Building an optical tweezer to investigate the membrane remodeling activity of srGAP proteins

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Neurodevelopmental disorders such as intellectual disability and autism spectrum disorder affect 4% of the population and have been tied to improper neuronal cell growth[1]. In order to develop healthy interconnections, brain cells undergo shape changes and form outward, finger-like protrusions known as filopodia. The energy required to stretch the cell membrane is significant and proteins like Slit-Robo GTPase Activating Proteins (srGAPs) have been implicated as helpers in this process[2]. Because the lack of proper srGAP3 expression has been linked to severe intellectual disability, understanding how srGAPs help brain cells develop is a key area of research[3]. The Henderson Lab seeks to understand srGAP activity by testing if/how srGAPs can sense and selectively bind to membrane protrusions of various curvatures. Proteins with similar compositions to srGAPs have been observed to cluster together and form "finger trap" like scaffolds that stabilize a region of stretched cell membrane[4]. We hypothesize that srGAPs will form similar scaffolds on filopodia. To test this, Henderson lab seeks to mimic the process of a cell membrane stretching outward during neuronal growth in the laboratory. Bubbles (vesicles) made of the same phospholipid bilayer that makes up cell membranes will be injected with srGAP protein to simulate cells. A tool known as an "optical tweezer" will then be used to manipulate a small plastic bead with focused laser light. By adhering this plastic bead to the surface of a phospholipid bubble and then pulling it away, we can "pluck" out a tubule that mimics a filopodia protrusion. The system will be conducted under a confocal microscope, which will be used to image the protein's response to the artificial protrusion. The other end of the vesicle will be aspirated by a micropipette. Changing the aspiration pressure will allow the regulation of vesicle surface tension and thus curvature of the resulting protrusion.

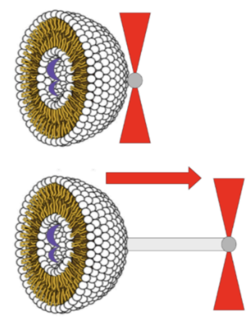

Figure 1: optical tweezer laser light (in red) is used move a plastic bead (in gray) to pluck a membrane tubule from a phospholipid vesicle containing srGAP protein (in purple).

This summer, I constructed an optical tweezer system. This technology leverages the principles of refraction to manipulate microscopic objects using focused laser light. Light bends as it passes through objects with different compositions. When it passes through a plastic bead, it will bend inward. Because light has momentum in motion, and momentum must be conserved, the bending of the light inward creates a force outward. This force holds the bead in place using laser light. Moving the center of focus will generate a force on the bead, moving it as well. Over several weeks, I worked to precisely guide a laser beam into the sample stage of a confocal microscope using 45 degree relay mirrors, lenses (to maintain the laser focus) and a simple telescope (to expand the beam to an optimal width at the sample stage). Another week was devoted to optimizing the position of a camera armature, which can be used to visualize the sample and the laser light at the sample time. The last three weeks of my research consisted of experiments in which I worked to trap plastic beads with laser light. After successful trapping, I used specialized video processing software to attempt to determine the force of the laser light on the bead based on variations in the bead's Brownian motion before and after being trapped in laser light.

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