Characterising atypical disulphide bond catalysing proteins in the pathogenic bacteria *Francisella tularensis*.

**Stephanie Penning**, Jason Paxman and Begoña Heras

1 Department Biochemistry & Chemistry, La Trobe Institute of Molecular Science, La Trobe University, Bundoora 3086, Australia.  
20371827@students.latrobe.edu.au

**Keywords:** Immunology, Redox Biology, Enzyme Kinetics

Multidrug resistant bacteria pose an imminent threat to human health. A promising and novel avenue to tackle antibiotic resistant bacterial infections are the disulphide bond catalysing proteins, known as Dsb proteins. Disulphide bonds provide stability and function to a vast array of proteins, including a range of virulence factors; the molecular weaponry bacteria use to cause infection [1]. Evidence has shown that disrupting disulphide formation by targeting Dsb proteins may be a viable novel antibacterial approach [2]. The multidrug resistant Gram-negative pathogen *Francisella tularensis*, the causative agent of tularemia, contains a unique disulphide bond catalysis system which previous work has implicated in its infectivity [3]. *F. tularensis* encodes for two DsbA-like thiol-oxidase proteins, denoted FtDsbA1 and FtDsbA2, however lacks an identifiable partner disulphide isomerase; DsbC, a key part of the classical Dsb pathway. This ongoing work aims to characterise this disulphide bond catalytic system in *F. tularensis* using a combination of structural biology, biochemical and molecular approaches.

Initial bioinformatic analysis of FtDsbA1 predicted several extended domains compared to the prototypical *Escherichia coli* DsbA including a lipid binding domain within an extended N terminus. This bioinformatic work was validated with a crystal structure of FtDsbA1 solved at 1.9 Å. Analysis of the experimental model reveals a novel DsbA architecture consisting of an insertion into the C-terminus, variations within the distinctive thioredoxin (TRX) domain as well as unique variations within the highly conserved active site. The contribution of these structural variations to FtDsbA1’s function is currently being characterised. Predictive structures for FtDsbA2 were generated using Alpha-fold and, in conjunction with bioinformatic analysis, predicts several unique structural features. In addition to the canonical TRX-like domain, FtDsbA2 is also predicted to contain domains that are uncommon in DsbA-like proteins. These domains resemble a lipid binding motif within an elongated N terminus and a peptyl-prolyl isomerisation domain as well as an extended C terminus, but their specific functions are yet to be uncovered. Crystallisation experiments for FtDsbA2 are currently ongoing. In parallel to structural analysis, the redox functions of both FtDsbA1 and FtDsbA2 are being investigated using a combination of biochemical and biophysical assays, including reduction potential determination, peptide oxidation and insulin reduction assays, stopped flow kinetics and lipid binding assays. The outcomes of this work will provide a comprehensive characterisation of *F. tularensis* DsbA proteins, which will expand our knowledge on Dsb systems in bacteria, inform their role in the virulence and infectivity of this pathogen, and provide tools for future research into the development of novel antibacterial agents against *F. tularensis*.

**Figure 1.** Ribbon representation of FtDsbA1 crystal structure (1.9 Å) highlighting the canonical thioredoxin domain (pink) harbouring the active site (green) and α-helical insert (grey) as well as the additional N-terminal (orange) and C-terminal (yellow) insertions

