Willamette University

Science Collaborative Research Program

Student Presentations

Friday 9/16/2011 – Ford 122

Science Collaborative Research Program
Research Conference
Friday 09-16-2011

3:20 – 3:35 COFFEE BREAK AND GROUP PHOTO

4:55 – 5:05 BREAK

Erik Reinhart. TYC-3144-595-1: A Study of a Suspected Double-Mode Pulsating Variable Star.

(Advisor: Professor Richard Watkins, Physics)

Pulsating variable stars are stars that radially and/or non-radially pulsate with an accompanied change in magnitude. The star that we studied, called TYC-3144-595-1(TYC), is a Delta-Scuti Type variable star that is located in the constellation Cygnus, was discovered by a Kepler Satellite Survey and is a suspected double-mode radial pulsator. For data collection, I pointed the telescope with the CCD camera attached at TYC and took 40 second exposures with both blue (B) and green (V) filters as well as calibration frames for 10 nights total. Then, by using the Image Reduction and Analysis Facility (IRAF), the images were reduced and the magnitudes for TYC and the comparison star (C1), as well as the Heliocentric Julian Date (HJD), were extracted. Finally, by plotting the HJD versus the magnitude difference between TYC and C1, a Fourier Transform program (Period04) was able to extract the pulsation frequencies. We confirmed that TYC is a double-mode pulsator with the fundamental and first overtone frequency being equal to the Kepler Survey values. We also confirmed that the sum of the two modes is also observed, which suggests mode interaction. More data is needed to confirm the difference frequency of the modes.

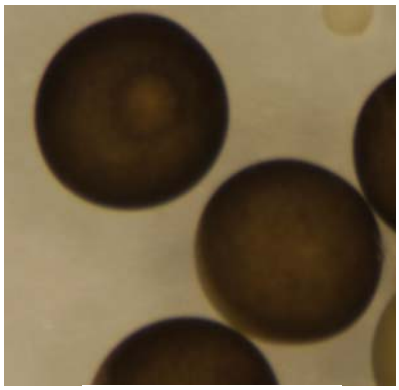
This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation, the M.J. Murdock Charitable Trust, and Willamette Alum Rick Baumann

Etienne Galbreath and Gwen Hryciw. Effects of the Hormonal Herbicide, 2,4-Dichlorophenoxy Acetic Acid, on Frog Oocyte Maturation.

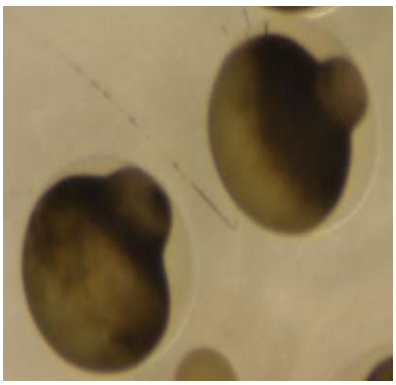
(Advisor: Professor Barbara Stebbins-Boaz, Biology)

Production of fertilizable eggs requires hormone-induced oocyte maturation. However, increasing evidence suggests that exposure to certain synthetic chemicals in industry, agriculture, and pharmaceuticals unintentionally disrupts normal hormone action, which can compromise reproduction and fertility. Oocyte maturation in the frog, *Xenopus laevis*, is induced by the hormone, progesterone. This can be conveniently recapitulated *in vitro* by surgically isolating the largest (stage VI) oocytes and incubation with progesterone. After several hours, a white spot forms in the pigmented pole of the oocyte, which is indicative of the breakdown of the nucleus, and the formation of the meiotic spindle. The signal transduction pathway that regulates maturation is well characterized and involves Mos/MEK/MAPK/MPF signaling. This convenient model system makes an excellent *in vitro* bioassay for identifying endocrine disrupting chemicals and their mechanism of action. Previous research showed that one of the most widely used herbicides, 2,4-D, blocks progesterone-induced maturation *in vitro*. We confirmed that 2,4-D causes abnormal oocyte morphology and signaling, which are correlated with an inability to undergo progesterone-induced maturation. Furthermore, we present preliminary data that support the hypothesis that 2,4-D induces an alternative pathway, Raf/MEK/MAPK, which may be functionally linked to the distinctive changes in oocyte shape.

***Xenopus* Oocytes**



Progesterone



2,4-D

This work was carried out as part of the Willamette University Science Collaborative Research Program with generous funding from the Mary Stuart Rogers Foundation (EG) and the Arthur A. Wilson Research Scholarship Award (GH).

Brian Mock. Weather In-Situ Deployment Optimization Method Visualization.

(Advisor: Haiyan Cheng, Computer Science)

The topic is the National Oceanic and Atmospheric Administration's (NOAA) Weather In-Situ Deployment Optimization Method (WISDOM) program. The WISDOM project is important in saving time, money, energy and lives from hurricanes. The current state of WISDOM balloon simulation is being improved for better choosing real potential WISDOM balloon launch sites. Simulation techniques are advancing with reverse time simulations and new visualizations which aid in verifying and shaping results.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation.

