**University of Leicester**

**BBSRC MIBTP Studentship Project 2025-6 entry.**

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**Section 2 – *Project Information***

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| **Project Title** | Activation of protein kinase G from Mycobacterium tuberculosis – uncovering molecular mechanisms by CryoEM |
| **Project Summary**  |
| This project will focus on fundamental mechanisms of environment sensing by the human pathogen *Mycobacterium tuberculosis*. We have identified a novel transmembrane protein complex for sensing external amino acids. It controls the activity of protein kinase G (PknG) which in turn controls bacterial central metabolism (whether amino acids are synthesised or broken down). This system is essential for the virulence of *M. tuberculosis* and may sense phagocytosis by macrophages. It is conserved in non-pathogenic Actinobacteria including organisms used in production of food and antibiotics.  The complex is made up of a periplasmic sensor protein and transmembrane transducer protein plus cytoplasmic PknG. We hypothesise that nutrient-sensing and signal transduction occur by a series of linked conformational changes leading to an active dimeric conformation of PknG that autophosphorylates.  The aim is to test this hypothesis by producing the complex for structural biology, protein interaction assay, and protein kinase assay.   The findings will have major implications for engineering industrial strains (PknG pathway is a validated target to improve productivity) and for developing new treatments for *M. tuberculosis* (PknG pathway is a validated target for drug development). Techniques that will be undertaken during the projectProtein structure determination by electron microscopy (Cryo-EM) and X-ray crystallography Membrane protein biochemistry Protein biophysical techniques to measure size, folding and interactions (including mass photometry, biolayer interferometry, intrinsic fluorescence) Molecular techniques: gene cloning, mutagenesis, genetic modification of bacteria for recombinant protein production and for phenotypic assay (gene knockout) Protein kinase assays  |
| **References** |
| An Aspartate-Specific Solute-Binding Protein Regulates Protein Kinase G Activity To Control Glutamate Metabolism in Mycobacteria (Bhattacharyya et al 2018) doi: 10.1128/mBio.00931-18.  |