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

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Abstract (250 word limit):

The NF-κB family of proteins mediate gene activation through protein-protein interactions and protein-DNA interactions, which helps regulate immune and inflammatory responses. Three members of the NF-κB family, Rel A, Rel B, and cRel, contain transcription activation domains—which are intrinsically disordered regions. These regions lack a fixed structure and have a range of conformation states that can recruit co-activator proteins to activate transcription. In addition, Rel B contains a Leucine Zipper domain that is also necessary for transcription activation. When compared with Rel A, there is limited research on these domains and how they affect how Rel B interacts with DNA. With the goal to characterize how Rel B interacts with DNA, we used Alphafold to predict Rel B's structural interactions and optimized purification protocols to obtain RelB for biochemical studies. Visualization of Rel B's structure identified an interaction between its Leucine Zipper and the coactivator CBP's KIX domain. A plasmid containing the gene for full length p50RelB human protein was introduced to E. coli cells, which were used to express our target protein. After expression, our protein was purified through column chromatography techniques and dialysis. The column chromatography purification steps were adjusted and changed to give better yields of our target protein. Optimization of our purification protocols will pave the way for future assays to investigate protein-protein and protein-DNA interactions by full-length RelB.