Jesse Sant. Analyzing the Force Dependent Function of Myosin 1c.

(Advisor: Professor David Altman, Physics)

Myosins are a superfamily of molecular motors that play a key role in many dynamic cellular processes, ranging from muscle function to various functions in the sensory cells of the ear and eye. This research aims to elucidate the force dependence of *Acanthamoeba* myosin 1c's function. This motor can act either as a cargo transporter or as an anchor, linking its cargo to the cell's actin cytoskeleton. By utilizing an optical trap to apply forces to the motor while it interacts with the filamentous protein actin, we will test the hypothesis that the function of myosin 1c is regulated by external forces within the cell.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Research Corporation, the Mary Stuart Rogers Foundation, and the M.J. Murdock Charitable Trust.

Andrew C. Rose and Brendan S. Zhang. Synthesis of an N-Heterocyclic Carbene Catalyst from N-Acetyl-D-Glucosamine.

(Advisor: Professor Andrew Duncan, Chemistry)

First isolated and characterized in 1991, N-heterocyclic carbenes (NHCs) are both potent organocatalysts and ligands for transition-metal catalysts. NHCs are strong sigma-donors, generally robust, and can be tailored for asymmetric catalysis in a variety of reactions. Our research this summer focused on developing a novel synthesis for nucleophilic triazolinylidene carbene catalyst (Figure 1) with chirality originating from a glucosamine backbone. Synthesis of the methyl 2-amino-4, 6-obenzylidene-2-deoxy D-glucopyranoside intermediate as well as the novel synthesis of the lactam intermediate (Figure 2) are reported. Further research will seek to: 1) Finish the synthesis of the target NHC; 2) Synthesize additional variants of the target NHC; and 3) Test the target NHC (and variants') catalytic abilities.

Figure 1.

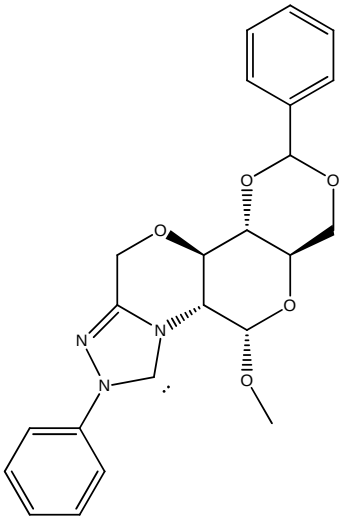
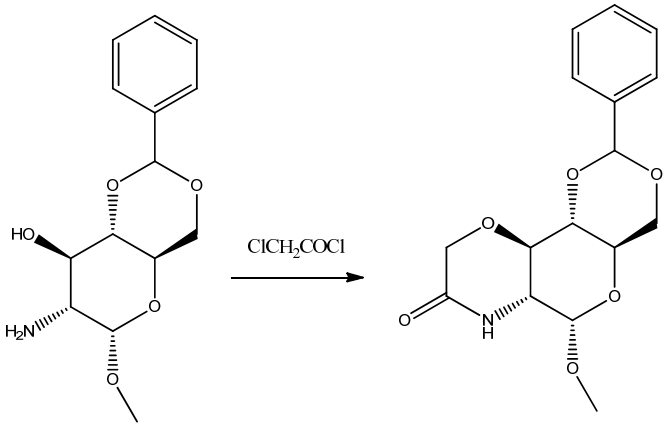


Figure 2.



Methyl 2-amino-4,6-O-benzylidene-2-deoxy-D-glucopyranoside

Lactam intermediate

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the American Chemical Society, the Petroleum Research Fund, the Mary Stuart Rogers Foundation, the Camille and Henry Dreyfus Foundation, the National Science Foundation, and the M.J. Murdock Charitable Trust.

Jenna Hermann. The Role of the Molecular Motor Myosin VIIa in Phagocytosis in Retinal Pigment Epithelium Cells.

(Advisor: Professor David Altman, Physics)

