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INTRODUCTION

This volume presents the research abstracts of Simons Fellows working at Stony Brook University during the summer of 2009. These abstracts will also be posted on the Simons Program Website: <http://stonybrook.edu/simons/>

Established in 1984, the Simons Summer Research Program enables academically talented high school students who have completed their junior year to come to Stony Brook University for a summer to engage in scientific research and experience life at a research university. Simons Fellows are given the opportunity to work with distinguished faculty mentors, learn laboratory techniques and tools, and become part of active research teams. All Simons Fellows have been nominated by their high schools, and have undergone a competitive selection process.

Take a look at what the students have accomplished in only seven weeks. You'll be impressed by what they have done, and what they have learned by working within a community of faculty, post docs, graduate students and undergraduates. We wish all the Simons Fellows well, and anticipate that these students will have great success with their future careers and future research endeavors.

We are grateful to all the Stony Brook University faculty research mentors, as well as the graduate students and post docs and lab colleagues who served as co-mentors, for devoting their time, energy and resources to the Simons Fellows. And we especially thank Dr. James Simons, Marilyn Simons and the Simons Foundation for their generous and ongoing support of this program.

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-Karen Kernan
Director, Programs for Research & Creative Activity
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Mario Albujar
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The activation of the Wnt Signal Pathway in regenerating *Nematostella vectensis*

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The Wnt signaling pathway, a pathway that, among other activities, regulates stem cell differentiation, has been known to be active in gastrulation of the cnidarian *Nematostella vectensis*, among other species, as well as in regeneration of another cnidarian, the Hydra. We used different known chemical activators of the Wnt pathway, 5-iodo-indirubin-3'-monoxime and Alsterpaullone, to "manually" activate the Wnt pathway in regenerating *Nematostella vectensis* and observed the morphological differences in a normally regenerating animal, as opposed to one regenerating in a Wnt pathway chemical activator. We have found that certain concentrations of both the 5-iodo-indirubin-3'-monoxime and the Alsterpaullone altered the regeneration of the *Nematostella vectensis*, as determined by regenerating tentacle morphology. With these results, we have concluded that proper activity of the Wnt pathway is required for normal regeneration in *Nematostella vectensis*. In the future, we plan to perform *in situ* hybridizations and antibody staining in order to discover more about the Wnt pathway's role in *Nematostella vectensis* regeneration, in greater detail.

Dylan Assael
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Mentor: Dr. Stephen Baines,
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The role of *Prochlorococcus marinus* and *Synechococcus marinus* in the ocean's silicate cycle

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Variations in the Si cycle have been linked to past and future climate change because Si is required by diatoms, which account for 50-70% of the CO₂ fixation in the ocean and 20% of that fixed on earth. The silica shells of diatoms also promote loss of CO₂ fixed by photosynthesis from the surface to the deep sea. Diatoms are presumed to account for most of the particulate Si in the ocean, but field data from the Eastern Equatorial Pacific (EEP) shows that Si in diatoms only amount to 20-30% of the total Si in particles. Single cell measurements indicate that small (0.2-2.0 μm) picocyanobacteria collected from the same region contain significant amounts of Si. Moreover additions of the Si analog Ge that were intended to inhibit Si uptake by diatoms also caused declines in the populations of the two dominant picocyanobacteria present, *Prochlorococcus marinus* and *Synechococcus marinus*. These two genera are the most abundant organisms on earth, and account for >75% of the photosynthetic biomass in the open ocean where productivity is low. Consequently, they exert substantial influence over the cycling of material in the ocean. Their role in the Si cycle and the importance of Si in their biology are completely unknown.

Cultures of each type of bacteria were grown in saltwater media with variable levels of silicic acid. Counts were conducted daily for comparisons of growth rates by either the use of a Coulter Counter (for *Synechococcus*) or epifluorescence microscopy (for *Prochlorococcus*). Silica dissolution in hot NaOH combined with a spectrophotometric analysis after complexation of Si(OH)₄ by acid molybdate gave an accurate representation of the cellular Si content for each treatment and species. Si content in the water was also measured using the acid molybdate method and compared to cellular Si content. These measurements were compared to x-ray fluorescence measurements of Si in individual cells collected from the EEP and to measurements of silica within particles from the same region. The cyanobacteria seemed to take up silicon readily as Si concentrations within the cells were 600 to 2000 times greater than the ambient silicic acid. Thus, the Si is not simply passively diffusing into the cells, but is being accumulated by the cyanobacteria. In addition, growth rates of *Synechococcus* increased with increased silicate concentrations. Based on the concentrations of Si found in the *Synechococcus* cells and the average population density of *Synechococcus* in the EEP, approximately 28% of the EEP's biogenic silica exists in *Synechococcus*.

This work was supported by the Simons Foundation.

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The potential Factor X: The effects of manipulating *E2f* gene activity on *sloppy paired 1* expression in *Drosophila* embryos

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The DNA-binding transcription factors encoded by the pair-rule segmentation genes play a significant role in establishing the segmented body plan in *Drosophila melanogaster*. The *sloppy paired-1* (*slp1*) gene serves as a reliable model for studying the development of both odd- and even- numbered stripes in response to pair-rule transcription factors during the blastoderm stage of embryogenesis. However, the factor or combination of factors involved in establishing the even-numbered stripes in the posterior cells of each parasegment remains unidentified. We analyzed the minimal proximal early stripe element (PESE C1+) that expresses even-numbered *slp1* stripes to determine if *E2f* is in fact the unknown Factor X responsible for this pattern. Preliminary results indicate that reporter gene expression is altered in *E2f* mutant embryos, although interestingly expression of endogenous *slp1* is only slightly perturbed. Conversely, complementary experiments indicate that ectopic expression of *E2f* has no effect on the expression of this reporter. Further studies will be needed to clarify the role of *E2f* in regulating PESE C1+ and the endogenous *slp1* gene.

Jason Chaves
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Inaccuracies in the single nucleus approximation due to the presence of light nuclei

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In the research of hot, dense matter, thermodynamic considerations must be made. The Equation of State (EOS) for hot, dense matter determines the thermodynamic properties and processes in such systems. Some models for the EOS, along with other components of hot, dense matter research is built upon the Single Nucleus Approximation (SNA), which represents the matter as an ensemble of neutrons, protons, alpha particles, and a single representative heavy nucleus. The use of a single representative heavy nucleus has been shown to not create any significant inaccuracies in bulk thermodynamic properties, as compared to retaining an ensemble of multiple nuclei. However, there is a possibility that the presence of several different light nuclei can be inaccurately represented by the light nuclei representative, the alpha particle, and could lead to significant errors in calculations employing the SNA. Correction of such errors could greatly improve the accuracy of supernova and neutron star calculations that are greatly dependent on the composition of matter, e.g. electron capture rates and neutrino emissions. Under nuclear statistical equilibrium, matter is treated as near-ideal, and the number density and abundance can easily be determined by minimizing the total free energy density subject to mass and charge conservation constraints. In this project we calculate the abundance of 326 total nuclei, $3 \leq Z \leq 20$, under conditions of $10^{-6} n_0 \leq n \leq 10^{-3} n_0$, $0.3 \leq T \leq 5$ MeV, and $0.01 \leq Y_e \leq 0.5$. We also evaluate the significance of these abundances and present a proposed modification to the SNA in order to account for these light nuclei. Additionally, we find that there are certain regions in n-T- Y_e space where the SNA is invalid. Although these regions tend to represent conditions that don't frequently occur naturally in the context of astrophysical simulation, the existence of such regions should be documented as we have done.

This work has been supported by the Simons Foundation.

Shivrat Chhabra
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Detection of antibiotic resistant bacteria using nanoliter droplets

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Sepsis, a whole-body inflammatory state, is the result of system inflammatory response syndrome and the tenth-leading cause of death in the United States. Today, most deaths caused by sepsis are the result of infection by bacteria with multiple antibiotic resistances. Diagnosis of such infections is often delayed by a twenty-four to seventy-two hour time frame to conduct blood cultures.

