Mass Spectrometry Imaging of Enzyme Activity in Brain and Beyond

Supervisory team:
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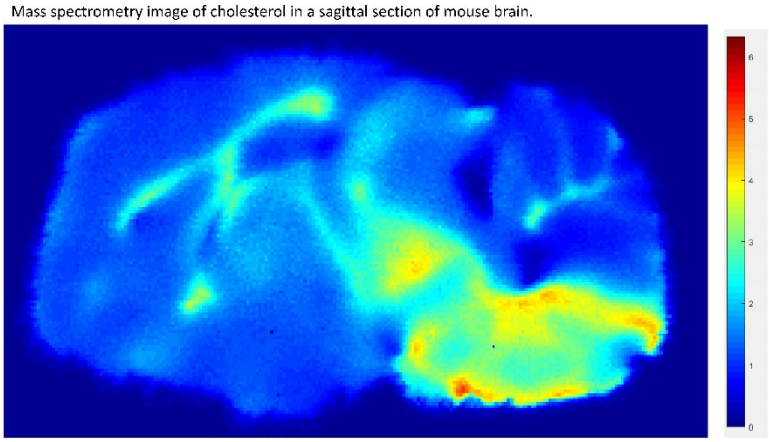
Host institution: Cardiff University, Swansea University

Submit applications for this project to Cardiff University


Mass spectrometry image of cholesterol in a sagittal section of mouse brain.

Project description:

The brain is the most cholesterol rich organ in the body. Despite this, details of cholesterol metabolism in brain are poorly understood. Defects in brain cholesterol metabolism are linked to disorders of aging, so the study of brain cholesterol is of wide interest both academically and to industry. The major enzyme that metabolises cholesterol in brain is the cytochrome P450, CYP46A1. CYP46A1 will also metabolise other compounds (e.g., cholesterol precursors) but very little is known about its activity towards different substrates in vivo.

In this project we will use mass spectrometry imaging (MSI) to determine how the activity of CYP46A1 varies towards different substrates to give different products according to spatial location in brain. We will perform these studies in mouse and investigate how spatial cholesterol metabolism varies during development from the foetus to newborn to adult to old adult. The results will not only inform about the rate of cholesterol turnover and transport between different regions of brain, but will also define the rate of production of biologically active metabolites e.g., modulators of neurotransmitter receptors (i.e., NMDAR, sigma-2) in different regions, and how cholesterol metabolites involved in development vary from the foetus to newborn to adult. Although we will initially target cholesterol metabolism by CYP46A1 in brain, the mass spectrometry imaging method will also be applicable to determining the activity of other CYP enzymes in brain and in other tissues e.g., liver, and will also be applicable to other enzymes families.

To complement the mass spectrometry imaging work, we will express and purify CYP enzymes, and investigate substrate specificity by determining the binding affinity of substrates (substrate binding constant (Ks)) and the turnover rate (catalytic constant, Kcat) in reconstituted cell-free enzyme assays including P450 oxidoreductase (POR). Products will be determined by mass spectrometry. Possible inhibitors and activators (including allosteric activators) will also be investigated as these may have potential therapeutic values. Importantly disturbed CYP46A1-mediated metabolism is implicated with disorders of aging and is linked with neuropsychiatric conditions.

Our aim as the SWBio DTP is to support students from a range of backgrounds and circumstances. Where needed, we will work with you to take into consideration reasonable project adaptations (for example to support caring responsibilities, disabilities, other significant personal circumstances) as well as flexible working and part-time study requests, to enable greater access to a PhD. All our supervisors support us with this aim, so please feel comfortable in discussing further with the listed PhD project supervisor to see what is feasible.