Isolation of NPR2 and determination of its Protein Binding Partners

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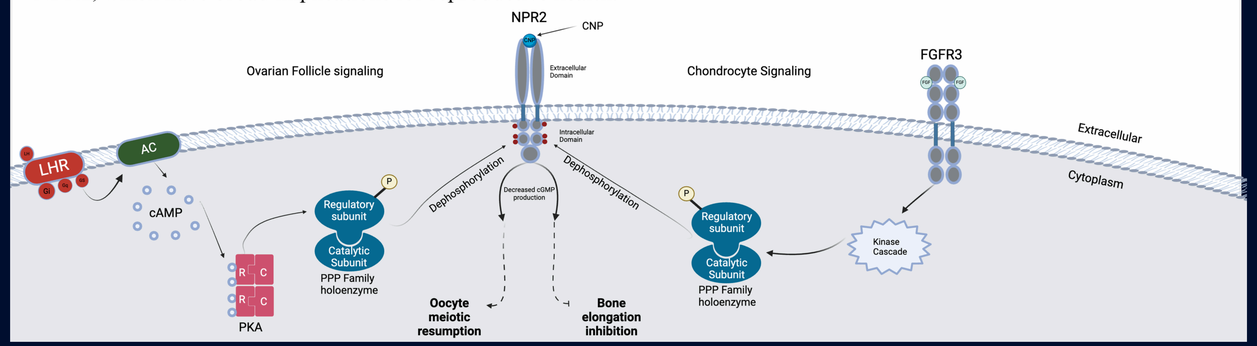
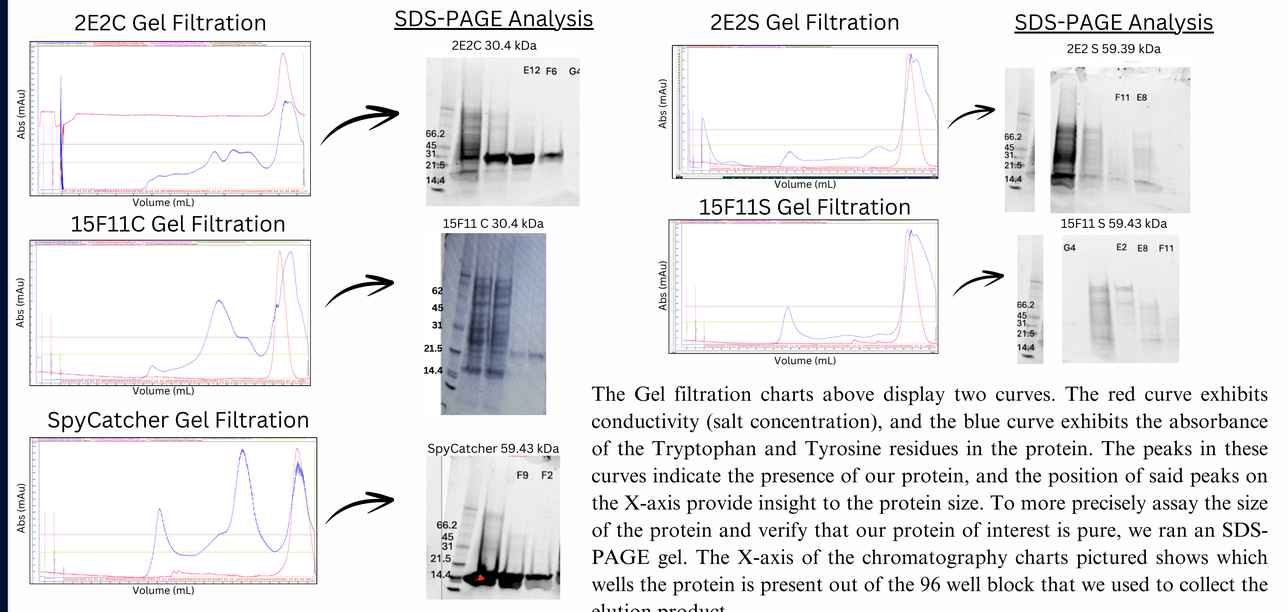
Introduction