For this reason, a device to rapidly identify the antibiotic resistances displayed by bacterial pathogens is urgently needed. Since antibiotic resistances are usually conferred by specific resistance genes, our device, to be maximally effective, would have to be able to detect the presence of these genes in individual bacterial cells. The purpose of this experiment was to create a model for such a device.

Working with a laboratory strain of *Escherichia coli*, we developed a system to amplify the gene for subunit A of DNA gyrase (*gyrA*) in nanoliter-sized aqueous droplets in oil, with each droplet containing one *E. coli* genome. We first lysed the bacteria using a combination of Triton X-100, Tris, EDTA, sodium acetate, and lysozyme. Upon lysis, we purified the DNA using phenol-chloroform extraction, followed by ethanol precipitation. We adjusted the concentration of DNA to obtain one bacterial genome per droplet, which, according to Poisson distribution, would result in the maximum number of droplets (37%) created containing a single genome. We also added PCR reagents, including primers for the *gyrA* gene and Sybr Green I for detection of amplification, to the solution prior to droplet formation. We formed droplets on a PDMS laboratory chip, and collected them to undergo PCR. We have so far observed amplification of the *gyrA* gene outside of the droplets, and expect to observe it shortly in the droplets formed.

This work was supported by the Simons Foundation, the National Science Foundation, and the United States Department of Energy.

Stephanie Chiang
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Characterization of a cyanobacterial bloom in Lake Agawam, NY

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Atmospheric aerosols impact the Earth's albedo and consequently major global climatic processes. They are considered to be important sources for cloud condensation nuclei (CCN) and ice nuclei (IN) which are essential for cloud formation and ice nucleation and consequently precipitation. Biological particles, such as microorganisms, are effective substrates for ice nucleation. Aerosols form across the world's bodies of water when air bubbles trapped underwater by breaking waves burst at the surface microlayer and release organic and inorganic particles including phytoplankton, bacteria, and debris. In the eutrophic waters of Lake Agawam, Southampton, New York, the cyanobacterium *Microcystis* sp. forms dense blooms of mucilage bound colonies in which bacteria and other particles are trapped. These *Microcystis* sp. cells have the potential to be aerosolized. The purpose of this study was to characterize particles aerosolized from *Microcystis*-dominated lake water through field collections and laboratory microcosm experiments. Particles were classified and enumerated by epifluorescence microscopy after staining. Both *Microcystis* cells and bacteria were aerosolized at an increasing rate in the laboratory microcosms as the blooms senesced. The presence of these aerosolized cells suggests that cyanobacterial blooms can provide potential sources for CCN and IN, critical for cloud formation.

This work was supported by the Simons Foundation, NSF Foundation, NOAA Atmosphere and Climate Program.

Demitri Dedousis
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The effect of gene expression levels on gene expression

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Several diseases are caused by abnormalities in the level of gene expression -- the rate that DNA is transcribed into RNA so that it can be translated into protein. By systematically altering gene expression in yeast, we can try to understand the quantitative relationship between gene expression and cellular pathologies. In the strain Th4927, the tet-off promoter regulates Lcb2, which is involved in cell membrane sphingolipid synthesis. The addition of doxycycline, a derivative of tetracycline, lowers the expression level of Lcb2. In our experiments, Th4927 competed against 41a, a green fluorescent reference strain not sensitive to doxycycline, at increasing doxycycline concentrations. As a control, R1158, the parental strain of Th4927 but without the tet-off promoter, was separately competed against 41a. Competitions were performed at concentrations of doxycycline between 0-3 μ g/ml. When the expression of Lcb2 is lowered with just a small amount of drug (0.5 μ g/ml), the strain grew at least 50% slower. However, the growth defect is progressively alleviated at drug concentrations above 2.0 μ g/ml, which suggests a complex relationship between the transcription of Lcb2 and cell growth. This provides a model for studying the tolerance of gene expression to perturbations and the evolutionary forces that have shaped promoter function.

Valentine Esposito
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Cellular *in vitro* evaluation of anti-oxidant reductant capabilities in MM6 cells: a preventative approach to reactive oxygen species

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Oxidative stress, which is induced by Reactive Oxygen Species (ROS), has recently been linked to an innumerable amount of diseases and ailments. Anti-Oxidants display reductant capabilities and therefore may also have the ability to deter ROS, potentially minimizing oxidative stress. This study was designed to determine the ability of an array of Anti-Oxidants to function in a human cellular *in vitro* model and prevent the escalation of ROS in addition to measuring their toxicity. First, different substances were tested as potential Anti-Oxidants by using an assay to determine the uric acid and copper reducing equivalents of said substances given that both uric acid and copper are established Anti-Oxidants. Once a variety of Anti-Oxidants were identified, a methodology was developed to measure the Anti-Oxidants' ability to prevent the production of ROS. A variety of concentrations of PMA, pyocyanin, and cell treatments were tested to determine which combination produced optimal levels of ROS in MM6 cells. Cells treated over night in serum free media and LPS and were additionally stimulated during the assay with 0.2mM pyocyanin produced sufficient ROS levels; this condition was established as the positive control for this experiment. Different concentrations of Anti-Oxidants were applied to cells under both positive and negative control conditions and DCFH, a reagent that fluoresces in the presence of ROS, was also applied. Plates with DCFH were read on a CytoFluor 2300 machine to measure the progression of ROS over the course of 90 minutes. Data from each concentration of Anti-Oxidant was compared to the positive control to determine if said Anti-Oxidant was able to suppress ROS production. Trypan Blue was applied to the plates at the close of each experiment in order to measure cell death and toxicity that the Anti-Oxidants may be accountable for. MTS was originally used to measure toxicity, however in this assay there was significant interference with its signal. Out of the Anti-Oxidants that have been tested thus far, Rosehip, Clove Extract, Pomegranate, Acai Extract, Golden Root Extract, and Clove *S. Aromaticum* have proven most promising as mediators of ROS. These Anti-Oxidants have displayed ability to reduce ROS levels in human cellular *in vitro* models by approximately 50% at only a 0.5 stock concentration. Future studies will not only identify additional Anti-Oxidants and measure their ability as preventive agents of ROS progression at various concentrations, but will also explore their potential as therapeutic agents.

This work was supported by a grant from the Simons Foundation.

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Department of Biomedical Engineering

An investigation into the physiological response to multiple exposures of hindlimb unloading in C57BL/6J Mice

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Bone loss occurs during exposure to microgravity with astronauts losing 1%-2% of their bone mass per month in space. Due to small sample sizes and the presence of confounding factors, current data from studies on astronauts do not provide enough information on the effects of microgravity on the musculoskeletal system. Nevertheless, a comprehensive risk assessment is required if astronauts are to continue being sent on multiple space missions. The objective of this experiment was to investigate the local and systemic effects of microgravity on bone. A rodent hindlimb unloading model was used to simulate microgravity in C57BL/6J (B6) mice, which have been previously shown to respond to hindlimb unloading (HLU) and reambulation (RA). Forty-four adult (4mo) male B6 mice were assigned to age-matched control and disuse groups subject to one, two, or three (n=11/group) HLU cycles. During each cycle, mice underwent tail suspension continuously for 2wk followed by 4wk of RA. Animals were sacrificed at 34wk and mandibles were removed and stored in 70% ethanol. Prior to scanning, the right side was isolated. *Ex vivo* scans (Scanco, micro-CT 40) of the first molar region were taken at 10 micron resolution. A 3D evaluation script provided data on bone volume (BV) and bone mineral density (BMD); similar data was mined from *ex vivo* scans of the two mouse femurs at the mid-diaphysis. There was no significant difference in BV or BMD among HLU groups in both the mandible and femur. The changes in BV and BMD in the femurs was primarily age related, the deterioration of the tissue was compounded by HLU, but in a statistically insignificant manner. The BV and the BMD of the mandibles and femurs was correlated with each other. The correlation was found to be statistically insignificant leading to the conclusion that there was no systemic effect due to HLU at the end of the 18wks. This claim supports the idea that astronauts may undergo multiple missions to the International Space Station if there is sufficient time in between missions to allow for recovery and prevent the harmful compounding effects from prior exposures.

