Bioscience for Health Project List for 2024 Recruitment

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How are some human skeletal muscles spared from atrophy-inducing signals?

Project Supervisor: Bethan Phillips

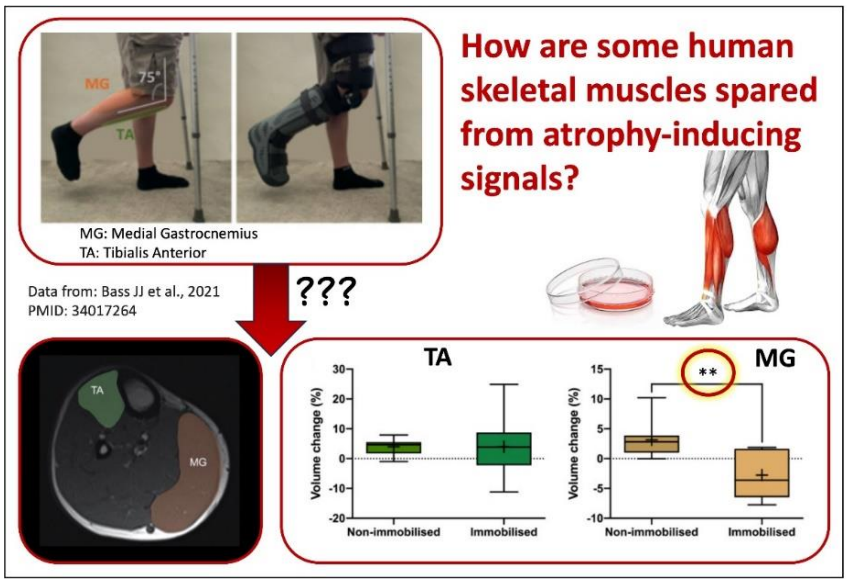
School: Medicine

Description: Existing resources facilitate rapid productivity in terms of investigating the myogenic features of stem cells derived from skeletal muscle biopsies from muscles exhibiting atrophy resistance vs. atrophy susceptibility. This is an entirely novel paradigm created by a recently completed BBSRC grant to the PI, and as such, has no competing molecular investigations in the literature. Using established techniques in the host lab, we would culture and explore pre-existing (BBSRC-funded) CD56+ stem cells from the medial gastrocnemius (MG; atrophy susceptible) and tibias anterior (TA; atrophy resistant) in respect to myogenic features and anabolism/catabolism.

In a realistic project in the context of 9-weeks, we hypothesise that exposure of these distinct TA/MG-derived muscle cells to a factor inducing catabolism, in this case dexamethasone (timely, as this is also used in Covid-19 as an anti-inflammatory treatment), will lead to greater catabolism of MG than TA cells; representing a "global-fingerprint" of atrophy susceptibility beyond loss of neural input through immobilization. The student will have the opportunity to work in an extremely well externally funded (UKRI, industry, charity) and multi-disciplinary lab, which is a thriving PGR environment, supported by UKRI/BRC-funded PDRA's, and with both clinical and non-clinical PGR student engagement- widening their perspective of team science at a very-early stage.

Location: Derby Royal Infirmary;

Full project description: Skeletal muscle wasting is a major public health problem linked to ageing, diseases, and physical inactivity. Until earlier this year, the proposed primary supervisor for this studentship held a BBSRC new-investigator grant aimed at understanding the mechanisms of muscle wasting in response to physical inactivity (e.g., with hospitalization/bed-rest/leg casting after fracture), and this studentship will build upon the findings of this grant. In this grant, we created a novel paradigm based on the premise of markedly differential muscle wasting rates across individual muscles; and in doing so, we are able to discern the "true" mechanistic underpinnings of muscle wasting, rather than processes uniformly dysregulated during muscle exposure to physical inactivity e.g., insulin resistance. This studentship forges new links to Nottingham Trent University (Piasecki) in complementary areas of neuromuscular function and cellular biology, that will build upon the knowledge and resources generated from the aforementioned BBSRC grant and remain wholly relevant in the coming years.



Year-1: The recent BBSRC grant showed that the tibialis anterior (TA; a shin muscle) is resistant to muscle wasting during 15-days of immobilization, whereas the medial gastrocnemius (MG; a calf muscle) is subject to significant muscle wasting. During this study we harvested muscle "satellite cells" from

both TA and MG muscles by a process of flow cytometry cell selection. These are specialised muscle-specific stem cells that are indispensable for maintenance and regeneration of muscle across the life-course. We hypothesize that distinct muscle wasting rates will be associated with distinct inherent regenerative capacities across TA and MG-derived stem cells. This element of the studentship would involve treatment of TA/MG-derived stem cells with cues that regulate muscle wasting and growth and elucidate the mechanisms by which some muscles are resistant to wasting.

Year-2: The recent BBSRC study conducted RNASeq in TA/MG following 15-days of immobilization. While the analysis of this is ongoing, it will undoubtedly yield data that will provide insights into the transcriptional basis of muscle wasting in atrophy-resistant vs. susceptible muscles. We hypothesize that a set of genes will be discretely regulated between TA and MG, homing in on the "master regulators" of muscle wasting programming. This part of the studentship would involve the follow-up of candidate genes using shRNA in human-derived muscle cells in vitro; the aim being to mechanistically link in vivo gene candidates to muscle wasting.

Year-3: The recent BBSRC grant focused on younger subjects, based on the need to understand the basic mechanisms of muscle wasting. However, a focus of BBSRC strategy is healthy-ageing across the life-course. Therefore, in this part of the studentship, a pilot clinical trial in older humans will be conducted, to determine the impact of age upon distinct muscle atrophy. We hypothesise that atrophy resistant muscles (i.e., TA) will not be spared in older people at risk of sarcopenia, unlike in younger people. This would involve short-term leg immobilization in a group of older people (>70 years) and would identify if loss of absence of atrophy resistance is a feature of sarcopenia.

Full project location: Derby Royal Infirmary;

Mitochondrial dysfunction in renal proximal tubule underpins kidney disease in all animals

Project Supervisor: David Gardner

School: Veterinary Medicine and Science

Description: In the rotation associated with this project the student can expect to get an experience of the types of work that the whole project will consist of: collection of fresh tissues, cell culture, bioinformatics. Some of this will be conducted in the vet school and some in BDI. We will hopefully get the student examining existing TEM images of mitochondria and devising a means to assess structural abnormalities. In addition, we will hopefullly get the student to experience cell culture with mitochondrial end-points in mind (in BDI). In addition, they will get an idea of the bioinformatics required, by working alongside existing post-docs, to understand renal transcriptomics.

Location: Sutton Bonington Campus;University Park;

Full project description: Background. Kidney cells are highly perfused and highly oxidative. As such, they have high mitochondrial density to support oxidative phosphorylation, but have an innate inability to switch to glycolytic metabolism when oxygen supply is limited. Kidney cells are therefore susceptible to damage (e.g. AKI, AKD) induced by reduced blood flow or blood pressure, nephrotoxic drugs, chemotherapy. Mitophagy, ferroptosis, apoptosis and necrosis are the mechanisms for such damage, all underpinned by mitochondrial dysfunction.

Males have much greater incidence of AKI than females, despite all mitochondria being derived from the oocyte. This suggests considerable mitochondrial heteroplasy within organs. Equally, treatments or drugs that support reduced mitochondrial function in the setting of AKI/AKD have potential to benefit many human or animal patients in acute and chronic illness, as acutely damaged kidney cells are replaced by fibrotic tissue increasing risk of chronic kidney disease. A hallmark of kidney damage is therefore mitochondrial dysfunction.

Experimental plan. UoN is uniquely positioned to support this project since in the vet school, academics can source fresh kidney tissue from a multitude of animals (feline, canine and porcine in particular) AND have access to mequipment for runnign mitochondrial functional assays. Working with academics based in the BioDiscovery Institute (BDI) then complex bioinformatics/genomics/proteomics/transcriptomics can be addressed. The student will first investigate AKI/AKD and CKD in relevant animal models, using mitochondrial read-outs as the underpinning factor. The student will therefore obtain experience in cell culture (feline, canine, porcine kidney cells), in measuring mitochondrial function in fresh tissue homogenates and in assessing mitchondrial structure using electron microscopy. For example, using the porcine model, we will quantify/outline a time-line (serial sampling, biopsy) of mitochondrial dysfunction that leads to increased renal interstitial fibrosis 8 weeks later. Experimental design will include male/female siblings (i.e. shared mitochondrial genome) vs non-sibs (i.e. non-shared mitochondrial genome). We will biomark the response in urine using mtDNA (milestone: mitochondrial dysfunction underpins AKI and increases susceptibility to CKD, urine mtDNA biomarks AKI). Plasma, tissue, urine transcriptomics, proteomics and assays of fresh tissue mitochondrial function (O2K Orobios) together with TEM will elucidate mechanism and provide a read-out/biomarker of dysfunction.

Similar human data will be obtained to establish translational relevance from a clinical AKI cohort (timed urine samples), together with fresh human kidney tissue obtained during urological surgery for ccRCC (milestone: mitochondrial dysfunction underpins AKI/CKD in humans, urine mtDNA biomarks recovery from AKI). Existing drugs or treatments that are known to support or prevent kidney mitochondrial complex I (or II-V, as appropriate) will be identified through screening and tested in the animal model, repeating the above protocols to determine efficacy in each sex.

Outputs. project will generate: 1) novel urinary biomarker of a) AKI, b) recovery from AKI; 2) unique paired datasets of mitochondrial function with renal transcript/proteomics, 3) potential treatments for specific patient groups where AKI was not foreseen (e.g. community-acquired AKI, unforeseen surgical adverse events e.g. neurosurgery)

Full project location: University Park;Sutton Bonington Campus;

Path analysis of connectomes

Project Supervisor: Jason Smith

School: School of Science and Technology (NTU)

Description: A connectome is a map of connections in a brain. This can be neuron to neuron connections in the microscale, up to region to region in the macroscale, and it can include structural and functional data. This project will look at analysing the structure of paths within these connectomes. A path from one node (neuron or region depending on scale) to another node is a sequence of nodes v1,…,vn such that vi connects to vi+1.

The lab rotation would begin by taking the C. elegans connectome (the smallest fully mapped microscale connectome) and computing the shortest directed paths between certain sets of neurons, and investigating the lengths and composition of these paths. Once these paths have been evaluated we can make slight changes to the connectome, which simulate neurodegenerative diseases, and see how this affects the paths.

The final topic of investigation is to take a simple simulation model of C. elegans using a neural simulator, and firstly investigate which paths are most frequently used for certain stimuli. And secondly, see how slight structural changes to these paths affects the functional propagation of signals.

Location: Clifton Campus;

Full project description: Connectomes are integral to our understanding of how the brain functions, and in recent years a wealth of data has become available on connectomes, across species and scales. This presents the new challenge of analysing this data. The aim of this project is to investigate the structure of paths in these connectomes and how they vary between individual, species, and scale, and the effect that neurodegenerative diseases have on these paths.

There are only two complete microscale connectomes: c. elegans and drosophila larva. Although a variety of partially mapped microscale connectomes exist for other species. There also exists some large microscale models such as the Allen Brain mouse and Blue Brain rat. At the macroscale there are many more connectomes of varying resolution for different species, and multiple individuals within species. Moreover, for the macroscale data we have connectomes for individuals that are suffering from certain neurodegenerative diseases, which can be compared to healthy individuals. Some of these connectomes are directed, however often this direction is missing in the data (due to imaging restrictions) or neglected in the analysis. This project would embrace the direction (when available) to investigate directed paths between node, which gives much clearer information on how data moves through the brain.

The distance between two nodes is the length of the shortest path between them. Connectomes are known to be well connected, in the sense that the distance between nodes is generally quite low, even in very large connectomes. This is because of "hub" neurons that allow two neurons that are spatial distant to connect to each other via hubs that connect across long distances within the brain. Hubs neurons are prevalent in the literature, though the definition varies significantly between cases. Part of this project is to identify hub neurons by looking at the paths to see which neurons are acting like hubs, as opposed to the existing approach of using structural properties of the neurons to determine which neurons are hubs.

The next stage is to investigate differences between paths in healthy vs unhealthy brains. For this we use the macroscale connectomes, where we have multiple connectomes from the same species (such as humans) including data about the individual, such as age and health. We investigate how the paths change between different individuals, and investigate if path structure changes with age, or if there is a detectable difference in paths between healthy individuals and those suffering from brain disorders, such as schizophrenia.

The final branch of investigation is to include functional data. Functional data is available for some of the connectomes, and for other connectomes we can simulate neuronal activity using neural simulators (such as NEST). We would investigate how the functional data passes through the paths, and link this back to the prior investigations. How does changing the structure affect the paths that are active in the functional data, how does

removal of hub neurons affect the speed at which signals propagate through the network?

Full project location: Clifton Campus;

Non-structural protein 6 as a pan-coronavirus immunomodulatory protein

Project Supervisor: Christopher Coleman

School: Life Sciences

Description: During the rotation the student will be introduced to the molecular and cellular biology techniques that will be used during the full project, including cloning, site-directed mutagenesis, Western blotting, fluorescent/confocal microscopy and others. Data generated during the rotation will be useful for the full project.

The student will also be given the opportunity to work with cell lines and possibly CL2 coronaviruses. We will also discuss future plans and requirements for CL3 work with the student so that they are fully aware of what this work entails.

A rough outline of plans for the rotation is as follows (primary focus, there will inevitably be overlap and changes of plans as the rotation progresses):

- Weeks 1-2: Computational comparison of nsp6 genes from multiple coronaviruses and prediction of key domains. Along with general lab induction and introduction to techniques such as tissue culture.
- Weeks 3-4: Transfection of wild-type nsp6 genes into cells and analysis of subcellular localisation and function.
- Weeks 5-7: Cloning of nsp6 mutations using overlap-PCR and analysis of subcellular location and function.
- Week 8-9: Data analysis and write-up. Discussions on future plans for the project and working at CL3.

Location: QMC;Sutton Bonington Campus;

Full project description: Coronaviruses (CoVs) are important pathogens of both humans and animals. The SARS-CoV-2 pandemic has highlighted the importance and potential of CoVs to emerge rapidly and cause severe disruption to human health and society, however there are also a number of less pathogenic human CoVs, highly pathogenic human CoVs and a wide-range of animal CoVs that, for example, can cause significant issues for farmers.

Prior to the development of SARS-CoV-2 treatments and vaccines, there were no approved treatments or vaccines for any human CoV and very small numbers for animal CoVs. One of the key problems highlighted by the emergence of the SARS-CoV-2 was this lack of vaccines/treatments for CoVs, which lead to delays in development of these interventions.

However, treatments and vaccines are often specific to individual CoVs and have limited utility against other CoVs. We propose that a key component of the future response to an emergent CoV could be use of interventions that are pan-CoV. That is, not specific to any particular human or animal CoV. Such a treatment could be used against all current CoVs as well as any future emerging CoV.

CoVs have a large positive-sense RNA genome that encodes three groups of proteins: non-structural proteins (nsps), structural proteins and accessory proteins. Although all CoVs have the same basic structural proteins, they tend to very different at the sequence level between different CoVs, accounting for differences in tropism etc. The accessory proteins are highly variable between different CoVs – with differences in

sequence, function and number in different CoVs. The nsps, in contrast, are reasonably well conserved between CoVs, in part because all of the key enzymes required for CoV replication are nsps.

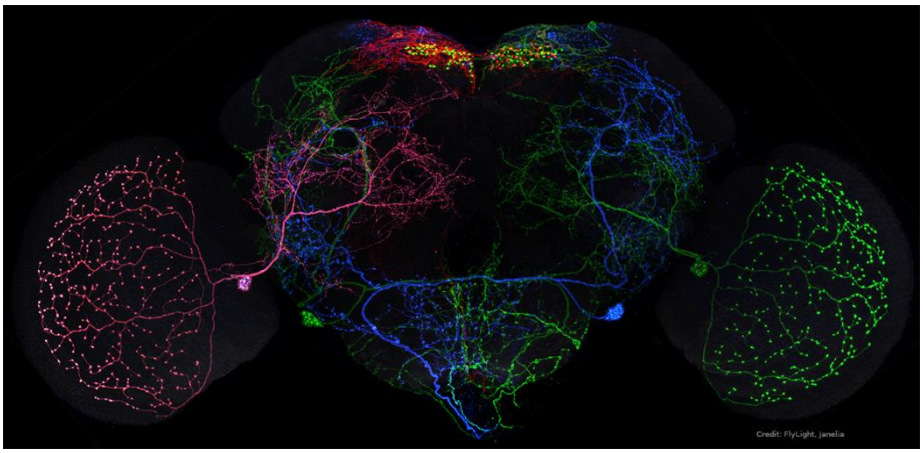
All viruses, have to overcome the host immune response in order to establish infection. One of the key innate responses to virus infection is the expression of restriction factors: host proteins that non-specifically block virus replication using a wide range of mechanisms. Successful viruses, therefore, have evolved many antagonists of restriction factors.

In this project, we will focus on nsp6 and a potential role in antagonising a key host restriction factor, tetherin, through the activation of the autophagy pathway. Furthermore, we predict is conserved across many (if not all) human and animal CoVs.

Previous work, by the lab and others, has shown that nsp6 proteins from a range of human and animal CoVs induce autophagy. We have further shown that these autophagosomes carry tetherin away from the site of viral restriction and, therefore, prevent restriction of the CoVs. This project will expand upon these preliminary studies to investigate the role of nsp6 in both autophagy and tetherin antagonism. We will utilise nsp6 proteins from a wide range of human and animal CoVs, including SARS-CoV-2 to discover if these mechanisms are truly pan-CoV.

The project will involve cloning CoV nsp6 genes, performing site-directed mutagenesis of CoV nsp6s, handling of CoVs at both CL2 and CL3 (again, including SARS-CoV-2) and creating nsp6 mutations with the genome of CoVs.

Full project location: QMC;Sutton Bonington Campus;



Towards the development of a pan-lineage vaccine: antigenic mapping of lassa fever virus glycoproteins

Project Supervisor: Janet Daly

School: Veterinary Medicine and Science

Description: The rotation will serve to introduce the student to studying the entry and replication of arenaviruses in a CL2 laboratory setting. The student will learn how to culture immortalised cell lines and use these for transfection and production of lassa fever virus (LASV) glycoprotein (GP) pseudotyped viruses. The student will work with receptor CRISPR-Cas9 knock out cell lines and use these for infection with LASV-GP pseudotyped viruses to assess differences in receptor requirements for entry. They will also assess how the susceptibility to host innate restriction factors, that can inhibit virus

entry and replication differs across the pseudotyped viruses tested. The student will also gain experience in using bioinformatics tools relevant to identifying the LASV virus lineage sequences to take forward for antigenic mapping. Key methodologies:

- Weeks 1-2: Cell culture techniques including transfection and production of LASV-GP pseudotyped viruses. Bioinformatic analysis of LASV lineage sequences.
- Weeks 3-4: Virus infection and titration by flow cytometry.
- Weeks 5-7: Infection of CRISPR-Cas9 receptor knockout cell lines, and host restriction factor overexpression cell lines. Western blot analysis to show receptor knockout and restriction factor overexpression.
- Weeks 8-9: Final data analysis and write-up

All relevant lab training will be provided and there will be the opportunity to meet virtually with collaborators in Nigeria and to present the project at lab meetings and to the wider One Virology group.

Location: Sutton Bonington Campus;

Full project description: Arenaviruses are a major cause of viral haemorrhagic fever. Endemic to West Africa and South America, arenaviral haemorrhagic fevers, such as Lassa fever (LF), are on the priority list of the World Health Organisation (WHO) as diseases requiring urgent research and development measures. Human transmission occurs through direct skin contact with infected rodent faeces or urine, ingestion of contaminated food, or through respiratory exposure to rodent excreta, resulting in illnesses ranging from mild flu-like syndromes to severe and highly fatal haemorrhagic diseases.

Annual outbreaks of the highly prevalent Lassa fever virus (LASV) across Western Africa have increasing health and socio-economic impacts, made worse by the lack of effective vaccines and treatments. Recent phylogeographical studies, in Nigeria, have highlighted the expansion of LASV outside traditional endemic regions and phylogenetic studies have revealed how genetically diverse this RNA virus is across the four major phylogenetic lineages. Thus, LASV genetic diversity presents a challenge for the development of a cross-protective vaccine candidate.

Previous GCRF-funded research has demonstrated differences in cell entry receptor usage by LASV glycoprotein (GP) sequences across these lineages, which provides evidence for distinctions in cell entry pathways. Related to this, we have demonstrated differences in LASV GP sequence susceptibility to entry restriction factors, which are innate immune factors that act to inhibit virus entry and replication. We have therefore hypothesised that emerging LASV sequence diversity could be altering virus cell entry routes as a mechanism of immune escape. We would like to expand on these findings to determine:

1. Whether the observed changes in cell entry are linked to innate immune escape and/or inherent properties of LASV glycoprotein (GP) processing and host cell interaction, and
2. Do these changes correlate with a particular LASV lineage and geographical area?
3. Strongly linked with the above, a major aim of this proposal is to understand how the effectiveness of LASV vaccines can be improved by providing a better understanding of the antigenic epitopes of the major antigen, the glycoprotein; particularly across circulating LASV lineages. To address this, we propose to:
4. Generate a panel of single domain antibodies (VHHs) against LASV GP-pseudotyped viruses by phage-display technology. More specifically, we will also pan selectively against LASV lineages that show differences in their cell entry mechanisms,
5. Engineer the lineage specific and pan-specific VHHs as bivalent Fc-fusions and perform virus neutralisation tests that focus on the entry step of the virus life cycle,

6. Generate antigenic maps and ultimately identify antigenic sites that could inform vaccine design.

The antibodies will be used to identify antigenic sites that are common across LASV lineages. We will determine correlations which are attributed to not only amino acid differences and properties but also to the changes in receptor usage and restriction factor susceptibility. The discovery of antigenic sites and subsequent generation of antigenic maps of LASV GP sequences could then be used for pan-vaccine development. This work will also aid in virus surveillance studies to highlight the antigenic variants that are prevalent and are emerging in a geographical region.

Full project location: Sutton Bonington Campus;

Does sweet tooth associate with the risk of developing type 2 diabetes?

Project Supervisor: Qian Yang

School: Biosciences

Description: The lab rotation will be a mini project to review the current methodology to examine sweet taste perceptions including the type of substances and concentration used in the literature. The students will receive training on health and safety, work in sensory with participants, ethical application and support on methodological development.

- Weeks 1-3: Conducting literature review and submitting ethical application.
- Weeks 3-6: Pilot study on exploring different methodologies to measure sweet taste sensitivities to develop hands-on experience working in the sensory lab, prepare samples and working with participants.
- Weeks 6-9: Data analysis on the pilot data to help the students to gain statistical analysis skills.

The mini project has been carefully designed so the student doing the lab rotation learns and develops skills required for the main PhD project that can be developed further and used throughout the PhD.

