Combined simulation and experimentation approach to study plasmid-mediated colistin resistance

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Project description:

This project seeks to understand how bacteria resist the action of colistin, a peptide antibiotic that disrupts the outer membrane of Gram-negative bacteria such as *E. coli* and is a last resort treatment used when resistance is encountered to other drugs. Colistin resistance was previously considered rare; however our team recently discovered plasmid-mediated resistance in an *E. coli* strain from a farmed pig in China. The gene responsible, *mcr-1*, has since been identified worldwide in bacteria from veterinary, environmental and human samples. MCR-1 protects bacteria from colistin by modifying their outer membranes; specifically it encodes a membrane-bound enzyme that catalyses transfer of phosphoethanolamine to the lipid A component of lipopolysaccharide. The mechanism of this reaction remains to be explored.

Recently we have succeeded in obtaining a crystal structure for the soluble, catalytic domain of MCR-1, revealing this to be a zinc metalloenzyme and suggesting a catalytic mechanism involving a single zinc ion. This differs from previous mechanistic proposals for related enzymes that involve two or three metal ions. We now seek to extend this work to study full-length MCR-1, comprising both soluble and membrane-bound domains, using a combination of computational and experimental approaches, and subsequently to exploit our findings to identify inhibitors of the enzyme. Based upon our structure the student will use expert biomolecular simulation methods (molecular dynamics) to build and evaluate a model of full-length MCR-1 in the context of the bacterial inner membrane. Subsequently this work will be extended to investigate its interactions with lipid A and phosphatidylethanolamine substrates. The results will generate a model for MCR-1 structure and function that will be tested experimentally using a combination of biochemical (assays of phosphoethanolamine transfer) and microbiological (assays of colistin susceptibility in recombinant *E. coli*) approaches. Subsequently this information will be exploited in computational studies aimed at identifying small molecules able to bind the MCR-1 active site and that thus disrupt its action upon bacterial lipid A. These will identify a panel of potential MCR-1 inhibitors that will be tested experimentally *in vitro* and for their effects upon colistin killing of MCR-1 producing bacteria.

This is a multidisciplinary project where the student will be involved in both laboratory and computational work for the majority of the award period. The student will gain training in state of the art computational methods and a wide range of experimental approaches to characterising bacterial membrane proteins, their function and interactions.