Structural biology of proteins responsible for biofilm regulation and dispersion

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A bacterial biofilm is a structured consortium of bacteria embedded in a self-produced polymer matrix consisting of polysaccharide, protein and DNA. More than 60% of microbial infections, enhanced by biofilms, are responsible for US productivity losses running into billions of dollars. Biofilm plays a significant role in endocarditis, osteomyelitis, dental caries, medical device-related infections such as ocular implant infections, and chronic lung infections in cystic fibrosis patients. Studies have shown, once established, biofilms are extremely difficult to eradicate since the pathogens can tolerate antimicrobial agents 10–1000 times higher concentrations compared to planktonic bacteria. Understanding the factors responsible for initiation, maturation and dispersion of the biofilm is essential for identifying a new class of anti-infective agents. One way to achieve this goal is by investigating the structural biology of proteins involved.

The biofilm life-cycle involves several stages, starting with a single and mobile bacterium, and the first step of this multi-faceted process is bacterial adhesion to a solid surface. Our lab had made significant contribution toward this by investigating bacterial surface proteins and pili components that play critical role in bacterial attachment. In this proposal I am targeting regulation and dispersion aspects of the biofilm by investigating S. *epidermidis* Esp involved biofilm regulation, and BdlA of *P. aeruginosa* that plays significant role in biofilm dispersion.

S. epidermidis Esp identified as the regulator/inhibitor of *S. aureus* biofilm belongs to the glutamyl endopeptidase family with specificity toward peptide bond after the negatively charged residues Glu and Asp. Crystal structure of active Esp was determined by us to explain the substrate specificity and found to have a trypsin-like fold but with significant differences around the N-terminus and also in and around the substrate binding site. Interestingly, Pro-Esp exhibits an unusually long pro-peptide compared to all other trypsin-like fold enzymes, and we would like to understand its role in the enzyme inhibition and also structural re-arrangements involved in Pro-Esp activation. We will probe the role of N-terminus with the help of shorter and stable N-terminal constructs and using x-ray crystallographic methods. We will also generate mutants of Esp and Pro-Esp to investigate role of the critical N-terminal Val67 and few other key residues involved in determining the efficiency and specificity of the enzyme.

We identified Autolysin (Atl) that plays significant role in the *S. aureus* biofilm generation as the substrate for *S. epidermidis* Esp. We revealed Esp cleaves the N-terminal AM domain of Atl, thereby inhibiting localization and the establishment of *S. aureus* biofilm. We intend to determine the crystal structure of S. aureus Atl and/or its fragments to explain the substrate specificity of Esp.

Bacterial dispersion from a matured biofilm, in response to a variety of environmental signals, is a dynamic and highly regulated process. A chemotaxis transducer protein BdlA has been demonstrated to be essential for the dispersion of the pathogen *P. aeruginosa*. BdlA gets activated by undergoing posttranslational modifications involving its cleavage into 2 fragments. Both the truncated polypeptide chains were shown to be needed for BdlA function, however, the mechanism by which they associate is not clear. We will use x-ray crystallographic techniques for better understanding the structure-function relationships of BdlA and its fragments in the dispersive role of *P. aeruginosa.*