UW Summer Research Poster Session

August 16th, 2007
9am – Noon
Mary Gates Hall Commons
Welcome

This poster session is a collaboration among several summer programs hosting UW and non-UW undergraduates from around the country, including the Amgen Scholars Program, Biostatistics Summer REU, Clinical Research Experience for Engineers, Genetically Engineered Materials Science & Engineering Center, Hooked on Photonics STC-MDITR, Intel Summer Research Experience, National Nanotechnology Infrastructure Network, and University of Washington Engineered Biomaterials.

We celebrate the accomplishments of this year’s summer undergraduate researchers and mentors.
Quotes from Students

Summer Program Researchers reflect on their UW summer research experience:

“I will always remember the great people I met and the amazing things I got to do during my stay at the University of Washington.”

“Our group was fantastic; we all got along and had an amazing time exploring the city.”

Summer Program Researchers give advice to future summer undergraduate researchers:

“[An] important thing I learned is that what you put into a project determines what you are able to get out of it, so the harder you work the more you learn.”

“BE FLEXIBLE!!! You may be given a task that you have no clue about, but you have to be willing to accept the challenge, and always put your best foot forward!”

Summer Program Researchers appreciate their mentors:

“Thank you for not giving up on me.”

“I would like to thank you for your time and patience, and all that you have done to help mold my mind in preparation for graduate school.”
Schedule of Events

Poster Session 1  (A – L)  9:00 am – 10:30 am
Poster Session 2  (M – Z)  10:30 am – 12:00pm

Please note: Abstracts are listed alphabetically by presenter’s last name

Refreshments available throughout Mary Gates Hall Commons
Creating an alternate specificity pair between mutated enzymes
Gerardo Alcazar, Senior, Biology, University of Houston
Mentor: Rachel Klevit, Biochemistry
Mentor: Devin Christensen, Biochemistry
Amgen Scholars Program

Breast cancer susceptibility can be attributed to inherited germ line mutations in BRCA1. The BRCA1 gene product serves a myriad of functions within the cell, including cell cycle, DNA damage repair, apoptosis, transcription, chromosomal segregation, and protein ubiquitination. Cells with little or no expression of BRCA1 display increased damage from DNA damaging agents. The conserved RING domains in BRCA1 and BARD1 form a heterodimer whose enzymatic activity is dependent on structure. The heterodimer holds structural conformation with the presence of BARD1 and forms a functional ubiquitin ligase complex by interacting with UbcH5. The BRCA1 protein is known to function with up to ten other conjugation enzymes. UbcH5 also appears to be promiscuous with its interactions in other biochemical pathways. The hypothesis of this study is that mutants of BRCA1 and UbcH5 will create a working conjugate enzyme exclusively in the enzyme pair. To test this hypothesis, a yeast two-hybrid screen was designed with point mutations on BRCA1 and a library of random mutations on a conserved region of UbcH5. The mutations in the enzyme pair will give insight into the structural interaction that makes the ubiquitin ligase complex active in ubiquitination. Further studies would want to establish whether the altered-specificity pair retained enzyme activity in the DNA-damaged repair cascade.

Controlled Stimulation of Toll-like Receptors by Nanoparticles
Saba Alniemi, Senior, Cell Biology and Neuroscience, Montana State University
Mentor: Hong Shen, Chemical Engineering
Amgen Scholars Program

Pathogen-associated molecular patterns (PAMPs) are molecular structures found on microbial pathogens. Eukaryotic cells have evolved toll-like receptors (TLRs) to recognize PAMPs and elicit immune responses. Antigen-presenting cells (APCs) are the main mediators of immune responses. Activation of TLRs on APCs leads to inflammatory responses and also to the development of antigen-specific adaptive immunity. For this reason, the use of synthetic TLR agonists to modulate and enhance immune responses has become a topic of research interest in vaccine development. Polymeric nanoparticles offer a convenient and versatile platform for the co-delivery of antigen and TLR agonists. In this study, the synthetic TLR agonists CpG oligodeoxynucleotide, polyinosinic: polycytidylic acid (poly I:C), and polyuridylic acid (poly U) were studied for their ability to enhance immune responses in nanoparticle formulations. Preliminary data show that delivery of TLR agonists by nanoparticles enhanced both class I antigen presentation and innate antiviral immunity.
A Comparison of Vitamin D Analogs in the Chronic Kidney Disease Mouse Model
*Bryan Bartley, Senior, Biochemistry, University of Washington*
*Mentor: Mohga El-Abbadi, Bioengineering*
*Mentor: Cecilia Giachelli, Bioengineering*
*UWEB-USIRP*

Chronic kidney disease (CKD) causes systemic derangements in mineral metabolism that manifest pathologically as two countervalent processes: the resorption of bone mineral from skeletal bone, and the abnormal deposition of bone mineral in cardiovascular tissues. A classic treatment for renal osteodystrophy is vitamin D, which acts as a powerful hormonal trigger for promoting bone formation. However, as an unintended side-effect, vitamin D treatment may also accelerate calcification of cardiovascular tissues, contributing to the high rate of cardiovascular disease in kidney patients. This study compares two commonly prescribed pharmaceutical forms of vitamin D and measures their relative contribution to vascular calcification in a mouse model of chronic kidney disease. Based on the results of the study, doctors may be able to make informed decisions about prescribing vitamin D to treat renal osteodystrophy while minimizing cardiovascular risk in their patients.

Probability, Uncertainty and Bayesian Networks: Robots and Predicting the Holding of Objects
*Leilani Battle, Sophomore, Computer Engineering, University of Washington*
*Mentor: Rajesh Rao, Computer Science and Engineering*
*Intel Research Experience*

Focus on robotics. Use of a Bayesian network to model and determine the probability of a robot’s having picked up an object, based on the difference in the change of pressure values in the robot’s feet. The resulting algorithm is then used to determine which of three set motions will lead to picking up several objects of specific size and shape within simulations. Using data tailored to one specific movement and shape was very successful in predicting probabilities for other shapes and movements in a limited environment.

Inexpensive Accelerometers Using IC Bondwires for Inertial Sensing
*Bettina Chen, Sophomore, Electrical Engineering, California Institute of Technology*
*Mentor: Brian Otis, Electrical Engineering*
*Intel Research Experience*

The current approach to producing integrated micromachined accelerometers is costly. However, the deflection of bondwires could be used for inertial sensing, resulting in a cheaper method of constructing accelerometers. Integrated circuits (ICs) are bound to a printed circuit board by short wires called bondwires. Since
these bondwires are not taut when soldered on to the bond sites of the chip or board, we can calculate the deflection these bondwires will experience when the board is subjected to an acceleration of ±1g from tilting the board. The deflection can then be used to determine the mutual coupling between wires and circuitry can thereby be designed such that the deflection can be detected. Thus, accelerometers can be made by using the deflection of bondwires as the determining factor for the acceleration a circuit board undergoes. In this project, I completed an initial feasibility analysis of this technique, demonstrating a deflection of about 1 nm for an acceleration of +1g. This deflection results in an approximately 0.8ppm shift in mutual inductance.

Evaluation of Quantum Dot Internalization by the bacterium Pseudomonas aeruginosa

Tiffany Chen, Senior, Biomedical Engineering, University of Texas at Austin
Mentor: Hongyan Ma, University of Washington, Chemical Engineering
Mentor: Kristy Katzenmeyer, Bioengineering
Mentor: James D. Bryers, Bioengineering
UWEB-REU

Biofilms cause a significant amount of all human acquired microbial infections. Pseudomonas aeruginosa is an opportunistic pathogen that often forms biofilms and contributes significantly to nosocomial (hospital acquired) infections. Investigations of the processes governing biofilm formation, persistence, and virulence require a new set of diagnostic tools that can be applied, non-invasively, to prevent destroying the biofilms’ structure. This project seeks to explore the prospects of replacing fluorescent stains with quantum dots (QDs); nanocrystals composed of periodic groups of II-VI, III-V, or IV-VI materials. Unlike traditional fluorochromatic stains, QD luminescence is photostable, with narrow emission spectra that are size tunable, and broad absorption spectra, which allow the excitation of multiple QDs with a single wavelength. Employing QDs to either label various bacterial species or to quantify genetic transfer between species requires background information on the natural uptake of QDs by bacteria. Studies in internalizing high quality polyethylene glycol (PEG) coated quantum dots using planktonic P. aeruginosa were carried out prior to future studies with P. aeruginosa in biofilms. We studied three distinct internalization methods: (1) bacterial incubation with quantum dots, (2) CaCl2 treatment transfection, and (3) electroporation. Using epifluorescence microscopy, all three methods appear to allow for some degree of internalization of quantum dots within the bacteria. Fluorescence scanning of supernatants and bacterial solutions were also performed to test for the presence of the quantum dots. Further studies will determine if in fact the quantum dots are inside the bacteria, or just associated with the surface. Results will provide a link to using quantum dots to study the internal mechanisms of the biofilm bacteria without photobleaching.
Correlations between Coronary Artery Diseases and Vibration
Yung-Chun Chen, Senior, Bioengineering, University of Washington
Mentor: Yongmin Kim, Bioengineering
Mentor: Steven Goldberg, Medicine
CREE

In the modern world, heart disease has become a very common disease. Currently, there are several different techniques for detecting coronary artery stenosis, but all of these techniques are limited to clinical facilities due to their size and invasiveness. For these reasons, a non-invasive detector for cardiovascular diseases is developed using Ultrasound. When a patient has a coronary artery disease, change of the blood flow causes formation of eddies downstream. The breakdown of eddies creates different magnitudes and directions of turbulence. When the turbulence hits the blood vessel wall, vibration occurs on the blood vessel surface. Therefore, detecting and analyzing the vibration information can help diagnose different coronary artery disease stages. Ultrasound can provide a low-cost and non-invasive technique to detect the vibration. Doppler ultrasound, unlike traditional B-mode imaging ultrasound, only needs one or two transducer elements, which would reduce the cost. Pulse Wave Doppler ultrasound, a Doppler ultrasound technique that sends and receives finite ultrasound sound pulses, can detect the position of the target tissues with a high sampling rate. Using Pulse Wave Doppler Ultrasound, the tissue movement information can be obtained and processed to determine the movement frequency information. The vibration amplitude from this can help diagnose coronary artery disease. This project is to analyze the frequency information from the vibration of localized heart tissues with coronary artery to find the correlation between the vibration and coronary artery diseases based on the reference from angiogram.

Imaging and Evaluation of Regenerative Tissue Implantation Technique for Myocardial Repair
Amy Clobes, Senior, Biology, University of Michigan
Mentor: Marc Takeno, Bioengineering
Mentor: Kip Hauch, Bioengineering
UWEB-REU

The exigent need for a clinical heart repair system motivated the Bioengineered Allogenic Tissue (BEAT) partnership to develop a multifaceted method using human cardiomyocytes seeded on poly(hydroxyethyl methacrylate) (pHEMA) scaffolding to be implanted directly into cardiac infarct tissue. To facilitate this novel repair approach, an implantation and imaging technique was developed and qualitatively examined in vitro to assess its potential for in vitro use. For implantation, degradable and non-degradable pHEMA scaffolds (ca. 400 x 2000 mm rods) were injected by a catheter needle into an excised chicken heart. After fixation, tissue containing implanted pHEMA was stained en bloc with acridine orange and eosin and embedded for Digital Volumetric Imaging (DVI), a tech-
nique which combines ultramicrotome sectioning, fluorescence microscopy, and digital imaging and processing to visualize tissues in three dimensions. The implantation technique showed that both degradable and non-degradable pHEMA were successfully injected into the heart tissue; however, the adhesive and fragile characteristics of non-degradable pHEMA indicated that it was unreliable for implantation use. Cell nuclei stained well with acridine orange, and cardiomyocyte nuclear orientation (which may serve as a proxy to indicate muscle fiber orientation in relation to the implanted scaffold), was observed in 3D images. Optimization of the staining protocol will be necessary to minimize over-saturation by acridine orange. Visualization of this scaffold implantation technique brings the field of tissue engineering one step closer to cardiac muscle repair.

**Protein fingerprinting of mitomycin C-resistant and non-resistant MCF-7 breast cancer cells**

*Jeff Cloutier, Junior, Molecular Biology and Biochemistry, Middlebury College*

*Mentor: Norman Dovichi, Chemistry*

*Mentor: Haley Pugsley, Chemistry*

*Amgen Scholars Program*

Breast cancer is the second-leading cause of female mortality in the United States. Currently, breast cancer prognosis is determined by a variety of pathological factors, including tumor size, tumor histology, mitotic index, axillary lymph node status, estrogen and progesterone receptor status, and Her2/neu status. The latter two factors are protein markers whose expression levels directly influence patient survivability and help guide treatment. However, due to the inherent variation in cancer, there is a need for a more comprehensive analysis of protein expression. To that aim, the overall goal of our research is to increase prognostic accuracy and provide a template for more personalized treatment by generating detailed protein fingerprints from breast cancer tissue. The focus of this project is to use 2-dimensional gel electrophoresis to generate protein fingerprints of mitomycin C-resistant and non-resistant MCF-7 breast cancer cells. Mitomycin C (MMC) is a chemotherapy drug used to treat breast cancer. The first objective of this project was to determine the appropriate concentration of MMC that kills 90% of a population of MCF-7 cells. The viable 10% is what has been defined as the resistant population. Various MMC concentrations were tested in dosing experiments, in which a certain concentration of MMC was incubated with MCF-7 cells for 24hrs, followed by a 24 hr recovery. Cell viability was determined using hemocytometry after staining with Trypan Blue. The data suggest that 30 µM MMC yields a resistant population, given a 24 hr recovery. However, when recovery time was increased to 96 hrs viability decreased to nearly zero, indicating that the population was initially resistant but was unable to replicate. Using 2-dimensional gel electrophoresis, a protein fingerprint was generated from a viable population of cells after a 30 µM dose and a 24 hr recovery. A protein fingerprint was also generated from an untreated MCF-7 population. Comparison of these two fingerprints led to the identification of notable differ-
ences in protein expression. Future work will focus on generating rich protein fingerprints of breast cancer using 2-dimensional capillary electrophoresis.

