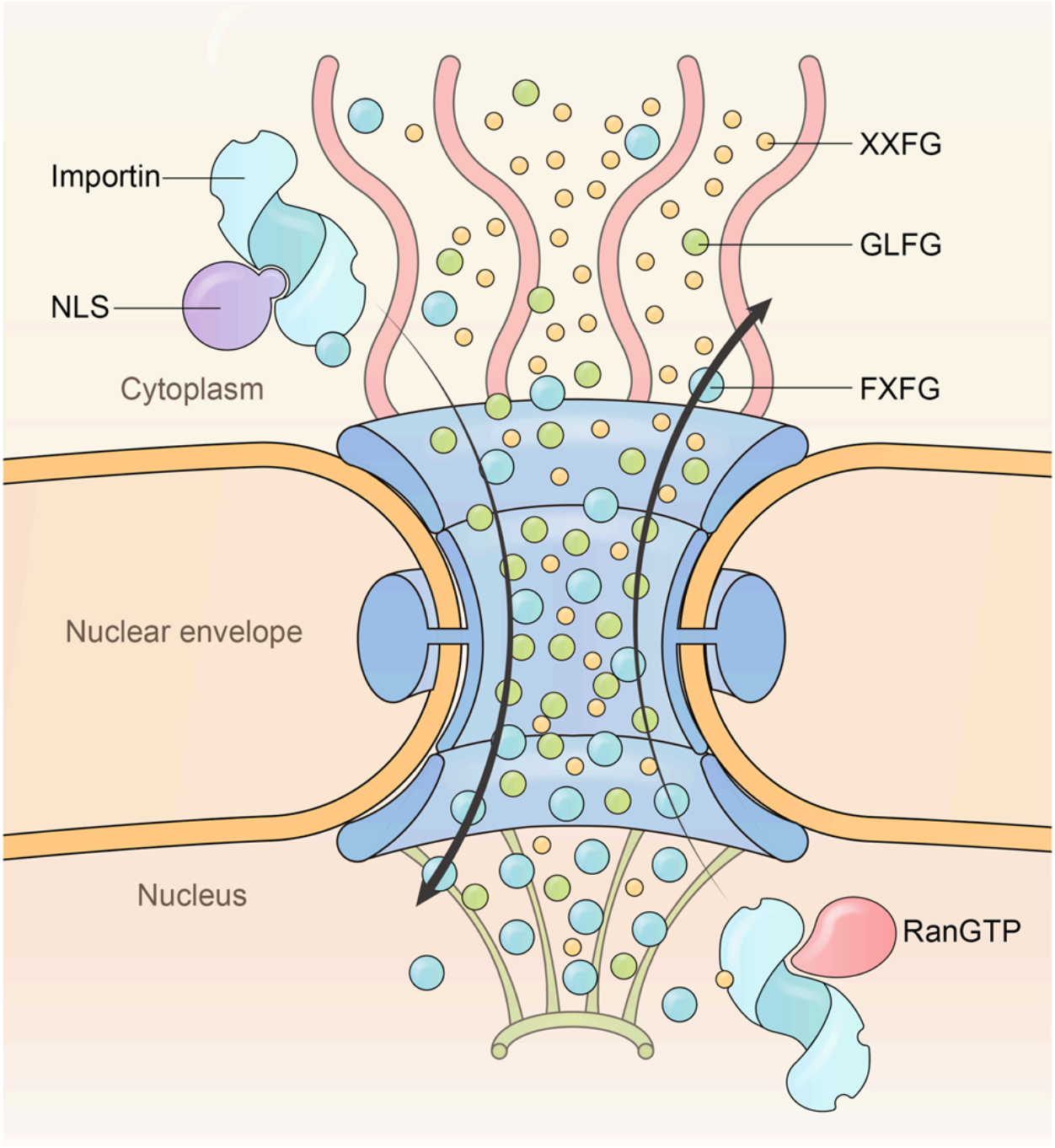
