Purification and Functional Characterization of the p50/RelB Intrinsically Disordered Region in NF-κB Signaling

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Introduction

The NF-ϰB pathway includes multiple transcription factors that activate cellular processes involved in immune response, proliferation, inflammation, and cell survival. One of these transcription factors is the protein dimer p50/RelB. The p50/RelB dimer contains intrinsically disordered regions (IDRs) that are essential for its function.

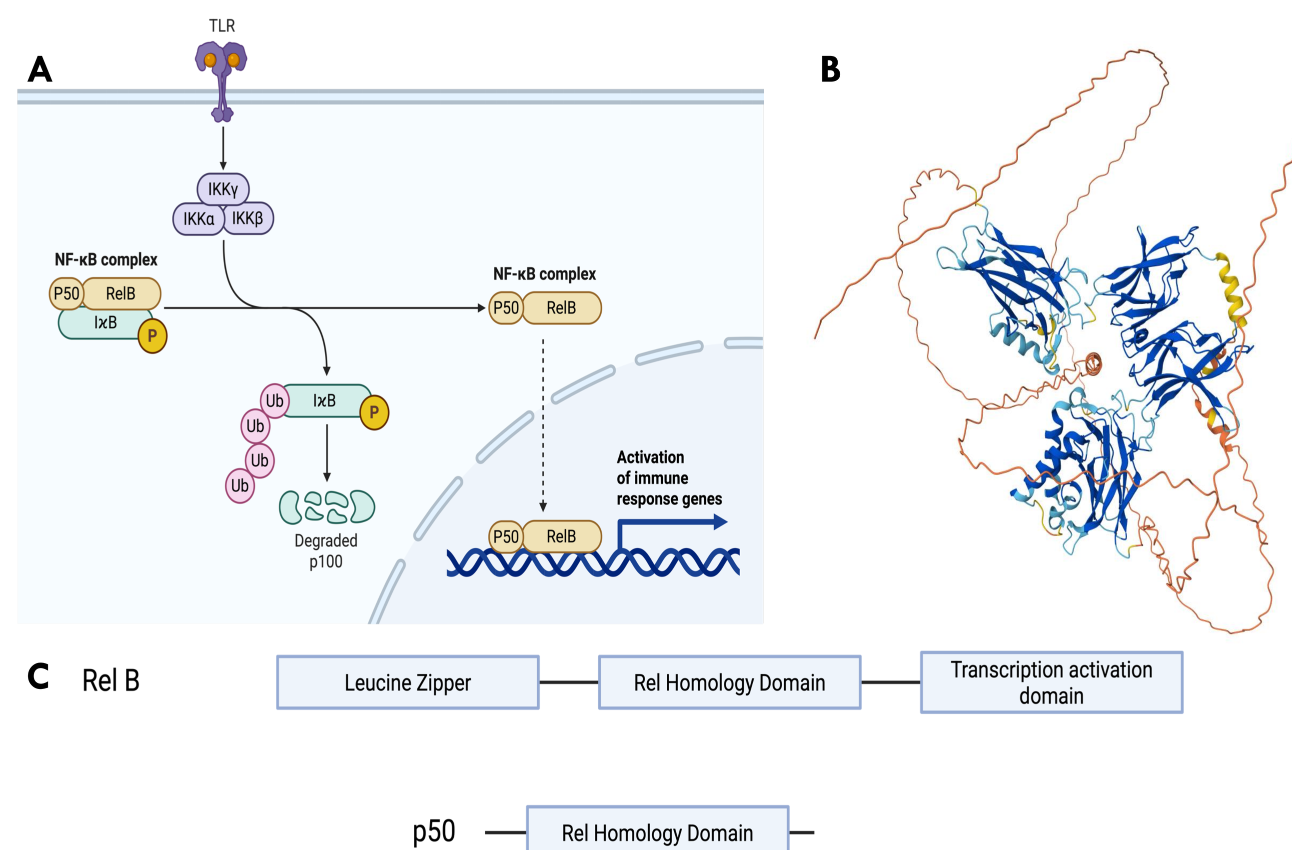
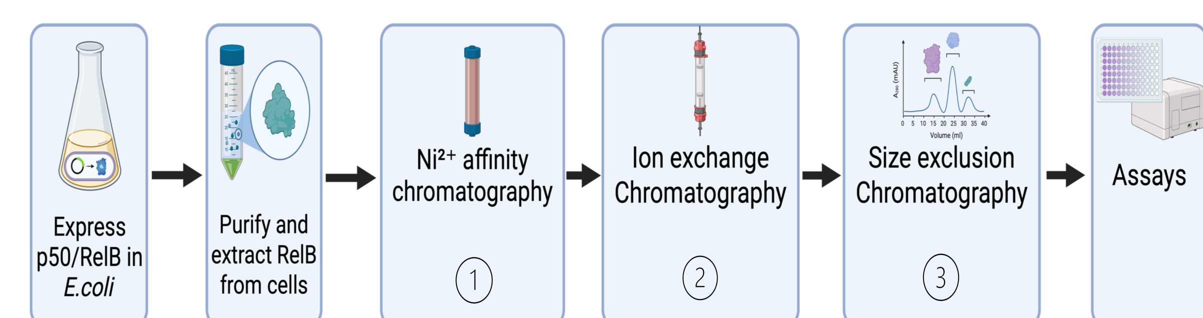


