I. Intellectual Merit

Thrombosis, a clot forming inside a blood vessel, is one of the leading causes of myocardial infarction (heart attack) in the U.S. Statistically, as many as 1-2 people per 1000 will suffer a blood clot to either an artery or vein. One of the main ways thrombosis is initiated is by platelet adhesion to collagen, a protein that makes up the structural portion of blood vessel walls. Typically, platelets are recruited to the vessel walls by the protein von Willebrand Factor (vWF), which tightly binds collagen. The mediated effect of vWF can be beneficial, such as bringing platelets to a blood vessel needing repair, however in certain conditions, thrombosis can occur from collagen's inability to remove vWF, and the resultant over-addition of platelets.

Over the past 10-15 years, scientists have reported the development of several peptides with anti-thrombosis activities that bind collagen and can displace vWF. Those particularly noteworthy were cyclic in nature, where the amino acid sequence of the peptide was held together through a relatively weak side-chain bond. Using our established strategy for improving peptide stability with "head-to-tail" cycles held together with a stronger bond, we began studies this semester that adapt and improve the compounds found in the literature.

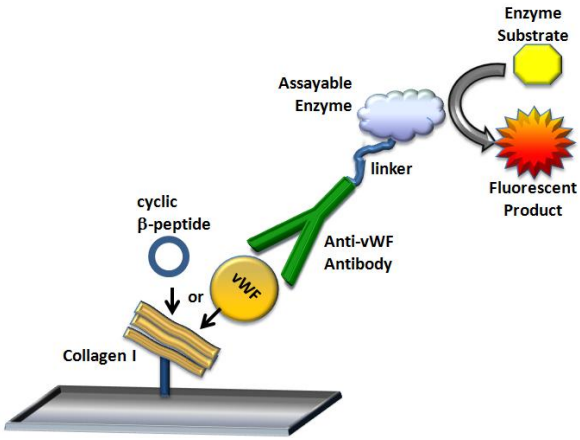

Fig. 1. ELISA diagramming the competition of vWF, our cyclic β-peptides and how results are visualized.

This summer we will build on our promising collagen-binding cyclic peptides. Ultimately, cyclic β-peptides, which are non-natural, folding oligomers, in place of the peptides currently developed in our lab, would offer many advantages as potential first generation therapeutics. β-peptides consist of chains of β–amino acids with an extra carbon along the amino acid backbone. As synthetic compounds, they resemble peptides found in nature, however contain several structural differences that make them intensely stable to the cellular environment, unrecognized by degrading factors. Their potential is vast: β-peptides have been developed into novel antibiotics, and cell permeable structures capable of inhibiting interactions found in cancer and infectious disease. **Our overarching goal is to synthesize cyclic β-peptides that bind collagen by displacing vWF in a dose dependent fashion.** This is the first step towards developing novel anti-thrombosis agents.

To achieve these goals, the students will perform the bioorganic synthesis of β-amino acids in two steps. Students will purify the final β-amino acids using column chromatography, as well as high performance liquid chromatography (HPLC), mass spectrometry and nuclear magnetic resonance spectroscopy (NMR) to identify the compound they made. Then, using standard solid phase peptide synthesis procedures, students will create the β-peptides from their synthetic β-amino acids, and will use our published protocol for cyclizing the products, characterizing their final peptides using the equipment described above. Once the compound preparation is achieved, students will use an Enzyme-Linked Immunosorbent Assay (ELISA) to quantify their β-peptide's ability to displace vWF from binding collagen (**Fig. 1**). A mixture of our cyclic β-peptides with vWF will be applied directly to plastic assay plates containing collagen immobilized in carefully separated wells. After incubation and washing, they will ascertain if vWf was able to bind collagen or if the β-peptides instead displaced vWF allowing it to be washed away. They will use a specific vWF antibody that elicits a fluorescent response to

detect the presence of vWF and will decipher the magnitude of this competition which will provide a useful screen for our β-peptides' potential as anti-thrombotic agents.

Weeks 1-2, students Susan Knox and Dylan Nguyen will synthesize, purify and characterize the individual β-amino acids and weeks 3-5 they will synthesize the three β-peptides for our study, purify and identify their products. Weeks 6-7 they will perform the ELISA study, and compare their results to the current peptide work both in my lab and the literature. Finally, the 8th week, students will complete their studies and perform stability assays, based on published work from my lab last year, to quantify the stability of the cyclic β-peptides in the cellular environment. These results will contribute to a future publication in a peer-reviewed journal such as *ChemBioChem* or *Biochemistry*. Additionally, I plan for this work to continue in our lab throughout the subsequent semesters, and these students would most likely present their results at the national American Chemical Society conference either March or August 2015.

II. Role of Students and Mentor

As there are many compounds to make and techniques to learn, the project is best divided among two students, with my leadership. Given my background in β-peptides, from graduate school and ELISAs, I will confidently lead the students to successful outcomes. Both students are rising Juniors and new to my research lab, so I will teach them each phase (synthesis, purification, and ELISA) and only after sufficient progress will allow them to work independently. In overall scope, they will learn chemical synthesis, how to use high level instrumentation and biochemical assay development. Each student will be assigned several β-amino acids and a β-peptide to work on by themselves. Additional compounds will be made through the collaborative effort of both students working together as a team. This allows them autonomy, individual ownership over their project, and yet team building and cooperation. Susan's previous research experience at Boise State University gives her an advantage as she already knows how to use many pieces of equipment and has a synthetic background. Dylan's skills in the classroom environment show that he will learn quickly and bring a fresh perspective to the project. I will set broad goals for each week and will interface with the students daily regarding the completion of their goals. Throughout the summer I will give them reading assignments from the primary literature and ask them to evaluate these critically so that they are fully immersed in understanding the project background as well as learning the lab techniques.

III. Broader Impacts

This is my fourth summer as a tenure-track Assistant Professor at TCNJ, and while I hope to obtain tenure this year, participation in the MUSE program will further my scholarship and help contribute to future promotion. Last summer, 2013, I did not participate in MUSE and as my current research undergraduates are graduating this May it is imperative that I have new students to expand my mentorship and who will continue to keep my established scholarship thriving and growing. This study will build on those begun this semester and will become part of a publication intended for submission by Spring 2015. Both students I wish to engage in research are highly motivated, high-achieving, with backgrounds in laboratory experience. Susan is interested in pursuing research and wishes to continue to a PhD program in biochemistry when she graduates. As a woman in science, she serves as a role model for younger students. Dylan is interested in a biomedical career, where biochemical research will enrich his path. As an underrepresented minority, Dylan serves as a role model, as well, for other similar students looking for an avenue into research. Performing MUSE in my lab is pivotal for students who have an interest in applying chemistry to problems in biology, and will be a springboard for both Susan and Dylan as they move forward in the field at large.