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

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. However, these regions have not been characterized before, limiting our understanding of how this dimer achieves its cellular function. We aim to expand on the function of these IDRs as NF-ϰB signaling could be a potential drug target for chronic inflammation. In order to test the function of these IDRs we aim to express and purify a functional full length p50/RelB construct. In this project we focused on troubleshooting the protein purification protocol, specifically on the ion exchange chromatography (IEX) step which removes bond DNA from the protein. First we compared multiple cation exchange columns, and observed the best separation with the SP-FastFlow strong cation column. Then we tested different buffers to optimize purification performance. Tris was used as a baseline, Sodium Phosphate as condition 1, and HEPES as condition 2, and the SDS-PAGE analysis confirmed that a majority of the protein of interest bound to the column when using both conditions. 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 disordered regions with DNA and other proteins to assess its functional role.