**University of Leicester**

**BBSRC MIBTP Studentship Project 2024-5 entry.**

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| **Project Reference** |  |

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**Section 2 – *Project Information***

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| **Project Title** | Molecular characterization of suicidal phage-resistance mechanisms in bacteria  |
| **Project Summary**  |
| **Importance** The proliferation of antibiotic resistance (AMR) is a formidable health challenge, accounting for 700,000 deaths annually1,2. There are several clinically important bacterial families where AMR has come to the forefront due to the emergence of severe drug resistance. Perhaps the most important example is gram+ *Mycobacteriaceae,* which includes *M. tuberculosis* (*M. tb*), responsible for significant infectious disease related mortality and AMR due to the emergence of multi-drug (MDR) and extreme drug resistance (XDR)-TB. MDR/XDR-TB is challenging to treat because *M. tb* resides deep within granulomas evading classical antibiotics. Consequently, using natural and engineered bacteriophages to target such persistent pathogens has recently gained popularity. Although utilizing phage therapies to treat bacterial infections and counter antibiotic resistance is promising3, there is a risk of promoting the emergence of phage resistant bacterial pathogens. Our awareness of the plethora of phage defence mechanisms is relatively recent and the molecular basis of most bacterial phage resistant mechanisms, even in well-known model phages is largely unexplored.  **Background** Bacteria resist phage infections by several strategies, interestingly as a measure of last resort, one strategy is by committing suicide upon phage attack by abortive infection (Abi)4. Although many Abi systems are known, the RexA/RexB system5 from *E. coli* is the most extensively characterized system where a *λ*-lysogenic prophage encoded RexAB complex inactivates the infection of other phages namely T4, T5 and T7. Despite being the first identified Abi system, their mode of action remains enigmatic. The RexA protein is thought to sense a poorly characterized T4-phage protein DNA complex and two copies of RexA activate one copy of RexB, an inner membrane ion channel resulting in severe loss of membrane potential and a drop in ATP-levels, eventually leading to cell death. RexAB-like systems have also been identified in *Mycobacteriaceae*. In mycobacteria, although mycobacteriophages have already been clinically shown to treat mycobacterial infections, the existence of phage resistance mechanisms remains a problem. The most interesting case is that of Sbash, a prophage that colludes with its host (*M. smegmatis* MC2155) to confer highly specific defence against infection by a mycobacteriophage Crossroads using a mechanism analogous to the RexAB system6.  **Objectives** The overarching vision of this research project is to characterize the molecular processes associated with abortive infection in bacteria with the eventual aim of designing robust mycobacteriophage based treatments against drug-resistant TB. This involves characterizing the RexA/RexB from *E. coli* and Sbash systems from mycobacteria using a combination of biophysical and structural methods. The immediate goals in this project are to first study the RexAB system in *E. coli* and decouple the RexA sensing and RexB membrane-integrated ion-channel functions by studying them separately using X-ray crystallography and cryo-EM.  The key questions in this project are (1) How does RexA sense phage-DNA-protein complexes? (2) How does RexA-phageDNA-protein complex activate membrane-integrated RexB? (3) How RexB causes pore formation, cation efflux and membrane depolarization?   **Environment** The project combines various biophysical and structural methods, utilizing the facilitates available within the University of Leicester's ecosystem (LISCB and Centre for Phage Research). It also leverages multidisciplinary collaborations across institutions: with Dr. Brian Ho, UCL (for live-cell imaging) and eBIC, and Research Complex at Harwell (for cryo-FIB, CLEM, and cryo-ET). The project will also utilize the expertise of Dr. Qian Cong (University of Texas, USA) in employing AI/ML methods within RoseTTAFold to identify targets for stabilization of protein complexes.  **Techniques that will be undertaken during the project*** Cloning, over-expression and purification of recombinant protein-complexes.
* Membrane-protein purification, solubilization and stabilisation procedures.
* Biophysical characterization of protein-complexes.
* Membrane-protein X-ray crystallography,  Single particle cryo-electron microscopy (cryo-EM), image processing, model building and integrative structural biology.
* *In-situ* studies on the RexAB system using Cryo-FIB and Cryo-ET.
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| **References** |
| 1. *IACG report on Antimicrobial Resistance*, 2019.
2. Mace M and Vadakkepat AK et al., *Nature*, 2022, 607, 191-196.
3. Dedrick RM et al., *Nat Med*. 2019, 25:730-733.
4. Lopatina A, Tal N, Sorek R, *Annu Rev Virol*. 2020, 7:371-384.
5. Parma DH et al., *Genes Dev.* 1992, 6:497-510.
6. Gentile GM et al., *mBio*. 2019, 10:e00196-19.
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