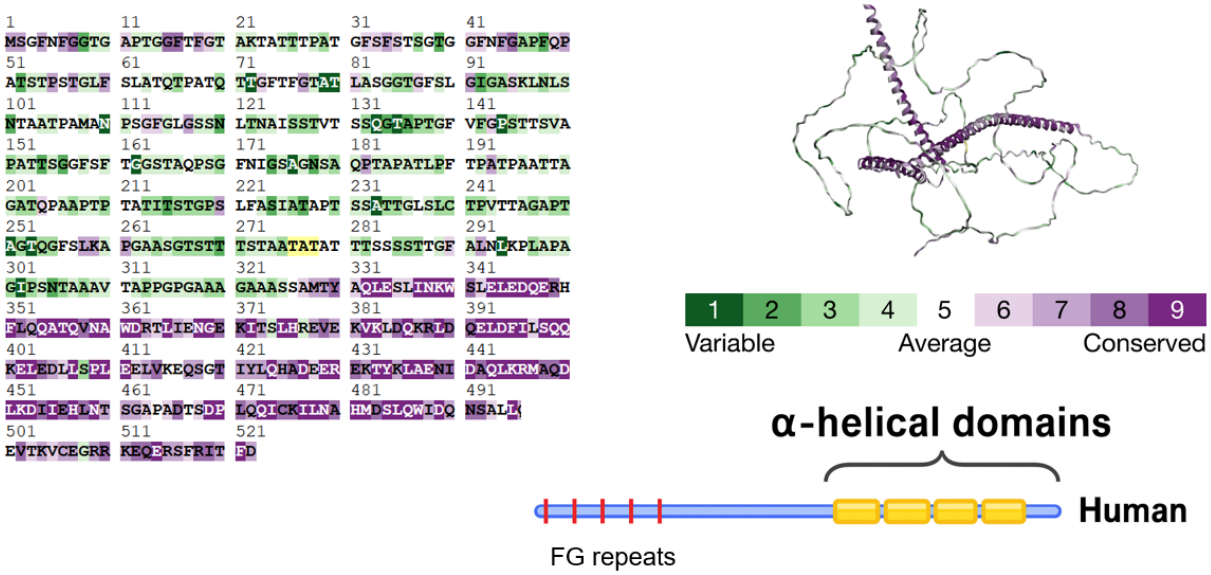
Description of Thesis Work

