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Optimization of the Human p50RelB Purification Protocol to Investigate Its Transcription Activation Domain and Leucine Zipper

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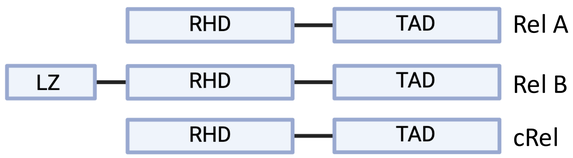
Introduction

The NF-kB family of transcription factors mediates gene activation through protein-protein interactions and protein-DNA interactions, which regulate immune and inflammatory responses.[5] Three members of the NF-kB family, Rel A, Rel B, and cRel, mediate gene activation through the use of their transcription activation domains (TADs) at the C-terminal regions, which are intrinsically disordered regions. These regions are necessary for interacting with other co-activators to activate transcription.

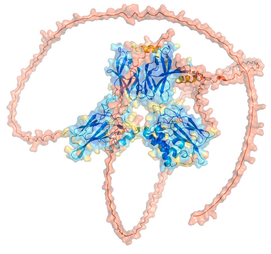


In contrast to Rel A and cRel, Rel B activates transcription with its transcription activation domain and the Leucine Zipper (LZ) at the N-terminal region.[2] We wish to elucidate Rel B's interactions with DNA and co-activators while performing comparative analysis to Rel A and cRel.

Alphafold Interactions

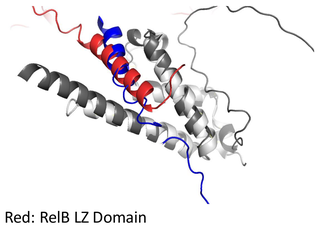
Rel B's structure was generated with Alphafold.[3] As plDDT score increases, the confidence in the generated region being a fixed structure is high. Our interest would be the orange-colored regions, which indicate intrinsically disordered regions.

- Very high (plDDT > 90)
- Confident (90 > plDDT > 70)
- Low (70 > plDDT > 50)
- Very Low (plDDT < 50)



