang Li, Ileana Streinu. KINARI-Web: A Server for Protein Rigidity Analysis, Nucleic Acids Research, 39 (Web Server Issue), 2011



## Modern Technology/Ancient Manuscripts

Rui Huang/2016 and Alice Yang/2017

The Modern Technology/Ancient Manuscripts project aims to create digital tools for analyzing handwriting in ancient Syriac manuscripts. Syriac writings include topics from daily life to early Christianity and provide insights to scholars of multiple disciplines. Unfortunately, many Syriac manuscripts are not documented by author or date. Writing programs to analyze multiple manuscripts and provide this information would further the identification of provenance, discovery of connections among texts, and framing of new historical questions.

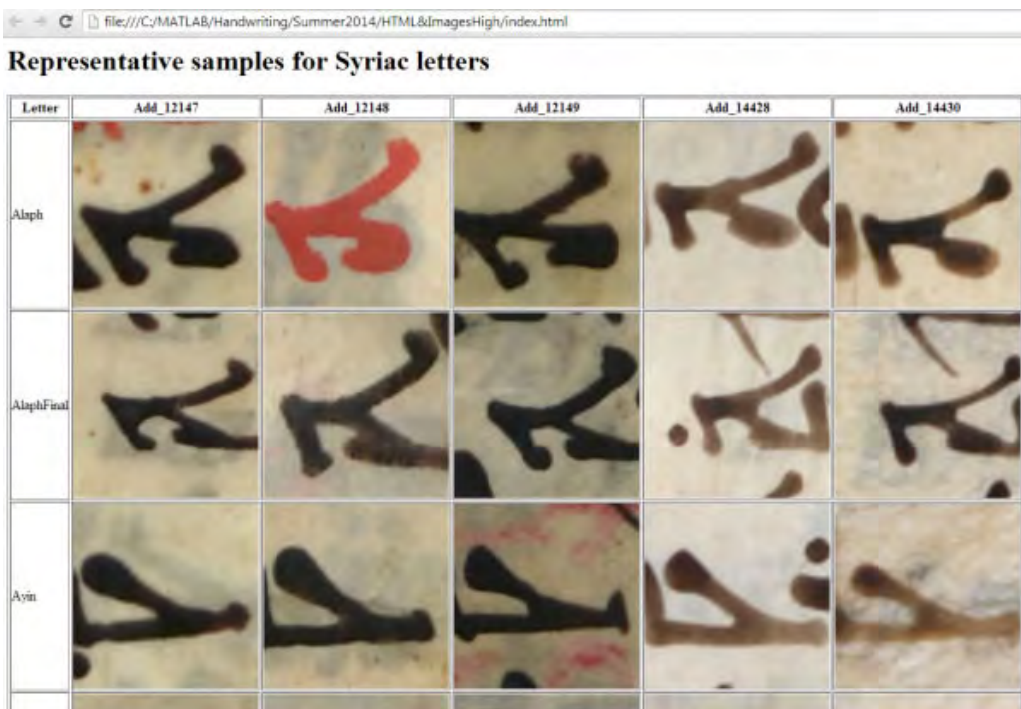
The research can be divided into two parts: creating a script chart of most representative samples for Syriac letters categorized by manuscripts and comparing the similarities between different sets of manuscripts. Using the programming language MATLAB, we revised and integrated programs written by Professor Howe and previous students as well as wrote new programs contributing to the whole framework. The programs were tested using a large database.

First, a script chart of the most representative samples was generated for Syriac letters categorized by manuscripts from a list of Syriac letter samples to produce a straightforward way to represent the handwriting styles for each manuscript and allow the scholars to work with greater speed and organization on a rough comparison between manuscripts. The tool illustrating Syriac letters can be used both in research and in teaching. Second, to process more specific identification of the manuscripts, such as author and year, a comparison program was written to generate a matrix of scores representing similarities between two sets of manuscripts. With these scores, we are hoping to be able to achieve further identification of manuscripts. Alice will present the summer work to the department at the beginning of next semester and during Collaborations next spring. She will also continue to work on the project during the next academic year, specifically on improving the manuscript comparison method.

The summer research experience was valuable for both of us. We learned a lot about code reviews and pair programming which are two important skills in the computer science field and are not easy to learn from class work. They will help us to be even better programmers, partners and researchers.

*(Supported by the 5 College Digital Humanities Grant, Huang and the Schultz Foundation and Provost's Office, Smith College, Yang)*

Advisor: Nicholas Howe, Computer Science





# Introduction to Java Online Course

Naomi Long/2015

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## Variables

### What is a type?

You may not have heard the word "type" before, but if you've coded in Python, you know (at least unconsciously) what a type is. A type is just a kind of data, like strings, integers, floating point numbers, or booleans. When we talk about the type of a variable, we're talking about the kind of data it's holding:

```
# python
x = 3          # x is type integer because it's holding an integer value
x = "abc"      # now x is type string
x = 5.5555     # now x is type floating point (the type for decimals)
x = True       # now x is type boolean
x = Point(0,0) # now x is type Point because it's holding a Point object
```

In Python, types change freely from one line to another; we can put a string in a variable that was previously holding an integer without any trouble.

Programming with Data Structures (CSC 212) is a core course in the computer science major that also happens to be one of the toughest. Students struggle to balance 212's two main topics - data structures and the Java programming language - with mixed results. Often, students end up focusing on learning Java to the neglect of data structures.

To address this issue, I decided to make an optional non-credit online course called Introduction to Java. Students could take the course in the summer or interterm before they took 212 to get a head start with Java, allowing themselves more time to focus on data structures while the class.

My teaching assistant experience taught me that students often struggled with basic Java syntax. They spend too much time looking up how to write a for-loop, forgetting to add semicolons, and poring over simple error outputs. Students also often lack the vocabulary to Google for solutions, and the help pages they find are filled with technical jargon they don't yet understand.

Thus, I designed the course with two main goals in mind: 1) teach students the vocabulary they need to Google for help, and 2) give them plenty of practice with basic syntax so they can spend less time during the course looking up print statements and more time formulating their program structure. Each chapter features a range of exercises that allow students to practice reading and writing Java.

While a student could purchase a textbook and go through its exercises before 212, the need still exists for an online course. This is because textbooks are expensive and too long; no one wants to read an entire textbook in addition to their regular course work. On top of that, most Java textbooks are too technical or focus too much on object-oriented programming theory, which is not what a 212 student needs.

My online course is ideal because it's short and tightly focused on practicing simple code. Because it's short, people are more likely to do it. And because it keeps students constantly coding, they'll get the practice they need and be more engaged than if they were reading a chapter in a textbook.

I plan to upload the course to the computer science department website and advertise it to students every semester. Once I've graduated, hopefully professors and students can continue to maintain and advertise the course.

*(Supported by the Online Learning Task Force, Smith College)*

Advisor: Nicholas Howe, Computer Science

# An Online Database for Computational Paremiology

Kaitlyn Stumpf/2016

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This summer I continued work on an online database for computational paremiology. The Global Proverbs Project is an ongoing attempt to create a comprehensive, interactive database of proverbs. This database allows us to organize and analyze the cultural data we collect in more innovative and interesting ways than ever before. The database we've created over the course of SURF will soon be online, supported by the community, and filled with proverbs from a range of cultures and languages. It significantly improves upon existing collections of proverbs in terms of its ability to categorize and study the proverbs it is given. As such it will be instrumental in furthering paremiology.

In order to finish the major portion of the database design by the end of the summer, in-depth understanding of the Django web framework, Javascript, JSON, and asynchronous programming was necessary. My research methodology thus revolved around the study of the above practices, through online tutorials such as *Getting Started With Django*<sup>1</sup>, and APIs such as the comprehensive *jQuery API*<sup>2</sup>.

This summer the following results were achieved: I added user registration and permissions to the database, so that website users now have the ability to register, log in and out, and change their password easily and safely. In addition, I extended the Django User model to contain a language field, which will be used in the future to organize the proverbs the user sees by the user's native language. I added a Twitter Bootstrap design to give the website's CSS a professional look. Finally, I created an asynchronous drop down menu in order to increase the detail with which a proverb's usage location can be described by the user who is entering the proverb's information into the database.

The development of this database will improve the online community's ability to organize and study proverbs. This is because while online proverb collections do already exist, they do not make use of the modern database and web technologies described above and as such they do not allow for computational studies. Once I have returned from study abroad, I will help the project move forwards by populating the database with the proverbs that were mined from online resources this summer, and research how best to display our proverbs based on the tags they are organized by.

*(Supported by the Schultz Foundation)*

Advisor: Eitan Mendelowitz, Computer Science

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1 Love, Kenneth. "Microblog Kitchen Sink." *Getting Started with Django*. N.p, 20 May 2013. Web. 22 May 2014.

2 *jQuery API*. N.p, n.d. Web. 13 June 2014.

# Web App For Visualizing Crystallographic Data

Vatasha White/2015

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Crystalline structures, specifically those belonging to the family of zeolites, are of interest because some are conjectured to exhibit auxetic behavior. Auxetic materials are materials that have a negative Poisson's ratio. Poisson's ratio describes the strain relationship of a material with respect to the lateral and axial direction. Auxetics are extremely relevant because they can help enhance material properties of other structures.

KINARI is a software for rigidity analysis of macromolecules developed by Professor Streinu and her students. The algorithmic backend of KINARI heavily relies on the pebble game which uses Laman's theorem for bar and joint frameworks (2D) and Tay's theorem (3D) for generic body bar hinge frameworks. Currently, Kinari works with proteins, but it can be extended to study rigidity and flexibility of other types of molecules. The long-term goal is to get Kinari to work with crystalline structures. To work towards this goal I have spent the summer developing a web app that will eventually be able to pass crystallographic data to KINARI.

Jmol is a Java Molecular visualizer that allows users to visualize proteins and crystals. Jmol provides a simple user interface via scripting commands and menu options. In addition, Jmol can be embedded as a web application through the usage of Jsmol, a JavaScript library.

Throughout the summer I worked on developing web app prototypes for the visualization of both crystals and proteins to explore/showcase Jsmol features. After becoming more familiar with Jsmol, I developed a web app that visualizes a crystal with interactive commands and extracts information from the cif or mmCIF file being loaded into the applet. There are three subroutines performed to help create the app – obtaining the file, visualizing the data stored in the file, and extracting information from the rendered file. The first subroutine is implemented in several different ways. One of the ways is by creating a query string that will fetch the file from the database. From the user's perspective, this is a simple input form that asks for a valid COD ID. The other ways that the file is obtained is by uploading a local file or by explicitly accessing local files via a dropdown menu of file selections. The second subroutine is achieved by loading the file in Jsmol and the third subroutine uses PHP and JavaScript to parse the file.

The next step in the project is to pass the parsed rigidity information and other crystallographic data to KINARI.

*(Supported by the National Science Foundation (4CBC))*

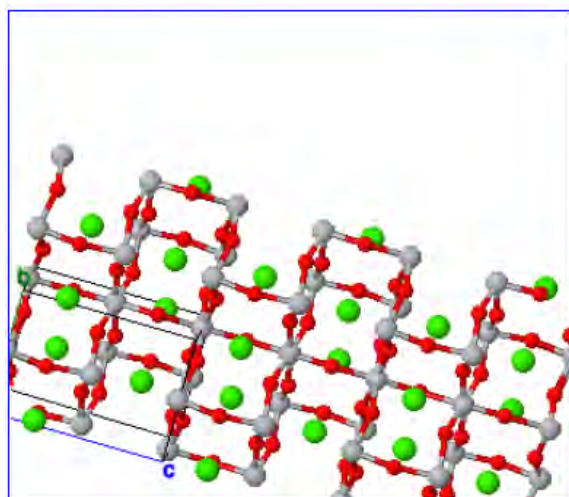
Advisor: Ileana Streinu, Computer Science

Select a Crystal ▾

Rotate:

☐ Box | ☐ Atom Labels | ☐ Axes

|   |                        |   |
|---|------------------------|---|
| <a href="#">Console</a><br><a href="#">Menu</a> | Select Scheme ▾        | Background: <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> |
| <a href="#">Reload</a>                          | <a href="#">Packed</a> | i: <input type="text" value="1"/> j: <input type="text" value="2"/> k: <input type="text" value="3"/>   |



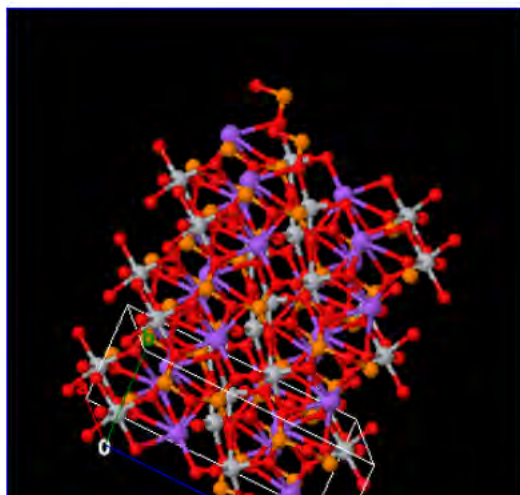
| Name                      | Value        | Extra Info |
|---------------------------|--------------|------------|
| Formula Structure         | 'Ca (Ti O3)' |            |
| Formula Sum               | 'Ca O3 Ti'   |            |
| Spacegroup                | 'P b n m'    |            |
| Symmetry Int Table Number | 62           |            |
| Symmetry Cell Setting     | orthorhombic |            |
| Symmetry Information      | -----        | -----      |
| a                         | 5.380        | (1)        |
| b                         | 5.440        | (1)        |
| c                         | 7.639        | (1)        |

Fig 1: Example of the developed web app loading Tridymite with applied symmetry of {1, 2, 3}

Rotate:

☐ Box | ☐ Atom Labels | ☐ Axes

|   |                        |   |
|---|------------------------|---|
| <a href="#">Console</a><br><a href="#">Menu</a> | Select Scheme ▾        | Background: <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> |
| <a href="#">Reload</a>                          | <a href="#">Packed</a> | i: <input type="text" value="2"/> j: <input type="text" value="3"/> k: <input type="text" value="1"/>   |



| Name                      | Value         | Extra Info |
|---------------------------|---------------|------------|
| Formula Structure         | 'Na Ti P2 O7' |            |
| Formula Sum               | 'Na O7 P2 Ti' |            |
| Spacegroup                | 'P 1 21/c 1'  |            |
| Symmetry Int Table Number | 14            |            |
| Symmetry Cell Setting     | monoclinic    |            |
| Symmetry Information      | -----         | -----      |
| a                         | 8.697         | (1)        |
| b                         | 5.239         | (7)        |
| c                         | 13.293        | (3)        |
| α                         | 90            | -          |

Fig 2: Cristobalite with applied symmetry of {2, 3, 1}





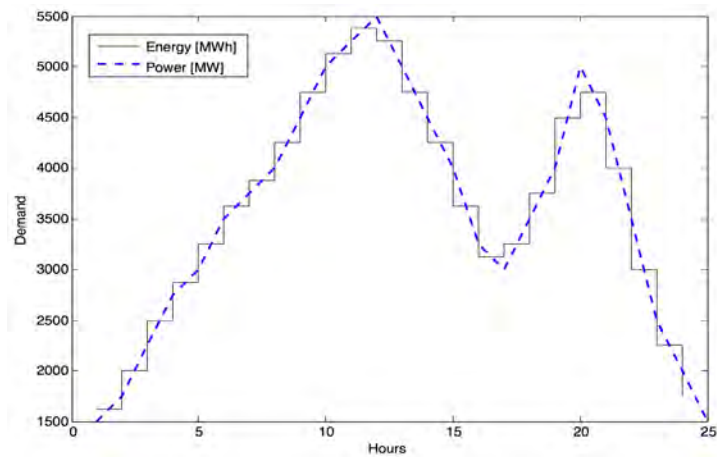


Figure 1. Hourly Demand in Power and Energy

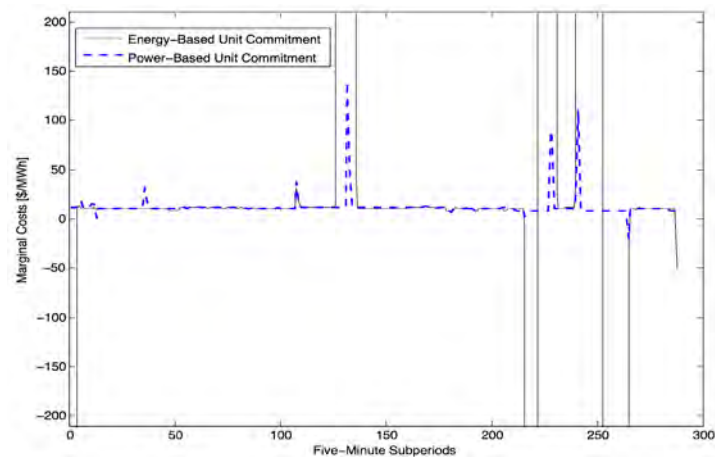


Figure 2. Comparison of Marginal Costs for Energy-Based and Power-Based Unit Commitment

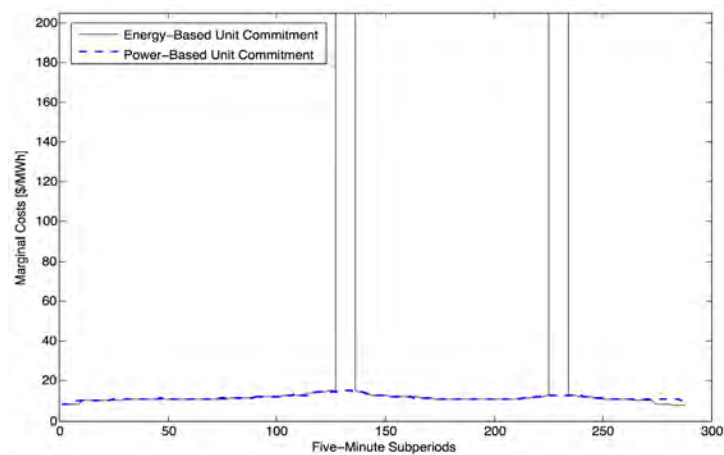


Figure 3. Comparison of Marginal Costs for Energy-Based and Power-Based Unit Commitment, Without Ramping Constraints



## Summer Research: The Flywheel Bicycle

Xi Jiang/2016

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My project is to mount a flywheel to a bicycle to store kinetic energy while coasting that will then be used in uphill climbs. A student at Copper Union also did this a few years ago but only a few papers on this have come out recently. Regarding this project as interesting and meaningful, I hope to reproduce that effort with a more sophisticated analysis and make a few design changes to increase the conversion efficiency under the guidance of Professor Denise McKahn.