FxFG domains are **intrinsically disordered protein regions** containing phenylalanine-glycine repeat motifs that serve as the primary determinants of nuclear pore complex (NPC) selectivity. These domains are strategically positioned within the central transport channel, where they form a **selective permeability barrier** that controls nucleocytoplasmic exchange



Some of the other FG domains, like NUP98, had already been established to form a biomolecular condensate. In this work, I have tried to explore if the Central transport channel Nups shows Phase separation.

Evolutionary Conservation in Intrinsically Disordered Regions



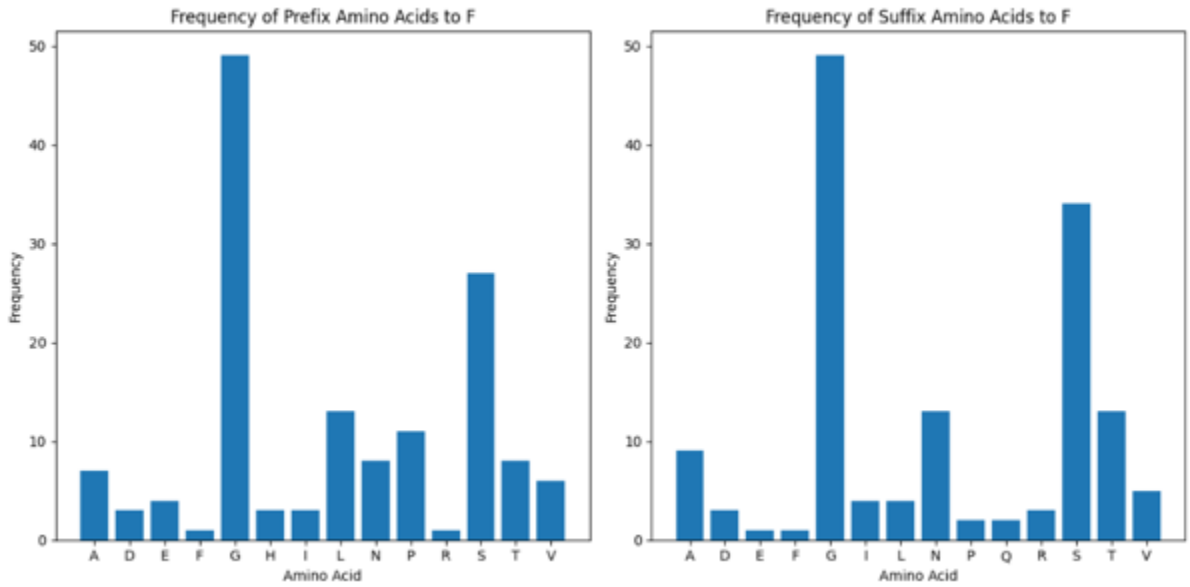
Our most striking finding challenges the fundamental assumption about intrinsically disordered proteins (IDPs). Despite the N-terminal region being highly disordered, we identified **unexpected evolutionary conservation across multiple species** (highlighted in purple sequences) upon closer observation, mostly those conserved sequences were FG

- **Functional significance**: Conservation in disordered regions suggests previously unrecognized functional constraints

- **Evolutionary pressure**: The maintenance of specific sequence patterns across species indicates critical biological importance
- **Novel regulatory elements** potentially controlling phase separation dynamics

- **Evolutionary signatures** of functional importance in nuclear transport

- **Therapeutic targets** for modulating NPC function in disease states



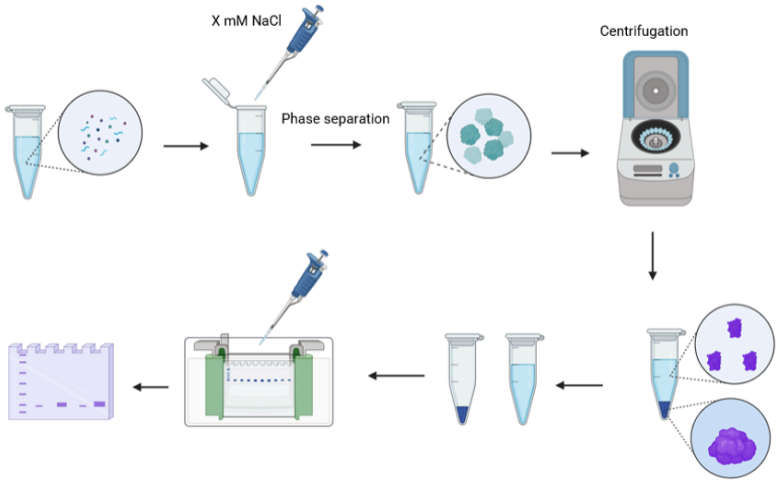
Upon further analysis, we found out these were indeed FG repeats, which were highly conserved across different species.