Location: Sutton Bonington Campus;

Full project description:

Diabetes is a global challenge. In the UK, the number of people affected by diabetes is expected to reach 5.5 million by 2030, 90% of which is Type 2 diabetes (T2D). Habitual high consumption of added sugars increases the risk of developing, or worsening T2D. A better understanding of the mechanisms that govern sweet taste and reward is crucial. While poorly characterised, lower sweet taste acuity and increased sugar consumption is reported in individuals with T2D. However, evidence of alterations in sweet taste acuity in people with prediabetes is lacking and our current understand of how sweet taste across healthy, pre-diabetes and T2D is limited. Here, this study aims to compare sweet taste sensitivity and preference in health, prediabetes and T2D, and relate this to sugar consumption habits. In addition, this project aims to determine how brain responses to sweet sensing differ among health, prediabetes and T2D groups.

This cross-disciplinary project brings together expertise from sensory and consumer scientist (Yang), diabetes clinicians (Idris) and MR imaging expertise (Eldeghaidy).

Study 1 (0-12 month): Sweet sensitivity and preference methodology selection. Literature review on the current methodologies of sweet taste sensitivity, sweet preference measurements. Pilot study to select the most popular used and robust and easy to perform methodologies that allow data collect for large number of participants.

Study 2 (12-30 month): Characterise sweet sensitivity and preference among healthy, prediabetes and T2D. At least 100 participants from each group will be recruited. Diabetes participants will be identified by local GP practices registers as having diabetes. Non-diabetes participants will be recruited by adverts. Eligible participants will be invited to screen their taste sensitivities, sweet preferences, record their sugar consumption habit, and invited to complete food preference and food frequency questionnaire.

Study 3 (30-48 month): Mapping the cortical response to sweet sensing among healthy, prediabetes and T2D. Thirty individuals matched for age, sex and weight for healthy, prediabetes and T2D will be recruited from Study 2. Eligible participants will undergo a taste task fMRI scan to map brain responses to sweet taste perception at different sucrose concentrations informed by Study 2's findings. Both sucrose (sweet + calorie) and sucralose (sweet + no calories) will be used.

Full project location: Sutton Bonington Campus;University Park;

Gene switches and treatment resistance in advanced cancer

Project Supervisor: Nigel Mongan

School: Veterinary Medicine and Science

Description: You will join a vibrant team comprising two postdocs (both former DTP students) and current DTP PhD students. During your rotation you will gain experience in all the relevant methods you will need for your PhD. You will be taught how to complete immunohistochemical evaluation of protein expression in human cancer specimens and how to assess the clinical and biological significance of the staining. You will gain experience in different approaches to knockdown or pharmacologically inhibit the pathway we are studying and will use qRTPCR and western blot to assess the effect on cancer relevant pathways. We will also train you how to complete bioinformatics analysis of RNAseq data and other genomics approaches. Collectively you will be equipped with all the skills required to complete a PhD. These skills are also highly transferable to careers in pharma and biotech.

Location: University Park;

Full project description: This project will investigate fundamental mechanisms of gene regulation and protein expression in the context of treatment response in prostate cancer. Prostate cancer is the most common cancer affecting men, with 1 in 7 white, and 1 in 4 Black men diagnosed with prostate cancer. There is no cure for advanced prostate cancer.

We have recently implicated RNA methylation in response to anti-androgen treatment, the most important approach to treat advanced prostate cancer. We found that RNA methylation is essential for response to treatment and new therapies that target RNA methylation can reverse treatment resistance. The exact mechanism whereby RNA methylation is involved in treatment response is the innovative focus of this proposal. This knowledge would enable the better use of these novel treatments in development for leukemia patients to be more rapidly translated to benefit prostate cancer patients.

You will be part of a friendly, successful and supportive team working collaboratively on related projects. Your project will be supported by existing genomics datasets and cell lines generated by our on-going grant funded projects. The specific focus of this project is to determine how the RNA methyltransferase complex function and composition changes in treatment sensitive and resistant prostate cancer. You will determine how

methyltransferase inhibitors already in clinical trial for both leukemia and solid tumours may benefit patients with prostate cancer. To do this you will use cultured cell lines and near-to-patient organoid models to understand how these drugs alter gene expression and thereby change treatment response. This information will help justify the repurposing of these drugs for prostate cancer patients.

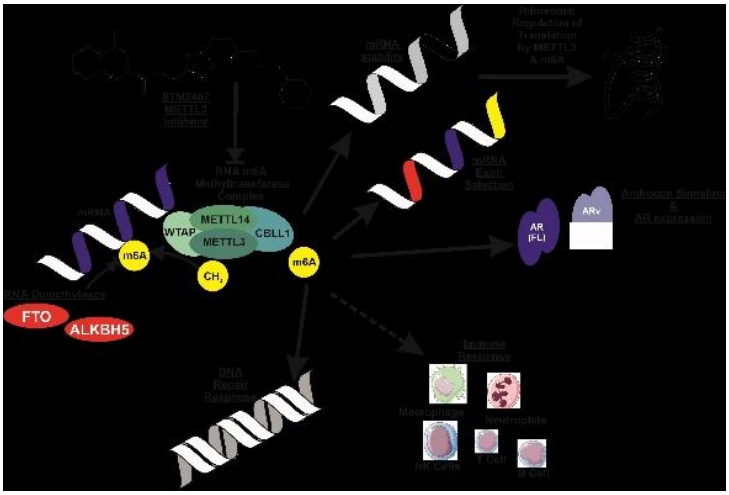
The skills you will obtained during your PhD are highly transferable and relevant to careers in academia, pharma and biotech. Specifically you will use

1. Immunohistochemistry to assess protein expression in human cancer specimens.

2. Bioinformatics to better understand how RNA methylation influences gene and protein expression and how RNA methylation inhibitors function in treatment sensitive and resistant cancer models.

3. Use different functional approaches such as siRNA and/or CRISPR-cas9 to disrupt gene function and determine the consequences on gene regulation in our models of treatment response.

4. Use in vitro functional assays to assess the effect of these inhibitors on cell proliferation, invasion and colony formation in respect of our commitment to the 3R principles.



Collectively you will obtain the experimental, bioinformatics, communication and leadership skills that are important in your career develop and you will be part of a supportive and vibrant team to do this.

Full project location: University Park;

Understanding the role of extracellular vesicles in the regulation of RNA

Project Supervisor: Victoria James

School: Veterinary Medicine and Science

Description: The rotation project will investigate our highly novel findings of how extracellualr vesicles produced by disease causing cells (in our model these are cancer cells) are able to regulate the fate of different types of RNA. This will involve learning some molecular biology techniques to isolate RNA and to look at speific patterns of regulation (i.e. splicing).

Location: Sutton Bonington Campus;University Park;

Full project description: The PhD builds upon the successful work of the group in identifying the mechanisms by which extracellular vesicles influence disease mechanisms, using prostate cancer as a model of complex disease.

Once thought to be little more than a way for cells to offload waste, extracellular vesicles (EVs) are now recognised to be a deliberate way for a cell to secrete cargos of RNA, DNA and proteins to reshape tissues and act as signal carriers. These somewhat overlooked

organelles may hold the key to understanding how tissues and systems in our bodies communicate, particularly within complex diseases.

The project will explore how EVs may play a role in regulating the processing of RNAs, affecting gene expression and ultimately the phenotype and behaviour of different cells. The project will involve a substantial amount of molecular biology, particualr in RNAs and cell culture skills alongside training in relevant bioformatic analysis, together with an opportunity to communicate and present the work to academic and external audiences. TFollowing the rise of RNA based theraeupetuics (e.g. COVID vaccines) these practical skills (particaurlyl RNA biology) and transferrable skill development through this project are increasingly attractive to employers of researchers both within and outside of academia.

We have limited the specific details of the project due to the novelty and sensitive commercial nature of some elements of the project, but we would be deligted to speak to interested candidates to ellaborate further if you have an interest in the area.

Full project location: Sutton Bonington Campus;University Park;

Understanding the role of lysine methyltransferases in ovarian cancer progression and treatment resistance

Project Supervisor: Jennie Jeyapalan

School: Veterinary Medicine and Science

Description: The lab rotation will be based at the multi-disciplinary Biodiscovery Institute, where you, the new researcher will be working within an international renowned, highly driven, and enthusiastic team of researchers, from molecular biologists, bioinformaticians, cell biologists and clinicians.

The project strives to understand how changes in histone modifications regulates transcription and DNA repair during cancer progression and treatment resistance. The question being 'Can we repurpose 'Epi-drugs', pharmacological inhibitors identified in other cancers, for ovarian cancer? You will start by learning the techniques that will be required for your project. Molecular biology techniques such as QRT-PCR for identifying mRNA levels of your gene of interest and immunoblotting (westerns) for looking at protein levels. You will start by familiarising yourself with ovarian cancer cell lines, how to grow them and using bioinformatic tools (web-tools and RNA-Seq analysis on publicly available datasets).

Location: University Park;

Full project description: Cancer is becoming a global issue within this aging population, with 1 in 2 of us developing cancer within our lifetime. Even though advances in cancer treatment have moved forward in recent years, the areas of drug resistance and no real treatment options for advanced cancers is where the current research is required. The updating of the hallmarks of cancer to include epigenetic alterations in cancer, is a fundamentally important for the cancer transcriptome, with histone modifiers altering the expression of oncogenes and tumour suppressors but also changing the alternative splice variants of genes to drive tumour progression.

Ovarian cancer is a deadly disease. Ovarian cancer research is an area of unmet need given its prevalence, aggressive nature, and crucial importance in the treatment of our patients. To address this critical need, in August 2023, we established a £4Million inter-

disciplinary centre of research excellence (NOVARC) at the Biodiscovery Institute (BDI), the University of Nottingham. NOVARC funding over five years is supported by a US-based donor, The University of Nottingham and Nottingham University Hospitals. The team led by Prof Srinivasan Madhusudan (co-supervisor on this application) comprises of clinicians and translational scientists with expertise in biological and clinical translational research. The centre includes experts in various aspects of biology and cancer therapeutics. The Supervisory team are all part of this exciting centre. The project will be investigating whether 'Epi-drugs', pharmacological inhibitors, could be repurposed for ovarian cancer?

The DTP student will undertake to;

- Assess the expression of histone modulators in publicly available datasets and within our human ovarian cancer specimens. This will indicate if the target is tumour promoting or suppressing, the protein location (nuclear or cytoplasmic) and clinical relevance.
- Identify which epigenetic modulators are altered during cancer progression and in cell-lines that are treatment resistant, by assaying cell proliferation, invasion and transcriptomic analysis.
- use of CRISPR technologies to knockout gene of interest and pharmacological inhibitors to target the epigenetic factors in ovarian cancer cell lines and patient-derived organoids. T
- use RNAseq and splicing analysis to determine the relative role of histone modifiers in alternative splicing and the cancer transcriptome.
- will compare the effects of functional depletion/over expression of histone modifiers on replicative stress and DNA repair in cancer cells.
- Collectively these aims will advance understanding of the role of H3K36me modulators in DNA repair, cancer transcriptomics in disease context and translational oncology.
- The DTP student will receive training in the following techniques:
- Clinical genomics and data interpretation: the student will learn how to utilise webtools that analyse publicly available datasets, complete immunohistochemistry on patient specimens and clinical correlations
- Cell culture (cell lines and PDO), CRISPR techniques, reporter assays, in vitro pharmacology
- Basic molecular biology, cloning, qRTPCR, western blotting
- Bioinformatics: our group has optimized pipelines and existing datasets already available for comparison. RNA-seq analysis and splicing analysis.

Full project location: University Park;Sutton Bonington Campus;

Antisense transcripts as novel biomarkers involved in the pathogenesis of ovarian cancer

Project Supervisor: Cinzia Allegrucci

School: Veterinary Medicine and Science

Description: In the lab rotation the student will be exposed to the different aspects involved in the research to gain an understanding of both the project and the environment. Training in different molecular techniques to assess LINE-1 and non-coding RNA transcripts both in silico (use of genome browser and bioinformatics data) and in the lab (quantitative PCR, western blotting, and immunohistochemistry) will be provided. The student will also learn cell culture techniques by culturing ovarian cancer

cell lines cultured both in 2D and 3D conditions and to use viability tests to assess cell proliferation.

The student will also participate to weekly lab meetings to learn about research projects undertaken by the lab and will learn to prepare presentations and written reports. Training in critically assessing published papers and in using graphical and statistical analysis packages will also be part of the rotation. Finally, the student will be fully integrated within the research group and the Nottingham Ovarian Cancer Research Centre to provide a full understanding of the research area that will form the PhD project.

Location: University Park;

Full project description: Ovarian cancer is the second most deadly cancer in women. This disease represents an important clinical challenge as tumour are detected at advanced stages and resistant to therapies. Therefore, there is urgent need to improve its clinical outcome and patients' poor prognosis.

Major attention has been given to the importance of discovering novel biomarkers for early diagnosis and effective treatment. This project addresses this clinical need by investigating the role of antisense transcripts in the development and progression of the disease. Antisense transcripts are RNA molecules that are generated by antisense transcription of DNA and involved in many cancer processes, including tumorigenesis, metastasis and therapeutic resistance. They include different classes of RNAs, including short (siRNAs, microRNAs) and long non-coding RNAs. Particularly interesting are the non-coding RNAs that are generated by the activity of the antisense promoter of LINE-1 sequences, a retrotransposon that is active in ovarian cancer and associated with poor patient outcome. Work in the lab has shown that these LINE-1 generated transcripts play an important role in cancer as they regulate long non-coding RNAs with oncogenic activity and contribute to the regulation of abnormal tumour cell proliferation, invasion and cancer stemness (manuscript in preparation). Therefore, this project will use this knowledge to further new developments in ovarian cancer to find novel biomarkers.

The project will take advantage of the latest bioinformatics, cellular and molecular techniques to study ovarian cancer using both cell line and patients' tumour tissue. Expression of antisense transcripts generated from LINE-1 will be assessed in a large patient cohort (608 samples) available from The Cancer Genome Atlas Program (TCGA) and samples for the Nottingham Biobank. These will be validated in ovarian cancer cell lines for functional studies. Gene editing of deregulated transcripts (RNA interference and CRISPR/Cas9) will be used to regulate expression and study the functional effect on genomic stability, cancer stem cell enrichment, cell proliferation, migration, tumorigenicity and resistance to therapy. The affected gene networks will then be studied by next-generation sequencing. Epigenetic regulation of antisense transcripts will also be undertaken to evaluate the effectiveness of epigenetic drugs in combined therapeutic strategies for ovarian cancer.

This project contributes to the important research that is undertaken by the Nottingham Ovarian Cancer Research Centre (NOVARC), an inter-disciplinary centre of research excellence based at the Biodiscovery Institute (BDI). The centre led by Prof Srinivasan Madhusudan (co-supervisor on this project) comprises of clinicians and translational scientists with expertise in biological and clinical translational research. The centre includes experts in various aspects of biology and cancer therapeutics relevant to this project including: i) Molecular & cell biology (Dr Cinzia Allegrucci: main supervisor), ii) Bioinformatics & cancer biology (Prof Nigel Mongan and Dr Jennie Jeyapalan: co-supervisors) iii) DNA damage signalling & repair biology (Prof Srinivasan Madhusudan,

co-supervisor), iv) Clinical ovarian cancer oncology and Clinical trials (Prof Srinivasan Madhusudan, co-supervisor).

A key priority of NOVARC is to nurture early career researchers by providing a dynamic research environment that will accelerate the development of novel diagnostics and therapeutics into the clinic.

Full project location: University Park;

Using neuroimaging to examine brain structural and functional connectivity.

Project Supervisor: Tracy D Farr

School: Life Sciences

Description: The candidate will undertake training surrounding visualisation and analysis of magnetic resonance imaging (MRI) data. This will begin with instruction with various software platforms routinely in our laboratories: FSLEyes, FSL, ANTS, and ITK-SNAP. Some of this software uses a Bash Unix shell command language and therefore it will be possible to learn some basic commands. Subsequently, we will provide training with different aspects of data pre-processing including how to judge image quality, correct for motion, smooth and segment the data. We want to encourage the candidate to explore different parameters to assist with understanding how the manipulations change the data. Subsequently, we will explore different types of image registrations. The candidate will learn when and how to apply a linear and a non-linear approach, and will gain experience using standard templates and atlases. The rotation will provide an essential foundation to image processing that would be of benefit for many different projects in the future.

Location: QMC;

Full project description: This project is focussed around using neuroimaging, specifically magnetic resonance imaging (MRI), to examine structural and functional connectivity in the brain; the big data name for this is connectomics. Diffusion MRI (dMRI) quantifies the movement of water throughout the brain and this information can be used to infer physical connections between structures. It is often used to visualise white matter. Functional MRI (fMRI) is sensitive to changes in oxygen metabolism, and because this correlates with brain activity, it can be used as an indirect measure of function. These techniques can be used to examine structure and function across the entire brain.

The lead supervisor has a strong background applying both of these techniques in rodents to investigate various different types of pathologies. She has recently developed a new collaboration with the University of Adelaide in Australia, and this group is one of the few world-wide that use sheep instead of rodents. They have collected various types of MRI data in sheep, including dMRI and fMRI, and some of the sheep have received a stroke.

This project proposes to build a pipeline for analysis of the sheep MRI data that includes preparation of the structural and functional connectome. We aim to interrogate connectome changes in response to age and sex, as well as stroke pathology. Structural information will be correlated with the functional data, and ultimately, with ex vivo tissue measures of biological mechanisms that are proposed to underpin aspects of ageing (such as neuroinflammation).

The secondary supervisor has a wealth of experience with the analysis of human MRI data, and a particularly strong knowledge of programming as well as statistical approaches to interrogate the data.

Depending on the interests of the student, the project could involve training with in vivo skills, preclinical MRI acquisition, behavioural testing, and tissue processing as part of the ongoing research program of the lead supervisor.

In summary, this project is at the interface of neuroscience, medical physics, and computational neuroscience and offers a variety of collaborative opportunities.

Full project location: QMC;

Investigating tRNA fragments as biomarkers of amyloidosis and alpha-synucleinopathies

Project Supervisor: Marion Hogg

School: School of Science and Technology (NTU)

Description: The rotation project will focus on investigating transfer RNA (tRNA) derived fragments and how their levels change in response to physiological stress conditions. tRNA fragments can be generated by the stress-induced ribonuclease angiogenin, and here we will examine how tRNA fragment levels reflect the underlying stress experienced by neurons.

We will differentiate induced pluripotent stem cells (iPSCs) into cortical neurons and confirm neuronal type using immunofluorescence with antibodies to cell specific markers. We will treat neurons with pharmacological agents to induce mild and moderate stress and confirm the stress response by immunocytochemistry with antibodies to stress-response factors. We will then purify RNA and quantify tRNA fragment levels using quantitative PCR techniques.

The student will learn a range of techniques from neuronal culturing and differentiation, immunocytochemistry, microscopy and image analysis, and RNA purification and quantification techniques. This will provide a valuable introduction to the methods required for the PhD project.

Location: Clifton Campus;

Full project description: Transfer RNAs (tRNAs) play a crucial role in protein translation where they bring amino acids to the ribosome to be added to a newly generated polypeptide chain. However, it has recently been discovered that tRNAs can be cleaved during stress conditions to generate a novel class of non-coding RNAs, called tRNA fragments. tRNA fragments are stable and detectable in blood making them ideal candidates as biomarkers, and they have been investigated in a range of neurological conditions including epilepsy (Hogg et al, 2019, Journal of Clinical Investigation) and Motor Neuron Disease (Hogg et al, 2020, Brain Communications). This project aims to identify differentially expressed tRNA fragments in plasma from patients with amyloidosis-associated disorders and alpha-synucleinopathies. We will then investigate the molecular mechanisms that underly tRNA fragment biogenesis and function using induced pluripotent stem cell (iPSC)-derived neurons.

The first year of the project will focus on Next Generation small RNA sequencing and analysis of small non-coding RNAs from plasma samples collected from people with amyloidosis and alpha-synucleinopathies, and age and sex-matched healthy controls to

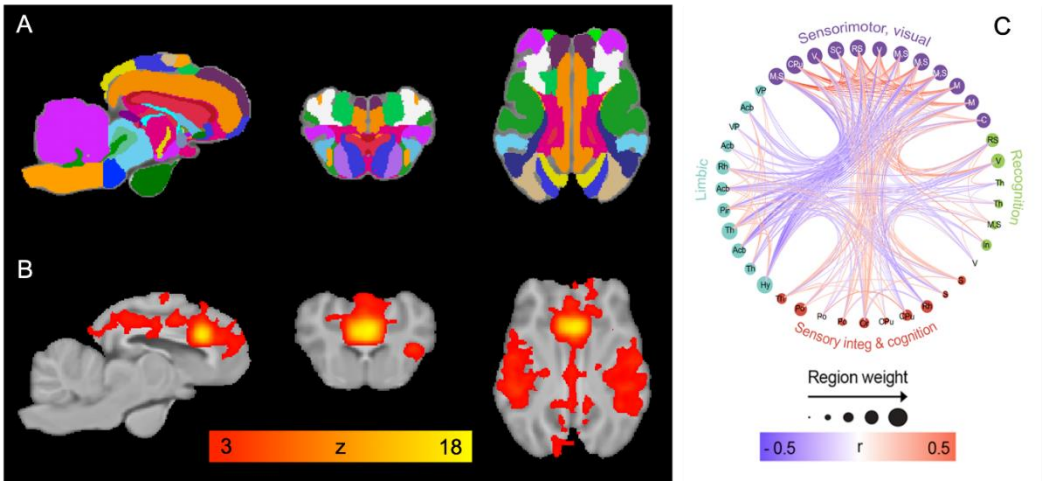
identify differentially expressed tRNA fragments. The student will isolate RNA and prepare libraries for small non-coding RNA sequencing on an exploratory cohort of samples, and differentially expressed tRNA fragments will be validated in a larger independent cohort using high throughput quantitative PCR and northern blotting techniques.

In the second year the student will investigate the molecular processes which lead to biogenesis of tRNA fragments using iPSC-derived human neuronal models. Dopaminergic and cortical neurons will be differentiated and validated using immunocytochemistry, then treated with pharmacological agents to induce amyloidosis, which is a key step in the pathology of many disorders, to investigate the underlying cause for dysregulated tRNA fragment levels. The ribonuclease(s) responsible for generating candidate tRNA fragments will be investigated and the effect of overexpression and depletion of tRNA fragments on neuronal activity will be explored.

Finally, in the third year the student will investigate the interactome of the identified tRNA fragments using synthetic tRNA fragments and standard biochemical techniques, and determine whether tRNA fragments interact directly with proteins and RNAs implicated in alpha-synucleinopathy or amyloidosis pathology both in vitro and in iPSC-derived neurons. Thus, this project aims to identify and characterise novel mechanistic biomarkers of neurological disease, and may reveal novel therapeutic targets.

The successful applicant will gain experience in a wide range of techniques including small RNA sequencing and data analysis, cellular and molecular biology, differentiation and maintenance of neuronal cultures, standard biochemistry techniques, RNA and protein analysis techniques, immunocytochemistry, live cell imaging and image analysis techniques. The student will join a vibrant neuroscience research environment at NTU with expertise in a range of different models and scientific approaches and the centre for dementia in the University of Nottingham. This project offers a great opportunity to join the newly established Neuroscience Research theme within the Centre for Healthy Aging and Understanding Disease based at NTUs Clifton campus, whilst benefitting from clinical expertise provided by the UoN supervisor and the Institute of Mental Health at UoN.

