

The NF- κ B family of transcription factors is fundamental in various biological processes, including inflammation, immunity, and cell proliferation, differentiation, and survival. While prior studies biochemically characterized the full-length mouse p50/RelA heterodimer, the analogous human NF- κ B heterodimer and other related family members remain largely unexplored. This work addresses that gap by examining and comparing human p50/RelA, p50/RelB, and p50/c-Rel. p50 and Rel proteins all share a highly conserved Rel Homology domain (RHD), while Rel proteins offer variability within their intrinsically disordered C-terminal transactivation domains (TADs). The intrinsically disordered region (IDR) on a protein pertains to a specific segment that is able to dynamically adopt multiple conformational states under normal physiological conditions. IDRs remain unexplored as they fundamentally shatter the structure-function paradigm and are difficult to characterize experimentally. By performing a comparison study, we can further characterize how intrinsically disordered regions behave across various dynamic states and how this can influence functionality within intracellular processes. In this project, I worked on optimizing the expression and purification of the human p50/c-Rel protein containing its intrinsically disordered domain. I successfully expressed recombinant p50/c-Rel in *Escherichia coli* and purified the heterodimer using Ni²⁺ affinity chromatography. SDS-PAGE analysis confirmed the isolation of both p50 and c-Rel at their expected molecular weights. This will enable us to investigate the binding of this protein to various DNA sequences and coactivator proteins, giving insight into its cellular role.