Reena Glaser
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Substrate induced osteoblast-like differentiation of stromal stem cells

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Typical procedures for growing bone grafts often lead to complications such as immunorejection, a long operation period, and excess costs. This study focused on the potential of Adipose-derived Stem Cells (ASCs) to differentiate along an osteogenic lineage, providing an autologous cell source that involves a non-invasive harvesting procedure. We have demonstrated that these cells can be induced to biomineralize and form calcium phosphate fossilization purely by plating the cells on a polybutadiene (PB) substrate created by spincasting. In contrast to ASCs that were grown on tissue culture plastic, ASCs grown on PB generated calcium phosphate deposits within 7 days in the absence of added dexamethasone. In addition, Confocal microscopy showed that the cells continued to proliferate and retain viability while biomineralizing. The most calcium phosphate crystallization was exhibited on the thin PB surface in a non-induced media as seen on the SEM/EDAX. Concurrently, the control setup—where the cells were plated on tissue culture plastic—did not produce any deposition. Moreover, biomineralization did not occur on the plastic surface in the absence of dexamethasone. In order to determine whether the induction was permanent or transient, we removed the cells exposed to polybutadiene after 14 and 28-day incubation periods, and then cultured the cells for another five days on plastic. These “educated” cells continued to form calcium phosphate mineralization on the plastic substrate, thus proving them to have become stably differentiated. ASCs were then grown on thin and thick PB fibers, where the moduli was unaffected by the thickness of the fibers. Nonetheless, there was an inverse relationship between the ASC modulus and the dynamic modulus of the PB surface; thus, demonstrating the ability of the cells to sense the mechanics of the substrate on which they were plated. Confocal imaging displayed cell growth along the fibers as calcium phosphate deposits formed. Further data is being collected using RT-PCR and immunohistochemistry for RNA and protein expression, X-ray Scattering microscopy, and continued use of the AFM, Confocal, and SEM/EDAX. Preliminary experiments revealed that *runx2* expression increased only in cultures exposed to dexamethasone and that its expression did not correlate with the increased calcium deposition promoted by ASC on PB. This suggests alternative mechanisms to those regulating biomineralization. Due to our positive findings regarding ASCs and their ability to promote calcium phosphate deposition, these easily isolated cells may be considered for development of adjunct therapy for the production of safe autologous bone grafts.

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Nataliya Gorbachuk

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The examination of the binding between gC1qR and Hyaluronic acid

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Hyaluronic acid, also known as Hyaluronan, is a non-sulfated member of the GAG (glycosaminoglycan) family of extracellular matrix components. It is ubiquitously distributed and abundantly expressed throughout the epithelial, connective, and neural tissues. Hyaluronic acid has been shown to play an important role in inflammation, cell migration and proliferation, reepithelization, and fetal wound healing and scarring. As a result of some of these roles, Hyaluronic acid is an important component of tumor cells. Therefore, while healthy individuals have a certain concentration of HA in plasma, individuals with particular diseases have elevated concentrations of this protein. An assay that would measure concentration of HA in body fluids using Hyaluronic acid binding protein (HABP) can help identify the condition of a patient's health. It has been shown that some of the HABPs of HA are CD44, RHAMM (Receptor for HA Mediated Motility), and ICAM-1 (Intracellular adhesion molecule-1). Furthermore, there is an assay on the market that uses HABP to identify concentration of HA. However, which HABP is used remains unknown, which questions the accuracy of such an assay.

On the other hand, gC1qR is a protein that binds globular heads of C1q and therefore, plays an important role in the complement system. This protein also binds high molecular weight kininogen (HK), and pathogen associated antigens. Its over-expression in tumor cells may suggest that it is an important factor in cancer development.

It has been reported before that HA is one of the ligands of gC1qR and therefore, gC1qR is a Hyaluronic acid binding protein (HABP). Based on this report, since gC1qR is a HABP, it should be possible to develop an assay that would detect concentrations of HA where gC1qR would be used as the HABP. However, using ELISA-based assay, we have shown that gC1qR has little interaction with HA. At first, we coated 50 μ l of gC1qR to the plate in carbonate buffer at concentration of 5 μ g/ml. Then we blocked it with 400 μ l of 1% BSA and then added 50 μ l of biotinylated HA that was put in different buffer solutions at either 5 μ g/ml or 10 μ g/ml. Then we added 50 μ l of alkaline-phosphatase neutravidin, and then added 50 μ l of pNPP. Between all these steps, the wells were washed 3x using TBST and all experiments were repeated at least 3 times in duplicates. This ELISA came out to be negative, showing no interaction between HA and gC1qR under different conditions. Then an ELISA was performed using different concentrations of biotinylated HA ranging from 0 to 5 μ g/ml (gC1qR coated to the plate at 5 μ g/ml) and of gC1qR ranging from 0 to 1.5 μ g/ml (HA coated to the plate at 5 μ g/ml). In all the experiments described above, the results showed no interaction between gC1qR and HA. Therefore, gC1qR is not a HABP. These results suggest that even though it has been shown that both gC1qR and HA are up-regulated in tumor cells these proteins play separate roles from each other in cancer development. However, it is possible that the results obtained may be due to the lack of HA nature. We will perform further experiments with new HA that will show whether the obtained data is accurate.

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Eddie Grove

Paul D. Schreiber (Port Washington) HS (NY)

Mentor: Dr. Bassem Allam,

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The expression of lectins in oyster larvae and its implications for oyster nutrition

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The goal of this research is to search for the presence and location of lectins in the mucocytes of oyster larvae at various stages of development. Lectins are known to have a role in recognition of non-self, much as antibodies do in higher animals. Lectins thus help the hemocytes of oysters recognize pathogens to engulf, and recent studies suggest that they help the oysters recognize which food particles to select for (Pales Espinosa et. al., 2009). If lectins are first expressed in oyster larvae around the same time that particle selection first occurs, i.e., in 10-15 day old larvae (Baldwin, 1995), this would suggest that lectins play an important role in particle selection in oyster larvae. This experiment makes use of a procedure known as *in situ* hybridization (ISH), which utilizes an antisense RNA probe that has the specific sequence of lectins in the mucocytes of the oyster. The probe hybridizes with complementary strands of mRNA that code for lectin synthesis. A sense probe, which will not hybridize with the lectin sequence is used as a control. An antibody is then used to attach to the RNA probe and substrates are used to identify the antibody. Following treatment with a counterstain, mucocytes that express the lectin sequence are visible under a light microscope. Antisense slides were examined under the light microscope for the purpose of localizing the positive signal, and they were compared with sense slides. The antisense slides were also examined side by side with H&E-stained slides, in which it is easy to identify mucocytes. Strong evidence for the positive signal was found in 40 day larvae. However, strong evidence for the presence of lectins in younger larval stages has not been found, as positive signal in the younger stages was not seen to come from specific cells, and as few, if any, mucocytes were found in these larval stages using H&E slides. The positive signal in stages younger than 40 days has, thus far been determined to be the result of nonspecific binding. If lectins are first expressed at the 40 day stage, this suggests that some other compound allows oyster larvae to select for particles at younger stages.

This work was supported by the Simons Foundation, the National Science Foundation, and the New York State Department of Environmental Conservation.

Jessica Hsu
Monta Vista HS (NY)

Mentor: Dr. Balaji Sitharaman,
Department of Biomedical Engineering

Photoluminescence of europium catalyzed single-walled carbon nanotubes for biomedical imaging

Jessica W. Hsu, *Monta Vista High School, Cupertino, CA*; Magdalena Swierczewska, Balaji Sitharaman, *Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY*

Single-walled carbon nanotubes (SWCNTs), with their various intrinsic optical properties, have been used as photoluminescent, Raman, and photoacoustic contrast agents for the imaging of cells and animals.¹ The SWCNTs show high optical absorbance spanning the ultraviolet to near infrared spectrum, have conjugated carbon bonds, and allow the encapsulation of metal ions widely used in medicine. In this work, we have synthesized and characterized single-walled carbon nanotube-based visible optical probes. These probes consist of europium (Eu) metal nanoparticles encapsulated within SWCNTs.² Eu's long-lived luminescence makes it a valuable tool for luminescent-based bioanalytical assays.³ However, Eu and lanthanoids in general have small extinction coefficients and weak optical absorbances ($1 \text{ M}^{-1} \text{ cm}^{-1}$, 100 fold smaller than those of conventional organic dyes). Thus, the SWCNTs serve as antennae or sensitizers to absorb the excitation light and transfer this energy to the Eu ions. The SWCNTs also serve several other purposes, which include sequestering the toxicity of the metal particles, shielding the Eu ions from the quenching effects of water, and acting as scaffolds for the attachment of biomolecules.