Full project location: Clifton Campus;Jubilee Campus;



Determining the influence of sex hormones on central and peripheral aspects of motor function across the lifespan

Project Supervisor: Jessica Piasecki

School: School of Science and Technology (NTU)

Description: The nine-week lab rotation associated with this project will be based at Nottingham Trent University. The methods involved will be directly related to the main PhD project. The student will be trained in state-of-the-art methods of electromyography (intramuscular and high density; iEMG, HD-EMG) and transcranial magnetic stimulation (TMS). These methods will be applied to a small sample of young men and women, for which ethical approval will be in place. The sample will be taken from the student population and will be an achievable milestone for the student. The student will also be trained in the latest signal processing and computational analyses procedures relevant to the high data outputs generated via these methods. All methods are currently operational within our labs, and all relevant training expertise is available within this supervisory team. This lab rotation will allow the student to familiarise themselves with the data collection and analysis methods necessary for the PhD, as well as collating important cross- sectional data that will later inform the initial steps of the main PhD project.

Location: Clifton Campus;

Full project description: The motor unit (MU) is the last functional element of the motor system and increases in muscle force production are mediated by recruitment of progressively larger MUs and an increase in MU discharge rate. These processes of muscle force production decline with age and contribute to age associated decrements of neuromuscular function and locomotor activity. This is particularly important in older females, whom despite living for longer, are disproportionately affected by disability in later life compared to older males.

The menstrual cycle prior to menopause is a natural process for most females, characterised by fluctuating levels of hormones. Oestrogen and progesterone are the predominant female sex hormones and are able to cross the blood-brain barrier potentially influencing the functionality of the central nervous system (CNS) and motor unit firing rate. Oestrogen elicits excitatory effects via potentiation of glutamatergic receptors while progesterone increases activity of GABA, causing inhibitory effects. Similarly, testosterone (T), the predominant male sex hormone, has an anabolic impact on skeletal muscle and is associated with electrophysiological characteristics in older men. Dehydroepiandrosterone (DHEA) and its sulphate derive (DHEAS), the precursors of T, as well as the dihydrotestosterone (DHT) synthesised from T, have been reported to progressively decrease with ageing in men, and we recently demonstrated positive associations between DHEA and MU firing rate in highly active and inactive older men.

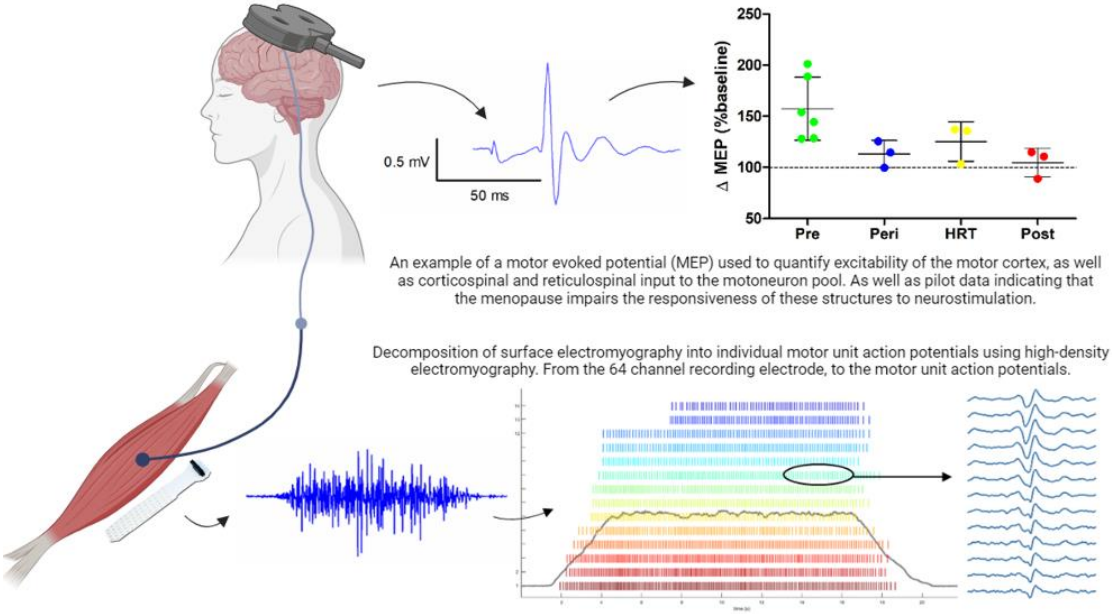
Previous findings from ourselves have demonstrated declines in MU discharge rate from middle to older age in women, which was not observed in men. Although direct mechanisms are unclear, these findings further highlight the potential contributions of altering sex hormones, in both men and women, on functional motor output and support further targeted assessment across the life course. The aim of this research project is to determine the influence of sex hormones on central and peripheral neuromuscular characteristics across the life-span. This will be achieved by combining novel, state of the art, methods of electromyography (intramuscular and high density; iEMG, HD-EMG) and transcranial magnetic stimulation (TMS) to explore central and peripheral MU characteristics and associated circulating sex hormone levels in pre-, peri- and post-menopausal women comparatively to young, middle and older age men. The project will

also seek to explore the cellular mechanisms by which these hormones interact with the muscle via skeletal muscle biopsies and imaging of the neuromuscular junction.

The research objectives for the project are to:
1. Utilise iEMG and HD-EMG techniques, combined with TMS, to assess MU characteristics in pre-, early and late peri menopausal, and post-menopausal women (n=60), and in young (18-30 yrs), middle (40-55 yrs) and old (70 yrs +) males (n=60), in the tibialis anterior and vastus lateralis muscles.
2. Quantify circulating levels of sex hormones, and neurotrophins across the male and female participant groups utilising mass spectrometry techniques.
3. Identify structural adaptation of the NMJ via histological imaging of targeted muscle biopsies.
4. Utilising cell lines garnered from skeletal muscle biopsies, in-vitro cell culture experiments will be conducted to explore cellular mechanisms of hormone and neuromuscular interactions.

Outcomes from this research may identify critical differences in sex-specific human ageing and has the potential to directly influence future research and clinical practice.

Full project location: Clifton Campus;



An example of a motor evoked potential (MEP) used to quantify excitability of the motor cortex, as well as corticospinal and reticulospinal input to the motoneuron pool. As well as pilot data indicating that the menopause impairs the responsiveness of these structures to neurostimulation.

Decomposition of surface electromyography into individual motor unit action potentials using high-density electromyography. From the 64 channel recording electrode, to the motor unit action potentials.

Behaviour and Addiction: Inhibition of Pavlovian-Instrumental Transfer

Project Supervisor: Charlotte Bonardi

School: Psychology

Description: Stimuli signalling rewarding outcomes trigger responding to obtain those outcomes – PIT, a phenomenon that underlies addictive behaviours. Conditioned inhibitors (CIs), stimuli which signal the omission of a specific outcome, suppress PIT in humans, but the specificity of these effects to specific rewards is unclear – will, say, an inhibitor for tobacco also reduce alcohol consumption? This rotation will explore this issue in mildly food-deprived rats, as their motivation is easily controlled, so the results more closely reflect human addictive behaviour.

Auditory or visual cues (X, Y) will respectively signal the omission of two qualitatively different outcomes, e.g. food and sucrose: S1-->O1, S1X-->nothing, S2-->O1, S2Y--

>nothing. The specificity of these inhibitors to their respective outcomes will be determined using transfer cues signalling the two outcomes (T1-->O1, T2-->O2). Inhibitors reduce the responding elicited by such transfer cues: but if inhibitors are outcome-specific, then X should suppress responding to T1 more than T2, and Y the reverse.

The rotation involves in vivo behavioural work with rats, and fosters many generic transferable skills, including critical thinking, project planning and organisation, data analysis skills, expertise with a range of statistical software (e.g. Med PC, Excel, SPSS, Jasp, R) and scientific writing.

Location: QMC;University Park;

Full project description: Pavlovian-instrumental transfer (PIT) is a phenomenon in which stimuli signalling rewarding outcomes trigger responding to obtain those outcomes – for instance, seeing a cigarette prompts a smoker to buy and consume tobacco. PIT is widely used in addiction research, underlying initiatives such as the curbs on tobacco branding and retail displays, and is also an important tool for designing therapeutic interventions to minimise relapse.

This project explores the effect of conditioned inhibitors (CIs), stimuli that signal the omission of a specific rewarding outcome, on PIT. We have evidence that CIs can suppress this elevation of responding by reward-related cues in humans (Alarcón & Bonardi, 2017; 2020), a finding with obvious therapeutic implications. However, many important questions remain.

First, the specificity of these effects to specific rewards is unclear: will an inhibitor for tobacco, say, also dampen the tendency to seek and consume other rewarding outcomes that have different sensory properties, such as alcohol? The boundary conditions under which such inhibitory cues are effective need to be established.

Second, we have preliminary evidence that inhibitory cues vary in their effectiveness as a function of time – being most effective at the exact point at which the omitted reward was expected. The temporal specificity of the effects of inhibitory cues on goal-seeking also needs to be determined.

The human paradigms in which we started this work cannot easily answer these questions, as they are limited in the motivational value of possible outcomes, and hence in the potency of the inhibitors they can generate. Thus we propose to look at these questions in rats. There is a long tradition of PIT research in rodents; as their motivation is more easily controlled, we can use more motivationally significant rewards, and the results will reflect more closely those of human addictive behaviour. We will use standard PIT designs, in which mildly food-deprived rats are trained that different Pavlovian cues (tones or lights; S1, S2) signal the delivery of qualitatively different types of food outcome (such as food or sucrose pellets; O1, O2). The rats will also be instrumentally trained to respond for these different outcomes, such as pressing levers or pulling chains (R1, R2). Finally the effects of these stimuli on responding will be assessed in the absence of any outcomes (Table 1).

Pavlovian	Instrumental	Test
S1-->O1	R1-->O1	S1: R1 > R2
S2-->O2	R2-->O2	S2: R2 > R1

The specific PIT effect we are exploring results in S1 elevating performance of R1 more than R2, because S1 and R1 share a common outcome while S1 and R2 do not (and vice versa for S2).

Pavlovian inhibitors will be established using standard training procedures, in which inhibitors (X, Y) signal the omission of an expected outcome (S1-->O1, S1X-->nothing, S2-->O1, S2Y-->nothing). Using these training techniques we can examine the outcome- and temporal specificity of the effects of these inhibitors on PIT test responding. This will form the basis of this PhD project.

Full project location: University Park;QMC;

Exosome biogenesis and organelle trafficking in neurodegeneration.

Project Supervisor: Alistair Hume

School: Life Sciences

Description: Aim – generate cell line producing fluorescent exosomes for analysis of exosome biology. In this rotation the student will generate neuronal model cell lines expressing a fluorescently tagged exosome marker protein (CD63-GFP). They will then use this use this to examine the role of small GTPases of the Rab family in exosome biogenesis and secretion using CD63-GFP localisation and release as a read-out. To do this the student will transfect neuronal cells with a vector allowing CD63-GFP expression, select for transfected/expressing cells and then use fluorescence microscopy and fluorimetry to examine the effects of altering Rab expression using siRNA depletion and over-expression. Rab GTPases are critical regulators of intracellular transport of membrane proteins including CD63 and thus examination of their function should give interesting insight into the mechanisms responsible for exosome biogenesis. Candidate regulators identified by this analysis will be taken forward for further study in the full PhD project.

Location: QMC;

Full project description: Intracellular transport pathways are fundamental for cell structure and function. Due to their longevity, complex morphology (long axonal and dendritic extensions) and requirement to maintain synaptic transmission, neurons are heavily dependent upon intracellular transport. Consistent with this, defects intracellular transport are directly linked to common forms of neurodegeneration. For example, altered function of the Rab activating protein C9orf72, a regulator of intracellular transport and subject of this project, are the major cause of genetic motor neurone disease (MND) and frontotemporal dementia (FTD).

Thus, this project will investigate how intracellular transport impacts neurodegeneration with the below aims:

1. Identify transport pathways controlling exosome biogenesis and secretion in neurones.
2. Investigate transport defects in C9orf72 deficient motor neurones.

Aim 1. Exosomes are a class of extracellular vesicle that derive from the intraluminal vesicles of multivesicular endosomes and may mediate intercellular communication important in health and disease. Recent data suggest that exosomes can spread pathological protein aggregates from diseased to healthy neurones thereby transmitting neurodegenerative pathology to healthy regions of the nervous system (Underwood, Valappil). For instance, recent data suggest that small GTPase Rab27b-dependent

exosome secretion allows pathological transmission of □-synuclein aggregates in Parkinson's disease. Thus, understanding the transport mechanisms regulating exosome biogenesis and secretion are crucial for the development of improved treatments for neurodegenerative diseases. To do this the student will generate model neuronal cell lines expressing labelled exosome marker protein CD63. This will allow high throughput measurement of exosome production and secretion and systematic screening and identification of proteins regulating these processes.

Aim 2. Intronic hexanucleotide (G4C2) expansion of the C9orf72 (chromosome 9 open reading frame 72) gene is a major cause of familial amyotrophic lateral sclerosis/motor neuron disease (ALS/MND, ~33%) and frontotemporal dementia (FTD, ~25%). C9orf72 colocalises with endolysosomal compartments, functions with SMCR8 (Smith-Magenis Syndrome Chromosomal Region Candidate Gene 8) and WDR41 (WD repeat protein 41) as a guanine nucleotide exchange (activating) factor for small GTPases Rab8a and Rab39b and regulates autophagic turnover of cellular proteins and compartments (references). Although it is thought that disease result from loss of function and/or toxic gain of function of aggregating expanded RNA and dipeptide repeat proteins the precise mechanism by which hexanucleotide expansion of C9orf72 causes disease is unknown. To better understand how C9orf72 contributes to neurodegeneration the student use fluorescence and electron microscopy and proteomics methods to characterise membrane transport defects in C9orf72 deficient motor neurones in culture.

Full project location: QMC;

Evolution and plasticity of human cognition

Project Supervisor: Deborah Serrien

School: Psychology

Description: In this lab rotation project, the student will gather supporting evidence from the literature for functional lateralisation patterns that reflect specialisation of the cerebral hemispheres for different types of information processing. Furthermore, the student will receive training to set-up and run behavioural experimental designs in order to test cognitive functions and their functional lateralisation profiles.

Location: University Park;

Full project description: Hemispheric lateralisation (or asymmetry) refers to the distinct organisation of the left and right side of the brain. One crucial aspect is functional asymmetry as each side is involved in the processing of different functions. Typical examples are left-hemispheric dominance for language vs. right-hemispheric dominance for visuospatial functions; a dichotomy that likely played an important role in the evolution towards behavioural specialisation. However, there are limited insights into the relationships between higher-order cognitive systems. In this PhD project, we will use a combination of behavioural and functional imaging methods (e.g., EEG, tDCS) for detailing the mechanisms that could support these associations. The findings will provide new insights into the complexity of human cognition and the evolution of strategic brain regions.

Full project location: University Park;

Understanding how particulate matter and respiratory viruses influence respiratory ageing using immune competent stem cell derived lung organoids

Project Supervisor: Nicholas Hannan

School: Medicine

Description: The student will differentiate human stem cells into fibroblasts, endothelial cells, macrophages and type II airway epithelial cells (TII AECs). Once generated the fibroblasts, macrophages, endothelial cells and TII AECs will be combined to create an 3D, immune competent, lung alveoli organoid. The alveoli organoids will then be exposed to particulate matter for 7 days. Following exposure, the alveoli organoids will be dissociated, and using flow cytometry, sorted into individual cell populations (fibroblasts, macrophages, TII AECs and endothelial cells) and gene expression profiles generated via QPCR. QPCR will develop a gene expression profile to assess inflammation, lung epithelium, fibrosis, cell death, and immune cell recruitment. This will be compared to control organoids that were not exposed to particulate matter.

To assess the effect of stretch on how cells respond, the experiment will be repeated using a cytostretcher to apply cyclic stretch to the lung organoids.

Location: University Park;

Full project description: The overall objective of our proposal is to bring together our recently developed hIPSC derived respiratory cells, with hIPSC derived macrophages, fibroblasts and endothelial cells to create a next generation model of the lung alveoli to better understand how inhaled particles and pathogens affect respiratory function as we age.

To achieve this we will create a multi-cell type, immune competent model of human lung alveoli that is capable of stretching and applicable to many areas of respiratory research. We will bring together stem cell derived type II alveoli epithelial cells with macrophages, fibroblasts and endothelial cells in a synthetic hydrogel developed by our industrial partner PeptiMatrix and using animal free cyclic peptides provided by PeptiDream. We will then demonstrate application of the model to understand how pollution and respiratory virus affect lung health.

The objectives and methodology below will provide an opportunity for development of a human specific, multi-cell-type and immune competent model of the human lung alveoli that could provide important mechanistic, diagnostic and therapeutic information across many areas of respiratory research.

Methodology:

1. Combine hIPSC derived lung cell types into synthetic animal-free hydrogels.We will bring together our stem cell derived type II alveoli epithelial cells, with macrophages, fibroblasts and endothelial cells in a synthetic hydrogel to generate a 3D multi-cell-type, immune competent organoid model of the human lung alveoli. We will then characterise gene expression and functional profile of individual cell types to establish baseline of a healthy lung organoid.
2. Understand the impact of cellular pollution and respiratory infection on lung alveoli tissue.

To demonstrate application of the model to ageing and respiratory health, we will expose the lung organoids to either fine particulate matter or human respiratory viruses, and use single cell mRNA sequencing to understand how cells respond individually and in combination to affect the gene expression and functional profile of other cells in the

aleoli tissue. We will perform these experiments both in static and stretching conditions to model normal lung mechanophysiology.

Together these objectives will create a new human specific model of human alveoli tissue applicable to a broad number a respiratory research areas. This includes inhalation studies which are a major focus in respiratory research, it includes understanding the effect of inhaled chemicals, cigarette smoke, infectious particles, carcinogens, fibrotic agents, drug delivery systems and environmental particulate matter. Importantly it will also provide a proof of principle model that a multi-cell-type respiratory organoid can be used for ageing related research.

Full project location: University Park;

Hippocampo-prefrontal-subcortical circuit in cognition and behaviour

Project Supervisor: Tobias Bast

School: Psychology

Description: Over the course of the lab rotation, students will be introduced to in vivo neuro-behavioural experiments in rodents on the basis of ongoing experiments.

- In vivo neurobiological studies: Depending on which studies are ongoing, students will be introduced to several of the following – stereotaxic brain surgery (e.g.to selectively manipulate or record from specific brain regions), intracerebral drug microinfusions, in vivo electrophysiology in rats. Depending on ongoing studies, students may also be involved in translational MR imaging studies in rats.
- Cognitive/behavioural studies: Students will receive training in the handling and cognitive/behavioural testing of rodents.
- Students will learn about the design and analysis of neuro-behavioural studies and software used for neuro-behavioural data collection (e.g. Ethovision, Plexon software) and for the statistical analysis of such data (e.g. JASP, Prism, SPSS).
- Introduction to ethical and legal frameworks relating to animal research: Students will learn about the important principle of the 3Rs and how this principle is translated into research practice.

Students will also attend bi-weekly lab group meetings and relevant neuroscience seminars.

Location: University Park;

Full project description: The brain circuit consisting of the hippocampus, prefrontal cortex and connected subcortical sites mediates and integrates important cognitive and behavioural functions, including memory, attention, cognitive control, emotional, motivational and sensorimotor processes. This circuit may play a key role in enabling the translation of every-day memories (e.g., of where you parked your car), which depend on the hippocampus, into adaptive behaviour (e.g., getting back to the car), for which prefrontal-subcortical circuits are vital (Bast, 2011, Curr Opin Neurobiol). Moreover, dysfunction within this hippocampo-prefrontal-subcortical circuit, especially within the hub regions – hippocampus and prefrontal cortex – may disrupt the wide range of cognitive and behavioural functions integrated within this circuit. Consistent with this, dysfunction in this circuit has been implicated in key cognitive and behavioural impairments characterizing neuropsychiatric disorders (Bast, 2011, Curr Opin Neurobiol; Bast et al., 2017, Br J Pharmacol).

Research questions: in this project, we will further examine the role of the hippocampo-prefrontal-subcortical circuit in adaptive and dysfunctional behaviour and cognition. The

specific research questions can be determined depending on the student's interest. Two main topics of our research include:

- Hippocampal learning-behaviour translation: Which prefrontal and subcortical regions contribute to behaviour based on hippocampus-dependent place learning, and by which mechanisms?
- Importance of GABAergic neuronal inhibition and of balanced neural activity: Imbalanced neural activity within the hippocampal-prefrontal-subcortical circuit, caused by changes in inhibitory GABA transmission, has come to the fore in important brain disorders, including age-related cognitive decline, Alzheimer's disease, schizophrenia, and Tourette's syndrome (Bast et al., 2017, Br J Pharmacol; Jackson et al., 2015, Trends Cogn Sci). What is the functional significance of GABAergic inhibition within this circuit and what are the cognitive and behavioural effects of imbalanced neural activity? Can they explain symptoms characterizing these disorders?

Methods

To address these questions, we will combine a wide range of neuroscience methods in rats. We will combine neuropharmacological modulation of specific brain regions by intracerebral drug microinfusions with translational tests of specific cognitive and behavioural functions (including learning and memory, attention, behavioural flexibility, fear, sensorimotor processes). In vivo electrophysiological methods will be used to characterise changes in neural activity patterns and interactions between relevant brain sites. A good overview of key methods can be found in our recent papers (Pezze et al., 2014, J Neurosci; McGarrity et al., 2017, Cereb Cortex; Gwilt et al., 2020, Hippocampus; Williams et al., 2022, eNeuro). Additionally, depending on interest and specific project objectives, students will have the opportunity to work with computational neuroscientists to synthesise experimental findings into neuro-computational models (e.g., Tessereau et al., 2021, Brain Neurosci Adv) or to use advanced analytical methods to analyse our experimental data (e.g., Maggi et al., 2022, bioRxiv); to apply 'translational' brain imaging methods to characterise neuronal network changes in a way that enables direct comparison to human brain imaging studies; to apply modern neural tract tracing methods (involving 'clarity' and light-sheet microscopy) and chemo-/pharmacogenetic methods for neuron-type specific manipulations (which we have recently set up).

For more info, see: https://www.nottingham.ac.uk/psychology/people/tobias.bast, 'Research' tab.

Full project location: University Park;

The use of Drosophila melanogaster neuronal imaging as a novel tool to test the efficacy of putative Alzheimer's disease drug candidates

Project Supervisor: Marios Georgiou

School: Life Sciences

Description: Alzheimer's disease (AD) is characterised by the presence of two major hallmarks, namely amyloid plaques and neurofibrillary tangles. Amyloid plaques are aggregates of β-peptides that are found in the brain interstitial fluid. These peptides originate from the APP protein, a glycoprotein normally found within the lipid bilayer, which in AD undergoes a series of cleavages by β-secretase and γ-secretase, leading to the production of β-peptides 40/42 amino acids long, which triggers neurodegeneration and the onset of the disease.

