EPSRC Centre for Doctoral Training (CDT) in Molecular Sciences for Medicine (MoSMed)

Synthetic antimicrobials based on natural toxins

Department of Biosciences, Durham University, UK

Supervisory Team	• Lead Supervisor: Prof Tim Blower, Department of Biosciences, Durham University, UK • Co-supervisor: Dr Jon Marles-Wright, School of Biology, Newcastle University, UK • Co-supervisor: Prof Steven Cobb, Department of Chemistry, Durham University, UK

Project overview/context

Tuberculosis remains the deadliest bacterial disease on the planet, killing around 1.5 million people each year. It can be treated, but antibiotic resistance is increasing. *Mycobacterium tuberculosis* can survive antibiotic treatment by slowing down its growth using naturally occurring toxins made inside tuberculosis cells. Some of these toxins target the essential process of untangling DNA after it replicates. We have characterised the topoisomerase enzyme that performs this untangling activity, and have shown that toxins inhibit its action. This project aims to generate atomic resolution models of toxin activity, so we can synthesise antimicrobial mimics for treating infections.

Research Project

Background

Mycobacterium tuberculosis (*Mtb*) infects one-third of the world's population and in 2021 accounted for 1.6 million deaths. *Mtb* is a bacterium encoding eighty different toxin-antitoxin loci, which perform multiple roles including antibiotic resistance and controlling dormancy inside host macrophages. These loci include two homologues of the widespread *parDE* toxin-antitoxin family that targets DNA gyrase, an essential topoisomerase necessary for DNA replication. DNA gyrase is also the target of multiple antibiotic families, such as the fluoroquinolones, which have annual sales of ~$8 bn. We have completed the first structural studies of *Mtb* gyrase:fluoroquinolone complexes that examined modes of fluoroquinolone action and host resistance. Now, we will apply this knowledge to structurally characterise gyrase:ParE complexes and develop ParE toxin mimics for use as novel antimicrobials against a range of bacterial pathogens. Students undertaking this project will receive inter-disciplinary training in molecular biology, microbiology, protein production, structural biology (X-ray crystallography and cryo-Electron Microscopy), and peptide synthesis.

Research Plan

This cross-institution, multi-disciplinary project will use a combination of biochemistry and structural biology to investigate gyrase inhibition by bacterial toxins. The student will examine multiple aspects; **1)** Identify residues determining gyrase:ParE interactions by AlphaFold modelling, site-directed mutagenesis, binding studies and biochemistry; **2)** Experimentally confirm the structural nature of gyrase inhibition, through both X-ray crystallography and cryo-EM; **3)** Generate peptide/peptoid mimics of ParE toxins that can be used as inhibitors of gyrase, and learn how to target other bacterial species dependent on cognate gyrase/ParE sequences. This project addresses a long-standing question regarding how the widespread ParE family is toxic, will provide insight into the biochemistry of the proven and essential antibiotic target, gyrase, and will develop insight into how naturally occurring toxins might be weaponised as novel antimicrobials.

Training & Skills

Methodology and Research Training

The proposed work will provide excellent inter-disciplinary training in diverse techniques:

1. **Molecular Microbiology and protein biochemistry:** The student will learn molecular biology, microbiology, protein purification techniques and biochemical assays, focussing on protein-protein and protein-DNA interactions, such as size exclusion chromatography, co-immunoprecipitation, fluorescence anisotropy, and gyrase activity assays (**Blower**)
2. **Structural biology:** The student will learn to prepare crystals for X-ray diffraction experiments (**Blower**), and how to prepare samples and grids for negatively stained and cryo- electron microscopy (**Marles-Wright**). The student will learn methods of structural data collection, data processing, and AlphaFold modelling (**Blower** and **Marles-Wright**).
3. **Peptide chemistry:** the student will learn peptide and peptoid chemistry for production of ParE toxin mimics to inhibit topoisomerases (**Cobb**).

This will prepare the student for research in both industry and academia, in the fields of microbiology, structural biology, biochemistry and drug development. The student will also have opportunities to work in multiple research groups, attend conferences, and develop professional writing, presentation and networking skills.

Further Information

Please contact the Project Lead for more information, Prof Tim Blower: timothy.blower@durham.ac.uk

Please also refer to:www.blowerlab.com, Twitter: @blowerlab

How to Apply

To apply for this project please visit the Durham University application portal to be found at: Home · Application Portal (microsoftcrmportals.com)

Please select the course 'PhD in Molecular Sciences for Medicine (EPSRC CDT)', which is registered in the Chemistry Department and indicate the reference **mos23_01 i**n the 'Field of Study' section of the application form. Please note that there is no need to submit a Research Proposal with your application, however we do require a Covering Letter, CV, academic transcripts, the contact details of two referees and proof of English language proficiency if relevant.

Within the MoSMed CDT we are committed to building a diverse community based on excellence and commitment. To that end in our recruitment of Doctoral Researchers we welcome applications from outstanding candidates of all backgrounds regardless of ethnicity, disability, gender identity, sexual orientation and will consider all applications equally based on merit.

Should you have any queries regarding the application process at Durham University please contact the Durham MoSMed CDT Manager, Emma Worden at: emma.worden@durham.ac.uk

Newcastle University Durham University UKRI Engineering and Physical Sciences Research Council