



Optimization of the Human p50/Rel A Protein Purification Protocol for Investigation of the Role of Its Intrinsically Disordered Domain

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INTRODUCTION

Background

- Nuclear Factor-kappa B (NF-κB) is a family of transcription factors with a wide role in the immune system (Zhang et al. 2017)
- p50/RelA, the most abundant member of the NF-κB family, is a heterodimer in this family (Fig. 1A)
- Both p50 and RelA have a Rel Homology Domain (RHD), which is well structured and where the protein dimerizes and binds DNA (Fig. 1B). RelA has a Transcription Activation Domain (TAD) which has IDRs and participates in protein-protein interactions (Fig. 1B)

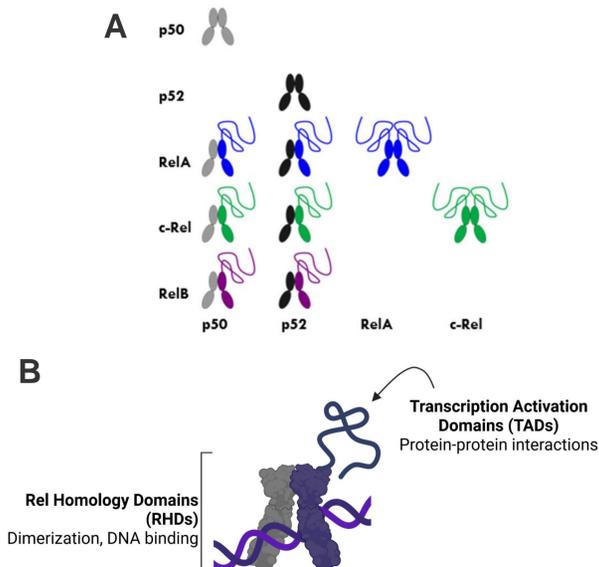
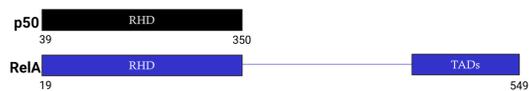


Figure 1. Members of the NF-κB family of transcription factors (A) and the main domains of p50/RelA (B). Created in <https://BioRender.com>

Previous Research

- RHDs and TADs were historically thought to be modular
- Baughman et al. found that presence of the TAD portion of p50/RelA affects both DNA binding affinity and specificity using shortened mouse constructs (Fig. 2) (2022)

Shortened Mouse Construct (prior work):



Full-length Human Construct (this research):



Figure 2. Diagram of p50/RelA Constructs

Goals of Project:

- Optimize purification of the full-length construct for efficient protein production so we can:
- Characterize the effects of the TAD of p50/RelA on DNA binding in the full-length human construct.

METHODS AND RESULTS

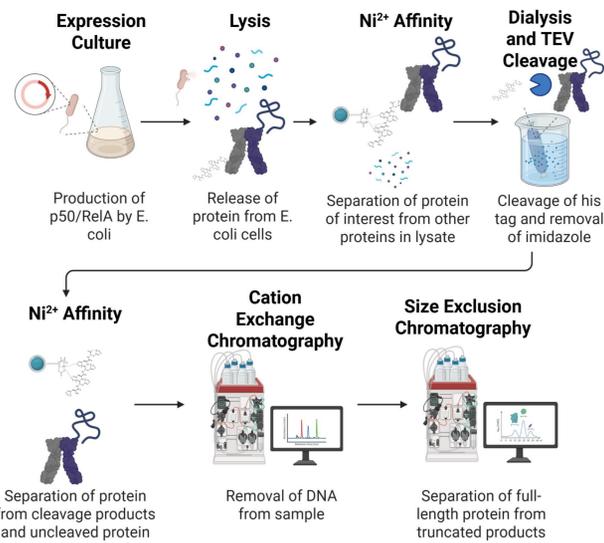


Figure 3. Flowchart of the purification steps taken. Created in <https://BioRender.com>

