Combatting Antimicrobial Resistance with Covalent Macrocycle Libraries

Supervisory team:
Main supervisor: Dr Scott Lovell (University of Bath)
Second supervisor: Maisem Laabei (University of Bristol)
Prof Jean van den Elsen (University of Bath)

Host institution: University of Bath

Project description:
Background: Infections with multidrug-resistant bacteria like methicillin-resistant Staphylococcus aureus (MRSA) are major threats to human health. Discovering novel targets for antibiotic development is a pivotal task to guarantee effective treatment in the future. WalKR is a two-component system present in S. aureus, consisting of a kinase (WalK) that senses environmental cues and relays this information to a response regulator (WalR), via phosphorylation, activating genes required to combat stress. WalK possesses an extracellular domain that must homodimerize for the enzyme to be functionally active. The WalKR system is an attractive antimicrobial target as it is essential for growth of S. aureus and mutations in the walK gene are linked to vancomycin resistance.

Aim and Overview: In this PhD project you will identify a targeted covalent macrocycle (TCM) inhibitor for WalK, which blocks homodimerization and prevents the formation of a functional WalKR system in S. aureus. TCMs combine the properties of a macrocyclic peptide and an irreversible inhibitor, binding to proteins with high affinity and selectivity by forming interactions over a large surface area and achieving permanent target engagement by covalent modification of a proximate nucleophilic residue. The Lovell lab have used TCMs to inhibit challenging viral, bacterial and cancer proteins.

Objective 1: Identification of a TCM inhibitor – Using intact protein mass spectrometry, we will identify covalent fragments that modify nucleophilic residues present at the homodimer interface of WalK by screening in-house libraries. Hit fragments will be 'grafted' onto diverse, chemically synthesised macrocyclic scaffolds to generate thousands of WalK dimer interface-directed TCMs. TCMs showing rapid covalent modification of WalK will be tested in SPR binding studies and in a size-exclusion chromatography assay to assess inhibition of homodimer formation. Co-crystal structures of hit TCMs bound to WalK will guide optimisation of selectivity and proteolytic stability. Chemical proteomics will be used to assess the proteome-wide selectivity of TCMs.

Objective 2: Lead TCM Validation – The lead TCM will be applied to MRSA isolates to assess toxicity and changes in growth rate. The Laabei lab will develop GFP transcriptional fusions for WalR target genes to enable activation of the WalKR system to be monitored upon treatment with our TCM. WalK engagement in vivo will be demonstrated by applying the TCM to Manduca Sexta larvae challenged with S. aureus.

Outcome: This research will validate WalK as a therapeutic target for treatment of MRSA infections and will provide an optimised TCM for further pre-clinical assessment.

Our aim as the SWBio DTP is to support students from a range of backgrounds and circumstances. Where needed, we will work with you to take into consideration reasonable project adaptations (for example to support caring responsibilities, disabilities, other significant personal circumstances) as well as flexible working and part-time study requests, to enable greater access to a PhD. All our supervisors support us with this aim, so please feel comfortable in discussing further with the listed PhD project supervisor to see what is feasible.