**BBSRC MIBTP Studentship Project**

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| **Project Title** | Revival of the immortals: investigation of molecular mechanisms of mycobacterial resuscitation from dormancy |
| **Project Summary**  |
| Antimicrobial resistance (AMR) is a significant global challenge. It is estimated that nearly 10 million people will die from infection with multi-drug resistant bacteria by 2050 if novel antimicrobials are not introduced. Drug resistant mycobacteria are amongstthe most important bacterial pathogens, mainly because of their incredible ability to hide in the human body from the immune system and antimicrobial agents. In addition, they can slow down their growth and become dormant and therefore gain tolerance to most drugs. Mycobacterial pathogens have evolved to rapidly respond to host-imposed stress (e.g. exposure to reactive oxygen and nitrogen species, acidic environment).  We have recently shown that sustained exposure of mycobacteria to nitric oxide (NO), an important signalling molecule produced by the immune system, does not kill them but triggers their transition into a special differentially culturable state. In this state, mycobacteria cannot grow in standard media and require resuscitation by resuscitation-promoting factor (Rpf). Rpf is a family of secreted muralytic enzymes (lytic transglycosylases) that play an important role in mycobacterial growth, regrowth from dormancy and virulence. Transcriptomic analysis of NO-treated mycobacteria revealed a dramatic reduction of expression of *rpfA, rpfB, rpfE*. Moreover, addition of Rpf inhibitors completely abolished resuscitation of NO-treated mycobacteria, thus confirming the importance of Rpf for resuscitation of NO-treated mycobacteria. However, the molecular mechanism of Rpf-mediated resuscitation remains unknown. Our previous work showed that the enzymatic activity of Rpf was essential for bacterial resuscitation; while others demonstrated resuscitation of dormant mycobacteria by muropeptides cleaved by RpfB and RipA from mycobacterial peptidoglycan. Muropeptides have been shown to play a signalling role in various bacteria and our recently published study demonstrated that mycobacteria can recycle muropeptides.  In this project we will employ a combination of molecular methods to reveal the resuscitation pathways in NO-treated mycobacteria. In particular, we will develop a high-throughput resuscitation assay for screening the *Mycobacterium bovis* BCG transposon library developed by Dr Moynihan. Mutants impaired in resuscitation will be further investigated to inform generation of reporter strains and biochemical experiments. The proposed project will include the following objectives: Optimisation of current resuscitation assay for high-throughput screening Identification of mutants impaired in regrowth of NO-treated mycobacteria on agar and resuscitation in liquid media Characterisation of these mutants and identification of molecular pathways involved in resuscitation Generation of complementation mutants and investigation of their phenotypes in resuscitation assays. Techniques that will be undertaken during the project* Resuscitation and growth assays using *M. tuberculosis* and *M. bovis* BCG
* Genetic manipulation of mycobacteria
* Genomic sequencing
* Enzymatic assays

BBSRC Strategic Research Priority: Understanding the Rules of Life - Microbiology |
| **References** |
| 1. **Moynihan PJ,** Cadby IT, Veerapen N, Jankute M, Crosatti M, **Mukamolova GV**, Lovering AL, Besra GS. (2019). The hydrolase LpqI primes mycobacterial peptidoglycan recycling. *Nat Commun* 10(1):2647.
2. Turapov O, FortiF, Kadhim B, Ghisotti D, Sassine J, Straatman-Iwanowska, Bottrill AR, **Moynihan PJ**, Wallis R, Barthe P, Cohen-Gonsaud M, Ajuh P, Vollmer W, **Mukamolova GV**. (2018). Two faces of CwlM, an essential PknB substrate, in *Mycobacterium tuberculosis*. *Cell Rep* 25(1): 57-67. e 5.
3. Rosser A, Stover C, Pareek M, **Mukamolova GV.** (2017) Resuscitation-promoting factors are important determinants of the pathophysiology in *Mycobacterium tuberculosis* infection. *Crit Rev Microbiol* 17:1-10.
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