Figure 1. (A) Simplified NF-κB signaling pathway illustrating activation of the p50/RelB complex.[2,3,5] (B) AlphaFold-predicted structures of the p50/RelB heterodimer[1,8] (C) Schematic representation of the domain architecture of the RelB and p50 constructs used in this study. Full-length RelB contains a leucine zipper domain, Rel homology domain (RHD), and transcription activation domain, while p50 contains the RHD.[3,4,5,7]

Objectives

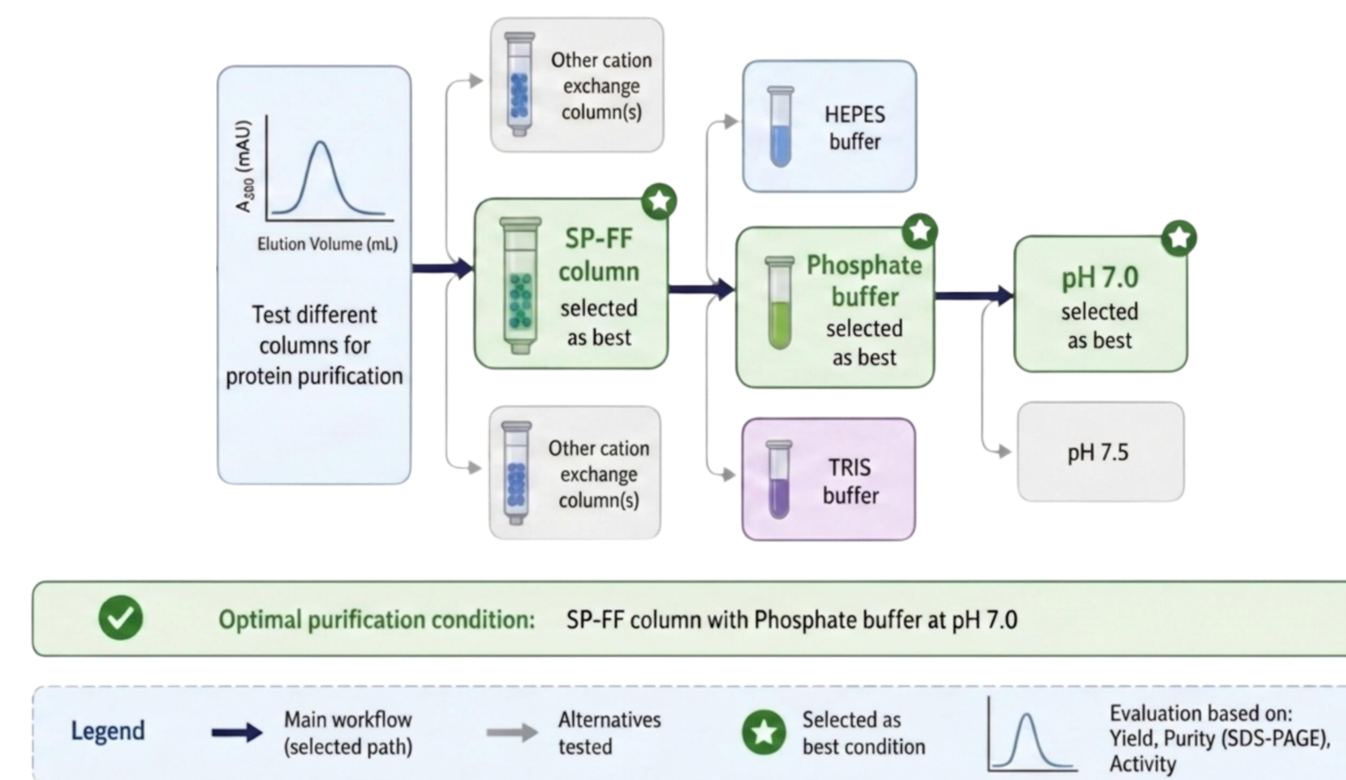
These regions were found to be essential for transcription activation but have not been characterized before, limiting our understanding of how this dimer achieves its cellular function[6]. We aim to expand on the function of these IDRs as NF-ϰB signaling could be a potential drug target for chronic inflammation. To test the function of these IDRs we aim to express and purify a functional full length p50/RelB construct, which we will use in biochemical assays.