The student will use fly genetics to generate three fly Alzheimer's disease models (see full PhD project description for more details), and to express GFP reporter constructs in specific neurones in the Drosophila brain to visualise these neurones in high resolution, using confocal microscopy. This will allow us to assess the effect of Aβ peptide on neuronal morphology and/or viability.

Using this methodology, the student will identify brain regions and/or specific neurones that are affected in our Aβ AD models. This will allow us to screen through novel drug candidates and identify those that reduce neurodegeneration and/or morphological abnormalities within affected brain regions.

Location: University Park;QMC;

Full project description: The hypothesis underlying this proposal is that Drosophila melanogaster has significant potential to accelerate the identification of novel agents that improve on current treatments for Alzheimer's disease (AD). Although the utility of transgenic mouse models in improving our understanding of AD is clear, questions are increasingly being raised of the validity of relying on mouse models alone in AD therapeutics, particularly in light of the very high failure rate of clinical trials, and consequently the sparse number of approved drug treatments for this disease.

We will develop a high-throughput, in vivo, screening platform for novel drug candidates, to identify those that reduce neurodegeneration and/or morphological abnormalities within the brains of fly AD models. Using sophisticated fly genetic techniques, we will generate numerous fly models of AD, visualise specific neurones in specific regions of the brain, and observe effects on neurone morphology and survival. We will then determine whether a panel of novel putative AD agents, as well as FDA approved drug treatments, lead to structural and molecular changes in the Drosophila brain, using high resolution in vivo confocal imaging and ex vivo mass spectrometry imaging.

GENERAL AIM: Using high resolution neuronal imaging in the Drosophila brain, together with behavioural assays, we will combine the power of Drosophila genetics with state-of-the-art cell biology to identify and characterise novel drugs for AD, for which, despite extensive investigation, efficacious treatments remain elusive.

SPECIFIC AIMS:

(1) To develop an assay to determine the effect of β-peptide overexpression on the morphology of individual and small subsets of neurones in the Drosophila brain.

We will image neuronal morphology using three distinct but related fly models of AD:

Model 1: Direct expression of Aβ42 peptide in specific neuronal populations. A simple and direct approach at modelling AD neurotoxicity, which we have used previously to study XJP-1 treatment.

Model 2: Expression of human APP and BACE1 in specific neuronal populations, thereby allowing us to generate Aβ peptides processed from human APP. This model will be used primarily to allow us to screen for novel selective β- or γ-secretase inhibitors (see Aim 3).

Model 3: Low expression of human APP and BACE1 in specific neuronal populations, generating a late-onset model of AD. We will use this model to more faithfully mimic late-onset AD, which accounts for 95% of reported AD cases.

(2) To determine how XJP-1, and other FDA approved drug treatment, affects neuronal morphology and/or survival in β-peptide-expressing brains.

Our recent work has shown XJP-1 to significantly improve lifespan, climbing deficits and reduce amyloid aggregates in the Drosophila brain (Uras et al, 2021). Once we have identified brain regions and/or specific neurones that are affected in our AD models, we will be able to see what effect XJP-1 treatment, and other FDA approved drug treatments, have on neuronal morphology/viability.

(3) To screen through novel putative AD agents using high resolution neuronal imaging together with behavioural assays in fly AD models.

We have recently developed numerous next-generation drugs that target AChE and BACE1, and plan to initially screen through these candidates.

Full project location: University Park;QMC;

Physiology, pathology and drug delivery opportunities associated with the lymphatic system of the rectum in animals and humans

Project Supervisor: Pavel Gershkovich

School: Pharmacy

Description: There is a substantial gap in current basic science knowledge and understanding of the anatomy and physiology of the lymphoid tissues and regional lymph nodes of the anal and rectal region in humans and animals.

During the rotation project, the student will be working together with and learning from our current PhD student who is looking into the physiology of the lymphatic system of the rectal region in rats. The project will involve histological evaluation of rectal and lymphatic tissues in rats, in vitro uptake of nanoparticles into rectal tissue, and in vivo studies in rats aimed to understand the uptake of fluorescent nanoparticles into rectal lymphatic tissues. The student will learn the principles of histopathology, hands on in vivo work, principles and ethics of research involving laboratory animals, fluorescent imaging, principles of pharmacokinetics and lymphatic transport, bioanalytical methodology. The student will have an opportunity to work both in the School of Pharmacy and in the School of Veterinary Medicine.

Location: University Park;Sutton Bonington Campus;QMC;

Full project description:

There is a substantial gap in current basic science knowledge and understanding of the anatomy and physiology of the lymphoid tissues and regional lymph nodes of the anal and rectal region in humans and animals.

Our initial histology assessment of the rectal tissues in rats, focusing on the lymphatic system of the region, revealed novel surprising findings of substantial lymphoid tissues in this area. This could have importance not only for rectal drug delivery approaches, but also for understanding the function of rectum and its lymphatic system in health and disease, in humans and animals. This PhD project is a direct consequence of these preliminary findings and realisation of the importance for deeper understanding of the function of the lymphatic system of the rectal region.

The fact that rectal mucosa contains a high number of Peyer's patches and other lymphoid tissues could allow the delivery of nano-scale drug delivery systems to the directly draining regional lymph nodes. Regional lymph nodes are involved in a number of medical pathologies. This includes anal and colon cancer, as well as progression of the

initial infection by Human Immunodeficiency Virus (HIV) following rectal exposure. Therefore, the focus of this work is development of a nanomedicine-based approach for delivery of therapeutics agents to regional lymph nodes following rectal administration. The ultimate aims of this project are:

1. Understanding of the physiological and pathological role of the lymphatic system of the rectum in health and disease, in animals and humans
2. Improvement of the treatment outcomes of anal and rectal cancer, as well potentially developing a novel post-exposure prophylaxis approaches for HIV prevention.

Specific objectives:
1. Detailed histological evaluation of rectal tissues of rats, mice, swine and human recta obtained from surgical resections, focusing on the lymphoid tissues and regional pararectal lymph nodes.
2. In vivo studies in rats and mice aimed to understand the nano- and micro-particles uptake into lymphoid tissues and lymph drainage pathways from anal and rectal regions.
3. Development, in vitro characterisation and in vivo proof of principle studies of nanoscale drug delivery systems for targeting anal and rectal lymphoid tissues and regional para-rectal lymph nodes.

Full project location: University Park;Sutton Bonington Campus;QMC;

Exploring the bio-instructive properties of glycosaminoglycans

Project Supervisor: Andrew Hook

School: Pharmacy

Description: This rotation will familiarise the student with key techniques to be used in the PhD project. The project is multidisciplinary in nature and this rotation will expose the student to cell biology, polymer chemistry and analytical aspects. During the project the student will print and characterise microarrays of poly(ethylene glycol) based hydrogels functionalised with peptides of cell binding motifs and glycosaminoglycans (GAGs). The microarrays will be cultured with cells to assess for cell binding.

The various techniques to be taught during this placement will be:
- Microarray printing (additive manufacturing)
- Cell culture
- Time of flight secondary ion mass spectrometry
- Polymer synthesis

Location: University Park;

Full project description: Cell behaviour is driven by reciprocal interactions between cells and the matrix that surrounds them. Signals encoded in matrix proteins and glycans (primarily glycosaminoglycans (GAGs)) remain a poorly understood and under exploited method for controlling cell behaviour. This project will systematically explore the response of pluripotent stem cells (PSCs) and cardiomyoctyes to combinations of ECM components to enable the design of artificial ECMs (aECMs) that can direct cell fate. Materials created using aECMs will ultimately have application in regenerative therapies and disease models.

The manner in which cells interact with the ECM, the key ECM factors associated with specific biofunctions, the role of the complexity observed in ECM and how much complexity is required to direct a specific cell fate is currently poorly understood[1]. This

project will address this problem through the creation of a material screening platform that allows for the rapid assessment of hundreds of individual and combinatorial aECMs. The project will focus on peptides with cell binding motifs present in the ECM and a variety of GAGs.

Research objectives - We hypothesise that combinations of ECM components can instruct the differentiation of PSCs and cardiomyocytes. This project will define how cells interact with and are controlled by their ECM. We refine this to 4 objectives.

Objective 1. Creation of a platform technology for screening a synthetic library of ECM components where both peptides and GAGs are mixed combinatorially, utilising additive manufacturing methodologies. This will enable thousands of combinations of GAGs and peptides to be screened in parallel for the first time. Using at least 40 base components, this project aims to screen more than 10,000 different materials, representing the most diverse set of aECM materials ever produced. This project will make use of unique GAG-like materials prepared from renewable sources by Brooke Farrugia at the University of Melbourne. The student on this project will be supported to apply for funding to travel to the University of Melbourne to prepare materials for screening.

Objective 2. Develop analytical tools to enable the robust and comprehensive surface characterisation of the chemical and physical composition of materials. This will make use of the extensive and world leading surface analytical capabilities at the University of Nottingham including the OrbiSIMS instrument.

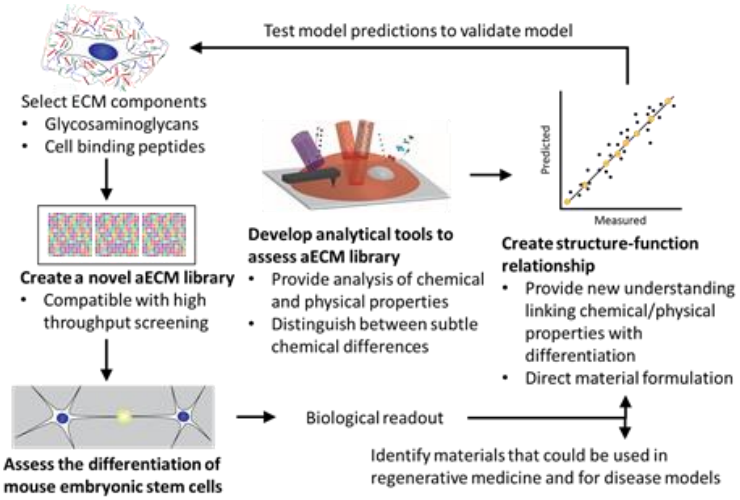
Objective 3. Assess the impact of the aECM environments on the earliest cell fate choices of PSCs and cardiomyocytes, for the purpose of identifying materials with potential for regenerative medicine applications. The project will make use of the world leading expertise in stem cell culture present at the University of Nottingham. Cardiomyocyte cell culture will be overseen by James Smith (University of East Anglia).

Objective 4. Establish a structure-function relationship between cell response and aECM formulations to extend the current understanding of the complex cell-ECM interaction utilsing machine learning and big data analysis approaches.

Reference -

[1] K. A. Kyburz and K. S. Anseth, Ann. Biomed. Eng. 2015, 43, 489

Full project location: University Park;



Genetic and biological rhythm drivers of molecular metabolism in fat and muscle

Project Supervisor: Rebecca Dumbell (NTU)

School: School of Science and Technology

Description: Rhythmic gene expression in multiple adipose tissue depots.

All cells in all tissues ever investigated have an innate daily rhythm, and these rhythms can be disrupted by interventions such as high fat feeding. These tightly controlled rhythms are essential for the normal functioning of cellular processes and coordinate processes within the whole body.

In this rotation you will carry out RNA extraction from multiple adipose tissues from mice fed a high rat diet or low-fat control, and tissues collected over 4h timepoints for 24h. Gene expression will be measured by qPCR and rhythmic gene expression calculated using statistical analyses such as 2-way ANOVA and sine-wave fitting using clock-lab software. This investigation will test for disruption of circadian clock genes as well as Zfhx3 and genes important for specific tissue physiology. This will form a discrete dataset that may contribute to the further research project and will provide the student with key molecular skills required for their PhD.

Additional training will be given in cell culture of preadipocyte cell lines, and differentiation to mature adipocytes, including processing and staining the cells for indicators of differentiation. This will establish the cell culture protocol to be carried forwardin the PhD.

Location: Clifton Campus;

Full project description: Links between disrupted biological rhythms and metabolic diseases like obesity, cardiovascular disease and type 2 diabetes are clear; with shift work, eating at the "wrong" time of day and jetlag all contributing to increased disease risk. Therefore, it is important to understand how disruption of daily biological rhythms (circadian rhythms) may lead to such diseases.

This project will investigate the transcription factor zinc finger homeobox-3 (ZFHX3) and its role in fat and muscle tissue, using mice and human cell culture systems. ZFHX3 is a regulator of circadian rhythm in mice, and rare genetic variants in ZFHX3 are associated with lower body mass index (BMI) people, and thus are protective against metabolic disease. CpG methylation of the ZFHX3 gene has been associated with the epigenetic (ageing) clock. Pilot data from the Dumbell lab demonstrate lower body weight, fat mass and metabolism in Zfhx3 mutant mice (Nolan et al BioRxiv 2022) and implicate ZFHX3 in in growth, metabolism, and healthy aging.

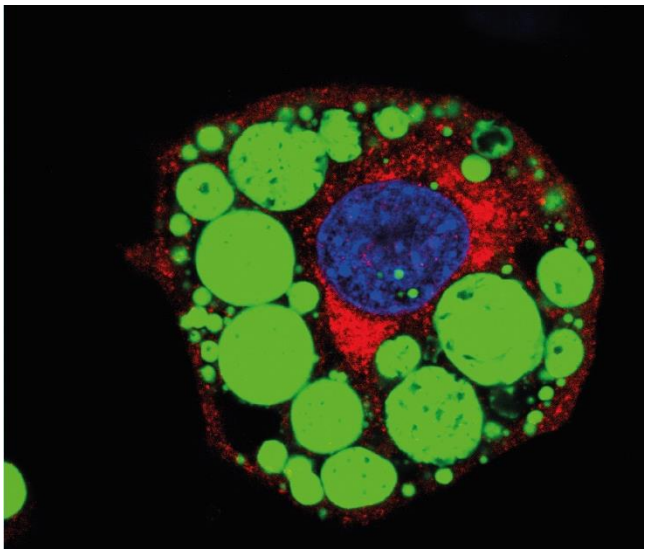
ZFHX3 is highly expressed in metabolically important tissues such as adipose and skeletal muscle, however its role in regulation of molecular processes in these tissues are completely unknown. ZFHX3 has been implicated in the regulation of JAK/STAT signalling component, signal transducer and activator of transcription-3 (STAT3). STAT3 has a known role in the regulation of cell growth and interactions with insulin, leptin and growth hormone signalling – all hormones involved in metabolic health.

The aim of this PhD project is to understand the role of ZFHX3 in circadian rhythm, differentiation and metabolic function in metabolically important cells, using molecular techniques, cell lines and tissues from mutant mice. This project is therefore composed of four main hypotheses:

1. ZFHX3 is required for normal circadian rhythm in peripheral cell lines
2. Knockdown or overexpression of ZFHX3 leads to altered differentiation of preadipocyte and muscle myoblast cell lines, through altered JAK/STAT signalling.

3. ZFHX3 plays different roles in different fat depots from mice.
4. Hypothesis: Human genetic variant of ZFHX3 alters action on circadian and/or metabolic processes in cell lines.

This project has the advantage of including molecular, in vitro (human and mouse cells), in vivo (mouse models) and human variant experiments, considering genetic and molecular regulation of this transcription factor. The student completing this project will gain skills in molecular techniques such as qPCR, CRISPR/CAS9 genetic modification of cell lines, signal pathway investigation using western blot and ELISA, histology, and image analysis, as well as the opportunity for mouse work. NTU is particularly well equipped to carry out metabolic experiments, from cell to whole animal, and this project takes advantage of these leading facilities. The student carrying out this project will be integrated into the national UK Clock Club and the Adipose Tissue Discussion Group national networks as well as learned societies such as the Society for Endocrinology and the Genetics Society and will have the opportunity to present their work regularly at such meetings to generate discussion and build their own professional networks.



Full project location: Clifton Campus;

Cracking the Code: Structural Basis for Regulation of Gene Transcription in HIV

Project Supervisor: Aditi Borkar

School: Veterinary Medicine and Science

Description: Regulation of gene transcription is a complex process involving multiple cellular factors. In the case of viruses, the pathogen hijacks the host machinery to propagate its own life cycle. Thus, there is significant interest in understanding the mechanism of this process for developing novel drugs and vaccines.

In this lab rotation, we will use a cell free expression system derived from tobacco sp. (commercially available as a kit) to express key proteins involved in regulation of gene transcription in the cell. The protein expression will be analysed using molecular biology and biochemistry techniques such as SDS PAGE Electrophoresis and Western Blot Analysis.

This lab rotation will equip the student with hands-on expertise in molecular biology, biochemistry and cell-biology techniques, which are fundamental and transferable skills for a wide variety of wet-lab projects.

The student will be placed at the dynamic and stimulating environment of Wolfson Centre for Global Virus Research, where a number of PhDs, postdocs and technical staff will provide plenty day-to-day assistance.

Location: Sutton Bonington Campus;

Full project description: The HIV virus exploits the human transcription machinery to replicate its genetic material, generating multiple copies of its own genome. This

process, called transactivation, plays a crucial role in the virus's infection cycle, making it a subject of intensive scientific investigation over the last two decades. Researchers are interested in understanding the intricate molecular mechanism of transactivation, as well as its potential implications for designing effective antiviral drugs.

Despite significant progress, there remains a knowledge gap concerning the complete transactivation complex (TAC), which involves a combination of human and viral proteins and RNA molecules. Our project aims to fill this knowledge gap by elucidating the structure and dynamics of TAC, thereby shedding light on the fundamental mechanisms governing the viral life cycle and fostering the exploration of innovative therapeutic approaches.

The primary objective of this research is to determine the structure of a functional TAC. To achieve this goal, the protein constituents of TAC will be obtained through recombinant expression within a plant-based expression system, while the RNA components will be synthesized in vitro using commercially available kits. Subsequently, the complex will be purified directly onto Electron Microscopy grids utilizing a specialised technique called affinity grid purification, originating and patented from the primary supervisor's laboratory (WIPO Patent No.),

For the final step of analysis, high-resolution cryo-Electron Microscopy will be employed, utilizing the state-of-the-art facility available at the University of Leicester under the guidance of second supervisor Dr. Emma Hesketh. This cutting-edge microscopy approach will enable the construction of a comprehensive three-dimensional model of the transactivation complex.

Ultimately, the outcomes of this investigation hold promise for advancing our understanding of the regulation of gene transcription, both in cellular health and viral disease.

Full project location: Sutton Bonington Campus;

Proteomic and electrophysiological features of the aged human neuromuscular junction

Project Supervisor: Mathew Piasecki

School: Medicine

Description: The PhD student will be based at purpose-built research labs, which are housed in the Derby designated MRC Versus Arthritis Centre for Musculoskeletal Ageing Research (CMAR). These labs are specifically equipped to combine human physiology with in vivo and ex vivo molecular biology providing an all-encompassing research perspective. The student will be trained in all methods specific to this project, with all relevant expertise within the supervisory team. This includes a range of applied human methods (electrophysiology, intramuscular and high density surface), computational approaches (signal decomposition), and molecular biology and proteomic techniques (PCR, immunohistochemistry, proteomics), for which full training will be provided. All ethical approval will be in place prior to commencing, and all human applied methods and signal decomposition analyses will be taught as part of ongoing experiments. Molecular aspects of the training will be performed using existing tissue banks (animal and human). Anything generated from this training will form pilot data for the remainder of the PhD project. The student will be immediately enrolled into our existing PGR community and will have access to a range of opportunities within our group and the wider lab.

Location: Derby Royal Infirmary;

Full project description: Average life expectancy has increased as a result of significant improvements in healthcare and pharmacological intervention, however this has not been equalled by an increase in healthspan, with the prevalence of chronic disease also on an upward trajectory. A large contributor to these age-related co-morbidities is the loss of skeletal muscle mass and function, known as sarcopenia, which can result in reduced mobility, increased social isolation and an enhanced propensity for falls. The age-related loss of function is largely attributable to altered neural input to muscle and dysregulation at the neuromuscular junction (NMJ). The NMJ is a chemical synapse which sits at the distal region of the motor neuron and bridges the communicative gap from nerve to muscle to initiate muscle fibre depolarization and contraction. Dysregulation can be characterised by increased variability of motor nerve discharge timings (discharge rate) and greater synaptic transmission instability. As such, the NMJ is essential for coordinated muscle contraction during activities of daily living and fine motor control, both known to deteriorate with advancing age. To delineate the role of the NMJ in human ageing, dysregulation must be considered at pre- and post-synaptic sites and encompass in/ex-vivo functional parameters. Recent methodological advances, partly developed in our labs, including high frequency sampling of intramuscular electromyography (iEMG) data and advanced decomposition, now enable in-vivo simultaneous generation of detailed imaging biomarkers of the NMJ to examine nerve-muscle interactions. Although ex-vivo analysis of the human NMJ has proven to be notoriously difficult, relying upon post amputee or cadaveric models, a relatively simple technique of applying low intensity percutaneous electrical stimulation and linear array electrodes has enabled mapping of NMJ-dense locations in muscle, allowing for targeted muscle biopsy sampling approaches towards enrichment of NMJ (e.g. biopsy sampling of the vasti showed a 15-fold increase in NMJ yield when compared to traditional techniques).

Herein, we propose a novel paradigm of in/ex vivo methodologies in which we will characterize the "physiol-OMICS" of the aged human NMJ in comparison to young, and the plasticity in response to a targeted supervised intervention. More specifically, the proposed PhD project will address three hypotheses:

H1: Motor nerve discharge variability and NMJ transmission instability will be greater in healthy older people compared to young.

H2: This abnormal function of the aged NMJ is underpinned by dysregulation of distinct protein signatures at pre and post synaptic sites.

H3: The structure, function, and proteome of the NMJ demonstrates a level of plasticity that is positively influenced by resistance training exercise.

These will be addressed via the recruitment of 24 young (18-35yrs) and 24 older (65-80yrs) men and women (50:50 ratio) who will undergo 16 weeks of unilateral quadriceps resistance training. Functional, electrophysiological, and proteomic analyses will be performed pre- and post-intervention on both vastus lateralis muscles, with the untrained serving as a control. Data generated here will engender minimally invasive techniques and technologies to quantify the mechanics and molecular aspects of the aged human NMJ, providing both mechanistic insight and translational interventional relevance to clinical practice.

Full project location: Derby Royal Infirmary;

Investigating the neurobiological mechanisms of psychedelics and their potential to treat affective disorders.

Project Supervisor: Claire Gibson

School: Psychology

Description: The student will be based within both supervisor's research groups. There will be time to undertake reading on the topic of psychedelics and psychiatric disorders whilst learning techniques of relevance to the main project. Initial studies will include the isolation of adult Drosophila brains. Brains will be imaged to second messenger molecules using genetically encoded sensors. We will teach how to record electrical activity by whole cell patch clamp of identified neurons (targeted expression of GFP in dopaminergic and serotonergic neurons) to study synaptic function and neuronal excitability. We will further introduce behavioural experiments to study locomotor activity, anxiety, circadian/sleep activity and learning and memory. This rotation will thus incorporate work to introduce approaches utilising flies but importantly also exposes the student to experimental strategies applied for the planned rodent work which covers a range of behavioural assays to study psychiatric disorders such as depression and anxiety.