At the end of this ten-week research, I was close to fabrication. My research started with information collection on applications of various flywheels through reading professional publications, watching animated videos, and talking with people expert at automobile and bicycles. I also presented my knowledge of flywheels and its applications on automobiles to my advisor and other students asking for advice concerning the next step I should take. Next, based on the draft used by the student at Copper Union, I started looking for suitable bicycle, and the transmission system and the flywheel appropriate for the bicycle. I utilized a bike discarded outside the Ford Hall with a big triangular frame in the front, where a flywheel with a diameter smaller than 11 inches could fit. I bought a flywheel weighed 20 pounds from an automotive salvage and a n360 Nuvinci transmission system online. Because the only flywheel available is too heavy for the bike, I worked with Eric Jensen from the Center for Design and Fabrication to trim the outer edge of the flywheel to reduce its size and weight to ideal condition. I also spent some time in the Center learning welding steel and aluminum as a preparation for fabrication.

Up until now, I have had almost all the crucial big parts needed for fabrication except for small connecting components that require accurate measurement and design. I will continue working on this project as a special study with Professor Denise McKahn. After fabrication, I plan to do some tests on its energy efficiency on inclination with different slopes within reasonable ranking of frequencies. And according to the analysis of test results, I would try to make some changes to the design to increase the practicality of the flywheel bicycle.

*(Supported by the Schultz Foundation)*

Advisor: Denise McKahn, Engineering

## Evaluation of the EOP Solution

Cecilia Lee/2015

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As the renewable energy technology is reaching a new high in the global energy market penetration, short term technology operation costs are accounting for more of the total system cost. These short term costs therefore have an increased influence on the production and investment decisions made by energy companies. To estimate these variables, including the investment and production decisions, an equilibrium problem (EP) via diagonalization method (DM) was solved. However, due to its relatively heavy computation burden, an equivalent optimization problem (EOP), modeled in another paper<sup>1</sup>, was utilized for this research.

One objective of this research was to determine whether the EOP solution is equivalent to the EP solution and that the computation time for the EOP is significantly less than that of the DM. Therefore, in this paper, the EOP solution, with 336 system states representing the system on an hourly basis, was evaluated and the computation time for the EOP and the DM was compared for various numbers of system states. The assessment of the accuracy of the EOP solution will help determine whether future researchers can rely on the EOP to obtain the solution to the equilibrium problem of the model. It is favorable to use the EOP model since it would decrease the computational burden and therefore, potentially the computation time.

To compare the computation time of the EOP to that of the DM, the system states for a period of a year for 2030 were studied to observe the relationship between the number of system states and the running time of CPLEX. The solutions solved via the EOP and the DM were compared and the percent error of the EOP solutions were calculated. Finally, the experiment was repeated for various number of system states for the same time period. The results were compared to that of the EOP solution with 336 system states. This was to examine how the accuracy of the EOP solution changes as the number of the system states increases closer to 336.

It was found that the EOP solution is indeed an adequate approximation with a percent error of around three percent. However, it is inconclusive whether the running time of CPLEX to solve the EOP is significantly less. It was anticipated that the accuracy would increase with the number of system states. However, there were mixed results. For the solutions of Firm 1, the percent error increased as the number of system states increased, but for Firm 2's, the results were as expected. It is unclear as to why this happens. Further studies will be conducted during the 2014 fall semester.

*(Supported by the National Science Foundation)*

Advisors: Judith Cardell, Engineering and Sonja Wogrin, Comillas Pontifical University Spain

<sup>1</sup> Gomez, N. A., "Including Short-Term Operation Details in Strategic Generation Expansion Models," Master's thesis, Universidad Pontificia Comillas de Madrid, 2014.

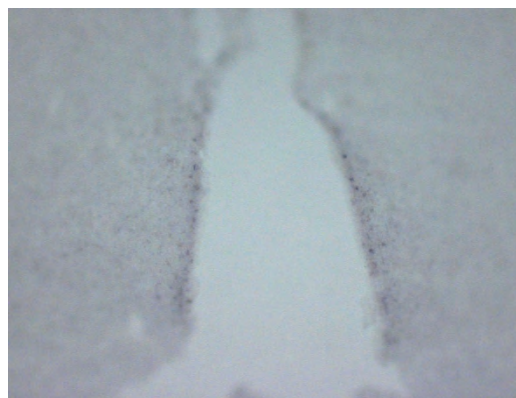
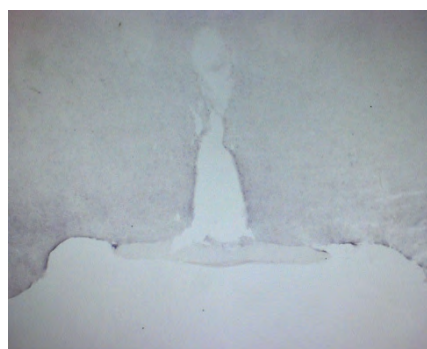
# Exploring Protein Therapeutics in the Central Nervous System

Tapiwa Lidzi Nkhisang/2016

Using large molecules such as proteins for the treatment of neurological disorders such as Alzheimer's disease, Parkinson's disease and brain tumors is a relatively unexplored area. This is due in part to the problems associated with their delivery to the central nervous system. Getting therapeutic drugs across the Blood Brain Barrier is an area of study that is under a lot of research. One question that arises is that of how protein based drugs get distributed throughout the brain to their therapeutic target once they have crossed this Blood Brain Barrier. If we can better understand these challenges, we can design protein therapeutics that target the central nervous system. Over the summer I conducted research under Professor Moore's lab in conjunction with Mary Harrington's lab. I was looking at the interleukin 1 beta (IL-1B) protein and tracking its activity in the brain to determine if the injected IL-1B is actually making it into the brain and where in the brain the IL-1B is travelling.

I did immunohistochemistry on the 40 um mice brain slices that were cut using a freezing microtome. Immunohistochemistry refers to the process of identifying antigens like proteins in tissue cells by using the idea of antibodies binding to antigens in the tissue cells. First I stained the slices with the primary antibody called Rabbit Anti-Flag then later with the secondary antibody Goat Anti-Rabbit, and then developed with Ni-DAB.

Most of the work I did was optimization and this included finding the correct concentration of the primary antibody which we found to be 1:5K. Through the optimization process I managed to decrease background staining using Hydrogen Peroxide, BSA (IGG free Bovine Serum Albumin) and PB (Phosphate Buffer).



1:5000 primary Anti Flag, 1:500 secondary GAR at 4x and 10x magnification

Throughout the summer, in addition to working on this project, I learned experimental methods in neuroscience which include brain slicing and brain staining. I got the opportunity to present my project to other labs. I also managed to answer the question I went into the research with. IL-1B can cross the blood brain barrier and for most of the project we were looking to see staining around the brain ventricles, of which we did. I hope to continue working on the same project doing a special studies.

*(Supported by the Schultz Foundation)*

Advisor: Sarah Moore, Engineering



# Engineering Diagnostic and Therapeutic Proteins Targeting Mesothelin-MUC 16 Interface

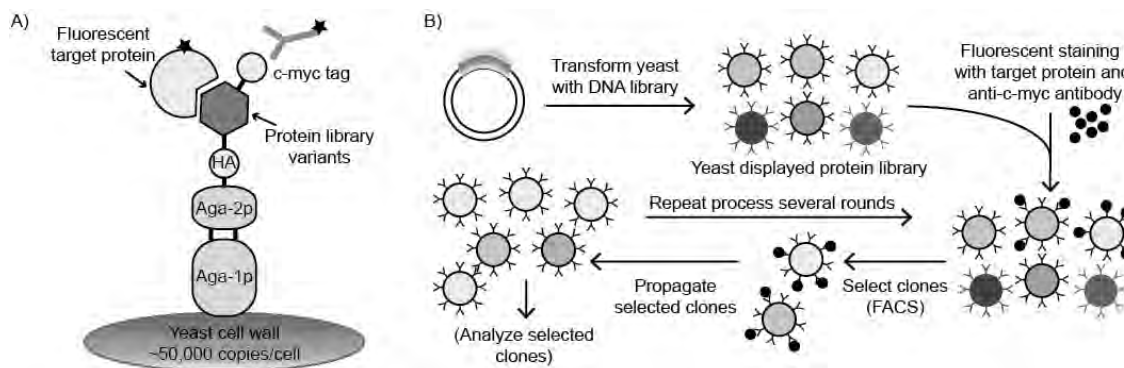
Allison Sirois/2015

In recent years, the role of targeted diagnostics and therapeutics for a several types of cancers has significantly progressed. As biomarkers are classified, it is imperative to understand and transform them into applicable diagnostic and therapeutic agents. Mesothelin (MSLN) is a cell-surface protein which exhibits potential as a tumor biomarker for several aggressive cancers. It has an advantageous expression pattern with which it is highly expressed on tumors while presenting at low levels on normal tissue.<sup>1</sup> MSLN is also known to bind another tumor marker, MUC16, and this interaction has been shown to facilitate metastasis and cell motility.<sup>2</sup> Our research aims to engineer proteins targeting this interface for use as both a targeted diagnostic and therapeutic. I specifically focused on identifying MSLN binding proteins from a naïve fibronectin library scaffold.

Soluble MSLN was produced using MSLN Flag-His YVH10 yeast cells and purified through His purification and size exclusion chromatography. A yeast surface display fibronectin library was received from Dr. Benjamin Hackel at University of Minnesota. Full-length expression of the fibronectin protein was confirmed using flow cytometry. The library was initially screened by magnetic-activated cell sorting (MACS) to enrich for weak affinity MSLN binders. The enriched library was further screened by fluorescent-activated cell sorting (FACS) labeled through a c-myc tag detection and binding with soluble protein (Figure 1A).

Results indicated that I was able to successfully produce and purify soluble MSLN for use in library screening. After two rounds of MACS it was observed that weak affinity MSLN binders were selected for as compared to a negative sort. Following two rounds of FACS, we successfully collected the top 3% of the double-positive clones.

The results signify that we are successfully selecting for clones that exhibit both strong protein display and binding to MSLN. I look forward to maintaining this project in the new academic year as I begin preparing my master's thesis. FACS will continue for several more rounds and resultant clones will be sequenced and mutagenized via error-prone PCR to encourage improved binding to MSLN (Figure 1B). It is our hope that continued work will lead to successful MSLN-MUC16 targeted diagnostics and therapeutics.



*(Supported by the Schultz Foundation)*

Advisor: Sarah Moore, Engineering

## References:

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# Perspectives in Engineering: A Multimedia Analysis

Greta Stacy/2015

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The “Through My Window” project works to create innovative educational materials for children and young teens. Whether these materials be for independent or classroom learning, “Through My Window” is a cutting-edge resource that works to change preconceptions about engineering through providing a deeper understanding of what engineering is and what it can be, while supporting the development of critical thinking and specific engineering skills. “Through My Window” utilizes the theory of Imaginative Education to create materials that engage students in the ways that help them learn best, rather than relying on “folk theories” of learning. This means the materials rely on the power of narrative and story-telling to communicate complex ideas and knowledge building to encourage students to think deeply about concepts. Since “Through My Window” is an online resource, a multimedia approach is the best method for sharing stories and ideas. Multiple forms of communication and opportunities for discourse allow students of different learning styles and backgrounds to access information, benefit from Imaginative Education, and share in the narrative of engineering.

This summer, videos were created that will become part of our online learning adventure about the engineering design cycle as well as marketing videos that tell the story of our team. The videos for the learning adventure will be used to introduce major concepts that are important to the narrative we have crafted around engineering design. The marketing videos are used to show our project to educators and other interested parties. Given the multimedia approach of our project, videos can best “show,” rather than “tell” the story of “Through My Window” and the importance of a new approach in STEM education.

Moving forward, video components of our online learning adventure about professional ethics in engineering will also be explored. Additionally, marketing videos will continue to be part of the project as we enter a video competition this fall.

*(Supported by the TE Connectivity Foundation)*

Advisor: Glenn Ellis, Engineering



# Design and Testing of DC/DC Converters for PEM Fuel Cell Systems

Yijin Wei/2016

A proton exchange membrane fuel cell (PEMFC) produces dynamic power from the chemical energy in hydrogen and oxygen with water as the only by-product. The fuel cell application of interest requires 3.7 V, but a typical single PEMFC produces 0.5-1 V. Fortunately, individual cells can be connected in series to increase the PEMFC output voltage, at the cost of increased weight. We consider the use of a boost DC/DC converter in series with a PEMFC to reduce system mass. A boost direct-current to direct-current (DC/DC) converter is capable of both increasing the PEMFC output voltage and regulating that voltage to a relatively constant value. This project involved the selection of an appropriate DC/DC converter, design and fabrication of a printed circuit board (PCB) to assemble the required electrical circuit, as well as static and dynamic testing of the integrated circuit (IC).

LTC3539 manufactured by Linear Technology was the DC/DC converter chosen for this work. Resistors, capacitors and inductors were sized and procured for the final integrated circuit. A PCB was designed to connect the external components to LTC3539 and Gerber files were generated for external PCB printing and assembly.

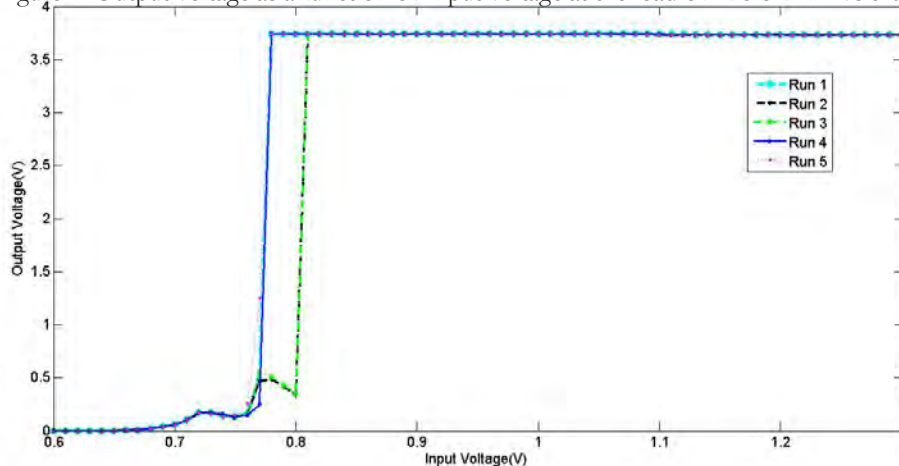
Static tests were conducted by connecting a DC power source to the input and a resistive load to the output. Load resistance was varied to relate input current and voltage to output current and voltage in steady-state. To assess reproducibility, the experiment was conducted five times with a 125.8  $\Omega$  load. As shown Figure 1, DC/DC converter's behaviors are reproducible at the normal operation and at the operation prior to turn-on, but the transition between the two modes is not reproducible.

For dynamic tests, an oscilloscope was used to measure the transient output response of the IC. The start-up and the shutdown response were adequately modeled with a sixth-order and a first-order transfer function respectively. However, this model was not adequate for the entire operational range. This work will be continued as a special studies project next semester. We will refine the PCB design for LTC3539 to reduce its size and mass as well as investigate alternative electrical models.

*(Supported by the Schultz Foundation)*

Advisor: Denise A. McKahn, Engineering

Figure 1. Output voltage as a function of input voltage at the load of 125.8 $\Omega$  in five trials



# Production and Development of Controlled Meteorological Balloons Atmospheric Research on Earth and Venus

Alex Widstrand/2017

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In many remote regions of the globe, it remains difficult to gather atmospheric data due to unforgiving climates and dangerous terrain. Yet these hazards do not diminish the importance of monitoring these areas, particularly in consideration of climate change. Light-weight remotely-controlled weather balloons provide a vehicle by which such data can be collected while vastly reducing undue risk to those interested in this data.

