After my junior year in high school I volunteered in a Chemical Engineering lab at Washington State University. Since then, my path has been directed by a passion for research. A variety of lab experiences provided excitement and challenge as I produced hydrogen from methane and recorded its combustion at over 600˚C, trudged waist-deep in lakes to collect samples of rust-producing bacteria, and used fluorescence microscopy to record domain formation on model cell membranes. I learned that through research I can give back to the academic community while building skills and exploring my curiosity. I experienced the potential for classroom knowledge to be directly applied and enhanced in the lab. Now, I am eager to apply scientific discoveries in the field which excites me the most, health care.

Cardiovascular disease is a chief concern for medical research worldwide. In the United States heart disease is the leading cause of mortality (about 27% of deaths), and a major cause of disability with projected costs of $304.6 billion for health care services, medications, and lost productivity in 2009.[1] Studies at the University of Washington's Heart and Muscle Mechanics (HAMM) lab examine the ability of stem cells and gene therapy to repair diseased and damaged cardiac tissue. As an undergraduate researcher in Dr. Michael Regnier's lab, I have significantly increased my understanding of the complexity of cardiovascular disease and the potential for research to alleviate health problems.

The cardiac cycle of contraction and relaxation is regulated by intracellular calcium ion concentration ($[Ca^{2+}]$). Specifically, contraction is initiated and regulated by the binding of Ca^{2+} ions to the myofilament regulatory protein troponin C (cTnC). Heart failure manifests as depressed contractility, which can be caused by reduced sensitivity to Ca^{2+} or a decreased number of active cells. Current pharmaceutical treatments are 'upstream' of the myofilaments, and have significant side effects such as increased intracellular $[Ca^{2+}]$ during diastole,[2] with the result of incomplete muscle relaxation and continued heart failure.[3] A novel long-term treatment is necessary to curtail the growth of casualties and costs associated with cardiovascular disease. For example, targeting the $[Ca^{2+}]$ 'sensor', cTnC, may

enhance contraction during systole without causing incomplete relaxation during diastole. The HAMM lab has engineered a cTnC with a single amino acid substitution, L48Q, which shows enhanced contractility at physiological calcium concentrations in demembranated rat trabeculae.[4,5] Thus, L48Q cTnC shows promise as an effective treatment for calcium insensitivity and cardiac disease.

Current studies at the HAMM lab are designed to more fully evaluate L48Q cTnC and the gene therapy strategies necessary to deliver treatment. This summer I took part in a project to determine the mechanism controlling [Ca^{2+}] sensitivity of cTnC. The project involved exchanging native cTnC proteins for L48Q proteins produced by E. coli. Native rat trabeculae and rat trabeculae which underwent an exchange with wild-type troponin were also tested as controls. Experiments at multiple sarcomere lengths were conducted to elucidate the length-dependent nature of the trabeculae containing the L48Q protein. By harvesting cardiac tissue from rats, clipping individual trabeculae, and testing the stiffness and force-generating capacity of altered trabeculae I learned valuable research skills.

I have now begun an independent project under the mentorship of Dr. Regnier to quantify the yield of L48Q protein exchanges. The goal of my project is to determine the amount of incorporation of L48Q cTnC within individual trabeculae, which can then be directly correlated to a shift in calcium sensitivity. Although changes in calcium sensitivity between native and L48Q cTnC-Tn exchanged trabeculae have previously been reported, the amount of L48Q cTnC-Tn that has been incorporated within the trabeculae during our exchanges must be quantified. Data from this study will document the effectiveness of L48Q exchange procedures and will quantify the relationship between L48Q exchanges and shifts in calcium sensitivity. It is important to determine the critical level of L48Q incorporation into the contractile filaments because gene therapy strategies such as viral/non-viral infection of cDNA that expresses the mutant will likely not replace all of the native TnC.