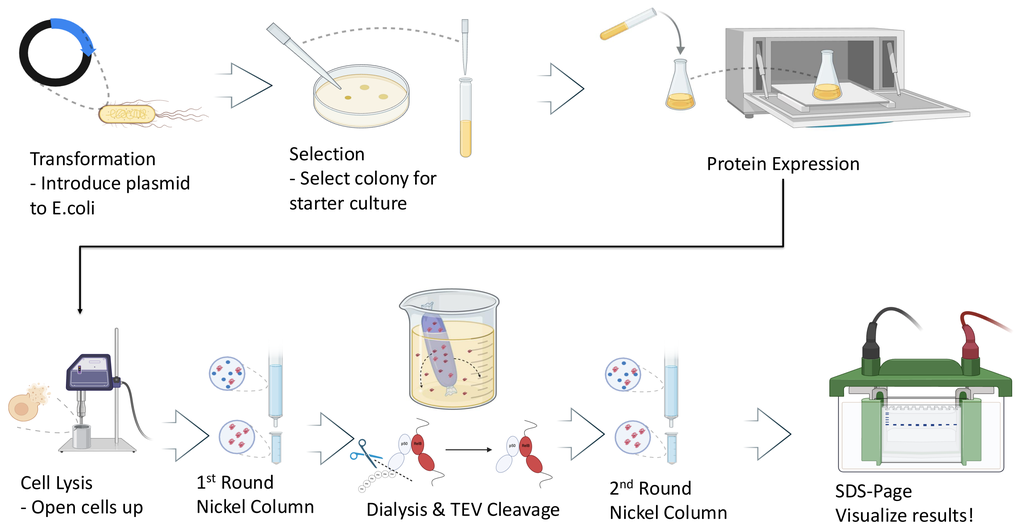
p50/RelB

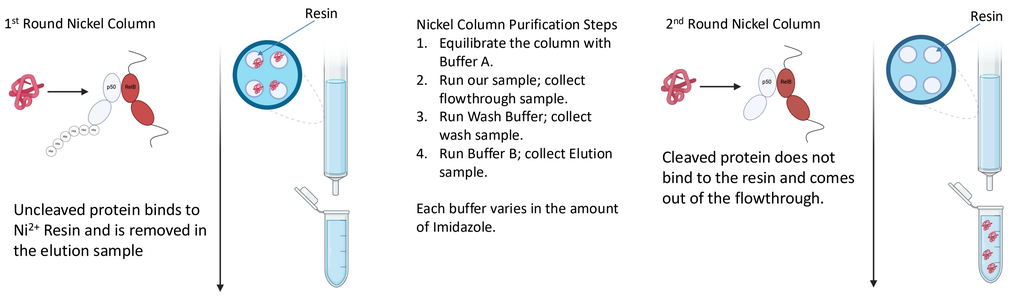
Additionally, we generated an interaction between Rel B with CBP's KIX domain. We found that 5/5 structures displayed Rel B's Leucine Zipper interacting with the KIX domain. This also indicated that this interaction was similar with Rel A's interaction with the CBP KIX domain.



Red: RelB LZ Domain
Dark Grey: CBP Kix Domain (Predicted)
Blue: Rel A (5U4K; from Protein Bank)
Grey: CBP Kix Domain (5U4K; from Protein Bank)

Methods





Prior Work

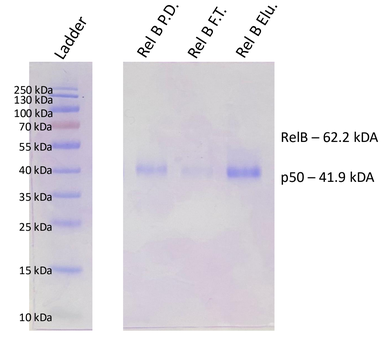
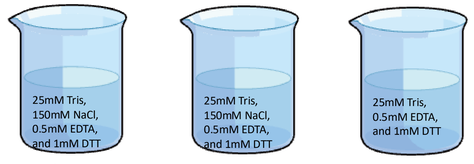


Figure 1. SDS-Page results of 2nd round nickel column showing protein band in the flowthrough (F.T.) and elution (Elu.) lanes. It is expected for the His-tag to be cleaved by TEV protease, and our target protein to elute out of the flowthrough lane. However, there are bands in the elution, indicating TEV Cleavage requires optimization.

Optimization Efforts

In light of our previous results indicating TEV cleavage required optimization, we hypothesized that adding more TEV will improve our results with more cleaved protein. To optimize the TEV cleavage, we set up three different Dialysis buffer experiments:



Control Experiment
- Protocol remains the same – 500µl of TEV

Experiment 1
- Doubled TEV amount → 1mL

Experiment 2
- Removed salt

Dialysis buffers had 7mL of elution each and TEV in 4°C fridge for 2 days.

Results & Conclusions

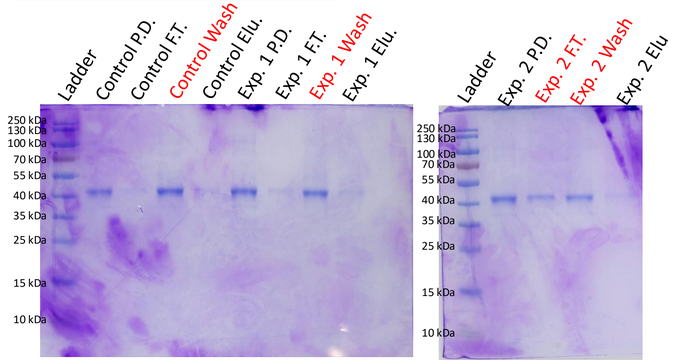


Figure 1. SDS-Page Results of Control ,Experiment 1, and Experiment 2. Protein band found in Control, Experiment 1, and Experiment 2 Wash lanes around the 40kDa band, indicating p50 (41.9kDA). Additionally, protein band found in Experiment 2 flowthrough (F.T.) around 40kDa band, also indicating p50 (41.9kDa). Finding protein band in either flowthrough or wash across all experiments indicated nothing was wrong with the TEV cleavage, but that weak-interactions between our protein and the resin had to be interrupted.

Future Directions

Taking that into account, the purification protocol will be adjusted to have an additional wash step:

Protein Expression & Purification → DNA and coactivator binding assays → Comparative Analysis Between NF-kB Proteins

This optimization will pave way for increasing protein expression yields to be used for future assays of full-length p50RelB to investigate its protein-protein and protein-DNA interactions.

Acknowledgements

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