The characterization of the Eu-SWCNTs reveals that they exhibit bright luminescence in the visible region (excitation wavelength 380-415 nm, emission wavelength 619 nm) and show excellent photoluminescence for use as optical imaging probes. Spectral characteristics of the Eu-SWCNTs are similar to those of lanthanoid chelates. The Eu-SWCNTs have long fluorescent lifetimes (sub-microsecond to millisecond range), sharply spiked emission spectra (<10 nm bandwidths), large Stokes shifts (>150 nm), and high quantum yields (~1). For applications in biological systems where background autofluorescence poses a problem, Eu-SWCNTs, with their long-lived luminescence, are advantageous over organic dyes and can be used as donors in resonance energy transfer experiments and as structural and functional probes. Eu-SWCNTs combine the benefits of the varied functionality of carbon nanotubes and the distinctive luminescence of europium to yield a novel optical probe.

This work was supported by the Simons Foundation and the Carol M. Baldwin Breast Cancer Fund.

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Pragya Kakani
Jericho HS (NY)

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Department of Neurobiology & Behavior*

The use of Fluorescence Resonance Energy Transfer (FRET) to observe apoptosis in glial cells

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Oligodendrocyte death plays a key role in the progression of multiple sclerosis as well as spinal cord injury. However, currently it remains unclear whether the mechanism guiding their death is apoptotic or necrotic. The purpose of this study is to test the ability of fluorescence resonance energy transfer (FRET) to detect oligodendrocyte apoptosis and thus potentially be used in application to such questions. CFP-DEVD-YFP fusion protein expression vectors in pcDNA3.1, cleavable by caspase-3 during apoptosis, were cloned in DH5-alpha E.coli cells. Extracted pCFP-DEVD-YFP plasmids were then used to transfect human embryonic kidney (HEK) 293 cells, PC12 cells, and mixed glial cultures containing oligodendrocytes. Apoptosis was induced through the use of 1 μ M staurosporine and cells were then observed 2 hrs, 3hrs, and 4 hrs through confocal microscopy. After being treated with staurosporine, YFP excitement of CFP decreased in glial culture suggesting that apoptosis was successfully detected through FRET. This finding may provide a basis for developing a ROSA26 reporter mouse to visualize oligodendrocyte apoptosis *in vivo* using non-invasive techniques as well as a new way to observe oligodendrocyte apoptosis *in vitro*. These techniques may be applied to address unanswered questions regarding multiple sclerosis and spinal cord injury with regards to oligodendrocyte apoptosis.

Jenny Kang
Belmont HS (MA)

Mentors: Drs. Yixian Qin and Wei Lin,
Department of Biomedical Engineering

Activation of the NMDA receptor in osteoblasts by low intensity pulsed ultrasound

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The dynamic equilibrium between bone resorption and formation is required for maintaining a healthy level of bone mass. An imbalance in these processes causes osteoporosis, a serious disease characterized by bone degeneration and susceptibility to fracture that currently afflicts 10 million Americans.

Scientists have found that bone cells can use mechanotransduction as a means of cell communication. With mechanotransduction, a mechanical stimulus (rather than a chemical ligand) induces a specific chemical signal to the nucleus. It is clear that mechanical loading generally helps bone formation—a lower force of gravity in spaceflight or less physical activity due to old age both cause overall deterioration. However, the exact molecular mechanism of this phenomenon is yet unknown. Medical researchers have tried *in vivo* therapies such as electromagnetic fields and ultrasound in order to stimulate bone growth.

The purpose of this study is to observe evaluate the effects of the Low Intensity Pulsed Ultrasound (LIPUS) on the activation of the N-methyl-D-aspartate receptor (NMDAR). NMDAR has a central role as a cation channel receptor in the depolarization of neurons. It was recently found on osteoblast cells, raising questions to its purpose on bone cells. Observing RNA transcription levels of c-FOS and c-JUN, genes that allow osteoblast differentiation, will show and quantify cellular activity.

For the first phase of the experiment, an optimal time for applying LIPUS was found. RNA transcription levels of c-FOS and c-JUN were highest at 20 minutes, showing it was the optimal treatment period for the MC3T3-E1 cell line.

The second phase of the experiment used this optimal time for treatment on all the groups. The goal was to delineate the cause of high transcription levels—in other words, whether the source of transcription was the presence of the chemical ligand, NMDA, or mechanical stimulus, LIPUS. Groups were as follows: pretreatment of NMDA and MK801 (an inhibitor of the receptor), pretreatment of NMDA, pretreatment of MK801, and no pretreatment. LIPUS was applied to all of these groups.

The expected results of this study are that cells pretreated with NMDA but not MK801 will show the most favorable results.

Joshua Kantharia
Farmingdale Senior HS (NY)

*Mentor: Dr. Robert Haltiwanger,
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How does glycosylation effect Notch binding to ligands?

Joshua Kantharia, Farmingdale Senior High School, Farmingdale, NY; Hideyuki Takeuchi and Robert Haltiwanger, Department of Biochemistry & Cell Biology, Stony Brook University, Stony Brook, NY

The Notch Signaling Pathway plays an important role in cell-cell signaling and differentiation. The Notch protein itself relays intercellular signals through its extracellular domain which is composed of 36 tandem epidermal growth factor-like (EGF) repeats, with EGFs 10 through 13 known to be critical to ligand binding via previous studies. EGF repeats can be glycosylated on serine or threonine residues with fucose and glucose. Glycosylation has been shown to modulate Notch's response to signals, but the molecular mechanisms for how glycosylation of EGF repeats affects Notch function is unknown.

The current study seeks to understand which EGF repeats are most critical to the binding of Notch to its major ligands, and how glycosylation of the EGF repeats affects binding. It is hypothesized that glycosylation of the EGF repeats will alter binding affinity. *Drosophila* Notch DNA encoding for this protein, the original Notch proteins identified, was used to create small fragments of its EGF repeats specifically EGFs 12, 11-12, 11-13, and 10-13. Using Mass Spectrometry analysis and other approaches, we will confirm creation of four EGF fragments, each with a different number of EGF repeats. Following glycosylation, binding assays will determine binding ability of the fragments.

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Brian Kelly
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*Mentor: Dr. Wolfgang Quitschke,
Department of Psychiatry*

The comparative effect of curcumin and curcumin sulfate

Brian Kelly, *Oceanside High School, Oceanside, NY*; Wolfgang Quitschke, *Department of Psychiatry, Stony Brook University, Stony Brook, NY*

Although metabolism abrogates the biological activity of most compounds, a small minority, such as the non-steroidal anti-inflammatory drug sulindac, becomes more biologically active. Curcumin, the yellow pigment of the spice turmeric that has been found to possess anti-inflammatory and anti-carcinogenic properties, is found in rat and human plasma primarily as the metabolites curcumin sulfate and curcumin glucuronide. Although there have been various attempts to increase the systemic bioavailability of curcumin, very few studies have investigated the biological activity of its metabolites. Thus, the aim of this study was to determine the comparative effect of curcumin and curcumin sulfate on cancer. Curcumin mixture of monosulfate and disulfate were synthesized according to a previously established method. While only two hours with ethyl acetate for several hours creates equal ratios of the monosulfate and disulfate, four hour incubation causes the disulfate to become over two times more abundant. Curcumin sulfate, even at 200 μM , only appears to stymie the growth of the astrocytoma cell line CRL-1718 while curcumin at 20 μM readily kills the entire population. In conclusion, curcumin is much more biologically active than its metabolite curcumin sulfate. Although one should be hesitant about making any conclusion concerning potential therapies without evaluating the other metabolites, the results provide preliminary evidence for inhibiting curcumin metabolism and finding ways to increase systemic bioavailability. Future research is necessary to determine the biological activity of curcumin glucuronide and other metabolites that may be present in vivo, and test for possible synergistic effects between curcumin and the metabolites.

