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

MycoTACs: Proteome targeting chimeras for *Mycobacterium tuberculosis*

Biosciences Institute, Newcastle University

Supervisory Team	• **Dr Jon Sellars, Newcastle University (Lead)** • **Dr Alistair Brown, Newcastle University** • **Prof Ehmke Pohl, Durham University**

Project overview/context

PROteolysis Targeting Chimeras (PROTACs) are a promising and recent technological development utilised in the degradation of proteins in eukaryotes. Application in bacterial pathogens however has been overlooked as protein degradation in this way was thought to be unique to eukaryotes. Recently it has become clear an exception to this exists, *Mycobacterium tuberculosis* and other actinobacteria, have been shown to possess a highly analogous degradation system. Consequently, this project will expand our current interest in *Mycobacterium tuberculosis* (*Mtb*) pathogen and focus on the development of MycoTACs, the first PROTAC like molecules for use in *Mycobacterium tuberculosis*.

Research Project

PROteolysis Targeting Chimeras (PROTACs) are a promising and recent technological development. PROTACs are hetero bi-functional molecules, capable of recruiting a protein of interest and E3 ligase to promote the degradation of proteins via the ubiquitin-proteasome system in eukaryotic cells.

Whilst being actively developed for eukaryotes, their application in bacterial pathogens has been overlooked. Recently it has become clear that actinobacteria, including *Mycobacterium tuberculosis*, possess a highly analogous system. As a result, this project complements our current interest in this deadly pathogen and aims to develop MycoTACs, the first PROTAC molecules for use in *Mycobacterium tuberculosis* (*Mtb*).

Project

The *Mtb* proteolytic pathway is known as the Pup-proteasome system (PPS) and mirrors that of eukaryotes through proteasomal degradation following conjugation of a prokaryotic ubiquitin-like protein (Pup), a small (7 kDa) and natively unstructured protein. Pup shares no homology to ubiquitin and where hundreds of ubiquitin ligases exist, only one enzyme, proteasome accessory factor A (PafA), conjugates Pup to bacterial protein targets.

Consequently, to develop our MycoTACs we will complete four objectives.
Objective 1: This will enhance our existing *in silico* library of PafA recruiting molecule(s) utilising computational modelling. To achieve this, *in silico* docking will utilise the Alphafold model of Mtb PafA, CCDC software (GOLD/Hermes) and ZINC online compound library.

Objective 2: Running concurrently with objective one, synthesis of PafA recruiting molecule(s) that have already demonstrated good *in silico* binding to PafA will be undertaken, alongside those identified in objective 1. Once prepared, the molecules will be screened for their binding efficiencies and biological properties against recombinant PafA and Pup in an established Pup conjugation assay.[1] Whilst this is the main objective, these molecules will also be screened for their antibacterial activity against *Mtb,* as inhibiting the proteasome has been shown to be an effective strategy.[2]

Objective 3: Having access to recombinant PafA enables us to undertake crystal trials and ultimately determine the structure. Being able to produce a crystal will have many benefits, such as substitution of the Alphafold model and

revalidation of our model compounds to crystal soaking experiments with molecules from objective 2 to validate the chosen *in silico* binding site.

Objective 4: With a PafA recruiting molecule(s) identified, we will conjugate these to existing drug molecules, identified for their ability to target essential pathways in Mtb. This will provide the final MycoTAC chimera suitable for antibacterial screening as in objective 2 to validate these tools as bacterial PROTACs.

Strategic vision
Tuberculosis is the deadliest human disease, with 1.5 million people succumbing to this disease in 2021. Identifying novel proteins involved in essential biochemical pathways is of the upmost importance in Mtb, the causative agent of tuberculosis. Recent studies demonstrating the Mtb proteome provides such opportunity validates our project aims and any results from this computational, chemical, and biological studies would is important to the field.

[1] O. Regev, M. Korman, N. Hecht, Z. Roth, N. Forer, R. Zarivach, E. Gur, *J. Mol. Biol.* **2016**, *428*, 4143-4153.

[2] a.) H. Zhang, H. C. Hsu, S. C. Kahne, R. Hara, W. Zhan, X. Jiang, K. Burns-Huang, T. Ouellette, T. Imaeda, R. Okamoto, M. Kawasaki, M. Michino, T. T. Wong, A. Toita, T. Yukawa, F. Moraca, J. Vendome, P. Saha, K. Sato, K. Aso, J. Ginn, P. T. Meinke, M. Foley, C. F. Nathan, K. H. Darwin, H. Li, G. Lin, *J. Med. Chem.* **2021**, *64*, 6262-6272; b.) R. Tyagi, M. Srivastava, B. Singh, S. Sharma, R. P. Pandey, S. Asthana, D. Kumar, V. S. Raj, *J. Biomol. Struct. Dyn.* **2021**, 1-11; c.) G. V. Janssen, S. Zhang, R. Merkx, C. Schiesswohl, C. Chatterjee, K. H. Darwin, P. P. Geurink, G. J. van der Heden van Noort, H. Ovaa, *ChemBioChem* **2021**.

Training & Skills

The project will provide invaluable training, as part of a multidisciplinary team, in organic synthesis, cell and molecular biology and computational chemistry that are sought after skills for future careers in chemistry and related fields.
You will join our multidiscplinary team and undertake research between Newcastle and Durham Universities, providing you an inspiring and vibrant working environment for your studies. At Newcastle, you will undertake the computational studies, synthesis and characterisation of novel MycoTacs and molecular biology screening of these compounds in *Mycobacterium tuberculosis*. At Durham, you will continue the computational studies alongside the purification and establishment of crystal studies on the protein of interest, PafA, with a view to crystallise this protein to expand our knowledge of the target.

Further Information

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How to Apply

You must apply through the University's online application system.

When applying to Newcastle University please select the Course Code **8207F (PhD in Molecular Sciences)**
You will need to:
- Insert the **programme code 8207F** in the programme of study section
- Select '**PhD in Molecular Sciences'** as the programme of study
- Input (only) the **studentship reference code (e.g. 22_08)** that you are applying for in the studentship/partnership reference field when prompted (all codes are outlined in the individual project adverts and can be found on the MoSMed website: https://research.ncl.ac.uk/mosmed/phdstudentships/)
- Attach all documents that are requested including a CV and cover letter. The cover letter must **clearly** state the project reference code, the full title of the studentship and state how your interests and experience relate to the project
- Attach degree transcripts and certificates and, if English is not your first language, a copy of your English language qualifications

Should you have any queries regarding the application process to Newcastle University please contact Selina McCarthy, MoSMed CDT Manager: Selina.McCarthy@newcastle.ac.uk or email mosmed.cdt@newcastle.ac.uk

Within the MoSMedCDT 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.

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