MRC Research Experience Placements Summer 2025

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- **Siddharth Banka** - Analysis of genomic variants in patients with unsolved genetic disorders.

T cell invasion in 3D cancer spheres

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Being able to engineer T cells to invade cancers will enhance their ability to suppress tumour growth. 3D in vitro models for measuring invasion will expedite the evaluation of strategies for increasing the invasiveness of T cells The aim of the project, therefore, is to assist a graduate student to develop models for evaluating T cell invasion in 1) 3D matrices and 2) into spheroids comprising established cancer cell lines.

1) Transwell inserts will be coated with a layer of collagen and inserts filled with solution containg T cells. T cell invasion into a lower chamber containg chemokine medium will then be ascertained using counting beads and flow cytometry.

2) Cancer spheroids will be formed in ULA plates and then transferred into collagen gel containing T cells stained with vital dye. The spheroids will then be washed and the fluorescence they contain quantified on a plate reader and imaged with a fluorescent microscope.
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Identification of novel hypoxia therapeutic targets in muscle-invasive bladder cancer.

Name of Supervisor: Conrado Guerrero Quiles
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Muscle invasive bladder cancer (MIBC) is a poor prognosis (21% 5-year overall survival) prevalent disease with >130,000 new diagnoses yearly worldwide. Radiotherapy and cystectomy are both standard-of-care treatments with similar outcomes. Radiotherapy allows bladder preservation, but hypoxia (<2% O_2) promotes radioresistance. Hypoxia is a common poor prognostic feature in cancer. In MIBC, 70% of patients express hypoxia markers. Hypoxia-modifying treatment (carbogen and nicotinamide [CON]) is the UK standard of care for MIBC, highlighting the therapeutic potential of targeting hypoxia. However, CON only temporarily increases tumour oxygenation. New targeted treatments are therefore necessary to improve the current hypoxia standard-of-care. Plasma membrane proteins (PMPs) are critical in cellular signalling and response to hypoxia. PMPs are also key to drug development due to their accessibility, comprising two-thirds of all protein-based drug targets.

Here, we will use proteomics to characterise the membrane protein composition of MIBC cells *in vitro*. Proteins will be extracted using the Pierce™ Cell Surface Biotinylation and Isolation Kit (Thermo Scientific, MA, USA), and then analysed by the University of Manchester proteomic facility. Identified proteins will be further confirmed by Western blotting and immunofluorescence. Finally, targets will be retrospectively validated *in silico*

using available internal (radiotherapy) and public (cystectomy) MIBC cohorts with outcomes and transcriptomic data (TCGA-BLCA, n=401; BCON, n=151; Christie-GemX=184)

The student will receive training in key molecular biology techniques, including: cell culture, protein extraction, western blot, and immunofluorescence. Additionally, training in RStudio will be provided to perform basic retrospective validation analyses. This project will be supported by Faris Alazani and Ammar Sharif (final-year PhD student) PhD projects. Faris and Ammar have expertise in the topic and routinely perform the techniques described in this proposal. Finally, the project will be allowed flexibility to support the student's development and research interests.

Air Pollution and the heart.

Name of Supervisor: Holly Shiels
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The mechanisms of air pollution-induced cardiotoxicity are complex and can be attributed in part to particulate matter that contain poly aromatic hydrocarbons (PAHs)[1]. Phenanthrene is the primary PAH in air pollution and is formed during the combustion of fossil fuels. It is highly lipophilic and cardiotoxic impairing both contractile and electrical activity of the mammalian heart [2]. We have recently shown that phenanthrene, slows heart rate and prolongs the cardio-electrogram (ECG) in mice when applied acutely, leading to a range of arrhythmias [3]. We have new evidence that this pro-arrhythmic phenotype also occurs in mice chronically exposed to phenanthrene and is worse in aged mice compared with young mice. Finally, we have shown that the mechanisms underlying these arrythmias are due at least in part to phenanthrene inhibiting sodium, calcium and potassium ion channels in the heart [2,3]. Thus, our findings to date indicate that exposure to realistic levels of this air pollutant causes major cardiac dysfunction in mice and may underly the incidence of cardiac dysfunction in humans living in areas of high air pollution globally.