This summer was spent scaling up production of controlled meteorological balloons that have previously been manufactured in very small quantities. Sixteen balloons were constructed with the goal of maintaining as light a system as possible without decreasing functionality. Materials and assembly techniques were chosen on the basis of strength, weight, and ability to withstand the extreme cold temperatures (-40 C).

Though the balloons manufactured over the summer were specially designed for a project in the in the Amazon scheduled for the fall of 2014, additional work, conducted in collaboration with engineers from the Jet Propulsion Laboratory, developed a new variation of the balloon for a possible mission to Venus. A small-scale model of this Venus balloon was built at Smith College; a video of the balloon deployment was created that was shared with JPL scientists. This work is actively continuing into the 2014-15 academic year.

*(Supported by the Schultze Foundation)*

Advisors: Paul Voss, Engineering and Jon Caris, Spatial Analysis Lab



Figure 1: Testing the high-pressure balloons.

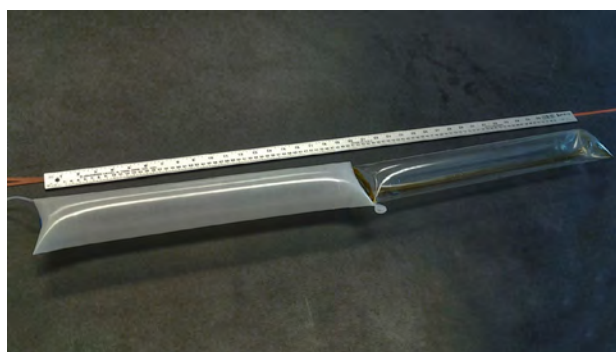


Figure 2: Venus balloon prototype, fully inflated.

## Great Lakes Ship-Based Science Communications Internship

Catherine M. Aguilar/2015

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My NOAA Internship with the Great Lakes Environmental Research Laboratory (GLERL) and Lake Michigan Field Station (LMFS) focused on the communication needs for the research and monitoring activities conducted along the shoreline of Lake Michigan and Lake Erie and aboard NOAA's research vessel Laurentian. This internship provided me the opportunity to increase the visibility and value of both the Great Lakes and the research that takes place at the Lake Michigan Field Station (LMFS). My projects included producing educational digital narratives, updating the NOAA Great Lakes Flickr and Facebook library, and submitting my original photos to the NOAA Facebook photo contest.

All of my projects included accompanying NOAA researchers in the field to capture action-shot pictures and videos using a GoPro Hero 3+ camera. To produce educational digital narratives, I used iMovie to edit the footage I took with the GoPro and to add appropriate background music to create a short video. I then sent my digital narrative to my supervisor for review. In addition to providing feedback on my videos, my supervisor would forward them to other NOAA officials for further review. Once the final edits were made and everyone who reviewed my video approved the final product, the video would be posted on the NOAA Great Lakes YouTube and Facebook page. To update the NOAA Great Lakes Flickr and Facebook library I would upload the pictures I took to iPhoto and a shared Google Drive. To submit photos to the NOAA photo contest, I would choose my top 3 to 5 pictures from my day's shoot and upload the pictures directly to the NOAA photo contest website.

Overall, project tasks for this internship emphasized communication and professional development skills. In addition to learning about the Great Lakes and how GLERL-LMFS crewmembers collect data for research, I also gained experience with a number of software programs including GoPro, iMovie, iPhoto, Apple Keynote, and Google Drive. Furthermore, the skills essential to completing the assigned projects included concise writing, video editing, and camera skills. Through these communication projects, I was able to contribute to NOAA's GLERL-LMFS public education and outreach efforts to keep the general public engaged with the NOAA Great Lakes research.

*(Supported by the Agnes Shedd Andreae 1932 Research Internship Fund)*

Advisors: Anne Wibiralske, Environmental Science & Policy and Margaret Lansing, National Oceanic and Atmospheric Administrations (NOAA), Great Lakes Environmental Research Laboratory (GLERL) – Lake Michigan Field Station (LMFS), Muskegon, Michigan



## Coral Reef Ed-Ventures 2014: An Environmental Education Program Conducted by Smith College Students for Youth in San Pedro, Belize

Kayla Clark/2014, Elena Karlsen-Ayala/2016, Riley Gage/2015, Kiara Gomez/2014, Victoria Dunch/2014, Clara Rosebrock/2016

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“R-E-S-P-E-C-T the earth, the reef, and the sea  
I’m gonna R-E-S-P-E-C-T myself, my friends, and family”

Local children from San Pedro, Belize started off their days singing this song at the 2014 Coral Reef Ed-Ventures Program and later performed it at their graduation ceremony in front of 250 friends and family.

This summer over 146 local children attended the Coral Reef Ed-Ventures Program taught by six Smith students in July. The children live on the island of Ambergris Caye, Belize, located close to the Meso-American Barrier Reef, the largest barrier reef in the western hemisphere. The town in which this program took place, San Pedro, heavily relies on its tourist attraction to the barrier.

A primary goal of the Coral Reef Ed-Ventures Program is to raise awareness of the environmental and economic benefits of a healthy reef system through research and environmental education. The program was founded in 2000 in collaboration with Hol Chan Marine Reserve on Ambergris Caye, and now has celebrated its fifteenth year this summer. The program is currently directed by the Smith College Environmental Science and Policy Program and led by three faculty advisors: L. David Smith, Denise Lello, and Allen Curran. The six Smith students represented several fields of study, including environmental science and policy, biological sciences, Spanish, geosciences, and sociology.

This year, the Smith students and faculty surveyed soft coral diversity and abundance at Mexico Rocks, an area designated as a future protected area. Using scuba, they photographed individual soft corals for later identification and took video transects of multiple coral mounds to map the spatial arrangement of the soft corals. They also surveyed individual sea fans to assess the frequency of disease, predation, and physical damage. These data will serve as a foundational baseline and help Hol Chan assess the effectiveness of the expanded marine reserve. Some of the students also traveled north to Bacalar Chico with biologists from Hol Chan Marine Reserve to do beach profiling and locate and map turtle nest sites using GPS. Smith students and faculty also visited the Belize National Zoo to learn about efforts to rehabilitate injured and abandoned native animals.

The Smith students offered two marine environmental education programs to children on Ambergris Caye in form of an advanced camp (ages 12-18) and a youth camp (ages 7-11). The one-week advanced camp program provided the older students with hands-on field research experiences based on their individual interests. Advanced camp students visited two mangrove sites, one on the beach side and the other on the lagoon side, where they compared sand composition, animal behavior, and naturalist observations through drawing. The advanced camp students then took the information they learned and offered their own lessons to the younger students of the youth program.

In the youth camp, the curriculum was designed to provide campers with multiple approaches (e.g., games, projects, songs) to understand the local coral reef environment, reef organisms, symbiotic interactions, and ecosystems. Students were encouraged to brainstorm ways to conserve the reef and other natural resources. Students participated in field trips to the reef and the beach to compare habitats and examine human impacts. They heard presentations from local conservation organizations, including ACES (American Crocodile Education Sanctuary) and Judy Waight from the Belize Coalition to Protect Our Natural Heritage. Highlights of the youth camp included a trip on the glass bottom boat to Shark Ray Alley (a marine protected area), the crocodile presentation by ACES, and the edible coral polyp activity.

Through place-based learning and critical analysis, the Belizean students were able to become better spokespersons for marine conservation. The research projects established benchmarks to test for future changes in coral reef communities and helped to strengthen our relationship with Hol Chan Marine Reserve.

*(Supported by the B. Elizabeth Horner Fund in the Biological Sciences, Agnes Shedd Andrea 1932 Fund, the Margaret A. Cargill Foundation, a generous gift from Linda Salisbury '73, the Environmental Science and Policy Program, and Smith College's Committee on Faculty Compensation and Development)*

Advisors: H. Allen Curran, Geosciences, L. David Smith, Biological Sciences, Denise Lello, Mathematics and Statistics, and Miguel Alamilla Jr., Director of Hol Chan Marine Reserve



*Clara Rosebrock and Kiara Gomez set up transect tapes along a coral mound for measurement*



*Kiara Gomez free dives approximately 12 feet with a transect tape*



# NOAA's Sentinel Site Program, Hawai'i Cooperative: Climate Change – From Observation to Stewardship

Jessica Lillquist/2015

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In 2011, the National Oceanic and Atmospheric Administration (NOAA)'s National Ocean Service initiated the Sentinel Site Program to address climate change issues in 5 major coastal areas in the US. One of these 5 sites is the Hawaiian Islands Cooperative, a unique collective of 4 locations that includes the Midway and French Frigate Shoals in the Papahānaumokuākea Marine National Monument in the Northwestern Hawaiian Islands, He'eia on the island of Oahu, and a portion Hawai'i Island. The Cooperative fosters collaboration between NOAA and other federal, state, and local partners to identify resilience-related activities, resources, and needs within the community. This summer I worked with site coordinator Doug Harper to support these efforts and met with local organizations to identify their needs and opportunities for new projects.

My first major task was to compile research, management, and planning documents for the Sentinel Site and Habitat Blueprint program on Hawai'i Island. This report will serve as a resource outlining work in the region that has been completed or that is underway and will help determine where other work can be done. It will continue as a 'living document' to be consistently updated as progress is made by program projects.

In addition, I met with local stakeholders at the He'eia sentinel site organizations to learn about their ongoing projects and to explore with them how GIS/GPS would be useful to them. Jan Yoshioka, chief financial officer and farm hand at Kāko o Ōiwi, requested assistance tracking the crops grown at the site. To help with this, I created a production map using ArcGIS online, defining their plots and labeling the content, quantity, and planting dates, so that she could track the rate of production and update the map with plot yields and new plantings.

For the NOAA Sentinel Site Program, I developed a chart of available grants for 2014 and 2015 that support climate change research and management. I included funds offered by NOAA and other government organizations that pertained to the needs of regional nonprofit and private sector groups.

The documents and maps I produced are tools for effective management and scientific research that will support the partnerships between NOAA and local communities to improve climate change resilience. The Hawaiian Islands Cooperative serves as a model for evolving collaboration between scientists and land managers to strengthen communities.

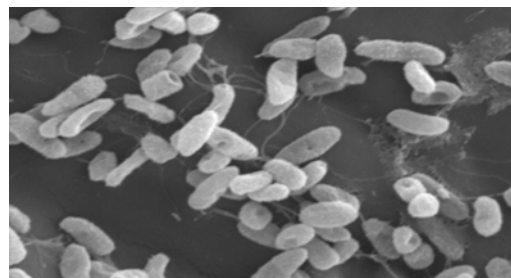
*(Supported by the Agnes Shedd Andreae 1932 Research Internship Fund)*

Advisor: Anne Wibiralske, Environmental Science & Policy and Douglas Harper, National Oceanic and Atmospheric Administration (NOAA), Pacific Services Center, Honolulu, HI

# Identifying Potentially New Virulence Genes in *Vibrio parahaemolyticus*

Katie Moshofsky/2017

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*Vibrio parahaemolyticus* (Vp) is a halophilic bacterium that naturally occurs in marine environments worldwide.<sup>1</sup> Certain strains of this bacterium cause severe gastroenteritis if ingested in raw seafood, most notably oysters.<sup>2</sup> The majority of strains that cause disease also encode hemolysins (thermostable direct hemolysin, tdh or the thermostable-related hemolysin, trh). Genome sequencing identified a new hemolysin gene, hlyA in a few clinical strains. HlyA is a known hemolysin in several pathogens such as *E. coli*.<sup>3</sup> The goal of this study is to characterize hlyA in Vp and determine its potential as a virulence marker, a gene that indicates the pathogenicity level of the bacteria.

In in-vivo and in-vitro studies, the hlyA strain (Vp3355) was compared with a strain containing a mutation in hlyA, ( $\Delta$ hlyA) and a second strain in which the mutation was complemented ( $\Delta$ hlyA+hlyA). Using a zebrafish model, I (along with other NOAA affiliates) inoculated zebrafish with the three strains at different bacterial concentrations. We then recorded the fish mortality over a 72-hour time period to assess virulence and calculate the median lethal dose (LD50). These studies, as well as experiments comparing strain virulence using in vitro lyses of hemocytes, are still in progress.

While in the lab, I analyzed several Vp strains for the presence of virulence or virulence-associated genes tl, tdh, trh, and ure<sup>4</sup> by PCR (Polymerase Chain Reaction) and gel electrophoresis. The trh gene has a highly variable nucleotide sequence and is difficult to amplify. Much of my PCR work involved using alternate primers in an attempt to find a single primer assay that can detect trh in all or most trh positive Vp strains. Accurate detection of trh is essential to identifying potentially pathogenic strains. As well, I compared Vp strains using a genetic fingerprinting method (Rep-PCR) to identify related strains with similar profiles.

I also participated in the first Ocean Sampling Day (OSD) on June 21. Water from 185 sites across 38 countries was sampled with the goal of creating a holistic view of the ocean's microbial diversity at a given moment in time. The samples were sent to the Max Planck Institute for Marine Microbiology in Bremen, Germany for analysis. If successful, OSD coordinators intend to make OSD an annual event. Next year, researchers will collect samples at the same locations and on the same day, providing a comparison of microbial diversity and evolution in varying environments over time. The results of these collections will be available in the European Nucleotide Archive, as well as the Smithsonian.<sup>5</sup>

*(Supported by the Agnes Shedd Andreae 1932 Research Internship Fund)*

**Advisors:** Anne Wibiralske, Environmental Science & Policy, and Rohinee Paranjpye and Gladys Yanagida, National Oceanic and Atmospheric Administration (NOAA), Northwest Fisheries Science Center, Seattle, WA

<sup>1</sup>Yi-Cheng, Su. "Pathogenic Vibrios and Food Safety." Nova Biomedical (n.d.): n. pag. Print.

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<sup>3</sup>Burgos, Ylanna, Karin Pries, and Antonio Antonio Fernando Pestana De Castro. "Characterization of Thea-haemolysin Determinant from the Human Enteropathogenic Escherichia Coli O26 Plasmid PEO5." *FEMS*, 10 Dec. 2008. Web. 19 May 2014.

<sup>4</sup>Thompson, Fabiano Lopes., B. Austin, and J. G. Swings. "*Vibrio parahaemolyticus*." *The Biology of Vibrios*. Washington, D.C.: ASM, 2006. 340-348. Print.

<sup>5</sup>Hoopin, Pertra T., and Guy Cochrane. "Ocean Sampling Day Handbook 2.0." *Ocean Sampling Day*. Micro B3, June 2014. Web. 16 July 2014

Photo by: Rohinee Paranjpye

# Coastal Ecology Research at the NOAA/Wells National Estuarine Research Reserve

## Emma Swartz/2016

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This summer I worked with four other undergraduate interns and some half-dozen research staff at the NOAA/Wells National Estuarine Research Reserve in Wells, Maine. We assisted researchers in the field and in the office on a variety of projects studying the ecosystem dynamics of Southern Maine's salt marshes and surrounding habitats with the aim of supporting safe and efficient ecosystem-based management. Specific projects included the study of larval fish communities, wading birds, diadromous fish, green crabs, and other marine invasive species. These projects investigated the spread of invasive species throughout the Gulf of Maine, marsh erosion, use of the local fish ladder, and general ecosystem stability; all significant concerns for the health of the creatures living in the rivers and marshes and for the economy of coastal Maine, which relies on these ecosystems for fishing, clean water, and tourism.

A key intern duty was to catch, measure, and tag fish with special attention to diadromous species such as *Salvelinus fontinalis* (brook trout) and *Petromyzon marinus* (sea lamprey). Fish were caught using fyke nets in stream and marsh habitats. Green crabs were also caught in crab traps in 24-hour cycles in three different locations: Wells, Yarmouth, and Damariscotta. The crabs were weighed and measured as part of a study to assess their contribution to marsh erosion. Interns counted wading birds such as herons, egrets, and ibis in the marshes and mapped their locations with GIS software as part of a 20-year study of ecosystem health in these birds' fishing grounds. Interns also helped monitor water quality and collected larval fish in a plankton net to identify which species of fish were using the estuary in the larval part of their life cycle. Interns also participated in the MIMIC program in which scientists and trained volunteers monitor the presence of marine invasive species such as tunicates and invasive algae, shrimp, and crabs and report their findings to a central organization.

Most of these projects are part of ongoing monitoring programs or the beginning of research projects that will continue for several years. Specific results from this summer's research are not yet available. However, these research projects contribute to our understanding of species dynamics essential for assessing coastal marine ecosystem health, and provide critical information showing the importance of estuaries to the people and organisms that live in them and to the ecosystems that surround them. This information will help policymakers and members of the community decide how to use and protect these valuable resources.

*(Supported by Agnes Shedd Andreae 1932 Research Internship Fund)*

Advisors: Anne Wibiralske, Environmental Science & Policy, and Kristin Wilson, Research Director, NOAA/Wells National Estuarine Research Reserve, Wells, Maine



# Understanding the SAR-ESP% Relationship with Peat from Hawley Bog

Hannah Francis/2016

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Hawley Bog (Hawley, MA) is an example of a bog with minerotrophic characteristics. There is a floating sphagnum moss peat mat, and sedges border a stream that flows through the wetland. The chemistry of the water in the peat mat is characteristic of acidic bogs with dilute concentrations of base cations (peat pH= 3.8–4.5, Ca= 0.8642 mg/L, Na= 0.8493 mg/L; ANC= 15.6  $\mu$ eq/L). Other bogs in New England are not as remotely located, and are often exposed to contaminants like road salt, which can contribute sodium and calcium (to a lesser extent) to cation exchange sites on organic material.

