Comparing the Motility of Myosin X with Parallel and Antiparallel Dimerization Domains
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This summer I worked in the lab of Dr. Yale E. Goldman under the mentorship of Dr. Matthew Caporizzo at the Perelman School of Medicine in the Department of Physiology. My project focused on myosin X, an actin-based molecular motor involved in filopodial formation, cell migration, adhesion, and mitotic spindle orientation. Myosin X is responsible for the metastatic nature of cancer by inducing filopodial formation by an unclear targeting mechanism that gives cells their ability to invade surrounding tissue. The goal of this project was to observe the processivity of isolated myosin X molecules on single and fascin bundled actin filaments \textit{in vitro}. These structural changes induced by ATP hydrolysis enable myosin X to generate force and do work inside the cell. Recently, it was discovered that myosin X has a unique coiled-coil domain that facilitates antiparallel dimerization. To test the implication of antiparallel dimerization on chemo-mechanical regulation, single molecule experiments were conducted with forced parallel and antiparallel constructs of myosin X dimers with spatiotemporal resolution capable of resolving individual stepping events.

In order to achieve spatial resolution approaching 1nm at speeds of 50ms, Total Internal Reflection Fluorescence Microscopy (TIRFM) was used to selectively illuminate active molecules labeled with quantum dots, fluorescent particles of a semiconducting material with diameters in the range of 2 to 10 nanometers, which allows tracking of their real-time motion. One challenge that arises when performing single molecule experiments is the need to eliminate background fluorescence from outside of the focal plane in order to improve the signal-to-noise ratio and consequently resolution of the experiment. TIRFM utilizes the evanescent wave to illuminate a confined space near the coverslip/sample chamber interface to obtain signal to noise levels sufficient for single molecule localization. TIRFM allows us to track the structural changes of myosin X, which are directly related to its processivity, step-size, velocity, and backward stepping frequency. These quantities are then used to infer the chemical kinetics of the motion, including ATP and ADP unbinding and rebinding rates of both the leading and trailing heads of the dimer.
My time in the Goldman Lab this summer was an overall educational experience. In addition to the research I performed in order to understand the complexities of the project on which I worked, I also had the opportunity to participate in a colloquium organized by Dr. Caporizzo with the other undergraduates in the lab. During these weekly sessions, we learned how to read and critically analyze recently published papers in the field of cell motility biophysics. This experience culminated with the opportunity to present a paper for the Department of Physiology at our weekly Journal Club. Overall, I thoroughly enjoyed my summer in the Goldman Lab. I have a newfound understanding of laboratory work that has caused me to rethink my future career path, and I look forward to continuing my research project in the fall semester.