Mechanism and inhibition of the plasmid-mediated colistin resistance determinant MCR-1

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Project description:

This project seeks to understand one mechanism by which bacteria resist the action of colistin, a peptide antibiotic that disrupts the outer membrane of Gram-negative bacteria such as *E. coli* and that is used as a last resort treatment when resistance is encountered to other drugs. Resistance to colistin was previously known, but rare; however our team recently discovered plasmid-mediated colistin resistance in an *E. coli* strain originating from a farmed pig in China. The gene responsible, *mcr-1*, has since been identified in multiple bacteria from veterinary, environmental and human samples and in worldwide locations. 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 the MCR-1 enzyme, 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. Hence we now seek to explore the mechanism of MCR-1 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 and quantum mechanics/molecular mechanics (QM/MM)) to investigate the MCR-1 zinc centre and its interactions with phosphoethanolamine. The results will generate a model for MCR-1 mechanism that will be tested experimentally using a combination of structural (X-ray crystallography), biophysical (spectroscopy), 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 we anticipate that 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 biological macromolecules, their function and interactions.