**Magnetic Levitation Prototype**

*Nick Colonnese, Senior, Mechanical Engineering, University of Washington*

*Mentor: Brian Fabien, Mechanical Engineering*

*Intel Research Experience*

Many modern day devices contain rotating parts that are held in place by bearings. There are many types of bearings, but one type is receiving a lot of interest: magnetic bearings. Magnetic bearings consist of both permanent magnets and electromagnets that suspend a rotating body in mid-air. Because the rotating body is held in air there is no friction, no wear on material, and if the bearings are designed properly, the body can rotate quickly with little vibration. A prototype of a magnetic levitation device suspended by magnetic bearings, or mag-lev device, was to be designed and built. Different designs for the device were considered, and a certain design was chosen. Different materials were considered, and the device was built. Conclusions are that a mag-lev device can in theory and in practice be constructed. The prototype serves as a proof of concept and shows that magnetic bearings can be a realistic solution for some applications. Recommendations include testing of the mag-lev prototype, as well as construction of larger and more advanced magnetic levitation devices.

**Characterizing a new line of transgenic mice with human toll-like receptor 9 on a murine toll-like receptor 9 knock out background**

*Amanda Crawford, Senior, Biology, Saint Mary’s College*

*Mentor: Christopher Wilson, Immunology*

*Mentor: Lynn Hajjar, Immunology*

*Amgen Scholars Program*

Antigen presenting cells (APCs) have internal and external toll-like receptors (TLRs) that recognize broad ranges of pathogens. TLR9 is found in an intracellular compartment and recognize DNA, but its expression in mice and humans differ. In mice, APCs that express TLR9 are B cells, plasmacytoid dendritic cells (pDCs), myeloid dendritic cells (mDCs), and monocytes while in humans, it’s only seen in B cells and pDCs. To see how the expression patterns influence immune responses and to get a better human model, a transgenic mouse was made with human TLR9 bred with a murine TLR9 knock out (KO) mouse. We were now looking to further characterize this new transgenic mouse line as compared to responses from wild type (WT) C57BL/6 mice and what we might expect in humans. In vitro responses from splenocytes and bone marrow derived dendritic cells (BMDCs) were analyzed by intracellular cytokine staining and flow cytometry. We found that delaying the addition of Brefeldin A (BFA), a reagent that allows for the intracellular accumulation of cytokines, resulted in stronger stimulation of APCs, perhaps due to the intracellular location of TLR9. In the
WT mice, we detected good IL-12 and TNF-α production in monocytes, mDCs, and pDCs in response to a wide panel of CpG oligodeoxynucleotides (ODNs). In contrast, we saw no response in the monocytes, a moderate response in mDCs, and a weak response in the pDCs of the humanized TLR9 mice. This transgenic mouse line is giving us a new way to understand how activation best occurs so we can utilize different ODNs to get a cytokine response from our own immune system that is known to lead to protective immunity through the TH1 lineage of T cells. This would then lead to potentially finding safer, more effective adjuvants and vaccines.

**User Interface Development for MobileASL**

*Omari Dennis, Senior, Computer Science, Norfolk State University*

*Mentor: Richard Ladner, Computer Science and Engineering*

*Mentor: Eve Riskin, Electrical Engineering*

*Intel Research Experience*

For Deaf people, the only means of communication using mobile phones has been text messaging. Many Deaf Americans prefer to use other methods of communication, other than Standard English. Using text messaging as a primary method is less natural and sometimes inconvenient. The purpose of the MobileASL project is to create an opportunity for real-time mobile communication for Deaf people, allowing them to use their native language, American Sign Language (ASL). One of the greater challenges is providing clear video transmissions on low-bandwidth signals using the limited processing power of mobile phones. Another challenge is creating a package that utilizes the video transmission capabilities and gives the user a comfortable and accommodating interface. A solid user interface and the functionality behind it are critical to give Deaf users the best experience possible. This research focuses on improving the user interface and building text messaging protocols. In addition to video, text messaging would be a helpful tool because it would be very useful in situations that are not conducive to video, such as dimly lit rooms or areas with weak connections, where there is a possibility of the video breaking down. The MobileASL project will provide Deaf people with a natural way to connect with each other, eliminating the restrictions of standalone text messaging methods.

**Isolating Human MCK to Regulate Muscle-Specific Gene Expression for Muscular Dystrophy Therapies**

*Melvin Donaldson, Junior, Bioengineering, University of Washington*

*Mentor: Stephen Hauschka, Biochemistry*

*Mentor: Robert Welikson, Biochemistry*

*Amgen Scholars Program*

The Human Muscle Creatine Kinase (MCK) gene holds great potential for use in gene therapies for muscle dystrophies. Our studies aim to characterize its activity and muscle specificity. Expression of muscle specific regulatory and struc-
tural genes during muscle cell differentiation controls striated muscle formation; transcription factors binding to DNA sequences in the 5'-promoter regions in part control gene expression. Using comparative gene analysis, the Hauschka lab has identified highly conserved regions across the human and mouse versions of mck. We are cloning an upstream promoter of the human gene (937 to +50) into a lentiviral vector driving the expression of a nuclear localized LacZ (nLacZ) reporter gene for subsequent transduction of rat and human cardiomyocytes and mouse skeletal myoblasts. Muscle cell-specific expression will be determined by imaging nLacZ and muscle cell markers. We will assay for relative transcriptional activity and muscle specificity in the different cells by qRT-PCR to detect bacterial b galactosidase- and muscle-specific mRNAs. We will then mutate transcription-binding sites in the human gene (specifically E-boxes) that have been previously shown to be critical for muscle-specific expression in the mouse mck promoter in order to assess the function of the most critical control elements in the human MCK promoter. If the human and mouse promoters behave similarly we expect to see a decrease in transcriptional activity in the human construct. The poster presents progressive steps in the molecular biology procedures that have been used to clone the human and mouse promoters and the nLacZ into the lentiviral vector plasmids.

Utility of FGF-10 in developing engineered epithelial tissue for surgical grafts
Kiran Dyamenahalli, Senior, Bioengineering, University of Washington
Mentor: James A. Bassuk, Urology
UWEB-USIRP

Fibroblast Growth Factor (FGF)-10 belongs to a family of 22 growth factors which regulate the proliferation and differentiation of their target tissues. Within the urinary bladder, FGF-10 is produced in the lamina propria and acts on urothelial cells that comprise the epithelium. In vitro and in vivo testing using recombinant preparations of human FGF-10 have shown that the protein dramatically increases the rate of proliferation of urothelial cells. This represents a remarkable departure from the low rates of regeneration observed during the normal state of quiescence. Recent advances in the field of urology have resulted in the ability to grow epithelial tissue from urothelial cells in the laboratory. We intend to characterize FGF-10’s effects on this engineered tissue in order to develop the protein as a treatment for lower urinary tract disorders. Our long-term strategy is to grow engineered epithelia on materials with characteristics that make them suitable for both cell culture and surgical implantation. This will allow us to develop autologous surgical grafts, negating the need for sub-optimal donor tissues from other parts of the body. The engineered epithelium’s barrier function was determined by measuring its trans-epithelial resistance (TER). Changes in gene expression, as a function of FGF-10 treatment, were identified using qRT-PCR. FGF-10 appears to augment the TER of engineered epithelia derived from both normal and diseased cell lines. Because natural bladder and
urethral epithelia act as barriers against the harsh urine environment and invasion of pathogens, FGF-10 could represent a means to bring the engineered epithelial model closer to natural urothelium. In addition, FGF-10 may be involved in the differentiation of these epithelia, as evidenced by changes in cytokeratin expression. If grown on a degradable scaffold, FGF-10-treated engineered epithelia could yield effective tissue grafts for the treatment of lower urinary tract disorders.

Characterization of Synthesized Chiral Compounds by X-ray Diffraction Analysis and Optical Imaging

Jose B Gallegos, Senior, Geology and Chemistry, New Mexico Highlands University

Mentor: Werner Kaminsky, Chemistry

Hooked on Photonics STC-MDITR

Understanding photonic materials on the basis of structural calculations is important for optimizing non-linear optical features in crystals. However, in crystals non-linear optical features are difficult to obtain experimentally since it requires rather large samples, whereas for a calculation of such properties only the atomic arrangement needs to be known and can be simulated or obtained from x-ray diffraction measurements on small specimen. Calculations based on quantum mechanics, which are sufficiently accurate for an isolated molecule, will neglect intermolecular interaction in crystals. Furthermore, classic calculations designed for inorganic crystals over simplify the orbital structure. Nevertheless, the classic model might be modified empirically to be applicable to organic crystals by reevaluating empirical parameters used in these calculations. For this task a series of synthesis’s was preformed of structurally related substances that must lack a center of symmetry: a necessary condition for being optically non-linear. Aryl iso-thiocyanates (ITC) were mixed with enantiopure chiral alcohols and amines. Here, two new compounds were synthesized from reacting 4-chlorophenyl ITC and 4-bromophenyl ITC with a-methyl benzyl amine (a-MBA). Also a continuation of the work that previously resulted in new compounds from reacting 4-nitrophenyl ITC and other ITCs with S-2-Butanol and a-MBA was preformed. This path was chosen due to the fact that by design of reacting any moieties with enatiopure moieties the new substances must be non-centrosymmetric. Uniquely, iso-thiocyanates feature a –R=S linkage, which builds out selected hydrogen bonds, allowing for a structured molecular packing as well as an enhancement of crystal growth. Additionally, the sulfur within iso-thiocyanates scatters well for x-ray structure analysis and shows sufficient anomalous diffraction to determine the hand of the chiral structures. Furthermore, aromatic rings within the compound provide a high number of mobile electrons, which is important for potential nonlinear optical applications. The synthesized materials where re-crystallized and then analyzed via x-ray diffraction resulting in new crystal structures which in turn were used for calculations.
Assessing the Validity of YQOL as a Psychometric Instrument of Injured Teens
Alexandra Goncharova, Post-Baccalaureate, Muhlenberg College, Mathematics and Economics
Mentor: Ming-Yu Fan, Psychiatry and Behavioral Sciences
Mentor: Xiao-hua Andrew Zhou, Biostatistics
Mentor: Douglas Zatzick, Psychiatry & Behavioral Sciences/Harborview Medical Center
Biostatistics Summer REU

Approximately 600,000 adolescents are hospitalized each year due to a severe injury. Few studies have investigated the association between post-traumatic stress disorder (PTSD), depressive symptoms and their effect on the quality of life outcomes of injured youth. Our study primarily focused on Youth Quality of Life Instrument – Research Version (YQOL-R), a relatively new psychometric instrument designed to assess specifically the socio-emotional well-being of the patient after an injury. Data was collected from 108 randomly chosen injured adolescents (ages 12-18) at University of Washington’s Harborview level I trauma center. Each patient responded to a series of questions from Child Health Questionnaire (CHQ), Center for Epidemiologic Studies Depression Scale (CES-D), PTSD Reaction Index (PTSD-RI) and YQOL-R. The data was collected at baseline, 2, 5 and 12 months after the injury. The baseline evaluation estimated the patient’s wellbeing prior to the injury. We used regression analysis to test the significance of CESD, PTSD and other variables in predicting YQOL scores. We used the two-sample t-test to evaluate the discriminatory power of YQOL between patients with and without PTSD and CESD symptoms. The investigation attained follow-up rate >80% at each post-injury assessment. Patients with CESD at baseline had a mean YQOL score of 71.05 (SD=19.10) and those without CESD had an average score of 85.69 (SD=12.79); with the standardized difference on the t-scale at -3.264 and p-value less than .001. Likewise patients with PTSD at baseline had an YQOL score of 79.56 (SD=14.9) and without PTSD 85.45 (SD=14.93); with the standardized difference at -2.02 and p-value of 0.021. The decrease in quality of life resulting from post-traumatic stress and depression is comparable to that of a permanent disability, such as allergies, vision problems, asthma and musculoskeletal problems. PTSD and depressive symptoms are independent predictors of the youth QoL at baseline and 12 month periods. Greater understanding of mental distress and its impact on the quality of life may improve the functioning of the acute care medical settings in regards to treating youth with a psychological trauma.
Development of a Transformable HPLC Column  
Brian Greene, Junior, Chemistry, University of Washington  
Mentor: Randy Salamon, Asemblon, Inc.  
UWEB-RECCS

High performance Liquid Chromatography (HPLC) is a common and effective way to separate and identify the individual components of a chemical mixture. We have set forth to develop an HPLC column that can be functionalized, at will, to serve various types of separations by modifying the surface of the packing material in a unique way. This novel process of column modification is not only cost effective, but also allows the user to custom tailor the surface chemistry of the column and enable separations that may not be possible using commercially available columns.

The Separation of Particles with Dielectrophoresis in a Microfluidic Channel  
Stanley Gu, Junior, Bioengineering, University of Washington  
Mentor: Lih Y. Lin, Electrical Engineering  
Mentor: Joseph Peach, Electrical Engineering  
Amgen Scholars Program

Dielectrophoresis (DEP) is a phenomenon that occurs when a particle is subject to a non-uniform electric field. The electric field induces a dipole in the particle and produces an interaction with it and the surrounding medium. Differently sized particles under different frequency of electric fields experience different amounts of DEP force. In our lab, our goal is to separate differently sized particles, such as DNA strands, by using this force. Our experiments involve flowing particles of interest suspended in solution through a microfluidic channel lined with various electrode arrays. Thus far, we have demonstrated that microbeads with diameters of 200 and 500 nanometers are able to be separated using DEP. By flowing these beads into an array of V shaped electrodes and setting the AC to 2.9 volts and 2.85 megahertz, we saw 200 nanometer beads drawn and trapped to the sides of the channel while the 500 nanometer beads continued to flow down the middle of the channel. These results show promise for separation of other particles, such as DNA, in the future.