Location: QMC;University Park;

Full project description: Background: The need for new treatments for psychiatric disorders cannot be underestimated given that major depressive disorder (MDD) has a population prevalence of 17% (UK) and the incidence is rising globally. Antidepressants such as selective serotonin reuptake inhibitors (SSRIs) act via increasing monoaminergic transmission, which is often depleted in MDD patients and work via prevention of serotonin reuptake within the synapse by blocking the reuptake transporter protein located in the presynaptic terminal. Despite their wide-ranging use, SSRIs are only effective for around 30%-40% of patients, their actions are not well understood, and they commonly produce intolerable side-effects.

Psychedelics and their incredible power to alter the perception of reality has been known and utilised for millennia. Their effects have been harnessed as sacramental tools to aid religious and spiritual ceremony; although within the last 70 years, their applications within neuropharmacology have initiated a renaissance in serotonergic and glutamatergic psychedelic research. We are yet to fully understand the details of their mechanisms of action and behavioural effects although evidence supports their role in altering consciousness and alleviating symptoms associated with MDD, anxiety, obsessive compulsive disorder and post-traumatic stress disorder. Through understanding their mechanisms of action may reveal novel therapeutic targets for more effective treatment of psychiatric disorders.

Importance:

1. Creating a new approach to elucidate the mechanisms of psychedelics' signalling in different subsets of neurons within a simple in vivo model. Typically, research related to psychiatric disorders involves use of rodents with time consuming and often aversive behavioural paradigms.
2. The use of Drosophila is novel approach allowing us to interrogate specific neurotransmitter pathways and neuronal circuits using highly sophisticated experimental methodologies coupled with available genetics. This way we will be able to expand and gain knowledge to apply a targeted confirmatory subset of experiments using rodents and exploring their mechanisms which may open up novel treatment targets.

Objectives:

1. Generation and characterisation of fly strains to test the effects of disrupted monoaminergic neuronal activity: A range of Drosophila lines will be established which exhibit altered monoaminergic and glutamatergic transmission. We will identify the effects of receptor and precursor knock-outs/knock-downs/overexpression (i.e. serotonin receptor, tryptophan hydroxylase and amino acid decarboxylase) in subsets of neurons to establish phenotypes assess at neuronal and behavioural levels. Electrophysiological and live imaging (calcium imaging: GCaMP6s, FRET imaging: cAMP) studies will investigate neuronal activity and be complement by behavioural studies.
2. Elucidation the target pathways of psychedelic actions: Measurements (physiology, imaging) of neuronal activities and whole animal behaviours will be assessed using the above lines in the presence of various psychedelics. This will define the circuits and subpopulations of neurons which are involved in responses to psychedelic actions.
3. Translational validation in mouse studies: The above information will allow us to interrogate specific neuronal networks and transmitter pathways in mouse which are affected by psychedelic actions. We will apply specific pharmacology to identify serotonergic signalling causing behavioural phenotypes of psychedelics and complement these findings with in vitro brain slice electrophysiology to characterise corresponding changes in neuronal activity.

Full project location: QMC;University Park;

Using machine learning to uncover patterns in the evolution of protein sequence and function across eukaryote genomes

Project Supervisor: Maria Rosa Domingo Sananes

School: School of Science and Technology (NTU)

Description: Our capacity to read the genomes of any living organism has recently increased dramatically. We now have whole-genome sequences for thousands of species and the numbers keep rising. In addition, thanks to powerful computers, we can compare the sequences of similar genes from many species to assess how they have changed during evolution. This has revolutionised our understanding of the diversity of life on our planet and how different species are related to each other. However, we still know little about how changes in DNA sequences relate to changes in the function and characteristics of living organisms. This project proposes to tackle this problem by analysing how gene sequences and functions have changed over time. To do this, we will first characterise patterns of how the sequences of many different genes have changed in many different species. During the short project, you will analyse rates of evolution for selected gene families across a curated set of eukaryotic genomes, and assess if there are significant differences for different gene classes. This short project will be completely computational. Previous experience with bioinformatics and programming is desirable, but training will be provided. You will gain skills in bioinformatics, data analysis and phylogenetics.

Location: Clifton Campus;

Full project description: During the last decade, we have experienced a dramatic increase in information about the diversity of life on our planet, largely due to the revolution in genome sequencing. We now have full genomes for thousands of species and more yet to come. Along with this, there has been great progress in reconstructing the evolution of sequence evolution and divergence, thanks to a combination of

increasingly sophisticated statistical models along with increased computational power. However, this increased understanding of the diversity of protein and genome sequences has not necessarily translated into understanding the evolution of function(s) (the 'genotype to phenotype' problem). The overall aim of this project is to develop a systems-level understanding of how protein functions change as sequences diverge. To do this, we will focus on analysing proteins from eukaryotic genomes to perform:

Aim 1. Computational analysis of protein sequence divergence across whole genomes to gain a systems-level view of protein sequence divergence in eukaryotes. We will bioinformatically infer sets of related genes in model species with fully sequenced genomes. We will then analyse how different gene sets have changed by investigating the relationship between the time of divergence between species and sequence divergence. We will classify different patterns using non-linear regression and machine learning approaches and analyse whether the observed patterns correlate with predicted functional properties, such as belonging to similar cellular processes or having similar roles in distinct processes (protein kinase, transcription factor, membrane protein). This analysis will provide a eukaryote-wide genome-level view of how proteins involved in different functions change and allow us to detect general evolutionary patterns.

Aim 2. Systematic complementation analysis of selected proteins to assess how function changes with sequence divergence. For this analysis, we will choose proteins that represent the diverse patterns assessed in Aim 1, and that have orthologs whose deletion or mutation causes fitness defects in our experimental model systems: the yeasts Schizosaccharomyces pombe and Saccharomyces cerevisiae. We will experimentally replace the endogenous gene with multiple sequences encompassing the diversity of each gene family and characterise how well the different sequences complement function. Like Aim 1, we will analyse if these patterns of function versus sequence vary for different proteins and whether they are related to functional properties.

This project approaches one of the most important outstanding questions in biology: how to link genotype and phenotype. These results will tell us if analysing evolutionary patterns can inform the prediction of function based on sequence information alone. The main novelty of this project is to attempt this at a systems level, by analysing multiple genes at different evolutionary scales, and by linking our computational and experimental analyses through patterns of sequence and function evolution across genes and species. During this project, you will acquire knowledge in advanced bioinformatics, data analysis, phylogenetics as well as lab skills in molecular biology and genetics.

Full project location: Clifton Campus;

Safeguarding DNA in Humans: Understanding Structure and Function in the Tumour Suppressor 'Hub' HELQ-RPA-POLD-CX3

Project Supervisor: Ed Bolt

School: Life Sciences

Description: This will welcome students to join PhD students, Post-Doctoral Researchers and the PIs in a collaboration using molecular biology, biophysics and genetic editing to understand human DNA repair. This is revealing how human cells safeguard against broken chromosomes that, if unrepaired, trigger neurological diseases, ageing and cancers.

The rotation is based in Prof Bolt's lab in the QMC Medical School. It will introduce lab techniques and principles for defining the molecular basis of protein-protein and protein-DNA interactions. In 9-weeks this will focus on interaction between the human HELQ protein and one of CX3, POLD or RPA. The rotation will provide knowledge and practical lab training covering:

- Alpha-Fold and in silico methods that visualise and manipulate protein structure (all weeks, ad hoc)
- Protein purification using FPLC. Assays for protein binding to DNA and to other proteins (weeks 2-6)
- Propagation of human cells and the principles for CRISPR 'Prime' editing (weeks 4-7)
- Use of a Lumicks C-Trap for real-time analysis of protein-protein and protein-DNA interactions (week 8)
- Week 9 will review the rotation experience, supported by the ongoing informal meetings and discussions between Bolt and Soultanas research groups. This will provide further expertise and insight into the full PhD project.

Location: QMC;

Full project description: The PhD project is part of a well-established collaboration to understand how human cells overcome the potentially catastrophic consequences of broken chromosomes, by repairing them without causing mutation. In the last 5 years we and others have identified a 'hub' of protein-protein and protein DNA interactions that are crucial for this, allowing our cells to survive and replicate: two examples of very recent published research from us and another international team can be viewed in the next section, and Prof Bolt will be happy to provide further examples and background about what we do (contact: ed.bolt@nottingham.ac.uk).

The full project will have three major components that each provide training in cutting-edge methods and will advance our knowledge of human DNA repair:

(A). Molecular biology of HELQ-RPA-POLD-CX3 in controlling DNA break repair. This HELQ interaction 'hub' modulates DNA unwinding, DNA synthesis and DNA annealing reactions, the three essential steps for repairing broken chromosomes. General background to this is provided in Reference 1 in the next section. This part of the full project will combine protein purification (HELQ, RPA, POLD, CX3) and assays in vitro, with a genetic experiment in human cells, together testing a new hypothesis for how human cells repair broken chromosomes without introducing mutations.

(B). Biophysical analysis of the molecular structure of HELQ-protein interactions using state-of-the-art methods on Nottingham University campus; C-Trap optical tweezers ("Lumicks-C") and Nuclear Magnetic Resonance (NMR). These define interactions of human HELQ with DNA (e.g., DNA annealing and unwinding) in response to its protein-protein interactions, and identify the atomic details of interaction between HELQ, RPA and POLD.

(C). CRISPR Editing of HELQ in human cells to translate knowledge gained in (A) and (B) into (predicted) defective DNA repair in human cells. This part of the project will deliver two amino acid substitutions into chromosomal HELQ using base- and prime-editing. We predict one substitution will inactivate HELQ-DNA function, and the other will inactivate HELQ-RPA interaction. Both therefore effectively dismantling the HELQ 'hub'. Cells lacking HELQ are highly sensitive to DNA damaging drugs such as mitomycin C, providing a ready genetic assay to test if our molecular discoveries have the same effect, validating them in human cells.

Additionally, through our existing collaboration with industrial/biotechnology companies in Cambridge and Glasgow there will also be opportunity to get involved in X-ray

crystallography as a route to solving molecular structures, as the 'PIP' component of the DTP PhD programme.

Full project location: QMC;

Multi-omics to identify placental epigenetic interactions associated with micronutrient deficiency in pre-eclampsia

Project Supervisor: Adaikala Antonysunil

School: School of Science and Technology (NTU)

Description: The lab rotation (LR) operating between NTU and UoN (over 9-week period) will provide the student with complementary experience in (i)clinical (ii)epigenetic and (iii)bioinformatic analyses (all techniques applicable to the main project).

The student will gain an understanding of the clinical samples from the cohort (based at UoN) through access of the database of participants recruited, the clinical variables already available and the logistics from Dr Mistry, KCL, CI of the original study. A small subset of human placental tissues from women with sufficient or deficient concentrations of B12 will be chosen and subjected to RNA isolation & cDNA synthesis. Gene expression and CpG methylation will be determined using qRT-PCR (Quantstudio7) and pyrosequencing (PyromarkQ48) in Dr Adaikala Lab, NTU. To determine the significance of the interaction of methylation/miRNA/gene and perform the integrated omics analyses, training will be given to the student by our collaborator Dr Daniel D'Andrea (Bio-informatics expertise, NTU) on machine learning approaches and the computational methods for the basic software. In Prof Gardner lab, UoN, we will determine levels of steroid metabolites in blood using GC-MS/MS and perform protein expression by immunohistochemistry/immunofluorescence.

Training in all methodologies including gene/protein expression, mass-spectrometry, pyrosequencing and data/bioinformatic analyses will be provided.

Location: Clifton Campus;Sutton Bonington Campus;

Full project description: Background: The rapid increase in obesity and rise in maternal age has led to growing population of women with pre-eclampsia (PE), the hypertensive disorder affecting between 2-5% of pregnancies and a leading cause of maternal/perinatal mortality and morbidity. PE is also associated with adverse pregnancy outcomes including preterm delivery and fetal growth restriction. This affects both mother and fetus, and have been associated with higher future risk of cardiovascular disease. Studies focusing on developmental origins of cardiometabolic manifestations suggest that micronutrients like vitamin B12 have a key regulatory role. Low maternal B12 has been associated with the development of PE and with higher cortisol and cardiovascular responses to stress in the offspring during adolescence. Studies also demonstrated associations between placental DNA methylation and increased maternal blood pressure during pregnancy, at genes implicated in cardiometabolic diseases. Our studies have shown that B12 deficiency in adipocytes, hypomethylated transcription factors of lipid regulation and altered the adipose-derived miRNAs targeting insulin resistance.

Hypothesis: The placenta generally tends to be less methylated to regulate fetal development. If placentae are subjected to further reduced methylated state in response to low B12 levels, this could lead to placental dysfunction. Although methylation can

regulate CpG islands on both gene and miRNA promoters, this coordinated action of methylation has greater functional impact on regulation of transcriptome. Therefore, we hypothesise that low B12 in the placenta could lead to aberrant methylation at gene/miRNA promoters which alters mRNA expression, representing an unexplored layer of gene regulation that is likely to influence metabolic dysregulation resulting in PE. We propose an integrative analysis to identify the epigenetic interactions of the methylation of CpGs on genes and miRNA encoding genes associated with transcriptomic changes in placenta of PE with low B12.

Experimental approach: Placental tissue and blood samples will be obtained from a cohort in collaboration with Prof Gardner (UoN) and Dr Mistry (KCL). The biobank has 559 pregnant women including normotensive and PE. The cohort is well-characterised with complete history of the mothers, blood collected in late pregnancy and placental tissue during delivery. Serum B12, folate, metabolic parameters (glucose, lipids) and steroid metabolites (GCMS, UoN) will be measured. Placental tissue samples will be chosen from mothers circulating B12 levels (low B12 <220pmol/L). Based on the rates of hypertension with B12 deficiency, a sufficient sample size (n=30) will be selected to adequately power (90%) the study (α-0.05, two-tailed). First, we will identify the differentially expressed mRNA, miRNA, and differentially methylated genes by analysing the omics data (mRNA-seq/miR-seq/RRBS). The multi-Omics and bioinformatic analysis will be performed by Novagene. We will then employ an integrated analysis of the omics/clinical/metabolites data to identify the epigenetic interactors between methylation-miRNA-gene network and its association with metabolic pathway. Next, we will validate these targets using qRT-PCR and pyrosequencing.

Outcome: Understanding these epigenetic events will be an effective tool in early prediction of PE and aid to develop targeting therapies to break the intergenerational cycle which would decelerate the risk of PE and cardiovascular risk in offspring.

Full project location: Clifton Campus;Sutton Bonington Campus;

Synthetic embryology: How to build a mammalian embryo

Project Supervisor: Ramiro Alberio

School: Biosciences

Description: During the lab rotation the student will learn how to grow embryonic stem cells and setup gastruloids to model early development in vitro. The student will also conduct embryo dissection and fluorescent staining to label cell membranes. Stained embryos will be images under a confocal microscope to acquire high resolution images for downstream analysis required for the modelling component of the project. Alongside the wet lab activities, the student will be introduced to single cell transcriptomic techniques for large scale transcriptomic analysis of developing embryos. The student will also be introduced to computational modelling approaches to create predictive computational models of development using Phyton and R environment. During this rotation the student will become familiar with to basic techniques used for gene expression analysis of sequencing dataset as well as gain basic understanding of modelling tools.

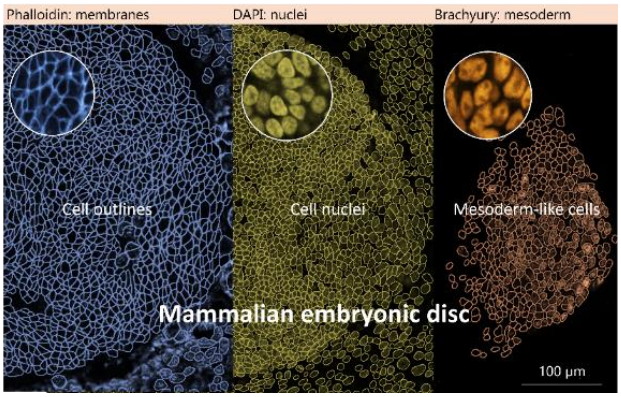
Location: Sutton Bonington Campus;

Full project description: Gastrulation defines a key developmental period in which epiblast cells undergo differentiation into the embryonic germ layers, and establish the basic animal body plan. This process is the result of multi-scale (from single cells to

tissues) events orchestrated by co-ordinated mechanical and biochemical cues. Although there is broad similarity in the gastrulation process in mammals, tissue organization and morphogenesis can vary considerably between species. This is most notably evidenced in species who display diverse embryo/extraembryonic geometries. Differences in extraembryonic tissues (EET) (e.g. extraembryonic ectoderm (ExE) of mice, extraembryonic mesoderm (ExM) in non-rodents mammals) and the conspicuous cup shape of the mouse epiblast compared with the flat bilaminar disc in non-rodent mammals (including humans) characterize some of the overt embryological differences. Remarkably, despite these differences, species convergence in a phylotypic stage (the period with highest morphological similarity across animals) appears to be reached soon after the completion of gastrulation, suggesting that disparities in tissue relationships within the mammalian conceptus may reflect alternative routes for attaining the same developmental process (e.g. such as symmetry breaking and Anterior-Posterior (A-P) patterning).

Embryo development is governed by morphogenetic processes across time, the integration of which has been conceptualized as "tissue tectonics". Here we apply this concept to investigations into the relationships between cell intrinsic (gene expression and cell geometries) and cell extrinsic (signalling) mechanisms governing onset of gastrulation in bilaminar disc embryos. This project will reveal conserved/divergent signals regulating these fundamental developmental processes in different mammals, with relevance to human development. A better understanding of co-opted developmental mechanisms will improve our understanding of mammalian phylogenetics and species diversity. Importantly, this new knowledge will be a platform for adapting conserved principles to synthetic embryology in the future, and for improving methodologies to model gastrulation in vitro, with applications in regenerative medicine and biotechnology. We hypothesize that symmetry breaking and the onset of gastrulation in the mammalian flat disc epiblast is determined by the interplay between biochemical signals, cell geometry and movement influenced by boundary conditions established by EET. The aim of the project is to integrate spatial gene expression and cellular geometry and dynamics information that characterizes the onset of gastrulation in a mammalian flat disc embryo to create a blueprint of the morphogenetic-signaling processes governing the establishment of the mammalian body plan. We will investigate the mechanisms of gastrulation in bilaminar disc embryos using multi-scale and multi-species comparisons combining: 1) spatial transcriptomics, 2) computational modelling and 3) functional analysis using in vitro (gastruloids) and in vivo embryo manipulations to understand the interplay between biochemical signals, cell geometry and movement during symmetry breaking and primitive streak (PS) formation.

The holistic, interdisciplinary investigations will establish the interplay between self-organization principles and tissue interactions during bilaminar disc embryo development. This project is closely aligned with aims of other BBSRC-funded projects in our laboratories.



Full project location: Sutton Bonington Campus;

Understanding lymph node determinants of therapy response

Project Supervisor: Jacqui Shields

School: Medicine

Description: The lymphatic network and lymph nodes act as sentinels of the immune system. Lymphatic vessels continually deliver tissue-derived signals for lymph node constituents to assess and initiate an immune response when needed. This system, however may be hijacked by tumours as a route of escape. We are keen to understand how therapy impacts this relationship, and lymph node function since therapeutic interventions perturb the tumour ecosystem, thus the signals and cells transported to lymph nodes will also change. We aim to identify the changes that occur in lymph nodes in response to treatment.

In this 9 week rotation, the student will:

a. learn to perform initial immunofluorescence staining of murine treated tumour draining lymph node tissues to characterise gross adaptations.
b. learn to conduct initial functional studies to show how factors released by tumour cells after therapy impact immune cell function. e.g. measurement of phagocytosis by macrophages

Location: University Park;

Full project description: The lymphatic network and lymph nodes act as sentinels of the immune system. Lymphatic vessels continually deliver tissue-derived signals for lymph node constituents to assess and initiate an immune response when needed. This system, however may be hijacked by tumours as a route of escape.

Tumour-associated lymphatic vessels drain material away from tumours to connected lymph nodes. The fluid, or lymph, they carry is rich in tumour-derived proteins, metabolites, DNA and immune cells. These factors induce significant lymph node remodelling, driving changes to immune composition and organisation. This ultimately disrupts anti-tumour immunity and provides a friendly niche for disseminating tumour cells.

With our growing understanding of the intimate relationship between tumours and draining lymph nodes as cancers develop, we are now keen to understand how therapy impacts this relationship, and lymph node function. Therapeutic interventions perturb the tumour ecosystem, thus the signals and cells transported to lymph nodes will also change. We aim to identify the changes that occur in lymph nodes in response to treatment, if these are specific to responsive tumours, and if we can then use the knowledge gained to boost therapy effects in non-responders.

The student will:

- Deeply phenotype the tumour draining lymph node immune and stromal microenvironment ; combining single cell transcriptomics and highly multiplexed imaging of therapy-treated murine and human tumours.
- Perform high dimensional assays such as mass spectrometry to identify contents of lymph from mice receiving therapy, revealing components specific to responders or non-responders (cellular, vesicle, soluble protein or exosomes)
- Mechanistically resolve the relationship between therapy response and lymph node adaptations in preclinical models, performing perturbation studies to identify interactions that may be disrupted to boost therapy response.

Full project location: University Park;

Investigations into the pangenomic diversity of the Prairie Epidemic Strain (PES) of Pseudomonas aeruginosa.

Project Supervisor: Fiona J Whelan

School: Life Sciences

Description: This project consists of two Aims with a focus on bioinformatic and microbiology methods. In the nine-week lab rotation, the goal will be for the student to get a sense of both research areas as well as the importance of this research to the cystic fibrosis community.

The rotation project will focus on a previously collected set of ~300 PES isolates and their genomes/metagenomes collected from an individual over 3 timepoints with the goal of quantifying the phenotypic and genetic diversity observed over time. Equal time allocation will be given to (1) microbial culture and in vitro investigations. The student will use high-throughput phenotypic assays (e.g., to identify mucosity, swarming/swimming ability, antimicrobial resistance) to quantify the phenotypic diversity of PES isolates. Results will be quantified using image analysis tools to, for example, quantify colony size and shape. In parallel, the student will (2) identify the genetic diversity of isolates collected from the individual over time. SNPs, insertions, deletions, and substitutions will be tracked across all isolates to build a picture of which indels are unique/shared between isolates collected (a) at the time or (b) at sequential timepoints. Finally, together we will identify any phenotypic observations which correlate with genetic variants.