We conducted a small study to determine the cation exchange behavior of  $\text{Na}^+ - \text{Ca}^{2+}$  on peat (equation 1) collected at Hawley Bog.



The experiment used six different sodium absorption ratio (SAR) solutions with an ionic strength of 10mM. This ionic strength mimics the groundwater characteristics of other peatlands in Massachusetts that receive road salt runoff. We determined the exchangeable sodium percentage (ESP) for peat equilibrated to different SAR solutions (at target strengths: 1, 5, 10, 20, 40, 60) (equation 2). The idea and methods were drawn from the paper by P. M. Kopittke et. al. (2006) that looked at Na-Ca exchange for clay minerals, but not for peat.

$$\text{SAR} = \frac{[\text{Na}]}{\sqrt{[\text{Ca} + \text{Mg}]}} \quad \text{ESP}\% = \frac{\text{Na}_{\text{meq/L}}}{\text{TotalCEC}_{\text{meq/L}}} * 100 \quad (2)$$

Cation exchange capacity ranged from 15.90-25.35  $\text{cmol}_c/\text{kg}_{\text{peat}}$  ( $n=12$ ). The ESP% values ranged from 1.3 for the lowest SAR solution to 16.5 for the highest. Even the SAR solutions with the highest [Na] and lowest [Ca] (up to 575.7 mg/L Na, and 2.92mg/L Ca) only received 16% sodium on cation exchange sites. While the plotted data appear to be increasing, the low ESP% values suggest that calcium is prohibiting Na from adsorbing to exchange sites.

The Vanselow selectivity coefficient ( $K_v$ ) for the cation exchange reaction equation is:

$$K_v = \frac{X_{\text{Na}} * a_{\text{Ca}}^{0.5}}{X_{\text{Ca}}^{0.5} * a_{\text{Na}}} \quad (3)$$

where  $X_{\text{Na}}$  or  $X_{\text{Ca}}$  are exchangeable Na and Ca concentrations and  $a_{\text{Na}}$  or  $a_{\text{Ca}}$  are activities of Na and Ca in the SAR solution. All  $K_v$  values for each SAR reaction are less than 1 (mean  $K_v = 0.14$ ), suggesting a strong preference for calcium on exchange sites. This implies that even as more sodium is put into the surrounding water, low concentrations of calcium inhibit its appearance on exchange sites. No apparent correlation exists between ESP and  $K_v$ , suggesting a constant selectivity coefficient. While the data collected provided insight into the cation exchange chemistry of peat, future work is needed to address the equilibration time between peat and the SAR solutions, and to see if  $K_v$  values change with higher ionic strength solutions.

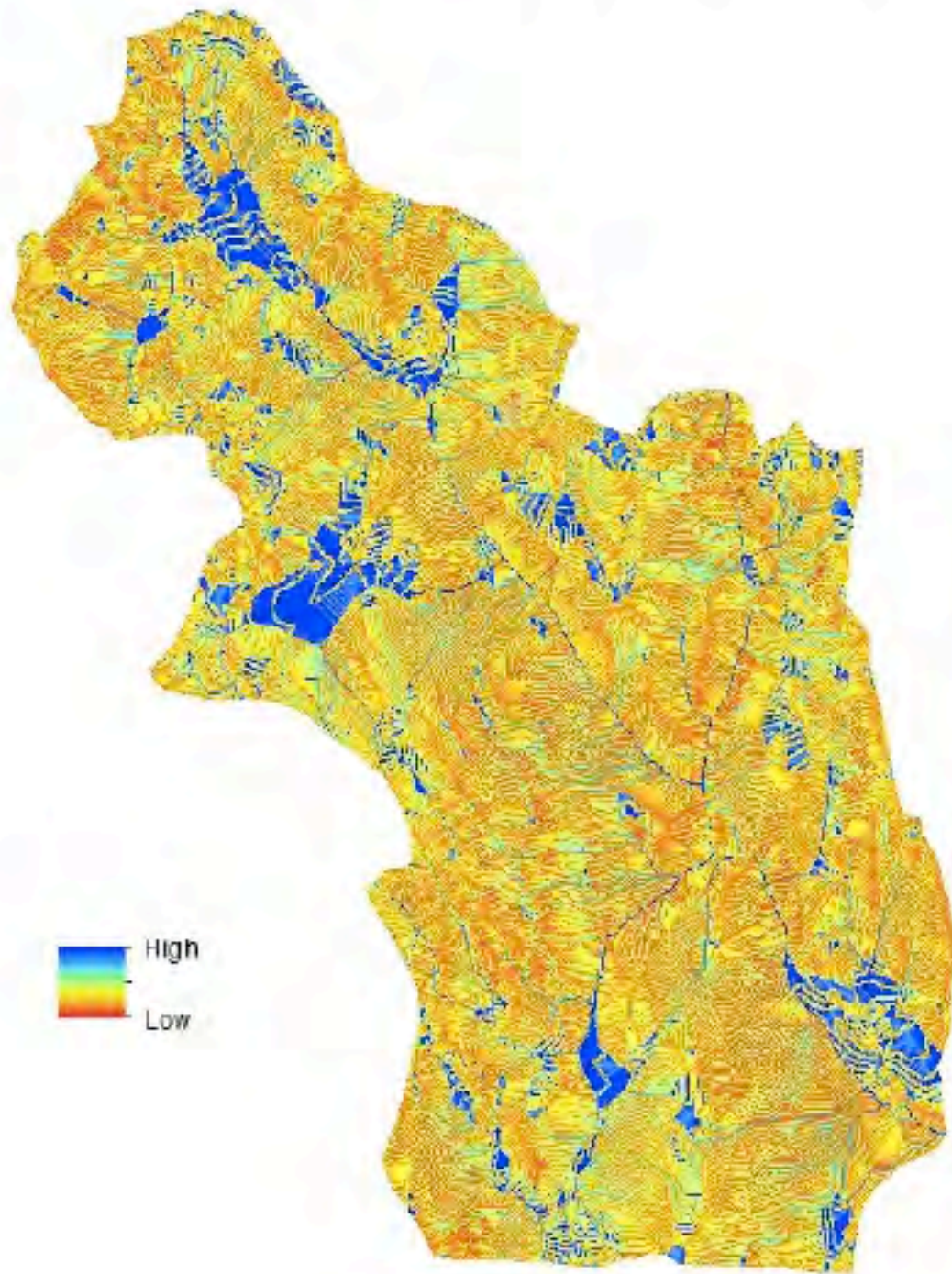
*(Supported by the Center for the Environment, Ecological Design & Sustainability (CEEDS))*

Advisor: Amy Rhodes, Geosciences





## Avery Brook Topographic Index





# Mapping of Pseudomorphs after Lawsonite in Blueschists and Graphitic Schists from the Island of Syros, Greece

Clementine Hamelin/2015

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Lawsonite is an index mineral of high-pressure low-temperature metamorphism.<sup>1</sup> It is rarely preserved on the surface after exhumation. On the island of Syros, Greece, prograde pseudomorphs after lawsonite are preserved in blueschists and graphitic schists, some bearing remnant lawsonite. Understanding the chemical reactions that occurred between the original lawsonite and other minerals in the matrix of the rock is critical to the understanding of regional scale metamorphism, as they can help us narrow down P-T conditions that these rocks experienced during metamorphism.

The samples used for this project were collected and described by previous KECK students and professors in 1999 and 2000.<sup>2,3</sup> Five different types of pseudomorph-matrix combination were described. During my five weeks in the lab, I selected and further analyzed four samples using the FEI Quanta SEM and EDS technology for mineral phase analysis. For each sample, mineral phases were determined, analyzed and used for phase mapping of the pseudomorphs and matrix surrounding them. Balanced chemical reactions that may have occurred to form the resulting pseudomorphs were determined.

Resulting modal maps show pseudomorph compositions that are either epidote dominant (zoisite, clinozoisite), white-mica dominant (phengite, paragonite), or about 50-50 epidote and white mica. The pseudomorphs generally contain albite. Remnant lawsonite is found principally in epidote-rich pseudomorphs. These pseudomorphs formed in two types of matrix: either glaucophane-rich (blueschists) or glaucophane-poor. Calculated reactions between lawsonite and glaucophane or white mica show how much epidote, white mica, chlorite would be produced in the pseudomorphs.

During the coming year, I will be generating maps of at least two other samples with different pseudomorph/matrix compositions. Phase quantification and analyses will be used for thermodynamic modeling calculations. Pseudosections (P-T diagrams) for estimated bulk compositions will be generated. The different types of pseudomorphs will each give us a piece of the geological story of their formation. We hope to understand the chemistry and thermodynamics behind their formation in order to understand the processes and conditions of their formation. This will further deepen geologists' understanding of low-temperature, high-pressure metamorphism in the Cyclades, as well as in other parts of the world where similar processes occur.

*(Supported by the Schultz Foundation)*

Advisor: John Brady, Geosciences

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<sup>2</sup> Able, Lindsey, 2001. Lawsonite Pseudomorphs in the Schists of Syros, Greece. Smith College Department of Geosciences, Special Studies Project. Faculty Sponsor: John B. Brady

<sup>3</sup> Sperry, A., 2000. Pseudomorphs after lawsonite as an indication of pressure-temperature evolution in blueschists from Syros, Greece in Mendleson, C.V., and Maciewicz, C. eds., Thirteenth Keck Research Symposium in Geology Proceedings.

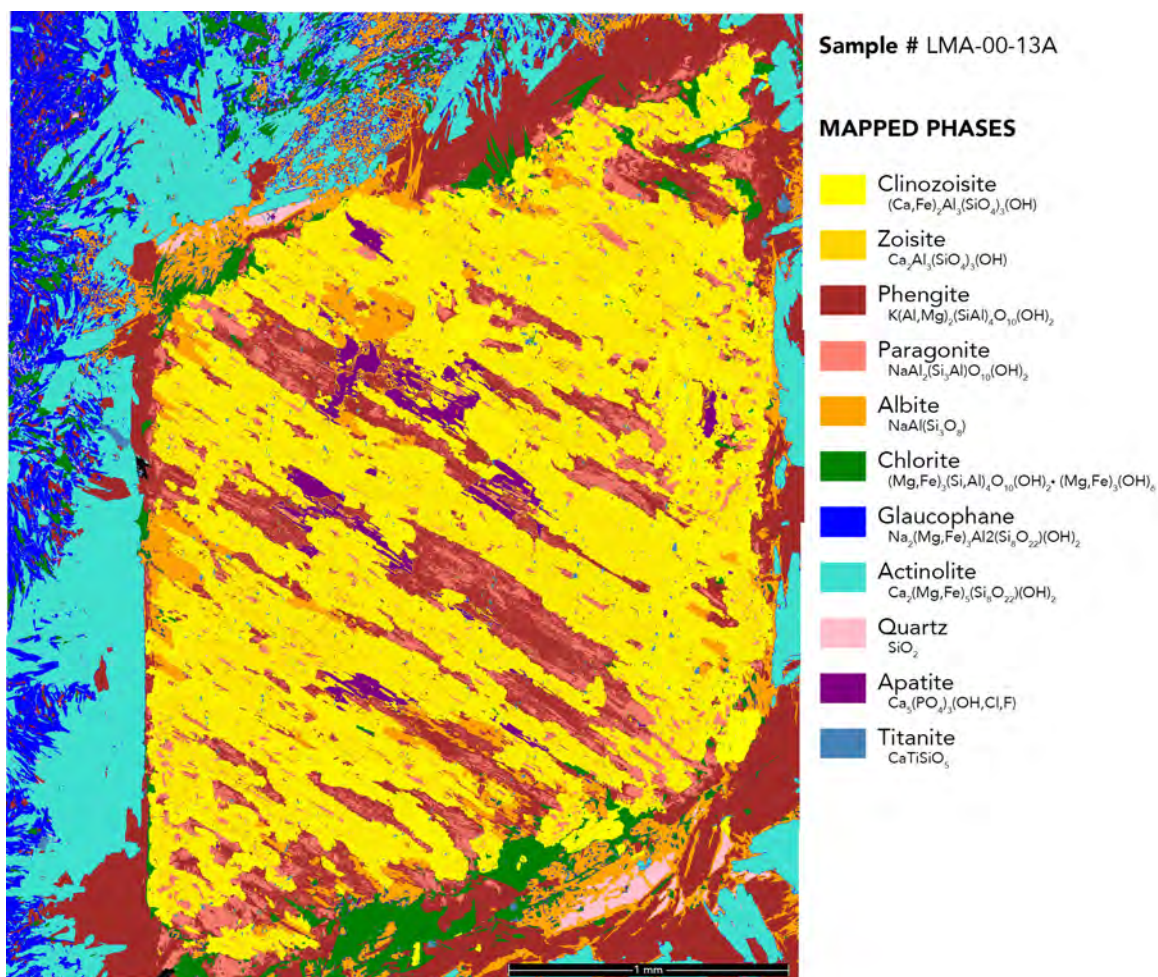


Figure 1. Sample LMA-00-13A – phase map of a diamond-shape pseudomorph in surrounding matrix. This pseudomorph is composed mainly of Epidote (clinozoisite + zoisite = 58%), with some large white mica crystals at the pseudomorph boundary and in the pseudomorph (phengite + paragonite = 28%), some albite (7%), chlorite (4%), quartz, titanite and apatite. The surrounding matrix is composed mostly of glaucophane (82%), actinolite (9%), albite (3%), phengite (3%), chlorite, titanite and quartz.

# Effects of Forest Succession on Nitrogen Cycling

Taylor Jones/2017

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*Tsuga canadensis* (Eastern Hemlock) trees at Smith College's MacLeish Field Station (West Whately, MA) were selectively logged about 25 years ago. The Eastern Hemlock is also seriously threatened by invasive insects, the *Adelges tsugae* (Hemlock Woolly Adelgid) and *Fiorinia externa* (Scales), which have caused widespread mortality of the hemlock trees. Deciduous trees such as the *Betula lenta* (Black Birch) commonly succeed hemlock, as is the case when selective logging at MacLeish created patches of Black Birch forest adjacent to mature Hemlock forest. This physical and temporal relationship presents an opportunity to study possible changes in soil geochemistry—nitrogen cycling in particular—25 years following forest succession.

This study continued seasonal monitoring of nitrogen mineralization rates in an adjacent Hemlock and Black Birch forest plot at the MacLeish Field Station, and it expanded the project with two additional Hemlock-Black Birch plots at MacLeish and a higher elevation hemlock- black birch plot located in Chesterfield, MA. The previous work, summarized in Zukswert et al., 2014, found patterns of ecological and ecosystem change associated with Hemlock removal in one of these adjacent plots, mostly observed with shifts with forest floor depths and forest floor community structure, however there was little evidence of major changes in nitrogen cycling between Black Birch and the Hemlock soils.

Nitrogen mineralization rates were determined by the incubation method, described in Zukswert et al. (2014). Organic soil horizons were wet sieved through a 2mm mesh and reacted with 0.01M  $\text{SrCl}_2$  to release  $\text{NO}_3$  and  $\text{NH}_4$  from the soil. Filtered extracts were analyzed using an ion chromatograph, and results were corrected for the dry weight of the soil. Plots were sampled for 2-3 incubation periods between May – July 2014, and each pair of plots was analyzed as a homogenized composite of 7 samples for the first incubation period, and then as individual cores (7 per plot) for the other incubation periods.

Results showed significant differences between several of the Black Birch and Hemlock plots. When comparing all black birch plots with all hemlock plots in a T-Test the mean nitrified mg/kg is significantly lower in black birch soil than the Hemlock soil ( $t = -4.526$ ,  $P < 0.0001$ ). The Black Birch's median is 0.069mg/kg with a mean of -0.0057mg/kg, while the Hemlock soils of all the plots have a median of -0.044mg/kg and a mean of -0.032mg/kg. However, when looking at net nitrogen mineralization rates between the Black Birch and the Hemlock forests, there is no significant difference found ( $t = -0.0474$ ,  $P > 0.9$ ).

While the data suggest a major difference in N cycling between Black Birch and Hemlock soils, every plot varies in how significant the difference is and which forest type has greater N cycling. Also, all the nitrified rates are very low, so even though results are significant, how much of an importance is this to the forest is questionable. Because we are observing the change in nitrogen cycling over time and we are seeing alterations in soil geochemistry every year, it is reasonable to suggest that to better understand how different a Black Birch community is from a Hemlock community will take many more years of research and continued sampling.

*(Supported by the Schultz Foundation)*

Advisor: Amy Rhodes, Geosciences





# Microfossil Assemblages in Cryogenian Cap Carbonates of Namibia, Zambia and Mongolia

Kelsey Moore/2015

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Cryogenian cap carbonates provide a critical record of evolution during one of Earth's most dynamic intervals of environmental change. Recent analyses of cap carbonates from multiple continents reveal the presence of fossil groups immediately after the ca. 716-663 Ma Sturtian Glaciation. Previous work on the cap carbonates of the Rasthof Formation of northern Namibia, has yielded diverse assemblages of agglutinated testate microfossils. This project focussed on the investigation of agglutinated testate microfossils found more recently in formations of equivalent age in Zambia and Mongolia, as well as comparison and further analysis of microfossils of Namibia.

