Investigating the Role of srGAP Protein Selective Lipid Binding in Cell Membrane Deformation

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Neurons in the brain communicate through synapses whose development relies on cellular sensing and mobility. This process requires high-precision cell membrane deformation. Irregular development of neural connections is linked to autism spectrum disorder and intellectual disability.[1] The formation of proper synaptic connections relies on tiny (up to 10 µm long and 200 nm wide) fingerlike protrusions of the cell called filopodia. Curvature of the cell membrane is moderated largely by Bin-Amphiphysin-Rvs (BAR) proteins, whose positively-charged banana-shaped dimers bind to the negatively-charged inner membrane leaflet, sensing and generating membrane curvature.[2] The Henderson Lab is researching the SLIT-ROBO GTPase-activating protein (srGAP) subfamily of BAR proteins (Fig. 1), a poorly-understood but important contributor to filopodial formation.[1]

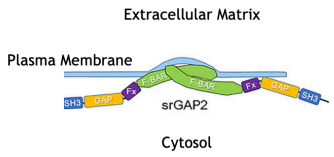

Figure 1. A membrane-adhering protein dimer forms between srGAP proteins. Adapted from Reference 3.

The Henderson Lab plans to determine srGAP's binding mechanism by determining its phospholipid binding partners in artificial model cell membranes. To do so, pure samples of aqueous srGAP must be produced. My project consisted in testing and refining procedures for srGAP production and purification using srGAP2 as a representative. To do so, *Escherichia coli* bacteria were transformed with recombinant DNA plasmids containing srGAP2's sequence; once grown into a culture, they were then stimulated to produce srGAP2 before destroying them and purifying the soluble protein using nickel-affinity chromatography. Unfortunately, as noted by honors student P. Spyrou '25, srGAP demonstrated low solubility in aqueous buffers, preventing its purification in solution.[4] Initial experiments, replicating his procedure as well as modifying it, confirmed this result (Fig. 2), suggesting the formation of inclusion bodies—insoluble masses of tangled protein in bacteria.

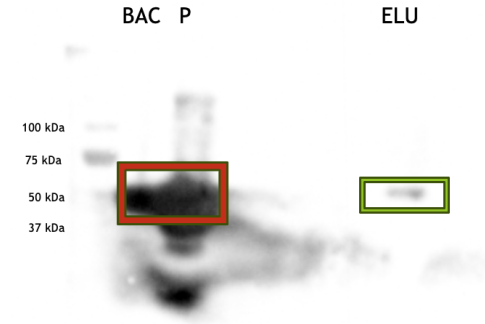

Figure 2. Color-inverted western blot showing lack of extracted protein due to insolubility. The BAR domain of srGAP2 weighs 59 kDa; thus, the protein bands at 59 kDa on the vertical axis should contain srGAP2. The heavy bands (red box) in the columns labeled BAC (Bacteria) and P (Pellet) indicate successful production of srGAP2 in the bacterial culture; however, almost all of this remained insoluble in the cell pellet. The faint band (green box) in the ELU (Elution) column shows the low quantity of soluble srGAP2 recoverable after purification.

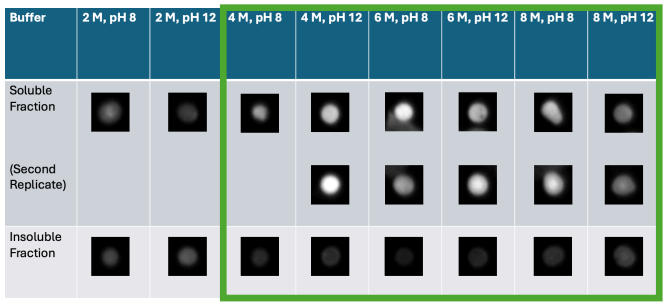

Figure 3. Dot blot results confirming solubility of srGAP2 in urea-based extraction solutions. The soluble fractions of most buffers containing between 4-8 M urea showed appreciable fluorescence (green box, Rows 1-2), indicating successful solubilization of the protein by high concentrations of urea. Meanwhile, very little protein remained insoluble (Row 3). Buffer pH had no visible relationship with extraction success.

To improve solubility, I tested multiple experimental procedures to forcibly denature the tangled protein and extract it, suspending it in a usable aqueous solution. An initial attempted extraction using guanidine hydrochloride as an extracting agent yielded minimal success, but unexpected extraction during a urea-based denaturation step pointed to urea as a potential extraction tool. Tests of several high-concentration urea buffers yielded promising data suggesting a high degree of successful extraction (Fig. 3). However, my project ended before the first full attempted extraction and purification of srGAP was complete. Further work is needed to confirm the success of my new procedure; if the procedure were a success, this would enable Henderson Lab to continue with the next stages of srGAP binding research, testing the binding of the purified protein on artificial membranes using a fluorescence microscopy assay.

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References

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