Sahil Khetpal
Texas Academy of Math & Science (TX)

Mentor: Dr. Iwao Ojima,
Department of Chemistry

Polyfunctionalized single-walled carbon nanotubes as a versatile platform for cancer detection and tumor-targeted dual therapy

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Despite the progress that has been made towards improving survival rates, cancer continues to be the second most devastating disease in the United States, taking nearly half a million lives each year. Consequently, it is essential to develop efficient methods for the early detection and treatment of cancer. In this research, single-walled carbon nanotubes (SWNTs) with their versatile physiochemical features have been explored as an efficient “theragnostic” platform for multimodal molecular imaging and simultaneous therapy.

The first aim of this project is an *in vitro* study that investigates the use of induced hyperthermia for the thermal ablation of cancer cells. Carbon nanotubes have been shown to perform as transducers, converting electromagnetic energy to heat in a radiofrequency field. Consequently, the designed conjugate contains a tumor-targeting module (biotin) and fluorescein to track the fate of the single-walled carbon nanotube using confocal fluorescence microscopy (CFM).

The second aim of this research study involves the use of functionalized SWNTs conjugated to the anticancer agent, a self-immolative disulfide linker, biotin, and technetium to monitor the fate of the carbon nanotube *in vivo*. After the SWNT is internalized, the drug will be released and then hyperthermia will be induced. This dual therapy has immense potential in the combat against cancer. Since the possible toxicity of carbon nanotubes in the body is a medical concern, the detection of the fate of carbon nanotubes is an important aspect of this research, which can be monitored using positron emission tomography (PET).

The final aim of this research involves the use of gadolinium and europium encapsulated within single-walled carbon nanotubes (M-SWNTs, where M = metal atoms) to be combined with the dual therapy based a carbon nanotube conjugate. This special nanotube can be used for molecular imaging to detect cancer at an early stage, and the dual therapy mechanism can be efficiently used to combat cancer.

Erica Lai
Holmdel HS (NJ)

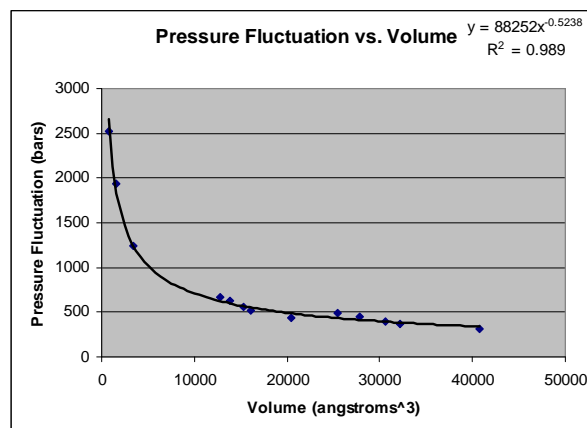
Mentor: Dr. Marivi Fernandez-Serra,
Department of Physics & Astronomy

Density and size effects in liquid water: the formation of nanobubbles, source of water anomalies

Erica Lai, Holmdel High School, Holmdel, NJ; Adrien Poissier, Jue Wang, Marivi Fernandez-Serra, Department of Physics and Astronomy, Stony Brook University

The purpose of this research project is to grasp a deeper understanding of liquid water and its unique properties. Specifically we have analyzed the liquid structure of water as a function of the system size and its pressure from a theoretical and computational approach. We perform molecular dynamics simulations of water molecules in a cubic box. We study five different pressures and five systems with different number of water molecules, from 32 up to 1024, accounting for a total of twenty-five different water systems. From these simulations, we analyze the radial distribution functions, diffusion graphs, orientational order parameter histograms, and graphs of hydrogen bond average coordination.

A plethora of information has been derived from this study, both confirmations of water anomalies under pressure and new discoveries. We conclude that 32- and 64-molecule water systems evidently are not good representations of liquid water; this has implications for theoretical studies where quantum-mechanical interactions between electrons in water are accounted for because they are always done for systems of these sizes. Pressure fluctuation in a water system is approximately inversely proportional to the square root of volume. As the density increases, interactions between water molecules are not relevant for molecules that are not first neighbors. Diffusivity increases with an increase in the number of molecules in a system, and diffusivity also increases with pressure in a localized region of the phase space. This allows us to speculate that liquid water is comprised of two different structures: tetrahedral and defective. The tetrahedral liquid is more prominent at room temperature and atmospheric pressure, but as the density increases the defective liquid starts to dominate; thus more molecules that do not have four hydrogen bonds appear. The novel aspect of this study is the new perspective for exploring water's structure. Instead of looking at the water molecules themselves, the areas lacking molecules were analyzed and coined "nanobubbles". Using this original approach, it was discovered that in the anomalies region, more bubbles emerged with increased pressure. Thus, more pressure results in more defects, more diffusion, and more bubbles. This work was supported by the Simons Foundation.



James Lauria
Northport HS (NY)

*Mentor: Dr. Mary Kritzer,
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The effect of androgen on mRNA and protein levels of NMDA receptor subunits in identified neurons of rat prefrontal cortex

James Lauria, Northport High School, Northport, NY; Conrad Tenenbaum, Fikri Birey, Mary Kritzer, Department of Neurobiology & Behavior, Stony Brook University, Stony Brook, NY

This research explores the glutamatergic influences on the dopamine system in response to alterations in steady state gonadal hormone levels in the Prefrontal Cortex (PFC) of rats. The PFC is important for cognition, memory, and executive function and operates normally only when it has correct levels of the neurotransmitter dopamine. Gonadal hormones like androgen are known to disrupt PFC function by abnormally elevating dopamine levels. My work investigates one possible mechanism of this deregulation; that essential dopamine circuits of the PFC are disrupted by hormone effects on the dopamine-regulating neurotransmitter glutamate. If this hypothesis is true, I would predict that the levels of glutamate receptors within the PFC are changed by hormone imbalance. To test this prediction I use in situ hybridization to determine the levels of mRNA, which transcribe glutamate NMDA receptor subunits and immunocytochemistry to determine the translation of these receptor proteins. First, this project involves preparation of the probes needed to conduct in situ hybridization. Riboprobes for each glutamate receptor subunit in the study will be synthesized based on published sequences of receptor encoding DNA. Thus far, I have produced probes for the NR1 and NR2C subunits and am testing them in tissue sections cut from the PFC of the rat. The NR1 probe is working correctly. I am also designing and preparing probes for the other glutamate receptor subunits, NR2A and NR2B. These are subunits that make up the NMDA glutamate receptors, which are localized on inhibitory interneurons in PFC. The second part of this project involves immunocytochemistry for the same NMDA subunits. This technique requires the use of antibody labels to quantify protein levels; in my case, the subunits NR1, NR2A, B, and C. To quantify hormones effects, immunocytochemistry and in situ hybridization will ultimately be examined in 3 groups of animals. Comparisons of Gonadectomized (GDX) rats, GDX rats with androgen, and a "sham" (control) group with intact hormone levels, will show if the presence of hormones has a quantifiable effect on the receptor protein or mRNA levels. I expect to show specifically that the lack of hormones in gonadectomized animals lowers the presence of glutamate receptors which causes the observed the increase of dopamine levels. Higher dopamine levels have been found in many cases of schizophrenia, autism and ADHD and these problems have also been correlated to low testosterone levels found 4 to 5 times more frequently in males. Therefore, by knowing the function of these hormones in relation to the dopamine system, a better treatment for such disorders may be produced. This work was supported by the Simons Foundation

Nicole Lee
Roseville Area HS (MN)

Mentor: Dr. Lilianne Mujica-Parodi,
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Ambiguity, fear, stress, and their effects on threat-perception and cognition

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Individuals can react to the same stimuli in many different ways, depending upon psychological state. For example, subjects who were induced to feel negatively were more likely to make higher estimates of death rates compared to those who were induced to feel positively¹. Discovering which emotional state causes which reaction is important in predicting human behavior. This is especially essential with high-risk conditions such as fear, acute stress, or ambiguity.