In order to carry out this investigation, I extracted and examined microfossils from the cap carbonates by dissolving the samples in a 10% buffered acetic acid solution. Once the microfossils were isolated, the Scanning Electron Microscope (SEM) allowed for closer examination of morphology and structure of the tests. Further EDS analysis provided key information regarding mineralogy of the microfossils, allowing for comparison between the various localities. Additionally, I made petrographic thin sections of all samples in order to confirm that the microfossils were found *in situ*.

Through this process, 10 out of 19 limestone samples of drill core material of the Rasthof-equivalent Kakontwe Formation of Zambia have produced abundant agglutinated testate microfossils similar to some forms from the Rasthof Formation and some rounded forms from the post-Sturtian Taishir Formation in northern Mongolia. SEM imaging, petrographic analysis, and EDS analysis reveal morphologic and mineralogical consistencies across assemblages from these three different basins; microfossils at all locations are spherical and ovoid, sometimes having blunt ends or containing slit-like apertures. Fossils found in the Kakontwe and Rasthof formations are generally more varied (ranging from spherical to ovoid with size ranges of ~50 to 120 microns), whereas Mongolian fossils are typically smaller in size (~50 to 90 microns) and spherical. EDS analysis demonstrates the presence of aluminosilicates, quartz, and iron oxides on the surfaces of all tests, and petrographic analysis confirms that the microfossils are preserved *in situ* at all locations.

These microfossils indicate that similar, shell-making eukaryotes were thriving globally in carbonate systems in the immediate aftermath of glaciation. Through these analyses, we are able to gain understanding of the fossil record of this period as well as of the types of communities that existed. This research will continue into the upcoming year as my senior thesis, and has provided me with incredible opportunities to gain experience with practical lab techniques as well as research processes.

*(Supported by the Schultz Foundation)*

Advisor: Sara Pruss, Geosciences

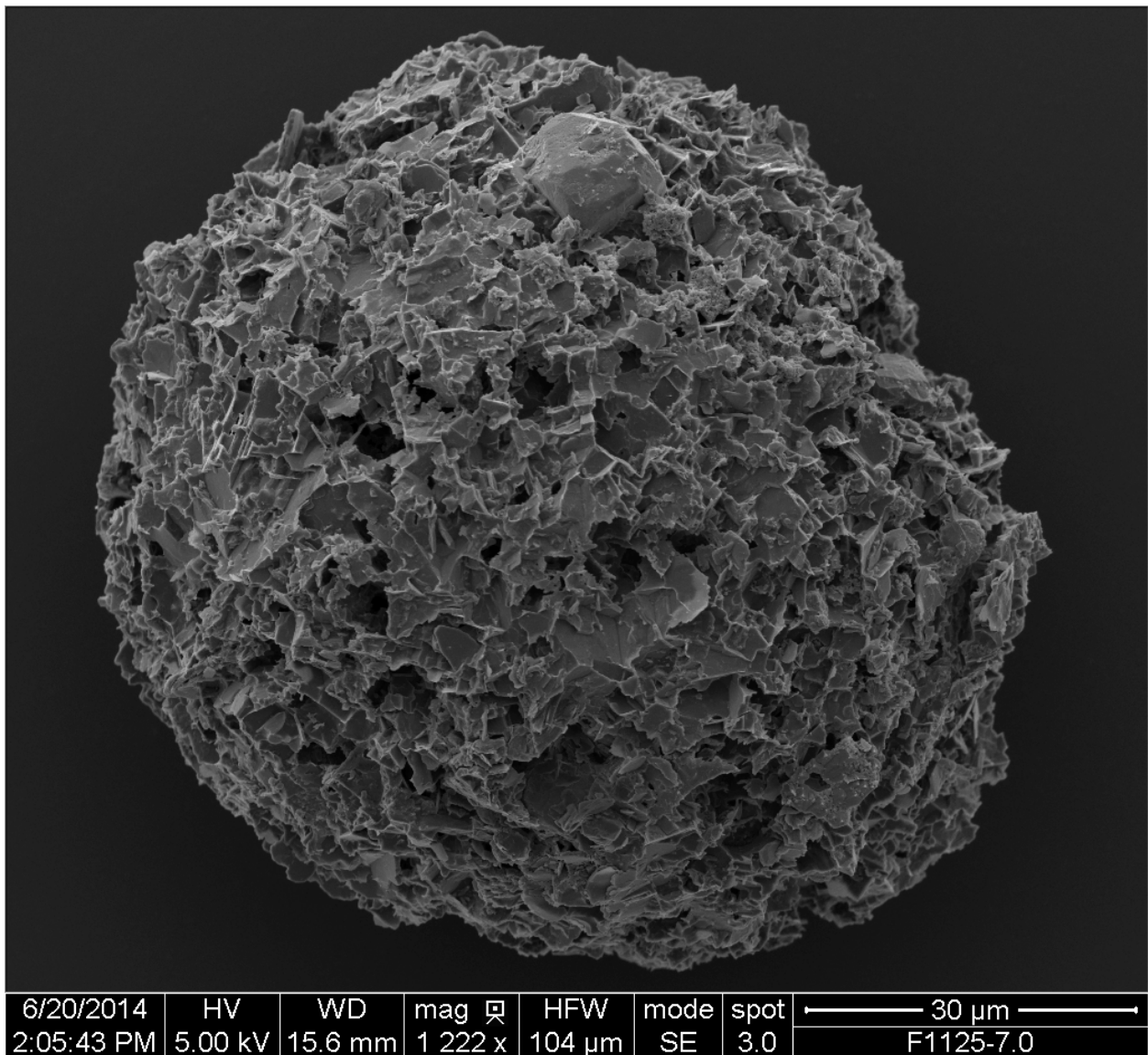


Image 1: Spherical test from the Taishir Formation of Mongolia





## Activities at the Ada and Archibald MacLeish Field Station and Research Regarding the Property's Namesakes

Jo Harvey/AC

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This summer's experience working as an intern at [Smith College's Ada and Archibald MacLeish Field Station](#) offered a variety of opportunities to share some of the truly unique and special aspects of the Whately, Mass., property with many diverse groups of people. From departments and offices such as the Smith Libraries staff and the Provost's Office, to groups of high school girls exploring STEM through [Eureka!](#), UMass's collaboration with Girls Inc., in Holyoke, Mass., and Smith's Field Studies for Sustainable Futures, visitors were able to participate in educational tours and hands-on activities. Tours included the [Bechtel Environmental Classroom](#) (one of five buildings worldwide to be certified as successfully completing the [Living Building Challenge](#)), the artwork of the field station's resident artist, [Dan Ladd](#), the [American Chestnut Foundations](#) tree orchard, as well as examples of student initiated projects such as the fruit orchard, a metal fabricated footbridge and a fire pit with planned pavilion.

I helped prepare for and facilitate hands-on activities for visiting groups to design experiments that demonstrate the use of insulation for energy conservation. I also helped groups to build their own composters, rain barrels, and carrying containers. My personal research project stemmed from my curiosity about the namesakes of the MacLeish Field Station, Ada and Archibald MacLeish.

In May of 2008 Smith College dedicated 243 acres in Whately, Massachusetts used for outdoor classes and research to Ada and Archibald MacLeish in recognition of the couple's close friendship with the school's first woman president, Jill Ker Conway, and their passion for the environment. My research was conducted with the goal of providing visitors to the Ada and Archibald MacLeish Field Station an understanding of the expanse of contributions the property's namesakes provided both in the arts and public service.

Archibald MacLeish was a lawyer, editor at Fortune magazine, appointed Librarian of Congress by FDR, Assistant Secretary of State and chairman of the American delegation of UNESCO. As a writer I was a three-time Pulitzer Prize winner for poetry and drama, as well as recipient of a Tony Award. None of these accomplishments would have been realized without the support of his wife of 65 years, Ada, who was herself an accomplished pianist and concert singer.

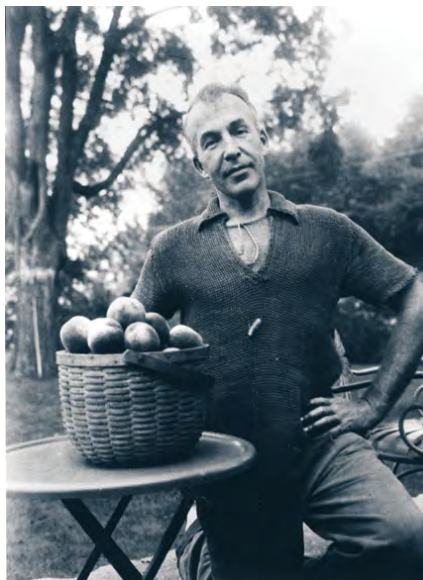
Upon Ada's graduation from the Westover School and prior to her marriage to Archibald, her parents sent her to Paris to study piano as a viable means of earning a future income. While there, she also studied voice. In the 1920's Ada returned to Paris with Archibald alongside many notable early 20th century writers, artists and composers such as Hemingway, Picasso, and Cole Porter. This trip proved advantageous for Ada as her singing career blossomed. When the MacLeishes returned to the United States, buying their home in nearby Conway, Mass., Ada essentially put an end to her professional singing career to support Archibald and raise their children.

Based on my interest in and research into Ada and Archibald's rich life histories and their close connection to Smith College, I plan to continue my work as an academic special studies project under the guidance of Reid Bertone-Johnson during the coming fall semester.

*(Supported by the Center for Environment, Ecological Design & Sustainability (CEEDS))*

Advisor: Reid Bertone-Johnson, Landscape Studies

Photographs of Ada and Archibald MacLeish on display at Ada and Archibald MacLeish Field Station, Whately, Mass.



# Development of the Fruit Orchard at the Ada and Archibald MacLeish Field Station

Jennifer Rioux/2015



A fruit orchard was installed at the Ada and Archibald MacLeish Field Station following various student design projects. Tia Novak ('13) created the original design for the orchard after taking a class at UMass on orchard design. Tia made a Collaborations poster based off of her orchard design project and President Carol Christ saw the design and decided to fund the orchard. Many students have worked with Reid Bertone-Johnson to modify Tia's project into an orchard perfect for Smith.

Three separate transects of trees: cider apples, keeper apples, and Mountain-Day-ready apples with a trial row of two Asian pear varieties were selected for the site. All trees are grafted on dwarf rootstock, meaning that the full grown trees will only be 6-8 ft. tall, making most apples able to be easily hand picked.

Establishing an orchard is a multistep process and the cyclical nature of college students makes it difficult to keep projects on track. I worked to maintain institutional knowledge through the creation of a map and research of past students' work versus what seemed most appropriate for the orchard now. I helped to reevaluate pest control methods, deciding that the deer fences installed around each individual tree should be replaced by an electric fence encasing the entire orchard. I also assisted Dan Ladd, artist in residence and arborist, to train and stake all 57 trees to hold the weight of their future fruits.

This summer we sheet-mulched an area around all of the trees with layers of cardboard and woodchips on top. This method suppresses the grass and weeds around the trees and allowing the trees to have more access to the nutrients in the ground without competition. We are a part of the Edible Ecosystem Research Network that began at Wellesley College and as a part of that research we will soon be planting an understory around the fruit trees. Finished compost will be added on top of the woodchips so that the new plants will get a boost of nutrients. We will be planting a blend of plants recommended by the network with each plant benefiting the orchard in a different way, adding nutrients to the soil, attracting pollinators, or deterring pests. The work I completed this summer set up the orchard for future student work and enjoyment.

*(Supported by Center for the Environment, Ecological Design & Sustainability (CEEDS))*

Advisor: Reid Bertone-Johnson, Landscape Studies

# Exploring Adinkras and Clifford Algebra Representations

Yixin Bao/2017, and Hana Foe/2017

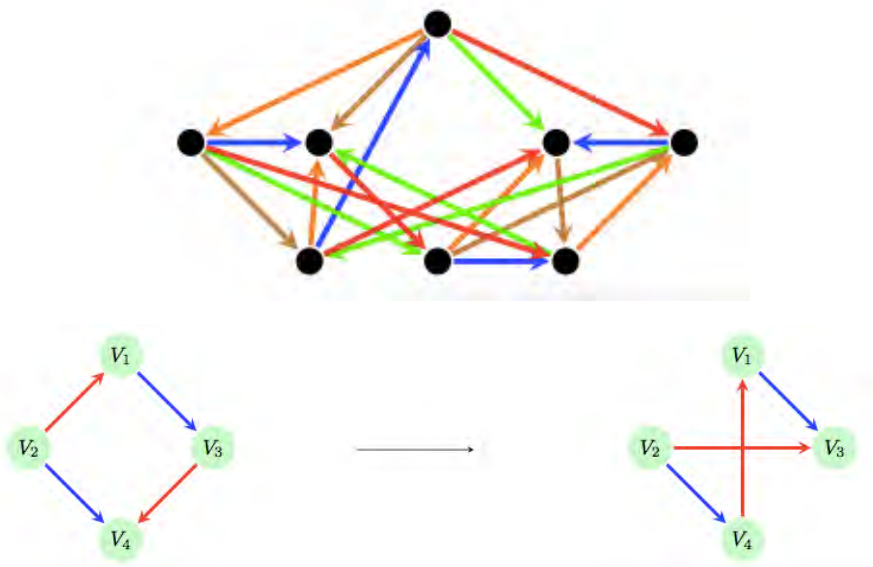
In our research (together with Leah Balay-Wilson/2015), we studied certain graphs called oriented chromotopologies and their relationship to Clifford algebra representations. Clifford algebras are certain algebraic structures that lie at the intersection of algebra, geometry, and physics. Examples of Clifford Algebras include the complex numbers and the quaternions. One of the ways to study Clifford algebras is by studying their representations. Essentially, a Clifford algebra representation is a set of matrices that “behave like” a Clifford algebra. Such matrices are often very large and thus very hard to work with, so it is useful to be able to describe them using graphs.

Certain types of Clifford algebra representations can be described by graphs called adinkras<sup>1</sup>. We modified the definition to obtain the notion of oriented chromotopologies, which describe representations for a different category of Clifford algebras. Then we described a method for producing oriented chromotopologies using error-correcting codes. We also described a process called color-averaging, by which one oriented chromotopology can be converted into another one. We proved that, after color-averaging, the corresponding representation is equivalent to the original one. Thus, color-averaging allows us to show that certain oriented chromotopologies are effectively the same, even though they might appear to be quite different.

We plan to present our work at the Women in Mathematics in New England conference later this year, and we plan to submit a paper for publication. The next step of our project will be looking deeper into the relationship between these graphs, codes, and Clifford algebras.

*(Supported by the Susan M. Rambo 1905 Fund, Bao and Ellen Borie Fund in Mathematics & Statistics, Foe)*

Advisor: Rajan Mehta, Mathematics and Statistics



<sup>1</sup>Faux, M., Gates, S. J. 2005. Adinkras: A graphical technology for supersymmetric representation theory. *Phys. Rev. D* 71.



# Modeling Actin Regulation During the Formation of Invadopodia in Metastatic Mammary Carcinoma

Jamie Cyr/2016

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Breast cancer metastasis is fatal due to dissemination of carcinoma cells and resulted in 40,000 deaths in the US last year. Over-expression of cofilin, an actin regulatory protein, has been correlated with metastasis occurrence. The formation of the cell motility structure invadopodia was studied. Invadopodia cell locomotion allows cells to degrade the extracellular matrix, and protrude through surrounding tissue. A mathematical model of the cofilin cycle and actin growth in invadopodia following stimulation by epidermal growth factor (EGF) was created to describe carcinoma cell metastasis. The model is used to explain experimental data taken from the lab of Professor John Condeelis (Albert Einstein College of Medicine) as well as test the sensitivity of various processes on the polymerization of actin filaments and cell motility.

The cofilin regulation model consists of a system of ordinary differential equations (ODE's) and takes into account different processes such as actin polymerization and capping, diffusion, phosphorylation and binding interactions of different actin regulatory proteins. The model assumes a two compartment geometry where the inner compartment represents the part of the invadopod precursor where actin growth is generated downstream of EGF stimulation. The system of ODE's describes a cofilin cycle in which the actin regulating protein shifts through various forms to influence actin filament polymerization. The ODE model was used to track cofilin and actin growth species concentrations within the cell over time. The model was then employed to determine the sensitivity of the processes involved in the cofilin cycle and the formation of free barbed ends. The model parameters were established by implementing the existing lamellapodia model as well as by fitting the modeled curves to data collected by our collaborator.<sup>1</sup>

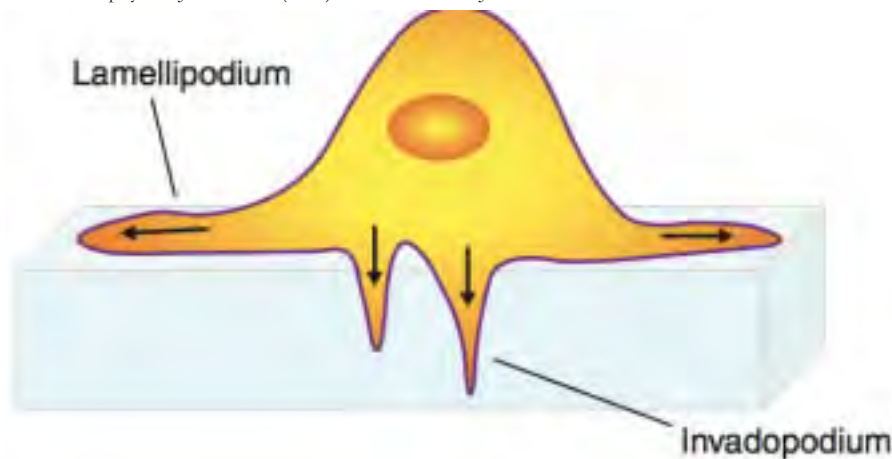
Conclusively the invadopodia cell motility model created accounts for many of the precursors to cofilin regulated actin growth in invadopodia cell motility, currently the model captures early actin growth profile (first 60 mins) however not the latter portion of the observed data provided by the Condeelis lab. In future work additional regulatory aspects that may modulate cofilin activity and actin growth will be considered in the hopes of creating a more complete model of invadopodia protrusion development post EGF stimulation. The finished model can be used to further quantify the process of actin regulation within the invadopodia and to generate new hypothesis that can be tested experimentally.

