2Q

Engineering Proteins and Cells

Session Moderator: Michael Regnier, Bioengineering

Mary Gates Hall Room 295

3:30 PM to 5:00 PM

* Note: Titles in order of presentation.

Novel Thermal-Responsive Conjugate System for Rapid Antigen Enrichment

Carlos Eduardo Estrada (Carlos) Alamo, Senior, Biochemistry, Bioengineering
Mary Gates Scholar, NASA Space Grant Scholar
Mentor: Patrick S Stayton, Bioengineering
Mentor: James Lai, Bioengineering
Mentor: Nuvala T. Fomban, Bioengineering

While point-of-care (POC), out-of-laboratory, testing enables reliable patient diagnoses in resource-limited environments, accurate detection of low concentration disease specific antigens in complex biological fluids (e.g., blood) remains a technological challenge. Current immunoassay-based rapid diagnostic tools, such as lateral-flow (LF), exhibit a limit-of-detection ca. $10^{-}9$ grams/mL, 2-3 orders of magnitude higher than relevant clinical concentrations of many disease biomarkers. Because LF devices lack signal amplification and have finite sample volumes, ca. 100μL, low-concentration samples that lack sufficient antigen mass to permit detection must be concentrated to provide a diagnostic result. Here we present the application of a soluble reagent system that incorporates nanoparticles and thermally-responsive polymers for antigen enrichment to facilitate detection of dilute samples using existing diagnostic tools. Nanoparticles exhibit unique properties that can be exploited to enrich antigens in solution. Poly(N-isopropylacrylamide) (PNIPAAm), a thermally-responsive polymer, undergoes a hydrophobic to hydrophilic reversible phase transition when triggered by a small temperature increase around a lower-critical-solution-temperature (LCST). Many studies have shown PNIPAAm functionalized FeO magnetic nanoparticles (mNP) exhibit this phase transition phenomena and form magnetically phoretic aggregates upon heating. In this study, PNIPAAm coated mNP and gold nanoparticles (functionalized with an antigen binding species) will be co-aggregated above the LCST to facilitate separation of captured antigens. Antigen enrichment from complex mixtures is achieved through a simple solution exchange. After decanting the biologically complex supernatant, the aggregate is resuspended in a small volume of clean buffer. The concentrated solution may then be examined using a variety of POC formats to detect antigen presence. This thermally-responsive reagent system has been shown to separate $>90\%$ of a gold-protein complex from a heated solution in a magnetic field. Sample processing via the proposed technology may help close gaps in early POC disease detection, reduce false negative results, and help prevent further propagation of infectious agents.

Design of a Small Molecule Drug to Rescue Function of p53 Cancer Mutants

Sara Erica (Sara) Calhoun, Senior, Bioengineering
Mary Gates Scholar, Washington Research Foundation Fellow
Mentor: Valerie Daggett, Bioengineering

The tumor suppressor p53 is a transcription factor involved in many important signaling pathways, such as apoptosis and cell-cycle arrest. Over half of human cancers lose p53 function by a single nucleotide polymorphism in its gene. Previous experimental studies suggest that p53 mutants that have overall lower stability are potential targets for drug rescue by a small molecule drug. Using computational methods, we are developing an anticancer drug to target p53 mutants and rescue their function by stabilizing the protein structure. Although structures of some p53 mutants have not been determined experimentally due to poor stability, the structural effects of a single amino acid change can be predicted using molecular dynamics (MD) computer simulations. In simulations of several p53 mutants, a large crevice on the surface of the protein forms where a drug may be able to bind. Virtual screening of a small molecule database is used to limit the number of potential drug candidates. Compounds that are predicted to have poor absorption and permeability based on chemical properties are screened out. Using computer docking programs, the binding modes and energies of drug candidates to the protein are predicted. Docking runs are per-

formed with drug candidates and p53 mutant protein structures taken from the MD simulations to identify and rank drug candidates by binding affinity. Based on these predictions, selected drug candidates will be tested experimentally to determine which can bind p53 mutants with high affinity and improve the overall stability of the mutants.

Designing A Gene Circuit To Produce Fusion Protein Serving As Noninvasive, *In Vivo* Tools

Jeehoon (Jee Hoon) Jang, Senior, Bioengineering
Mary Gates Scholar
Mentor: Herbert Sauro, Bioengineering

Synthetic biology is a new and growing field of study where existing organisms are engineered at the DNA level. This project investigates the use of fusion proteins in synthetic biology research. Such proteins involve the fusion to two different proteins where one of the proteins is often a fluorescently active protein such as Green Fluorescent Protein (GFP) so that the fused protein can be easily tracked using light microscopy. The project will use fusion protein technology to monitor the level of enzymes in vivo. As a result the fusion protein will be used as a tool for research and provide valuable data to help re-engineering metabolic pathways. In this project, fusion proteins from enzymes (such as Phosphoglucoisomerase - PGI) found in the glycolytic pathway will be designed. In addition, the use of standardized biological parts will be explored in creating the genetic circuit that will control the fusion protein once in the cell. To design such a "gene circuit", PCR (Polymerase Chain Reaction) and In-Fusion Assembly will be used once the PGI gene sequence is located on the chromosome. The In-Fusion method is a more efficient technique of connecting two different gene parts together than the traditional method of restriction digests. After creating the gene circuit on a plasmid, it will be inserted into the native chromosome of E.coli using an isogenic construction method, which allows one to insert the circuit at a precise point in the E.coli genome. Finally, the transformed E.coli will be tested using fluorescent microscopy where we expect to observe the glycolytic enzyme levels in the metabolic pathway as the organism grows.