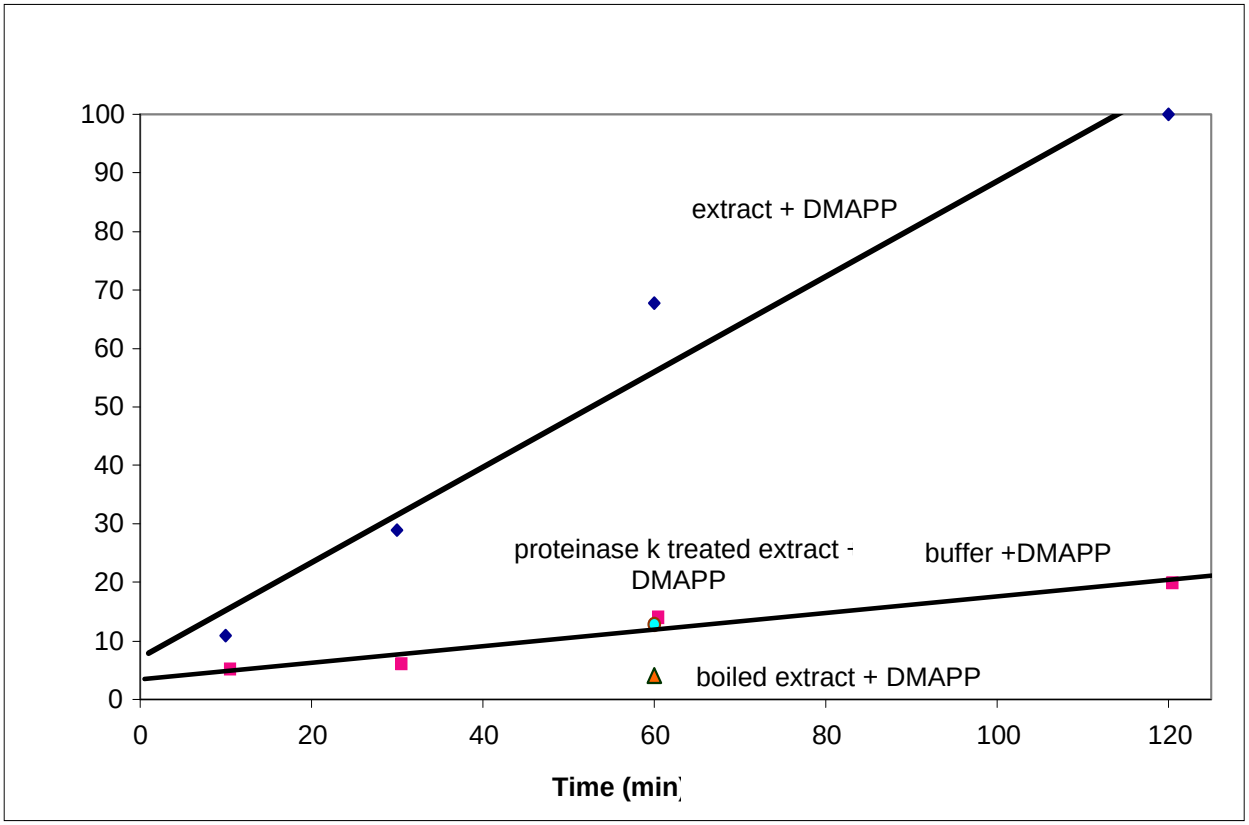
Myosins are a superfamily of actin-based motors whose force generation is involved in a variety of processes including muscle contraction, trafficking of vesicles, and growth. Mutations in the unconventional myosin VIIa result in the disease Usher's syndrome (USH), which results in deafness and retinis pigmentosa. Myosin VIIa is found within the retinal pigment epithelium (RPE), the monolayer of cells responsible for phagocytosing membrane disks shed by rod cells. Because a failure in RPE phagocytosis results in retinis pigmentosa, myosin VIIa is thought to play a role in this phagocytic process. While its involvement in phagocytosis has been studied in mice and *Dictyostelium discoideum*, the specific role of myosin VIIa in the RPE has not been unequivocally defined, nor how mutations in the motor may lead to USH. The goal of our research is to test the hypothesis that forces generated by myosin VIIa are required for RPE phagocytosis.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Research Corporation, the Mary Stuart Rogers Foundation, and the M.J. Murdock Charitable Trust.

Joseph F Cardiello. Enzymatic Synthesis of Isoprene in a High Temperature Moss.

(Advisor: Professor Alison Fisher, Chemistry)

Isoprene is the most abundant volatile molecule produced by plants. The enzyme isoprene synthase (IS), which has been found in several plants, catalyzes the conversion of dimethylallyl diphosphate (DMAPP) into isoprene. A number of moss species have been found to emit isoprene, but to our knowledge no lab has isolated IS from a moss. We successfully extracted and partially purified IS from *Campylopus introflexus*, a thermophilic moss. We used a very sensitive gas chromatograph with a reducing gas detector to assay extract samples for isoprene production from DMAPP. Our results confirmed that a protein extracted from the moss catalyzed the synthesis of isoprene. We used ion exchange chromatography to partially purify the extract. Future studies will focus on the further purification and characterization of the *C. introflexus* IS in order to compare this novel enzyme to known plant isoprene synthases.