The aim of this 6-week project is to investigate inflammation of cardiac, gut and liver tissue using histopathology and immunohistochemistry from the the young and old mice from this set of experiments (under control conditions and following chronic phenanthrene exposure).

The student will learn skills in histology and immunohistochemistry and be able to recognise markers of inflammation and immune cell infiltration in 2 key target organs for air pollution – the heart and liver. The student will work closely a PhD student, and as part of a larger team investigating PAH cardiotoxicity.

[1] Marris CR, Kompella SN, Miller MR, Incardona JP, Brette F, Hancox JC, et al. Polyaromatic hydrocarbons in pollution: a heart-breaking matter. J Physiol 2020 Jan;598(2):227-47.

[2] England E, Morris JW, Bussy C, Hancox JC, Shiels HA. The key characteristics of cardiotoxicity for the pervasive pollutant phenanthrene. Journal of Hazardous Materials. 2024 May 469:133853.

[3] Yaar S, Filatova TS, England E, Kompella SN, Hancox JC, Bechtold DA, Venetucci L, Abramochkin DV, Shiels HA. Global air pollutant phenanthrene and arrhythmic outcomes in a mouse model. Environmental Health Perspectives. 2023 Nov 1;131(11):117002.

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Brain-Specific Spatial Transcriptomics: Multimodal Benchmarks and Disease Model.

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Background:

Research in spatial transcriptomics has advanced significantly, enabling high-resolution gene expression analysis in tissues. However, brain-specific spatial transcriptomics faces a critical shortage of benchmark datasets that account for the brain's unique architecture. While general spatial transcriptomics datasets exist, they lack comprehensive multimodal data integration across brain regions and conditions, particularly in combining MRI, histology, and transcriptomics data. Additionally, current models predominantly focus on normal brain structure, with limited adaptation for neurological diseases.

Project Description:

This project addresses the lack of brain-specific spatial transcriptomics benchmarks and develops disease-adaptive multimodal models. We will curate a comprehensive benchmark dataset tailored to brain research, integrating transcriptomics, histological data, and MRI scans across various brain regions in both healthy and diseased states. Building on this dataset, we will develop multimodal models capable of detecting molecular and spatial changes related to neurological conditions.

Aims and Objectives:

1. Create a multimodal brain-specific benchmark dataset integrating spatial transcriptomics, MRI, and histological data across diverse brain regions in both healthy and pathological states.

2. Develop and validate disease-specific models (e.g., Alzheimer's, Parkinson's, and brain cancers) for detecting molecular and spatial alterations associated with neurological conditions.

3. Build a standardized evaluation framework enabling consistent assessment of brain-specific spatial transcriptomics models.

Student Involvement and Skill Development:

Each student in this project will focus on one of the objectives described earlier. For example, a student working on Objective 1 will curate, preprocess, and integrate multimodal datasets to create a brain-specific benchmark dataset. A student focusing on Objective 2 will collect and validate existing machine learning models tailored to specific neurological conditions. For Objective 3, the student will design a standardized evaluation framework to ensure consistent and reliable assessment of brain-specific machine learning models.

Through this work, students will acquire specialized skills in computational techniques and machine learning algorithms for spatial transcriptomics data. Additionally, they will gain expertise in dataset curation, algorithm development, and evaluation framework design, alongside essential skills in scientific communication and interdisciplinary collaboration.

Ferroptosis and small airway disease in COPD

Name of Supervisor: James Baker
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Background

Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of mortality and is characterised by progressive airflow limitation, inflammation and small airway remodelling. We have shown alveolar macrophages have increased iron levels in COPD. Increased cellular iron is a hallmark of ferroptosis, a type of cell death triggered by lipid peroxidation and iron accumulation. Others have demonstrated the importance of iron overload in COPD pathogenesis in driving ferroptosis in mouse models and *in vitro* studies. Iron and lipid peroxidation (ferroptosis marker) have been observed, but not quantified, in COPD epithelial cells. Small airway disease is known to be an early indicator of COPD pathogenesis, however, the relationship between small airway disease and ferroptosis has not been assessed. Further work is needed to characterise patient phenotypes that may be responsive to ferroptosis-targeting therapeutics.