Natriuretic peptide receptor 2 is a transmembrane signaling receptor and guanylyl cyclase that produces cyclic GMP molecules when active, triggering the arrest of the meiotic cycle involved in oocyte maturation. When dephosphorylated, NPR2 becomes inactive and allows oocyte meiosis to continue, and thus oocyte maturation to occur. This process is mediated by PPP family phosphatases, the identity of which are unknown. NPR2 is also integral in bone growth, as the dephosphorylation of the receptor as a downstream affect of FGF family membrane receptor activation leads to inhibition of bone elongation. Additionally, A homolog to NPR2, NPR1, has applications in blood pressure regulation. An understanding of the identity and binding interactions of the PPP Family phosphatases is critical towards understanding how bone growth and oocyte maturation occur. As such, the goal of our experiment is to understand the structural and regulatory dynamics of NPR2. An understanding of these mechanisms can provide valuable insights into cellular events related to the dynamic regulations of NPR2, which have broad implications for reproductive health.



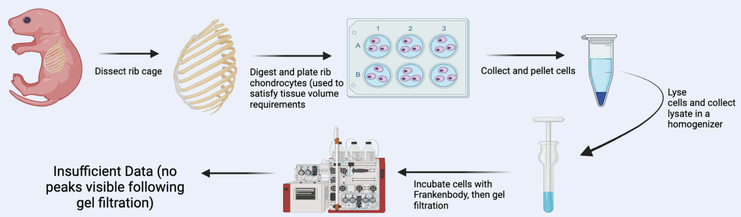
Results



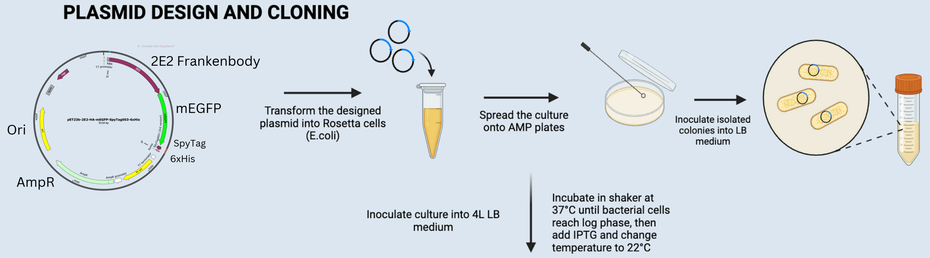
The Gel filtration charts above display two curves. The red curve exhibits conductivity (salt concentration), and the blue curve exhibits the absorbance of the Tryptophan and Tyrosine residues in the protein. The peaks in these curves indicate the presence of our protein, and the position of said peaks on the X-axis provide insight to the protein size. To more precisely assay the size of the protein and verify that our protein of interest is pure, we ran an SDS-PAGE gel. The X-axis of the chromatography charts pictured shows which wells the protein is present out of the 96 well block that we used to collect the elution product.

Methods

NPR2 TISSUE EXTRACTION (Research Foundation)



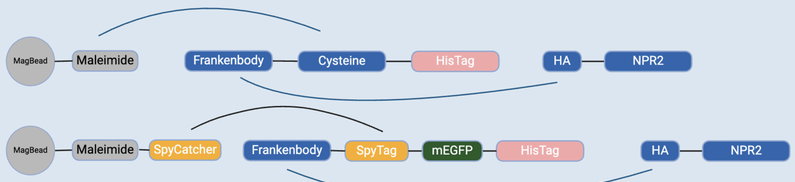
PLASMID DESIGN AND CLONING



The Plan

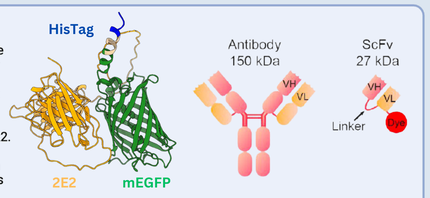
In order to achieve the goal of isolating NPR2, we will experiment with two different methods:

- Utilize SpyCatcher and SpyTag to covalently bond to each other and associate the magnetic bead with our designed protein
- Utilize the cysteine residue on the Frankenbody to bind the maleimide on the magnetic bead and form a bond

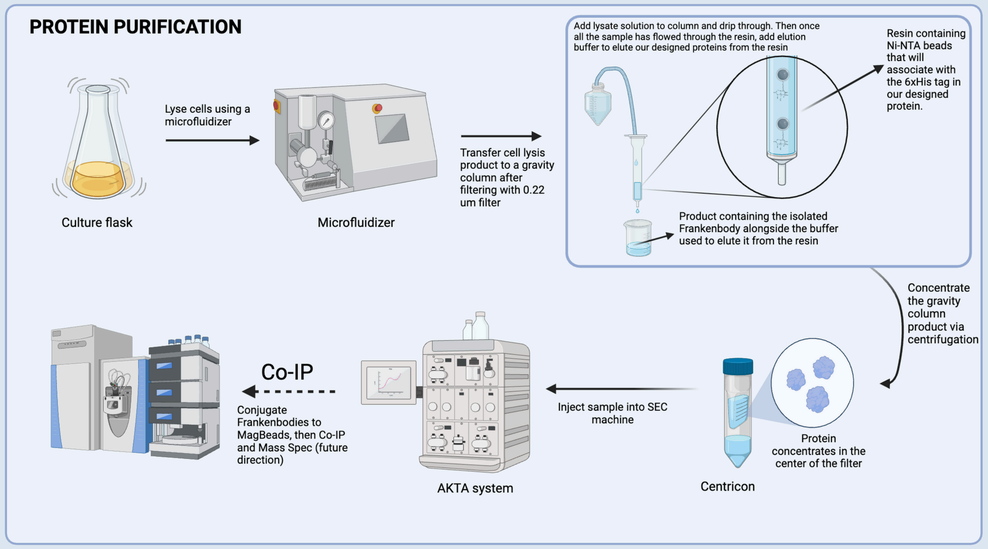


The Tool: Frankenbodies

Frankenbodies are chimeric antibodies that contain only the variable single chain fragment part of the antibody, in other words the domain that interacts with the protein that the antibody has an affinity for. Frankenbodies are able to exist stably in live cells, which make them the ideal tool for our objective. Our designed Frankenbodies contain a HisTag, which we are able to conjugate with the HA residue on NPR2. The first schematic on the right displays the predicted protein structure of the Frankenbody, and the schematic on the left provides a generic illustration of how Frankenbodies are structured.

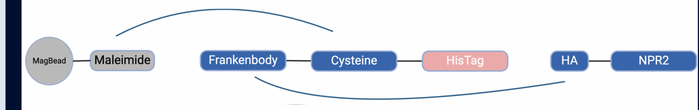


PROTEIN PURIFICATION



Results

The SDS page results show clear bands that indicate the correct size for the 2E2C protein, the 15F11C protein, and the SpyCatcher protein, but not the two SpyTag proteins. This indicates that we should first conjugate the Frakenbodies (2E2C and 15F11C) directly to maleimide activated MagBeads and use these MagBeads to isolate NPR2 alongside its binding partners in future experiments including Co-Immunoprecipitation.



Next Steps

- Connect magnetic beads to these fragments using the maleimide group to bind to the cysteine labels in the Frankenbody
- Test the binding efficiency of our magnetic bead conjugated Frakenbody to HA-GFP
- Isolate and purify mouse NPR2 and associated proteins (binding partners) from transgenic mouse tissue
- Send the conjugated sample for Mass Spectrometry analysis at UConn Storrs campus
  - Depending on yield, use directly for EM structural analysis. If insufficient yield, perform protein over-expression
- Focus on identification of cytokines that will allow us to fix mutations in NPR2

Acknowledgements

- Thank you to the HRP program for allowing me this opportunity
- Thank you to the Chou Lab for providing support and guidance