To determine the amount of L48Q cTnC incorporated within trabeculae I will need to implement a relatively novel technique. The standard method of separating proteins by molecular weight is not sensitive enough in this situation because the differences between the proteins are slight. In addition, the incorporation of a protein tag, or label, could affect the properties of the protein and would therefore require extensive control studies. The alternative method I will employ utilizes the location of cTnC within the troponin complex. Since our procedure exchanges the entire troponin complex instead of simply cTnC we are able to use properties of the entire complex in order to quantify our exchange. One of the other two components of troponin, cTnI, can be phosphorylated by protein kinase A. [6] The protein we exchange is a recombinant protein derived from E.Coli, which lack the phosphokinases present in mammalian myocardium. Therefore, our recombinant protein is completely dephosphorylated. Knowing this, we can determine the amount of recombinant protein exchanged by western blot analysis of native and exchanged trabeculae, using a specific phosphoprotein antibody, and subsequent image densitometry. The amount of exchanged protein incorporated within trabeculae will then be correlated to the shift in calcium sensitivity. I am also assisting with control experiments to determine the separate effect on dephosphorylated TnI on the contractile properties of trabeculae with exchanged troponin. I appreciate the significance of this challenging project which will test and hone the skills I have already developed, produce valuable information, and allow me to meaningfully contribute in the lab.

As my research progresses, I plan to become involved in additional experiments that will determine if L48Q cTnC can improve the contractile properties of stem cell-derived cardiomyocytes and help them mature towards the adult form. This line of experiments merges the HAMM lab's efforts in gene therapy and cell replacement/tissue engineering to treat patients following a heart attack (myocardial infarct). Combining these novel and cutting-edge approaches is an exciting new direction for the HAMM lab and will allow me to use a variety of novel bioengineering techniques.

I knew from my first day in the HAMM lab that my academic and career goals would be enhanced. As I was handed my homework assignment, a differential equation modeling muscular force redevelopment, I realized the importance of education to successful research. After months in the lab, I know that hands-on experience provides interest and depth to classroom concepts. In the lab I perform intricate procedures and become proficient with new equipment and techniques under the guidance of an impressive team. I am grateful to be one of only four undergraduates in the lab and have benefitted immensely from the mentorship of both professional and academic researchers. I am inspired by my lab experiences and conversations with my mentors. I have seen first-hand that successful research depends on a solid educational background and look forward to applying my education through research.

My goals of a PhD in Bioengineering and career in medical research require continued commitment to exploring human biology. Increased knowledge at cellular and genetic levels will provide more effective treatment programs and the potential to delay or eliminate onset of disease. Providing quality health care without enormous expense is critical for the future of our nation. Involvement in the HAMM lab allows me to better understand the research and development necessary to produce safe, effective therapies that can reduce suffering and costs. With a focus on stem cells and gene therapy, the HAMM lab remains on the cutting edge of medical science. As I intend to develop cost-effective technologies and therapies that can be employed in developed and developing countries, proficiency in novel research is critical.

Conducting increasingly independent research in my field adds valuable depth to my experience at the UW. Work in the lab not only inspires my pursuit to find innovative solutions, but teaches other important lessons. In the mentorship I receive from Dr. Regnier, Dr. Razumova, and the rest of the lab I recognize the importance of giving back through education. I look forward to a career which allows me to both teach and lead a lab so that I can pay forward the time and energy dedicated to me.

Figure 1 - Sample of Comassi blue stained SDS-PAGE gel
Lanes (Left to right): 1 - Control (protein ladder); 2, 4 – Wild Type Troponin Complex; 3, 5 – cTn with cTnI L48Q

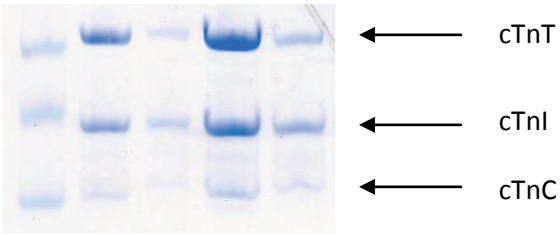
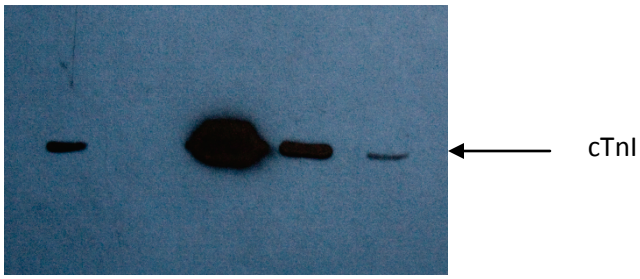


Figure 2 – Sample of Western Blot
Lanes show variation in cTnI fluorescence due to relative phosphorylation



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