Quantitative Analysis of FMISO PET Imaging of High Grade Gliomas  
Jennifer Hadley, Senior, Biomedical Engineering, Washington University in St. Louis  
Mentor: Kristin Swanson, Pathology  
Amgen Scholars Program

Gliomas are aggressive primary brain tumors with a 100% fatality rate, independent of treatment. Magnetic resonance imaging (MRI) reveals anatomical features of the tumor and positron emission tomography (PET) reveals biochemical
processes. PET scans utilizing the [18F]-Fluoromisonidazole (FMISO) radiotracer can be used to image areas of hypoxia which often occurs in high grade gliomas. The relative activity level of FMISO in tumor versus normal brain (tumor:blood ratio, TB) has not yet been verified relative to regions MRI abnormality. Current methods of FMISO PET imaging analysis scale the PET image to the baseline FMISO activity in the blood, using an arbitrary TB ratio of 1.2 to determine the volume of hypoxic cells in the tumor (HV). Twenty-five patients with high grade gliomas were imaged using FMISO PET, gadolinium-enhanced T1-, and T2-weighted MRI. FMISO PET images were then scaled using a range of TB ratios (0.8--1.6); total FMISO intensity and HV obtained from the scaled FMISO PET images were taken relative to tumor volumes obtained from T1Gd and T2 MR images. FMISO intensity outside the MRI-defined tumor volumes for each TB ratio showed rapid decay at ratios above 1.2, indicating that FMISO activity at or above the 1.2 TB level is restricted to areas of MRI abnormality, and is in fact a good measure of HV within the tumor, as the ideal ratio would allow no intensity in regions outside the tumor volume. FMISO intensity decayed approximately linearly as the TB ratio increased inside the T1Gd and T2 volumes, as expected; 1.2 yielded a representative nonzero intensity. We found the TB ratio of 1.2 to be accurate in determining HV, in glioma patients confirming the validity of the 1.2 TB ratio.

Analysis of self reactive T-cell populations in mice lacking transcription factor Foxp3 or TGFB-receptor
Kyle Hansen, Senior, Biochemistry and Cell Biology, University of California San Diego
Mentor: Adrian Liston, Immunology
Mentor: Alexander Rudensky, Immunology
Amgen Scholars Program

During T-cell development, multiple mechanisms are required to protect against self reactive T-cells. Although these developmental processes are very efficient, a small population of self-reactive cells T-cells still escapes the thymus. When left unchecked, these pathogenic cells infiltrate the organs and tissues, leading to auto-immune associated lesions and eventual mortality. Previous studies have found that a subset of CD4 + Foxp3 + Tcells known as regulatory T-cells are vital for the maintenance of self tolerance. In addition, Transforming Growth Factor (TGF-) signaling in T-cells has been shown to be crucial in restricting the proliferation of self-reactive cells. Although it has been established that Foxp3 and TGF- work through two distinct pathways, it is not fully understood which self-reactive populations they target. One possibility is that both Foxp3 and TGF- work together to restrain the same population of self reactive T-cells. Alternatively, they may act independently to inhibit the proliferation of two discrete populations of pathogenic cells. Our aim is to isolate, clone and sequence T-cell receptors from mice lacking Foxp3 and mice with a T-cell specific ablation of TGF- receptor II. Employing this method will allow us to compare the
T-cell receptor repertories from both the Foxp3 and TGF-β receptor II knock-out models. Further investigation of the interactions between these two pathways and their targets will shed light onto the mechanisms required for immunological tolerance and give insight into future therapies for auto-immune related diseases.

**Collective Investigations at the Center for Collaborative Technologies**

*Jennifer Hanson, Senior, Computer Engineering, University of Washington*  
*Mentor: Richard Anderson, Computer Science and Engineering*  
*Mentor: Natalie Linnell, Computer Science and Engineering*  
*Intel Research Experience*

Work this summer at the newly established Center for Collaborative Technologies at University of Washington entailed much cooperative effort. The center focuses on distance learning technologies and high-tech classrooms, more specifically developing and expanding Conference XP, a platform program enabling multi-location collaboration and lectures. Two projects were performed to further larger programs in the educational technological discipline. The first program is a speech recognition program that will be used primarily to transcribe university lectures on computer science theory and algorithms. The project for the speech recognition program dealt with processing raw data in the form of Latex formatted Computer Science theory papers for the purpose of obtaining appropriate text for training data. Processing involved removing latex commands as well as selectively modifying math content to formulate comprehensible text. The second program is Classroom Presenter, an educational tool for distance learning, as well as increased interaction between the lecturer and students. Classroom Presenter (CP) utilizes tablet PC’s to allow for direct and instantaneous contact via inking with a stylus on decks of slides. Extensive code files were reviewed and specific issues addressed as a main task after widespread experimentation with CP. The first project was given in part to learn the differences between C# and Java as well as to work with Microsoft Visual Studio, the environment employed to create CP. The outcome constitutes of a Latex cleanup program being written, tested, and finished. With the second project, greater understanding of a large multi-class event based program was accomplished. Fixing minor issues within an extensive program is a stage of software development; therefore, program applications require much testing and modification to perfect results and the overall experience for the end user. Further testing and revision is in progress for CP.

**Investigating inter-domain regulation of von Willebrand factor interactions with platelets**

*Ryan M. Harrison, Junior, Biomedical Engineering and Economics, Johns Hopkins University*  
*Mentor: Wendy Thomas, Bioengineering*  
*National Nanotechnology Infrastructure Network REU Program*
Von Willebrand factor (vWF), a large multimeric blood plasma protein, is integral to in vivo platelet aggregation and clot formation. Of particular interest are the shear-dependent interactions between the A1-domain of vWF and platelet glycoprotein (GP) Ib. Weak, transient bonding between these two partners anchors platelets and vWF long enough for other glycoprotein and integrin mediated bonds to form. Disruption of these transient bonds, such as mutations that abolish the shear-dependence of the interaction, lead directly to clinical illnesses such as von Willebrand disease, the most common hereditary blood clotting disorder. To detect the presence of inter-domain regulation within vWF, we investigated the interaction of platelets with the isolated A1, A1A2A3 and D’D3A1A2A3 domains of vWF under flow conditions. Ristocetin Induced Platelet Aggregation (RIPA) assays showed that isolated A1 and A1A2A3 inhibit platelet aggregation in solution, corroborating evidence that isolated A1 is sufficient for platelet interaction. In addition, ELISA assays demonstrate similar surface absorption for both isolated vWF domains and multimeric vWF. While we have recovered shear-dependent platelet adhesion to multimeric vWF, we have failed to recover specificity in domain-platelet interactions. This is indicative of either an occluded platelet (GP Ib) binding site upon surface absorption or the improper refolding of our isolated domains.

Incorporation of DNA Particles into Chitosan Nanofibers for Localized Tissue Regeneration

Johanna Hayenga, Junior, Bioengineering, University of Washington
Mentor: Ashleigh Cooper, Materials Science and Engineering
Mentor: Miqin Zhang, Materials Science and Engineering
UWEB-REU

Controlled DNA delivery to a localized target is an important tool for tissue regeneration. A problem associated with controlled DNA delivery is that naked DNA may degrade, causing a loss of function and decreased control of the release rate. Encapsulation of the DNA may protect it from such degradation. A sufficient DNA delivery vehicle and incorporation method are necessary for delivery of DNA to a localized target. DNA encapsulation was performed by mixing with a cationic polymer, polyethyleneimine, which surrounded the negatively charged phosphate backbone of the DNA via charge neutralization. A DNA delivery vehicle of a chitosan-based nanofibrous scaffold was investigated. Nanofibers have been investigated to possess high surface area and high porosity which maximizes cell interaction and tissue regeneration. Chitosan, a biodegradable and biocompatible polymer, was mixed with polyethylene oxide and nanofibers were prepared through an electrospinning process. DNA-PEI particles were mixed into the chitosan based solution and applied for the electrospinning. To this end, encapsulated DNA was successfully incorporated into the chitosan-based nanofibers. Further investigation of the degradation of the nanofibrous scaffolds and well as the DNA function is recommended.
Parametric Investigation of Picoliter Droplet Interfacial Tension

Joel Herness, Senior, Chemistry, University of Washington
Mentor: Daniel Chiu, Chemistry
Amgen Scholars Program

The study of microfluidic droplets as microscale reaction systems has been essential to the study of subcellular biochemistry. To better understand the droplet formation process, a thorough understanding of interfacial tension (IFT) dynamics is necessary. This project seeks to correlate changes in IFT as a function of oil type, ion concentration, surfactant concentration and surfactant type. System conditions were varied and measurements of the IFT were made by flowing droplets through a microfluidic channel constriction and observing the deformation and relaxation. Fast imaging techniques were used to gather images which were then analyzed using software coded with the program Labview. We expect to see a positive correlation between oil viscosity and IFT and a negative correlation between both surfactant concentration and ion concentration and IFT. These results should provide general trends for IFT as system variances are made, allowing future researchers to anticipate necessary changes in microfluidic geometries as the system being investigated changes.

A Fluorescence-Based Assay for Investigating Intracellular Trafficking of Gene Carriers

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Mentor: Suzie Pun, Bioengineering
UWEB-REU

Gene therapy is a promising therapeutic technique that could be used to treat diseases such as cancer and hemophilia by correcting abnormal cell function at the genetic level. Currently, the majority of gene therapy clinical trials utilize virus-based gene carriers due to their high efficiency of gene transfer. However, virus-based carriers can cause insertional mutagenesis, illicit immunogenic responses and are difficult to manufacture. Nonviral gene therapy is a safer alternative with the added benefits of being cost effective and easy to manufacture in large quantities; however current nonviral delivery systems are limited by low transfection efficiency. Endosomal escape has been shown to be a major barrier to the delivery of DNA to the nucleus. Quantitative measurements of endosomal escape would help identify inefficiencies of current nonviral gene therapy agents and would allow for the development of rationally designed delivery vehicles. We investigated two commonly used, commercially available nonviral delivery systems: Lipofectamine 2000 (L2K) and polyethylenimine (PEI). DNA was labeled with highly fluorescent 7-nitro-2,1,3-benoxadiazol-4-yl (NBD), which can be quenched by the membrane impermeable reducing agent sodium dithionite. We investigated the dithionite reduction of L2K and PEI complexed DNA in three cell lines: HeLa, NIH3T3, and CHO-K1. It was found that CHO-K1 cells
have the least background autofluorescence in the relevant wavelengths. Triton X-100 was used to disrupt the cell membranes to show that the NBD fluorescence could be quenched to background level. The development of this method would allow for a better understanding of the intracellular distribution of different nonviral delivery vehicles.

**Home Monitoring of Drug levels in Saliva Using a Microfluidic System**

*Benedict Hui, Senior, Bioengineering, University of Washington*

*Mentor: Paul Yager, Bioengineering*

*Mentor: John Miller, Neurology*

*CREE*

Currently, the method of TDM (therapeutic drug monitoring) is via blood samples. This results in infrequent use for outpatients due to the need for phlebotomy, and the need for the patient to visit a doctor. The current method is also slow, with the delay between blood sampling and determination of drug levels taking as long as 48 hours. The overall result of this is improper drug dosing, and thus an increase in morbidity and mortality. This problem can be solved by moving the TDM from the laboratory to the home. This process must therefore become automated, inexpensive, convenient, and use a sample that can be easily and willingly obtained by the patients themselves. A practical home TDM system based on saliva would have the potential to change how TDM is done, and also improve the quality of healthcare for tens of millions of people in the US.

**Genetic diversity in the interior introns of PfCRT in chloroquine-resistant (CQR) *P. falciparum* from Kilifi, Kenya**

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*Mentor: Carol Sibley, Genome Sciences*

*Amgen Scholars Program*

Chloroquine resistance in the malaria parasite *P. falciparum*, responsible for increased malaria mortality worldwide, is coded by mutations in the gene PfCRT. Previous research has shown little diversity among genetic markers flanking PfCRT across samples of CQR *P. falciparum* due to heavy chloroquine selection pressure in the past. Because they have remained homogenized, these current markers do not appear to reflect evolution occurring since the last chloroquine sweep; in the current context where CQR is well-established, new markers with gene diversity representing ongoing evolution are needed. Promisingly, DaRe et al. (2006) reported elevated diversity in newly-discovered interior intronic markers within PfCRT in samples from Papua New Guinea – diversification in interior introns that is unrepresented in traditionally-monitored markers flanking the gene. To test DaRe et al.’s findings in a geographically distinct population, we aimed to assess the diversity of interior intronic markers in PfCRT relative to flanking markers in 27 patient blood samples from Kilifi, Kenya. The markers are microsatellites (MS), stretches of DNA repeats which mutate only by adding
or deleting repeats, and so different polymorphisms of the interior intronic markers were conveniently discerned by length. Agarose gels and an automated capillary sequencer were used to measure the lengths of the PCR-amplified interior intronic MS, and gene diversity (H) across the samples calculated per intronic region. The results suggest elevated diversity in the interior intronic regions that is comparable to those suggested by DaRe, corroborating his observations even given the geographical difference and differences in local CQR alleles. H is currently being calculated for the flanking markers of the Kilifi samples to provide a direct comparison between the markers in the interior introns vs. those flanking the gene. In further work, the diversity of progressively older samples might be examined to confirm these changes as truly representing increasing intronic diversity following the last chloroquine sweep, and thus their potential value in monitoring continued evolution of PfCRT.

**Parametric Investigation of Picoliter Droplet Interfacial Tension using a Microfluidic Device**

*Kevin Kelley, Junior, Chemistry and Physics, Pomona College  
Mentor: Gavin Jeffries, Chemistry  
National Nanotechnology Infrastructure Network REU Program*

The transport and detection of nanoscale objects has become an essential part of sub-cellular biochemical research and with it the use of droplets as controllable confined volumes. To fully understand and utilize the chemical environment of droplet systems, it is necessary to elucidate the physical properties of the droplet interface, namely through studying the interfacial tension. The capillary number (Ca), which depends on interfacial tension, is an important physical factor for designing and calculating fluidic dynamics in microfluidic droplet systems. This project seeks to investigate the Ca by measuring changes in interfacial tension as a function of the following factors: ion concentration, surfactant type, surfactant concentration, and oil type. The interfacial tension of numerous individual droplets was measured by examining the deformation and restoration dynamics of the drops under extensional flow. Droplet generation parameters were independently varied and results were captured using fast imaging techniques. This video data was analyzed using a mathematical model of droplet dynamics, coded with the program Labview, which calculated the interfacial tensions. This project will attempt to further clarify the quantitative relationship among the key factors, and how they govern droplet formation when used in microfluidic devices for bio-analytical applications.

