Isolation and Purification of *H. sapiens* and *M. musculus* NF-κB proteins

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The NF-κB pathway is critical for regulating inflammation and immune responses. In diseases such as cancers and autoimmune disorders, this pathway is often aberrantly activated. The most common NF-κB protein is the RelA/p50 heterodimer, an essential transcription factor and promising therapeutic target. However, obtaining a detailed biochemical understanding of the function of RelA/p50 remains challenging due to intrinsically disordered regions (IDRs) within the RelA subunit. Historically, IDRs have not been studied well given their complex nature and lack of a stable structure, but understanding their function could be vital to treating NF-κB dysregulation. Our first experimental stage aimed to isolate and purify *H. sapiens* RelA/p50 using protocols established for the *M. musculus* version. SDS-PAGE analysis revealed low protein yield following cell lysis, suggesting issues with the protocol. Our second stage focused on improving NF-κB protein yield through optimizing sonication by altering tip depth and increasing on/off cycles, based on scientific literature. SDS-PAGE analysis showed decreased protein yield in the altered protocol compared to the control, indicating that more aggressive sonication may further compromise purification results. These findings highlight the complexity of protein extraction and the importance of balancing cell lysis efficiency with protein preservation. Future work could explore additional sonication parameters or alternative lysis methods to improve yield. Once an optimized protocol is established, downstream studies can examine the structure and function of RelA/p50's IDRs. This work may contribute to understanding NF-κB proteins and creating therapeutic strategies targeting RelA/p50 to treat dysregulation.