Project Details	
Project Code	MRCIIAR25Ex Harmer
Title	Developing new therapies against the most dangerous antibiotic resistant bacteria
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Antimicrobial resistance is an increasing societal issue, contributing to ~5,000,000 deaths in 2019. The highest priority bacteria are the ESKAPE pathogens that account for over half this mortality and morbidity. This project will contribute to fighting antimicrobial resistance by developing new high-throughput in vivo ESKAPE pathogen infection models in the wax moth Galleria mellonella. These will be used to test resistant clinical isolates with our novel compounds that make bacteria more susceptible to conventional antibiotics and facilitate further development of the compounds. The project will accelerate the development of our compounds and provide new in vivo models for antimicrobial discovery.
Description	The so-called “ESKAPE pathogens” (Enterobacteria, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterococcus faecium) are the most serious antimicrobial resistant bacteria. In 2019, over 3,000,000 deaths worldwide were estimated to be associated with antimicrobial resistant strains of these bacteria alone. The likely number of deaths will continue to increase without development of new treatment options and may make some existing medical procedures non-feasible. One approach that has been proposed is to develop potentiating medicines that weaken ancillary functions of specific bacteria, rendering them more susceptible to conventional antibiotics. The effectiveness of this approach needs to be demonstrated in a whole organism during the lead development/lead optimisation phase of drug discovery. This is particularly the case for academic projects as such drug development will not be fundable without these proof-of-concept data. The model organism Galleria mellonella has been used for over a decade as a model for infection. Galleria larvae are cheap, have easy husbandry, and replicate results in the mouse well. Both acute and chronic infections can be modelled, and treatments can be delivered both prophylactically and post-infection. The Galleria Mellonella Research Centre at the University of Exeter has pioneered genetic engineering of Galleria. Importantly, this provides access to fluorescently labelled larvae. Combining these with labelling of bacteria allows highly effective detection of infection progression through imaging flow cytometry of larva haemolymph. However, this has only been demonstrated for infections of a small number of bacteria. We have developed compounds that inhibit the Macrophage Infectivity Potentiator (Mip) protein that is found in almost all infectious bacteria. Our compounds significantly potentiate standard of care antibiotics in mice against Klebsiella pneumoniae. They have shown strong effectiveness against many bacteria (e.g. Acinetobacter baumannii, Neisseria gonorrhoeae, Burkholderia pseudomallei) in cell based models. We expect that the compounds will show effectiveness against all the ESKAPE pathogens as co-treatments with the standard of care. These

	compounds would act as an excellent demonstration of the value of the Galleria model. The Mip proteins have been crystallised in complex with compounds. However, the crystals are all dependent on the compound to form part of the lattice, requiring bespoke crystallisation for each experiment. It would be highly valuable to develop a generic crystal form. Using the latest AI protein design tools, we will develop such crystals for the Mip orthologues from each of the ESKAPE pathogens. This will assist compound development alongside the in vivo work. The key aims of the project will be: 1) To develop the Galleria mellonella cytomics model for all ESKAPE pathogens 2) To demonstrate the effectiveness of Mip inhibitors against ESKAPE pathogens 3) To use AI protein design to develop a Mip crystal that will support drug development Specific objectives will be: 1a) Learn to culture laboratory strains of one example of each ESKAPE pathogen 1b) Learn husbandry, handling, injection, and humane termination methods for Galleria 1c) Establish suitable infection models for each pathogen, determining suitable infection doses for acute and chronic infections 1d) Learn cytomics methods for each ESKAPE pathogen and use these to determine suitable sacrifice points for each pathogen 1e) Learn Galleria antibiotic treatment methods 2a) Determine suitable concentrations of Mip inhibitors to use in Galleria 2b) Demonstrate co-treatment of Galleria with SOC antibiotics and Mip inhibitors 2c) Test clinical strains with Mip inhibitors 3a) Learn to use AI-based protein design tools 3b) Learn to apply AI-based tools to inpainting of existing proteins into a scaffold (for crystallisation) 3c) Learn to prepare proteins 3d) Learn protein crystallisation and to determine structures with compounds 3e) Use protein-compound structures to inform design of improved compounds This plan will provide significant opportunities for the student to take ownership. The project will be most impactful with success of all three aims. However, each aim has clear opportunity for extension and development. We will encourage the student to expand on aims that align most with the student's interests and longer term aspirations. We will expect the student to focus literature investigation on areas of their greatest interest and to develop proposals to extend the project according to their preferred area of expansion.

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