**Fabricating Sphere-templated Chitosan-alginate Scaffold for Replacement Human Digit Tissue Engineering**

*Arnold Kim, Junior, Bioengineering, University of Washington  
Mentor: Christopher Allan, Orthopedics and Sports Medicine  
Mentor: Buddy Ratner, Bioengineering  
CREE*
Digit and limb loss are frequent and debilitating healthcare problems. Current
Treatment options for finger loss are very limited and each has its set of signifi-
cant complications: replantation in selected cases, implanting non-biological
prosthesis, or toe-to-hand transfer. The ultimate goal of this project is to design
an implantable, multi-scaffold construct for tissue-engineering a replacement
digit for finger amputees. In the duration of the CREE grant, much time and ef-
fort has been put into manufacturing chitosan-alginate scaffold using the sphere-
templated approach. Chitosan-alginate has been shown to be a good scaffolding
material for cartilage and bone tissue engineering. Although chitosan-alginate
scaffolds can be readily prepared using the lyophilization method, it is extremely
difficult to control pore-sizes with this method which may result in suboptimal
healing characteristics. A chitosan-alginate scaffold prepared using the sphere-
template approach will allow precision control over pore-sizes which may result
in a superior healing property in comparison to the conventional chitosan-algi-
nate scaffold. Once the sphere-templated scaffold with desirable characteristics
is prepared, the healing properties of the sphere-templated and the conventional
scaffolds will be compared via a subcutaneous implant study in wild-type mice.

**Rapid Synthesis of Silver Nanowires**
*Kylee Korte, Junior, Chemistry, Bradley University*
*Mentor: Sara Skrabalak, Chemistry*
*National Nanotechnology Infrastructure Network REU Program*

The presence of various ions has been shown to influence the shape and size
of metallic nanostructures produced via the polyol method. A study of copper
salts has shown that the presence of copper(II) chloride in the polyol reduction
of silver nitrate allows for the production of silver nanowires. Silver nanowires
have applications in many areas, including electronics and catalysis. These wires
are produced quickly (in approximately one hour), and the synthesis may be eas-
ily performed in disposable glass vials and using pipettes to deliver reagents. In
this synthesis, silver nitrate is reduced in the presence of poly(vinylpyrrolidone)
(PVP) and copper(II) chloride. PVP acts as a capping agent. The role of
copper(II) chloride is under investigation, but results from controls indicate that
both copper(II) and chloride ions are necessary to synthesize the wires. Electron
microscopy has been used to characterize the wires.

**Characterization of Culture-derived Megakaryocytes from Cord Blood
CD34+ Hematopoietic Progenitors**
*Hoyin Lai, Senior, Bioengineering, University of Washington*
*Mentor: JoAnna Reems, Medicine*
*Mentor: Diana Gilligan, Medicine*
*Mentor: Shahram Vaezy, Bioengineering*
*CREE*

Megakaryocytes are produced in vivo in the bone marrow from hematopoi-
etic progenitors, which express the CD34+ antigen. Populations of CD34+ hematopoietic progenitor are not only found in the bone marrow, but can also be isolated from umbilical cord blood (UCB). The purpose of this study was to characterize the in vitro generation of megakaryocytes from UCB CD34+ cells after culturing the progenitors in serum-free media with cytokines for 10-15 days. Development of megakaryocytes from CD34+ progenitor cells was characterized by performing total nucleated cell (TNC) counts, colony-forming unit assays, and immunophenotyping. The TNC count expands by two orders of magnitude from Day 0 to Day 10. Immunophenotyping shows decrease in CD34 antigen expression from an average of 93.2% ± 7.4% (n = 2) to 11.9% ± 0.8% while expression of megakaryocyte lineage markers CD41, CD42a, and CD61 increases to 52.1% ± 26.2%, 49.1% ± 34.1%, and 45.6% ± 28.4% respectively, and further to 61.1% (n = 1), 57.0%, and 82.5% by Day 15. In the meantime, colony-forming assays show decrease in number of colonies and non-mega-karyocytic colonies. These combine to show increased differentiation of CD34+ cells into the megakaryocyte lineage and decreased clonagenic potential of committed cell lines. This will aid future studies in optimizing conditions for megakaryocyte proliferation ex vivo; and will further enable studies to produce functional and transfuseable platelets to treat thrombocytopenia.

Msx-1 expression in cells isolated and cultured from human fetal digits
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Mentor: Christopher H. Allan, Orthopaedics and Sports Medicine
UWEB-REU

Digit regeneration is possible in humans, but is restricted to a very distal level of amputation and a young age. Digit tip cells beneath the nail unit showing Msx-1 expression are closely associated with digit regeneration. These cells appear at the site of regrowing tissue after tip amputation in fetal digits. In this study, cells from human fetal digits were cultured to assess cell growth and Msx-1 expression. Distal digit tips ranging from 53-59 day estimated gestation age (EGA) were used. Cells were dissociated from the digits and plated for culture. Rapid and vigorous cell growth in vitro was noted. Following establishment of successful cell culture, these populations were immunostained to determine whether the regeneration-associated Msx-1 expressing cells were represented. Significant binding of antibody was observed, suggesting either that the culture conditions favor growth of the desired population of Msx-1 expressing cells or that Msx-1 expression is upregulated in response to cell culture. This is the first report, to our knowledge, of the culture and characterization of human fetal digit-derived cells. Further characterization of these isolated cells is necessary. If these cells represent a form of digit tip stem cell as defined by Msx-1 expression, delivery of these cells to wounds may promote regeneration rather than repair.
The Escherichia coli lac repressor (LacI) is a DNA-binding protein that regulates the production of proteins involved in lactose metabolism. Permissive sites within LacI have been previously identified. Short sequences can be inserted at these sites without affecting the normal function of the protein. The sequence for the inorganic silica binding motif, QBP3, was inserted into a permissive site residue 33 of the E. coli lac repressor, endowing LacI with the ability to bind both DNA and inorganic silica. After PCR screening for the QBP3 insert six candidates were sequenced and two were chosen for continued characterization. Western blot analysis of these constructs showed good protein expression, and β-galactosidase assays indicated LacI clones maintain normal function. The constructs showed some binding to fine silica and minimal binding to coarse silica. These analyses suggest the insertion at residue 338 binds less favorably to silica than the previously successful insertion at residue 317. Engineered proteins like these, that bind both DNA and inorganic compounds, can be utilized to arrange nanostructures in predictable patterns, using DNA as a scaffold.

Quasi-living Synthesis of Semiconducting Polymers
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Mentor: Christine Luscombe, Materials Science
Hooked on Photonics STC-MDITR

Scientists have taken an interest to organic semiconducting polymers (OSPs) and their applications ever since their discovery in the late 1970s. In more recent years, conducting polythiophenes have shown promising prospects for use in economical all-polymer transistors, nanooptical and nanoelectronic materials, and highly conductive plastics. However, scientists oftentimes have little control over the production of OSPs due to their complex mechanism during formation. OSPs are usually made by polycondensation reactions which typically eliminate small molecules during synthesis. Furthermore, the step-wise nature of the reaction contributes to the slow rate of attaining a high molecular weight. Because of both the elimination and the step-wise mechanism, homogeneous polymers formed by polycondensation reactions tend to be difficult to attain. If OSPs could be synthesized in a quasi-living manner, then the reactive site could be kept intact. Additionally, there would be improved control over the morphology and property of the polymer thin films, which would help to improve the performance of electronic devices. Starting with fluorene, we have been working to improve the process of synthesizing OSPs in a controlled manner. Because changes in molecular structure inevitably affect the visible and functional properties of the material, a controlled chain-growth polymerization would allow for the opportunity to influence the creation of new materials.
Creating an altered specificity pair of a tumor suppressor protein and associated enzyme

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Mentor: Devin Christensen, Biochemistry
Amgen Scholars Program

BRCA1, a breast and ovarian tumor suppressor protein, is implicated in the recruitment of repair machinery to sites of DNA damage. Protein ubiquitination is the post-translational covalent attachment of the small peptide ubiquitin, and regulates a multitude of cellular processes. A trio of enzymes is responsible for ubiquitination. E1 activates ubiquitin, ubiquitin conjugating enzyme E2 delivers it, and ubiquitin ligase E3 covalently attaches the tag to the targeted protein. BRCA1, one of thousands of human E3s, associates with many E2s including the human E2 examined here. The promiscuity of BRCA1 and the E2 necessitates isolating the pair to find their target. To perturb its interaction with E2s, selected BRCA1 residues were mutated. A library of randomly mutated E2 variants was then generated and screened, using the yeast two hybrid assay, for regained ability to bind the BRCA1 mutants. The discovery of an altered-specificity pair will be helpful in elucidating the BRCA1-mediated DNA-repair mechanism.

Synthesis and Characterization of Oligoacenes for Organic Electronics and Optoelectronics

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Organic semiconductors are currently of great interest for applications in organic electronics, such as thin-film field-effect transistors, photovoltaic cells, and light-emitting diodes. Organic field-effect transistors offer a potential low cost alternative to inorganic semiconductors. Among the well studied p-type organic semiconductors are oligoacenes, exemplified by pentacene. Oligoacenes, and particularly pentacene, have resulted in impressive performance from both thin-film and single-crystals transistors and are considered as a benchmark for OFETs. Development of new building blocks based on oligoacenes is essential to optimizing the performance of organic electronic devices and investigate the correlation between structure-property. In this study, oligoacene-based organic semiconductors 5,12-dihydroquinoxalino[2,3-b]phenazine (1), a mixture of 1, 2- Dithia–cyclopenta[fg]napthacene and Napthaceno[5,6-c,d] -1,2-dithiole (tetracyanoquinodimethane) (2), and 1,2,3,4-tetrachloro-benzo(b)phenazine (3) were synthesized. Oligoacene (1) was obtained by a condensation reaction in
poly(phosphoric acid) (71%). Compounds (2) were synthesized via substitution reaction in dimethylformamide and oligoacene (3) was obtained in a condensation reaction in chloroform (58%). The molecular structures were confirmed by 1H NMR, UV/PL/CV, and GC-MS. The absorption maximum for (1) was 522, while (3) displayed absorption maximum 520 nm. Photoluminescence shows an orange emission for (3) with emission maximum at 605 nm, while a red emission was observed for (1). Field-effect transistors will be fabricated to explore their initial electronic properties.

**Effects of Octadecanethiol on CdSe Quantum Dot Photoluminescence**

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Quantum dots are semiconductor nanocrystals with high photoluminescence efficiencies and narrow, size-tunable photoluminescence. They are promising candidates for use in applications such as light-emitting diodes. Due to their small size, quantum dots have a high surface-to-volume ratio, and their properties are sensitive to their surface chemistry. Understanding the effects of surface chemistry on quantum dot photoluminescence and how specific ligands bind to quantum dots surfaces is important to tailor the properties of quantum dots for specific applications. We study ligand binding to CdSe quantum dots by measuring changes in the photoluminescence intensity and lifetime of the quantum dots as a function of octadecanethiol concentration in solution. We will fit the thiol titration data to a model by Tachiya, in order to determine the thiol adsorption binding constant and the average number of binding site per quantum dot.

**Creation of a Soluble Polymer for an Organic P-N Conductor**

*Kyle Lynch-Klarup, Sophomore, Physics and Religious Studies, Grinnell College*

*Mentor: Meghana Rawal, Chemistry*
*Mentor: Glenn Bartholomew, Chemistry*
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Organic semiconductors are rapidly gaining interest in both the scientific and commercial sectors as a replacement for their pre-existing inorganic counterparts because of their potential for cheap manufacturing costs and easy creation. Self-assembling light-emitting electrochemical cells (LECs) with a fixed charge distribution have already been designed. However, these LECs required polyethylene oxide (PEO) to keep the emissive polymer and ion-pair monomers from separating when placed into solution. This complicated the device and led to lower than desired yields. We have been working on developing an emissive polymer with the PEO directly attached to simplify the device and improve its quality. Various versions are being synthesized in an attempt to create a polymer.
soluble with the ion-pair monomers.

Analysis of Suppressive Regulatory Elements of the mouse Muscle Creatine Kinase gene in striated muscle

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Mentor: Stephen Hauschka, Biochemistry
Mentor: Phillip Tai, Biochemistry
Amgen Scholars Program

This study investigates the mechanisms by which the gene Muscle Creatine Kinase (MCK) is transcriptionally regulated. MCK, which is abundantly expressed in all striated muscle, is an ideal model for studying muscle gene transcription. MCK has three known regulatory regions: 1) the proximal promoter, 2) the upstream enhancer, and 3) the poorly characterized Modulatory Region 1 (MR-1). Recent studies have shown that MR-1 contains a 95-bp sequence that is a potent enhancer for muscle specific gene transcription. We have now identified another highly conserved 161-bp sub-region of MR-1 called MR-1A. When MR-1A is removed from an expression construct containing MR-1 and assayed in cultured mouse skeletal muscle cells, a two-fold enhancement in transcriptional activity is observed; suggesting that MR-1A represses gene transcription. We predicted that MR-1A may also suppress the powerful upstream enhancer, due to the common elements found in the upstream enhancer and the 95-bp enhancer. However, placement of MR-1A in conjunction with the upstream enhancer, does not result in a significant repression of transcription. This study suggests that the suppressive nature of this unique region specifically represses the 95-bp enhancer of MR-1 and does not repress other positive MCK regulatory regions. Importantly, this finding suggests that functional differences between transcriptional activities of the upstream enhancer and the 95-bp enhancer in MR-1 may be due to the sensitivity of the MR-1A suppressor region to developmental, nutritional, or environmental factors. Further studies are necessary to identify the DNA elements within MR-1A and the transcription factors that bind these regions to fully characterize this complicated mechanism.

Design for ‘X’

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Mentor: Sashi Komandur, Industrial Engineering
Intel Research Experience

In the past, products and systems were designed solely to fulfill a need, with little or no consideration to other design factors. Product functionality was the primary concern of designers until just recently. In the 1990s many studies emerged that analyzed the effects of new design philosophies that took other factors into consideration. These studies are collectively described as “design for x” (DFX). The “x” can stand for any criterion, with some popular ones being “manufactur-
ability,” “assembly,” “affordability,” “reliability,” “disposability/recyclability,” and “life cycle.” By employing the design guidelines of DFX, companies will likely improve their product quality and also see a reduction in cost. This paper presents the concepts and guidelines of several different DFX philosophies as well as the potential benefits of incorporating them.