Aim: Investigate the relationship between ferroptosis and small airway disease in COPD.

Objectives:

- Quantify small airway epithelial lipid peroxidation levels in Non-smokers, Smokers and COPD patients.
- Assess small airway disease measurements relationship with lipid peroxidation

Study design

Archived resected lung tissue sections from COPD patients (n=30), Smokers (n=20) and Non-smokers (n=10) will be stained with a 4-HNE antibody to assess lipid peroxidation. The association of lipid peroxidation to small airway disease measurements (airway diameter, epithelial area and small airway attachments) will be assessed. Smoking status and disease severity (GOLD stage I-III) will be investigated to assess if ferroptosis is associated with early-stage disease and control for effects of smoking status.

The candidate will learn key laboratory skills in the discipline of lung histopathology, including staining of human lung tissue, light microscopy, identification of microscopic lung architecture, image acquisition and analysis, and data handling with statistical analysis.

This research will help generate novel understanding of cell-specific roles in ferroptosis and their association with COPD disease mechanisms

Applying an in vitro model to understand adipose-liver communication in obesity
Name of Supervisor: Louise Hunter
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Introduction: Obesity is a multi-organ disease. In health, fat cells (adipocytes) act as a site for lipid storage and release. Adipocyte dysfunction in obesity is associated with the development of obesity-related pathologies in other organs, including the liver (e.g. steatosis and inflammation), which are the cause of significant human morbidity.

Aim of project:

- This project aims to determine whether an *in vitro* model can be used to mimic the effect of adipocyte lipid overload on liver cells, either through the use of co-culture or conditioned media techniques.

Learning objectives:

- The project will equip the student with widely-used laboratory skills (e.g. cell culture, RNA extraction, RT-qPCR, protein extraction, Western blotting) which should be valuable for future postgraduate research.
- The project will provide the student with experience in experimental design, data analysis and presentation.
- The project will also provide the student with an opportunity to be embedded in an active research laboratory team, with the option to shadow researchers undertaking a variety of experimental techniques, and thus broaden their research experience.

Proposed plan of work:

- We will use the mouse 3T3 L1 MBX pre-adipocyte and AML12 hepatocyte cell lines. The student will learn how to differentiate 3T3 L1 MBX cells into adipocytes, and will treat these will insulin, glucose and fatty acids to drive maximal lipid storage.

- The student will then employ co-culture or conditioned media techniques to apply adipocyte-derived signals to AML12 hepatocytes.
- The student will assess the effect on hepatocyte biology through quantifying expression (gene and/or protein) of key inflammatory and metabolic markers, and quantifying hepatocyte lipid content with Oil Red O staining. These outcomes can be compared with what is published in the literature about liver pathology in obesity, to determine whether this *in vitro* model is a valuable tool.

Testing the safety of Channelrhodopsin optogenetic therapies.

Name of Supervisor: Nina Milosavljevic
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Loss of photoreceptors, caused by inherited or acquired outer retinal degenerations, is an irreversible, and currently incurable, cause of severe loss of vision. Restoring photosensitivity to the surviving inner retina is a viable strategy for providing some recovery of vision function. The new exciting biological approach of optogenetic therapy achieves this by viral delivery of photosensitive proteins (photopigments) to remaining cells in degenerated retinas. Following promising results in mice, clinical trials employing microbial-type light-gated ion channels

(channelrhodopsin-2 (ChR2) variants e.g. ChrimsonR) as photopigments for optogenetic therapy are currently underway. While these photopigments restore visual responses in acute experiments in mice with retinal degeneration and have shown efficacy in early-stage clinical trials, questions remain over long-term efficacy and safety. In our lab, we have data suggesting that long-term viability is a concern for ChR2 and its variants because, as non-selective cation channels, they seem to cause great physiological stress to cells by impacting ion balance. One particular concern, which will be investigated during this summer research placement in our laboratory, is the calcium conductance of ChR2 which is known to trigger apoptosis in cells.

Aim: Identify how targeted cells expressing ChR2 cope with their non-selective ion flux, in particular, Ca^{2+}

Objective 1. Assess viability of cells expressing ChR2 in cell culture.

Objective 2. Measure intracellular Ca^{2+} concentrations during the light stimulation of ChR2 in cell culture.