The purpose of the study was to find the effect of fear, acute stress, and ambiguity on the perception of threat. This was done by presenting 14 subjects with a computerized series of threatening and non-threatening faces (angry/neutral) that started out with low signal to noise with increasing signal over time. Subjects were then told to indicate if they thought the faces were angry or neutral in the shortest time possible. This experiment was performed twice: under fear conditions (immediately prior to jumping out of a plane) and under control conditions (in the laboratory), which were counter-balanced for order. Dependent variables were accuracy and response time.

With the data thus far, analysis shows that when subjects are in a state of fear, they become more efficient with their use of time. Their response times in fear were not significantly quicker than the times for the control situation throughout the entire spectrum of angry/neutral faces ($F=1.821$, $p=0.197$), but when the faces were angry, the subject's response times were significantly faster ($F=4.367$, $p=0.027$). In terms of accuracy, the subjects were equally accurate when shown angry faces in fearful and control states ($F=1.480$, $p=0.243$). However, when presented with neutral stimuli, the subjects, when fearful, were more likely to label the face as a threat ($F=5.557$, $p=0.033$).

Our results suggest that fear tends to make people more efficient in their use of time in actual situations of threat, but also increases their tendency to perceive threats in harmless situations. People become "trigger-happy" when frightened, reacting aversely before an accurate assessment of the situation can be made. Further studies in this topic will be done by adding more subjects and observing the effect of other variables such as age, gender, and social dominance on response.

This research was supported by the Simons Foundation, the Laboratory of Emotion and Cognition, and funding from the Office of Naval Research.

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Joshua Lieber
Oyster Bay HS (NY)

*Mentor: Drs. John Noé & Harold Metcalf,
Department of Physics & Astronomy*

Synthesizing a cylindrical vector beam with a Mach-Zehnder interferometer

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A cylindrical vector beam (CVB) is a beam of monochromatic light whose linear polarization varies symmetrically with respect to the beam's axis of propagation. The two basic CVB's are either radially or azimuthally polarized. The many interesting applications of CVB's include enhanced laser cutting and drilling, high-resolution microscopy, and particle acceleration.

CVB's can be generated by precisely combining the two orthogonal first-order Hermite-Gauss laser modes (denoted $HG_{1,0}$ and $HG_{0,1}$) while also giving them orthogonal linear polarizations. The first-order HG modes resemble two spots of light an equal and opposite distance from the center of the beam; they differ by being aligned along either a vertical or horizontal line. A correctly synthesized CVB will look like a bagel. When the bagel is analyzed with a linear polarizer it turns back into an HG mode which rotates as the polarizer is turned.

In this project, a radial CVB was generated within a Mach-Zehnder interferometer, a device which first divides and then recombines a beam using two mirrors and two beamsplitters in a rectangular configuration. One HG mode was obtained from an open-cavity HeNe laser, and the second was generated by rotating the beam in one arm of the interferometer 90° with a Dove prism. Similarly, the two orthogonal polarization states were obtained by re-orienting the initial polarization direction using polarizers in each arm of the interferometer.

We were successful in creating a radial CVB that passed the test described above, although the recreated HG patterns were somewhat irregular in shape. Very precise adjustments were needed to make the re-combined beams perfectly collinear and coaxial, and to have the correct phase relationship. In the future we hope to create a mathematical model of the synthesis process that will help guide the challenging alignment procedure.

We would like to thank the Simons Foundation for funding this research, and Prof. Harold Metcalf for establishing and supporting the Laser Teaching Center.

Naveen Murali
Staples HS (CT)

Dr. Miriam Rafailovich,
Department of Materials Science & Engineering

Engineering a 2D potentiometric biosensor for protein detection

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¹Staples High School, Westport, CT, ²Roslyn High School, Roslyn Heights, NY, ³University of California – Berkeley, ⁴Carnegie Mellon University, ⁵SUNY Stony Brook

There is an increasing need for precise protein detection as a diagnostic tool for identifying diseases, pathogens, and abnormal protein levels in the body. An ideal biosensor for proteins is one that has both specificity and sensitivity. One common technique for constructing such protein biosensors is molecular imprinting (MI). Existing MI techniques for biosensing application are three dimensional, making use of bulk polymer matrices. This technique is unfavorable because of its long response time and low protein reoccupation of polymer cavities.¹

Alternatively, molecular imprinting on two dimensional surfaces has been proposed through a self-assembled monolayer (SAM) of thiol (11-mercapto-1-undecanol) and gold. Shown in Figure 1, this SAM is comprised of a Si wafer, a gold layer formed through chemical vapor deposition, an additional layer of thiol, and the proteins desired for templating embedded in the thiol. Proteins in aqueous solution are polyelectrolytes whose charges are dependent on the intrinsic isoelectric point of the protein as well as the ionic composition of the solution.¹ Charged protein adsorption into geometrically complementary cavities of the thiol and gold layer yields noticeable electric potential differences due to gold's conductive properties. We hypothesized that an effective electrochemical biosensor capable of identifying specific protein concentrations, selectively sensing proteins, and *in vitro* protein detection could be engineered through the use of gold-thiol SAMs.

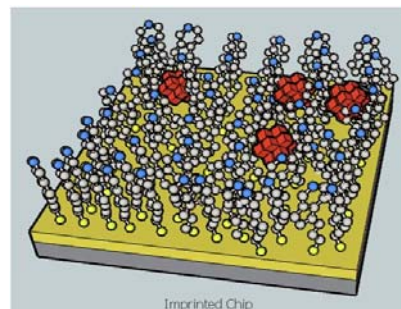


Figure 1: the SAM with organic thiol molecules and proteins for templating (shown in red).

In order to demonstrate the ability of the chip for determining specific protein concentration in an unknown solution, the chip was incubated in a 6% fibrinogen solution. A linear standard curve of fibrinogen concentration vs. voltage difference was produced and found to be $y=1.2877x + 3.6674$ for fibrinogen concentration x in $\mu\text{g/mL}$ and voltage difference y in mV. The selectivity of the sensor was also evaluated by cross testing the biosensor with hemoglobin and fibrinogen. Chips were imprinted with hemoglobin and fibrinogen separately and tested in solutions of fibrinogen, hemoglobin, or a combination of the two. Results indicated that for fibrinogen imprinted chips, the combined proteins produced the highest voltage response as the charged hemoglobin protein occupied cavities intended for the uncharged fibrinogen molecules, therefore producing a larger voltage response.

At the same time, previous research has shown that Au nanoparticles are toxic to human dermal fibroblasts and disrupt the integrity of the cell. Fibronectin is a glycoprotein essential for processes like cell growth and adhesion. Therefore, it was hypothesized that exposure of these cells to Au nanoparticles would reduce fibronectin production. Cell media was extracted from human dermal fibroblasts exposed to 45 nm and 13 nm particles as well as no particles (control). Voltage response curves from the potentiometer confirmed this hypothesis as the control media had the highest response for fibronectin while 45 nm was lowest due to extensive cell damage from these large particles.

¹ Wang, Y., Zhou, Y. "A potentiometric protein sensor built with surface molecular imprinting method." *Biosensors and Bioelectronics*. 2008; 24:162-166

Purumeh Michelle Nam

Choate Rosemary Hall (CT)

*Mentor: Dr. Robert Haltiwanger,
Department of Biochemistry & Cell Biology*

Generation of truncation mutants to study ER retention of mPofut2

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Glycosylation is a post-translational modification wherein a glycan is attached to a protein, hence becoming important for a protein's structure and function. There are several types of glycosylation, including *N*-linked, *O*-linked and *C*-linked. *O*-linked fucosylation occurs on serines or threonines nested within consensus sequences found in cysteine-rich motifs. There are two well-studied motifs, Epidermal Growth Factor (EGF) repeats and Thrombospondin type 1 repeats (TSR). TSRs have 6 cysteines that form three disulfide bonds. The consensus sequence for *O*-fucosylation in TSRs is C¹X₂(S/T)X_{2/3}C². Protein *O*-fucosyl transferase (Pofut2) is the enzyme responsible for the addition of *O*-fucose to TSRs. Pofut2 knockout mice are embryonically lethal but the mechanism of this is unknown. Pofut2 localizes to the ER and modifies only properly folded TSRs. Pofut2 lacks a KDEL sequence and hence the mechanism of its ER retention is unknown. There are two known mechanisms of ER retention- KDEL sequences wherein receptors retrieve soluble ER proteins and "piggybacking" in which a protein attaches itself to another protein that has KDEL. We attempted to study which one of these mechanisms is involved in the ER retention of Pofut2 by generating truncation mutants. These mutants will help identify a possibly novel retention signal or the region of Pofut2 that interacts with its piggybacking partner. Truncations were subcloned into pCDNA4.0/TO backbone and will subsequently be transfected to animal cells to study their ER retention.

