# Undergraduate Research Symposium May 18, 2018 Mary Gates Hall

# Online Proceedings

## POSTER SESSION 1

**MGH 241, Easel 147**

*11:00 AM to 1:00 PM*

**Thermodynamic Properties of the High-Affinity Binding of Glutathione to Glutathione S-Transferase Quantified by Isothermal Titration Calorimetry**

*Clay Renshaw, Junior, Biochemistry, University of Nevada Las Vegas*

*McNair Scholar*

*Mentor: Ronald Gary, University of Nevada, Las Vegas*

Glutathione S-transferase (GST) is an enzyme that is present in most organisms. Its primary function is the detoxification of xenobiotic compounds. In mammals, some GST isoforms have a role in modulating the MAPK signaling pathway. The GST protein from *Schistosoma japonicum* (26 kDa) is also an important tool used in biochemistry labs as a tag for the isolation of proteins via affinity chromatography. Reduced glutathione (GSH) is a substrate for GST that binds to its G-site with a high affinity, and this interaction can be exploited to purify GST-fusion proteins on agarose beads containing a GSH moiety. In the research presented, GST was expressed in *E. coli*, purified, and dialyzed to remove GSH ligand completely. The protein concentration of the purified, dialyzed GST sample was quantified using Qubit$^{TM}$ fluorescence spectroscopy. Isothermal titration calorimetry (ITC) was utilized to measure the dissociation constant and thermodynamic properties of the binding of GSH to GST. ITC is a highly sensitive analytical method that monitors changes in heat produced from noncovalent chemical associations. In order to obtain accurate ITC measurements, various concentration ratios of GSH/GST were explored. Quantifying the binding affinity and thermodynamic properties of GSH binding with GST provides useful information for investigators who use GST as a fusion tag or who are interested in reactions between the GSH-GST complex and its substrates.

## POSTER SESSION 1

**MGH 206, Easel 177**

*11:00 AM to 1:00 PM*

**Cross Species Analysis in Zebrafish and Rats to Determine Differences in Gene Expression Relating to Bone Regeneration**

*Eric Christopher (Eric) Katzung, Junior, Bioengineering*

*Mentor: Ronald Kwon, Orthopaedics and Sports Medicine, UW School of Medicine/Institute for Stem Cell and Regenerative Medicine*

Zebrafish have a higher regenerative potential than mammals, but the genetic differences between the groups that lead to their respective responses are not fully understood. Cross species analyses with mammalian models allows comparison of these genes with their mammalian orthologs to determine which genes show similar or different enrichment during bone regeneration processes. Information from microarray datasets for both rats and zebrafish provide information about the genome-wide transcription during regeneration in each model; the regenerative process in rats has a different timeline than zebrafish, so our lab has developed software to relate the two timelines so that gene expression can be directly compared. Using this information, I am performing two sets of analyses. In the first analysis, I am determining which genes show conserved expression in both models to identify deeply conserved genes essential for regeneration in both systems. In the second analysis, I am finding genes that are differentially expressed between rats and zebrafish during regeneration; pathway analyses of these genes can provide a better understanding of the differences in regenerative response, and potential ways to manipulate this response in both models. Determining the processes that involve these genes can help identify genetic targets associated with important regenerative functions, which can be applied to a mammalian model to potentially improve its regenerative response. Preliminary results for the similarly expressed genes' ontology of shows involvement in skeletal development, phosphate transport, and organogenesis; the differentially expressed genes' ontology shows involvement in morphogenesis, peptide linking, and organogenesis. The genes with similar expression affect ECM-receptor interactions and focal adhesion pathways, while the differentially expressed genes are affect nicotinate and nicotinamide metabolism and carbon fixation. Manipulating these pathways in zebrafish can show their distinctive roles in the regenerative process and provide a better understanding of the differences between the zebrafish and mammalian regenerative processes.

# POSTER SESSION 1

**MGH 206, Easel 178**

*11:00 AM to 1:00 PM*

**Geometric Models and Image Analysis Algorithms for Detecting Skeletal Phenotypes in Zebrafish to Identify Osteoporosis Causal Genes**

*Rehaan M. Bhimani, Sophomore, Pre Engineering*
*Mary Gates Scholar*
*Mentor: Ronald Kwon, Orthopaedics and Sports Medicine, UW School of Medicine/Institute for Stem Cell and Regenerative Medicine*

More than 40 million people in the United States have or are at risk for osteoporosis, a condition characterized by traits including low bone mineral density (BMD) and high fracture risk. Though genome-wide association studies have identified many candidate genes associated with genetic risk factors, genetic variations that are associated with osteoporosis-related traits, the causal genes underlying these risk factors remain undiscovered. My research centers around developing tools to assess bone morphology in a zebrafish model to identify specific genes that cause osteoporosis or other skeletal abnormalities. Because a trademark characteristic of osteoporosis is expanded bone diameter due to accelerated bone remodeling and increased resorption in the endosteum, I have developed tools to assess bone expansion in zebrafish vertebral structures. By using integral calculus and basic machine learning with the R programming language, I developed a predictive model for calculating approximate diameters for the centrum foramen, the hole in the zebrafish vertebral body. As a more direct alternative, I have also developed a program using image analysis tools in MATLAB that identifies the foramina of the centra, neural (superior) arches, and haemal (inferior) arches in binarized microCT stacks of zebrafish skeletons and calculates the cross-sectional area of each foramen. I am using these tools to retroactively analyze previously scanned mutant zebrafish to identify genes that have phenotypes consistent with abnormal foramina of the vertebral body and arches. I have determined that mutants with the bmp1a and plod2 genes knocked out separately have expanded centrum foramina. After compiling and analyzing a zebrafish data archive with data from other previously imaged mutant samples, my results will indicate previously undetected phenotypes. This data will aid in my lab's efforts to determine the causal genes underlying osteoporosis genetic risk factors. This research may lead to discoveries that are used in future osteoporosis gene therapies.