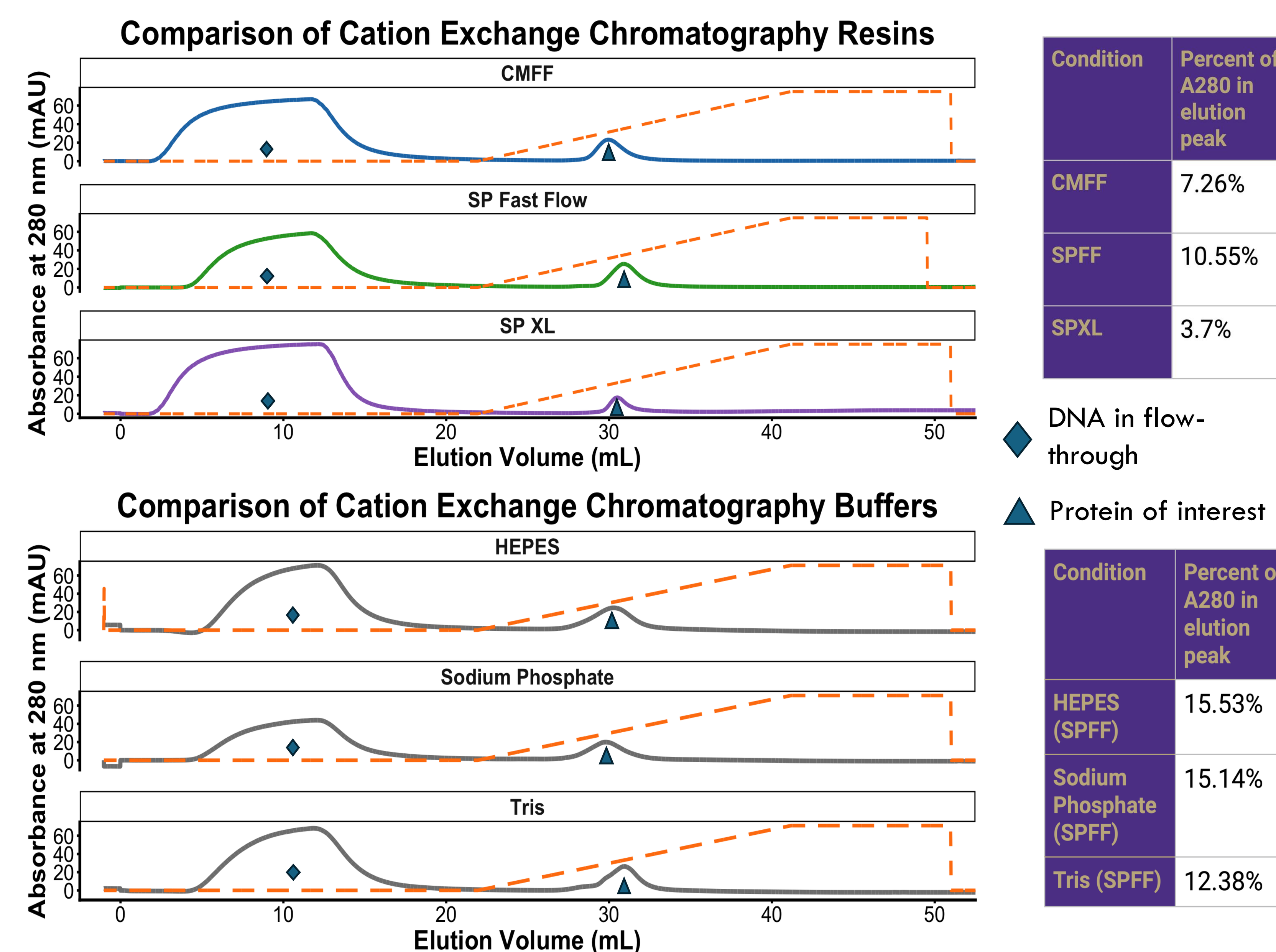
1. Separates E.coli proteins from target protein.
2. Separates DNA bound to the protein.
3. Separates full-length p50/RelB heterodimers from truncated proteins that lost (IDRs) and p50 homodimers.

