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

Mass spectrometry approaches to address capability gaps in drug discovery for membrane transporters

Newcastle University (Biosciences Institute)
Durham University
GlaxoSmithKline (GSK) Stevenage, UK

Supervisory Team	• **Prof Matthias Trost, Newcastle University (Lead)** • **Dr Maria Dueñas, Newcastle University** • **Dr Andrew Brown, GSK** • **Prof Steve Cobb, Durham University**

Project overview/context

Transporters are a large family of proteins which convey sugars, amino acids, and other solutes across biological membranes. Some drugs target this family but in general transporters are sparsely exploited for therapeutic agents. This project aims to address two capability gaps in transporter drug discovery by applying state-of-the-art mass spectrometry (MS) techniques to detect solutes directly with the aim for assay development for drug discovery. The student will develop cell-based assays for membrane transporter function and apply them in the context of drug discovery projects for novel inhibitors with the industrial partner. Secondly, the student will develop MS-based whole-cell binding assays for transporters and other membrane proteins.

Research Project

Membrane transporters regulate solute concentration across biological membranes, vital for homeostasis of cytoplasm and intracellular organelles. They are important targets of marketed medicines and drugs of abuse, and a common cause of drug-drug interactions (DDIs), though only a tiny proportion of this large and diverse family are targeted therapeutically. Recently, with the increasing statistical power of variant-to-phenotype association, multiple transporters have shown remarkable and unexpected association to human disease, suggesting this family holds significant promise for the development of novel therapeutics.

Mass spectrometry (MS) approaches are widely applied in the arena of drug screening, where "label-free" measurement is ideally suited to direct quantification of substrate/product for soluble enzymes. The same principle – direct measurement of ionisable solute – holds for characterisation of transporters. This project will adapt established protocols measuring intracellular analytes (Mateus et al (2017), DOI:10.1073/pnas.1701848114) to characterise transporter-expressing cells. The industrial partner, GSK, has four active early drug discovery programs on novel transporter targets; the student will initially focus on one of these. Using plasmid and baculovirus reagents supplied by the partner to express transporters in cell culture, methods will be developed to quantify transporter-mediated solute flux (cellular accumulation or efflux), utilising ^{13}C or other suitable tracers where necessary. Where non-proprietary small-molecule inhibitors are available, these will be used to show functional inhibition at the cellular level.

Having established initial methods, the student will complete a thorough mechanistic evaluation of transporter flux rates and substrate (or co-factor) dependence. Next, the student will spend 3-6 months at GSK (Stevenage, UK) where MS platforms have been adapted to higher-throughput (HT; Rapidfire-MS, Echo-MS and MALDI-MS). Methods established at Newcastle University will be adapted onto HT platform(s) to generate data in quantity. A key objective will be to compare MS to alternative transporter assays. Finally, the student will apply mutagenesis to develop mechanistic models for protein conformational changes driving solute transport and, where appropriate, inhibitor binding sites. These will be facilitated either by

homologous published structures or structure-determination efforts ongoing at the partner.

A secondary objective of the project will be to evaluate MS applications to cell-based binding for target engagement assays. These approaches, which have just started to appear in the literature (Chen et al (2022) https://doi.org/10.1016/j.slasd.2022.08.005), rely on high-affinity probes to cell-surface proteins. The method is applicable to transporters but also GPCRs and ion channels: low temperature "freezes" probes in the cell-bound state and washing and probe detection by high sensitivity-MS forms the basis of a competition-binding assay.

In summary, this project aims to address two significant capability gaps the application of MS in drug discovery: 1) functional cell-based assays for inhibition of membrane transporters and 2) cell-based binding assays for membrane proteins. The student will interact with scientists from diverse disciplines within active drug discovery projects and impactful publications should be achievable from both themes.

Further Information

Please contact Prof Matthias Trost for details: matthias.trost@ncl.ac.uk

How to Apply

If applying to a **Newcastle project**, you must apply through the University's **Apply to Newcastle Portal**. Once registered select **'Create a Postgraduate Application'.**

Use 'Course Search' to identify your programme of study:

- search for the 'Course Title' using the programme code: **8207F**
- select **'PhD Molecular Sciences for Medicine (SNES)'** as the programme of study

You will then need to provide the following information in the 'Further Questions' section:

- a 'Personal Statement' (this is a mandatory field) - upload a document or write a statement directly into the application form. Please include the full title of the studentship, the studentship code, and how your interests and experience relate to the project.
- the relevant studentship code **(mos23_10)** in the 'Studentship/Partnership Reference' field. If you wish to apply for additional studentships, please make sure to add the relevant studentship reference each time, before submitting each separate application. For example, you may wish to apply for mos23_10 AND mos23_11. **You must include the relevant code for your application to be considered.**
- when prompted for how you are providing your research proposal - select 'Write Proposal'. You should then type in the title of the relevant research project. You do not need to upload a research proposal.
- An up to date CV.
- Please upload all documents in PDF format.

Training & Skills

The student will receive training in a wide range of techniques, including mass spectrometry on a wide range of instruments, cell culture, biochemistry, molecular biology and medicinal chemistry.
Within the CDT, the student will have a number of programmes available to learn new "soft" skills, including a taught training programme and the opportunity to complete a "mini-MBA".

Equality, Diversity and Inclusion (EDI)
Within the MoSMed CDT we are committed to building a diverse community based on excellence and commitment. To that end in our recruitment of Doctoral Researchers we welcome applications from outstanding candidates of all backgrounds regardless of ethnicity, disability, gender identity, sexual orientation and will consider all applications equally based on merit.

Should you have any queries regarding the MoSMed application process to Newcastle University please contact Craig Hinds, the MoSMed CDT Manager: **mosmed.cdt@newcastle.ac.uk**

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