Location: University Park;

Full project description: The Prairie Epidemic Strain (PES) is a clonal strain of Pseudomonas aeruginosa that infects the lungs of individuals with cystic fibrosis (CF). Individuals who are chronically colonized by PES have increased morbidity and mortality compared to individuals colonized by non-epidemic strains of P. aeruginosa. Currently, we do not understand how PES – or other epidemic strains including the Liverpool Epidemic Strain (LES), Manchester Epidemic Strain, and Australian Epidemic Strains, types 1 and 2 (AES1, AES2) – transmit from patient-to-patient or why their infections are correlated with increased mortality in this patient population. Previous analyses conducted in our group on 200 PES isolate genomes and 9 culture-enriched metagenomes has revealed an astonishing level of stability in PES's pangenome structure (i.e., no evidence of gene gain or loss) in contrast to an incredible amount of intra- and inter-patient genetic variation (i.e., single nucleotide polymorphisms (SNPs), insertions, deletions, and substitutions). We hypothesize that understanding this juxtaposition between gene content and variation in the context of other epidemic strains will allow us to uncover the mechanisms by which PES is able to spread in this patient population.

In this project, the student will combine bioinformatic methodologies – including population genetics, comparative genomics, and pangenomics – with classic microbiology to study the intra- and inter-patient diversity of PES. The precise focus of the project, and allocation between in silico and in vitro methods, will be determined in consultation with the student but will be based upon the following aims:

1. Use culture-enriched metagenomics and shotgun genomics to study the population-level diversity of PES in people with CF. Clinical samples collected from individuals with CF will be cultured on an agar that is selective for the growth of P. aeruginosa. A previously developed PCR assay designed to detect PES will be used to screen for its presence in clinical samples collected in the UK (where it's prevalence is unknown)

and Canada (where the prevalence is ~30%). Culture-enriched metagenomic and genomic sequencing will be conducted on PES positive individuals; the student will use these data to compare the intra- and inter-patient diversity of this strain and to identify any correlations between phenotypes observed in vitro and genetic variation observed in silico.
2. Build a pangenome of P. aeruginosa to identify genes, SNPS, and genetic co-occurrence relationships unique to epidemic strains. Recent research on the Escherichia coli pangenome has identified genetic signatures that are uniquely present in unrelated, phylogenetically diverse pandemic strains (Connor C et al. 2022 bioRxiv). Here, we will interrogate the P. aeruginosa pangenome to ask similar questions of the CF epidemic strains, including PES. We will use new software recently developed in our research group as well as cutting-edge machine learning approaches pioneered by the project's second supervisor.

This project will provide the student with a unique combination of bioinformatic and microbiology skills, which will be widely transferable to other areas of biological research. Further, the project can easily be shaped by the student's interests to focus more heavily on either the computational or laboratory-based aspects of the research.

Full project location: University Park;

Role of Glial Fibrillary Protein (GFAP) in the response to Neuronal Degeneration and Injury.

Project Supervisor: Claire Friel

School: Life Sciences

Description: During this rotation you will begin to develop the skills and generate the reagents required for the full PhD project. You will use the better studied intermediate filament protein vimentin as a model to learn how to assemble intermediate filaments from purified subunit protein. You will then use a cutting-edge optical trapping and microfluidics system to trap individual filaments and measure their response to stretching forces. In parallel, and based on protocols previously established for vimentin, you will generate an expression system for GFAP and begin to develop a purification and filament assembly protocol for this astrocyte-specific intermediate filament subunit. There will also be an opportunity to observe the imaging techniques used to observe the localisation and function of GFAP in cultured-cells and whole organisms. Therefore, this rotation will give a full view of the PhD project and set up the experimental systems and protocols necessary for the full project.

Location: QMC;University Park;

Full project description: Glial Fibrillary Acidic Protein (GFAP) is a Type III intermediate filament protein and the characteristic cytoskeletal protein in astrocytes. GFAP filaments are responsible for maintaining the structure and mechanical strength of astrocytes. Upon traumatic brain injury, expression of GFAP increases and astrocytes proliferate. Increase in GFAP expression is also a feature of neurodegenerative diseases such as Parkinson's and Alzheimer's. It is hypothesised that GFAP induction may promote neuro-regeneration. Several single point mutations to GFAP cause Alexander disease: a rare disorder that progressively damages the nervous system for which no treatment is currently available. Research to date suggests that the intermediate filament system of astrocytes, primarily composed of GFAP, acts as a crisis command centre that coordinates responses to cellular stress, such as caused by neurological diseases and

injury. The assembly and physical characteristics of GFAP intermediate filaments is poorly understood, this project will uncover the mechanical properties of GFAP filaments, the impact of mutations on these properties and how this relates to the in vivo functions of GFAP filaments and their role in cellular responses to neuronal disease and injury.

This work aims to:

1. Develop an expression and purification protocol for GFAP isoform 1 and assemble GFAP filament in vitro.
2. Characterise the mechanical properties of GFAP filaments and the impact of mutations.
3. Relate the in vitro mechanical properties of individual GFAP filaments to the role of GFAP in vivo in responding to neuronal stress, such as neurodegenerative disease or injury.

Aim 1)
You will use existing protocols for the expression and purification of other type III intermediate filament proteins, such as vimentin, to develop an expression system and purification protocol for GFAP. You will use established conditions for intermediate filament assembly to generate isolated GFAP filaments. This will be the first time GFAP filaments have been assembled in vitro.

Aim 2)
You will use a state-of-the-art optical trapping and microfluidics system to assemble and GFAP filaments with an integrated fluorophore. Using this instrument, you will trap individual filaments and apply a stretching force to determine the force extension relationship of GFAP intermediate filaments. The protocol and data analysis methods developed will then be used to measure the impact of disease-causing single point mutations to the GFAP protein.

Aim 3)
You will apply the knowledge generated in Aims 1 & 2, to better understand the function of GFAP in the nervous system of organisms and the ability of astrocytes neurons to respond to injury. This will be achieved through a combination of mouse behaviour and in vivo microscope of astrocyte and neuron interactions.

GFAP is a critically important protein in the function of astrocytes. This work will use experimental systems ranging in complexity from single molecules to whole organisms to uncover how the physical characteristics of GFAP filaments support the role of the astrocyte intermediate filament system in coordinating the nervous system response to disease and injury.

Full project location: University Park;QMC;

Understanding the lifetime accumulation of antimicrobial resistance genes in the human gut microbiome

Project Supervisor: Dov Stekel

School: Biosciences

Description: The lab rotation will give the student exposure to building a simple agent based model of human gut microbiomes. The purpose of the model will be to understand the impact of lifetime exposure to antibiotic resistance genes and antibiotics on the gut microbiome. The model will be coded in Python or other suitable programming language,

using a suitable agent based modelling framework (e.g. agentpy or mesa). This will give the rotation student exposure and skill development in programming, model development, simulation and analysis. The student will be located in Dov Stekel's computational biology laboratory in the School of Biosciences, benefitting from more experienced computational biology students and postdocs also working in antimicrobial resistance, and co-located in an open plan office environment with other (experimental) microbiology students and postdocs who are part of the Division of Microbiology, Brewing and Biotechnology. The rotation student will also benefit from regular meetings with Fiona Whelan, with expertise in bioinformatic analysis of microbiomes.

Location: Sutton Bonington Campus;

Full project description: The human gut is a complex ecosystem containing a diverse array of microorganisms. While most gut microbes are beneficial and contribute to our health, some bacteria (e.g. Enterobacteriaceae and Enterococcaceae) can become opportunistic pathogens if they migrate to other organs, such as the urinary tract or blood; this is particularly a problem for older or immune compromised people.

The human gut contains antimicrobial resistance genes (ARGs). These become established through lifelong exposure to ARGs and use of antibiotics; indeed it has been shown that the numbers of ARGs in human gut microbiomes increase with age. Thus older individuals are at a heightened risk of resistant infections associated with gut organisms, such as urinary tract or blood infections. Most resistance genes predominantly reside within non-pathogenic and unclassified bacterial species in the gut. However, the ARGs can be located either within the chromosomes of gut bacteria or on mobile genetic elements, and so are able to transfer to potential opportunistic pathogens and thus lead to drug resistant infections.

We have recently published a paper that used an agent-based simulation model to understand how resistance genes accumulate within an individual's gut over their lifetime. This paper received significant global press coverage. We showed that reduced exposure to resistance genes in food and water, coupled with reducing medical antibiotic use, can reduce the long-term increase in resistance within gut microbiomes. Reduction in ARG intake is especially important during periods of antibiotic use.

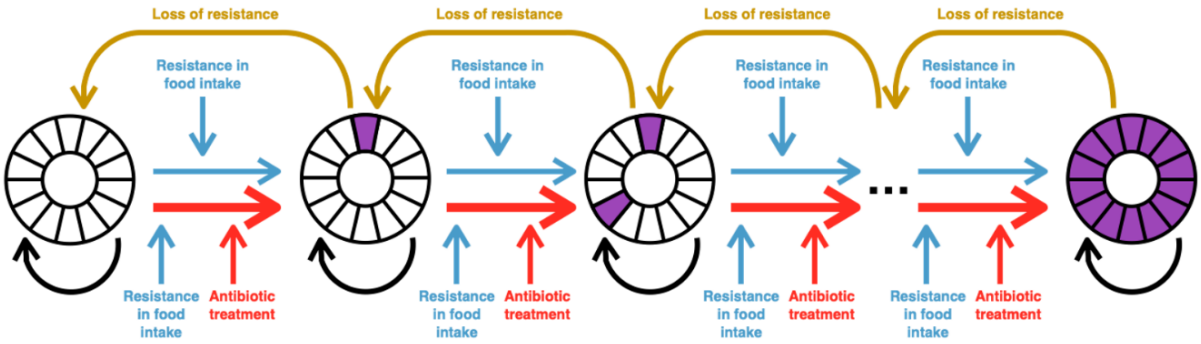
However, that model is somewhat abstract, treating antibiotics and resistance classes as simplified variables. The aim of this project is to construct and analyse a much more biologically informed and detailed model. This comprehensive model will include specific resistance genes, both individually and in combinations, alongside their associations with specific mobile genetic elements, commensal gut bacteria, and opportunistic pathogens. It will also include specifics of lifetime antibiotic usage.

This ambitious project will be facilitated by the wealth of publicly accessible human gut metagenomic data and antibiotic usage records from various countries. An important aspect of the project will be to analyse these metagenomes to calibrate the model to align with real-world data. Once the model is established, it will simulate numerous individuals within a population, accounting for individual variations, differences in exposure to resistance genes, and variances in antibiotic use.

Through these simulations, our objective is to understand how diverse lifetime exposures can culminate in specific drug/bug resistance combinations. This, in turn, will enable us to quantitatively evaluate impact of altered ARG exposure or antibiotic regimes on the likelihood of developing specific drug-resistant infections in old age, such as urinary tract infections or bloodstream infections.

This is a computational project that will develop skills in agent-based models, Monte Carlo simulations, and bioinformatic analysis of genomic data. It is ideal for a student with a keen interest in computational biology and some coding experience.

Full project location: University Park;Sutton Bonington Campus;



Neural and behavioural mechanisms of cognitive flexibility: integrative neuroscience studies of the overtraining reversal effect

Project Supervisor: Silvia Maggi

School: Psychology

Description: Based on the student's interest, we offer a variety of lab rotations relevant to the full project. The main goal of the project is to understand how training on a task and prefrontal-cortical regions contribute to behavioural flexibility when changes in reward contingency occur. This goal requires a varied set of skills ranging from experimental to computational procedures. The mix of training in experimental and computational methods can be determined based on the preferences of the student.

Experimental procedures may include:

- training in cognitive/behavioural testing in rodents (e.g., automated operant task);
- training in in-vivo neuroscience procedures (e.g., stereotaxic brain surgery, intracerebral drug microinfusions, in-vivo electrophysiology);
- training on ethical and legal frameworks required for animal research (i.e. 3Rs, replacement, refinement and reduction of the use of animals in research)

Computational procedures may include:

- training in statistical analysis of behavioural data available in the lab;
- training in coding with Matlab or Python programming languages;
- training in using behavioural modelling (i.e. Reinforcement Learning and Bayesian models) to extract insights from the data

The student will join in with ongoing experimental and/or computational work in the supervisors' lab. They will also attend weekly lab group meetings and relevant neuroscience seminars.

Location: University Park;

Full project description: Responding flexibly to ever-changing environments is a vital skill for survival and daily functioning. Conditions such as obsessive-compulsive disorders (OCD) and schizophrenia often manifest as behavioural inflexibility,

characterized by repetitive thoughts and behaviours. This often leads to misalignment between an individual's actions and their intended outcomes, such as falling into habits. This form of flexibility can be studied in humans and animals with varied learning tasks. The prevailing theory suggests that flexible behaviour is supported by a goal-directed system which drives actions based on desirable goals (e.g. reward maximisation), compared to habit formation, which lacks sensitivity to outcome value (e.g. compulsive responding) (Gillan et al., 2011). According to this dual-system theory, at the behavioural level, the switch between goal-directed and habit formation occurs by overtraining (i.e. repeated exposure to the same task's rule).

However, an intriguing discovery from the late '50s and '60s, known as the overtraining reversal effect (ORE), challenges this notion. This effect suggests that overtraining can facilitate flexible adaptation of behaviour, as reflected by a faster switch to a new rule when rules are reversed (Mackintosh, 1969; Reid, 1953). This phenomenon was shown in humans and rats (Reid, 1953; Sitterley & Capehart, 1966). Yet, due to contradictory results and inconsistencies, this line of research lost momentum in the late '70s. Nevertheless, recently published (Dhawan et al., 2019) and our own (in preparation) results suggest that overtraining can indeed promote flexibility, confirming the initial findings of the ORE.

This project aims to delve into this apparent contradiction. We aim to elucidate the role of overtraining in engaging the goal-directed system through the ORE. By using behavioural testing (e.g., automated operant tasks) in rodents, this project seeks to gain a deeper understanding of the impact of amount of training on behavioural flexibility. Furthermore, in the brain, prefrontal cortical (PFC) regions have been implicated in flexible behaviour based on rapid learning from actions (Banerjee et al., 2020; Floresco et al., 2008; Powel and Redish, 2016; our own recent findings, in preparation). Alterations of PFC regions have also been associated with OCD- and schizophrenia-like symptoms (Ahmari and Rauch, 2021). However, it is unclear how PFC contributions change over learning and extended training.

To fill these gaps, we will use experimental procedures, such as cognitive and behavioural testing with neuropharmacological manipulation in specified regions. We may also use in-vivo electrophysiological methods to determine the neural impact of pharmacological manipulations and to link changes in task performance to neural changes resulting from these manipulations. The neuro-behavioural data produced by these experiments can be analysed by means of statistical approaches, including recently developed Bayesian trial-by-trial strategy analysis (Maggi et al., 2023), to estimate the impact of overtraining and neuropharmacological manipulations on flexibility.

In summary, this project will investigate how training and prefrontal-cortical regions influence flexible adaptation when reward contingency shifts. We will combine experimental and computational approaches according to the specific research questions that most interest the student. Our findings, in addition to enhancing our grasp of fundamental neuro-behavioral mechanisms, could inform the development of novel therapies for clinical conditions characterised by behavioural inflexibility, such as OCD and schizophrenia.

Full project location: University Park;

Developing nanoscale structural and compositional techniques for vascular pathologies.

Project Supervisor: Kenton Arkill

School: Medicine

Description: The aim of the rotation is to develop imaging and analytical skills that and benefit biological and physical sciences backgrounds.

The rotation would be emersed in the nanoscale and microscale research centre to use transmission electron microscopes to collect structural data on the glomerular and endothelial filtration barriers. We have a multitude of blocks ready for imaging across a variety of kidney (and other) pathologies. Skills and knowledge acquired include:

- Ultrastructural imaging by transmission electron microscopy
- Capillary wall structures
- Image analysis and quantification
- Introduction to glycosaminoglycans and extracellular matrix
- Options to include fluorescent histochemistry
- Options to include building a initial mathematical model for multilayer vascular permeability.

Location: University Park;

Full project description:

Skillset: Whilst the project is multiskilled with training across the skillset, comfort with numeracy and computers would make it more enjoyable.

Background: Tissues require the correct molecules to reach them from the vasculature. This molecular transport is controlled by the capillary walls which include a combination of the endothelial glycocalyx layer, junctions, basement membrane and underlying cells. These work in tissue dependant combinations to allow the correct combination of molecules to transport at various hydrostatic pressures.

This project will define the physiological structure, tissue specific composition and the physical and biological mechanisms behind it. This will form the bedrock to elucidate how pathological states need to be corrected.

Hypothesis: The endothelial glycocalyx fibres defines vascular permeability as the primary filter except in tissues with particularly tortuous tight junctions (e.g. neuronal vessels)

Experimental Methods: Nottingham has received ~£5M investment in cryogenic nanoanalysis equipment over the last 3 years. The project will "lift-out" samples from biopsies to analysis by 3D cryo-transmission electron microscopy or wall layer specific cells/structures for transcriptomic/proteomic analysis.

Research Plan

Aim 1: Development of tissue cryo-preparation and transfer techniques for cryo transmission electron microscopy and nanoscale biopsies. High Pressure Freezing can immobilise molecules up to 200µm depth of tissue suitable for transfer into various imaging and transcriptomics machines. This work will be performed on physiological tissue characterised for its permeability, including tissue generated with correlative physiological or biochemical parameters known

Aim 2: Using the skills and methods developed in Aim 1 determine the pore sizes and shapes for each (sub-)layer in kidney and neuronal capillaries. This will be paired with transcriptomic/proteomic data acquired from the individual layers. Cutting edge RNAi techniques will be used to validate identified pathways and efforts for in vitro and ex vivo modelling can be made.

Aim 3: Correlate the structure and the permeability parameters, predict changes from manipulation and test using pathologies (e.g. Diabetes/Alport Syndrome/Denys-Drash Syndrome), to form the definitive comprehensive understanding of blood vessel permeability.

Expected Outcomes: A technical breakthrough in structural analysis in tissues, that can immediately be used in combination with ongoing projects. A determination of the layer contribution to permeability including spatial location and variable molecular structure in a tissue dependent manner. A clear link between dynamic physiology, structural biology and mathematical first principles for vascular permeability in both normal and exemplar pathological cases.

Impact: This project will be text-book defining physiology that is the basis for the vast majority of vascular pathologies and treatments. This project builds on extensive ongoing pathological in-vivo experimentation but can be used as a basis for other diseases (e.g. cancer and dengue) and therapeutics (e.g. fluid therapy and drug delivery).
Full project location: University Park;

Assessment of protease and ligand-induced PAR2 conformational changes and its subcellular trafficking in living cells – how is inflammatory pain initiated?

Project Supervisor: Raphael Silvanus Haider

School: Life Sciences

Description: During the lab rotation, the student will gain critical hands-on experience in the maintenance of mammalian cells, while using the cultured cell lines for experiments commonly performed in cell biology and pharmacology labs, as well as in modern drug discovery processes. The student will become familiar with experimental design and workflow, including transfection of biosensors, the utilisation of different measurement platforms (PHERAstar plate reader and confocal fluorescence microscope) and the evaluation of recorded data using respective analysis pipelines. These skills not only contribute to an advanced understanding of GPCR signalling dynamics but also provide a valuable toolkit for future research in molecular and cellular biology. In detail, the student will:

- Express chromophore-labelled PAR2 fusion proteins in combination with subcellular compartment markers to analyse receptor trafficking (via bioluminescence resonance energy transfer (BRET)) and confocal microscope (co-localisation).
- Utilise arrestin and GPCR kinase knockout cells to investigate the impact of these proteins on the subcellular PAR2 trafficking behaviour.
- Assess the recruitment of miniG proteins and arrestins to PAR2 following activation with various activators, to evaluate signalling profiles.

Location: QMC;

Full project description: This project will focus on the protease-activated receptor 2 (PAR2), a G protein-coupled receptor (GPCR) of established physiological relevance as a

mediator of inflammation and pain. Despite this clear physiological role, no PAR2 targeted therapy has yet reached the clinic. This is likely due to our incomplete understanding of its signalling function.

GPCRs constitute the largest family of membrane proteins and fulfil important roles in most physiological processes. They act to sense extracellular biological, chemical and physical agents to elicit an intracellular signalling response. In humans, they tightly control the action of hormones and neurotransmitters and enable the perception of odours and even light. In the case of PAR2, this receptor regulates pain in response to inflammatory events. GPCR signalling is initiated by these diverse extracellular activators via the induction of conformational changes within the receptor at the plasma membrane. These conformational changes convert the receptor into an active state, enabling it to interact with its main downstream signalling transducers: G proteins, GPCR kinases and arrestins.

We now know that a given GPCR can activate these transducers in distinct patterns, depending on which activator initiates the signalling. This characteristic, termed biased signalling, is mediated by the GPCR being able to adopt a spectrum of different active conformational states. While multiple PAR2-activating proteases have been identified, which elicit distinct PAR2 signalling responses, not much is known about the specific conformational states these activators induce. Similarly, there is still a large gap in our understanding of conformational changes (or lack thereof) caused by clinically relevant PAR2 antagonists. Moreover, recent reports have shown that PAR2 signalling at the plasma membrane and especially from intracellular compartments is responsible for different types of pain, although the molecular machinery enabling this signalling remains unknown. Answering these integral questions about fundamental PAR2 signalling characteristics will be crucial to inform modern drug discovery processes that target the treatment of inflammatory bowel and rheumatic diseases. Most importantly, the tools and methods designed and employed throughout the project will expand our spatio-temporal understanding of GPCR signalling.

The aim of the project is to characterise the conformational landscape of the protease-activated receptor 2 upon activation with different agonists & antagonists to link this information with changes in localised signalling and trafficking responses.

The student will use structure-informed design to generate biosensors via molecular biology techniques, such as Gibson Assembly and Site-Directed Mutagenesis. This will enable the assessment of activator-induced PAR2 conformational changes via intramolecular bioluminescence resonance energy transfer (BRET). Furthermore, the student will complement these cutting-edge cellular measurements with a comprehensive assessment of the PAR2 trafficking and subcellular signalling cascade using advanced fluorescent microscopy methods, such as confocal microscopy. The project will convey expert knowledge in molecular and cellular signalling processes, ranging from receptor activation to the ultimate termination of its signalling. The goal is to create a subcellular map of PAR2 signalling with high spatial and kinetic resolution to understand the initiation of nociceptive signalling and aid drug discovery efforts to relieve pain in diverse inflammatory pathologies.

Full project location: QMC;

Design and development of Gi peptide biosensors for the interrogation of signal transduction processes in G protein-coupled receptors (GPCRs).

Project Supervisor: Charlie Laughton

School: Pharmacy

Description: Class A GPCRs primarily signal through Gas, Gai/o, Gaq/11 or Ga12/13 containing G proteins, each with distinct functional outcomes. We have recently shown (Farmer et al, FASEB J. 2022 36:e22576) that small peptides that mimic the Gas G protein C-terminus are novel broad-spectrum biosensors for the characterisation of GPCR activation and ligand pharmacology, when combined with resonance energy transfer techniques to detect receptor-sensor interaction. Such sensors would also be beneficial to assess Gi coupled receptor function, our knowledge from current structures suggests more variability in Gai C terminal binding site. Thus rational design of Gi biosensor peptides will require an accurate understanding of the three-dimensional structure of the GPCR/Gai interaction, but currently this is not available. Though a number of crystal and cryoEM structures are available, their resolution and/or artifacts introduced to enable structure determination render them unsatisfactory for reliable peptide design and optimisation.