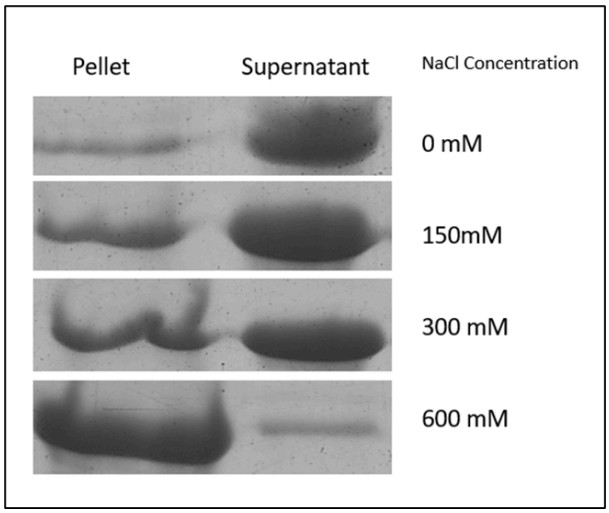
Overcoming the "Invisible Protein" Challenge

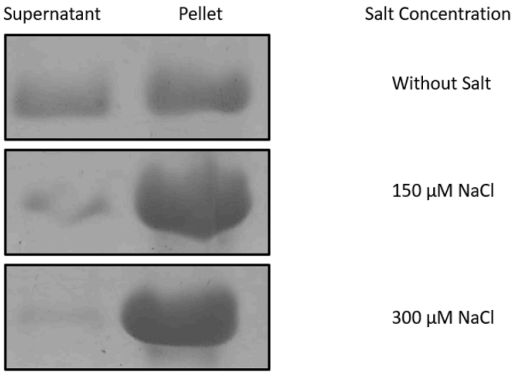
I had optimized the purification and expression protocol multiple times. As these domains have the molar extinction coefficient (ε) nearly 0, making them very tedious to estimate the concentration and track them during chromatography.

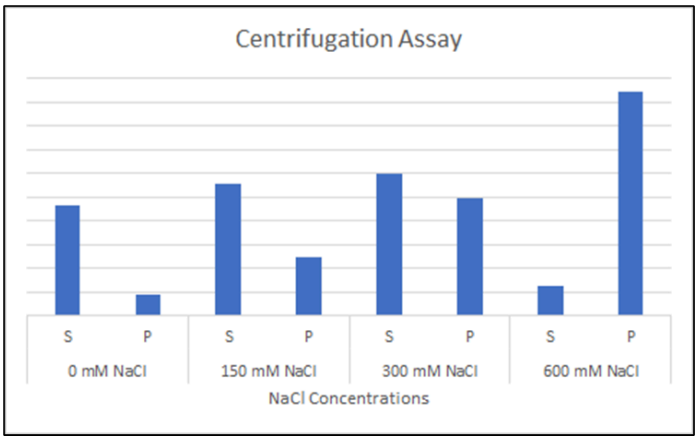
Quantitative Phase Separation Assays using centrifugation.

The following is the schematic for the assay. The phase-separated protein, being higher in density than the buffer, tends to pellet down during phase separation







