Project Details	
Project Code	MRCIIAR24Br Spencer
Title	Penicillin Antibiotic Action and Resistance in a Bacterial “Superbug”
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Beta-lactams (penicillins and related drugs) are the most important antibiotics. Beta-lactams inhibit penicillin-binding proteins (PBPs) but in resistant bacteria are hydrolysed by mechanistically related beta-lactamase enzymes. This project combines cutting-edge methods in structural biology and molecular simulations to study reactions of beta-lactams with both types of enzyme and inform new strategies to overcome beta-lactam resistance.
Description	Antimicrobial resistance (AMR) is a global public health emergency that is already directly responsible for 1.3 m deaths worldwide. Beta-lactams (penicillins and related agents) are the most widely used antibiotics in human medicine and remain essential for treatment of healthcare-associated infections by a range of bacterial pathogens. As alternatives remain limited beta-lactam resistance makes such infections severe and sometimes life-threatening. Beta-lactam resistant strains of Gram-negative bacteria (GNB) such as Klebsiella pneumoniae are among the commonest AMR pathogens and considered by the World Health Organization as the highest priority for antibiotic research and development. Beta-lactams inhibit penicillin-binding proteins (PBPs) that catalyse the final step in biosynthesis of the rigid cell wall that is essential to bacterial viability. While PBP alterations can be associated with beta-lactam resistance, in GNB the major resistance mechanism is production of beta-lactamase enzymes that hydrolyse the beta-lactam amide bond to abolish antimicrobial activity. Beta-lactamases form four distinct mechanistic groups, of which three are, like PBPs, active-site serine enzymes (SBLs). Both SBLs and PBPs react with beta-lactams to form a covalent acylenzyme; in PBPs this is inhibitory and long-lived whereas for reaction of beta-lactamases with good substrates this may be very transitory. Understanding acylenzyme formation is of profound importance to both antibacterial development and combatting resistance: small molecules (including beta-lactams) that efficiently acylate PBPs are likely to possess antibacterial activity, and to evade resistance if SBL acylation is inefficient; whilst agents that form long-lived acylenzymes with SBLs can be co-administered with beta-lactams to overcome resistance. This project investigates the acylation mechanisms of PBPs and SBLs through a combination of structural, computational and kinetic approaches. Focusing initially on enzymes from K. pneumoniae, for which recombinant expression has already been achieved and (for SBLs) crystallisation conditions are established, the student will obtain crystal structures of PBPs and SBLs bound to beta-lactam antibiotics used to treat human infections. These structures will be used as the basis for molecular simulations using molecular dynamics and hybrid quantum mechanics/ molecular mechanics (QM/MM) approaches to elucidate the energetics and mechanism of the acylation reaction and identify properties (of enzyme and substrate) that contribute to efficient acylation. Results and predictions from simulations will be verified by experiment: measuring the rates of the acylation reaction using steady- and pre-steady state kinetic approaches;

	and verifying the contributions of individual residues within the active site to acylation using site-directed mutagenesis. The computational components of the project exploit discipline-leading expertise in methods development (co-supervisors van der Kamp and Mulholland) supported by the High-Performance Computing (HPC) facilities at the University of Bristol and across GW4. The crystallographic work will exploit our ongoing collaboration with Diamond Light Source to apply developing serial methods to investigate transient, as well as metastable, states in the acylation reaction. The student will thus receive training in state-of-the-art methods in the distinct, but connected, disciplines of biomolecular simulations and X-ray crystallography. Knowledge transfer and impact opportunities arise through the Bristol and GW4 AMR networks, that bring together AMR researchers, including clinicians, across multiple disciplines; as well as involvement of the supervisory team in national collaborative projects in biomolecular simulations (CCP-BioSim) and dynamic structural biology (UK XFEL Hub).
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