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

Developing novel chemical probes to interrogate the allosteric mechanism of MTHFR and explore its therapeutic potential

Newcastle University, Chemistry

Supervisory Team	• **Dr Celine Cano (Lead), Newcastle University** • **Professor Wyatt Yue, Newcastle University** • **Professor Ehmke Pohl, Newcastle University**

Project overview/context

The folate and methionine cycles are critical metabolic pathways for cell survival. The enzyme MTHFR represents a key regulatory connection between these cycles, hence exerting a strong influence on an array of diseases. Loss or reduced MTHFR function results in a life-threatening metabolic disorder called homocystinuria whilst on the other hand, overexpression of MTHFR has been observed in certain cancers (e.g. prostate).
We propose to develop proof of concept chemical probes to modulate MTHFR and explore the therapeutic potential of both, inhibiting and activating the enzyme**.** The studentship will provide a wealth of experience in medicinal chemistry design and organic synthesis. This would be ideal training for someone wishing to pursue a career in medicinal chemistry or chemical biology.

Research Project

Background

Previous work in W. Yue's team determined the crystal structure of human near-full-length MTHFR (Figure 1).

Following screening of SAM mimetics against the MTHFR regulatory domain, 4 compounds were found to bind to the allosteric domain, some (e.g. (S)-SKI-72) inhibiting the enzyme in a similar manner as SAM (Figure 2).

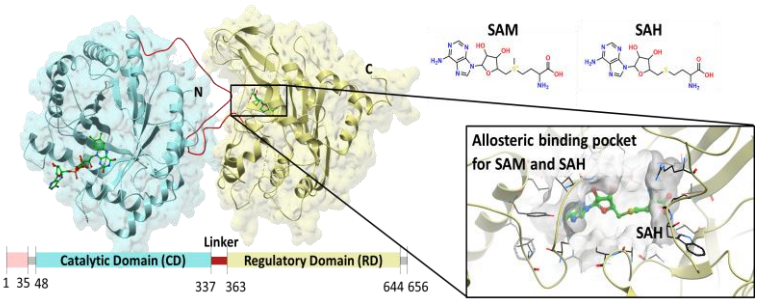

Figure 1. Crystal structure of human MTHFR, featuring a regulatory domain with novel SAM/SAH-binding pocket.

Research Plan

Through chemical synthesis, coupled to biophysical, structural and cellular characterisation, we will **develop novel chemical probes to the study and manipulation of MTHFR activity** and provide a means to evaluate both, inhibition and dis-inhibition routes for potential therapeutic applications.

1 - Development of allosteric modulators of MTHFR (collaboration with W. Yue and E. Pohl)

The student will tailor the starting hits of MTHFR to become more potent, selective and cell-permeable. The student will synthesise derivatives of (*S*)-SKI-72 and the other hits by initially retaining the adenosyl moiety and focus on introducing modifications on the side chain. In collaboration with E. Pohl, *in silico* design of compounds will explore several substructures off the chemical backbone. Once compounds have been synthesised, biophysical (activity assay, SPR) and structural (x-ray,

cryo-EM) characterisation will be performed in collaboration with W. Yue.

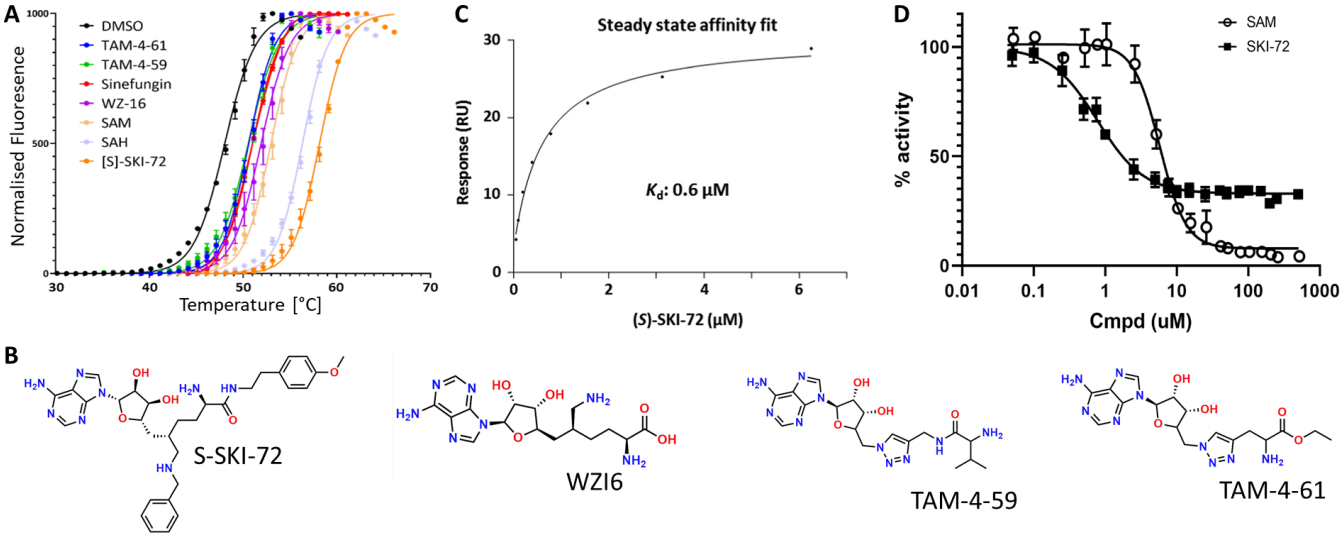

Figure 2. Screening of MTHFR by DSF (A) against a library of SAM analogues yielded four hits (B) which were validated by SPR (C) and activity assay (D).

For the most promising compounds, endogenous activity will be measured in HEK293T cell lysates expressing wild-type or genetically modified MTHFR, to assess in-cell potency (collaboration with Dr. Sean Froese, Children's Hospital, Zurich).

2 - Fragments that inhibit MTHFR

The mapping of MTHFR binding sites by screening the Newcastle FragLites library will also be explored with a view to provide an invaluable proof-of-concept to assess MTHFR druggability and establish whether MTHFR inhibition/activation is tractable with a fragment-based approach. FragLites are a set of halogenated compounds expressing paired hydrogen-bonding motifs that are able to identify productive drug-like interactions within a protein.

X-Ray screening of the FragLites library will be conducted in Yue's group and the **follow-up development of hit compounds will be carried out by the student (medicinal chemistry).**

Training & Skills

The student will be based in a dynamic multidisciplinary drug discovery and translational research environment. The Newcastle Medicinal Chemistry Group is a fully integrated drug discovery group, consisting of 30 researchers. The group hosts regular group meetings to discuss progress, as well as medicinal and synthetic chemistry literature reviews. We also hold monthly multidisciplinary project reviews at which the student will be expected to present results to colleagues in Biosciences. Our laboratories house state of the art equipment, including a dedicated 500MHz NMR spectrometer, modern microwave synthesisers, automated chromatography, preparative HPLC and an Agilent 6550 iFunnel QTOF mass spectrometer. Each year, the PhD student will also undertake short (circa 2 months) placements within the Structural Biology/Biophysics labs (Yue, Pohl)) to provide further multidisciplinary training.

Further Information

Please contact Lead Supervisor for project enquiries, celine.cano@ncl.ac.uk

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_03)** 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/)

Newcastle University · Durham University · UKRI Engineering and Physical Sciences Research Council

- 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.

Newcastle University Durham University UKRI Engineering and Physical Sciences Research Council