**Development of a Mathematical Model of Glioma Tumorigenesis and Evolution Mediated by Platelet-Derived Growth Factor (PDGF)**

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Amgen Scholars Program*

Gliomas are the most prevalent type of primary brain tumor in adults. Despite all possible treatment attempts, including aggressive surgical resection, these tumors are uniformly fatal. This is due in part to glioma’s highly diffuse invasion of the surrounding normal brain well below the threshold of detection for clinical imaging. Dr. Peter Canoll at Columbia University has demonstrated that rats develop brain tumors that resemble human gliomas when their corpus callosum are injected with a retrovirus expressing platelet-derived growth factor (PDGF). Most notably, 17 days post infection only 30% of the tumor cells are infected glial progenitor cells—the other 70% are malignant non-infected glial progenitor cells, recruited to the tumor by interactions with PDGF. We used this result to guide our development and analysis of two distinct mathematical models of the complex interactions between infected progenitors, recruited progenitors, other progenitors, and the remaining normal cells over time and space. Of the two models investigated, one includes a population of progenitor cells that have neither been infected nor recruited and a recruitment parameter, while the second lets recruitment depend upon the concentration of infected cells at each time point. Our preliminary work with the model focused on exploring parameter values to obtain accurate representation of the experimental rat model data. Only the first of the two models we created empirically reflects the data, and a sensitivity analysis of our parameter space suggests that, assuming equal diffusion rates for all cell populations, the doubling time for the recruited progenitor cells must be greater than (roughly twice) that of the infected progenitors, contrary to what we expected. We anticipate that further analysis of our model and additional data from upcoming rat experiments will help us determine the reasons for these surprising results.

**Alternative Methods for Collecting and Analyzing data from Surface Plasmon Resonance and Fluorescence Technology**

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Hooked on Photonics STC-MDITR*

During the recent decades there have been a great number of uses found for
Surface Plasmon Resonance and Fluorescence technology, with the most interest being focused on biological sensors and chemical detection. Both aspects of this technology could provide great insight into providing the correct post-devastation relief, such as following a hurricane, tsunami, or flooding. The major concern is the large amounts of food and water contamination that follows these events. This technology provides a way of detecting and monitoring such things, but due to the delicacy and expense of the equipment, it is not practical. This research will introduce innovative spectrometer designs and techniques for the collection and analysis of data from different system setups. The main focus is to remove the expensive optical arrangement and provide an equivalent electronic circuit, at a much lower cost and smaller size. These changes will provide the reliability and accuracy of the current arrangements, but will allow for greater mobility to the user, and will be of great use in many different environments.

Localization of Epileptic Seizure Onset via High-Density Non-Invasive Scalp EEG

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Mentor: Ceon Ramon, Electrical Engineering
Mentor: Mark Holmes, Harborview
Mentor: Eric Chudler, Bioengineering
CREE

Current methods for localizing epileptic seizure onset areas within the brain are highly invasive and involve the placement of intracranial electrodes followed by waiting for one or more seizures to take place while recording an electroencephalogram (EEG). Clinicians can then use the recorded information to determine the location of interest. Recently, Monto et al. (2007) used detrended fluctuation analysis (DFA) to uncover a relationship between long-term temporal correlations in intracranial EEGs taken during interictal sleep and the locations determined to be the onset sites of epileptic patients using traditional methods. This implies that it may be possible to determine the location of probable seizure onset without the requirement that a patient endure a seizure. This project attempts to replicate the results of Monto et al. using high-density scalp EEG recordings, which are entirely noninvasive. Replicating the results would mean that clinicians could localize epileptic areas of interest within a patient’s brain non-invasively and without the patient enduring a seizure, an advance that would greatly benefit the diagnosis of epileptic patients. Future work will be to move beyond the DFA techniques used so far to include phase information from the recorded EEGs to better localize areas of interest and provide additional diagnostic insight into the onset of the epileptic seizures.
Summarization and Optimization of the Modified-Hachinski Ischemic Score
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Mentor: Xiao-Hua Andrew Zhou, Biostatistics
Mentor: Nathaniel Mercaldo, National Alzheimer’s Coordinating Center
Amgen Scholars Program

Vascular Dementia (VaD) is the second leading cause of dementia in the United States. Currently, interest exists in distinguishing VaD from Alzheimer’s Disease (AD) and one of the most popular tools for distinguishing these patient groups is the modified-Hachinski Ischemic Score (m-HIS). Our research sought to describe the distribution of m-HIS across clinical diagnosis groups and find the variables in the m-HIS which would improve the classification of VaD. The data used in the study came from the National Alzheimer’s Coordinating Center, the data repository for 29 Alzheimer’s Disease Centers from across the United States, which used a standard set of forms, the Uniform Data Set. Boxplots and frequency distributions were used to describe the distribution of the total m-HIS total and item scores, respectively. Sensitivity and specificity were estimated for each possible total m-HIS score thus resulting in a receiver operating characteristic (ROC) curve; these estimates were also calculated for each individual item. The last portion of this study implemented three model building methods, all subsets logistic regression, classification trees, and random forests. The initial boxplot displayed that definite distinction between diagnostic groups could be identified. ROC analysis was performed on the total m-HIS and it was found that the optimal cutpoint should be 2. The model building methods indicated that history of stroke is the most important variable in distinguishing AD patients from VaD when the diagnosis is based on clinical measures. These results indicate that history should be the primary tool for classifying VaD and that history of stroke and history of hypertension discriminate as well as the total m-HIS score. However, our future work should include neuropathological diagnosis and mixed forms of dementia (VaD + AD) to provide more generalizable results.

Atomic Force Microscopy Characterization of Cross-Specificity of AuBP1 on Four Inorganic Surfaces
Ankita Mishra, Sophomore, Chemical Engineering (Biomolecular), California Institute of Technology
Mentor: Christopher So, Materials Science and Engineering
Mentor: Hanson Fong, Materials Science and Engineering
Mentor: Mehmet Sarikaya, Materials Science and Engineering
GEMSEC REU Internship

Molecular biomimetics is an emerging research field inspired by nature’s unique ability to fabricate materials and functioning devices precisely controlled by protein recognition and assembly. Combining the knowledge of specific interactions between proteins and associated materials synthesized with molecular
biology techniques to manipulate and replicate desired proteins, functional materials can be fabricated and controlled at the nanometer scale. Techniques have been developed to engineer peptides that selectively interact and bind to certain inorganics. A protein that binds specifically to gold has applications as a "messenger protein" that guides biomaterials within the body, for patterning and bifunctionalizing of surfaces, and in producing biosensors. The cross-specificity – or ability to bind to unselected materials – of Au-binding peptide (AuBP1), a peptide genetically engineered through cell-surface display and showing strong gold binding characteristics, was studied by atomic force microscopy (AFM). The goal was to compare the binding characteristics of AuBP1 on gold and other solid, inorganic materials. Experiments were done to examine the strength and quality of binding of AuBP1 to highly ordered pyrolytic graphite (HOPG), muscovite mica, and silicon wafer. Analysis of AFM scans showed over 50% more binding to gold than to mica, HOPG, or silicon. Results demonstrated that AuBP1 exhibited high specific affinity for gold. Future experiments will involve similar binding studies with these cross-specific surfaces to see if surface saturation can be attained, as well as studies with other metals and ceramics. Studies of AuBP1 binding to HOPG, especially, will also continue as even minimal binding to HOPG suggests the peptide’s ability to “intelligently” recognize the physical orientation of surfaces.

Molecular dynamics analysis of PrPC and PrPSc structure including cross-species variations

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*Mentor: Valerie Daggett, Medicinal Chemistry*
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*Amgen Scholars Program*

The prion protein (PrP) is an ubiquitous protein that is linked to transmissible spongiform encephalopathies (TSEs) such as mad cow disease and Creutzfeldt-Jakobs disease. Research indicates that infection is caused by aggregates of misfolded cellular PrP. This conversion is thought to occur in vivo in cellular compartments with low pH, and to vary across species, leading to what is known as the species barrier. To gain an understanding of the mechanisms of disease progression and toxicity, it is thus necessary to study the structure of both cellular PrP (PrPC) and converted PrP (PrPSc) in a variety of species. We have used molecular dynamics (MD) simulations to study early intermediates of converted PrP structures in both human and Syrian hamster, focusing specifically on acidic, basic, and polar amino acid contacts within the protein. To induce conversion, a low pH environment is simulated, and comparisons are made of residue contacts between converted structures and control simulations run at neutral pH. Additionally we have examined contact differences between human and Syrian hamster PrP to gain insight into interspecies infectivity.
New Observations of the Air-Water Interface

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UWEB-REU

The air-water interface is a ubiquitous chemical, physical, and biological entity that is surprisingly poorly understood. Many studies suggest that liquid water at the interface may maintain an ice-like surface structure at temperatures well above its freezing point, though the depth of the structured region is unknown. An “exclusion zone” has been observed at the air-water interface in which polystyrene-latex microspheres tend to migrate away from the interfacial region. This microsphere-free zone appears to have similar properties to the previously studied exclusion zone that forms next to various hydrophilic surfaces. Reported here are various properties of the microsphere-free region at the air-water interface. The exclusion zones next to hydrophilic surfaces and at the air-water interface form regardless of microsphere size, functionalization, or surface charge. Adding solutes decreases the rate of formation of the exclusion zone, but does not necessarily prevent it from forming. The presence of free radicals in solution does not significantly affect exclusion zone formation. Basification of the water can completely prevent the formation of the microsphere-free region at high concentrations, but at concentrations less than 1 mM NaOH the hydroxide anions greatly enhance the rate of exclusion zone formation. Perhaps the most interesting finding at the air-water interface is the “tent phenomenon.” This occurs when a capillary tube is touched to the surface of the water above a microsphere-free region and thousands to millions of water layers beneath the capillary tube, the microspheres are pulled up. The fact that the microspheres beneath the microsphere-free region are pulled up suggests that there could be vertical structuring within the microsphere-free region. This, in turn, could provide evidence that the water at the air-water interface is more extensively structured than in bulk. The mechanism for this structuring is largely unknown, and physical properties of this exclusion zone require further elucidation.

Characterization of Plasma Polymerized Immunosensor by XPS, SPR and AFM

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CREE

Surface treatment of well plate with plasma polymerized acrylic acid (ppAA) has been used to fabricate an antibody array. This study examines both the amount and the bioactivity of the immobilized antibody. A comparison of ppAA – based strategy to the traditional physical adsorption of antibodies was done using X-ray photoelectron spectroscopy (XPS) and surface plasmon resonance (SPR). XPS results for the ppAA surfaces indicate that 1) ppAA can be depos-
Adaptive Evolution of Mouse Seminal Proteins

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Male seminal fluid proteins have numerous effects on reproductive responses, including female behavior modification, pathogen defense, and sperm storage. Due to competitive interactions between these proteins and those in the female reproductive tract, it is highly likely that strong pressures for positive (as opposed to purifying) selection will occur, resulting in rapid adaptive evolution. A set of mouse prostate proteins potentially under positive selection was selected for analysis of non-synonymous (changing protein sequence) versus synonymous (silent) mutations between mouse and rat, generating a dn/ds ratio. A dn/ds ratio above 1.0 is indicative of positive selection, which has been shown in genes involved with immunity, reproduction, chemosensation, and toxin degradation. These DNA sequences were then PCR-amplified using primers designed to function in both mouse and rat, and template DNA from six mouse species: Mus m. domesticus, Mus m. musculus, Mus m. castaneus, Mus spretus, Mus caroli, and Mus pahari. Together these species provide a comprehensive sampling of the Genus Mus. The same genes in Rattus norvegicus were also used as outgroup sequences. Sequencing of these PCR products allowed for construction of multiple alignments of orthologous protein-coding sequences. These alignments will be analyzed to determine whether these genes are evolving as predicted by neutral, positive, or negative models. The program codeml will be used for this analysis, as it allows for identification of specific amino acid residues under positive selection. Since disease genes are often associated with positive selection, the results should provide insights into the mechanisms of disease evolution, as well as clarify which reproductive genes are most functionally important.
The differentiation of group B streptococcus serotypes using ToF-SIMS and XPS

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UWEB-REU

The ultra high vacuum surface analytical techniques Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) and X-ray Photoelectron Spectroscopy (XPS) are extensively used in semiconductor and biomedical research, but only limited cases where these techniques are applied to the analysis of single cells. In this work, the surface of group B streptococcus (GBS), also referred to as streptococcus agalactiae, is examined. GBS is the most common cause of bacterial septicemia, pneumonia, and meningitis of neonates in the United States. The surface of GBS is well characterized by standard microbiological techniques. For this reason, a direct comparison can be made between the known surface structures by ToF-SIMS and XPS. The surface of GBS is layered with a capsule composed of 5 different polysaccharides (glucose, galactose, N-acetylneuraminic acid, N-acetylglucosamine, and rhamnose), which makes up 10 to 30% of the dry weight of the microorganism. In this work, the five polysaccharides, pure capsule from type III GBS, and UV killed GBS strains COH1 and COH1-13 were investigated. Principal component analysis was used to determine representative peaks from the pure polysaccharides. Atomic Force Microscopy was used to verify the presence of GBS on the surface. XPS was used to determine the surface elemental composition for all sample types. Amino acid and polysaccharide fragments were identified in the ToF-SIMS spectra of the GBS samples, and it was found that high mass peaks around 630 dalton were responsible for the greatest difference between the two strains.