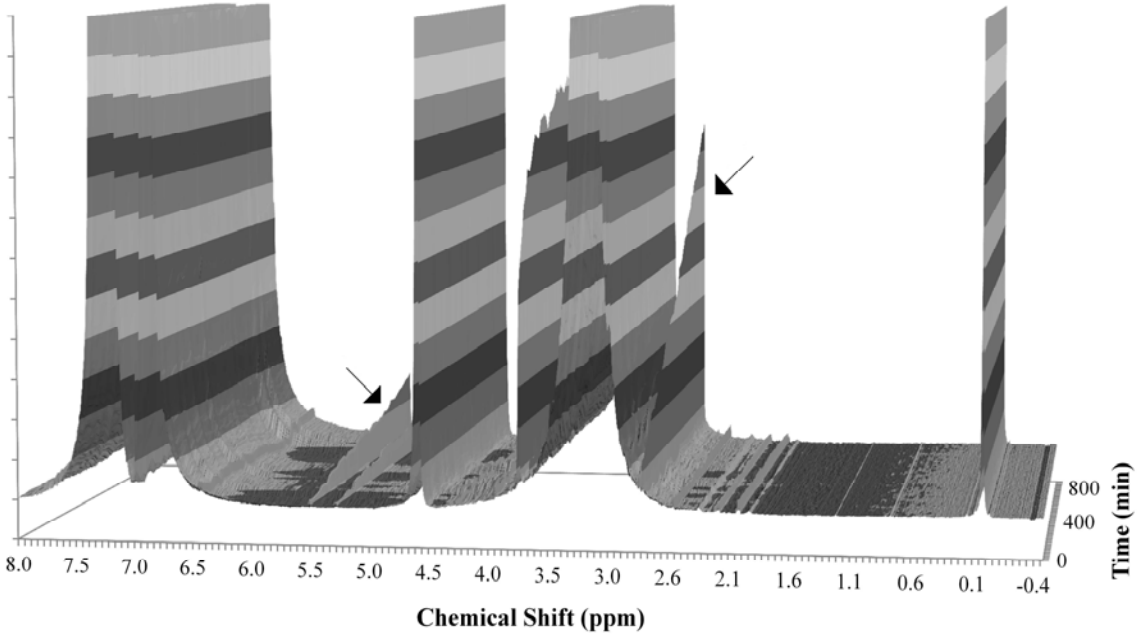


This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation and the M.J. Murdock Charitable Trust.

Ryne K. Smith. Dimerization Kinetics of 3-Butene-2-one Observed Using Quantitative Nuclear Magnetic Resonance.

(Advisor: Professor Chuck Williamson, Chemistry)

The 3-butene-2-one + water binary liquid system is partially miscible and exhibits a rare circular coexistence curve. The curve changes over time due to the spontaneous formation of dimerization and hydrolysis products which affect the overall solubility of the system. Quantitative nuclear magnetic resonance techniques (NMR) were developed in order to measure the rate of product formation over the course of the experiment. Preparation included: collecting T_1 relaxation data on the species involved to determine the maximum NMR sampling rate for experiments; employing the globally optimized alternating phase rectangular pulse decoupling scheme to prevent ^{13}C splitting from appearing in the ^{1}H NMR spectra; setting up the necessary 3-D shims to maintain the resolution of the spectra without spinning the sample; making a 2,2,3,3-tetradeutero-3-trimethylsilyl propionic acid sodium salt reference sample of known concentration; setting up an air flow heater and cooler to control the instrument temperature to ± 0.05 °C; and developing an automated data collection routine, as well as a serial processing program in order to expedite the process. The degradation of pure samples of 3-butene-2-one was then examined over a series of temperatures, and the rate of dimer formation was found to range from 2.57 μM / min at 15 °C to 51.1 μM / min at 45 °C. The activation energy of the dimerization reaction was calculated to be 82.6 kJ / mol.



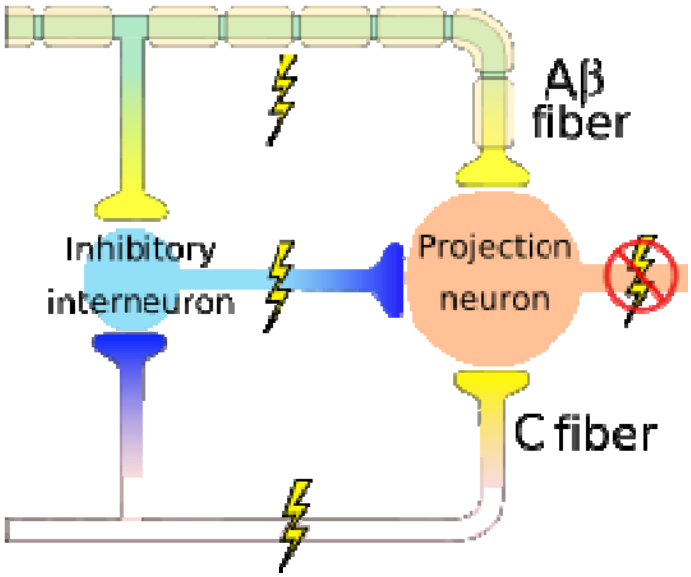
Representative spectra of observed dimer growth over the course of an experiment at 37.5 °C. Dimer peaks are indicated by the arrows.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation and the National Science Foundation.

Shannon J. Palmer and Wyatt O. Briggs. Intra and Inter Day Reliability of Presynaptic Inhibition in the Soleus of Diabetic Peripheral Neuropathy Patients.

