1G

Towards Better Understanding of Human Diseases through Molecular Biochemistry

Session Moderator: Valerie Daggett, Bioengineering

MGH 238

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Investigating the Role of Smooth Muscle Cell Type II Transforming Growth Factor-ßReceptor Deletion on Early Onset Aortopathy

Donya Derakshani, Junior, Biochemistry
Mentor: Jay Zhu, Department of Surgery

Abnormalities in TGF-ßsignaling are thought to cause aortic aneurysms and dissection in people with inherited syndromic illnesses such as Marfan's and Loeys-Dietz Syndrome. However, the mechanisms linking changes in TGF-ßsignaling to aortic disease evolution are poorly understood. We developed a mouse model in which the type II TGF-ßreceptor was deleted to characterize and study the effects of this perturbation in TGF-ßsignaling. My research focused specifically on characterizing any histologic changes in the aorta in 7-8 week old mice, 1 week after deleting the TGF-ßreceptor. To quantify changes in aortic architecture, we employed methods such as H&E staining and fluorescence microscopy. Our results showed that the ascending aortas of the mice with the TGF-ßreceptor knockout had significantly larger medial thickness and medial area, with p-values of 0.0001 and less than 0.0001, respectively. However, we did not find a significant difference in elastic breaks between the two experimental groups, although previous research has shown increased elastic breaks in aortas of mice with the TGF-ßreceptor deletion when measured at a later point (14 weeks post-deletion). Together, these results suggest that there are subtle but significant aortic changes as early as one week following receptor deletion. This suggests that losing TGF-ßsignaling causes early and immediate changes in the aortic smooth muscle cells and matrix proteins that are quantifiable by histology. More research is needed to understand the precise mechanisms behind why these changes occur and how they might be related to the development of aneurysms.

Modeling Chronic Gut Motility Deficiency in Autistic Children Using Patient-Specific Induced Pluripotent Stem Cell-Derived Enteric Neurons

Kyle James Curtis, Senior, Biochemistry, Chemistry (ACS Certified)
Levinson Emerging Scholar, UW Honors Program
Mentor: David Mack, Rehabilitation Medicine & Bioengineering, Institute for Stem Cell and Regenerative Medicine

Many children with Autism Spectrum Disorder (ASD) suffer from decreased gut motility that manifests as debilitating chronic constipation, leading to an unhealthy aversion to food. We hypothesized that the same neurological defects that cause the hallmark cognitive impairments in ASD also negatively impact the enteric nervous system controlling gut peristalsis. Within the autism spectrum, we chose to study one particular condition called Phelan-McDermid Syndrome (PMDS), because every patient with the diagnostic chromosome 22 micro-deletion has a dramatic decrease in gut motility. Evidence suggests that the gene-of-interest in the deleted region is SHANK3 because mice with SHANK3 haploinsufficiency show decreased postsynaptic density of neurotransmitter receptors. The overriding goal of this project is to develop novel drug treatments for patients with PMDS and ASD. Finding new compounds that can increase (or bypass) postsynaptic receptor density could alleviate these patients' gut motility problems. However, models to study the enteric nervous system either involve invasive biopsy procedures to remove enteric tissue from patients or the use of animal models that fail to replicate the pathology observed in humans. Therefore, the objective of this study is to create a human disease-in-a-dish model of enteric nervous system dysfunction by generating stem cells from PMDS patients and differentiating them into enteric neurons (ENs). We predict that molecular characterization of SHANK3-deleted enteric neurons will identify aberrant signaling pathways suitable for therapeutic intervention. Preliminary experiments from our

laboratory have demonstrated the feasibility of differentiating patient-derived induced pluripotent stem cells (iPSCs) into neural crest (NC) cells, which are necessary precursors to making enteric neurons. NC plus smooth muscle co-culture experiments will then be used to drive fluorescently labeled normal and SHANK3-deficient NC cells to enteric neurons, thus enabling phenotypic characterization of the disease phenotype *in vitro.*

Design of an Alpha-Sheet Peptide for the Inhibition of Aggregation in AL Amyloidosis

Lauren Nicole Martini, Senior, Computer Engineering, Bioengineering
Mary Gates Scholar
Mentor: Valerie Daggett, Bioengineering
Mentor: Matthew Childers, Bioengineering

The misfolding and aggregation of free light-chains into amyloid fibrils is the hallmark of antibody light-chain (AL) amyloidosis, a fatal disease associated with the accumulation of amyloid species in tissues throughout the body, including the heart and kidneys. Current treatment options, including chemotherapy and bone marrow transplant, do not address the causes of aggregation on a molecular level. Molecular dynamics (MD) simulations were used to investigate misfolding pathways in the aggregation of two light chain monomers, Jto and Wil. These simulations showed that under amyloidogenic conditions, conversion from beta-sheet to alpha-sheet secondary structure was observed in both Jto and Wil. Misfolded conformations, obtained from the MD simulations, were used to guide the design of alpha-sheet peptides, which have been used previously to inhibit amyloid formation in diverse systems. The designed peptides were evaluated computationally by docking them against misfolded conformations of Wil, and the best performing peptide was chosen for future experimental work to explore its potential to limit aggregation.