Expression, Lysis, Ni²⁺ Column Chromatography, and TEV Cleavage Steps Analyzed by SDS-PAGE

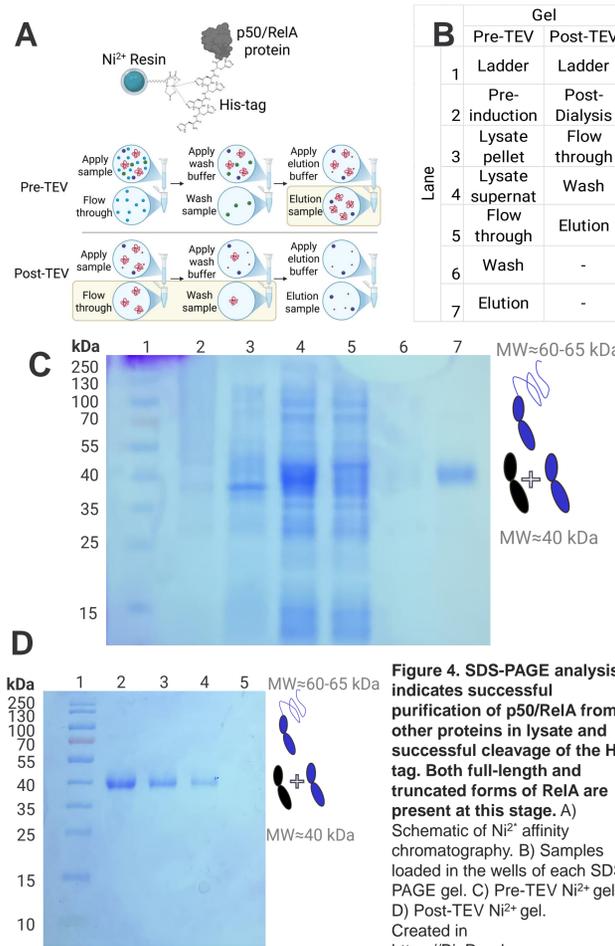


Figure 4. SDS-PAGE analysis indicates successful purification of p50/RelA from other proteins in lysate and successful cleavage of the His tag. Both full-length and truncated forms of RelA are present at this stage. A) Schematic of Ni²⁺ affinity chromatography. B) Samples loaded in the wells of each SDS-PAGE gel. C) Pre-TEV Ni²⁺ gel. D) Post-TEV Ni²⁺ gel. Created in <https://BioRender.com>

Cation-Exchange Chromatography Step Analyzed by Chromatogram

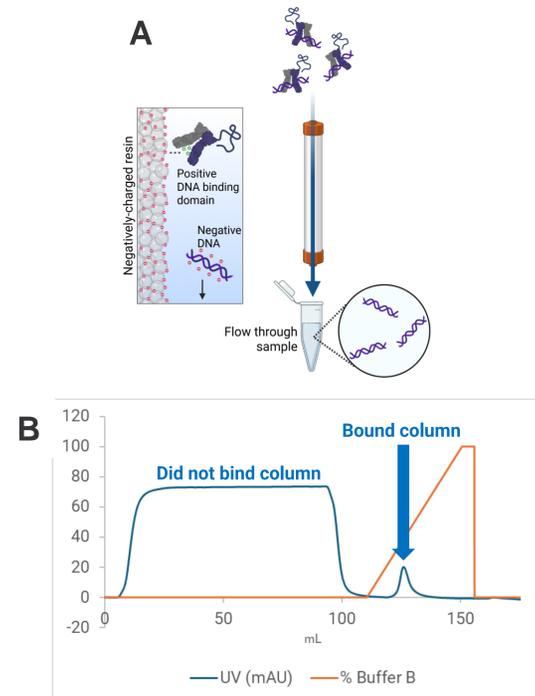


Figure 5. Most p50/RelA did not bind the cation exchange column in the DNA removal step, indicating more optimization necessary for this step. A) Schematic of cation-exchange chromatography. B) Chromatogram of cation-exchange chromatography collecting flow through. Created in <https://BioRender.com>