(*Advisor: Professor Junggi Hong, Exercise Science)*

The purpose of this study was to determine the reliability of presynaptic inhibition as a criterion for evaluating the spinal cord circuitry in diabetic patients with neuropathy. Eight subjects with either type I or II diabetes and peripheral neuropathy (58.24 ± 6.38 yrs, 161.56 ±8.22 cm and 71.51 ± 4.13 kg), were recruited for the study. A paired-reflex depression protocol was used to assess presynaptic inhibition, with a pair of pulses at equal intensity at 35% of the soleus Mmax, separated by 80 milliseconds. A series of 10 paired-reflex depression trials were completed on the dominant leg in a prone position. The mean percent change of the conditioned relative to the unconditioned H-reflex was assessed. An intraclass correlation coefficient (ICC) was used to determine intra and intersession reliability. The ICC for intra-day reliability was .94 and .87 for inter-rater reliability between day 1 and day 2. Measures of presynaptic inhibition measured as paired reflex depression in the soleus muscle of diabetic peripheral neuropathy patients are consistent and reliable within and inter day.
Keywords: H-reflex, soleus, presynaptic inhibition, intraclass correlation coefficient

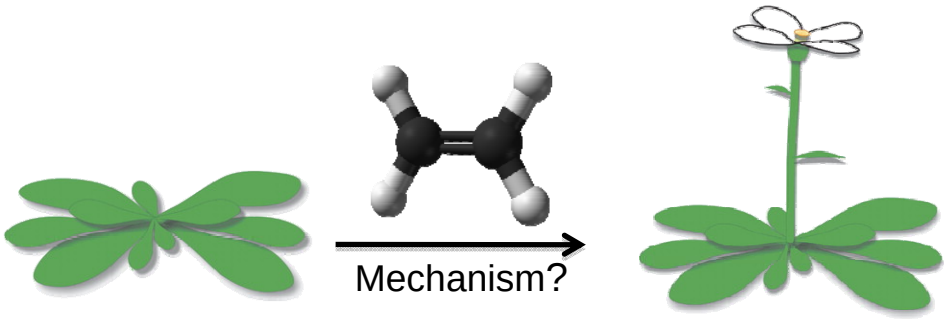


This work was completed as part of the Willamette University Science Collaborative Research Program with generous support from the Mary Stuart Rogers Foundation.

Garrett Eickelberg. Ethylene Promotes the Floral Transition in *Arabidopsis Thaliana* through the Attenuation of FLC.

(Advisor: Professor Alison Fisher, Chemistry)

The timing of the floral transition in *Arabidopsis thaliana* plants depends on a network of regulatory genes and pathways. The most notable genes involved in the transition are the floral repressor *FLOWERING LOCUS C* (*FLC*), and the two floral promoters *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANTS 1* (*SOC1*). Some evidence suggests that ethylene signaling through the ETR1 receptor leads to the promotion of the floral transition, and the goal of this research was to determine the mechanism through which this happens. To do this, we analyzed the expression of *FT*, *FLC*, and *SOC1* in three *Arabidopsis* mutants with varying ethylene sensitivity (*ctr1-1* > WT > *ein3* > *ein2* = *etr1-1*). Using Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) we found the relative expression of *FLC*/*UBQ* to be higher and *SOC1*/*UBQ* to be lower in ethylene insensitive mutants. However, the expression of *FT* was elusive in our analysis and will require further research to quantify. These results suggest that ethylene works to promote flowering in a dose dependent manner through the inhibition of the floral repressor *FLC*.



This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation, the National Science Foundation, and the M.J. Murdock Charitable Trust.

Alfredo Zuniga and Tori Pagel. The Microtubule Regulatory Protein Stathmin is Essential for Axonal Transport.

(Advisor: Professor Jason Duncan, Biology)

Neurons utilize a microtubule-based transport system to bidirectionally transport proteins, vesicles, and organelles between the cell body and the synaptic terminal through the axoplasm. We have identified the protein stathmin (stai), which regulates the dynamics of the microtubule cytoskeleton, as a component required for axonal transport in *Drosophila*. We have isolated hypomorphic mutations in the *stai* gene that cause neuronal dysfunction resulting in phenotypes consistent with severe defects in axonal transport. Mutant third instar larvae exhibit a posterior paralysis, or 'tail flip' phenotype, visible after each peristaltic contraction of the body wall musculature during the crawling cycle. In addition, immunostaining of the axons of the longitudinal segmental nerves that emerge from the brain and bilaterally innervate the body wall musculature of each larval segment identify focal swellings and accumulations of transported components. Despite this neurological dysfunction, a small percentage of *stai* mutants survive to the adult stage but exhibit severe movement defects, often dragging their hind limbs as they walk. The viable adult *stai* mutants also have a significantly reduced lifespan. Unexpectedly, adult *stai* mutants also exhibit a progressive, age-dependent seizure phenotype characteristic of the class of 'bang-sensitive' mutants that have altered neuronal excitability. Electrophysiological analysis indicates *stai* mutants have a lower evoked seizure threshold than wildtype animals. We demonstrate directly that all observed phenotypes are due to loss of *stai* function. First, mobilization of the mutagenic transposable element in the *stai* gene reverts all phenotypes to wildtype. Second, genetic rescue of the phenotypes by introduction of an exogenous copy of the *Drosophila stai* transgene ameliorates all observed phenotypes. Interestingly, we are also able to partially rescue the observed phenotypes in our *Drosophila stai* mutants with an exogenous copy of the human stathmin gene STMN1, indicating that the *Drosophila* and human stathmin proteins are functional homologues. Collectively, our data identifies a novel, evolutionarily conserved role for *stai* in the regulation of microtubule-based axonal transport.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Research Corporation, the Mary Stuart Rogers Foundation, the National Science Foundation, and the M.J. Murdock Charitable Trust.

