**Elucidating Protein-DNA Recognition by the Bacterial Transcription Factors MarA and VirF by Homology Modeling and DNA-binding Studies of Wild-type and Mutant MarA and VirF**

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*Shigella flexneri*, a gram-negative pathogen, is the main cause of bacterial dysentery in humans. Infections by *Shigella*, known as shigellosis, lead to approximately 200,000 deaths globally each year. Current treatments include ciprofloxacin and azithromycin, but the resistance rates to these antibiotics have risen significantly worldwide. This highlights a critical need for novel treatments for bacterial infections. One such approach that may potentially circumvent the raise of resistance is to target bacterial tools for infection, rather than bacterial viability. This approach is known as antivirulence inhibition.

*Shigella* relies on various virulence factors that are essential to macrophage apoptosis and escape, intestinal epithelial cell invasion and cell-to-cell spread. These processes rely on a main transcriptional regulator, VirF, to activate transcription of the virulence genes *virB* and *icsA*. While several AraC proteins have been studied, almost none have reported using native VirF, and the three-dimensional structure is yet to be solved. We hypothesize that VirF would make an ideal antivirulence target, and here, we set out to better understand how VirF interacts with DNA in order to gain insight as to how this interaction can be probed as a target for inhibition.

To work with VirF *in vitro*, researchers have relied on a massive maltose binding protein tag that solubilizes the protein. This leaves the question of whether any of results from these studies would be different with a more biologically relevant form of the protein. To address this, we attempted to optimize the expression and purification of a N-terminal Histidine-tagged VirF and a truncated form of the VirF DNA binding domain (DBD). We further characterized the VirF DBD using the structures of two *E. coli* VirF homologs, GadX and MarA•*marRAB*, to generate homology models of the VirF DNA-binding domain in free and DNA-bound conformations. We conducted an alanine scan of seven residues in MarA and VirF that make base-specific interactions to identify residues important for binding to the *marRAB* and *virB* promoters, respectively. We continued to probe the VirF•*virB* interaction by developing chimeric proteins of MarA and VirF, hoping to induce binding to *virB* with the goal of using the chimeric protein as a model to study the VirF DBD inhibitor, 19615.