Recombinant expression and purification of *NF-κB* p50/c-Rel heterodimers for DNA-binding biochemical studies

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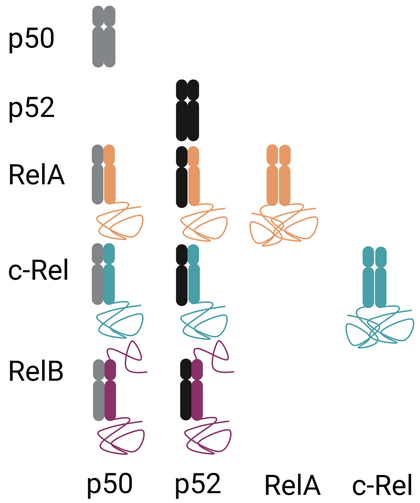
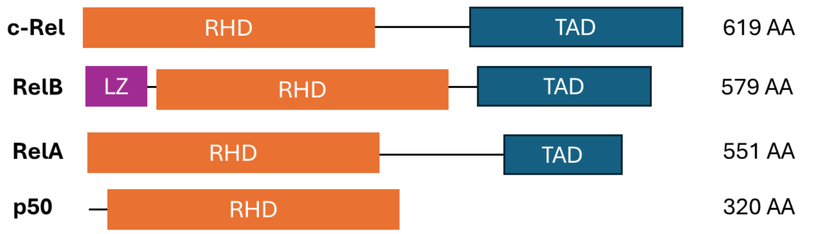
Introduction



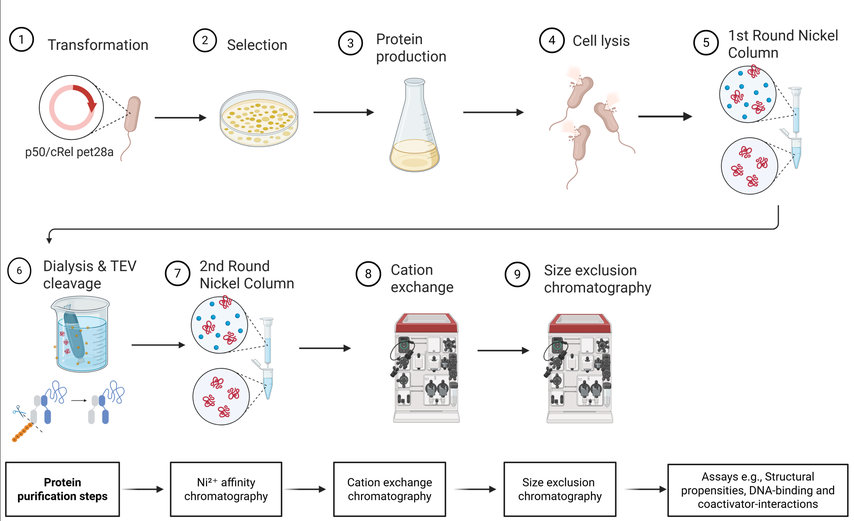
- The NF-κB family of transcription factors is fundamental in various biological processes, including inflammation, immunity, and cell proliferation, differentiation, and survival.
- The NF-κB family of transcription factors contain ten members that can form as either homo or heterodimers. Interestingly they all share a highly conserved Rel Homology domain (RHD), while Rel proteins offer variability within their intrinsically disordered C-terminal transactivation domains (TADs).
- Prior studies biochemically characterized the full-length mouse p50/RelA heterodimer, the analogous human NF-κB heterodimer and other related family members remain largely unexplored.

SEQUENCE COMPARISON



- Previous biochemical studies revealed that the RelA TAD increases DNA-binding affinity while reducing sequence specificity, highlighting the need to examine full-length NF-κB transcription factors.
- By performing a comparison study, we can further characterize how intrinsically disordered regions behave across various dynamic states and how this can influence functionality within intracellular processes. In this project, I worked on optimizing the expression and purification of the human p50/c-Rel protein containing its intrinsically disordered domain. I successfully expressed recombinant p50/c-Rel in *Escherichia coli* and purified the heterodimer using Ni^{2+} affinity chromatography. SDS-PAGE analysis confirmed the isolation of both p50 and c-Rel at their expected molecular weights. This will enable us to investigate the binding of this protein to various DNA sequences and coactivator proteins, giving insight into its cellular role.

Methods

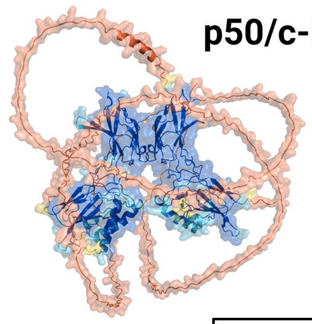


Results

Computational predictions and experimental validation of *NF-κB* p50/c-Rel *H.S* heterodimers

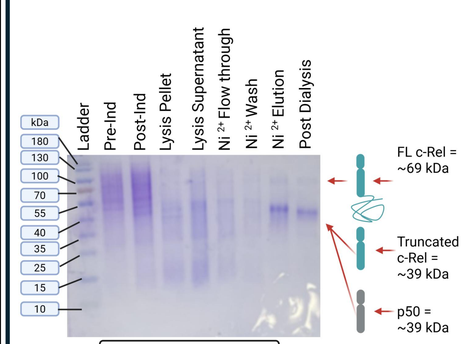
3D predicated structure of p50/c-Rel using AlphaFold 2 indicating *NF-κB* heterodimer contains intrinsically disordered regions. Structure is colored according to pLDDT confidence scores.[4]