James A. Neal. A Medicinal Approach to Preventing Blindness: The Design and Synthesis of Novel Tetracaine Derivatives as Ion Channel Blockers.

(Advisor: Professor Sarah Kirk, Chemistry)

Cyclic nucleotide-gated (CNG) ion channels play a major role in the complicated process of human eyesight. Retinitis pigmentosa is a disease triggered by a genetic mutation in the eye that causes CNG channels to remain open. Over time too many cations pass through the channel that eventually kills rod cells and leads to blindness. Tetracaine is a known small molecule that blocks the open channel with moderate affinity and prevents the influx of cations. Previous research has shown that increasing the lipophilicity in the tail or head region improves channel binding. Our research focused on further optimization of tetracaine and resulted in the design and synthesis of nine novel derivatives that prevent degradation in the body and improve the blocking of CNG channels. The head group was altered by replacing an ester group with amide or thioamide functional groups which are more resistant to hydrolysis *in vivo* (**1**-**5**). Furthermore the central aromatic core was modified with electron withdrawing groups to create tighter binding (**3**-**5**). Finally, two derivatives focused on changes to the tail region (**6**,**7**). The novel derivatives will be assayed using patch clamp to assess their ability to successfully block CNG channels and ameliorate the effects of retinitis pimentosa.

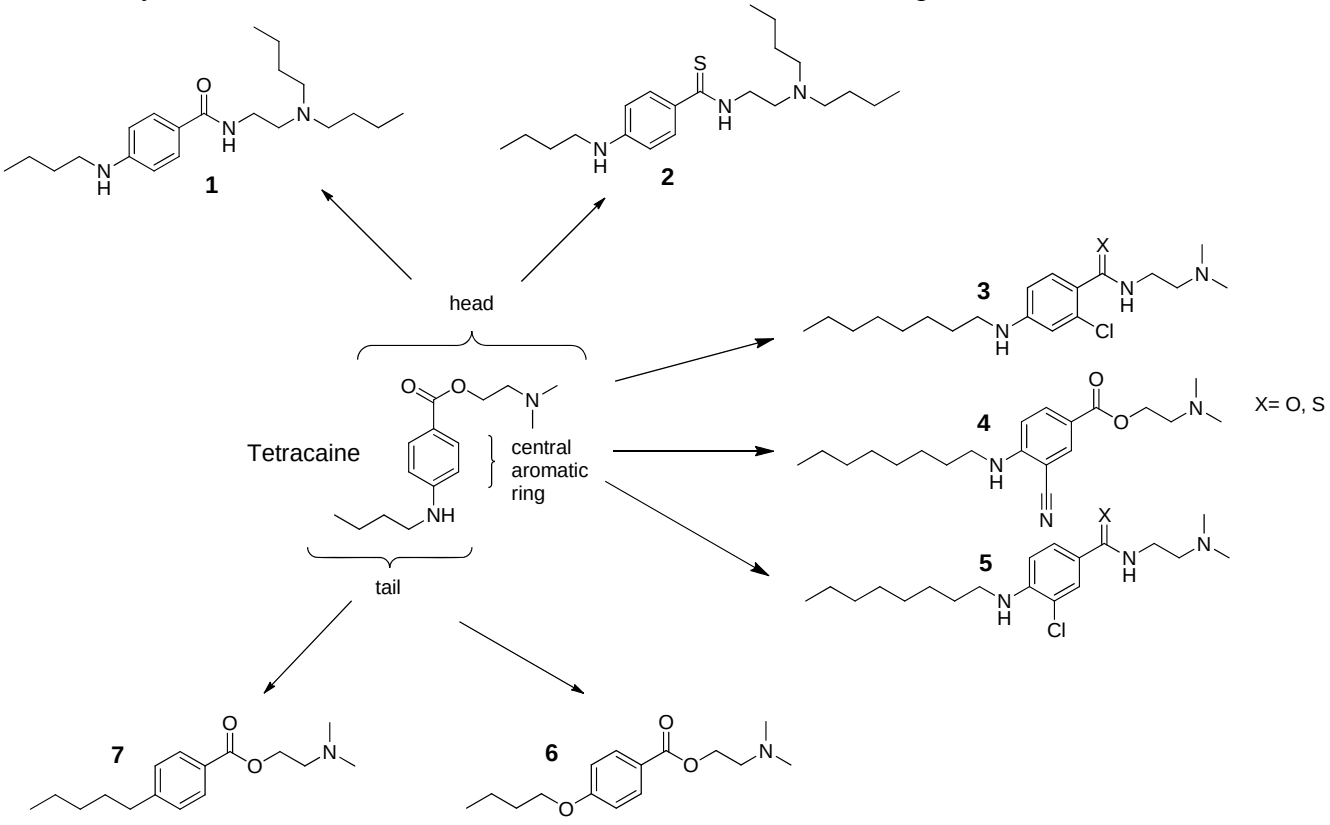


Figure 1: Tetracaine Derivatives

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation, the National Science Foundation, and the M.J. Murdock Charitable Trust.

