The uncontrollable rise in resistance to current antimicrobial agents is a worldwide concern. Beyond traditional approaches to develop novel antibiotics, targeting bacterial virulence is considered a promising approach. It is thought that by targeting virulence, there will be a weaker selective pressure on the bacteria to develop resistance while also displaying little effect on the normal microbiota in the environment and within a host. Specifically, antimicrobial resistance in the bacterial genus, *Shigella,* is a significant problem requiring novel therapies to lessen its rise. These organisms, particularly *Shigella flexneri*, are responsible for the diarrheal disease Shigellosis which leads to approximately 200,000 deaths globally every year. A promising virulence target is the transcription factor, VirF. VirF is an AraC-family protein, that contains an N-terminal dimerization domain and a C-terminal DNA-binding domain (DBD) harboring two helix-turn-helix (HTH) motifs. This protein activates transcription of two major virulence genes, *virB* and *icsA*, which allows the pathogen to invade and spread within colonic epithelial cells. Previous drug discovery campaigns identified VirF inhibitors which exhibited anti-virulence effects *in vivo* but failed to improve upon their efficacies for testing in animal models.

 This thesis presents results focused on elucidation of both the VirF DNA-binding and dimerization domains to aid future virulence-targeted drug discovery. First, we developed homology models for the VirF DBD using structures of VirF-homologs from *E. coli*, GadX and MarA. We conducted alanine-scanning mutagenesis on seven residues within VirF which were based on an alignment with MarA residues that contributed base-specific interactions with its cognate promoter, *marRAB*. We elucidated VirF DNA-binding activity for its three cognate DNA promoters (*pvirB*, *picsA*, and *prnaG*) using wildtype and seven DBD mutants in *in vitro* DNA-binding assays. Upon testing with *pvirB*, mutations to the N-terminal HTH exhibited significant reductions in DNA-binding, while the effects of mutations in the C-terminal HTH varied. When expanded to *picsA* and *prnaG*, WT MalE-VirF bound to these promoters through multiple binding shifts with the DBD mutants displaying similar binding trends compared to *pvirB*. Specifically, the VirF•*picsA* interaction was more sensitive than the other promoters where all mutations, except I189A, caused reductions in DNA-binding activity.To study VirF dimerization, we employed a LexA monohybrid β-galactosidase reporter assay which confirmed WT VirF dimerizes and identified alanine-mutations to Y132, L137, and L147 significantly affected dimerization. These mutations also significantly affected protein stability, but we successfully purified Y132A MalE-VirF which was capable of binding to the *virB* and *rnaG* promoters, albeit with weaker affinity or reduced protein:DNA ratio, respectively. I-TASSER generated full-length VirF structures which predicted the location of these residues and the potential contributions of Y132 for dimerization activity as well as L137 and L147 to protein stability.

 To discover VirF-targeted inhibitors, compound screening was performed. Our previously optimized VirF-driven β-galactosidase reporter assay was used to screen a set of *S. flexneri* virulence inhibitors identified at GSK. However, none of the hit compounds exhibited activity against VirF so it is likely they are interacting with virulence targets outside of VirF. In addition, the LexA reporter assay was used to screen lead compounds, which previously exhibited inhibition of VirF transcriptional activation, against VirF dimerization but none displayed dimerization inhibition. Despite these unsuccessful screens, the in-depth functional analysis of the VirF DNA-binding and dimerization domains will be crucial for the success of future VirF-targeted inhibitor discovery and design.