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Discerning diet from dental morphology in the Alligatorid genus *Paleosuchus*

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Both Schneider's Dwarf Caiman (*Paleosuchus trigonatus*) and Cuvier's Dwarf Caimain (*Paleosuchus palpebrosus*) inhabit the Amazon Basin of Brazil and Colombia, along with other members of the Order Crocodylia such as *Caiman yacare* and *Caiman crocodylus*. According to the fossil record, the two species of the *Paleosuchus* genus shared a common ancestor until relatively recently when compared to the divergence of the genus from Family Alligatoridae, at which point they began to diverge into the two extant species. Both species are able to inhabit the same general region because each species inhabits its own ecological niche; specifically, the two species have vastly different diets. *P. palpebrosus* eats mainly fish, crabs, and terrestrial invertebrates such as Araneida, Hymenoptera, and Diplopoda. *P. trigonatus* also eats terrestrial invertebrates; however, it eats only Hymenoptera. Unlike its cousin, *P. trigonatus* supplements its diet with shrimp and common terrestrial vertebrates such as lizards, frogs, snakes, and small mammals. *C. crocodylus* actively consumes fish, snakes, rodents, shrimp, and snails, a diet that is more similar to that of *P. trigonatus*.

The purpose of this investigation is to determine whether or not the dental morphology of the two species of *Paleosuchus* is distinguishable, and, if so, whether or not it is reflective of dietary proclivities. I took eight linear measurements of 81 *P. trigonatus* teeth, 121 *P. palpebrosus* teeth, and 42 *C. crocodylus* teeth using the Zeiss Axiovision Microscopy unit and the corresponding software. All teeth were photographed in situ from two angles and all measurements are in micrometers. I then computed ratios as described by Smith et al. (2005) to decrease the variability in the dental morphology due to age of the animal upon death and tooth position in the jaw. Based on the linear measurements a total of four angles were also computed. The ratios and angles were run through a discriminant function analysis using SPSS software. The results show a distinct separation between the teeth of *P. palpebrosus* and *P. trigonatus*. There was 92.2% accuracy in the classification of teeth within the original group and 91.4% accuracy when the same teeth were cross-validated. The two variables that best distinguish between the three species of Caiminae are MCL/QCL and CH/QCL. The two species most closely associated with each other are not the two *Paleosuchus* species but rather *Caiman crocodylus* and *P. trigonatus*. Overlap in the data occurs in the premaxillary and mesial maxillary tooth positions. This indicates that the differences in dental morphology are not related to their common ancestry but rather to their differences in feeding ecology.

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Super Kähler-Ricci flow

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The Ricci flow, the smooth deformation of the metric of a Riemannian manifold, has profoundly impacted the study of geometry and topology in recent decades. This geometric evolution equation was used by Grigori Perelman in 2002 to prove the Poincaré Conjecture, one of most difficult and important unsolved problems in the history of mathematics. Another new and exciting field is the theory of supermanifolds, which has important applications in string theory. We modified the Ricci flow equation for supermanifolds, and obtained a second-order partial differential equation that resembles the Ricci and Calabi flow but has its own set of solutions, which we continue to explore.

This work was supported by the Simons Foundation.

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Cosmic ray and geomagnetic field flux: an elusive correlation

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Atoms and subatomic particles called cosmic rays constantly bombard Earth's atmosphere. When a cosmic ray with enough energy enters the atmosphere, it collides with air molecules to create a self-perpetuating shower of particles, primarily muons and neutrinos. These muons emit photons due to their speed when as they pass through the atmosphere. This light is the basis of the vast majority of ground-based cosmic ray detector systems. Each muon detected, referred to as a count, is assumed to represent one cosmic ray.

Many factors affect the cosmic ray incidence and cosmic ray count flux. The purpose of this study was to identify what, if any, correlation exists between the Earth's magnetic field flux, also known as the geomagnetic field flux, and cosmic ray incidence. In order to achieve this, scintillator count and weather data were taken at Roosevelt High School in Roosevelt, New York from January 1st to June 24th and geomagnetic field strength data was taken at the United States Geological Survey magnetic field observatory in Fredericksburg, Virginia. After barometric pressure and temperature, which affect the energy a cosmic ray must possess to produce light and therefore the accuracy of scintillator detection method, were accounted for with a logarithmic regression, the magnetic field strength flux was compared to the cosmic ray count flux. There appeared to be a weak relationship between cosmic ray counts and geomagnetic field strength over the course of this study, but a much stronger correlation was observed between the diurnal dip of the vertical component of geomagnetic field strength and a similar dip in cosmic ray counts. Because a strong daily but only a weak seasonal correlation was observed, it may be that the diurnal geomagnetic field flux has some strong effect on muons in the atmosphere but little effect on the overall cosmic ray flux, meaning that the detection method must be corrected for magnetic field strength to yield truly accurate cosmic ray flux data. In order to test this hypothesis, however, scintillator detectors must be placed more closely to a magnetic field strength detector to ensure accurate and synchronized data, as the geographic disparity between New York and Virginia may yield inaccurate correlations when examining hourly changes. Additionally, the angle of incidence of shower-created muons might be studied to see if it changes with the magnetic field, thereby changing count rate.

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The effect of N-terminal mutations on Rad54 function

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Homologous recombination (HR) is the process during which similar genetic sequences are exchanged. In mitotic cells, HR between sister chromatids facilitates the repair of DSBs caused by stalled replication forks, mutagenic substances, or UV radiation. In meiosis, HR between homologous chromosomes serves the dual function of ensuring even segregation at Meiosis I and of increasing the genetic variation of a population by creating new allele combinations. Meiotic cells have therefore developed numerous mechanisms to ensure that HR takes place between homologs. *Saccharomyces cerevisiae* is a model organism for studying recombination because numerous proteins required for recombination in yeast have orthologs in humans. Understanding the function of these proteins may help explain certain genetic disorders and cancer.

Rad54, an evolutionarily conserved DNA motor protein, interacts with the RecA ortholog Rad51 to create functional nucleoprotein filaments that mediate strand invasion. While these proteins exist in both meiotic and vegetative cells, certain meiosis-specific proteins contribute to an interhomolog bias for meiotic recombination. Dmc1, also a RecA ortholog, performs similar functions to those of Rad51. A key difference is that it interacts with the Rad54 paralog Rdh54. Previous studies from the Hollingsworth lab have shown that Rad54-T132 is phosphorylated in meiotic cells to reduce Rad54's ability to interact with Rad51, thereby enhancing Dmc1-mediated strand invasion of homologous chromosomes.

It has been observed that Rad54-S318 is phosphorylated *in vivo* in meiotic cells. Because phosphorylation of Rad54-T132 has been identified as a regulatory mechanism to limit interaction with Rad51, phosphorylation of Rad54-S318 may also be functionally important. Both T132 and S318 are located in the amino terminal part of Rad54 which is known to bind to Rad51. My project was to test the effects of various N-terminal mutations on RAD54 function in mitotic and meiotic cells. RAD54-S318A mutations prevent phosphorylation because alanine lacks a hydroxyl group. RAD54-S318D mutations can mimic phosphorylation because both aspartic acid and phosphate groups are negatively-charged. RAD54-S318R substitutes a positive charge in place of the negative charge that would be conferred by phosphorylation. Lastly, I tested a mutant that has a frame shift mutation causing only the first 153 residues of Rad54 to be translated.