The student will join a young and enthusiastic group and contribute to the characterisation of the implications of long-term ChR2 actions for ionic balance and cell viability in cell culture. The student will receive training in a range of cutting-edge techniques for live-cell imaging and cell viability assays. We anticipate that the successful research placement will

lead to a dataset that will be part of the ongoing project in the group and a planned publication.

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Image-based computational modelling to unravel structure-function relationships in the human placenta.

Name of Supervisor: Richard Mcnair
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Two million babies are stillborn every year. In addition to personal loss, stillbirth has wider costs and negative effects for society. This placement is part of the Manchester-led international research project "Multi-modal studies to understand pregnancy and prevent stillbirth", which itself is part of Wellcome Leap's *In Utero* program, an ambitious program driving translational research to reduce global rates of stillbirth. The project aims are to identify clinically important structure-function relationships in the human placenta through a range of medical imaging and measurement during gestation and *ex vivo* placental imaging and histology, with an end-goal of developing a digital twin of pregnancy for the sensitive detection of stillbirth risk.

The aims of this project are:

1) To develop mathematical models of blood flow and solute exchange (particularly oxygen) using structural information extracted from state-of-the-art 3D *ex vivo* imaging
2) To use these models to characterise the structure-function relationships in different tissue samples representing a range of placental pathologies linked to risk of stillbirth
3) To suggest candidates for new structural biomarkers

The student will be working with data from Synchrotron-Computerised-Tomography scans of placental tissue, which gives high-resolution measurements of the complex structure of the placenta. The project will consist of applying principles from fluid mechanics and transport to characterise the (in)efficiency of oxygen exchange in these samples. This will involve learning to use specialised computational software, such as COMSOL Multiphysics and Avizo. The student will also gain experience in analysing complex data and modelling outputs.

The outcomes of this analysis at the microscale will feed into macroscale models used by collaborators across the *In Utero* program. Therefore, the student will also gain experience working in a large multi-disciplinary team and develop their communication skills through interdisciplinary knowledge exchange.

Understanding reactivation of neurogenesis during Spinal Cord Regeneration.

Name of Supervisor: Ximena Soto Rodriguez
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Mammals, including humans, cannot replace lost neurons after spinal cord injury (SCI), whereas zebrafish can regenerate neurons to replace those that are lost after injury. Upon injury, neural stem cells initiate proliferation and generate new neurons in species with regenerative capacity, but in mammals, only cells that contribute to scar formation are generated.
My previous research has shown that during development the generation of new neurons (neurogenesis), genes are not simply on or off. Instead, the levels of some genes pulse dynamically over time (ultradian oscillations), influenced by specific factors, and control whether the cells decide to stay as proliferating neural stem cell or become new neurons. It is known that the same genes are important during SC regeneration but how they work isn't known. Therefore, it is not clear whether during SC regeneration and the generation of new neurons are also controlled by the pulses in gene activity. **Elucidating the dynamic signals and mechanisms leading to successful SC regeneration in an animal with regenerative capacity will generate important and valuable outcomes that can be tested in higher organisms**.
Zebrafish is a powerful, tractable and robust animal model able to achieve functional neural regeneration following SCI, characterised by de novo neurogenesis and regrowth of neuronal connections. Taking advantage of its regenerative capacity and its amenability to genetic manipulation you will be using larvae zebrafish as an experimental model.

You will:

(1) Use state-of-the art live imaging technique to observe gene pulsatile expression in real time upon SCI.

(2) Investigate how changes on gene dynamic expression can affect cell-fate decisions during SC regeneration.

Addressing the functional importance of pulsatile gene activity during cell-fate decisions in SC regeneration, will provide insights with potential translational implications for SC injuries in animals with no regenerative capacity, such as mice and humans.

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Towards development of novel anti-cancer agents against oncogenic microRNAs: synthesis and assessment of their binding and catalytic activities.