Size Exchange Chromatography Step Analyzed by Chromatogram

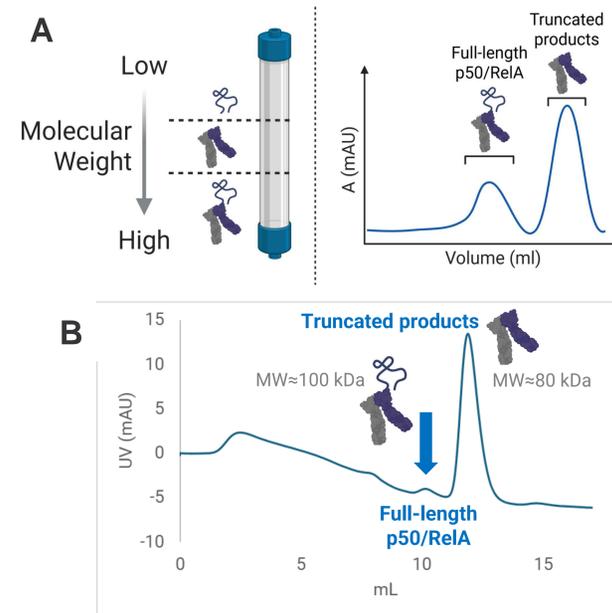


Figure 6. Size exclusion chromatography (SEC) separated full-length p50/RelA from truncated products and indicated a smaller ratio of full-length to truncated p50/RelA. A) Schematic of how SEC works and example chromatogram showing approximate ratios. B) Chromatogram of SEC of our sample. Created in <https://BioRender.com>

CONCLUSIONS

- Full-length human p50/RelA is expressed, but further optimization is needed to reduce truncation during expression.
- Protein is successfully purified by Ni²⁺ chromatography and cleaved by TEV protease.
- Cation-exchange chromatography removes some DNA from sample, but further optimization is needed to increase yield of purified protein.
- Size exclusion chromatography step is effective in separating purified, full-length protein from truncated products from expression.

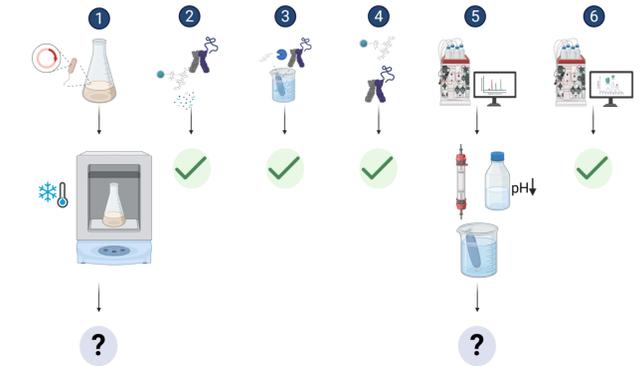


Figure 7. Conclusions on the purification steps. Created in <https://BioRender.com>

FUTURE DIRECTIONS

- Once these protocols are optimized, they will be scaled up to produce protein for assays evaluating DNA binding as affected by the TAD.
- Protein will also be produced to characterize protein-protein interactions undergone by the TAD.

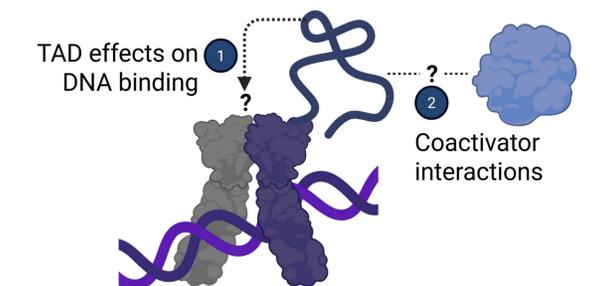


Figure 8. Future directions of this project. Created in <https://BioRender.com>

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REFERENCES

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