CdSe Quantum Dot Photodiodes

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National Nanotechnology Infrastructure Network REU Program

The emission and absorption spectra of CdSe quantum dots are highly tunable making this one of the most studied nanocrystalline semiconductors. TiO2 is widely used as an anode material in photovoltaic cells due to its superb performance as a charge carrier. Therefore, a CdSe/TiO2 coupled system is expected to be an efficient and tunable photoanode. In the past, CdSe/TiO2 systems have been grown from their precursors on a substrate. Such methods yield a densely packed, well-connected nanocrystalline structure, in which the CdSe absorbs photons, while the TiO2 network acts as a charge transfer system. However, the size and shape of the CdSe quantum dots cannot be precisely controlled. In contrast, direct deposition of as-prepared quantum dots on TiO2 coated sub-
strates fail due to poor CdSe-TiO2 coupling. Here, we demonstrate an alternative method to assemble an effective photoanode using colloidal CdSe quantum dots and an intermolecular linker, 3-mercaptopropionic acid. The thiol group on the linker binds to the CdSe quantum dots, while the carboxylic acid group attaches to the TiO2 network. With this approach, photocurrents up to 0.5mA were detected under room light in simple photodiode device configurations. In addition, the diode photocurrent action spectra closely resembled the absorption spectrum of the colloidal CdSe quantum dots, indicating CdSe-TiO2 coupling.

**Needle “Microscope” for Optical Biopsy of the Pancreas**

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*Mentor: Xingde Li, Bioengineering*

*Mentor: Joo Ha Hwang, Gastroenterology*

*CREE*

A needle imaging probe is being developed to allow minimally invasive, highly accurate diagnosis of pancreatic cancer. Optical Coherence Tomography (a type of laser imaging) will be used to take microscopic images from the tip of an optical fiber inside a hypodermic needle. Using this probe will give doctors enough information to make a diagnosis without introducing the risks of a major biopsy. This is important because pancreatic cancer is almost always diagnosed after it has advanced to an incurable stage. Better diagnostic technology will lead to earlier diagnoses and more cured patients.

**Specific Ablation of Regulatory T cells Induces Activation and Expansion of Myeloid Antigen Presenting Cells in Non-lymphoid Organs**

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*Mentor: Alexander Rudensky, Immunology*

*Amgen Scholars Program*

Regulatory T cells (Treg) are a subset of CD4+ T lymphocytes that suppresses the body’s immune responses against self-antigens, thereby preventing autoimmune diseases. Our laboratory has identified the X-chromosome-encoded transcription factor Foxp3 as a Treg lineage specification factor and a definitive marker for these cells (J. Fontenot et al., Nat Immunol. 2005). However, molecular understanding of Treg’s suppressive function and the mechanisms by which Treg control immune responses remain unclear. Recent microscopy studies have suggested that the interaction between antigen-presenting cells (APC) and Treg is central to the regulation of immune response. In support of this, myeloid APC including dendritic cells and macrophages increase in absolute numbers in the lymph nodes and spleen of Foxp3DTR mice subjected to toxin-induced Treg ablation. In these “knock-in” mice, DNA sequence encoding the human diphtheria toxin receptor (DTR) was inserted into the Foxp3 locus. Specific ablation of the Treg population can be induced in Foxp3DTR mice by acute diphtheria toxin
treatment (J.M. Kim et al., Nat Immunol. 2006). By taking advantage of this model, our study aims at understanding the mechanism(s) by which Treg control the response of myeloid antigen-presenting cells. We examined the status of myeloid cells in secondary lymphoid and non-lymphoid organs after inducible ablation of Treg in Foxp3DTR mice. Near complete elimination of Treg resulted in the activation and expansion of dendritic cells and macrophages in the lung and the skin, but to a lesser degree when compared to the spleen. We will next analyze the transcriptional profile of the sorted dendritic cells and macrophages to identify target genes sensitive to the presence or absence of Treg in these organs. The study will provide new insights into the molecular basis of Treg suppressive function and further elucidate how Tregs modulate APC’s function to maintain immunologic tolerance.

Multiple nitric oxide targets in the tricarboxylic acid (TCA) cycle of Salmonella Typhimurium

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Nitric oxide (NO·) is produced by human immune cells to fight infections caused by microorganisms. NO· is known to inhibit bacterial growth by targeting a variety of protein prosthetic groups including Fe-S clusters, thiols and heme-iron. The gram-negative bacterium Salmonella enterica serovar Typhimurium (S. Typhimurium) is a major source of food-borne illness worldwide. NO· exposure of S. Typhimurium via administration of 2 mM SperNO results in ~20 hours of bacteriostasis. However, this NO·-induced “lag” in growth can be significantly reduced by supplementation of all 20 amino acids. NO· rescue is not seen when the amino acids methionine (M) and lysine (K) are excluded from the supplementation mix. This suggests that S. Typhimurium is unable to synthesize M and K de novo in the presence of NO· and therefore require exogenous supplementation for maximal growth. The experimental goal was to determine the NO· targets that result in the conditional M and K auxotrophies, and to investigate the regulation of NO· response in S. Typhimurium. It was determined that NO· targets the tricarboxylic acid (TCA) cycle resulting in the cells inability to synthesize succinyl-CoA, a precursor specifically for the biosynthesis of M and K. This inhibition occurs by at least two mechanisms: the biochemical inhibition of a-ketoglutarate dehydrogenase, as well as the mis-regulation of the succinate dehydrogenase (SDH)/fumarate reductase (FRD) reaction. We propose that the latter mechanism results from the NO--sensitivity of Fnr, a central regulator of SDH and FRD expression.
Surface-modified Listeria monocytogenes as a carrier for the intracytosolic delivery of therapeutics

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The delivery of therapeutic proteins, peptides, or nucleic acids into cells would benefit from a delivery system that efficiently enters the host cell cytosol. Listeria monocytogenes is a facultative intracellular pathogen that rapidly enters the cytosol after invading host cells. Attenuated strains of Listeria are of interest for intracellular therapeutics delivery. Here, we demonstrate a surface conjugation strategy that allows modular attachment of therapeutic cargo to Listeria. Listeria were surface-modified by reaction with NHS-biotin, enabling the attachment of biotinylated cargo through a streptavidin linker. Surface biotinylation of Listeria was confirmed through a plate binding assay. Biotinylated Listeria bound to a plate displaying surface-adsorbed streptavidin, while unmodified Listeria failed to bind. The attachment of a biotinylated, fluorescently-labeled model protein cargo to the surface of biotinylated Listeria was examined, and the invasion of host cells by this Listeria-protein conjugate was investigated using fluorescence microscopy. Listeria-drug conjugates could be applied toward nucleic acid and protein delivery.

Dry Storage of a Gold-Conjugated Antibody: Fulfilling the Need for Portability in the DxBox Project

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In many third world countries, medical diagnosis technology is often limited in distribution, unsophisticated and inaccurate. To remedy this problem, we propose a point-of-care diagnostic system, which brings this technology directly to the patient. The device tests for a panel of six different fever-causing diseases using nucleic acid recognition and immunoassay techniques in a microfluidic format, which requires only a drop of blood. In this way, we will produce rapid, high quality results using only a minute sample while functioning in a low-cost and portable format. The device’s portability requires that the reagents be stored in a form that maintains their functionality and viability in an unrefrigerated, non-temperature-controlled environment. One way to do this is through dry storage. Specifically, work focused on the preservation of the secondary gold-conjugated antibody used in a sandwich immunoassay for the detection of Plasmodium falciparum malaria. This reagent was stored in dry form with different amounts of preservatives (sucrose and trehalose) on a porous substrate (polyester) and stored at different temperatures (4°, 20° and 45°C). The antibody
was rehydrated with a buffer and then used in the assay to test the viability of the reagent after rehydration at different time points. The efficacy of rehydration coupled with a full assay in a microfluidic format was explored as well. This research found that sucrose and trehalose preserved a large portion of the function of the gold-conjugated secondary antibody for at least sixteen days at the three temperatures. Furthermore, we found that rehydration in a microfluidic format, as well as implementation of this method in a microfluidic immunoassay gives viable results. Thus, this design supports the DxBox endeavor.

**Investigation of bacterial growth motility of Pseudomonas aeruginosa on agar substrates with varying dehydration properties and nutrition concentrations**

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*Amgen Scholars Program*

Pseudomonas aeruginosa is an opportunistic bacteria that is known to inflict infections of the pulmonary tract, urinary tract, in burns and wounds, and can also be the cause for blood invects. It affects in particular patients with cystic fibrosis, burn wounds, cancer, or patients that are immunocompromised. Recent research of bacterial growth on test surfaces has shown that P. aeruginosa exhibits swarming motility at low nutrient concentration, and a lack of motility at higher nutrient concentrations. Specifically, on agar, a common growth medium for bacterial cultures, the bacteria exhibited swarming motility at a 0.4% agar-water concentration prior to gelling. Significantly reduced growth motility was found at 0.% agar concentration. The growth medium, along with agar and water, also included glutamate, an amino acid which acts as a carbon nutrition source required for bacterial growth, and a mixture of dissolved minerals to support growth. Considering that bacterial surface motility on agar is strongly affected by the availability of water (“surface wetness”) and nutrient concentration, in this study the growth process is examined as a function of gel dehydration, and the surface adhesive properties and morphology of agar. Agar’s surface properties were studied on the nanoscale by atomic force microscopy. Thereby, adhesion forces of agar were found to depend on the degree of dehydration, and the growth motility of P. aeruginosa was found to depend both on dehydration and the initial agar concentration prior to gelling.

In collaboration with Ursula Koniges, Senior, Chemical Engineering, University of Washington.
Comparing Statistical Models Using Coronary Artery Calcium Scores
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Mentor: David Yanez, Biostatistics
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Biostatistics Summer REU

In the U.S. today, 1/3 of all deaths are attributed to cardiovascular diseases, making it the number one cause of death. It is believed that coronary artery calcification (CAC) levels can be a promising measure or surrogate for cardiovascular disease endpoints (e.g., stroke, heart attack, etc.), however, standard statistical techniques are inadequate for analyzing relationships between Agatston scores (a common measure of CAC) and risk factors of disease (e.g., age, cholesterol, etc.) because Agatston scores are very highly skewed. To add to the difficulty, CAC is a mixture of two processes: discrete (no CAC vs. >0 CAC) and continuous (Agatston scores). Due to this, even more advanced statistical techniques such as the Tobit Model (Tobin, 1958) can be inadequate because model assumptions do not account for skewed data. This research extends the Tobit Model to allow for skewed data using a nonparametric transformation of CAC scores, and also allowing for the predictors to be different for the two processes. We employ likelihood based methods on the nonparametrically transformed CAC scores. Parameter estimates are obtained using the Newton-Raphson method. Standard error estimates are obtained using Fisher’s information matrix. For the CAC data, we compared the likelihood based estimates from Zhou (2007) to the more restrictive Tobit Model. We tested whether the two processes (discrete vs. continuous) were independent. The Zhou method provides a more robust alternative for modeling semi-continuous response data. This methodology can easily be extended to account for repeated measures or clustered data.

Satellite Cell Proliferation in Culture
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CREE

Satellite cells are known to be the cell population that resides in skeletal muscle that regenerates the muscle after damage; however, it is still unknown if they can be considered stem cells. For a cell to be considered a stem cell, it must be able to reconstitute a given tissue, differentiate into multiple cell types, and be capable of self renewal. The condition of being able to regenerate a tissue has been answered; satellite cells are capable of regenerating skeletal muscle. The second two are dependent on the ability to culture satellite cells in the lab. Satellite cells were first isolated from murine muscle tissue via flow cytometry. The enriched satellite cell population was then cultured as a single cell deposition on three different surfaces: gelatin (the traditional growth surface), a neutral
nanofiber coated surface (NANS), and a positively charged nanofiber coated surface (SANS). The single cell deposition allowed for quantitative analysis of the number of cell doublings that the satellite cells go through in culture, which then showed their ability for self renewal. To demonstrate that the isolated population of cells is truly a population of satellite cells, after culture, the cells were allowed to differentiate into myotubes.

Specific peptide binding to inorganic substrates with the use of micro-contact printing

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GEMSEC REU Internship

Polydimethylsiloxane (PDMS) stamping is proven to be effective in peptide adhesion to inorganic surfaces. Genetically Engineered Peptides which have specific binding to inorganic substrates (or GEPI’s) are useful tools in the industries of nanoscience, molecular biology and medicine by serving as mediators for biosensors. Peptide sequences specific to inorganic materials such as silica, glass and gold can be used to pattern surfaces of inorganic substrates with the use of PDMS stamps. These peptides can in turn be used bi-functionally, to not only bind to the inorganic surfaces but also to photo-visual devices, other peptides, and even proteins. A method of PDMS stamping is presented to optimize peptide binding to inorganic surfaces, as well as a self-assembly mechanism for micro-arraying surfaces. PDMS stamps with quartz binding proteins (QBPs) (Emre) have been found to bind to surfaces and bi-functionally display the patterns of a PDMS stamp. Incubation with a second peptide leads to specific binding in areas not previously stamped. This in turn can be visualized by two different photo markers, such as quantum dots, specific to the peptide of choice. The importance of finding a cheap and effect method of immobilization of bi-functional peptides is a necessary element in expanding the abilities of bionanotechnology today.

Preparation of azobenzene monolayers for use as a light driven molecular pump

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UWEB-RECCS

Azobenzene compounds have been shown to photo-switch, or change conformation from the more stable trans position to the cis position when placed in certain wavelengths of light. The energy from the light is thought to initiate a radical reaction in which the double bond between the nitrogens becomes a single bond, thus allowing the molecule to rotate. This change in conformation is reversible, making azobenzene notable for its ability to “wag” or continually
change between the trans and cis conformations. This project seeks to harness the movement of the azobenzene in order to run a light-driven molecular pump. This pump will be formed as a self-assembled monolayer on gold and could be applied in areas of microfluids, drug-release or data-storage.

