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

Harnessing tuberculosis toxins to manipulate bacterial growth

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Project overview

Tuberculosis remains the most deadly human disease on the planet, killing around 1.6 million people each year. Many factors contribute to the success of *Mycobacterium tuberculosis* as a pathogen. Of all studied bacteria, *M. tuberculosis* has the largest complement of "toxin-antitoxin" systems. Though counter-intuitive, activation of these toxins inside the bacteria may allow *M. tuberculosis* to survive attacks by the immune system and antibiotics. By studying how the toxins work, we can better understand how to control bacterial growth, and identify new antibiotic targets, with long-term impact towards the development of new therapies.

Research Project

Background. *Mycobacterium tuberculosis* is the biggest infectious disease killer worldwide, with 1.6 million deaths per annum and increasing rates of antimicrobial-resistant infections. Strategies to manage tuberculosis require fundamental research to drive innovation in therapeutics, but the World Health Organisation has highlighted the scarcity of new approaches[1]. An opportunity exists to address these points by harnessing "toxin-antitoxin" (TA) systems in bacteria that target essential steps such as translation, DNA replication and cell wall synthesis. The abundance of TA systems throughout bacteria, and *M. tuberculosis* in particular, suggests these systems represent a rich source of new biochemistry and antibacterial targets.

TA systems encode two components, a toxic protein that targets an essential cellular process, and an antagonistic antitoxin, which blocks toxin activity when cells are growing under favourable conditions[2]. Although the processes that lead to toxin activation remain under debate, it has been proposed that under certain stress conditions, increased toxin transcription and synthesis may lead to activation[3,4]. This in turn reduces growth rate, which can provide a means to survive with minimal metabolic burden until favourable conditions return[5]. TA systems are remarkably abundant in *M. tuberculosis*, which encodes more than 80 putative systems that are thought to contribute to the success of *M. tuberculosis* as a human pathogen[6–8]. Many of the putative *M. tuberculosis* toxins tested thus far were shown to inhibit bacterial growth, and the highly toxic nature of some toxins suggests that their antibacterial mechanisms could be developed into antimicrobials[9].

Research Plan. In our recent work, we established and characterised the MenAT TA family of nucleotidyltransferases, which were proven to be active in killing tuberculosis[10,11]. Toxin $MenT_3$ targets and modifies all four mycobacterial serine tRNAs. The MenAT family also represents a new class of type VII TA systems, regulated through phosphorylation by the cognate MenA antitoxin[12,13].

These discoveries prompt two new routes for controlling the growth of *M. tuberculosis*; (i) modulating toxin activity and (ii) inhibiting amino-acyl charging of serine tRNAs. This project aims to explore these opportunities through a combination of biochemistry, structural biology and *in silico* methods encompassing protein dynamics modelling, docking studies and structure-based drug design.

Objective 1 – Toxin inhibition. Use our high resolution structures of toxin $MenT_3$ and $MenT_4$ with VirtualFlow[14],

to screen and identify potential small molecule inhibitors. Hits will be screened for toxin inhibition through established biochemical assays, microbiology and X-ray crystallographic studies.

Objective 2 – Dysregulation of toxin-antitoxin interactions. Structural and biophysical studies will be performed to build models for the control of MenT toxins through phosphorylation by cognate MenA antitoxins.

Objective 3 – Target toxin targets. $MenT_3$ modifies serine tRNAs, indicating the seryl-tRNA synthetase as a potential antibiotic target. Structural studies of the seryl-tRNA synthetase from *M. tuberculosis* will be combined with *in silico* screening methods to identify and characterise potential inhibitors.

Strategic vision. Studying the recently identified MenAT toxin-antitoxin family is timely and addresses an unmet research need, by characterising new potential antibiotic targets in a relevant human pathogen through a combination of biochemical and computational methodologies.

References. [1] WHO (2020) Global Tuberculosis Report. [2] Page, R. & Peti, W. (2016) Nat Chem Biol 12, 208–14. [3] Song, S. & Wood, T. K. (2020) Adv Biosystems 4, 1900290. [4] LeRoux, M. et al. (2020) Mol Cell 79, 280-92. [5] Hall, A. et al. (2017) Curr Opin Microbiol 36, 102–10. [6] Akarsu, H. et al. (2019) PLOS Comput Biol 15, e1006946. [7] Keren, I. et al. (2011) MBio 2, e00100-11. [8] Sala, A. et al. (2014) Toxins 6, 1002–20. [9] Freire, D. et al. (2019) Mol Cell 73, 1282-91. [10] Beck, I. et al. (2020) Biochem J 477, 2401–19. [11] Cai, Y. et al. (2020) Sci Adv 6, eabb6651. [12] Yu, X. et al. (2020) Commun Biol 3, 216. [13] Wang, X. et al. (2020) Trends Microbiol doi: 10.1016/J.TIM.2020.12.001. [14] Gorgulla, C. et al. (2020) Nature 580, 663–8.

Further Information

For informal enquiries, please contact Dr Tim Blower (timothy.blower@durham.ac.uk).

Training & Skills

This project, led by Dr Tim Blower (Durham), provides training in molecular microbiology, protein biochemistry and structural biology. Second supervisor Dr Agnieszka Bronowska (Newcastle) will provide training in *in silico* methods including ligand screening and structure-based drug design. At later stages, Dr Danny Cole (Newcastle) will provide further training to employ hit-to-lead optimisation workflows. This multidisciplinary training will be further supported by external courses (ie. Diamond Light Source), producing a scientist with the broad range of in-demand skills that are needed to tackle ongoing societal problems such as antimicrobial resistance and discovery of novel drug hits.

How to Apply

To apply for this project please visit the Durham University application portal to be found at: https://www.dur.ac.uk/study/pg/apply/

Please select the course code F1A201 for a PhD in Molecular Sciences for Medicine and indicate the reference MoSMed21_09 in 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, an academic transcript, the contact details of two referees and proof of English language proficiency if appropriate.

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

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