Project Details	
Project Code	MRCIIAR26Br Oliveira
Title	Exploring Communication Networks in DNA Gyrase: A Pathway to Next-Generation Antibiotic Discovery
Research Theme	IIAR
Project Type	This is a computational led project using biomolecular simulations, but will include laboratory experiments to test/validate predictions arising from the simulations.
Summary	Bacterial antibiotic resistance is a global public health crisis. New antibiotics for resistant organisms are urgently needed, but attempts to identify new molecular targets in bacteria have been largely unsuccessful. One alternative approach is to identify new routes to disrupting the function of known, validated targets. This project applies state of the art computational methodologies, developed in our group, to investigate communication networks in the multisubunit protein machine and antibiotic target DNA gyrase. We will explore how gyrase activity is coordinated and affected by mutations, and use this information to identify new binding sites for small molecules/potential antibacterial candidates.
Description	Antibiotic resistance is a global public health crisis exacerbated by the weakness of the antibacterial development pipeline. Given the challenge of identifying new unexploited targets for antibacterial drugs, new approaches to disrupting the biological activity of known targets are increasingly important for antibacterials discovery. Bacterial type II DNA topoisomerases, enzymes that modulate DNA topology to counter torsional stresses imposed by processes such as DNA replication or transcription or that separate daughter chromosomes during cell division, comprise one such known target. Type II topoisomerases (DNA gyrase, DNA topoisomerase IV) are multisubunit molecular machines that break, translocate and religate bound DNA in a cycle driven by binding and hydrolysis of ATP. This complex activity presents multiple opportunities for disruption by small molecules. At present, however, only DNA breakage/reunion is targeted by inhibitors, in particular fluoroquinolones, used in the clinic as antibacterials. Other methods of targeting type II topoisomerases that evade current fluoroquinolone resistance mechanisms provide routes to new antibacterials acting on these validated targets. This project seeks to understand how communication through the topoisomerase II enzyme structure connects different biochemical events (e.g. ATP binding/hydrolysis and DNA binding/cleavage/translocation/religation) in the overall mechanism and, potentially, how such communication networks might be disrupted through small-molecule binding to cryptic yet druggable sites. The resulting understanding can then be applied to identify alternative methods of DNA topoisomerase II inhibition that may ultimately be exploited in novel antibacterials. We have recently developed a computational approach, dynamical non-equilibrium molecular dynamics (D-NEMD) simulations, to identify intramolecular communication networks in biological systems. The D-NEMD approach introduces a perturbation (e.g. removal of a bound ligand) to a simulation of the target protein at equilibrium, and compares the response to this perturbation over time with a parallel

	simulation of the non-perturbed system. Monitoring the propagation of this response through the protein structure then identifies pathways by which different parts of the structure communicate with one another. We have used the D-NEMD approach to investigate communication networks in multiple disease-relevant target proteins, including nicotinic acetylcholine receptors and the SARS-CoV-2 spike and main protease. Additionally, D-NEMD simulations of beta-lactamases involved in bacterial antibiotic resistance uncovered residues remote from the active site whose mutation was predicted to affect activity due to their participation in such networks, a finding we subsequently validated by experiments. Recently, D-NEMD simulations of DNA gyrase from the TB pathogen Mycobacterium tuberculosis revealed the mechanism by which the ATP site is connected to the rest of the DNA gyrase ATPase domain, identifying two distinct routes through which such connections can occur. In this proposal, the student will undertake D-NEMD simulations of larger portions of DNA gyrase, including its complexes with DNA and small-molecule inhibitors, to identify communication networks operating both within and between subunits. Our initial target will be M. tuberculosis DNA gyrase, due to (i) its high clinical relevance as a target for second-line anti-TB drugs; (ii) the existence of relevant crystal structures on which simulations can be based; and, (iii) our previous application of D-NEMD to the ATPase domain, which facilitates initial training by providing an established system and pre-existing data, with which the student can learn to set up and analyse these simulations. After such training, the student will use the prep period to select new target gyrase structures for investigation by D-NEMD, studies that we anticipate will include investigation of the networks connecting the DNA breakage/relegation site to both the ATPase site and to other small molecule binding sites such as that for the recently identified allosteric inhibitor evybactin. Based on these simulations, we anticipate predicting residues in M. tuberculosis DNA gyrase where mutation would disrupt such communication networks and, identifying surface regions (such as the evybactin binding site) that present potential binding sites for novel small molecule inhibitors. The effects of identified mutations, including those at candidate small-molecule binding sites, will be tested experimentally in gyrase activity assays. We will also use docking and virtual screening approaches to identify potential small ligands binding at such sites. The project applies a state-of-the-art computational approach, pioneered by the lead supervisor, to a mechanistically complex molecular machine and key antibacterial drug target, and exploits the worldclass High-Performance Computing (HPC) facilities available at the University of Bristol. Our current objectives are to focus on communication networks in M. tuberculosis gyrase that connect: (i) the ATPase and breakage-reunion active sites; and (ii) the breakage-reunion site to other small-molecule binding sites; and on this basis (iii) explore the effect of residue substitutions and/or small molecule binding on such networks. We will strongly encourage the student to take ownership of the project by e.g. identifying the most relevant gyrase structures/conformations/complexes for investigation by simulation;

	establishing the most appropriate balance between simulations and experiments; and determining whether to extend investigations beyond M. tuberculosis DNA gyrase to encompass type II DNA topoisomerases from other bacterial pathogens.
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