This rotation project will involve using state of the art computational methods, including AI and molecular dynamics simulations, to refine the available experimental data and provide high-quality models of GPCR/Gai interaction that subsequently can inform experimental peptide design, synthesis, and evaluation studies. Biosensors of this type offer the opportunity to discover novel allosteric ligands that bind to the G protein region of GPCRs with future therapeutic potential.

Location: University Park;

Full project description: We have recently shown (Farmer et al, FASEB J. 2022 36:e22576) that fluorescently labelled peptides related to the sequence of the C-terminal region of Gas G proteins have wide applicability as biosensors of GPCR activation and as sensors for novel druggable binding sites. For example, they provide a route to the identification and development of non-competitive intracellular allosteric modulators (IAMs), a class of GPCR ligand of particular current interest. While most designed biosensors are very specific for a particular protein target, by design we have demonstrated that these molecules are applicable to several GPCR systems that signal through Gas. It is therefore of obvious benefit to extend the arsenal of such biosensors to those based on the structurally related Gai/GPCR interaction, so that a second class of GPCR systems becomes amenable to investigation by the same techniques. However, we know from currently available cryoEM structures that different Gi coupled receptors show subtle differences in the nature of the Gi C terminal binding cleft which need to be incorporated into broad spectrum biosensor design.

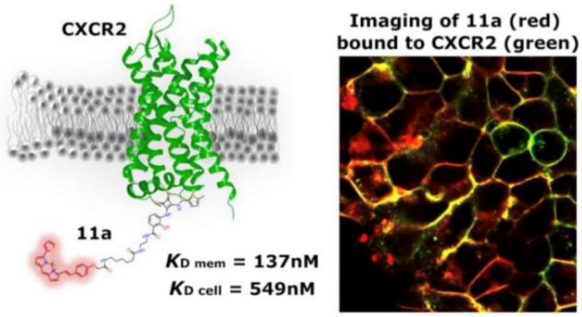
The project will begin with the development of a validated model for the three-dimensional structures of established GPCR/Gai systems. Initial models will focus on examples such as the NPY Y1 receptor, DP2 prostanoid, CXCR2 CCR2 / CCR9, dopamine D2 and / or mu-opioid receptors as these show a particular preference to coupling to Gai [Inoue et al., 2019, Cell 177, 1933–1947]. Work will extend what has been achieved through the associated rotation project. Methodologies will include ab initio structure determination using AI-based tools such as Alphafold2, remediation/computational mutagenesis of experimentally determined structures using molecular graphics methods such as Isolde, and validation through prediction of peptide-protein binding affinities with molecular dynamics simulations.

With the knowledge generated in the computational studies, the next phase will take peptide sequences predicted to bind well to selected Gi coupled GPCRs (procured through external custom synthesis) and characterise them pharmacologically, for example by evaluating their ability to influence the binding of known orthosteric agonists (e.g. dopamine) through stabilisation of active or inactive GPCR conformations. Notably, we have a lead biosensor peptide with appropriate activity for the NPY Y1 receptor that can be used as a lead for both modelling and rationale design. Peptide ligands with confirmed binding will then be developed by the student to generate novel fluorescent biosensors (e.g. using Cu(I)-catalysed Huisgen azide-alkyne 1,3-dipolar cycloaddition (CuAAC) chemistry to selectively attach fluorescent dyes of interest).

For receptor-Gi peptide combinations identified by initial screening, we will then generate a NanoBRET assay (Farmer et al., 2022). This is a powerful technique to assess the affinity and efficacy of orthosteric agonist drugs. Furthermore, these peptides provide suitable fluorescent probes for measuring ligand binding at the intracellular receptor binding site, which has been successfully targeted to generate NAMs for a few GPCRs to date (e.g. CXCR2, Casella et al 2023). Using a Gi-GPCR biosensor assay in this manner offers a platform to screen against our in-house compound library of ~80,000 ligands, with a view to discover new chemical scaffolds as intracellular allosteric modulators of different Gi coupled GPCRs.

Our success with the discovery of Gs-coupled GPCR fluorescent biosensors offers an excellent foundation from which to continue this work with a focus on Gi-coupled GPCRs.

Full project location: University Park;QMC;



How the spinal cord talks to the brain: Illuminating the sensory output system of the spinal cord

Project Supervisor: Kim Chisholm

School: Life Sciences

Description: The goal of this rotation will be to develop an understanding of spinal cord biology and develop technical expertise to allow students to reveal the role of spinal cord networks in healthy and pathological sensory experiences. The student will learn relevant microscopy skills (confocal, two-photon and epifluorescence microscopy), clearing techniques (to improve the transparency of tissues) and preparation for RNA sequencing. This will allow students to label, visualise and probe spinal cord networks to start answering questions about their role in sensory processing and provide essential laboratory experience and transferrable skills.

Throughout the rotation the student will have opportunities to develop their skills in various topics, including experimental design (e.g. experimental design assistant), power calculations (GPower), data and image analysis (Fiji, Excel, R) and statistical techniques (SPSS, R).

As such the rotation will include training in a number of translatable and vulnerable skills, including work with complex data sets, communication of experimental findings (written and oral), in vivo (patho)physiology and optical microscopy.

The rotations will be based in the labs of KC and RH to optimise exposure to a wide range of skills and research approaches.

Location: QMC;

Full project description: This studentship will explore the role of spinal cord neurons in sensory processing, by focusing on one of the most critical pathways connecting the sensory stimuli from our bodies to their perception in our brains: spinal cord projection neurons. These neurons are a critical output pathway of sensory signals reaching our brain and as such regulate the flow of signals that ultimately shape our perception of the world around us. Therefore, they play a pivotal role in conditions marked by aberrant sensory experiences, such as chronic pain or persistent itching.

This project will use mouse models, recently developed in vivo fluorescence microscopy methods, tissue clearing, as well as novel neuronal silencing/stimulation approaches, to probe this pivotal spinal cord output system and its role in aberrant sensory processing. The types of questions that will be addressed include a) whether spinal cord projection neurons form subgroups (defined by their function/genetics) based on which brain region they communicate with and b) whether their involvement in sensory dysfunction differs depending on which subgroup they are part of. The knowledge gained by this studentship will advance our understanding of spinal cord projection neurons in sensory abnormalities and how we may be able to harness their pivotal role therapeutically.

This studentship will suit individuals interested in the interaction between different areas of the nervous system, relevant to many neurobiological processes, including spinal plasticity associated with chronic pain states. We will provide training in innovative imaging techniques which are relevant to many areas of neuroscience, preparing the individual for their future research career.

Objective 1: Develop skills in genetic tools, such as AAVs and transgenic mouse lines, to express calcium indicators in neurons and to visualize calcium responses in mice in vivo and other relevant culture systems.

Objective 2: To establish in vivo skills in the induction and behavioural assessment of mouse models of spinal cord plasticity, including mouse models of chronic pain.

Objective 3: To use pharmacological and genetic (optogenetic/chemogenetic) tools to modulate the activity of neurons (increase or decrease their activity) and to determine the behavioural measures of neuroplasticity in vivo.

With these skills the student will develop and investigate research questions related to the role of spinal cord networks in sensory processing and pathological sensory conditions. These may include:

1. Do spinal cord projection neurons respond differently to sensory stimuli, depending on which area of the brain they communicate with and does this link to differences in the genes they express?
2. How does inhibition of projection neuron subsets in models of spinal cord plasticity alter any phenotypes we see in rodent models?
3. How do other neuronal populations interact with spinal cord projection neurons?

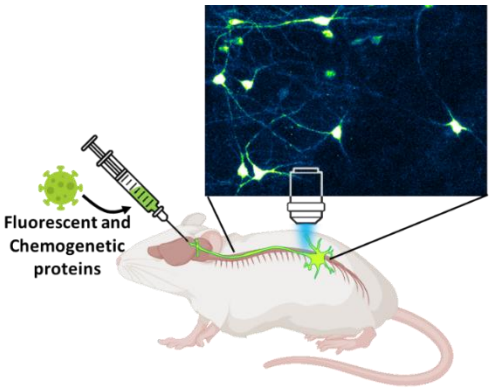


Figure legend: Spinal cord projection neurons (in green) project from the spinal cord directly to the brain, transmitting important sensory signals from our bodies to our awareness. They therefore form a crucial communication network that shapes our sensory perception of the external world.

The supervisory team have expertise in the techniques, facilities and the research focus of this studentship to ensure successful PhD supervision and research outcomes. The supervisory team have access to state-of-the-art facilities and provide dynamic research approaches, embedding inclusivity into their lab ethos. We encourage students to help shape project direction as part of a research team.

Full project location: QMC;

Telling tails: using computer-based approaches to understand the roles poly(A) tails in gene expression.

Project Supervisor: Keith Spriggs

School: Pharmacy

Description: Textbooks tell us that poly(A) tails regulate translation and mRNA stability. However, when the first methods to examine this genome wide were developed, there appeared to be no correlation between the size of the poly(A) tail, the stability of the mRNA and its ability to direct protein synthesis. Recent work in our laboratories indicates that this may indeed be the case in unstimulated cells, but that poly(A) tail metabolism is very important when cells respond to signals or stress. After many years of trying, we now finally have high throughput data that will allow us to test these ideas.

In this rotation, you will investigate RNA sequencing datasets to:

- Determine which genes change poly(A) tail size during growth factor stimulation
- Determine if different transcription variants have different poly(A) tails
- Determine if particular characteristics of genes determine poly(A) tail size
- Correlate poly(A) tail sizes of mRNAs with mutations from haploid genetic screens for a polyadenylation inhibitor to identify resistance genes regulated by polyadenylation
- Use Ingenuity Pathway Analysis to characterise groups of genes emerging from these analyses

This project will require you to use computer-based approaches. While training will be offered, an interest in computational analysis is essential. The work will contribute to the fundamental understanding of gene expression and to the development of polyadenylation and deadenylation inhibitors as medicines.

Location: University Park;

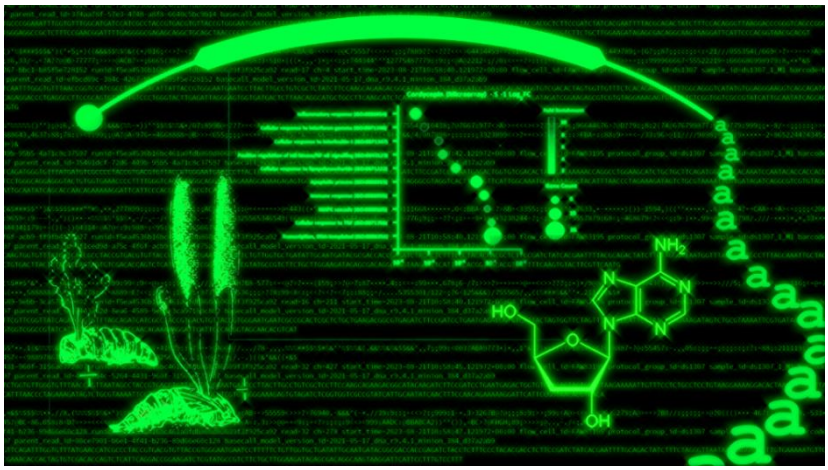
Full project description: Work in our laboratories suggests that poly(A) tail length correlates with gene expression under conditions such as inflammation and growth factor stimulation. Indeed, the polyadenylation inhibitor cordycepin and its derivatives are

showing promise as anti-inflammatory and anti-cancer therapies (a derivative of cordycepin is currently in a clinical trial). Very little is known about the role of poly(A) tail metabolism in these processes because key tools to address these questions have been lacking. That's why we are now using haploid genetic screens, which identify mutations that convey resistance to a drug to investigate the mechanism of action of this type of treatment. In addition, we have recently acquired several large Nanopore (long read) sequencing datasets of cells treated with growth factors as well as polyadenylation inhibitors that allow us to determine changes in RNA expression and the size of poly(A) tails and to characterise transcript variants. These datasets match large datasets on mRNA translation and stability available from our collaborators at the Beatson Institute in Glasgow.

In this PhD project you will:

- Develop and refine methods for analysing Nanopore sequencing data, comparing poly(A) tail size distributions and mapping the mutations in haploid genetic screens
- Analyse and compare long read Nanopore datasets
- Analyse and compare data from haploid genetic screens
- Compare data on mRNA polyadenylation with mRNA abundance, translation and stability
- Analyse mRNA sequences to identify elements that regulate poly(A) tail metabolism
- Integrate different high throughput data (transcriptomics, proteomics, metabolomics, poly(A) tails, genetic screens) to explore the effects of cordycepin in model cell lines.

The findings of your computational work will mostly be tested experimentally by others, but if you are interested you can do some laboratory work yourself. This project will require you to become proficient in coding, learn to use large computer systems, manipulate large datasets and tackle complicated computational and statistical problems. We will offer training, but this is a very fast developing field and a degree of independent thinking as well as a willingness to identify outside sources of help is essential for success. The work is likely to advance the fundamental understanding of gene expression as well as lead to the development of polyadenylation inhibitors as medicines.



Full project location: University Park;

The regulation of multipotent stromal cell functions in inflammageing; a focus on miRNA roles

Project Supervisor: Jehan El-Jawhari

School: School of Science and Technology (NTU)

Description: According to the plan described below, the student will spend six weeks at NTU (Dr. El-Jawhari's lab) and three weeks at UoN (Dr. Dajas-Bailador's lab).

NTU lab rotation at interdisciplinary biomedical sciences labs, Department of Biosciences, Clifton campus, and it will include training on:

- In vitro human cell culture techniques, including specific protocols for multipotent stromal cells.
- Cell proliferation and differentiation assays using specialised kits.
- Cell surface phenotype characterisation of human cells using flow cytometry and related analysis.
- Cell secretome assessment using the ELISA platform.
- Using the Qiagen Ingenuity Pathway Analysis (IPA) software, an outstanding tool for analysing, integrating, and interpreting data derived from 'omics experiments, e.g., miRNA experiments, to understand the cellular signalling pathways.

UoN lab rotation at the School of Life Sciences will include training on:

- Molecular biology assays, particularly qPCR/miRNA quantification.
- Microscopy techniques related to cell biology.

In both rotations, there will be an introduction to the research team, various lab facilities, and communication activities.

Location: Clifton Campus;QMC;

Full project description: The inflammageing, a process of chronic inflammation associated with ageing, is the drive of several autoimmune and degenerative diseases and delayed healing of traumatic tissue damage, all causing significant health and socioeconomic burdens.

Multipotent stromal cells (MSCs) are classically involved in tissue regeneration/degeneration. Furthermore, these unique cells can have essential roles in modulating immune response. MSCs display immune suppression in normal conditions, limiting the inflammatory phase that precedes any normal healing process. Conversely, these cells can have pro-inflammatory roles under chronic inflammation, as shown in rheumatoid arthritis and autoimmune disorders.

miRNAs have regulatory roles in several physiological processes, and recent research has indicated a link between miRNAs and some aspects of MSC biology, e.g., differentiation and immunomodulation. Still, it is unclear how miRNAs respond to the effect of inflammatory mediators and if they alter MSC functions, contributing to inflammageing progress.

This project aims to investigate in detail the roles of miRNAs in regulating various functions of MSCs within inflammageing mimicking microenvironment (i.e. cells exposed to inflammatory mediators, such as complement systems and pro-inflammatory cytokines).

The main objectives will be to quantify the expression levels of miRNAs and their role in regulating human MSC functions when treated with inflammatory mediators. Confirmation of miRNA roles will be analysed using specific inhibitors. The project will also include a detailed assessment of the related molecular mechanisms underlying cell function changes.

This project will offer the candidate researcher valuable research experience and extensive training using up-to-date laboratory and analytic techniques. Tissue culture, cell survival, proliferation and immunomodulation functional assays will be performed. Quantification of miRNA, secretome, transcriptome and proteomics quantifying assays will be utilised in this project. Other data analysis and statistical methods will be included in this project.

The supervisory team will include MSC biology (JEJ) and miRNA biology (FDB) expertise. Dr. JEJ's previous research showed that inflammatory mediators can distinctively affect MSC functions, and these changes are linked to the pathogenesis of inflammatory and ageing-related bone and joint diseases. Her expertise will be essential for investigating MSC functions and the molecular basis for these functions, allowing a unique opportunity for a deep understanding of a new rule for MSCs in norm versus inflammageing. Dr. FDB's research has proven that miRNAs have significant roles in neuronal cell growth and communications. His supervision will be essential for investigating miRNA biology in human cells, providing an outstanding opportunity for training on techniques and analysis to study the role of these regulatory RNA molecules.

This work programme will enrich the research on the molecular and cellular basis of human ageing via investigating new mechanisms of inflammageing. In addition to known immune changes, this project will explore how MSCs could contribute to this process and related ageing diseases. The research could also pave the way to identify new therapeutic targets and improve cell-based therapies for such inflammatory conditions and related tissue damage.

Full project location: Clifton Campus;QMC;

Understanding the role of actin nucleation in the cellular stress response

Project Supervisor: Amanda Coutts

School: School of Science and Technology (NTU)

Description: The lab rotation will investigate nuclear actin dynamics in live cells under a variety of different conditions (e.g., DNA damage and other stress responses). We have a variety of cell lines where we stably express a GFP-tagged nuclear localised actin chromobody (developed by Prof R. Grosse). The lab rotation will provide the student with key skills related to this project including, cell culture, imaging techniques including live and fixed cell immunofluorescence-based time-lapse imaging and flow cytometry.

Location: Clifton Campus;

Full project description: Actin is a cytoskeletal protein that plays essential roles in many cellular processes, such as adhesion, intracellular trafficking, membrane dynamics and motility. Monomeric actin (globular, G-actin) polymerises into filaments (F-actin) to provide actin with its unique roles in many dynamic cellular processes. While traditionally considered a cytoplasmic protein, it is now clear that actin is also found in the nucleus where it participates in many essential processes such as DNA replication and repair, chromatin remodelling and transcriptional regulation. The polymerisation state of actin is controlled by actin-binding and nucleation promoting proteins and, in the cytoplasm, controlled actin polymerisation lays the foundation for its roles in regulating vital cellular processes such as motility, intracellular trafficking and adhesion. While transient nuclear actin filaments have also been described in a variety of processes, such as during the cell cycle and DNA damage/stress response, we have limited knowledge of the key regulators and the specific role of actin filaments in nuclear structure and function.

We have uncovered roles for nuclear actin in the DNA damage response where it influences transcriptional regulation and splicing. We want to understand how nuclear F-actin is regulated during stress responses and how this impacts on nuclear function and cell outcome. This research is expected to uncover novel pathways related to cell survival during the stress response and reveal new components involved in nuclear activities and structure. Understanding how actin and actin-nucleation impacts processes

such as the regulation of gene expression and splicing and how stressors such as DNA damage influence this is essential to our understanding of fundamental aspects of cell and molecular biology and the relation to human diseases such as cancer.

This project will include a wide variety of cell and molecular biology techniques including cell culture, genetic manipulation including CRISPR technologies, siRNA, cell imaging, nanobodies, and biological assays to define phenotypic outcomes related to the DNA damage response.

Full project location: Clifton Campus;

The striatum as a recurrent neural network

Project Supervisor: Mark Humphries

School: Psychology

Description: The rotation project will introduce the student(s) to the cutting-edge of systems neuroscience, by tasking them with tackling the question of how a neuron population encodes movement.

They will analyse a session of imaging data from 300+ neurons in the striatum during tracked, spontaneous behaviour. We will introduce them to the basic methods for handling and visualising these data. We will then task with them with understanding the basic structure of the population's activity during movement: of how many neurons are active and when, and of the correlations in their activity. This will lead them into performing basic dimension reduction on the population activity, to answer the question of how redundant is the coding of movement – whether it is shared among many neurons, or sparsely distributed across the population.

This rotation will thus provide students a taste of the cutting-edge of neural activity recordings, and introduce them to ideas of how machine-learning and data science techniques can be used to tackle questions of brain computation and coding.

Location: University Park;

Full project description: The massive, silent striatum controls our behaviour. When it falters, movement disorders ranging from Parkinson's disease to the tics of Tourette's result. Keeping its two output pathways in balance seems key to maintaining our ability to control our behaviour. The canonical model for the striatum predicts that these dual output pathways compete to respectively select or suppress behaviours represented by cortical inputs. But recent advances in cell-specific imaging and optogenetics have brought strongly dissenting data: both pathways are similarly co-active during behaviour, and stimulating either pathway both lacks the predicted opposing effects on downstream neurons and does not have the predicted effects on behaviour. A new model of the striatum is thus essential.

Understanding the striatum, and how those two output pathways are controlled, is difficult thanks to its complex internal architecture. In this project, we will test the hypothesis that the striatum is a special class of recurrent neural networks that use purely inhibitory connections. We will build and analyse this class of networks, deriving from them predictions for the activity of neuron populations in the striatum during movement. We will then test these predictions in two large-scale datasets of population recordings from striatum in freely-exploring mice.

The student will build a two-layer inhibitory recurrent neural network (iRNN) that captures the key elements of the striatum's circuit, synthesising a wide range of anatomical and neural data. Particularly key will be capturing the two output populations, defined by their neurons' respectively expressing the D1 or D2 receptors for dopamine. With this model, we will then derive predictions for the activity of those output populations during movement, in particular for their granularity – how many things can be encoded and how discretely - and their dimensionality – how complex the activity will be.

We will also use the model to make predictions about the contributions of the striatum's circuit to its output, which can be tested in future experiments. These include the contributions of dopamine activating the D1 and/or D2 receptors, and of the wiring within and between the two output populations, which is asymmetric.

We already have available imaging data-sets of the activity of large populations of D1 and D2-expressing neurons in mouse striatum during free behaviour, coupled to full tracking of that behaviour, from the studies of Klaus et al (Neuron, 2017) and Markowitz et al (Cell, 2018), shared with us by the those labs. We will use the tracking data to determine the types of movement used by the mice during exploration of their arena, then use the neural data to test model-derived hypotheses of how that movement was encoded in the striatum.

Klaus, A.; Martins, G. J.; Paixao, V. B.; Zhou, P.; Paninski, L. & Costa, R. M.
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Neuron, 2017 , 95 , 1171-1180
Markowitz, J. E.; Gillis, W. F.; Beron, C. C.; Neufeld, S. Q.; Robertson, K.; Bhagat, N. D.; Peterson, R. E.; Peterson, E.; Hyun, M.; Linderman, S. W.; Sabatini, B. L. & Datta, S. R.
The Striatum Organizes 3D Behavior via Moment-to-Moment Action Selection.
Cell, 2018 , 174 , 44-58
Full project location: University Park;

Investigating regulatory mechanisms of the AP1 transcription factor in hypoxic (low oxygen) cell adaptation

Project Supervisor: Alan McIntyre

School: Medicine

Description: Regions of low oxygen (hypoxia) are an environmental stress found in about half of colorectal tumours. Clinically hypoxia is associated with poor patient outcome, metastasis and therapy resistance. Therefore new approaches to targeting these regions of tumours are required to enable improved patient survival. Hypoxia induces dramatic changes in the transcriptome, a key response which drives adaptation to hypoxia. Induced changes include increased expression of genes that regulate stem cell capacity, metabolism and metastasis.

