Understanding mechanisms of bacterial lipid A modification through computation and experiment

Supervisory team:
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Project description:

Gram-negative bacteria such as E. coli possess an outer membrane external to their cell wall that plays a key role in regulating entry of materials into the cell, contributes to the ability of the organism to cause damage and can be targetted by antibiotics (such as colistin) and antimicrobial peptides produced as part of host defence against infection. The outer layer of this membrane is composed of lipopolysaccharide, which in turn consists of a lipidated dimer of N-acetylglucosamine phosphate (lipid A) decorated with carbohydrates. Enzyme-catalysed addition of phosphoethanolamine (PEtN) or arabinose (Ara) to lipid A affects outer membrane structure and interactions with both antibiotics and defence peptides, but the mechanisms by which this is achieved are not well understood.

This cross-disciplinary project will apply computational and laboratory-based methods to establish mechanisms of lipid A modification, in so doing developing experimental tools that may be also applied to discovery of inhibitors of these enzymes with potential use in overcoming antibiotic resistance, and computational methods, including use of virtual reality, of use in exploring interactions of proteins, particularly those utilising zinc, with small molecules.

Computational work will use molecular simulations at both classical and quantum mechanical levels of theory to explore the structure and dynamics of the PEtN and Ara transferase enzymes responsible for lipid A modification. These simulations, which also include the membrane environment, will also be used to develop models for the complexes with the respective lipidated PEtN and Ara donor, and the lipid A acceptor, substrates. This process will involve our interactive molecular dynamics in virtual reality (iMD-VR) environment, enabling us to create models for the lipid A modification reaction mechanism. Predictions from these models will be tested experimentally using microbiological, biophysical and enzyme activity assays in combination with enzyme variants and mutants to establish the likely roles of specific amino acid residues in lipid A modification. The results will identify how transferase enzymes achieve lipid A modification, information that may ultimately be exploited through targeting with inhibitors designed to sensitise producer bacteria to membrane-acting antibiotics.

The student will work on both computational and experimental strands for the full duration of the project, acquiring skills in molecular simulations, including use of high-performance computing (HPC) and iMD-CR environments, and the design and execution of enzyme activity assays as applied to membrane protein systems.