Uncovering the structures and mechanisms for the largest group of bacterial surface virulence factors

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We know so little about how bacteria utilise surface virulence factors to colonise, infect, persist and cause disease in their hosts. The largest group of these virulence factors are the autotransporters, where although they employ a simple process for translocation to the bacterial surface, their functional passenger domains show a diverse range of pathogenic functions such as promoting adhesion, biofilm formation, invasion and tissue destruction. Despite extensive international efforts at the genotype-phenotype level that have confirmed the association of autotransporters with bacterial pathogenesis, less than 0.6 % of their structures have been determined with very little information on their molecular mechanisms of action.

With 10 new structures of autotransporter passenger domains over the past few years our group has been leading this area of research. Taking advantage of many autotransporter passenger domains being based upon large >500 residue β-solenoid structures, we have successfully employed Xenon derivatisation at the Australian Synchrotron to acquire anomalous signal for structure determination by single isomorphous replacement. More importantly, we have used our crystal structures to inform a comprehensive array of biophysical, biochemical and microbiological approaches to uncover the mode of action of the autotransporters and their roles in bacterial pathogenesis. Using this approach, we were the first to determine the molecular mechanism of an autotransporter adhesin[1]. We found that this Ag43 adhesin from Uropathogenic *E. coli* (UPEC) promoted bacterial biofilms through a self-association mechanism between neighbouring *E. coli* cell surfaces. This knowledge on biofilms is critical given their contribution to bacterial chronic infections and the development of antibiotic resistance.

Here we present the first crystal structure and mechanism of action of an autotransporter adhesin that binds to host tissue to facilitate bacterial colonisation[2]. The crystal structure of UpaB from UPEC was found to display significant modifications to its β-helix that creates two different binding sites, allowing it to interact simultaneously with both host surface proteins and polysaccharides. As shown in live animal models, both sites co-operate to achieve bacterial colonisation. In contrast to Ag43 that forms self-associations that lead to biofilms, UpaB through directly binding host factors to facilitate colonisation creates a second mechanistic group of the autotransporter adhesins.

Returning to Ag43, we also investigate the conservation of its self-association mechanism with 3 new crystal structures of Ag43 homologues from widespread *E. coli* pathogens[3]. We show that adaptations to this mechanism of action alter the kinetics of bacterial aggregation and biofilm formation, presumably to suit the different *E. coli* pathogens to their specific infection sites. Even more importantly, we are using our molecular knowledge on autotransporters such as Ag43 to develop new classes of anti-bacterial inhibitors. To date we have developed and patented a successful inhibitor that targets Ag43 to prevent pathogenic *E. coli* biofilms[4]. Again using X-ray crystallography, we have determined the structure of the first autotransporter adhesin-inhibitor complex to fully understand how this novel inhibitor interacts with Ag43 and blocks its function.

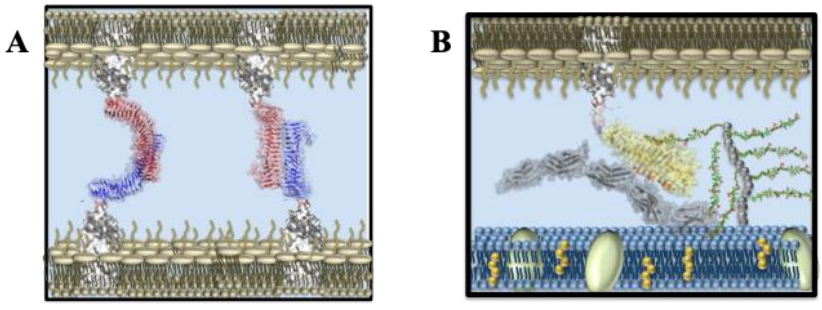

Figure 1A: Ag43 self-associates between *E. coli* surfaces to promote aggregation and biofilm formation. **B.** UpaB directly binds both host proteins and carbohydrates to promote UPEC colonisation.

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