Investigating the Lipid-Binding Affinities of srGAP

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Background: The purpose of this project was to analyze Slit–Robo GTPase-activating protein's (srGAP) precise lipid binding affinities and how specific lipid affinities can organize srGAP assembly on membranes necessary for their formation of filopodia. To do this, we aimed to assess srGAP binding to specific membrane compositions developing a fluorescence binding technique.

Filopodia, which are finger-like, actin-rich membrane protrusions, an essential cellular structure responsible for cell migration, neurite outgrowth, and tissue closure, and serve as precursors for synaptic maturation[1]. To induce cellular filopodial formation, precise control of membrane-deforming Bin/Amphiphysin/Rvs (BAR) proteins initiate actin filaments into three dimensional structures[1,2]. srGAP, which is a subfamily of BAR proteins and is our protein of interest, is connected to filopodia formation[2]. srGAPs, due to their specialized F-BAR domain, when bound to the plasma membrane, induce outward membrane bending which leads to filopodia initiation[2]. srGAP protein-associated mutation in the human genome can result in dysregulation leading to neural developmental issues, cognitive deficits, contribution to a wide variety of cancers, and early infantile epileptic encephalopathy[2,3]. The mechanisms behind the regulation of srGAP proteins and their precise binding affinities to membranes are relatively unknown, and the understanding behind these mechanisms could be pivotal towards our understanding of many neurological processes including intellectual disability and Autism Spectrum Disorder.

It is hypothesized that changes in surface density of phospholipids can elicit srGAP binding. By developing a fluorescence-based tryptophan assay to quantify the lipid binding specificities of srGAP to artificially prepared membranes in the form of vesicles having user-defined compositions, one can identify precise lipid binding affinities of srGAP, which could be key in understanding the formation of filopodia structures.

Methodology: srGAP contains a tryptophan residue in their F-BAR domain which can be strategically used to quantify srGAP binding onto membranes. Because tryptophan's fluorescence undergoes a spectral shift when moving from a hydrophilic to hydrophobic environment, a tryptophan fluorescence binding assay detecting tryptophan's change in environment can quantify the movement of srGAP proteins from aqueous to hydrophobic bilipid layer environment[4]. To develop this technique, Annexin V protein, which has well documented lipid binding specificities for a fluorescence-based tryptophan assay, was used as a control[5].

Exogenous expression in *E. coli* was used to produce Annexin V. *E. coli* was then lysed using sonication and chemical lysis. To isolate Annexin, centrifugation followed by nickel affinity chromatography and size exclusion chromatography via the fast protein lipid chromatography machine was utilized. To verify that our protein we produced was Annexin V, SDS-PAGE and Western Blots were used. Once verified, the Annexin quantity was assessed via a bicinchoninic assay and upscaled using the Vivaspin 20 and centrifugation.

Once Annexin V was purified, a tryptophan fluorescence binding assay was used to quantify Annexin V binding onto membranes. To do this, Annexin V was introduced to calcium, which was done to induce an outward shift of Annexin V's tryptophan residue from hydrophobic to hydrophilic environment. Then, the fluorescence was measured while titrating in small quantities of lipid to identify the precise lipid binding affinities. The development of this technique is still ongoing.

Results and Future Directions: Annexin was successfully produced and purified. The final concentration of protein achieved was 3.41 mg/mL and the total protein was 2.91 mg. The expected spectral shift of Annexin V was observed when Annexin V was introduced to calcium. The determination of Annexin V's spectral shift when introduced to lipids is still ongoing. In the future, once the fluorescence tryptophan binding assay is fully developed, srGAP lipid binding affinities will be assessed using this technique.

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