*(Supported by the Four Colleges Biomathematics Consortium)*

Advisor: Nessay Tania, Mathematics & Statistics

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# An Introduction to Oriented Chromotopologies

Hana Foe/2017

We studied graphs of physical representations called “oriented chromotopologies.” These oriented chromotopologies consist of colored edges with arrows and vertices in order to map Clifford algebra representations. Clifford algebras are important concepts in the realm of mathematics, and we can create representations of these Clifford algebras using matrices. However, these representation matrices can become large and complicated, thus making them difficult to work with. We are also influenced by similar graphs called “adinkras.” Many of the traits of oriented chromotopologies are equivalent to adinkras, such as the colored edges and the ability to map Clifford algebra representations. Nevertheless, adinkras must follow a greater set of rules that make it more restrictive than those of oriented chromotopologies. By using oriented chromotopologies, we can depict the Clifford algebra representations while being able to work with them more freely.

In order to explore oriented chromotopologies, we looked at the process of constructing them as well as investigating the characteristics that make them special by reading literature pertaining to Clifford algebra representations and adinkras. We then create oriented chromotopologies from codes and Clifford algebra representations. We use proofs to justify our methodologies and found interesting results. We used a method called color-averaging, unique to oriented chromotopologies, to allow us to translate one graph to another and therefore one Clifford algebra representation to another. We were also able to prove the legitimacy of the creation of oriented chromotopologies by proving that there is a relationship between oriented chromotopologies and certain Clifford algebra representations and proved that there is an oriented chromotopology for every irreducible Clifford algebra representation.

From our results, we can say that an oriented chromotopology is a way to map Clifford algebra representations. Because these are closely related to adinkras, oriented chromotopologies have strong physics applications. In the field of mathematics, oriented chromotopology graphs simplify the use of Clifford algebra representations by making them easier to look at. Our research is just an introduction to a more complicated concept. Further, we want to look deeper into the relationship between these graphs, codes, and Clifford algebras. We are hoping to present our findings at an external conference.

*(Supported by the Ellen Borie Fund in Mathematics & Statistics)*

Advisor: Rajan Mehta, Mathematics & Statistics

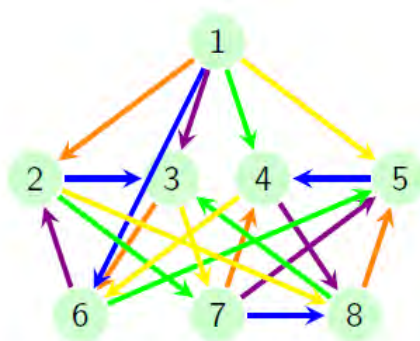


Figure : A (5,2) oriented chromotopology

# Fair Weather Friends: The Role of Oxytocin in Female Meadow Vole Seasonal Sociality

Jenn Christensen/2015

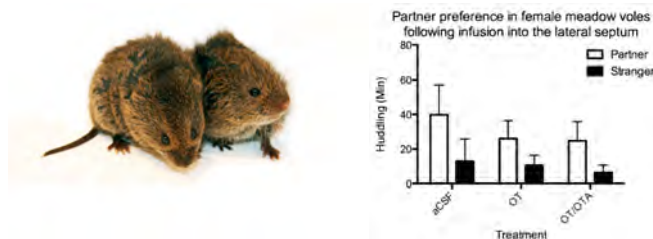
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Research regarding the neuropeptide oxytocin has gained momentum in the last decade. As a peripheral peptide hormone, oxytocin was first associated with uterine contractions during labor. This association led to its implication in maternal bonding; moreover, recent findings indicate it has influence on anxiolytic behavior, pro-social behavior, and pair bonding.<sup>123</sup> Our research focuses on the role of oxytocin on female-female affiliative bonds.

Meadow voles (*Microtus pennsylvanicus*), a new model in social behavior research, are ideal for our research. Female meadow voles are territorial during the summer months, demonstrating aggression toward other females.<sup>4</sup> Winter marks a change in social behavior, as they live communally throughout the season. While the affiliative behavior can be elicited in laboratory settings by simulating short daylight conditions, the question of neuronal mechanism remains.

To determine the role of oxytocin in a specific brain region, we investigated the lateral septum, a region that has high oxytocin receptor density.<sup>5</sup> Using females housed singly in winter light conditions, we infused oxytocin, oxytocin receptor antagonist, or artificial spinal fluid into the lateral septum. Following infusion, animals were cohoused for twenty-four hours with a conspecific female. Then focal animals were subjected to a partner-preference test, which allows animals to choose to huddle with its cohabitation partner or with a stranger.

Preliminary data indicate a trend toward decreased huddling when oxytocin and oxytocin antagonist are administered into the lateral septum, which could indicate the oxytocin pathway is indeed involved in affiliative behavior. The next step in our research will be to explore the effect of oxytocin in other high receptor regions.



*(Supported by the Howard Hughes Medical Institute)*

Advisor(s): Annaliese Beery, Neuroscience and Psychology and Allison Anacker, Neuroscience

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# T-maze: An Effort Based Task to Assess Fatigue in Middle Aged Mice

Tatenda D Mahlanza/2015 and Fortunate F Chifamba/2016

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Fatigue is commonly described as an overwhelming feeling of tiredness that can not be reversed by increased amounts of sleep or rest. It is a common symptom of several chronic illnesses including cancer, Parkinson's disease and depression.<sup>1</sup> Fatigue greatly reduces the quality of life of those who suffer from it. Currently, there is no valid neural basis of this phenomenon; however, the neuro-inflammation caused by pro-inflammatory cytokines such as interleukin 1 $\beta$  has been implicated in several studies.<sup>2</sup> The development of animal models can help to identify neural substrates which can ultimately direct clinical efforts to formulate treatments.<sup>3</sup>

Our research lab has developed a mouse model of fatigue by administering 400ng interleukin, an optimal dosage of the cytokine. Building on this model, we assessed the effects of fatigue in an effort-based task in the T-maze, a structure consisting of a starting arm, and two perpendicular arms which are designated as the high density arm and low density arm. The high density arm contains 3 reward pellets blocked by a 13cm barrier while the low density arm contains one pellet and is left exposed. Choosing the high density arm results in a greater reward but also requires more effort. Our hypothesis was that the induced fatigue would result in a decrease in high density choices. After satisfying the training criteria, middle-aged C57b1/6J mice (n=4) were injected peritoneally with 18ug/kg IL-1 $\beta$  and later counterbalanced with 0.1mg of saline. Assessment 3 hours after treatment showed that, on average, interleukin administration resulted in a decrease in HD arm choice (67.5 +/- 0.92)% from the pretest HD choice (97.5 +/-0.25)%. This was greater than the decrease in HD arm choice after saline injections (22.5+/-0.70)%.

In a second experiment we assessed the effects of interleukin on food restricted mice on the wheel. Although food-restriction dramatically changed normal baseline activity for each mouse, interleukin administration resulted in a greater reduction in both wheel and motion activity than saline administration.

In the fall semester, we hope to assess whether voluntary exercise reduces or reverses the effects of fatigue in the T-maze. This work will form the basis of Tatenda's honors project.

*(Supported by the Frances Baker Holmes Internship Fund, Mahlanza and the Howard Hughes Medical Institute, Chifamba)*

Advisor: Mary Harrington, Neuroscience and Psychology

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# Electrophysiological Analysis of Novel Anesthetic Cyclohexanols on Mammalian GABA-A Receptors

Angela Tai/2015

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General anesthetics are therapeutic agents that play a crucial role in surgery; however, they can cause side effects such as metabolic acidosis and liver complications. There are specific protein receptor targets in the brain that are modulated by general anesthetics. More specifically, the Hall lab investigates GABA receptors using electrophysiology to learn more about the receptor modulation by anesthetics. Many derivatives of the plant monoterpene menthol (a cyclohexanol) are positive modulators of GABA receptor currents. By examining structures of menthol and cyclohexanol derivatives and determining which are most useful for positive modulation of GABA, the lab is attempting to generate better, lower risk anesthetics.

For my project, WSS-1 cells from the Human Embryonic Kidney cell line (HEK) were used due to their expression of human GABA-A receptors with a subunit composition of  $\alpha 1\beta 3\gamma 2s$ . This summer I maintained the tissue culture for the WSS-1 cells and learned the patch clamp technique, with which I will be able to measure the GABA receptor currents and their modulation by anesthetic cyclohexanols in the coming year. This research is directed at identifying novel agents that will provide the required anesthesia during surgery without the complications of unwanted side-effects.

*(Supported by the Howard Hughes Medical Institute)*

Advisor: Adam Hall, Neuroscience and Biological Sciences

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# Environmental and Behavioral Influences on Pro-Social Behavior in Rats

Sarah Winokur/2015

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Empathy behavior, a subcategory of pro-social behavior, is crucial for the functioning of societies in which we live. Understanding the neurobiology underlying empathy can translate to better treatment of people with neurodevelopmental disorders characterized by social impairment, such as autism. A recent model devised by Dr. Inbal Ben-Ami Bartal is thought to assess empathetically motivated pro-social behavior in rats. Bartal concluded that rats learn to intentionally and quickly open a restrainer to free a trapped cagemate, even when social contact between the two rats was prevented, and when opening the restrainer meant sharing a food resource with the restrained rat.<sup>1</sup> It was also found that rats who were familiar with the strain of a stranger restrained rat behaved pro-socially, while rats who were unfamiliar with the strain of the stranger did not.<sup>2</sup> The goal of the current study is to build on Bartal's model by analyzing various behavioral traits and environmental stimuli that may contribute to the animals' pro-social behavior as indicated by the new model. I hypothesize that a correlation exists between certain rat behavioral traits and performance on the pro-social behavior test. To see if this is true, I designed a study that looks at anxiety, social interaction, social motivation, and learning and memory in relation to pro-social behavior in rats. I also hypothesize that free rats who are familiar with the restrained rats will show more pro-social behavior than free rats who are paired with strangers during the pro-social test. The study I created to address this will run pairs of familiar and unfamiliar rats through the pro-social behavior test. Additionally, I hypothesize that certain environmental stimuli may affect the free rat's behavior during the pro-social test. To test this, I designed a study that manipulates Bartal's model by removing visual, olfactory, or auditory stimuli in order to isolate these potential variables and assess their relationship to pro-social behavior.

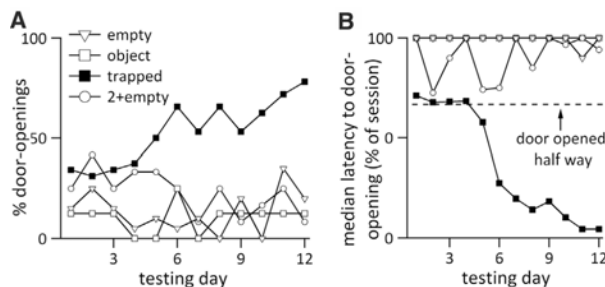
This summer I designed my study from the ground up with the aid of my advisors, which was a tremendous learning experience. I composed an Animal Subjects Approval Form for review and approval by the Institutional Animal Care and Use Committee at Smith, researched and prepared protocols, and built apparatuses with guidance from the Smith Center for Design and Fabrication. I am fortunate to be collaborating with Dr. Bartal, who is currently at UC Berkeley. We plan on sharing data between our labs in hopes of publishing a paper together.

*(Supported by the National Science Foundation)*

Advisors: Annaliese Beery, Neuroscience and Psychology and Allison Anacker, Neuroscience

<sup>1</sup> Ben-Ami Bartal I, Decety J, Mason P (2011) Empathy and pro-social behavior in rats. *Science* 334:1427-1430.

<sup>2</sup> Ben-Ami Bartal I, Rodgers DA, Sol Bernardez Sarria M, Decety J, Mason P (2014) Pro-social behavior in rats is modulated by social experience. *eLife*.



**Figure 1.** Expected outcome of the pro-social behavior in rats paired with trapped cagemates (black squares). It is shown in 1A that rats will increase their overall percent door openings during the duration of the test. 1B illustrates that the latency to door opening will decrease by the end of the 12 days of testing (figures originally from Bartal et al., 2011).

# The Search for Novel Anesthetics

Naina Zaman/2016

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Health facilities and hospitals world-wide use general anesthetic compounds to produce a loss of consciousness during procedures. Propofol is commonly used as a general anesthetic due to its effectiveness and rapid onset/offset. However, there have been countless incidences of adverse health dangers associated with propofol (such as its role in Michael Jackson's death). Negative health outcomes include decreasing blood pressure and depressing breathing<sup>1</sup>.

How do these general anesthetics affect the brain? Neuronal GABA<sub>A</sub> receptors are crucial due to their role as anesthetic targets in the mammalian brain<sup>2</sup>. Anesthetics are positive modulators of GABA<sub>A</sub> receptor currents. Propofol, one of the strongest positive modulators of GABA<sub>A</sub> receptor responses strongly enhances inhibitory transmission resulting in unconsciousness.

The goal of my research was to find a novel anesthetic that provided a similar enhancement of GABA responses as propofol without the negative adverse effects. To do so, we looked at stereoisomers of a specific compound, 2,6 dimethylcyclohexanol (DMCH). Studies have shown the importance of stereoselectivity of anesthetics in order to reach optimal drug effects.

Recordings were performed by measuring the GABA currents via the patch-clamp technique with whole cell recording. The patch-clamp is an electrical recording technique that allows for the measurement of the GABA receptor currents and their modulation by anesthetics. WSS-1 cells from Human Embryonic Kidney cell-line (HEK) were used due to their large size and expression of human GABA<sub>A</sub> receptors with the subunit composition of  $\alpha 1 \beta 3 \gamma 2s$ .<sup>2</sup>

My research focused on the chemical isomers cis,cis-2,6-dimethylcyclohexanol and trans, trans-2,6-dimethylcyclohexanol which are somewhat similar in structure to propofol. With increasing concentrations of GABA (1, 3, 10, 30, 100, 300  $\mu$ M) co-applied with 30  $\mu$ M of either cis,cis-2,6-DMCH or trans, trans-2,6-DMCH modulator, the percent enhancement of the GABA currents for each drug were measured at each concentration. The results showed that the cis, cis isomer had significant greater enhancements in the receptor currents compared to the trans, trans isomer, leading us to believe it may be a better anesthetic. Future research includes looking at stereoisomers of 2, 6-diisopropylcyclohexanol (DIPCH) which is closer in structure to propofol. I also spent some time in Professor Shea's chemistry lab to synthesis cis, cis and trans, trans 2,6 DIPCH which we will examine in the future and compare to cis, cis 2,6 DMCH and see which stereoisomers are the most potent anesthetics.

*(Supported by the Howard Hughes Medical Institute)*

Advisor: Adam C. Hall, Neuroscience and Biological Sciences

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<sup>2</sup> Davies, P. A., Hoffman, E. B., Carisle, H. J., Tyndale, R. F., & Hales, T. G. (2000). The influence of an endogenous B3 subunit on recombinant GABAA receptor assembly and pharmacology in WSS-1 cells and transiently transfected HEK293 cells. *Neuropharmacology*, 39, 611-620.

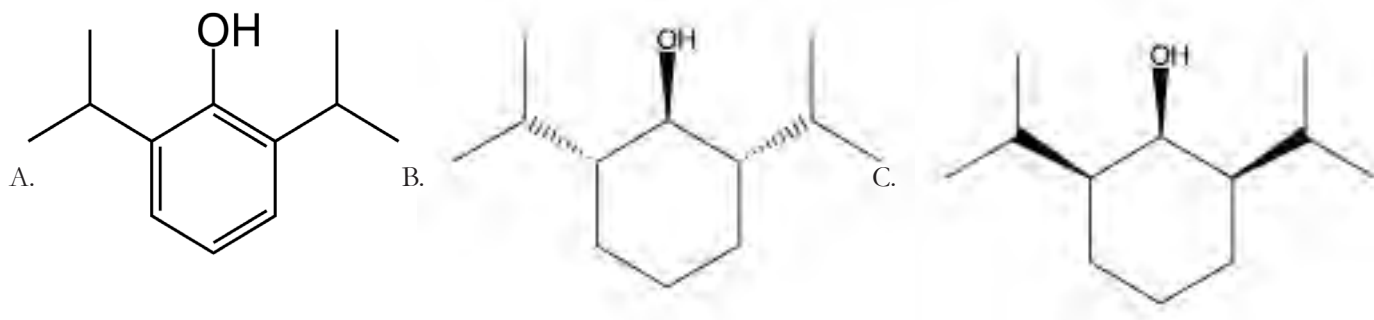


Figure 1. Structure of A.) propofol, B.) trans,trans-dimethylcyclohexanol, C.) cis,cis-dimethylcyclohexanol.