Optimizing the Passive Mechanical Properties of a Novel Microporous and Microchanneled Fibrin Scaffold for Cardiac Tissue Engineering

Gabrielle Domonique (Bree) Robinson, Senior, Bioengineering
Mary Gates Scholar, NASA Space Grant Scholar
Mentor: Michael Regnier, Bioengineering
Mentor: Kassandra Thomson, Bioengineering

The increasing occurrence of cardiac damaging events in the United States has led to a dire need for new methods to regenerate wounded heart tissue. The surgical implantation of engineered tissue grown on cell delivery devices is one proposed method for cardiac repair. A promising example of one such device is the novel microporous and microchanneled fibrin scaffold being developed by the Heart and Muscle Mechanics Lab at the University of Washington. This scaffold has been shown to direct proper cardiomyocyte alignment and promote cell adhesion to the scaffold material. It is my goal to optimize the passive mechanical properties of these fibrin scaffolds for tissue engineering applications by investigating parameters such as scaffold stiffness and rate of degradation. Because fibrin is a tunable polymer, slight variations in the way the scaffolds are constructed can affect their material properties. In order to determine which method of construction provides the most viable tissue engineering scaffolds, a selection of fibrin scaffolds are being made by varying parameters in the fibrin polymerization and scaffold preparation processes. The mechanical properties of these scaffolds are then assessed using stress-strain analysis. Of the parameters being varied, the concentration of fibrinogen (fibrin monomer), the extent of fibrin cross-linking, and the length of acetone exposure are expected to have the greatest influence on the mechanical properties of the scaffolds. Both scaffold stiffness and degradation time are expected to increase with increases in these factors. Duration of alcohol exposure was found to have no significant impact on scaffold stiffness, indicating alcohol as a potentially viable storage solution. Optimizing the passive mechanical properties of these fibrin scaffolds will further the development of a novel scaffold for cardiac tissue engineering and help prepare them for use in future *in vitro* studies.

Perfusable 3D Cell Culture for the Evaluation of Drug Delivery Vehicle Penetration and Transport

Nicholas Andrew (Nic) Roehner, Senior, Bioengineering
Mentor: Suzie Pun, Bioengineering

Mammalian cell culture systems are widely used *in vitro* models for the evaluation of drugs and drug delivery vehicles such as nanoparticles, mostly because they can help make subsequent animal tests more predictable and less costly. By and large, however, these systems have been formulated as 2D monolayers of cells which fail to take into account both extracellular delivery barriers and phenotypic differences of cells *in vivo*. While nanoparticles delivered in a bulk solution to a 2D monolayer tend to reach and bind to cells with little interference, the same is not true for nanoparticles delivered *in vivo* to a 3D environment characterized by smaller pore sizes of the extracellular matrix (ECM). Furthermore, cells in 2D monolayer cultures are exposed to a relatively uniform environment in culture medium, whereas cells grown in 3D tissue mimicking ECM gels are subject to gradients of pH, nutrients, and waste products that exert both stimulatory and inhibitory influences on cell phenotype and proliferation, much like cells in actual solid tumors. These differences between 2D and 3D cell culture systems highlight

the need for 3D models that can more accurately capture *in vivo* conditions. The Pun lab has developed a 3D cell culture perfusion chamber that can be used to evaluate the penetration of fluorescently labeled nanoparticles into a cancer cell-hydrogel tumor mimic. Improvements have been made to the chamber system in regards to recovering live cells for post-experimental flow cytometry, a cell counting process to determine the percentage of cells associated with fluorescent nanoparticles and therefore the percent penetration into tissue. Recently, the system has been used to compare the penetration of polystyrene beads with that of pluronic micelles, the latter being a promising new vehicle for delivering anticancer agents to tumor tissue.

Application of DNA for improvement of Loading Parameter Optimization in Organic NLO Materials

Terry Angelo (Terry) Villarreal, Junior, Biochemistry
Mentor: Meghana Rawal, Chemistry
Mentor: Philip Sullivan, Chemistry and Biochemistry, Montana State University

While current high-speed electronics are reaching their limits of the potential bandwidth, the demand for electronics to manage larger amounts of data at greater speeds continues to increase. One potential solution is organic non-linear optical materials; however, in order to become commercially viable, the materials must possess a high electro-optic coefficient (r33) as well as thermal and photochemical stability. One of the ways to tackle this problem is to improve dipole order. Electric field poling is currently used to align the molecules; this however is far from ideal due to intermolecular forces that are present. Our current investigation involves attaching the chromophore to a surfactant which has a quaternary ammonium salt on the other end. This chromophore-surfactant will then be titrated with low molecular weight sodium-DNA where it will undergo an ionic exchange at the quaternary ammonium salt end and then allow the chromphore to intercalate into the DNA. The DNA will not only act as a buffer and reduce dipole interaction but it will also limit the movement of the chromophore along one axis. Chromophores consist of three main parts: a donor, bridge and acceptor. For our particular material, the two places to attach a surfactant is either the donor or bridge section. Our first attempt was a Sonogashira Coupling of the acceptor side of the chromophore to a surfactant, which did not produce the desired molecule. The next line of pursuit is to attach a surfactant to the donor part of the chromophores through an esterfication reaction. Once these target molecules have been synthesized, they will be analyzed with UV-Vis spectroscopy to measure the interaction of the pi conjugated systems of the base pairs on DNA and the pi conjugated systems of our organic non-linear optical materials that should occur when intercalation happens.