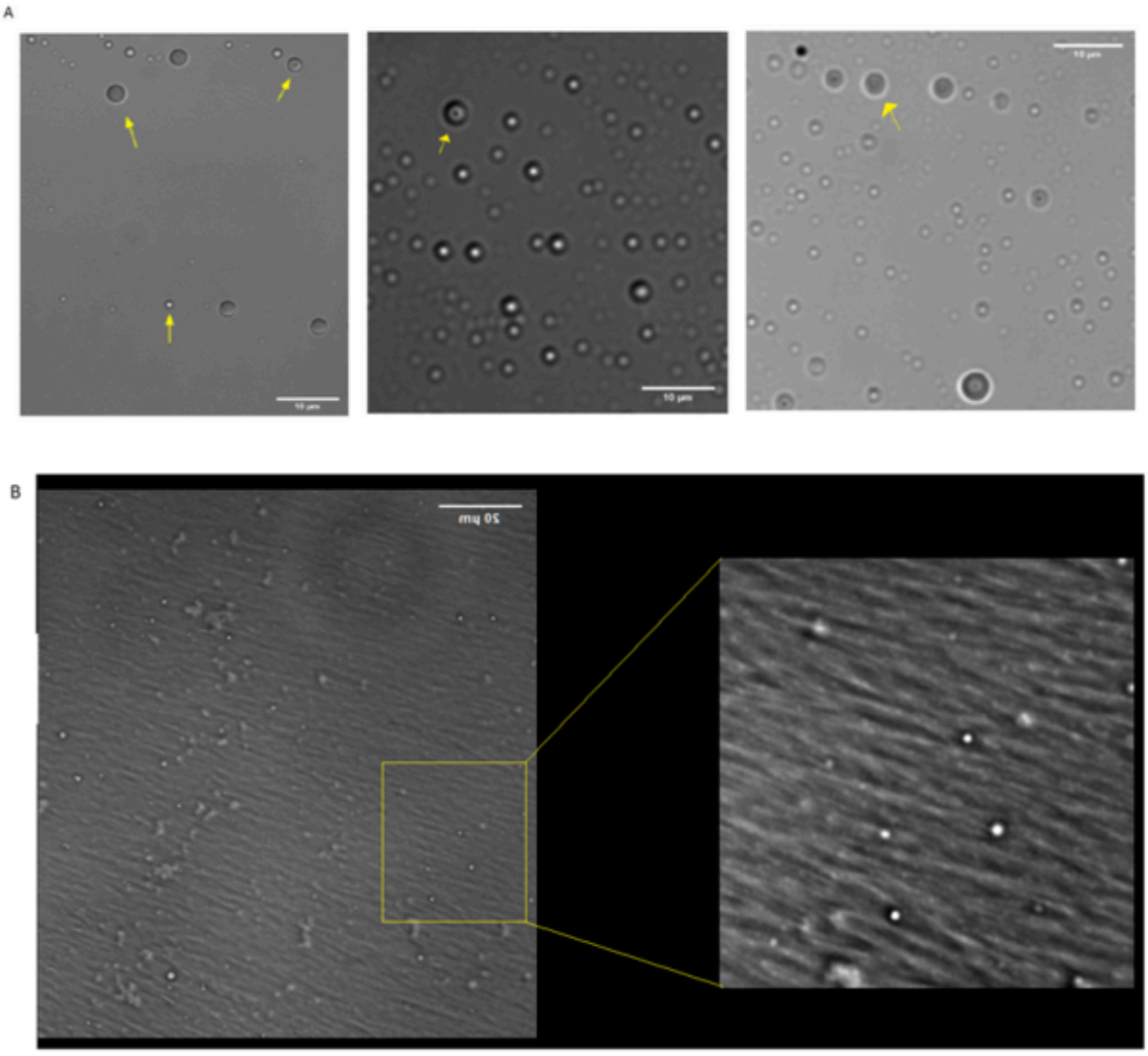


On quantification via Image J, we found that NaCl was inducing phase separation; thus, the protein concentration in the pellet fraction is increasing, and the supernatant fraction is decreasing.

Microscopy Protocol optimization to visualize phase separation

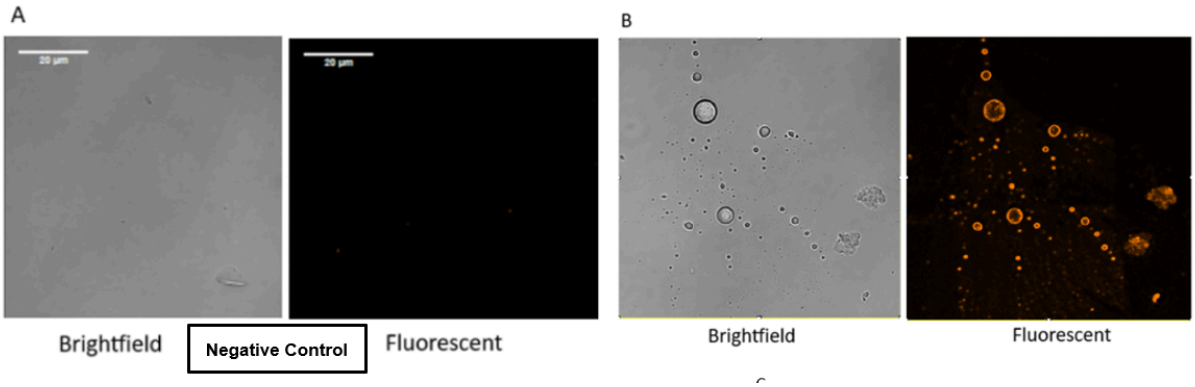
We further went for optimizing the protocol for visualization of phase separation under Microscopy, but we were still working on it by the end of my thesis tenure. The final results showed protein aggregation.