RAD54-S318A and *rad54-153Δ* were tested for their ability to complement *rad54Δ* for sensitivity to the DNA damaging agent, MMS, in vegetative cells, as well as reduced sporulation in meiotic cells (presumably indicative of unrepaired double strand breaks). RAD54-S318A fully complements both phenotypes, indicating that phosphorylation of S318 is not required for Rad54 function. In contrast, *rad54-153Δ* is completely sensitized to MMS, demonstrating that the N-terminal fragment of Rad54 cannot replace normal Rad54 function. *rad54-153Δ* mutants sporulate worse than do *rad54Δ* mutants, exhibiting a phenotype similar to *rad54Δ rdh54Δ* diploids. This result suggests that the N-terminal fragment may compete with Rdh54 for Rad51 binding-sites. For all assays, further testing with double mutants and phosphomimic mutants will allow more conclusive information to be gathered. This work was supported by funding from the Simons Foundation and by a grant provided by the National Institutes of Health.

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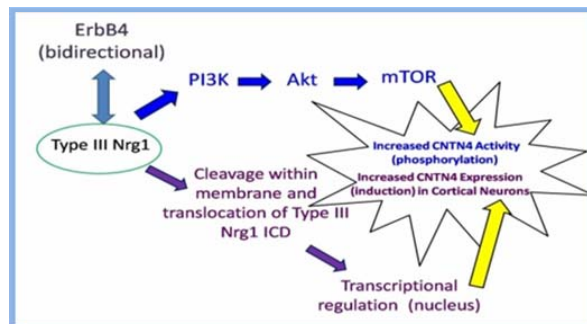
The role of Type III Nrg1/ErbB4 signaling in regulating cortical synaptogenesis via activation of CNTN4 expression

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Type III Neuregulin-1 (Nrg1) is a schizophrenia susceptibility gene and a member of the epidermal growth factor (EGF) family of signaling molecules. Its unique structure consists of an EGF domain, where it interacts with the receptor tyrosine kinase ErbB4 via binding. This signaling relationship between Type III Nrg1/ErbB4 is bidirectional, as it elicits forward and back signaling. In forward signaling Nrg1 acts as a ligand for ErbB4, while in back signaling Nrg1 acts as a receptor for ErbB4. Back signaling between Type III Nrg1/ErbB4 regulates transcription through nuclear translocation of Type III Nrg1 intracellular domain (ICD) and activates the phosphatidylinositol-3-kinase (PI3K) intracellular signaling pathway, which regulates neural development. Defects in Nrg1 are suspected to inhibit these vital intracellular processes, which may contribute to neurological disorders.

Similarly, Contactin4 (CNTN4) is a candidate gene for autism that regulates cell adhesion and the formation of neuronal networks. The similar roles of Nrg1 and CNTN4 in neural development led to the hypothesis that Type III Nrg1/ErbB4 back signaling may influence the expression (Nrg1 ICD translocation) and activation (PI3K pathway) of CNTN4 within the cortex of the brain. This relationship was initially tested in Human Embryonic Kidney (HEK293) cells, measuring Nrg1 and CNTN4 expression as well as PI3K activation via phosphorylation of a downstream serine-threonine kinase, Akt. The cells were transfected with three forms of Type III Nrg1, two of which contained mutations in the transmembrane domain, enabling a test of the effect of mutations on signaling. By adding soluble ErbB4 (sErbB4) to a set of cells, Type III Nrg1/ErbB4 back signaling was induced. As a result, CNTN4 and the three forms of Type III Nrg1 were expressed in the cells. CNTN4 expression was greatest in cells treated with sErbB4, indicating a potential link between CNTN4 and Nrg1/ErbB4 signaling. However, Akt phosphorylation (pAkt) was not detected, suggesting specificity of the pathway in neurons. Upon conducting the experiment in murine neuronal tissue of Nrg1 wild-type, heterozygous and knockout mice, it was found that CNTN4 and Nrg1 were simultaneously expressed. Collectively, these findings suggest potential regulation of CNTN4 expression and activity via Type III Nrg1/ErbB4 back signaling. This work was supported by the Simons Foundation.

Proposed Model of Nrg1/ErbB4
Back Signaling and CNTN4 Interactions



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Confocal pulse amplitude-modulated ultrasound stimulation of osteoblast integrin pathways

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Osteoporosis is a severe health issue that affects an estimated 44 million Americans, or 55 percent of the people 50 years of age and older. Low bone density patients, such as those with osteoporosis, are more liable to suffer fractures, particularly in the hip and spine. Such fractures typically require major surgery and may result in the long-term debilitation of normal human movement. Low intensity ultrasound, a non-invasive treatment method, has been shown in many studies to accelerate fracture healing, especially in delayed unions and nonunions.

Despite evidence of a biological response to mechanical stimulation, the specific intracellular mechanism is unknown. Several studies have hypothesized that low-intensity pulsed ultrasound (LIPUS) stimulates proliferation and differentiation of bone cells through gene expression triggered by mechanotransduction signaling pathways. Integrins, which are cell-surface transmembrane protein receptors, may play a key role in cell signaling regulation as one of the promising candidates.

The purpose of this study was to investigate the effects of ultrasound stimulation on integrin pathways in cultured MC3T3-E1 osteoblast mice cells in order to examine optimal functionality of integrin pathways. This experiment investigates effects of specific integrin receptors by blocking integrin combinations $\alpha 5\beta 1$ and $\alpha v\beta 3$ with specific antibodies that bind to the integrin subunits. A control is treated with ultrasound, but does not block any integrins. The sham is not blocked and is not treated with ultrasound. This study uses a novel ultrasound setup with two focused beams to create a confocal signal. In addition, this study uses an amplitude-modulated signal, which has varied amplitude that can exceed the intensity of the consistent pulsed amplitudes of LIPUS. The MC3T3-E1 osteoblast cells were treated with pulse amplitude-modulated ultrasound (PAMUS) transducers at 100 kHz for 15 minutes and observed at standard time markers of 30 min, 1 hr, 3 hr, and 6 hr to evaluate changes in transcription levels. qPCR was used to analyze mRNA levels of c-fos, c-jun, and osterix, which are transcription factors that play a significant role in the differentiation of bone cells and bone remodeling.

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Crucial Interactions in the Base Excision Pathway of 8-oxoguanine by Formamidopyrimidine DNA Glycosylase (MutM)

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The genomes of all organisms are under constant attack, creating thousands of damaged lesions. Cells cannot properly function if DNA damage corrupts the integrity of essential genetic information, leading to cell senescence, apoptosis or even cancer. Among the most extensively studied lesions is 8-Oxo-7,8-dihydroguanine (8-oxoguanine or 8OG), formed by reaction with endogenous reactive oxygen species (ROS) [1]. Failure to remove the 8OG lesion before replication occurs will result in G to T transversion, the most virulent of point mutations [2]. DNA glycosylases in the base-excision repair (BER) pathway are every organism's natural defense against cancer. In bacteria *E. coli*, MutM (or Fpg) is responsible for recognition and excision of 8OG:C lesions. Base excision repair begins with interrogation of the DNA by the Fpg lesion recognition complex, resulting in the "flipping" or eversion of 8OG out of the duplex and into the enzyme's active site, where the damaged base is catalyzed and excised. By utilizing the AMBER 10 molecular dynamics (MD) program, our goal is to investigate the structural and energetic pathway of the eversion process in which MutM "flips" the 8OG lesion out of the DNA duplex [5]. The intrahelical and extrahelical reference structures were generated from the two x-ray crystal structures 2F5O and 1R2Y [3,4].

Examination of the PMF (potential of mean force) allows us to determine the energetic pathway and assay the structural changes along the reaction coordinate. Our simulations indicate a significant energetic difference exists between the 8OG and the G eversion pathway. The energetic landscape of 8OG is relatively flat with small local minima, favoring eversion. In contrast, the PMF profile of G has a significant energy barrier to extrusion, rendering eversion difficult. Our analysis demonstrates that entrance of Arginine111 into the duplex is responsible for major conformational changes in the DNA backbone and hydrogen bond formation. This may contribute to the energetic differences of eversion between 8OG and G. Insight into the crucial interactions of the 8OG excision pathway will lead to the development of new therapeutic approaches to cancer utilizing molecular modulators of hOGG1 activity, the human functional analogue of MutM.

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