Cation Exchange Chromatography Optimization

In this project we focused on troubleshooting the protein purification protocol, specifically on the cation ion exchange chromatography (IEX) step which removes bound DNA from the protein.



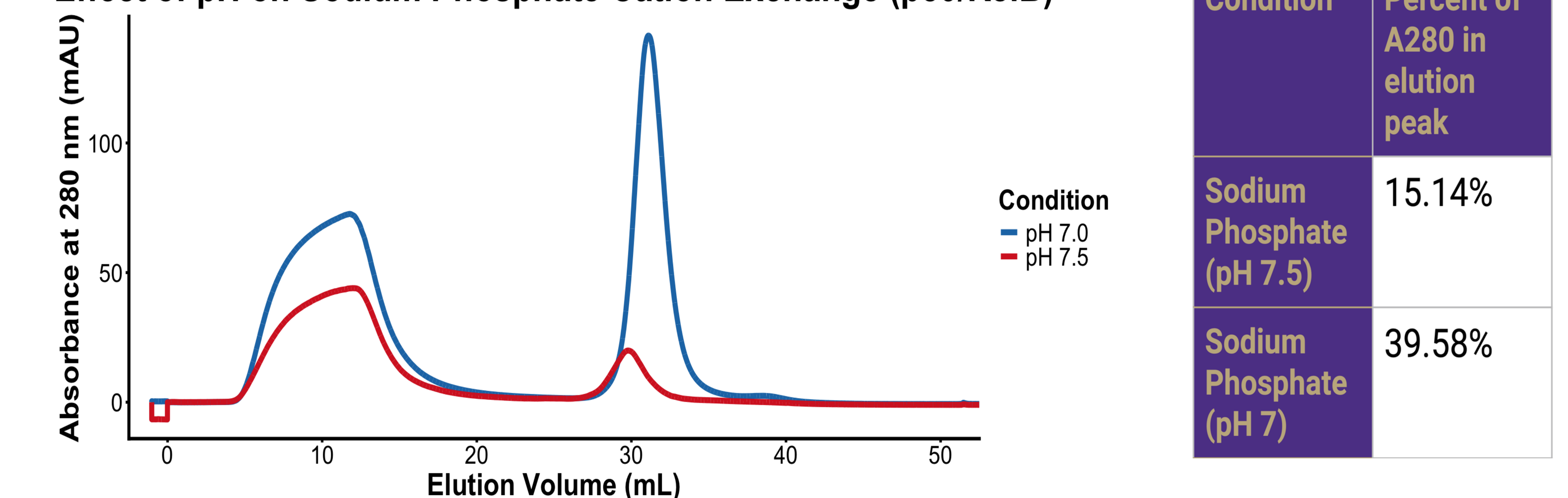
First, we compared multiple cation exchange columns and observed that p50/RelB bound the best to the SP-FastFlow strong cation column. Then we compared different buffers and we decided to use sodium phosphate.



Condition	Percent of A280 in elution peak
CMFF	7.26%
SPFF	10.55%
SPXL	3.7%

Condition	Percent of A280 in elution peak
HEPES (SPFF)	15.53%
Sodium Phosphate (SPFF)	15.14%
Tris (SPFF)	12.38%

Figure 2. Comparison of cation exchange chromatography conditions for p50/RelB purification. SP Fast Flow (SPFF) showed the greatest retained elution peak enrichment among tested resins, while HEPES and sodium phosphate buffers outperformed Tris buffer.



Condition	Percent of A280 in elution peak
Sodium Phosphate (pH 7.5)	15.14%
Sodium Phosphate (pH 7)	39.58%

Figure 3. Overlaid cation exchange chromatograms of p50/RelB purified in sodium phosphate buffer at pH 7.5 and pH 7.0. Lowering the pH from 7.5 to 7.0 resulted in increased elution of the retained p50/RelB peak, with the percentage of A280 signal in the elution peak increasing from 15.14% to 39.58%.

After selecting our resin and buffer combination, we compared two pH conditions. Lowering the pH from 7.5 to 7.0 resulted in approximately a 2.6-fold increase in protein in the elution peak relative to pH 7.5.

Future Directions

Next, we will repeat the optimized protocol, and complete purification with size exclusion chromatography. After obtaining a purified protein, we will test binding affinity and interactions of the full-length protein including its disordered regions with DNA and other proteins to assess its functional role.

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