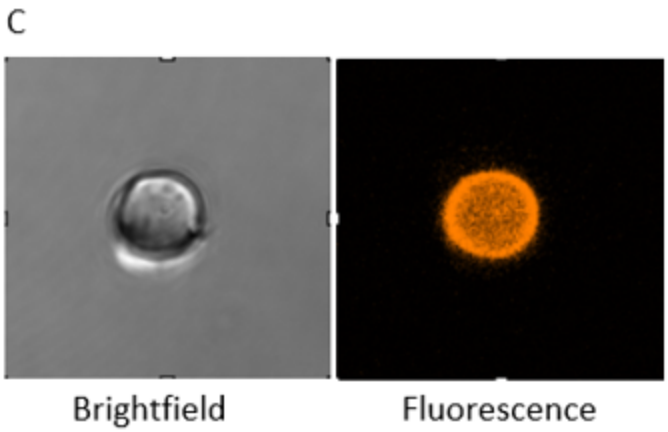
On the side, we tried to reproduce phase separation from an established protocol in which they have shown BSA phase separate under higher PEG(Polyethylene glycol) concentration as a crowding agent.



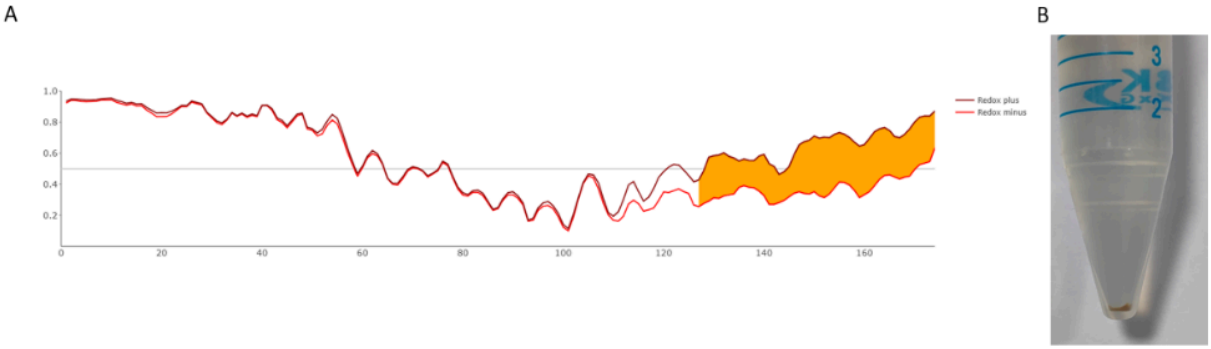
DIC micrograph of BSA(40μM) condensates under 12% PEG (Polyethylene Glycol)

The following is phase separation visualized by non-covalently labeled BSA using SYPRO ORANGE Flourophore.





Redox-Sensitive Condensate Dynamics



Our IUPred analysis revealed **dramatic sensitivity** of FxFG condensates to redox conditions:

- **Environmental responsiveness**: Condensates show remarkable sensitivity to reducing agent concentrations
- **Physiological relevance**: May represent a regulatory mechanism for NPC function during cellular stress
- **Therapeutic implications**: Redox modulation could provide new approaches for treating transport-related diseases