Very high (pLDDT > 90)
High (90 > pLDDT > 70)
Low (70 > pLDDT > 50)

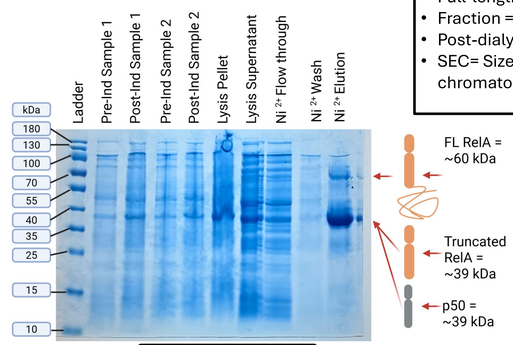


SDS-PAGE validation of *NF-κB* heterodimers

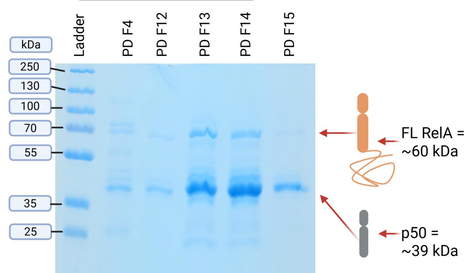
KEY
- Full-length = FL
- Fraction = F
- Post-dialysis = PD
- SEC= Size-exclusion chromatography



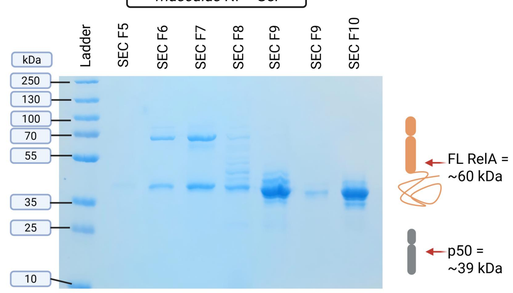
A.) p50/c-Rel FL *Homo sapiens* Ni^{2+} Gel



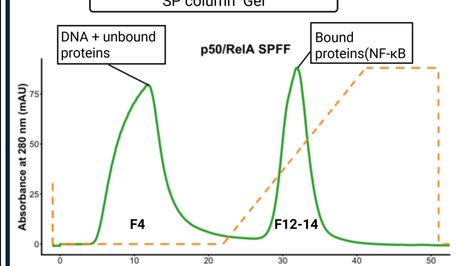
B.) p50/Rel A FL *Mus musculus* Ni^{2+} Gel



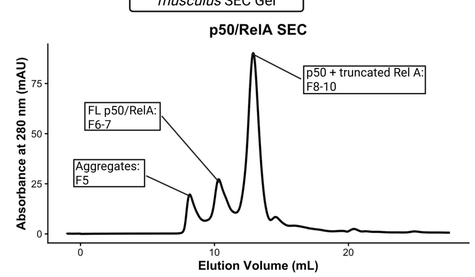
C.) p50/Rel A FL *Mus musculus* SP column Gel



D.) p50/Rel A FL *Mus musculus* SEC Gel



E.) p50/Rel A FL *Mus musculus* SP column *chromatograph*



F.) p50/Rel A FL *Mus musculus* SEC column chromatograph

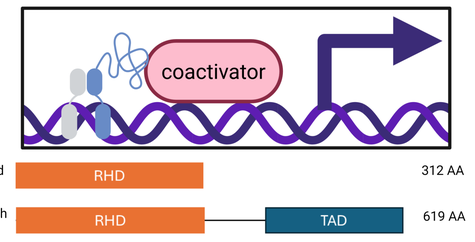
A. SDS-PAGE analysis of p50/c-Rel expression and Ni^{2+} purification at 18ºC. Showing full-length c-Rel (~69 kDa), truncated c-Rel (~39 kDa), and p50 (~39kDa).
B. Positive-control Ni^{2+} purification of full length p50/RelA *Mus musculus* (MM) heterodimer, included to replicate prior expression and purification results under a new laboratory setting.
C. SP column fractions showing bands at the expected heterodimer molecular weight.
D. Size-exclusion chromatography (SEC) fractions (SEC F6, SEC F7) display clean co-elution of RelA and p50, consistent with a properly assembled heterodimer.

Results illustrate the separation and final purification of *Mus musculus* heterodimer as a positive control here at UW Tacoma campus. Still need to work on optimization of analogs human construct p50/c-Rel purification.

Conclusion

- Alpha fold computational data supports target domains are likely to be intrinsically disordered.
- TEV cleavage and secondary Ni^{2+}affinity chromatography confirmed successful removal of His-tag.
- Further optimization is needed to express and purify the full-length *Homo sapiens* version.
- Full-length P50/RelA *Mus musculus* version successfully purified at UW Tacoma campus.

Future Directions



Schematic of p50/c-Rel heterodimer bound to a *κB* DNA site. The c-Rel transcription domain (TAD) recruits a coactivator protein to initiate transcription.

- This work provides a foundation for biochemical characterization of the p50/c-Rel heterodimer, including analyses of DNA-binding properties and protein-protein interactions relevant to immune signaling. Future studies will examine how DNA-binding specificity and regulatory interactions shape *NF-κB* mediated gene expression and contribute to immune responses, cancer, and inflammatory disease.

Acknowledgements

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References

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4. Mirdita, M., Schütze, K., Moriwaki, Y. *et al.* ColabFold: making protein folding accessible to all. *Nat Methods* **19**, 679-682 (2022).

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