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Analyzing the Inhibition of NADase Activity via the F174 TcpC Mutant

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The innate immune signaling pathway is essential to host defense and protecting from bacterial invasion. Our protein of interest, TcpC, plays a vital role in the TLR-TIR domain which recognizes pathogens and generates a response. From previous studies, we understand that TcpC is a uropathogenic TIR domain identified in Escherichia coli CFT073 that is analogous to the human TIR domain and promotes bacterial survival. TcpC prevents TLR and MyD88 specific signaling adaptor proteins, which in turn downregulates innate immunity. Previous studies also show that the SARM1 TIR domain is capable of cleaving NAD+, which provides evidence that this TIR domain serves as an ancient enzyme motif that plays a key role in regulating metabolic and bioenergetic pathways via NAD+. We analyzed previous research on wild-type (WT) and mutant (EA) proteins and decided to mutate an amino acid in the same vicinity that we located using Chimera. We hypothesized that our chosen F174 mutant will block NADase activity. With the use of our pair of complementary primers in addition to our F174 mutation, we were able to use quick-change mutagenesis to create our mutant plasmid. In order to purify our protein, we used affinity chromatography by isolating His-tagged TcpC through adding nickel beads. We demonstrated through SDS-Page analysis that we were able to express and purify our mutant protein. The WT version of TcpC has NADase activity, which breaks down NAD+. The EA version of TcpC is the version that we already recognize blocks NADase activity. Our mutant, F174, had variable results in regards to NADase activity. We are repeating this experiment to better determine our mutants effect on NADase activity. If it does affect NADase activity, it may have profound effects on the metabolism of the cell.