Nicole B. Rodgers. The Creation of a Breeding Zebrafish Colony at Willamette University to Determine the Effects of 2,4-D on Neurodevelopment.

(Advisor: Professor Barbara Stebbins-Boaz, Biology)

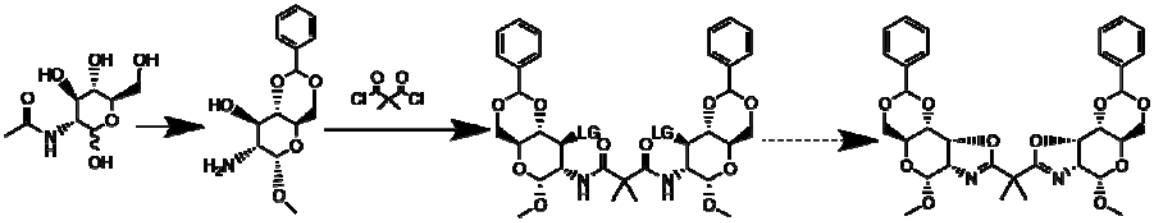
Zebrafish (*Danio rerio*) are a popular vertebrate model for studies in multiple biological disciplines. Their development is easy to follow, making developmental toxicology experiments feasible. Their most useful characteristics are the translucency of developing offspring and their relatively rapid maturation. Zebrafish are also fairly easy to maintain in a laboratory environment. The primary goal of this project was to establish a breeding colony of zebrafish at Willamette for future research purposes. This was achieved, and with the resultant embryos, preliminary studies were carried out to determine if exposure to the widely used herbicide, 2,4-D, would adversely affect neurodevelopment, specifically Mauthner cells. These are large neurons that control the fast escape response in fish and amphibians. Embryos were exposed to various concentrations of 2,4-D at specific developmental windows and observed over time for defects in morphology and motility. Details about effective techniques and conditions to support a breeding zebrafish colony, rear the young and conduct embryonic exposure experiments will be presented as well as key obstacles.

This work was generously supported by the Arthur A. Wilson Research Scholarship Award.

Aaron Bilbao. Novel Bisoxazolines Derived from Carbohydrate Aminoalcohols for Symmetric Catalysis.

(Advisor: Professor Andrew Duncan, Chemistry)

Bisoxazolines are asymmetric catalysts consisting of a chiral organic ligand complexed with a metal in order to form a reactive catalyst. Bisoxazolines in principle consist of two oxazoline joined by a separator such as carbon or pyridine. The majority of these compounds are formed from amino alcohol materials. Our goal was to create a novel bisoxazoline made from a carbohydrate starting material. To this purpose we used glucosamine. We successfully synthesized bis-amide precursors but so far have been unable to isolate a full carbohydrate based bisoxazoline. Various conditions are being optimized in order to successfully close the oxazoline rings; these include experimentation utilizing a variety of bases, solvents and temperatures. After a successful glucosamine based bisoxazoline is synthesized we plan to experiment with different complexed and catalytic options.

