**Self funded project**

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| **First Supervisor** | Dr Amanda Chaplin  |
| **School/Department** | Molecular and Cell Biology  |
| **Email**  | Ac853@leicester.ac.uk  |

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| **Second Supervisor** | Prof Galina Mukamolova |
| **School/Department** | Respiratory Sciences  |
| **Email**  | Gvm4@leicester.ac.uk  |

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| **Additional Supervisor** |  |

**Section 2 – *Project Information***

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| **Project Title** | Deciphering the non-homologous end joining (NHEJ) DNA-double strand break repair mechanism in *Mycobacterium tuberculosis*  |
| **Project Summary**  |
|  In all kingdoms of life, DNA damaging agents can jeopardize the integrity of the cellular genome, resulting in a range of developmental defects. A break across both strands of DNA is the most critical form of DNA damage and if left unrepaired can ultimately result in cell death 1. Non-homologous end joining (NHEJ) is considered to be the major repair pathway of DNA-double strand breaks (DSBs) in all organisms 2. NHEJ in bacteria is a simplified system compared to humans and mechanism and protein interplay are not well understood. The intracellular human pathogen *Mycobacterium tuberculosis* (*Mtb)* causes the respiratory disease Tuberculosis (TB), which affects one quarter of the world population 3. Understanding the fundamental process of DNA-repair in *Mtb* will help us to determine the importance of this mechanism for infection and long-term survival of the bacteria within the human host, ultimately revealing potential novel targets for future antimicrobials. The NHEJ mechanism in *Mtb* involves Ku and the multifunctional protein LigD 4. Previous studies have shown that Ku and LigD directly interact in order to repair DSBs 5,6. Although multiple cryo-EM structures have recently been solved of human NHEJ proteins, there is limited structural information in bacteria 7,8. This PhD proposal will aim to understand how only Ku and LigD can fulfil the multiple roles of NHEJ that are fulfilled by multiple different proteins in humans and will provide mechanistic insights into how this system has evolved from prokaryotes to higher eukaryotes.Within this PhD proposal, cryo-EM will be used to solve structures of Ku and LigD in complex with various DNA substrates. Once solved, the importance of key interacting protein residues within the structures will be determined using mutagenesis and biophysical assays. Furthermore, the importance of these proteins within *Mtb* and the findings from structural work will be further validated using complementation studies with *Mtb*, *Mycobacterium smegmatis* and *Mycobacterium marinum*. This will allow the student to be trained in containment level 2 and 3. Overall, this PhD proposal will aim to determine the precise mechanism of NHEJ in *Mtb*, which will have huge benefit for understanding the mechanism of NHEJ in bacteria, and other organism, and lead to potential development of novel antimicrobials against *Mtb*.  |
| **References** |
| 1 Aplan, P. D. *Trends Genet* **22**, 46-55, (2006).2 Zhao, B., Rothenberg, E., Ramsden, D. A. & Lieber, M. R. *Nat Rev Mol Cell Biol* **21**, 765-781, (2020).3 Bagcchi, S. *Lancet Microbe* **4**, e20, (2023).4 Della, M., Palmbos, P. L., Tseng, H. M., Tonkin, L. M., Daley, J. M., Topper, L. M., Pitcher, R. S., Tomkinson, A. E., Wilson, T. E. & Doherty, A. J. *Science* **306**, 683-685, (2004).5 Weller, G. R., Kysela, B., Roy, R., Tonkin, L. M., Scanlan, E., Della, M., Devine, S. K., Day, J. P., Wilkinson, A., d'Adda di Fagagna, F. *et al.* *Science* **297**, 1686-1689, (2002).6 Gong, C., Bongiorno, P., Martins, A., Stephanou, N. C., Zhu, H., Shuman, S. & Glickman, M. S. *Nat Struct Mol Biol* **12**, 304-312, (2005).7 Chaplin, A. K., Hardwick, S. W., Liang, S., Kefala Stavridi, A., Hnizda, A., Cooper, L. R., De Oliveira, T. M., Chirgadze, D. Y. & Blundell, T. L. *Nat Struct Mol Biol* **28**, 13-19, (2020).8 Chaplin, A. K., Hardwick, S. W., Stavridi, A. K., Buehl, C. J., Goff, N. J., Ropars, V., Liang, S., De Oliveira, T. M., Chirgadze, D. Y., Meek, K. *et al.* *Mol Cell* **81**, 3400-3409 e3403, (2021). |
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