Name of Supervisor: Elena Bichenkova
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SIGNIFICANCE AND POTENTIAL IMPACT. A major biomedical challenge is highly-selective therapy against abnormal gene expression in disease states (*e.g.* cancer, inflammation) where combination therapies, including comparatively toxic drug cocktails, are otherwise indicated. Novel therapeutic strategies for selective treatment of disease states can be facilitated by targeting of upstream cellular components (*e.g.* messenger RNA, small non-protein-coding RNAs) to achieve controlled translational arrest of pathogenic proteins and

thus trigger a desired therapeutic response. Indeed, short functional non-coding microRNAs are implicated in many types of cancer, and thus can be used as biological targets for development of more selective and powerful anticancer therapies to allow minimising adverse drug reaction and severe toxicity.

AIMS OF THE PROJECT. This project will be focused on synthesis of the peptidyl-oligonucleotide hybrids to achieve selective targeting of oncogenic microRNAs (*e.g.* miR-21, miR-17, miR-155) with abnormal expression profiles in cancer. These chemically engineered RNA-targeting molecules will be generated by conjugation of short, catalytically inactive peptides with DNA recognition motifs to produce novel biologically-active molecules capable of recognising and cleaving disease-relevant microRNAs.

SUMMER PLACEMENT SPECIFIC OBJECTIVES

During this summer placement, the recruited student will:

(a) be introduced to the core expertise in supramolecular chemistry for site-directed functionalisation of peptides and oligonucleotides, developed in the host group;
(b) learn techniques for their post-synthetic conjugation;
(c) receive training in analytical characterisation of the synthesised bioconjugates by HPLC, UV and NMR spectroscopy;
(d) be exposed to the fluorescence-based screening methods to monitor cleavage of oncogenic microRNAs.

TRAINING THAT WILL BE PROVIDED TO THE STUDENT

This cross-disciplinary project will be carried out at the interface of structural biology, cancer research, supramolecular and analytical chemistry. The training will encourage the recruited student to appreciate collaborative and coordinated multidisciplinary approaches necessary to achieve a deep, integrated understanding for addressing biological grand challenges. The bioanalytical and quantitative skills in HPLC, NMR, UV and fluorescence detection, acquired during the training, will be essential for the future experimental design and data analysis in order to provide solutions to complex biomedical and clinical problems.

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Radiation-Induced Oxidative Stress and Microvascular Damage in Human Hair Follicles
Name of Supervisor: Talveen Purba / Matt Harries
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Hair loss is a distressing and potentially permanent consequence of life saving cancer-treating radiotherapy, however both therapeutic interventions and research into damage mechanisms are limited, especially within clinically relevant human tissue models. Our group is developing a novel model for radiation-induced alopecia using human hair follicles and is using this unique opportunity to learn more about how exactly radiation damages the follicle to cause hair loss. The successful student will join the Manchester Hair Research

Group to conduct a project that will specifically investigate how oxidative stress, induced by radiation, impacts the small blood vessels that provide nutrients to the hair follicle. To achieve this, immunohistochemistry parameters for oxidative stress markers (e.g. 8-OH-dG, 4-HNE, GSH and PRDXs) will be systematically examined within CD31+ endothelial cells on tissue slices obtained from human hair follicles subject to radiotherapy ex vivo. The student will be trained in relevant laboratory-based approaches (i.e. immunostaining, fluorescence microscopy) as well as quantitative analysis and critical interpretation of research data.

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Analysis of genomic variants in patients with unsolved genetic disorders
Name of Supervisor: Siddharth Banka
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Rare genetic disorders affect up to 5% of the population and cause a substantial burden to patients and their families. Genome sequencing is used to diagnose rare genetic disorders as standard of care in England. However, more than 50% of patients that undergo genome sequencing remain undiagnosed. We hypothesise that a subset of unsolved cases is due to variants which are challenging to identify from the sequencing data or due to variants with impacts that are difficult to predict. The overall aim of this project is to improve the diagnosis of rare diseases through the analysis of sequencing data. This aim will be achieved through the following objectives:

1. Use bioinformatic tools to prioritise a list of disease-causing rare genetic variants from a cohort of undiagnosed patients.
2. Use available clinical data to perform genotype-phenotype correlation analysis

Expected research outcomes: Complete the analysis of a set of rare variants, which will facilitate new diagnoses and/or the identification of novel diseases.

Expected learning outcomes: Exposure to a range of bioinformatic tools to analyse genomic and clinical data.