Project Details	
Project Code	MRC21IIRBr Van der Kamp
Title	Countering antimicrobial resistance: investigating activity of β-lactamase inhibitors using atomistic simulation and experiment
Research Theme	Infection, Immunity & Repair
Summary	Antibiotic resistance threatens human health. β-lactamases cause resistance to β-lactams, the most widely used antibiotics. Some, but not all, β-lactamases can be countered with inhibitors, of which some may also halt bacterial growth. We will investigate these differences by simulation and experiment, develop simulation protocols to predict inhibitor activity, and use these to guide inhibitor design.
Description	Rising antibiotic resistance is a major problem for human health. Resistance to β-lactams, the single most important antibiotic class, often arises through their breakdown by β-lactamases (BLs). Many BL producing bacteria are multi-drug resistant and may cause untreatable infections. A clinically important class of BL inhibitors to combat this resistance are diazabicyclooctanes (DBOs), used together with β-lactams (e.g. avibactam-ceftazidime, relebactam-imipenem) to treat complicated secondary care infections. Interestingly, new DBOs in development (e.g. Nacubactam, Zidebactam) show inhibition of penicillin binding proteins (PBPs, the cellular targets of the β-lactams) as well as BLs, leading to a so-called "enhancer" effect upon β-lactam action and possible use as 'dual mode' antibiotics. To inhibit BLs and PBPs, DBO inhibitors form stable covalent acyl-enzyme complexes. Using multi-scale computer simulations, we have calculated the efficiency of β-lactam acyl-enzyme formation and breakdown for a number of BLs, thereby predicting whether individual enzymes can confer resistance to specific β-lactams (Chem Comm, 50, 14736; ACS Catal, 10, 6188) and their susceptibility to BL inhibitors (Biochemistry, 57, 3560). Crystal structures of complexes of key BLs with multiple inhibitors are now available (J Mol Biol, 431, 3472) and experimental data provide evidence that in some cases these complexes can slowly degrade to regenerate active enzyme, potentially providing a route to inhibitor resistance. This multidisciplinary project aims to address why certain DBOs inhibit both BLs and PBPs, whereas others inhibit BLs only. Computational assays based on multi-scale simulations will be used to assess formation and breakdown of the acyl-enzymes formed by a range of DBOs with clinically relevant serine BLs (e.g. KPC-2, OXA-48 and variants) as well as PBPs from target bacteria. This is challenging, as different reaction mechanisms will need to be explored. The accuracy of these assays will be validated by experimental determination of DBO inhibition of example BLs and PBPs using steady-state, stopped- and quenched-flow kinetic methods (Spencer). The BL test set will include new variants identified from an extensive collection of clinical isolates collected worldwide, including regions with endemic resistance (Pakistan, Thailand, India), and characterised by genomic and phenotypic approaches (Toleman; collaborator Walsh). Although challenging (due to multiple techniques and targets, and the complex mechanisms), the expertise of the supervisors makes the project feasible. By interrogating recent clinical isolates, the BLs selected will be of the highest relevance. The project is inherently multi-disciplinary, with the student being trained in multiple techniques. In addition to the co-

	supervisory team, interactions with key collaborators from this team are envisaged, such as with the Schofield group (Oxford, cutting-edge experimental techniques for antibiotic/beta-lactamse interaction mechanisms) & the Walsh group (Oxford). The project will provide training in cutting-edge techniques in multiple disciplines (computational chemistry, molecular biology/biochemistry, clinical microbiology/genomics) using state-of-the-art facilities in the context of a highly collaborative AMR research environment. The project will benefit from Bristol's excellent high-performance computing resources (incl. one of the UK's largest university computer clusters). Insights obtained into DBO inhibition will inform development of new DBOs with desirable characteristics. Synthetically tractable DBO modifications will be identified and evaluated in silico to assess their promise as BL and PBP inhibitors. Potential exploitation will be discussed with companies active in this area (e.g. Selcia, with whom we already collaborate). We will also exploit current interest in antimicrobial resistance through public engagement.
Supervisory Team	
Lead Supervisor	
Name	Dr Marc Van der Kamp
Affiliation	Bristol
College/Faculty	Life Sciences
Department/School	Biochemistry
Email Address	marc.vanderkamp@bristol.ac.uk
Co-Supervisor 1	
Name	Dr James Spencer
Affiliation	Bristol
College/Faculty	Life Sciences
Department/School	School of Cellular and Molecular Medicine
Co-Supervisor 2	
Name	Dr Mark Toleman
Affiliation	Cardiff
College/Faculty	
Department/School	School of Medicine
Co-Supervisor 3	
Name	Professor Adrian Mulholland
Affiliation	Bristol
College/Faculty	Faculty of Science
Department/School	School of Chemistry
Co-Supervisor 4	
Name	
Affiliation	
College/Faculty	
Department/School	