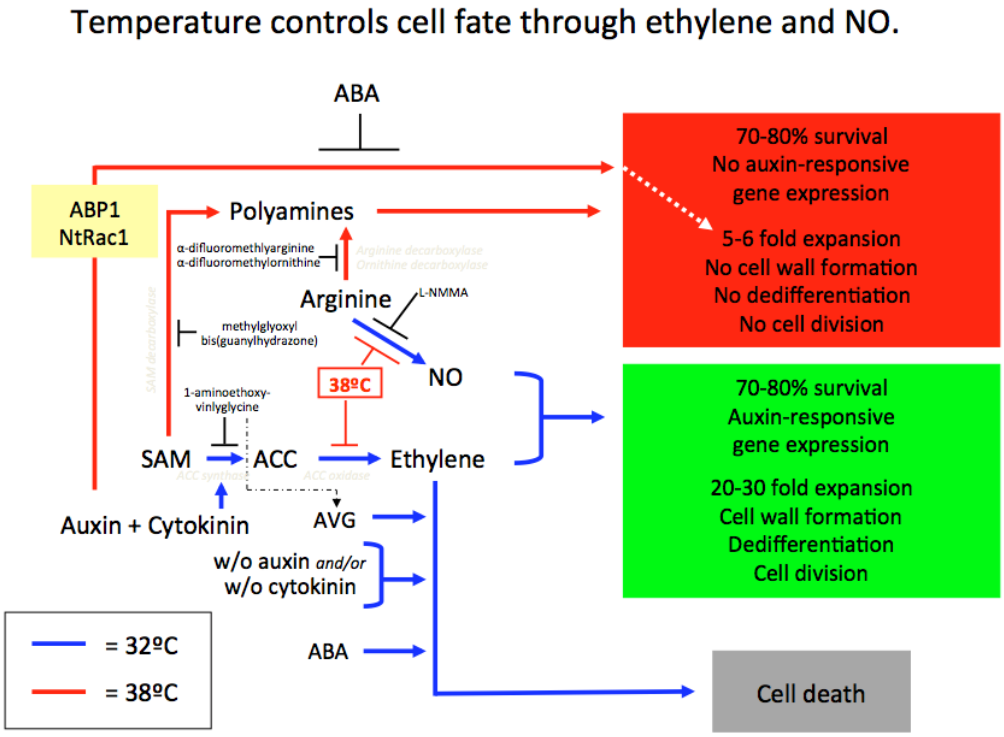


This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation, the National Science Foundation, the M.J. Murdock Charitable Trust and the Camile and Henry Dreyfus Foundation.

Logan K. Blair. Sequencing Polyamine Biosynthetic Genes to Study the Effect of Heat-Stress-Induced Arginine-Dependent Biosynthesis on Arginine Dependent Nitrous Oxide Production.

(Advisor: Professor Gary Tallman, Biology)

Global climate change is expected to cause more frequent and sustained periods of high heat. Little is known about the cellular and molecular mechanisms responsible for heat-stress-induced physiological plant responses. In cultured guard cell protoplasts (GCP) of *Nicotiana glauca*, heat and inhibitors of nitric oxide (NO) biosynthesis block signaling by the plant growth hormone, auxin. NO and polyamine biosynthesis both require arginine as a substrate. I hypothesize that heat reduces arginine-dependent NO production by inducing genes for arginine-dependent polyamine biosynthesis. I will test this by measuring levels of polyamine biosynthetic gene transcripts in heat-treated and non-heat-treated GCP by real-time quantitative PCR (qRT-PCR). While *Nicotiana glauca* possesses a high basal thermotolerance the *N. glauca* genome has not been sequenced. Prior to design of qRT-PCR primers I am developing and optimizing protocols for isolating and sequencing *N. glauca* reference and polyamine biosynthetic genes using primers designed for the closely related *Nicotiana tabacum*.



This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the National Science Foundation.

Kate Gadek. Ethylene's Regulation of the Floral Transition in *Pharbitis nil*.

(Advisor: Professor Alison Fisher, Chemistry)

Ethylene is a common plant hormone involved in many different plant processes from seed germination to fruit ripening. At high concentrations ethylene has been shown to inhibit the flowering of some plants, while the role of endogenous ethylene in flowering is not well understood. To investigate the role of ethylene in plant flowering, we examined the effects of endogenous and exogenous ethylene on specific genes involved in the flowering pathway of *Pharibitis nil* 'violet' (Japanese morning glory). We treated plants with 1-aminocylcopropane-1-carboxylic acid (ACC), aminoethoxyvinylglycine (AVG), and silver thiosulfate complex (STS) to increase exogenous ethylene, decrease ethylene production or decrease ethylene signaling, respectively. Previous data has shown that ethylene regulates the expression of the *FLOWERING LOCUS T* gene homologs, Pn*FT1* and Pn*FT2* in *Pharbitis*. We measured the relative expression of two key genes in the flowering pathway *GIGAGTEA* (Pn*GI*), a gene upstream of the floral stimulator Pn*FT1/2*, and *APETALA-like2* (Pn*AP2-like*) a gene downstream of Pn*FT1/2*. Expression levels were examined using RT-qPCR and analyzed by the $\Delta\Delta C_t$ method. Our results indicate that ethylene does not regulate Pn*GI*. Data showed a reduced amount of Pn*AP2-like* transcripts in both induced control plants and AVG-treated plants compared to non-induced plants. Our results suggest that Pn*AP2-like* may act as a repressor of flowering, and endogenous ethylene may promote flowering of *P. nil* through direct or indirect effects on *PnAP2-like* expression.

This work was completed as part of the Science Collaborative Research Program and supported with generous funding from the Research Corporation, the National Science Foundation and the M.J. Murdock Charitable Trust.

Paul Gaudin. Analysis of Polyamine Levels in the Guard Cell Protoplasts of *Nicotiana Glauca.*

(Advisor: Gary Tallman, Biology)

In my 2011 summer research I practiced and focused on developing a procedure to analyze polyamine levels in guard cell protoplasts (GCPs) of *Nicotiana glauca*. Procedural aspects included cultivating the GCPs, extracting polyamines from the GCPs, and analyzing the polyamines against a generated standard curve of benzoylated polyamines through high performance liquid chromatography (HPLC). Polyamines that will be analyzed include: spermine, spermidine, putrescine, agmatine, and L-arginine. Future experimentation will include assessments of polyamine levels in GCPs exposed to high temperatures or to polyamine-specific degradation enzymes.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the National Science Foundation.