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

Translating fragment maps to medical impact

Translational and Clinical Research Institute, Newcastle University and Department of Chemistry, Durham University

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Project overview/context

Crystallographic fragment screening provides a "map" of interaction hotspots on a protein surface and provides leads for subsequent chemical probe and drug discovery. This studentship will be employed to define a playbook for how fragment-binding "maps" of a protein family can be exploited to define biological mechanisms and actionable approaches to selective protein inhibition. Our chosen target family is the cyclins, key activators of protein kinases that regulate gene expression and the eukaryotic cell cycle and are possible targets for drug discovery.

Research Project

The streamlining of crystallographic fragment screening enables structural biology to seed the dissection of the molecular basis of disease via rapid development of chemical probes. Realizing this opportunity requires associated technologies to be enhanced, ranging from imaging and assays for validating binding events to applying structural information and computational tools to analyze the fragment "maps" and convert them into synthetically achievable molecules suitable as probes in cellular assays. This project leverages an existing partnership with DLS, to develop the most useful downstream experimental, synthetic and computational techniques into a coherent strategy that can be deployed to efficiently develop tool compounds to assist in target validation.

Cyclin-dependent kinases (CDKs) are proven actionable targets in diverse disease conditions. Primarily mediated by the cyclin, the CDK-cyclin module is incorporated into complexes of diverse composition that govern cell-type specific activities. Cyclins are structurally conserved but diverse in sequence and offer an opportunity to develop highly selective inhibitors. We have used an in-house developed library of fragments, termed FragLites, to generate comparative fragment maps of cyclins A, K and T. These maps reveal multiple "hotspots" distributed over the cyclin surfaces. Encouragingly, certain fragment-binding hotspots overlap with sites of known interaction partners while others are novel and may identify sites for as-yet unknown binding partners. These three rich datasets represent the first opportunity to evaluate FragLites as tools to dissect the distinguishing features of members of a protein family and are a starting point to develop cyclin-specific probes.

The research project is structured to first expand the XChem (fragment) / FragLite cyclin dataset. Cyclin A and cyclins T and K regulate respectively the cell cycle and transcription. To help elaborate their conserved and distinguishing features and direct inhibitor development, fragment datasets will be collected for cyclins B and H (a cell cycle and transcriptional cyclin respectively). These cyclins have been selected as they are tractable to structural studies and as partners respectively of CDK1 and CDK7, they regulate CDKs undergoing validation as cancer drug targets.

The second objective is to develop computational tools, in collaboration with Professor Frank von Delft (DLS), to analyze the fragment "maps" and convert them into synthetically achievable molecules. Robust protocols are required to deduce libraries of targets for synthesis downstream of fragment campaigns that are compatible with manual and robotic approaches. This work will benefit from a partnership between CRUK and DLS to maximize the impact of both organizations in translating basic science into improved health outcomes in the area of structure-based drug discovery.

To validate these computational approaches, elaborated fragments designed to bind to at least three proposed cyclin target sites will be selected based on their potential to provide functional insight for first stage

fragment elaboration/fragment-merging campaigns. Imaging and orthogonal biophysical assays will then be applied to confirm the relevance of crystallographic fragment hits to solution-state binding. The project will benefit from established biophysical platforms within the groups at Newcastle and Durham, and access to cell-based approaches at Newcastle. Taken together, they will enable the utility of different approaches to site validation to be explored.

Training & Skills

This studentship will provide an opportunity to gain experience in fragment-based drug discovery and work towards optimizing the technologies and developing the workflows to streamline downstream fragment development. Training will be multi-disciplinary at the interface between the biological, chemical and structural sciences, in areas that are fundamental to driving our understanding of drug development and disease at the molecular and cellular level.

There will be an opportunity to acquire skills in molecular biology to develop and optimise heterologous (E. coli and insect cell) protein expression systems. Training in chromatographic techniques to generate proteins suitable for biochemical and biophysical assays and structural studies to support chemical probe development will be provided. Established biophysical and imaging techniques include surface plasmon resonance (SPR) isothermal titration calorimetry (ITC) and homogenous time-resolved fluorescence (HTRF) at Newcastle, and differential scanning fluorimetry (DSF) at Durham. Structure determination by X-ray crystallography will identify and verify Fraglite binding and by cryo-EM will characterise multiprotein complexes. Mass spectrometry will be used together with other biophysical techniques to emphasise the importance of orthogonal experimental validation.

Skills in computation and data science will be developed to explore the utility of fragment campaigns as starting points for the design of allosteric protein-protein interaction inhibitors.

A key training, however, will be in the development of innovative science and problem-solving approaches to address a major bottleneck in the generation of novel small molecule therapeutic agents.

The nature of the data will require excellence in various quantitative skills, for example in analysis of assay data, and of X-ray and cryo-EM data sets. The student will be part of a large, highly collaborative team. Regular project meetings will provide opportunities to train in presentation skills and manage collaborations. We work closely with colleagues in other academic units and in industry providing an opportunity to experience best practices from academia and industry.

Further Information

Please contact Jane Endicott:
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How to Apply

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

When applying to Newcastle University please select the Course Code **8207F (PhD in Molecular Sciences)** 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. 22_06)** that you are applying for in the studentship/partnership reference field when prompted (all codes are outlined in the individual project adverts and can be 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 state 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

Should you have any queries regarding the application process to Newcastle University please contact Selina McCarthy, MoSMed CDT Manager: Selina.McCarthy@newcastle.ac.uk or email mosmed.cdt@newcastle.ac.uk

Within the MoSMedCDT 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.

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