# SESSION 1I

## MULTIDISCIPLINARY APPROACHES TO MEDICAL RESEARCH

*Session Moderator: Gwenn Garden, Neurology*

**MGH 248**

*12:30 PM to 2:15 PM*

* Note: Titles in order of presentation.

**Time-Lapse Imaging of Cell Dynamics During Zebrafish Bone Regeneration**

*Micaela L Everitt, Senior, Bioengineering*
*Mary Gates Scholar, Washington Research Foundation Fellow*
*Mentor: Ronald Kwon, Orthopaedics and Sports Medicine, UW School of Medicine/Institute for Stem Cell and Regenerative Medicine*

It has long been known that certain species possess the capacity to regenerate bony appendages following amputation through a process called epimorphic regeneration. For instance, zebrafish possess the ability to regenerate their tail fin bones. Early regeneration involves the formation of a blastema, similar to the blastema that mediates limb regeneration in salamanders. After the blastema forms, the bone regeneration process resembles the bone developmental process in humans. Thus, a better understanding of epimorphic regeneration holds promise to enhance our understanding of regenerative biology, allow for medical advances in bone tissue engineering, as well as increase understanding of the skeletal developmental processes. A challenge in understanding the regeneration process is the inability to immobilize fish to generate time lapsed images of various stages of regeneration. Typical methods for anesthesia in zebrafish only enable 10-20 minutes of sedation. Several studies (including those by our lab) have developed specialized methods to do long-term imaging with the use of Tricaine Methanesulfonate and Benzocaine. However, both anesthetics are sodium channel inhibitors, which inhibit the regeneration process itself. In order to circumvent this problem, we designed a chamber for lower anesthetic plane imaging with a restraint system, as well as a glass-bottom window to facilitate imaging. The chamber is coupled to peristaltic pumps so water and anesthesia can flow in and out. In pilot studies we have found the zebrafish quickly acclimate to the chamber, and have remained in the chamber for two hours. We have been able to image sp7, NADH and birefringence simultaneously for the first 2 hours of regeneration and have found stability in these signals over time. Our studies indicate that this long-term imaging chamber may allow us to see details of dynamic processes that unfold during bone regeneration, which currently can only be seen through snapshots of short-term imaging.

# POSTER SESSION 3

**MGH 206, Easel 168**

*2:30 PM to 4:00 PM*

**RNA Interference Screen for Genes Involved in JNK Dependant Basal Cell Extrusion of *Drosophila melanogaster***

*Catherine Baoanh Pham, Junior, Business Administration*
*Katherine Kaidi Zhao, Senior, Microbiology*
*Shannon J. Hu, Junior, Pre-Sciences*
*UW Honors Program*
*Sung Ahn, Sophomore, Pre-Social Sciences*
*Brittney Renee Spooner, Senior, Biochemistry*
*Mentor: Jiae Lee, Biochemistry*
*Mentor: Young Kwon, Biochemistry*

Previous research on JNK-mediated stress signals demonstrated that stem cells in the posterior midgut of Drosophila Melanogaster only undergo compensatory proliferation or apoptosis. However, our group discovered that the stem cells can also undergo the process of basal extrusion and dissemination, resulting in the cells being eliminated from the tissue into the hemocoel, the blood containing intertissue body cavity. The JNK signal promotes the cells to exit the epithelium of the gut, move through the muscle layer, and be released to the hemocoel, which resembles the process of metastasis in human cancer. In order to understand the mechanism of this extrusion process, we carried out an RNA Interference (RNAi) screen and sought to find genes that are necessary for the stem cell extrusion in the JNK activator, HepCA, expressed flies. We used the ESG-GAL4, UAS-GFP, TUB-GAL80TS(EGT) genetic system to study the knockdown effect from the RNAi of each gene. We selected 215 lines of kinases and phosphatases of flies to test if the knocked-down gene results in suppression of cell elimination by the JNK stress signal. After 4 days of inducement, which is the sufficient time for the JNK signal can promote complete extrusion of the intestinal stem cells, the intestines were dissected, fixed, mounted, and examined with a fluorescent microscope for any presence of stem cells. Through this screen, we could find 33 lines that had strong suppression of cell elimination, 39 lines that had a moderate effect. Our results suggest that the knocked down genes with strong suppression of cell elimination are involved in the mechanism of basal cell extrusion, and future research dictates an investigation into the molecular function of each of these genes.