Project Details	
Project Code	MRCIIAR25Br Spencer
Title	Small-Molecule G-Quadruplex (G4) Ligands as Candidate Antibacterials for Resistant Bacteria
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Increasing resistance makes developing new antibiotics, that kill bacteria by different mechanisms from existing agents, a public health priority. G-quadruplexes (G4s) are specific nucleotide structures, formed by guanine-rich sequences, that represent potential antibacterial targets as their stabilisation by small-molecules disrupts cellular processes (DNA replication, transcription). We have identified several small molecules with antibacterial activity that bind G-quadruplex sequences in vitro. This project combines imaging, whole-genome sequencing and proteomics, together with antibacterial susceptibility assays and investigations of resistant mutants; to investigate their cellular targets and killing mechanisms and assess the potential of G4 ligands for development of new generations of antibiotics.
Description	Bacterial antibiotic resistance is a global public health emergency that already causes 1.3m deaths annually. The problem is exacerbated by continuing failure to develop new antibiotics, especially drugs with novel modes of action that evade resistance to agents in current use. G-quadruplexes (G4s) are distinctive nucleic acid structures that form when guanine-rich sequences associate, and can be stabilised by binding of small-molecule ligands. G4 structures can disrupt biological processes such as DNA replication and transcription, are over-represented in portions of eukaryotic genomes such as telomeres, and as such are recognised as potential therapeutic targets for conditions such as cancer; but are less studied in bacteria. This project investigates interactions of G4-binding ligands with bacteria and with bacterial G4 sequences in vitro, seeking to understand the antibacterial activity of specific G4 ligands and how this might be exploited in new generations of antibacterial drugs. In previous work we have identified several series of G4 ligands with variable activity towards Gram-negative (e.g. Escherichia coli) and Gram-positive (Staphylococcus aureus) target species, and that vary in their in vitro interactions with oligonucleotides containing bacterial G4 sequences. This project applies a range of approaches to investigate the mode of antibacterial action and cellular targets of these agents and related molecules, their susceptibility to resistance development; and ultimately assess the potential for G4 ligands to provide the basis for new antibacterial drugs. Initial experiments will use antibacterial susceptibility assays to establish the spectrum of activity of our panel of G4 ligands against laboratory (type) and clinical strains of target bacteria. Based on the results of these experiments, the most active combinations of small molecule and bacterial target will be further investigated using a combination of imaging and multi-omic methods to establish mode of action. We will first use Bacterial Cytological Profiling (BCP) to compare the morphological response of bacteria challenged with G4 ligands to those to agents with known modes of action; and combine this with proteomic investigations of bacteria challenged with G4 ligands, compared to

	unexposed controls, to establish effects upon gene expression. Subsequently we will use whole-genome sequencing of G4-ligand exposed bacteria to look for binding sites in bacterial genomes (that we expect to identify through e.g. changes in short-read density); and RNAseq to validate and extend findings from proteomics and establish the extent to which effects on protein expression operate at the transcriptional and/or translational levels. Based on the results of these whole-cell studies we will identify G4 sequences that are the strongest candidate targets for ligand binding and test their interactions with G4 ligands in vitro (measuring ligand binding to synthetic oligonucleotides by spectroscopic methods) and in bacterial cells and extracts using reporter constructs containing candidate G4 sequences. These data will identify G4 sequences targeted by our small-molecule ligands, and establish the cellular consequences of these interactions. In parallel we will investigate the susceptibility of G4 ligands to resistance development by target bacteria, based on the hypothesis that G4 ligands may bind to multiple nucleotide sequence targets in the bacterial cell, and so be at reduced risk of resistance development as multiple mutations would be required to abolish activity. We will use serial passage (exposure to increasing concentrations of antibiotics) to generate strains of bacteria with reduced susceptibility to antibacterial G4 ligands, and whole-genome sequencing to identify mutations potentially associated with resistance. Involvement of such mutations in resistance can be confirmed by complementation experiments reintroducing the wild-type gene to mutant strains, or by generating the equivalent knock-outs or mutants in susceptible (wild-type) backgrounds. Collectively these experiments will constitute a rigorous investigation of the mode of action of antibacterial G4 ligands and provide a basis on which to assess the development potential of the G4-targetting strategy and/or specific G4 ligands. The project provides training in imaging, multi-omic, biophysical and (molecular) microbiological methods in the context of a multidisciplinary environment of established collaboration between synthetic chemistry (Galan) and bacteriology (Spencer) research groups, with Feil providing expertise in next-generation sequencing. We expect students to develop the direction of the project by inputting into key decision points including the choice of ligand, pathogen (Gram-positive versus Gram-negative) and methodology (imaging/multi-omics/reporter assay) on which they will primarily focus. Knowledge transfer and impact opportunities arise through opportunities to participate in the Bristol and GW4 antimicrobial resistance (AMR) networks, that bring together local and regional AMR researchers, including clinicians, across multiple disciplines.

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