Toward a Peptide-Based Therapeutic for Type 2 Diabetes

Steven Hsu, Senior, Bioen: Nanoscience & Molecular Engr
Mary Gates Scholar
Mentor: Valerie Daggett, Bioengineering

Type 2 diabetes (T2D) is a disease associated with pancreatic islet β-cell failure; especially, the loss of β-cell mass. Approximately 75% of patients who start with one medication will need multiple to control the progression of this disease. With no cure and numerous secondary complications - blindness, kidney failure, heart attack, and stroke - T2D places an enormous burden on our society and healthcare system today; The disease is projected to be the seventh leading cause of death by 2030. Islet amyloid polypeptide (IAPP) deposition is observed in approximately 90% of T2D patients. While human IAPP (hIAPP) is amyloidogenic, forming amyloid fibrils and deposits, IAPP from rodents is not. Our group's previous findings suggest that T2D and other amyloid diseases form soluble toxic oligomers through a non-standard α-sheet secondary structure. We propose a peptide-based therapeutic to combat T2D via utilizing synthetic α-sheet compounds complementary to the amyloid-associated α-sheet structure to target the toxic oligomer form of hIAPP. In this study, we addressed the efficacy of this peptide-based therapeutic through inhibiting the aggregation of hIAPP via a Thioflavin-T assay and neutralizing the hIAPP oligomer cytotoxicity via a MTT cell viability assay in a human-derived pancreatic cell line. The preliminary results suggest that our peptides are effective in reducing hIAPP aggregation and oligomer cytotoxicity. Moving to a more biologically relevant model, we evaluated the effects of our compounds on reducing amyloid formation in the islet of Langerhans from transgenic mice; the preliminary results are encouraging. The α-sheet platform provides a novel potential therapeutic for treating T2D and other amyloid diseases.

Alpha-Sheet Peptides Inhibit Functional Amyloid Formation of *Streptococcus mutans* Biofilms Adhered to Artificial Salivary Pellicles

Natasha Anay Paranjapye, Senior, Bioengineering
Mary Gates Scholar
Mentor: Valerie Daggett, Bioengineering

Streptococcus mutans is an acidogenic bacterial species that predominates in the oral microbiome. *S. mutans* binds to the salivary pellicle, a layer of proteins that forms on the tooth surface and forms acids after metabolizing sugars. Accumulation of *S. mutans* biofilms leads to cavity formation, and when the bacteria travels to and infects the heart, can lead to infectious endocarditis. Therefore, decreasing accumulation of *S. mutans* is a key concern. Recent evidence suggests that *S. mutans* is among the bacterial species that utilize functional amyloid fibrils in its biofilms. Amyloids are insoluble fibrillar protein aggregates with a cross-ßstructure, and functional amyloids are used by bacteria to provide structure and strength to their biofilms. While functional amyloid systems in bacteria such as *E. coli* and *S. aureus* have been investigated, very little is known about the mechanism or purpose of *S. mutans* functional amyloids. Polyphenolic small molecule epigallocatechin gallate, or EGCG, is an amyloid inhibitor in S. mutans biofilms. Previous results from our lab suggest that amyloid fibrillization progresses via an intermediate that adopts a unique secondary structure, an alpha-sheet. Alternating L- and D- amino acid peptides adopt an alpha-sheet secondary structure and have been shown to inhibit amyloid formation in multiple mammalian systems by binding to soluble alpha-sheet-containing oligomeric species. Inhibition of amyloid formation by alpha-sheet peptides suggests presence of an alpha-sheet intermediate species on the pathway to functional amyloid formation. To investigate the mechanism of functional amyloid formation in *S. mutans*, alpha-

sheet peptide inhibitors were compared to EGCG for their ability to inhibit fibril formation of *S. mutans* adhered to an artificial salivary pellicle.

Inhibition of a Synthetic Amyloid's Aggregation and Toxicity by Multiple Native Amyloid Species

Timothy Mark Bi, Senior, Bioengineering
Washington Research Foundation Fellow
Mentor: Valerie Daggett, Bioengineering

Alzheimer's disease affects millions of individuals worldwide, yet there is an astounding lack of marketed treatments that can effectively slow the neurodegeneration associated with the disease. In the past few decades, evidence has emerged that small, soluble aggregates of beta-amyloid (Aβ) peptide known as oligomers are primarily responsible for toxicity in the brain, which has sparked research to develop inhibitors targeting the toxic oligomers. However, the actual structure of Aβ oligomers remains unknown, in large part because traditional methods used to determine protein structure are ineffective due to the dynamic and heterogeneous nature of these oligomers. This in turn has greatly hindered therapeutic development. Interestingly, a designed α-sheet peptide known as AP3 displays striking behavioral similarities to Aβ under low pH, making it an ideal model for understanding amyloid protein behavior. In addition, AP3 aggregation and toxicity are potentily inhibited by other amyloid species. The interactions between this synthetic amyloid and naturally-occurring amyloid species, including Aβ, are being explored both experimentally and via computer simulation, and the data are being used to design a de novo peptide inhibitor. This inhibitor is being tested for its ability to inhibit amyloid aggregation and toxicity, as well as whether it can specifically bind to heterogeneous populations of Aβ even at low concentration. The successful completion of this project wil result in significant progress towards understanding amyloid behavior and aggregation. This may eventually lead to novel applications of the α-sheet structure in treatments and diagnostic assays for Alzheimer's.