# Collective Modes of Shell-Shaped Bose-Einstein Condensates

Frances Yang/2015

Bose-Einstein condensates are a collection of atoms trapped and cooled to low temperatures such that all atoms are in the same state and quantum mechanics dominates. For this project, these atoms that are trapped to occupy a region akin to the surface of a sphere. There are two spherically symmetric modes for a shell-shaped condensate: the “balloon” mode, in which the mean radius oscillates, and the “accordion” mode, in which the shell thickness oscillates. It is generally difficult to find the frequency of these oscillations analytically, thus numerical methods are used. The goal of this project was to see the evolution of the modes as a shell condensate deforms to a spherical condensate.

The ground state function for an initial trapping potential was found using the numerical method by Chifalo et al.<sup>1</sup> Oscillations were induced by evolving this state through time in a different trapping potential using the method by Cerimele et al.<sup>2</sup> By using different potentials, oscillations of a shell to a sphere can be seen. Frequencies were found by performing a Fast Fourier Transform on the maximum value of the function through time.

Figure 1 shows the oscillation for one shell arrangement; the envelope suggests a coupling between the two modes. The evolution of the frequencies is shown in Figure 2. The “balloon” mode appears to be roughly constant for a shell, while the “accordion” mode decreases as the mean radius of the shell decreases. It is unclear if the two shell modes converge to the single sphere mode as limited data was obtained for when the condensate density becomes appreciable within the shell. Comparisons with thin shell and sphere frequencies obtained analytically suggest that we are closer to the weak interaction limit for our largest shell and closer to the strong interaction limit for our sphere. This is due to the variation in the density of the condensate as the size of the shell changes.

This work will be continued as an honors thesis, in which the data collected this summer will be refined and analyzed, and the coupling between these two modes will be further explored. Other work could look at non-spherically symmetric shell oscillations or oscillations of an spheroidal shell condensate.

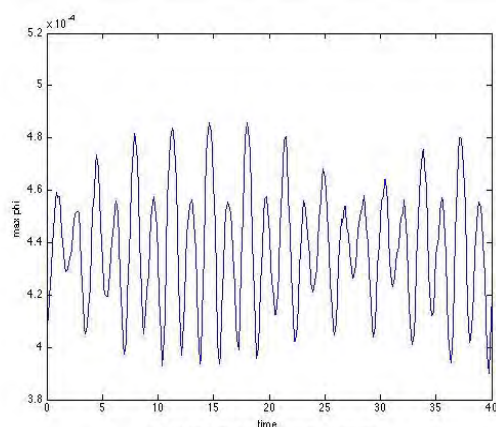


Fig. 1: Oscillation of the maximum of the function

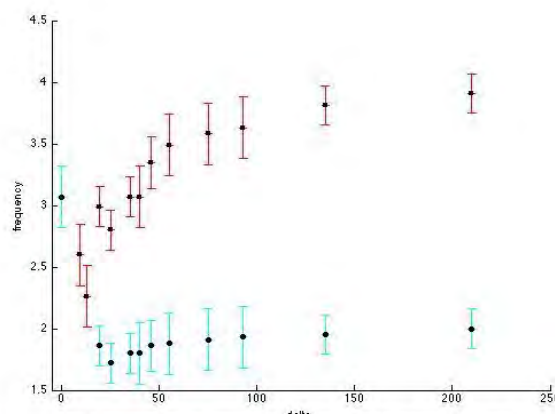


Fig. 2: Balloon mode (red/square) and accordion mode (blue/circle) from sphere to shell

*(Supported by the National Science Foundation)*

Advisor: Courtney Lannert, Physics

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<sup>2</sup>M. M. Cerimele, M. L. Chiofalo, F. Pistella, S. Succi, and M. P. Tosi, Numerical solution of the Gross-Pitaevskii equation using an explicit finite-difference scheme: an application to trapped Bose-Einstein condensates, Phys. Rev. E 62 1382 (2000).







# Charting the Relation Between “How” and “Why” Children Draw: A Case Study of the Path to Artistry

Noah Blohm/2017

This longitudinal case study of the 183 drawings of “Emma”, who attended the Prospect School from 1976 to 1985, from age 5 to 13 years describes her path to artistry as a function of “how” and “why” she draws.<sup>1</sup> Drawing is identified with Personal Drawing Style (PDS), one of three functions: narrative, telling a story; descriptive, representing without action; and graphic, abstractly exploring form and color. How Emma draws narrative and descriptive images is described within a Levels of Drawing (LOD) scale identifying milestones in development: Scribbles, Shapes, Symbolic, Schematic, Conventional (Figure 1), Realism (Figure 2), and Beyond Realism (Figure 3). Artistry emerges at the level of Beyond Realism, when representations express impressions, physical resemblance, and individual style.

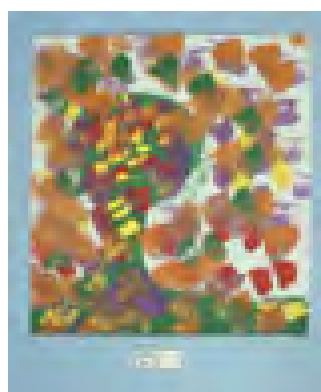
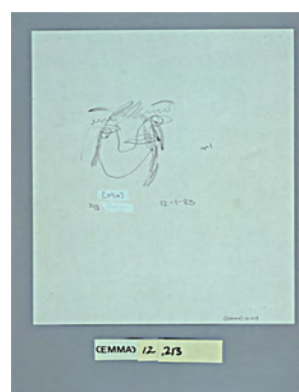


Figure 1: Emma’s drawing of falling leaves at age 5 represents space Conventionally but depicts falling leaves as splotches of color typical of Scribbling.



Figures 2 (left) and 3 (right): In these two drawings Emma (age 12) represents her friend Mia within distinctively different levels of drawing: Realism (left) and Beyond Realism (caricature on the right).



Information about the “why” and “how” of Emma’s drawing is related to general development contained in weekly Narrative Records of teachers on Emma’s academic achievement; personal and interpersonal functioning; artistic performance; and craft, play and drama.

By studying the Narrative Records, we gained a clearer understanding of why Emma draws the way she does across development. Emma explores various methods of depictions, particularly in her descriptive works. Also reflected in her academic and craftwork, this shows the diversity in her artwork comes from her personal enjoyment of exploration and experimentation. Early on, Emma creates many narrative drawings, but this weans to more descriptives and graphics. Narratives likely diminish because Emma enjoys creating conflict/resolution stories about interpersonal relationships; Emma finds difficulty creating such stories for Narrative drawings and discovers more fitting methods, such as writing and drama. As Emma grows increasingly independent in her academics and interpersonal relationships, she becomes more self-determined in her artwork; she cultivates experimental graphic works and Narratives and Descriptives that are Beyond Realism.

Coding for the highest level of development reveals that within one year Emma typically draws at two or more levels for narratives and descriptives, with greater diversity in the latter. This may be because Emma draws more Descriptives as she gets older, leading to more room for experimentation. As she progresses in her development, Emma uses the resources of various LODs to develop a sort of toolbox, which she then utilizes in various manners according to her desired function and presentation of a work.

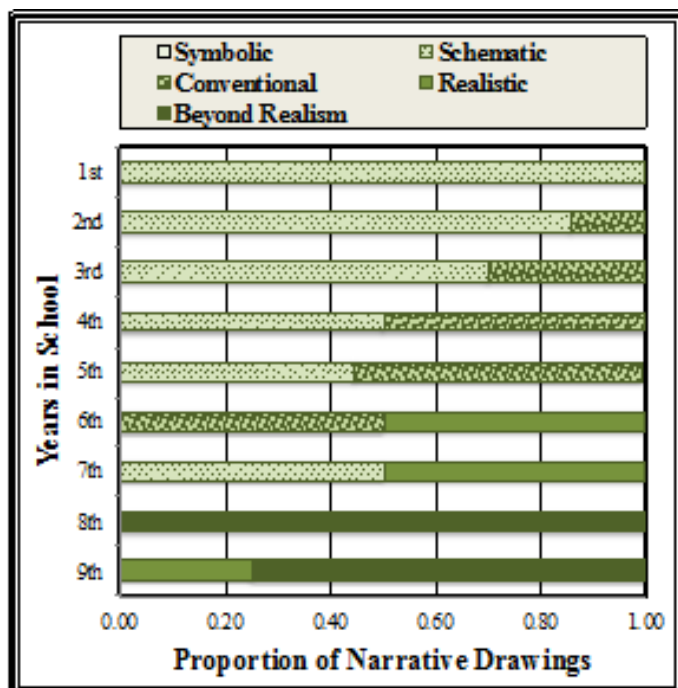


Figure 4: The proportion of Emma's yearly Narrative drawings expressed in terms of the highest Level of Drawing they reflect.

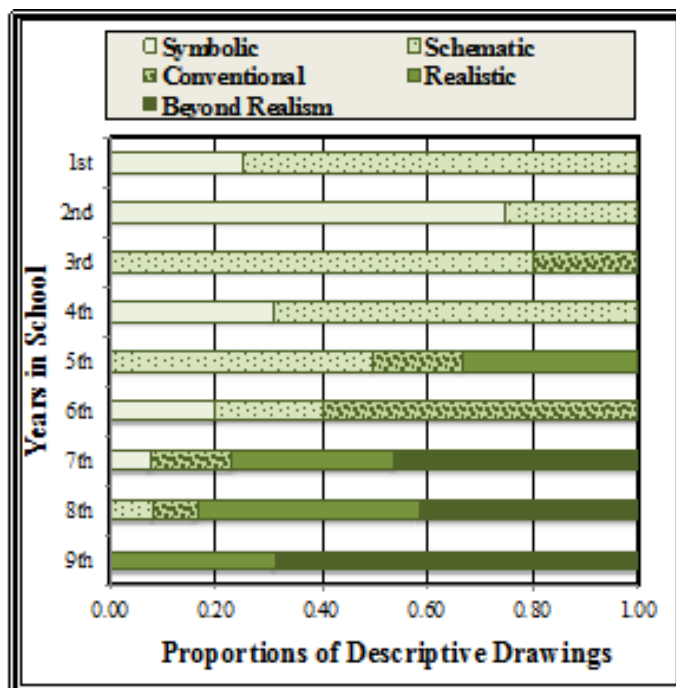


Figure 5: The proportion of Emma's yearly Descriptive drawings expressed in terms of the highest Level of Drawing they reflect.

*(Supported by the Frances Baker Holmes Internship Fund)*

Advisor: Peter Pufall, Psychology

<sup>1</sup>Prospect Archive of Children's Work. Center for Digital Initiatives. University of Vermont, 1984. Web. 1 July 2014.

# Eye Fixations and Backtracks During Reading

Janelle Gagnon/2015

When we read a sentence, our eyes do not travel in a smooth line across the page. They jump from spot to spot. This motion has two basic components: fixations and saccades. Fixations are when the eye stops on the page, and saccades are the quick jumps between fixations. When a saccade is directed backward in a sentence to fixate on an earlier word, this is a backtrack, or regression. These eye movements are illustrated in the diagram.

An eye tracker is used to study these movements. The eyetracker looks like a computer screen with a tiny camera in the front, and records where a participant is looking on the screen.

In this study, short narrative paragraphs were presented on the eyetracker, which participants were instructed to read as they normally would read a story. The goal of this study was to determine if eye movements while reading could tell us about the cognitive processes happening while processing the story, especially in terms of readers' expectations about the narratives.

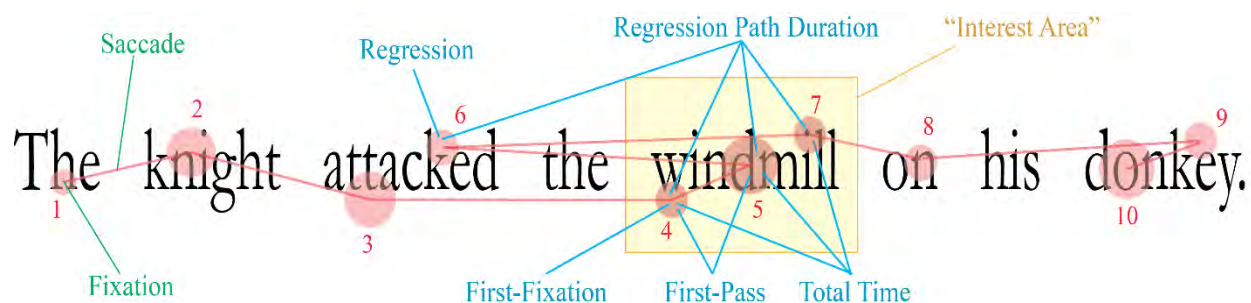
This research was a continuation of a project started during the Regarding Images Kahn Fellowship in Spring 2014. The major goals during this internship were to collect new participants, code the collected data, and plan the next study. Because there has not been a lot of research on this topic, the coding criteria for the gaze data were created this summer in order to quantify the data in a way that would accurately reflect the research question.

The data analysis is ongoing, and the research done this summer has already suggested improvements that can be made to the stimuli for future studies on this topic, as well as provided new directions for the follow-up study. Analysis will continue throughout this year as an honors thesis.

*(Supported by the Frances Baker Holmes Internship Fund)*

Advisor: MJ Wraga, Psychology

References: <https://wiki.brown.edu/confluence/display/kertzfab/Eye-Tracking+While+Reading>









# The Role of Reward Feedback Cues on Task Engagement of Individuals High in Trait BAS

Marisa Kubik/2015

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To make and evaluate everyday choices, we rely on a balance between two motivational systems, the Behavior Activation System (BAS) and the Behavior Inhibition System (BIS), which guide approach and withdrawal behaviors, respectively. Beyond approach behavior, the BAS is involved in reward seeking, goal oriented and positive-incentive behavior with neural connections to dopaminergic and reward pathways.<sup>1</sup> Previous research has shown that BAS individuals demonstrate heightened responsiveness to reward cues<sup>2</sup>. This project aimed to compare task engagement in high and low BAS individuals across conditions of reward and non-reward cues.

Young adults (18-24 years) categorized as high or low in trait BAS<sup>3</sup> via questionnaire (n=40) were asked to perform a modified flanker task, in which they needed to “feed” the central fish, out of a row of five, by pressing the corresponding buttons on a button box. The central fish faced the same (congruent) or opposite direction (incongruent) as the flanking fish. All participants completed two task conditions, reward gain and reward loss. Conditions were counter-balanced across participants and both accuracy and reaction time were recorded.

Consistent with previous flanker task findings, a main effect emerged for trial type with greater accuracy ( $F(1,40)=131.83, p=.00$ ) and faster reaction times ( $F(1,40)=417.33, p=.00$ ) on congruent, compared to incongruent, trials. A two-way condition by order interaction ( $F(1,40)=8.56, p<.01$ ) revealed that seeing reward gain cues followed by reward loss cues increased overall accuracy. When exploring BAS and accuracy, a trend-level three-way interaction emerged between trial type, condition and trait BAS ( $F(1,40)=3.61, p<.07$ ) such that high BAS individuals had greater accuracy on incongruent trials during the reward loss versus reward gain condition ( $t(20)=7.26, p=.00$ ). A trend-level three-way interaction also emerged between trial type, BAS and order ( $F(1,40)=3.81, p<.06$ ) for reaction times following correct and incorrect responses, with high BAS individuals displaying a significantly slower reaction time after errors when the reward loss condition is introduced first ( $t(12)=-2.34, p<.04$ ).

Combined, these results show high BAS individuals have increased task engagement when feedback cues indicate a loss of reward. Specifically, high BAS participants had heightened sensitivity to strategies that increased their performance for response accuracy and reaction time. This pattern expands understanding of how high trait BAS individuals respond to feedback in differential reward contexts. Future research directions include the addition of examining physiological measures of neural reactivity and ultimately to expand the subject group to developing children.

*(Supported by the Howard Hughes Medical Institute)*

Advisor: Mary Harrington, Psychology, and Jennifer Martin McDermott, Psychological and Brain Sciences (University of Massachusetts, Amherst)

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<sup>3</sup>Gray, J.A., 1989. Fundamental systems of emotion in the mammalian brain. In: Palermo, D.S. (Ed.), *Coping with Uncertainty: Behavioral and Developmental Perspectives*. 173-195.

