EPSRC Centre for Doctoral Training (CDT) in Molecular Sciences for Medicine (MoSMed)

Sleeping superbugs: exploring functionality of key proteins in *C. difficile* spore formation

[Newcastle University, Biosciences Institute – Molecular Mechanisms of Life]

Supervisory Team	• **Dr Paula Salgado (Lead)** • **Dr Karrera Djoko** • **Dr Julia Hubbard**

Project overview/context

C. difficile is an opportunistic and highly antibiotic-resistant pathogen that relies on disruption of the gut microbiota to cause disease. We urgently need to develop species-specific antimicrobials that kill *C. difficile* without impacting the gut microbiota.
The infectious agent of *C. difficile* is a dormant cell form (spore) that is resistant to physical and chemical agents. Spore formation is a promising but unexploited target for new therapeutics but many functional details, particularly for early stages, are poorly understood. Using a multidisciplinary approach, we will explore the functionality of key proteins required for sporulation, opening new therapeutic avenues.

Research Project

In this project, we will to explore the functionality and enzymatic mechanism of key proteins involved in the early stages of formation of the spores. We will combine screens of library compounds with biochemical and biophysical characterisation of these proteins, in parallel with microbiological studies. The process of engulfment of the future spore by the mother cell during early sporulation involves several proteins and remodelling of peptidoglycan (PG), the main cell wall component. We have identified two essential enzymes and now aim to understand their functionality in more detail.
In the first part of the project, we will identify new binding sites in the two proteins of study by screening a library of small compounds – FragLites – developed by colleagues in Newcastle. These compounds have unique characteristics that allow identification of sites beyond catalytic motifs. Alosteric or specificity regions as well as potential interaction pockets are of particular interest. Initially, we will screen the compounds using protein crystallography, combined with isothermal calorimetry, surface plasmon resonance and nuclear magnetic resonance. The project also involves developing methodology to expand the usability and application of the FragLites hits which could then be applied to multiple projects, enhancing the widespread use of this library.

Once identified, we will design point mutations in these new sites to test their effect in protein function and structure using recombinantly expressed and purified protein. Mutations resulting in altered function will also be introduced in *C. difficile* to probe their role in sporulation. Finally, sites that alter sporulation of the bacteria will be tested in our animal disease models to understand the effects on disease transmission and recurrence.

In parallel, we will investigate the enzymatic mechanism of the enzymes. We are particularly interested in the metal requirements for activity. We will investigate the role of the metal-coordinating residues, as well as the metal specificity involved. Any potential effect of FragLite fragments on metal binding and/or requirements will also be screened.

Understanding the fundamental properties of the engulfasome is crucial and will open new therapeutic avenues for future development of C. difficile-specific drugs. Targeting spores will carry a reduced risk of development of new resistance mechanisms by the bacteria, decrease transmission and environmental

contamination, which would be of great societal benefit.

Training & Skills

The project will provide invaluable training in biochemical and biophysical techniques, structural biology as well as molecular biology and microbiology, which will be highly desirable for a future career in research into antimicrobial resistance (AMR), both from a fundamental and applied, translational perspective. AMR is an incresingly global problem and new therapeutics and approaches are needed. The proposed work will provide a particular focus on development and exploration of new compounds and methods to assess functionality and drive drug development.

You will join our multi-disciplinary team between Newcastle and Durham Universities, an inspiring and supportive environment for your PhD studies. At Newcastle, you will work alongside other students and postdoctoral scientists studying proteins involved in *C. difficile* sporulation as well as experts in structure-based drug discovery with many years' experience in industry. Work at Durham University with expert metalloprotein biochemists will complement your training. You will also have the possibility to access the state-of-the-art facilities at Diamond Light Source, the UK synchrotron.

Further Information

Dr Paula Salgado
paula.salgado@ncl.ac.uk
@pssalgado (Twitter)

How to Apply

You must apply through the University's online postgraduate application system

You will need to:

- Insert the **programme code 8207F** in the programme of study section
- Select '**PhD in Molecular Sciences'** as the programme of study
- Input (only) the **studentship reference code (e.g. 21_02)** that you are applying for in the studentship/partnership reference field when prompted (all codes are outlined in the individual project adverts found on the MoSMed website: https://research.ncl.ac.uk/mosmed/phdstudentships/).
- Attach all documents that are requested including a CV and cover letter. The cover letter must **clearly** state the project reference code, the full title of the studentship and set out how your interests and experience relate to the project
- Attach degree transcripts and certificates and, if English is not your first language, a copy of your English language qualifications
- Email: mosmed.cdt@ncl.ac.uk once you have submitted your application to confirm the project you have applied for. Please include the studentship reference code and full project title.

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