**Using Cloning and Transfection to Develop Traceable Pit1 and Pit2 Proteins**

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**UWEB-REU**

Vascular calcification is a prevalent and deleterious disease occurring as a natural side effect of aging, diabetes, menopause, and osteoporosis and contributing to cardiovascular disease and the failure of native and bioprosthetic cardiac valves. One important regulator of calcification is phosphorous. It has been found that elevated extracellular phosphorus levels cause vascular smooth muscle cells (VSMCs) to calcify and that phosphorous is transported into human VSMCs by the type III sodium-phosphate cotransporters Pit1 and Pit2. While antibodies to these cotransporters have been developed, attempts to characterize them using Western blots and cell lysates expressing endogenous levels of the cotransporters have yielded inconclusive results. This study created traceable Pit1 and Pit2 proteins by cloning their full-length cDNA into pIREShrGFP2a, a vector with a hemagglutinin (HA) fusion protein and green florescence protein (GFP) coexpression. By transfecting these traceable plasmids into human HELA and 293 cells, readily transfectable cell lines, cells expressing higher levels of these cotransporters were created. Since the HA tag and cotransporter proteins are fused together, the reliable and well-characterized anti-HA antibody should recognize the same protein as the cotransporter antibodies, thus providing a way to verify the specificity of the cotransporter antibodies. Western blots were run on these transfected cells comparing the HA antibody with Pit1 and Pit2 antibodies. While cell lysates containing HA (positive control) revealed a distinct band at 55 kD, only nonspecific bands were detected in HELA cell lysates. Although Pit1 antibodies did not identify specific bands in cells expressing higher levels of Pit1, the Pit2 antibody potentially recognized three specific bands between 90 and 100 kD. Definitively characterizing these antibodies enables future lines of study which could provide valuable insight into the prevention of Pit1 and Pit2 mediated vascular calcification.

**Anisotropic Kink Absorption of 2’, 7’-Dichlorofluorescein in Potassium Acid Phthalate (KAP)**

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*Mentor: Theresa Bullard, Physics*

**Hooked on Photonics STC-MDITR**
In this study dyed crystals were used as tools for understanding crystal growth and surface chemistries at a molecular level. Selective recognition of dyes for specific surface features during crystal growth from solution can be exploited for locating and identifying hillocks, even though the mode of entry into crystals is as yet unknown. Dyed crystals have also been applied towards spectroscopic and photonic purposes, crystal dye lasers, chiroptical investigations, single molecule photophysics, and understanding orientation of single molecules in ordered media. This research focused on identifying how a dye becomes incorporated into a model crystal system, known as potassium acid phthalate (C6H4-COOH-COOK, also known as KAP), during growth from aqueous solution via spontaneous nucleation, and how impurities influence the kinetics and morphology of hillocks that appear in the crystals. This research project built off of studies performed by Hottenhuis et al., where intentional trivalent cationic impurities were shown to have specific step and kink inhibiting effects when adsorbed into KAP hillocks. Ce3+ and Fe3+ in particular were chosen because each one influences the shape of the hillock in a very distinct manner through selective kink adsorption. With the addition of our dye, 2', 7'-dichlorofluorescein, we were able to observe how the dye’s behavior was altered by the impurities’ influences on hillock morphologies and kink blocking. Results from the compilation of various spectroscopy and microscopy methods helped to identify, for the first time, selective recognition of molecular dye additives for specific kink sites, demonstrating high chemical specificity of dye inclusion.

A Quantum Dot-based Microfluidic Immunoassay

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Mentor: Neil Geisler, Bioengineering
Mentor: Paul Yager, Bioengineering
Amgen Scholars Program

New tools for point-of-care diagnostics are needed for more effective treatment of disease. We present a rapid and sensitive assay, with potential for simultaneous multianalyte detection on a microfluidic platform. Using antibody-linked quantum dots (QDs) as fluorescent reporters, a sandwich immunoassay is carried out inside a poly-laminate plastic card. QDs are well suited for point-of-care diagnostics due to high brightness and photostability. A CO2 laser system was used to cut a microfluidic channel into thin (25 µm) Mylar plastic with adhesive on both sides. A layer of optically clear PMMA was similarly cut to form the top of the microfluidic channel. A gold-coated slide was patterned with a self-assembled monolayer of biotin-alkyl-thiol using a piezoelectric spotter to form immunoassay regions of interest ~500 µm in diameter. The remainder of the surface was passivated with non-fouling polyethylene glycol-thiol. The microfluidic channel layer was then applied to the slide, followed by the PMMA top layer and additional laminate layers for interfacing to an off-chip pump system. Dimensions of the final channel were 60 mm by 2.5 mm by 0.064 mm (total channel volume ~10 µL). Small volumes (100µL) of streptavidin, biotinylated
capture antibody, mouse IgG as a target analyte, and QD-antibody conjugates were then sequentially flowed into the channel. Multispectral imaging was used to analyze QD fluorescence of the sandwich immunoassay spots (peak emission 655nm). Using this assay, we have currently achieved detection as low as 70 pM of model analyte in PBS buffer.

**Exploring the Feasibility of Machine Learning in Low-power Embedded Systems**

*Anthony Seo, Senior, Electrical Engineering, University of Washington*

*Mentor: Anthony LaMarca, Intel Research Seattle*

**Intel Research Experience**

The purpose of this project was to investigate the feasibility of using a low power microcontroller to do real time inference. Currently, Intel Research in Seattle created a device called the Multi Sensor Platform (MSP). This device captures data with the sensors and processes the information to infer activity in real time. However, due to the power consumption of the current processor, the run time is very short. Therefore, it has been proposed to use a low-power processor, to replace the current processor. Much of the devices produced today such as MP3 players, smart phones, PDAs have the potential to do real time inference. If this is successful, then it is possible to integrate these features onto such devices.

To do this study, a simple configurable microprocessor made by ARC International was used. All of the software needed, such as the integrated development environment and the simulator for the microcontroller, was provided by the company. With these tools, simple programs were created which performed the Fast Fourier Transform and matrix inversion. The simulator was then used to get the cycle count of each program. From the simulations, it was determined that it is possible to do real time inference on a low-power embedded system with such a microprocessor.

**Electronic Control of Peptide Binding on Silicon Chips**

*Sunny Sharma, Senior, Electrical Engineering, University of Washington*

*Mentor: Babak Parviz, Electrical Engineering*

*Mentor: Ranjana Mehta, Electrical Engineering*

**Intel Research Experience**

Binding of bio-molecules to microfabricated structures is of great interest for a wide range of applications. For protein array chips and biosensors functionalized with specific antibodies, there is a need to selectively bind bio-molecules, especially proteins and polypeptides, to inorganic templates made via solid-state microfabrication. This project aims to electronically control the binding of genetically engineered inorganic-binding peptides onto metal structures micro-patterned on silicon devices. We use the biotinylated Gold Binding Peptides (GBP) that exhibit specific binding onto gold surface in contrast to other metals such as silver, platinum and chromium. A bias voltage is applied to the gold pads mi-
crofabricated on a silicon/silicon dioxide (Si/SiO2) chip to obtain differentially biased gold pads through a circuit. The adsorption of biotinylated GBP onto the gold electrodes is examined by delineation of the electrodes using streptavidin-conjugated fluorescent quantum dots. Qualitative data analysis is performed by employing fluorescence microscopy. Our measurements exhibit a correlation between the polarity and magnitude of the applied electric field and peptide binding. This level of selective control presents the ability to electronically program an array of microelectrodes with different polypeptides by sequentially exposing the pads to the peptide solutions and applying the appropriate bias voltage. We aim to develop a protocol for using polypeptides for the bottom up self-assembly of nano-scale quantum dots onto microfabricated patterns; and achieving a further level of control over the binding of the polypeptide to microstructures via application of a bias voltage. Our approach opens a new venue for bridging the biological and inorganic domains, guiding self-assembly of structures and devices from the bottom up, and presents a powerful new tool for fabrication of nano-scale array structures and hybrid organic/solid-state devices.

Magnetic Nanoparticles for Brain Tumor Treatment
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Mentor: Conroy Sun, Materials Science and Engineering
Mentor: Miqin Zhang, Materials Science and Engineering
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The targeted delivery of therapeutic drugs to tumor cells will provide a significant advantage over conventional chemotherapeutic approaches in the treatment of cancer. Current treatment regimens are not always successful due to adverse effects of chemotherapeutic drugs on healthy tissue. Targeted delivery to cancer cells will reduce the exposure of healthy tissue to chemotherapeutic drugs. In our previous work, a multifunctional nanoparticle conjugate was developed to specifically target gliomas, a brain tumor diagnosed in approximately 17,000 Americans annually. This nanoparticle consists of an iron oxide core coated with a layer of biocompatible poly(ethylene glycol) (PEG) polymer, which is covalently linked to chlorotoxin (CTX), a peptide with high affinity for tumor cells of neuroectodermal origin. In addition to serving as a targeting agent, the superparamagnetic nature of the iron oxide core provides significant MRI contrast enhancement. In our present work we have developed a new nanoparticle conjugate to serve as a drug delivery vehicle capable of carrying the therapeutic cancer drug, methotrexate (MTX), specifically to brain tumor cells. The surface of the nanoparticle core was modified with (3-aminopropyl)-trimethoxysilane and a PEG diacid to form a self assembled monolayer. The modified particles were subsequently conjugated with MTX via an amide bond and CTX via a thiol ether bond. The successful conjugation of MTX with nanoparticles was determined through Fourier transform infrared (FTIR) spectroscopy. The cell targeting ability of the nanoparticle conjugate was determined through in vitro
iron uptake studies using the ferrozine assay. D23 (medulloblastoma) and 9L (glioma) cells were shown to have significantly higher iron content per cell than healthy rCM (rat cardiomyocyte) cells demonstrating successful cancer cell targeting. By attaching MTX to the nanoparticles and harnessing the targeting capability of CTX, we have created a drug delivery vehicle that will someday circumvent current limitations of chemotherapy. Furthermore, through MRI scans, this nanoparticle conjugate will allow for real-time monitoring of therapeutic drug delivery to target cells.

**Synthesis of Two Lipid-Soluble Two-Photon Dyes for Use in Precision Biological Stimulus Transfer**

*Isaac Stormer, Junior, Chemistry, University of Washington*

*Mentor: Phil Sullivan, Chemistry*

*Mentor: Josh Davies, Chemistry*

*Hooked on Photonics STC-MDITR*

Synthesis of two highly conjugated, lipid-soluble organic compounds for the purpose of forming a long wavelength absorbing two-photon dye to be contained within a vesicle membrane will be performed in this project. The applications of these two photon dyes are numerous. One of the more important possible uses would be using the dye in conjunction with drug delivery in vesicles. Since the dye is lipid-soluble it can easily associate with nonpolar vesicles. These vesicles contain both the dye and the drug can readily interact with biological structures with lipid bilayers, i.e., membranes. Once inside the biological structure, the vesicles can then be opened by irradiating the biological structure with long wavelength (and thus low energy) light. The two photon dye will then absorb the photons of radiating light and cause the vesicle to explode and to release its contents. The synthesis steps of these dyes will be shown along with UV/vis spectroscopy results.

**Development of a Biofilm Based System to Simulate Antibiotic Treatment of Chronic Cystic Fibrosis Lung Infection**

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*Mentor: Sam Moskowitz, Pediatrics*

*Mentor: Paolo Vicini, Bioengineering*

*CREE*

Cystic Fibrosis (CF) is a fatal genetic disease that affects about 60,000 individuals worldwide. The mutated CF gene is manifested as an impaired epithelial salt transport. This causes dehydration and accumulation of mucus plaques in the lungs. This mucus causes breathing difficulties and promotes chronic bacterial infections that lead to premature death. The bacterium Pseudomonas aeruginosa (PA) is the most common pathogen in these chronic lung infections. PA grows in the lungs as a biofilm. The biofilm increases PA’s resistance to antibiotics, and thus allows PA to persist even during exposure to high doses of antibiotics.
Today, due to the lack of appropriate animal models, antibiotic regimens and dosing used in CF patients are based on bacterial antibiotic susceptibility testing that is of uncertain relevance and efficacy. The goal of this project is to develop an in vitro device that models in vivo biofilm infections and their corresponding antibiotic treatments. This requires that the device accurately mimic the biofilm growth, the appearance and disappearance time course of an antibiotic at the target site upon systemic administration (pharmacokinetics or PK), and the effects this antibiotic exposure time course has on the biofilm (pharmacodynamics or PD) found in the lungs of CF patients. Such a device could be used to tailor dosing for individual patients with less guesswork and to test new treatments for CF.

Creating Electrical Engineering Educational Tools
Jessica Tran, Senior, Electrical Engineering and Dance, University of Washington
Mentor: Eve Riskin, Electrical Engineering
Intel Research Experience

We are developing and creating an interactive website for the EE 341 Discrete Time Linear Systems class with the intent to educate EE students nationally. Currently, there exists an interactive webpage for the preceding class, EE 235, entitled Continuous Time Linear Systems. The EE 235 website complements the concepts presented in the current textbook being used. Positive comments have been expressed by students using the EE 235 website, and thus a need for an interactive website for the EE 341 class was recognized. We are developing the EE 341 website using Adobe FLASH CS3 Professional, Adobe Dreamweaver, and LaTeX2HTML software. The website will present another perspective about EE341 concepts such as convolution, the Z-transform, and the Discrete Fourier Transform (DFT). Upon completion of this website, future research on the usability and effectiveness of interactive websites may be considered in conjunction with the Center for Engineering Learning and Teaching Department (CELT).

