Project Details	
Project Code	MRCIIAR26Ba Tooke
Title	Understanding β-lactam antibiotic action and resistance with developments in dynamic structural biology
Research Theme	IIAR
Project Type	This project combines both wet and dry lab elements. The primary focus will be on wet-lab methods (enzyme purification, biochemical characterisation of enzymes, structural biology) but will feature dry lab approaches (biomolecular simulation).
Summary	β-Lactams, including penicillins, are the world's most commonly used antibiotics, but resistance is rising—particularly in opportunistic bacterial pathogens like *Klebsiella pneumoniae*. This project investigates how β-lactams bind and react with bacterial enzymes called penicillin-binding proteins PBPs and β-lactamases, which are key to both antibiotic action and resistance. Using advanced techniques—including time-resolved X-ray crystallography at synchrotrons/XFELs (X-ray Free-Electron Lasers), biomolecular simulations, molecular biology and structural biology—we aim to evaluate and capture "molecular movies" of these reactions in action. This project provides interdisciplinary training to understand antibiotic resistance mechanisms to ultimately support the design and evaluation of new drugs.
Description	**Background** β-Lactams (penicillins and related agents) are the most prescribed class of antibiotics worldwide. These drugs function by binding to and inhibiting penicillin-binding proteins (PBPs), enzymes responsible for cross-linking the peptidoglycan cell wall (transpeptidation)—an essential component for bacterial survival. PBPs represent a large and structurally diverse enzyme family with critical roles in peptidoglycan synthesis, rigidification, and regulation. Carbapenems are last-resort β-lactam antibiotics used to treat severe, multidrug-resistant bacterial infections. Carbapenem-resistant Enterobacterales, such as Klebsiella pneumoniae (Kp), are classified by the World Health Organization as Priority 1: CRITICAL pathogens. In 2019, over 1.27 million deaths were directly attributed to drug-resistant infections, with K. pneumoniae the third most significant contributor, responsible for over 20% of these deaths. In Gram-negative bacteria, β-lactam resistance primarily arises through β-lactamase (BLA) production—enzymes capable of hydrolysing and inactivating β-lactam antibiotics. Both PBPs and active-site serine BLAs form covalent acyl-enzyme complexes with β-lactams, but with very different outcomes: PBPs are inactivated by stable acylation, whereas BLAs can efficiently deacylate and inactivate the antibiotic. Mutations in the PBP transpeptidase active site have also been associated with reduced β-lactam susceptibility and clinical resistance. Despite their clinical importance, the processes of acyl-enzyme formation and breakdown in PBPs and BLAs remain poorly understood, particularly at the atomic level and on catalytically relevant timescales. Key Research Question How do β-lactams bind to (acylate) and react with both PBPs and BLAs? **Specific Objectives**

	1. Characterisation of K. pneumoniae PBPs (PBP2 and PBP3) Biochemical and structural data on K. pneumoniae PBPs are currently limited. Soluble constructs of PBP2 and PBP3 will be expressed and purified, building on existing protocols from the Tooke and Spencer labs. Enzymatic activity will be characterised using a combination of kinetic and gel-based BOCILLIN competition assays with β-lactam antibiotics. Structural characterisation will be carried out by X-ray crystallography, supported by access to the South West Block Allocation Group at Diamond Light Source (UK). Crystallisation approaches will include soaking and/or co-crystallisation to investigate antibiotic and inhibitor binding. Natural variants of PBP2 and PBP3 identified from clinical isolates (from K. pneumoniae and other Gram-negative bacteria), and sequence data from collaborators at the Universities of Bath and Bristol, will guide the selection of mutants for study. Site-directed mutants will also be generated to produce crystallographically more tractable systems. Available structures (from this project, the PDB, or Alphafold predictions) of wild-type and clinical variants of PBP2/PBP3 will be explored using biomolecular simulations to investigate the dynamic properties of these systems. Molecular dynamics and hybrid quantum mechanics/molecular mechanics (QM/MM) approaches will be applied to investigate differences across variants that contribute to β-lactam resistance or reduced susceptibility. 2. Use of dynamic structural biology to investigate the shared mechanism of PBP and BLA acylation To gain new mechanistic insights, this project will apply serial time-resolved crystallography at both XFELs (X-ray free electron lasers) and synchrotrons to capture intermediate states in enzyme catalysis on timescales (milliseconds to seconds) previously inaccessible. These data will be integrated with high-level QM/MM biomolecular simulations to generate “molecular movies” of PBPs and BLAs engaged in catalysis. Crystals of PBPs and BLAs will be optimised into microcrystalline slurries suitable for studies at microfocus beamlines at Diamond Light Source (e.g., I24) and XFELs. The Tooke and Spencer labs already maintain several tractable microcrystalline BLA systems, which the student can further develop. These will be studied alongside PBPs from Objective 1 to investigate β-lactam and inhibitor acylation across both enzyme classes. Crystal structures obtained from Objective 1, new XFEL studies and using existing crystallographic data (Tooke lab, unpublished) will provide snapshots of Michaelis and acyl-enzyme complexes. These structures will feed into combined QM/MM modelling (Tooke, Van der Kamp, and Mulholland) to simulate and evaluate acylation reactions of antibiotics and inhibitors in silico. This PhD project will therefore combine cutting-edge techniques in molecular microbiology, structural biology, and biomolecular simulation to study β-lactam binding, affinity, and reactivity within PBPs and BLAs. On structurally tractable systems, the student will apply novel developments in time-resolved crystallography to visualise enzyme mechanisms in unprecedented detail.

	Structural and biochemical information on PBPs from K. pneumoniae is still sparse. Detailed atomic-level insights into acyl-enzyme formation and breakdown will underpin developments in PBP understanding, inform on new strategies to overcome β-lactam resistance and guide the design of next-generation β-lactam antibiotics and inhibitors. **Student Ownership and Development** This project integrates both wet and dry lab approaches, allowing the student to tailor the direction of their work to align with their methodological and scientific interests. For example, they may choose to focus more heavily on experimental microbiology and crystallography or develop a greater emphasis on computational simulation. The student will also have the opportunity to take ownership over the selection of PBP variants and BLA systems to study, and to lead the development and optimisation of mechanistic assays.
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