Project Details	
Project Code	MRCIIAR26Ba Preston
Title	Bacterial Hunger Games: Targeting Nutrient Transport for Novel Antibiotic Development.
Research Theme	IIAR
Project Type	Wet lab
Summary	Most current antibiotics are derivatives of previous drugs and resistance adapts quickly to the new derivatives, greatly limiting their useful lifespan. New antibiotics with novel bacterial targets are desperately needed. In this project we will develop bacterial nutrient transporters as novel antibiotic targets. Inhibiting nutrient uptake will slow bacterial growth, allowing immune responses to clear the infection, and/or augmenting current antibiotic use. As proof-of-principle for this approach we will use metabolic modeling to identify target transporters, genetic engineering and novel peptide inhibitors to drug them and single cell imaging to test the effects of this on cell growth and viability.
Description	The widespread resistance of bacteria to current antibiotics demands that novel antibacterial compounds are developed (1). Most existing antibiotics are iterations of previous compounds to which resistance arises quickly. Identification of genuinely novel targets is required to generate a much wider portfolio of antibiotics. In this project we provide proof-of-principle that bacterial nutrient transporters are novel targets for antibiotic development. All bacteria take up nutrients from their environment to fuel metabolism required to survive, grow and replicate. Transport systems are used to take molecules across the otherwise impermeable cytoplasmic membrane. This essential role suggests that inhibition of bacterial transporters will inhibit bacterial growth, with inhibitors acting as antibiotics. To do this we will develop a computational approach to use genome scale tools to identify the most promising transporters to prioritise for inhibitor development and test their drug target vulnerability. While bacterial metabolism has been discussed as a source of novel antibiotic targets, the intracellular location of most metabolic enzymes poses major barriers to development of exogenously applied inhibitors. Here, we take advantage of transporters having external facing components to avoid this major hurdle. There are numerous different types of transporters, but all transport specific substrates from the periplasm, through the cytoplasmic membrane, into the cytoplasm for entry into metabolism. The breadth of exchange of molecules between the cell and external environments is extensive, with predictions of hundreds of exchange reactions involved in bacterial metabolism (see below), involving dozens of different transporters. Compared to metabolic enzymes, transporters are much more accessible to small molecule inhibitors, having entire components, or protein segments on the periplasmic face of the cytoplasmic membrane. Also, whereas central metabolic enzymes are highly conserved across species, particularly in the case of closely related bacteria of the Enterobacteriacea, transporters are less tightly conserved, particularly the transmembrane channels where the maintenance of the amphipathic nature of the channel can be maintained by different uncharged amino acids, enabling greater

	tolerance of sequence variation compared to e.g. enzyme active sites. This improves the chances of developing narrow spectrum antibiotics targeted to pathogens that do not affect other members of the microbiota. Genome scale metabolic models (GSMMs) are mathematical models that match genes (and thus their predicted protein products) to metabolic reactions, curated from literature, experimental studies and GSMMs developed for other bacteria and organisms {Gu, 2019 #11}. A biomass objective function (BOF) is defined for a particular bacterium, describing the molecular composition of the cell in terms of protein, carbohydrate, nucleic acid, lipid and if known, other specific components, determined by experimental measurement of these components in cells. The GSMM produces a stoichiometrically balanced set of reactions that produces the BOF-defined cellular composition and is used for in silico exploration of metabolism, simulating biomass formation for different media formulations and, of relevance here, simulating the effect of knocking out specific reactions on the generation of biomass to identify reactions and thus metabolic pathways important for growth of the bacterium under different conditions. Here, we will test the hypothesis that bacterial nutrient transport systems are novel, druggable targets for antibiotic development. To do this we have several specific aims: 1. To use a Klebsiella GSMM to systematically simulate the substrates required for growth under different conditions and computationally match specific transport systems to the uptake of key substrates. 2. Experimentally validate the role of candidate target transporters in Klebsiella growth under different conditions and across strains using genetic modification to knock out or knock down genes encoding target transport systems. 3. Design transporter-specific peptide inhibitors and screen peptide libraries. We will rationally design peptide inhibitors and screen semi-rational libraries to identify peptide sequences that: bind to and disrupt oligomerisation interfaces, preventing functional assembly; mimic key interaction motifs, competitively displacing native partners; target extracellular domains, avoiding permeability and efflux issues associated with intracellular inhibitors. 4. Test the drugability of the transporters to inhibit growth. The effect of genetic modification and peptide-based inhibition will be tested using single cell imaging incorporating measurement of labelled metabolite uptake and cell growth and division. Analysis of individual cells allows assessment of heterogeneity of response among a population of cells, something prevalent in the response of bacteria to antibiotics. While here we propose Klebsiella for proof of principle of our approach, the student will explore other high priority AMR pathogens during the prep period, and may decide on other targets, creating ownership of the project from an early stage. The project addresses an urgent need for novel antibiotic development, and will develop and test an approach that is applicable to pathogens generally. The student will benefit from a project incorporating

	computational, biophysical, and genetic approaches, providing wide ranging training. 1. Pariente N. 2022. PLoS Biol. 20:e3001918. doi: 10.1371/journal.pbio.3001918. 2. Gu, C. et al. 2019. Genome Biol. 20:121. doi: 10.1186/s13059-019-1730-3.
Supervisory Team	
Lead Supervisor	
Name	Professor Andrew Preston
Affiliation	Bath
College/Faculty	Faculty of Science
Department/School	Department of Life Sciences
Email Address	a.preston@bath.ac.uk
Co-Supervisor 1	
Name	Professor Stefano Pagliara
Affiliation	Exeter
College/Faculty	Faculty of Health and Life Sciences
Department/School	Living Systems Institute
Co-Supervisor 2	
Name	Professor Jody Mason
Affiliation	Bath
College/Faculty	Faculty of Science
Department/School	Department of Life Sciences
Co-Supervisor 3	
Name	
Affiliation	
College/Faculty	
Department/School	