

# Structural and Functional Studies on a F-like Type IV Secretion System Protein TrbB

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The vast diversity of bacterial phylogeny is attributable to their elaborate genomic recombination capabilities. One mechanism for genomic recombination is conjugation, mediated by the multi-protein type IV secretion system (T4SS) in gram-negative and other bacterial species. T4SS-mediated conjugation is central in the dissemination of genetic elements that confer increased survivability in many bacterial populations, and one crucial consequence is the spread of antibiotic resistance genes. Structural and functional data on the the T4SS will provide avenues to understand bacterial genomic recombination at a fundamental level and provide overt direction for the rational design of novel therapeutics. Here, we report studies on F-like T4SS protein TrbB. An *in vitro* fluorimetric activity assay confirms the putative disulfide isomerase activity of TrbB, which is previously reported to play a mediating role in pilus extension as a protein chaperone. Small-angle X-ray scattering analysis on GST-TrbB yields a low-resolution model with a Guinier radius of gyration of 41.1 Å. AlphaFold backed by circular dichroism provide strong evidence that GST-TrbB is composed primarily of alpha-helices. Circular dichroism at 222 nm was also monitored as a function of temperature to gauge the stability of GST-TrbB by determining its apparent midpoint of unfolding (T<sub>M</sub>) to be 57 degrees C. Circular dichroism and Size-Exclusion Chromatography were also used to characterize the putative interaction between TrbB and TraW.