PURIFICATION OF THE NATIVE THIN FILAMENT WITH POST TRANSLATIONAL MODIFICATIONS AND MEASUREMENT OF CALCIUM MEDIATED ACTIVATION WITH THE *IN-VITRO* MOTILITY ASSAY

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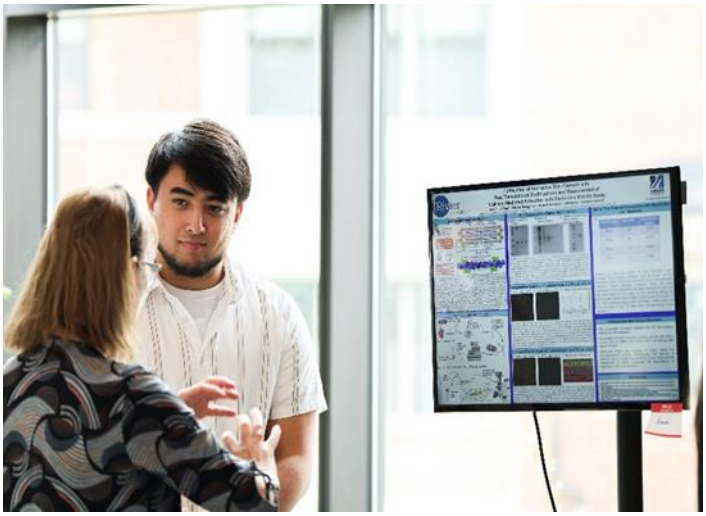

Aaron LePoer '24, a senior Biology major, *graduating in May 2024,* presents at the Transformative Learning Conference (TLC) in April 2024.

"I was fortunate to be introduced into this field beginning with Dr. William Schmidt. He opened my eyes to the biophysical and biochemical world of proteins and how we can alter their functions through post translational modifications. These types of modifications and studies are steppingstones to potential therapeutics in the medical field to treat certain diseases like hypertrophic cardiomyopathies.

Luckily, I have my mind set on medical school, but this type of research even strengthened my hopes of pursuing medical school. Having been able to shadow an orthopedic surgeon while having the laboratory experience has shown me how amazing the human body is. I now know there are so many complex mechanisms that line the idea of muscle contraction, and how these force generating functions create movement in our body. This type of research has developed my levels of persistence, tenacity, and even maturity. Research through Rivier is something that will stick with me for life and has bettered me as an individual."

Abstract

The sarcomere is the basic contractile unit of cardiac muscle consisting of the thin filament that contains actin, and the thick filament that contains myosin. Myosin interacts with the thin filament through complex cycles of attachment and detachment of myosin heads; myosin hydrolyzes adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate and isomerizes to the pre-power stroke conformation, where the head is primed by calcium (Ca^{2+}) to generate force. As a result, the rate at which cooperative activation between the thick and thin filament occurs drives muscle contraction. Native thin filaments (NTFs) can be used to examine the mechanism of muscle contraction. The molecular regulation may vary within isolated NTFs. We intend to study the proteins that are involved in modulation of muscle fibers using *in-vitro* assay that can mimic the *in-vivo* cardiac proteins interactions. In this study, dissected porcine cardiac tissue was used to purify NTFs and at the same time to enhance purity of NTFs by removing connective tissue. The NTFs were isolated through a series of

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centrifugations and homogenization. Flow cells containing a nitrocellulose membrane coat were probed with myosin to allow for the passage and interaction with the isolated NTFs. Then, purified NTFs were fluorescently labeled and examined under epifluorescence microscopy via *in-vitro* motility assays (IVMAs) to assess the velocities of the thin and thick filament cycling of attachment and detachment under varying concentrations of Ca^{2+}. The present results show the successful purification of NTFs and their Ca^{2+} mediated activation in the IVMAs.

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