Role of the Isthmus in Regulating Spontaneous Synchronous Activity in Chick
Wendy Tse, Senior, Biology, Dartmouth College
Mentor: Martha Bosma, Biology
Amgen Scholars Program

Spontaneous synchronous activity (SSA) is widespread electrical activity that originates in the absence of sensory input. During gestation, SSA plays a role in determining neuron differentiation and migration, axon growth, and establishing the proper connections between synapses. It is still not completely understood how SSA sets up the critical wiring of the central nervous system necessary for proper development, which when fully understood would set the foundation for understanding developmental disorders in the brain. The developing embryonic
chick brain is divided into the forebrain, midbrain, and hindbrain, each of which will develop into distinct components of the mature brain. My work focused on elucidating how the isthmus, which is a physical boundary between the midbrain and hindbrain, acts as a gatekeeper, conducting some transients, while blocking others from propagating from the hindbrain on through to the midbrain. Immunocytochemistry (ICC) was performed on brain slices to investigate the morphology of the isthmus. Initial ICC findings suggest that the isthmus is not devoid of cells as previously thought because positive nuclei staining was present throughout transverse slices of the chick brain from midbrain through the hindbrain. To establish the function of the isthmus, calcium imaging was performed on the midbrain, isthmus, and hindbrain to quantify the hindbrain-driven SSA which is able to cross the isthmus to reach the midbrain. Midbrain activity had not been previously investigated in chick. My findings suggest that midbrain activity is present in the chick and in a manner of frequency comparable to mouse, not occurring in all brains with active hindbrain activity. This finding suggests conservation across species, emphasizing the importance of both SSA and the isthmus as a regulator of proper signal transmission.

poly(2-Hydroxyethyl Methacrylate) Scaffolds for Cardiomyocyte Regeneration
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Mentor: Sarah Atzet, Chemical Engineering
Mentor: Scott Curtin, Bioengineering
Mentor: Buddy Ratner, Bioengineering
UWEB-REU

The viability and morphology of cells implanted to replace nonfunctional collagenous scar tissue in cardiac muscle can be dependant on the cell scaffold and its properties. Therefore, the physical properties of scaffolds for cardiomyocyte regeneration were studied. Mechanical properties and degradation rates were evaluated for degradable scaffolds composed of 10kDa and 20kDa poly(2-hydroxyethyl methacrylate) (pHEMA) crosslinked with polycaprolactone (PCL) dimethacrylate. These hydrogels were crosslinked at three different densities: 4.5, 9.0, and 13.5 mol%. pHEMA copolymerized with a non-degradable crosslinker (tertaethylene glycol dimethacrylate) served as a control. The degradation rate of these gels was evaluated in 0.007 M NaOH, 1.0 mg/mL lipase, 0.5 mg/mL lipase, and phosphate buffered saline. Statistical analysis of samples submerged in NaOH concluded that as crosslinking density increases, degradation time increases. The non-degradable control showed no measurable degradation and the 13.5 mol % gels did not fully degrade within the experiment timeframe. Interestingly, preliminary results of samples in lipase solutions did not display sufficient degradation. An activity assay of the lipase solutions concluded that it is indeed functional. The enzyme either cannot degrade the gels or sufficient time has not elapsed for a trend to display itself. A higher enzymatic concentration could be used to determine whether the lipase is either slow or ineffective.
at degrading the scaffold. Mechanical tests indicate that the Young’s modulus is decreasing over time for the degradable gels in 1.0 mg/mL lipase solution while the non-degradable controls remain constant. MTT and the elution method cytotoxicity tests of the scaffolds used 3T3 mouse fibroblasts. After sufficient rinsing with PBS both the non-degradable and degradable gels showed no cytotoxicity by either method. We have shown that changing the amount of crosslinking density affects degradation in a predictable manner and the hydrogels are suitable for future in vivo studies.

Study of the role of the protein p120 catenin on the movement of early cells in zebrafish embryos

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Mentor: Merrill Hille, Biology
Amgen Scholars Program

This project focuses on how cells in early zebrafish embryos move, specifically the role of the protein p120 catenin in this process. It is proposed that this protein controls when cells move: when p120 catenin is present, it strengthens the junctions between cells and regulates the direction of cell protrusions. The protein p120 catenin was down-regulated with an antisense RNA Morpholino (MO), which binds to the AUG-start site of p120 catenin mRNA, thus, blocking the translation of this protein. Freshly spawned eggs were injected with MO and the development of the embryos was followed for the next 24 hours. Photos of the embryos taken during their development often showed cells dissociating from the dorsal side of the embryo. Embryos were also staged, fixed, and hybridized with DIG-labeled RNAs to observe the location of migrating mesodermal cells. For the in situ hybridizations, embryos were processed and stained as described by Westerfield (1993). Changes in morphology between the controls and the MO-injected embryos were compared. The photographs of the embryos show differences in the location of the mesodermal cells. The neural plate and notochord cells from the MO-injected embryos were more separated than cells in the controls at the Bud stage (10 h), which is what would be expected from cells lacking the propose movement controlling protein, p120catenin. There was also a large difference in the length of the first 6 somites of MO-injected embryos and those of the controls at 12 hours. The somites from the MO-injected embryos were narrower and longer than the control embryos. These observations suggest that the normal movement of the cells toward the dorsal side of the embryo was slower after ectopic injection of MO, which knockdowns p120 catenin mRNA. The cells find it harder to move in the absence of the p120 catenin protein.
Locating the source of neuropathic pain in current medical practice has been difficult and many times unsuccessful. Patients suffering from neuropathic pain may lack physical detriments to indicate an obvious cause of the pain and can suffer without treatment or assistance. Our animal model for neuropathic pain was created by partially ligating the sciatic nerve to one hind leg; the remaining hind leg was used as a control for the same animal. This creates a painful sensation in the animal’s paw and hind limb. Using High Intensity Focused Ultrasound (HIFU), we can identify the location of pain in animals with a partial sciatic nerve ligation injury, as compared to the control limb. When compared with traditional pain indicators such as the Von Frey Method or the Hargreave’s Plantar stimulation, evidence suggests that HIFU may be more sensitive as an indicator of the presence of pain. The HIFU extends a physician’s manual palpitation to deeper structures, as shown by results generated after a topical anesthetic was applied to numb the skin. To determine a threshold for non-painful stimulation in humans, we have been testing various intensities and durations of HIFU on healthy human phalange tissue. If HIFU is shown to be a safe and sensitive method for stimulating deep tissue, it may be used to determine the source of pain in future studies.

The Molecular Swiss Army Knife
Kathy Y. Wei, Junior, Bioengineering and Computer Science, University of Washington
Mentor: Jamie M. Bergen, Bioengineering
Mentor: Suzie Pun, Bioengineering
Amgen Scholars Program

Gene therapy promises to treat neurological disorders, such as Alzheimer’s disease, Huntington’s disease, and Parkinson’s disease, that currently have limited or no available treatment. Non-viral gene delivery vehicles offer several advantages over viral-based vectors because they are potentially safer and more customizable. The specific non-viral vehicles used in this experiment are polyplexes, which are polymer/DNA complexes. The major challenge faced by these materials is the inefficiency of polyplexes at overcoming barriers to gene delivery, especially in non-dividing cell types such as neurons. Intracellular barriers to nuclear delivery of foreign DNA include targeting (getting to the cell), uptake (getting into the cell), endosomal escape (getting out of the endosome), retrograde transport (getting to the nucleus), and nuclear localization (getting into the nucleus). This project focuses on attaching peptide ligands that
target specific barriers to the surface of polyplexes to increase DNA delivery efficiency. The DNA incorporation efficiency, polyplex size, and polyplex charge were measured for various formulations of a virally-derived peptide conjugated to polyethylenimine, a cationic polymer that is widely used to deliver DNA. This peptide, which may have endosomal escape as well as retrograde transport capabilities, increased gene delivery in a model cell line by up to a factor of 26-fold. Also, the synergistic effect of combining the peptide and Tet1, a neuron targeting peptide based on tetanus toxin, is being studied in cultured neuron-like cells. Success in increasing gene delivery with safe, customizable polyplexes will bring us closer to treating not only neurological diseases, but also diseases like cancer.

**Improving the Power Conversion Efficiency of Titanium Oxide/ P3HT Bi-layer Devices by Doping with Iron Nitrate**

*Natalie Wilhelm, Sophomore, Undeclared, Brown University*

*Mentor: Dan Liu, Material Science and Engineering*

*Mentor: Christine Luscombe, Material Science and Engineering*

*Hooked on Photonics STC-MDITR*

The motivation for this research was to create a more efficient bi-layer organic/inorganic solar cell device by doping the inorganic layer, TiO2, with iron (Fe3+). When photons strike the surface of the device, charged particles called ‘excitons’ are created. They separate into positive holes and electrons at the boundary of the two layers and are transported through the layers to the two conductive electrodes, creating an electric current. The organic material, poly 3-hexylthiophene (P3HT), acts as a p-type semiconductor. The inorganic titanium oxide acts as an n-type semiconductor. In order to elevate the efficiency of the TiO2, the diffusion and collection of the electrons within the TiO2 must be improved. The excited electrons in the conduction band will only exist for a few nanoseconds before they recombine within the TiO2. Also, the electrons can only travel around ten nanometers before recombination so an electron acceptor must be immediately available. By doping the TiO2 lattice structure with transition metal ions, such as Fe3+, the photocatalytic activity of TiO2 increases. The ions take in the electrons and reduce the number of electron-hole recombinations. The metal ions are added as nitrates into the titania solution [titanium (IV) ethoxide and butanol], which is then spin-coated onto indium tin oxide (ITO) coated glass. After it has been calcinated, the substrates are coated with the P3HT solution in an argon glovebox. Silver electrodes are applied by vacuum deposition. The devices are tested by connecting the two electrodes (ITO and silver) into an electric circuit and applying a light source while measuring the current and voltage.
Engineering Mycobacterium smegmatis porin A (MspA) for DNA analysis
Risa Wong, Junior, Physics, University of Washington
Mentor: Tom Butler, Physics
Mentor: Jens Gundlach, Physics
Amgen Scholars Program

We are engineering a nano-scale biological pore, Mycobacterium smegmatis porin A (MspA), to analyze DNA at the single-molecule level. In nanopore analysis of DNA, a voltage difference is applied across a lipid bilayer in which a single protein pore is inserted. This generates a flow of ionic current through the pore and also drives single-stranded DNA to thread through the pore. When the DNA is present in the pore, it physically blocks some of the ionic current. If we can resolve the differences in current blockage between each of the four DNA bases, nanopore analysis of DNA has the potential to become a quick and inexpensive direct sequencing technique. MspA is a promising nanopore for DNA analysis because it has an advantageous structure and high stability. However, DNA does not pass through wild-type MspA, most likely because of electrostatic repulsion between negatively charged amino acids on the surface of the pore and the negative charges on DNA. We have engineered both a triple-mutant and a sextuple-mutant MspA with some of the pore’s excess negative charge removed. I have been working to characterize both of these mutants and to test them for DNA interaction. Promisingly, both MspA mutants demonstrate clear current blockages when single-stranded DNA is present.

Utilizing a PCR-Based Assay to Study the Prevalence of the Four Species of Malaria in Kenya
Lianna Wood, Senior, Biochemistry and History, University of Washington
Mentor: Carol Sibley, Genome Sciences
Amgen Scholars Program

Human malaria, which takes the lives of at least one million people each year, is caused by four species of Plasmodium parasite. Of these, Plasmodium falciparum is the most lethal and the dominant species throughout Africa. However, the rate of infection for the three other species is not well known. Even when species identification is performed, microscopy is generally employed, which underestimates mixed species infections and is generally more subjective than other methods. Therefore, we intend to identify and utilize a more accurate PCR-based method of species identification to assess the infection rate for each species in 307 blood samples taken from malaria patients in Kilifi, Kenya. Only one study has been published using PCR to look for all four malaria species in Kenya, but the rates of infection of the two less common species of malaria, P ovale and malariae, were surprisingly high while no P vivax was found. Such high infection rates for P ovale and malariae are incongruous with previous data from other regions of Africa, and, therefore, we do not expect our study to have the same findings. Additionally, we expect to see some P vivax since it
seems likely that there are susceptible individuals in the Kenyan population. In searching the literature, we targeted semi-nested PCR assays that utilized the small subunit ribosomal RNA sequence to maximize assay sensitivity and species specificity. We identified three well described and commonly used assays, but one protocol had been shown to miss some strains of P ovale. Initially, we employed a single-tube protocol, and a set of ten known P falciparum and two P vivax patient samples were tested. However, concerns were raised about this assay’s ability to detect P vivax and P falciparum coinfections. In addition, the available plasmid positive controls did not work with the assay, and we moved to the third protocol, which has worked well with the positive controls, despite contamination issues. In the future we will use this latest protocol to assess the samples from Kilifi, Kenya.

Using Genetic Markers to Estimate Relatedness
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Mentor: Bruce Weir, Biostatistics
Biostatistics Summer REU

Genetic relatedness is fundamental to many aspects of life such as marriage laws, identifying human remains and mapping disease genes. This study estimates relatedness using various numbers of SNPs to determine whether a greater number of markers will produce better estimates or if there is a threshold number. Two data sets were organized, each consisting of 13 unrelated Utah families made up of 12 individuals: four grandparents, two parents and six children. A computer program written in C++ calculates the probabilities k0, k1 and k2 given the genotypes using initial values and Bayes’ theorem to find intermediate values at each locus. These are then averaged over the total number of loci to give new values for k0, k1 and k2 which are then substituted back into the original equation. This entire process continues until the log-likelihood changes by less than 10^-6. The final result is an estimation of the probabilities k0, k1 and k2 for each possible pair of people. This study was successful in reproducing the results of a previous study by Bruce Weir and Amy Anderson. It also showed that when greater numbers of SNPs are used, the estimates appear to change very little and the estimates in the parent-offspring case get worse. When looking at 49 pairs of people genotyped on 8000 or more SNPs, the results exhibited sufficient estimations of parent-offspring and unrelated pairs, but grandparent-grandchild estimates still seemed variable about the true values. It might be valid to conclude that 2800 SNPs is the greatest number required to provide sufficient estimates for certain relationships. Furthermore, the use of 8000 SNPs does not estimate the grandparent-grandchild relationship any better. This study made some progress in the area of estimating relatedness using genetic markers, but more work will need to be done to fully validate these results.
Optimization of process parameters for micropart capillary assembly with precision positioning
Andrew Zhou, Junior, Bioengineering, University of Washington
Mentor: Raji Baskaran, Electrical Engineering
Mentor: Shaghayegh Abbasi, Electrical Engineering
Mentor: Karl Böhringer, Electrical Engineering
UWEB-REU

The assembly of microdevices is currently done mainly with the pick and place method. However, this method is slow and expensive for assembly requiring high precision. Thus, the development of a new fast, yet highly precise method of assembly is the focus of a lot of current research. Self-assembly in liquid medium, where microdevices spontaneously arrange into more complex systems using capillary forces, has been shown to be a potential candidate for parallel batch assembly with precision positioning. However, studies of how the design and process variables affect the precision of the method have yet to be investigated. The main barrier to achieving the desired precision in the x-y direction is the tilting of parts during the assembly process. In this project, we investigated the effect the volume of polymer has on tilting and developed a theoretical model for the tilting. Our data showed that the magnitude of tilting increases as the volume of polymer used increases, in accordance with the model. In addition, we studied a method using vertical vibration with a speaker to correct the tilting. Our data showed that this method is practical under certain conditions of polymer coverage on the binding sites. With further investigation, tilting should be able to be corrected consistently. In the future, the horizontal position alignment of this method can be studied when the tilting problem is solved.
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