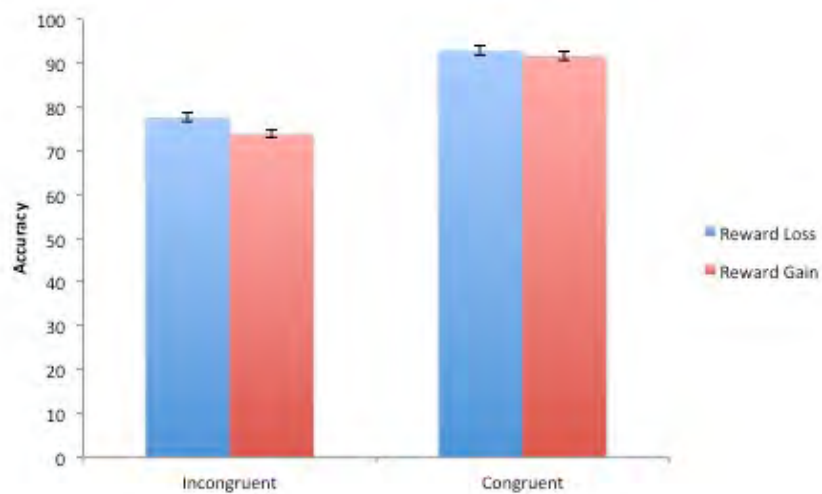


Figure 1. Individuals with the high BAS trait had higher accuracy on incongruent trials during the reward loss condition than the reward gain condition ( $t(20) = 7.26$   $p < .04$ ).

# Language Acquisition Has Separate Direct Effects, Both Concurrent and Longitudinal, on False Belief Reasoning

Elizabeth Lindley/2015

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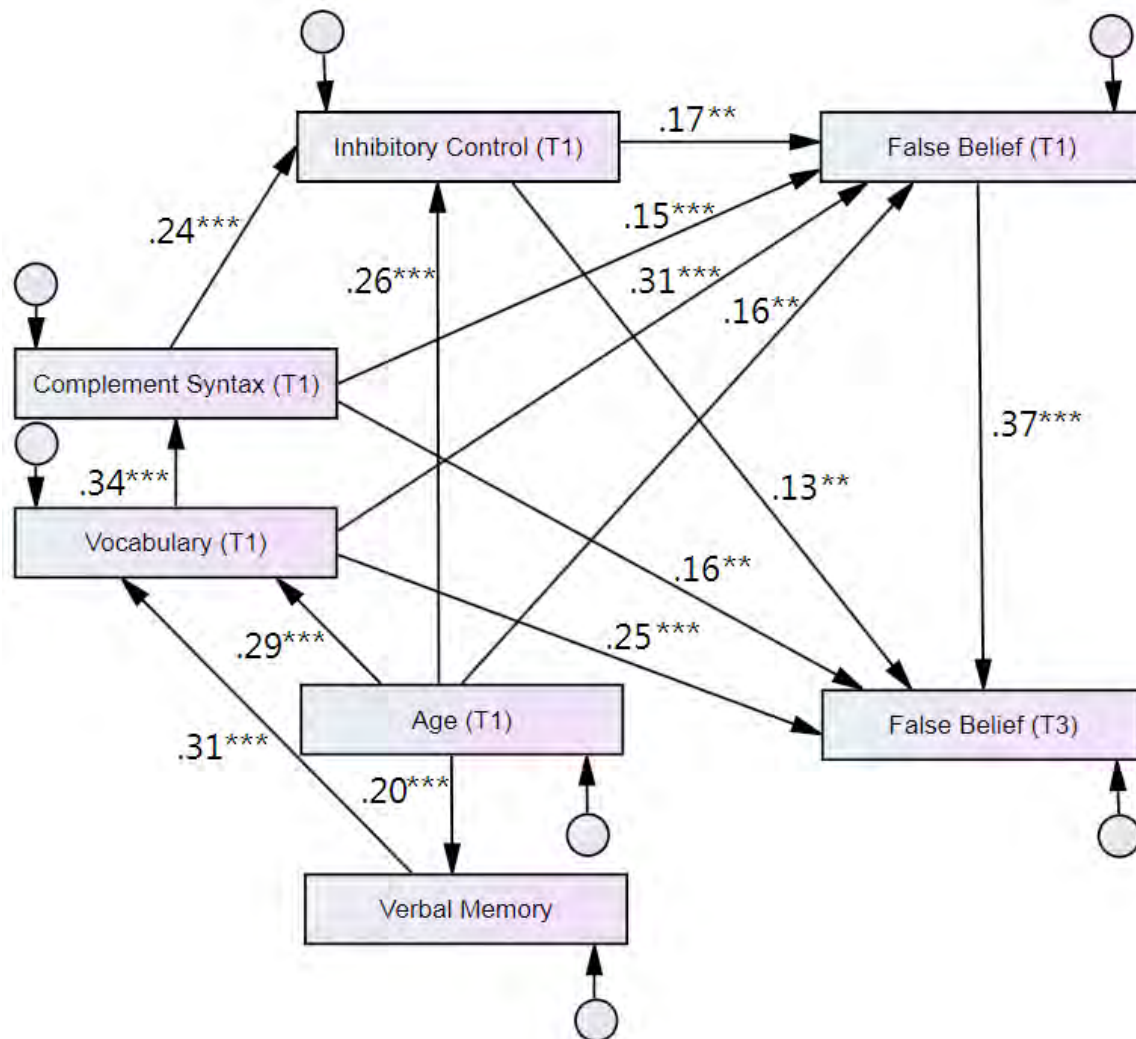
Children's inhibitory control and language acquisition have both been shown to contribute to their explicit reasoning about false beliefs (FB) (Devine & Hughes, 2014; Milligan, Astington & Dack, 2007). But the separate effects of each and any interactions between these variables have proven difficult to tease apart because of small sample sizes, insufficient variance in false belief outcome measures, and inadequate measures of language acquisition. In addressing such shortcomings in the literature, under Professor Peter DeVilliers, I was able to render results from a large-scale longitudinal study of 325 children tested on a battery of language, inhibitory control (IC), and both verbal and low-verbal tests of false belief reasoning (FB) early in the preschool year (age = 4.5 (3.0 to 5.5); Time 1) and six months later (Time 3). Nonverbal IQ and verbal memory span were collected in-between (Time 2).

A structural equation model (SEM), created using SPSS AMOS, examined the relationships among vocabulary (EOWPVT) scores, memory for false complements (de Villiers & Pyers, 2002), and IC as concurrent and longitudinal predictors of FB scores (See Figure 1). Each language measure and IC had significant concurrent direct effects on FB scores at Time 1. Significant direct effects also held longitudinally for vocabulary, complement comprehension and IC on FB scores at Time 3, even controlling for the effects of FB1 on FB3. In addition, vocabulary had indirect effects on FB through significant effects on complement comprehension; and complement comprehension had indirect effects on FB through its significant effect on IC. Background variables of Age and Verbal Memory Span also contributed significantly to the model, although the effects of memory were only indirect through vocabulary. The model was an excellent fit to the data, with fit indices (IFI and CFI) > .97, and RMSEA = .05. We conclude that specific aspects of language acquisition have separate direct effects on explicit FB reasoning, both concurrently and longitudinally. Likewise, inhibitory control contributes directly to performance on FB tasks. Language also contributes indirectly through effects on IC. The longitudinal SEM supports both general theories of the effects of language on FB reasoning (Astington & Baird, 2005), and the specific effects of complement syntax (de Villiers & de Villiers, 2009).

*(Supported by the Frances Baker Holmes Internship Fund)*

Advisor: Peter de Villiers, Psychology

Figure 1. Structural Equation Model of concurrent and longitudinal relationships between language measures, inhibitory control, and explicit false belief understanding in 325 preschoolers.



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# Gender, Ethnicity, and Drinking Games Involvement in a Large, Multiethnic Sample of College Students

Janelle R. Olsen/2015

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A drinking game (DG) is a high-risk social drinking activity designed to promote alcohol intoxication and has been associated with increased negative drinking consequences.<sup>1-2</sup> To date, research examining gender and DGs remains unclear, with some studies suggesting that men and women are just as likely to play and others reporting that men are more likely to play than women.<sup>3-6</sup> As far as DGs participation and its association with alcohol-related problems, Pederson and LaBrie<sup>7</sup> found a higher association between DGs participation and alcohol-related problems among women compared to men, while Sheehan et al.'s found that the positive association between gaming consumption and alcohol-related problems are similar between genders.<sup>8</sup>

Research examining DGs has been conducted primarily with White college students, with only a handful of studies examining ethnic group membership and its relevance to DGs. Haas et al.<sup>5</sup> found a small positive correlation between non-White ethnicity and rates of DGs participation. Although Pedersen and LaBrie<sup>7</sup> found that White students were more likely to have both played drinking games and drank more while gaming than non-White students, the relationship between gaming and alcohol-related problems was higher among non-White students.

The present study examined gender and ethnicity and their relevance to DGs and addressed the following research questions: (1) Does frequency of DGs participation or the amount of alcohol typically consumed while playing drinking games differ by gender or ethnicity? (2) Are the associations between (a) frequency of DGs participation and alcohol-related problems and (b) the typical amount of alcohol consumed while gaming and alcohol-related problems similar in men and women and across different ethnic groups?

Students (n=7,533) from 30 U.S. colleges/universities completed demographic questions, AUDIT<sup>9</sup> (a measure of alcohol-related problems), and DGs participation (frequency and amount of drinks consumed while gaming). Chi-square tests indicated that among those who reported playing DGs monthly, a higher proportion of women reported drinking 5+ drinks while gaming than men. Compared to other ethnic groups, a higher proportion of White students reported participating in DGs and consuming 5+ drinks while gaming. Finally, the association between DGs participation and alcohol-related problems was similar for men and women and across all ethnic groups. However, the correlation between the typical amount of alcohol consumed while gaming and alcohol-related problems was higher for (a) women compared to men and (b) White students compared to Black and Asian students.

Women metabolize alcohol more slowly than men, thus if women drink more alcohol than men while playing, women will achieve considerably higher BACs, putting them at elevated risk for experiencing negative drinking outcomes.<sup>9-10</sup> Providing education about the risks of DGs, especially to women<sup>11-12</sup> could prove useful in helping reduce their risk for harmful drinking consequences. Psychoeducational efforts targeting college women who play DGs could incorporate Protective Behavioral Strategies<sup>13</sup> within the context of the DGs.

Presented as a poster at the 2014 American Psychological Association (APA) Convention.

*(Supported by the Frances Baker Holmes Internship Fund)*

Advisor: David Palmer, Psychology

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## Race and Trauma Studies

Hannah Young/2015

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As a member of Nnamdi Pole's Posttraumatic Stress Disorder (PTSD) Lab, Professor Pole involves us in various research endeavors related to the field of traumatic studies. Posttraumatic Stress Disorder or PTSD is a psychiatric disorder that develops after witnessing or experiencing a potentially traumatic event. This includes actual or threatened death, serious injury, or sexual assault (American Psychological Association, 1994). PTSD is characterized by three subsets of symptoms: re-experiencing symptoms, avoidance, and hyperarousal. In Professor Pole's lab we explored various aspects of what PTSD research looks like. From data checking on a longitudinal police study to presenting posters at the International Society for Traumatic Studies; Professor Pole's lab has offered me a variety of opportunities. This summer has been no different.

The work I did for SURF this summer was two-fold. My first project was to serve as a teacher's assistant for Professor Nnamdi Pole's class at the Smith Social Work School called "Racism in the United States: Implications for Social Work". My second focus was the continuation of a study we began in Professor Pole's PTSD Lab last year, exploring the efficacy Acceptance and Commitment Therapy (ACT) as an inpatient treatment for PTSD at the VA Central Western Massachusetts Healthcare System, in Leeds MA.

For my first task, working as a TA, I helped put finishing touches on the moodle website as well as maintaining it. I uploaded papers, entered grades, and gave students back their papers. Throughout this process I was also able to read papers and give Professor Pole my feedback. I also assisted Professor Pole in proofreading papers that were submitted to Psychological Bulletin, of which Nnamdi is an editor. These processes of editing informed one another. Working as a TA gave me a view of what kind of work Social Work students were asked of. I saw the great introspection necessary when working towards an MSW, as well as the importance for introspection required in the field at large, in this case pertaining to race.

My second undertaking was to continue entering data that Professor Pole obtained from the VA study. I was entering data from the patients Professor Pole surveyed them on their feedback and expectations through several measures at the beginning of the inpatient program and the end of treatment assessment. My job was to enter this data, and begin to organize what had been previously entered.

Each academic and research oriented endeavor offered me insight on the various ways in which I could interact with the subject matter. As a rising senior I am considering various clinical PhD programs and as a PhD student, one is asked to work on a range of assignments. This SURF opportunity granted me a taste of the variety of work I will be doing in my near academic future.

*(Supported by Frances Baker Internship Holmes Fund)*

Advisor: Nnamdi Pole, Psychology

References: American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC: Author.

# Decision-making Simulation Study Using Mechanical Turk

Louise Huafeng Yu/2016

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“Hindsight Bias” (Cartoon by Peter Nicholson, *The Australian*, October 16, 2002)

My SURF activities focused on designing and implementing an online survey-like experiment about the influence of hindsight bias on people's post hoc evaluations of an event's feasibility. The decision-making simulation study is a continuation of two previous studies conducted by Professor Blanchard's lab. Instead of using conventional Survey Monkey and a Smith student sample, we designed the current survey using Qualtrics, an online survey-service provider that has not been widely used at Smith; we also plan to launch the survey on Amazon Mechanical Turk, a crowdsourcing Internet marketplace.

Consistent with previous studies, we kept the fictional character “Amanda,” a company leader who makes investment decisions, in the current version of study. We increased the number of dependent variables from 2 to 4. Specifically, the study condition that subjects will be randomly assigned to comprises 2 types of project choice (same/different project from Amanda's) and 2 kinds of role (observer/decision maker). In this way, we will be able to investigate a comprehensive set of conditions in one study. Also, the outcome information receiving was changed from “receive only the outcome of Amanda's project” to “receive the outcomes of both project.” We predicted that knowing the outcome of non-chosen project would exaggerate or attenuate the extent of hindsight bias, based on the valence of outcome. Moreover, we added several post tested questions, which assess self-perceived responsibility, surprise level and the way people attribute causes for the success/failure. Previous studies found that hindsight bias will affect people's post hoc evaluation of the project's feasibility, and, therefore, exaggerates the perception of escalation of commitment, especially for observers in failure condition. It is suggested by Pezzo & Pezzo (2007) that negative outcome triggers a “motivated” sense-making process, in which people tend to seek external causes first. We hypothesized that this tendency implies self-serving bias, which may influence the magnitude of hindsight bias. Through this experiment, we hope to find some initial insight about self-serving bias, which may point out a direction for future designs.

The experimental use of Qualtrics and M-Turk will be a good try-out for other social science studies. Other research studies have shown both advantages and disadvantages of using M-Turk. It will provide a more effective to generate a large and gender-balanced sample in a short time, which will increase the power of the statistical analyses and external validity, respectively. I have learned many of the intricacies of Qualtrics and will use this knowledge again in future investigations.

*(Supported by Frances Baker Holmes Internship Fund)*

Advisor: Fletcher Blanchard, Psychology

Pezzo, M. V. & Pezzo, S. P. (2007). Making Sense of Failure: A Motivated Model of Hindsight Bias. *Social Cognition*, Vol. 25, No. 1, 2007, pp. 147-164.

# Ethnic Identity among Asian Ethnicity

Yiwen Zhu/2016

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A sense of ethnic identity development among Asian Americans has been recognized as a factor connected with subjective well-being, interpersonal relationships, marriage patterns, and acculturation. Our study focused on the correlates of ethnic identity for Asian International students. As a comparison group, we also examined the same correlates for Asian domestic students.

In 2014 we surveyed all students of Chinese, Korean, or South Asian ethnicity attending a highly selective women's liberal arts college on the East Coast of the U.S.. Fifty percent of those contacted answered the electronic survey. For this particular set of analyses we split the sample into international students ( $n = 97$ ) and U.S. citizens ( $n = 120$ ).

As expected, international students ( $M = 3.97$ ) scored higher on ethnic identity than Asian American students ( $M = 3.62$ ) ( $t[214] = 3.14, p < .05$ ). In terms of correlational patterns, International students who had higher ethnic identity also scored higher on authoritarianism ( $r = .24, p < .05$ ), but the correlation did not hold for domestic Asian students ( $r = -.09$ ). It's possible that ethnic identity manifests as a form of nationalism among international students. As expected, a sense of collectivism appeared to be an indicator for higher ethnic identity in both groups ( $r = .28$  and  $r = .26$  for international and domestic students, respectively,  $p < .05$ ) whereas a sense of individualism did not necessarily imply lower ethnic identity ( $r = .08$  and  $r = .05$ ).

Interestingly, ethnic identity was correlated with life satisfaction for international students ( $r = .34, p < .001$ ), but the same pattern did not hold true for Asian American domestic students ( $r = .15$ ). This suggests that ethnic identity serves as a buffer for well-being among Asian international students adjusting to life in the U.S. Ethnic identity was positively correlated with the importance of Asian ethnic friendships in the lives of both international ( $r = .23, p < .05$ ) and domestic students ( $r = .23, p < .05$ ).

Asian international students continue to come to the U.S. for higher education. In order to help international students adjust to life in the U.S., we are studying how ethnic identity relates to important psychological variables such as life satisfaction. Comparing results with Asian American domestic students may have implications for how college administrators structure orientation sessions for international and domestic students.

*(Supported by the Frances Baker Holmes Internship Fund)*

Advisor: Bill Peterson, Psychology

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