Our recent data identifies that the AP-1 transcription factor as a key regulator of the hypoxic transcriptome that supports growth, survival and metastasis of hypoxic tumours. These molecules are difficult to target therapeutically however by inhibiting the kinase molecules that regulate their hypoxic activity we could develop a therapeutic approach to targeting therapy resistant hypoxic tumours.

In this rotation, we will use clinically relevant kinase signalling inhibitors to:

- Assess the impact of kinase inhibition of reducing AP-1 activity and expression and the regulation of hypoxia induced genes and proteins (Q-PCR, Western Blot, Luciferase assay)
- Assess the impact of the kinase signalling inhibitors that modulate AP-1 activity on cell phenotypes including 3D spheroid growth (2D and 3D tumour cell culture) and/or invasion (invasion assay).

Location: University Park;

Full project description: Low oxygen tension (hypoxia) is an environmental stress that is part of normal development and stem cell maintenance and is induced in pathological settings including, inflammation and cancer. Hypoxic regions are found in about half of breast and colorectal tumours and are associated with poor patient prognosis, metastasis and therapy resistance. Therefore new approaches to targeting these regions of tumours are required to enable improved patient survival. Hypoxia induces dramatic changes in the transcriptome, a key response which drives adaptation to hypoxia. Induced changes include increased expression of genes that regulate stem cell capacity, metabolism and angiogenesis.

The majority of research has focused on HIF1α and HIF2α, transcription factors (TFs) which are stabilised in hypoxia. Our recent data suggests that the transcriptomic changes that occur are regulated by a number of TFs and epigenetic regulators. Our evidence suggests that the AP-1 transcription factors (a heterodimer of proteins belonging to the c-FOS, c-JUN and ATF families) are key regulators of the hypoxic transcriptome and support growth survival and metastasis of hypoxic tumours. These molecules are difficult to target therapeutically however by inhibiting the kinase molecules that regulate their hypoxic activity we could develop a therapeutic approach to targeting therapy resistant hypoxic tumours.

Reactive oxygen species (ROS; by-products of celluar metabolism) are increased in hypoxic tumours and ROS in turn can modify amino acids of proteins inside and outside of the cells. These are known as oxido-reduction-mediated post-translational modification of proteins (redox-PTMs). Redox-PTMs are essential regulators of protein structure and function. When excessive levels of ROS are produced in cancer, aberrant redox-PTMs can negatively impact the normal protein function.

We plan to test the hypothesis that components of the AP-1 transcription factor are activated in response to key hypoxic signalling pathways and reactive oxygen species (ROS) induced post translational modifications.

Specifically we will:
(1) Identify key hypoxic signalling pathways that regulate AP-1 protein activity in hypoxia using a CRISPR CAS9 knockout screen of kinase signalling molecules.

We will use a CRISPR CAS9 knockout screen of all known kinases and identify kinases and their signalling pathways that regulate AP-1 activity in hypoxia.

We will validate the results of the screen by testing the impact of CRISPR knockouts and therapeutically relevant inhibitors against identified kinases on cell survival (2D and 3D cell culture models) and transcriptional regulation (RNA-SEQ) in hypoxia.

(2) Investigate the impact of ROS induced-redox PTMs on AP1 subunits and HIF proteins.

We will identify PTMs on AP-1 subunits using CoIP and in colorectal cancer tissue using immunohistochemistry. The impact of Redox-PTMs on AP-1 function will be investigated at a activity level (Reporter assay, western blot), transcriptional level (RNA-SEQ, QPCR)

and phenotypic level (3D cell culture models, immunofluorescence etc), using mutational studies.

Full project location: University Park;

Signal boost: Exploiting the solvent effect to optimise signal and functional performance in genetically expressed fluorophores and FRET sensors

Project Supervisor: Kevin Webb

School: Engineering

Description: "Heavy" water, containing an extra neutron within the hydrogen nucleus, affects the fluorescence properties of certain fluorophores through a "solvent effect". This has been poorly characterised in the case of genetically expressed fluorophores, particularly in the case of FRET partners used in modern FRET-based biosensors.

This project aims to characterise and optimise the response of genetically expressed fluorophores by exploiting the solvent effect. This lab rotation will teach technical skills in cell culture, transfection, imaging, and data analysis. You will establish cultures of human-derived epithelial cell lines (e.g. Calu-3) and use these to express a subset of genetically expressed fluorophores (e.g. eGFP, YFP, CFP, RFP). The resulting fluorescence signals will be analysed using a mix of live cell imaging (epifluorescence, confocal laser scanning microscopy) and compared with fluorescence plate reader measurements. You will determine excitation and emission spectrum and lifetime of the fluorescence state in the presence of a defined range of [D2O]. The resulting data will be analysed using GraphPad Prism to extract quantitative measurements of absolute signal intensity and lifetime vs [D2O]. High-resolution microscopy will then allow dynamic measurements of these signal alterations during D2O exposures in real time at the subcellular scale.

Location: QMC;University Park;

Full project description: "Heavy" water, containing an extra neutron within the hydrogen nucleus, affects the fluorescence properties of certain fluorophores through a "solvent effect". This has been poorly characterised in the case of genetically expressed fluorophores, particularly in the case of FRET partners used in modern FRET-based biosensors. The ability to tune the quantum efficiency and lifetime of fluorescence excited states is attractive due to the potential to optimise signals for high-resolution mapping, dynamic measurements of real-time protein-protein interactions, and the dynamic mapping of diffusional and signalling events at the intercellular to subcellular scales. This signal enhancement is expected to arise through an increase in brightness, shift in frequency, alteration in lifetime, or alteration in FRET efficiency.

This PhD project will provide thorough interdisciplinary training in the technical skills of cell culture, transfection, imaging, and data analysis. You will establish cultures of human-derived epithelial cell lines (e.g. Calu-3) and use these to express a subset of genetically expressed fluorophores (e.g. eGFP, YFP, CFP, RFP) and FRET constructs. The resulting fluorescence signals will be analysed using a mix of live cell imaging (epifluorescence, confocal laser scanning microscopy) and compared with fluorescence plate reader measurements. You will determine excitation and emission spectrum and lifetime of the fluorescence state in the presence of a defined range of [D2O]. The resulting data will be analysed using GraphPad Prism to extract quantitative measurements of absolute signal intensity and lifetime vs [D2O]. High-resolution

microscopy will then allow dynamic measurements of these signal alterations during D2O exposures in real time at the subcellular scale.

The Universities of Nottingham and Birmingham collaborate in the COMPARE project to create and apply novel tools and methods to the study of GPCR signalling in living cells and tissues. These experiments use a range of functional fluorescent probes, such as genetically expressed indicators (e.g. for [Ca2+]i) and FRET biosensors to detect and follow GPCR signalling at the cellular to subcellular scale in a variety of cell types. Learnings from the study of individual fluorophores above will be applied to characterise the effect of [D2O] on these important indicators and biosensors of signalling dynamics. These experiments will be conducted in individual cells and ultimately in a combination of tissue-on-chip (μSIM) and in vivo (Drosophila 3rd instar larvae) experiments to demonstrate the effect of solvent effect optimisation on the performance of these tools in living cells and tissues.

The outputs from this project will inform the rational design of incubation solutions and methods to optimise the photonic performance of genetically expressed fluorophores, indicators, and FRET biosensors. By exploiting the solvent effect to improve signal strength and stability, it may be possible to minimise exposure of samples to cytotoxic LED/laser radiation, minimise reagent concentrations and costs, and improve acquisition speed in a range of industrial and research-level experimental contexts. This gentle treatment and improvement in spatiotemporal resolution of measurements may be expected to translate into optimised experimental paradigms that more accurately transduce the real-time behaviour of physiological processes in high-resolution, high-content imaging.

Full project location: University Park;QMC;

The role of potassium ion channels in central nervous system cell division and stemness

Project Supervisor: Stuart Smith

School: Medicine

Description: Large conductance calcium activated big potassium channels (BK/MaxiK channels) are suggested as having a critical role in control of cellular proliferation and homeostasis. Aberrant ion channel expression has been linked to cellular differentiation, proliferation, migration, and appears to accompany oncogenesis. This project will examine these roles with regard to the development and proliferation of central nervous system cells (astrocytes and neurons).

The rotation project will utilise highly proliferative glioblastoma cell lines as an exemplar of proliferation and tumorigenesis in CNS cells. Expression levels of BK channels in normal CNS cells and glioblastoma will be examined using bioinformatics from established gene expression datasets. The expression levels of these in normal astrocytes and GBM cells will be evaluated using real time PCR and Western blotting to study expression at RNA and protein level.

If time allows, the levels of BK expression in neural stem cells will also be examined using similar techniques, critically assessing whether there is change in expression when cells transition from stem cells to their differentiated state as mature astrocytes or neurons.

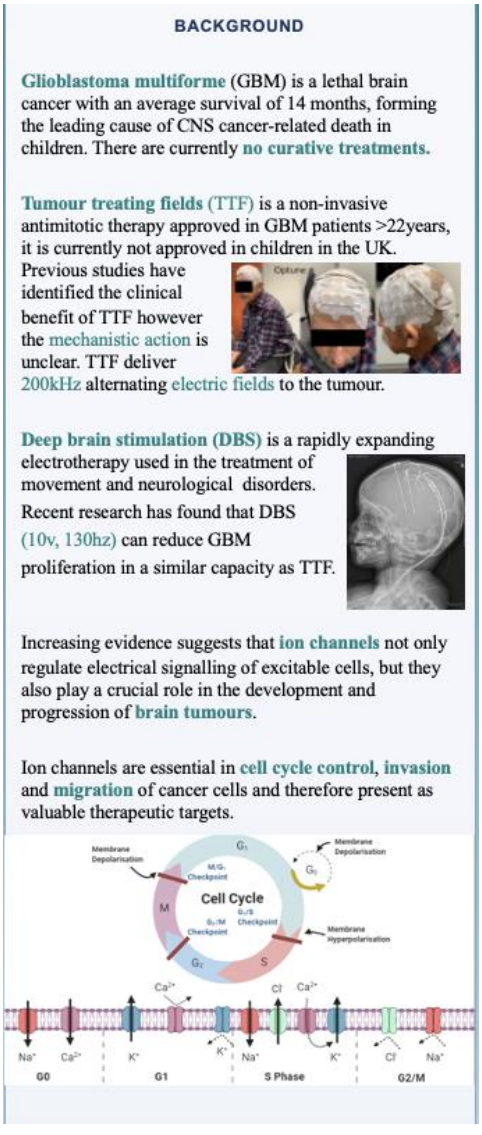
This project will allow selection and initial validation of BK channels as modulators of interest in CNS cell development and proliferation.

Location: University Park;QMC;

Full project description: The full PhD project will also focus on the role of BK channel function in the growth and development of CNS cells, using both normal CNS cells and the highly proliferative CNS tumour glioblastoma as model systems to study these processes.

Further validation of BK channel targets will be undertaken, examining expression levels in cell lines as in the rotation project, but extending this to additional cell lines and a broader range of normal and abnormal CNS tissues. Techniques utilised will include cell culture, PCR, immunohistochemistry, immunofluorescence, Western blotting, cell cycle analysis, electrophysiology and live cell imaging. Functional analysis will also be undertaken, examining whether cells in the process of migration exhibit changes in BK channel expression.

Previous data from our groups and others has implicated the functional electrophysiological role of ion channels in the control of cellular proliferation in CNS and other cell types. Ion channel function seems to have key roles in determining cellular behaviour including properties such as proliferation and cellular motility. A further major component of the PhD project will therefore be to examine the electrophysiological role of BK channels in normal astrocytes, neurons and in GBM cells. Patch clamping techniques will be undertaken to evaluate channel function and membrane potential in these cells. Pharmacological and genetic (e.g. siRNA) manipulation using known agents that open or block BK channels will be used to study the role of disrupting BK channel function. The effects of channel modulation will be assessed on properties such as proliferation and migration, key components of the development of the nervous system and the progression of tumours of the brain.



The effects of BK channel manipulation will also be studied in the context of cellular differentiation, using neural stem cells in their stem state compared to after they have undergone differentiation to mature astrocytes or neurons. We hypothesise that the 'stemness' of these cells is directly related to the membrane potential and ion channel conductance of their membrane, with BK channels acting as a key control mechanism.

The supervisors for this project include expertise in the role of ion channels in cellular behaviour with specific expertise in BK channels (RK), specialist experience in electrophysiological techniques and the functional assessment of ion channels (PS) and the development of the CNS in

health and disease (SS). We would aim to develop a high impact publication as a result of this project and to deliver national and international conference presentations.

Hypotheses:
1. The expression of BK channels varies between central nervous system cells in different states of proliferation, differentiation and health and disease
2. The function of BK channels in CNS cells is linked electrophysiologically to key cellular properties such as control of proliferation and migration.
3. Manipulating BK channel function in normal and abnormal CNS cells will alter cellular properties such as proliferation, motility and stemness.
Full project location: University Park;QMC;Derby Royal Infirmary;

Deciphering the functional solution structure and structure-activity profile of the macrocyclic antibiotic zelkovamycin

Project Supervisor: Weng Chan

School: Pharmacy

Description: Aim 1 (week 1-3): Literature search and reading of background science.

Aim 2 (week 2-5): To examine the in vitro antimicrobial activity of zelkovamycin. A focused study to determine the antimicrobial activity of zelkovamycin against several pathogenic S. aureus, including the highly virulent S. aureus USA300 strain and an intermediate-vancomycin resistant strain, using both MIC assays and our recently established dose-response studies.

Aim 3 (week 4-9): To determine the solution structures of zelkovamycin. The solution structures of zelkovamycin will be determined using high-field NMR studies, including NOE and temperature coefficient studies, in [D6]DMSO and [D6]DMSO–CDCl3 mixture. Our preliminary studies already established that two conformers of zelkovamycin exist in DMSO.

Location: University Park;

Full project description: Antimicrobial resistance (AMR) poses a global threat to human health. In a recent analysis, it was estimated that over 1.2 million deaths are directly attributable to bacterial AMR. In fact, the overuse of broad-spectrum antibiotics has resulted in the emergence of multi-drug resistant S. aureus strains that are refractory to most, if not all antibiotics used in the clinic. Hence, novel antimicrobial agents with unique modes of action are desperately needed to treat infections caused by multidrug-resistant (MDR) bacterial pathogens

Zelkovamycin is a 24-membered macrocyclic antibiotic with a unique narrow spectrum of antimicrobial activity (1,2). The antibiotic appeared to display potent activity against Staphylococcus aureus and Staphylococcus epidermidis, whilst broadly inactive against other Gram-positives and most Gram-negatives. In our studies, zelkovamycin was found to be highly potent against S. aureus SH1000. Zelkovamycin is the only naturally occurring antibiotic that is structurally related to argyrin. However, argyrin is inactive against S. aureus but showed acceptable activity against the Gram-negative Pseudomonas aeruginosa.

The objective of this studentship proposal is to apply chemical biology approaches to unravel and determine the pathogen specificity of zelkovamycin. We have recently established for the first time that zelkovamycin exists in two solution structures or

conformers. Thus, chemically engineered analogues of zelkovamycin will be utilised to test structure-activity relationship and conformer stabilising models, which would lead to the identification of novel pre-clinical lead compound(s) for the treatment of staphylococcal infection. The overall objectives would be achieved by prosecuting the following work packages:

*WP1: To determine the functional solution structure of zelkovamycin

The aim here is to fully determine the two solution structures or conformers of zelkovamycin. The structure of the major conformer in non-aqueous and aqueous buffers will be determined. The experimental data form this study will inform on the mechanism of action (MOA), resistance development pathway, and the design of novel zelkovamycin analogues outlined in WP2.

*WP2: To design and chemical synthesis of novel zelkovamycin analogues

The chemical architecture of zelkovamycin is related to argyrin. We have already established robust and scalable chemical methods for the synthesis of key building blocks and diverse argyrin analogues. The established methodologies will be applied for the total chemical synthesis of novel zelkovamycin analogues. In the initial instance, we will focus on structural/chemical changes in the dehydrobutyrine, 4-methoxytryptophan and the adjacent 2-methyldehydrothreonine residues.

*WP3: To evaluate the antimicrobial potency and scope of selectivity

A detailed antimicrobial activity of zelkovamycin and analogues thereof against several pathogenic S. aureus, including the highly virulent S. aureus USA300 strain, an intermediate-vancomycin resistant strain and a panel of clinical strain will be determined using both MIC assays and our recently established dose-response method. The antimicrobial activity against a wide range of both Gram-positives and Gram-negatives will also be evaluated to determine the scope of specificity.

(1) Tarantini, et al. (2021) Actinomadura graeca sp. nov.: a novel producer of the macrocyclic antibiotic zelkovamycin. PLOS One, 16, e0260413.

(2) Hao et al (2020) Zelkovamycins B−E, cyclic octapeptides containing rare amino acid residues from an endophytic Kitasatospora sp. Org Lett, 22, 9346.

Full project location: University Park;

Characterisation of potentially pathogenic enteric viruses from the River Trent, Derbyshire, UK.

Project Supervisor: Kenneth Mellits

School: Biosciences

Description: The student will first sample unprocessed wastewater samples from the Severn Trent Wastewater Treatment Plant, Sawley, Derbyshire. Nucleic acid will be isolated from these samples, and the student will probe, by PCR, for enteric virus likely to cause disease and known to be transmitted faecal orally also known as Faecal Indicator Virus (FIV), these include Hepatitis A and E, Rotavirus, Norovirus Genotype II, Enterovirus,. Type F Adenovirus. We will also use Next Generation Sequencing (NGS) specifically RNA-Seq to take a holistic approach and determine what RNA viruses are present without bias. Both the PCR and RNA-Seq will enable us to characterize the serotypes of these viruses, to correlate with diagnosed clinical gastroenteritis and other

disease such as respiratory in humans. Our final goal is to model and extrapolate the contribution of these environmental FIV to the contact populations incidence rates risks, using the Severn Trent detection data. Techniques used: Nucleic Acid extraction, RNA-Seq and associated Bioinformatics, infection modeling.

Location: Sutton Bonington Campus;

Full project description: Faecal indicator organisms (FIO) that can potentially cause a myriad of diseases are always present in wastewater. One group of such organisms are the enteric viruses which have been associated with a wide range of infections including gastroenteritis, respiratory infections, hepatitis, conjunctivitis, and meningitis. Their ubiquity and ability to persist in the environment for several months has been a subject of public health concerns globally. These viruses are mainly transmitted through the faecal-oral route via contaminated water and food, but also by person to person spread. Commonly studied Faecal Indicator Viruses (FIV) include: Adenovirus (Type F), Norovirus, Sapovirus, Enterovirus, Hepatitis A and E viruses, Rotavirus, and Astrovirus. Some of the viruses, such as Norovirus, with faecal-oral spread, cause human outbreaks with seasonal trends. Moreover, respiratory viruses such as SARS-CoV-2 and influenza have been found in wastewater, as well as zoonotic viruses, Rabies, and Jingman Tick Virus and other viruses. Thus, virus contamination of water sources and the environment have been linked to anthropogenic activities such as industrial, agricultural, and sewerage treatment works. These activities have made the water courses vulnerable to breakdown and subsequent infiltration by these viruses, resulting in the possible intake of these biological contaminants in addition to the physicochemical contaminants if not properly controlled. As a result, this could lead to significant consequences for human and animal health, food and water security, and ecosystem functioning.

In the present study, samples of waters from the Water Treatment works at Severn Trent Water Treatment Works, Sawley, Derbyshire, which will be collected at input. Five different enteric virus serotypes will be monitored by PCR, comprising of Human adenovirus type F (HAdV-F), a DNA enteric viruses and RNA enteric viruses including, Hepatitis A Virus (HAV), Hepatitis E Virus (HEV), Rotavirus (RoV) and Noroviruses (NoV) of genogroup GII. Moreover, to determine the overall load of all (RNA) viruses, we will extract RNA from input and subject this to RNA-Seq to determine the types of virus nucleic acid present without bias. Previous studies have shown a wide range of virus present (1). Finally using clinical strain data available from the National Health Service (NHS) and the Animal Health and Veterinary Laboratory Agency (AHVLA) we will attempt to model transmission of these viruses to humans and animals.

References

1. Stockdale R.S. et al., 2023 RNA-Seq of untreated wastewater to assess COVID-19 and emerging and endemic viruses for public health surveillance. Lancet Reg Health Southeast Asia Jul;14:100205. doi: 10.1016/j.lansea.2023.100205. Epub 2023 May 1

For more information see:
Wang H, Kjellberg I, Sikora P, Rydberg H, Lindh M, Bergstedt O, Norder H. 2020. Hepatitis E virus genotype 3 strains and a plethora of other viruses detected in raw and still in tap water. Water Res 168:115141.

Full project location: Sutton Bonington Campus;

Advances in genetic mechanisms and pathway analysis towards diagnostic, prognostic and treatment opportunities for osteosarcoma

Project Supervisor: Catrin Rutland

School: Veterinary Medicine and Science

Description: You will be joining a vibrant and successful research team with a proven track record in cancer research. Our supervisors and collaborators cover all of the techniques and skills required within your studentship. You will start your rotation by learning some of the essential techniques involved in the long-term project such as histology, immunohistochemistry, microscopy, and PCR. You will also learn bioinformatics and comparative skills to unravel the One Health links between different species with osteosarcoma. You will undertake background reading into osteosarcoma, and also mechanisms and pathways in differing cancer types. You will focus your reading by producing a systematic literature review paper, which would be you first publication (of an expected 6-8 within your PhD within our group) and will be the basis for your first year report for annual progression. Therefore, you will learn all of the skills necessary to produce this research and to start learning your laboratory-based skills. The skills and techniques you will learn are directly translational to many research areas.

Location: Sutton Bonington Campus;University Park;

Full project description: Cancer is a leading cause of non-communicable morbidity and mortality throughout the world. Osteosarcoma (OSA) is a rare bone cancer in people. However, OSA incidence rates in dogs are 27 times higher than in people. Prognosis in both species is relatively poor, with 5 year OSA survival rates in people not having improved in decades. For dogs, 1 year survival rates are only around ~ 45%. Improved and novel treatment regimens are urgently required to improve survival in both humans and dogs with OSA. Utilising information from genetic studies could assist in this in both species, with the higher incidence rates in dogs contributing to the dog population being a good model of human disease1-5.

This project and rotation is laboratory-based with some bioinformatics and modelling, focusing on identifying specific proteins that are expressed in canine osteosarcoma, these will also be compared with existing human osteosarcoma data. Histological stains such as H&E and immunohistochemistry will be used to identify regions of protein expression in osteosarcoma samples in comparison to controls. The proteins chosen will be based on those identified with our RNASeq work on N=~300 tissue samples and N=2100 DNA swabs.

Our preliminary analysis of available genome-wide OSA datasets has identified a network composed of 32 differentially expressed genes that are associated with shorter survival time in canine OSA1,2,3,5. However, two key questions remain unresolved, how do these 32 genes cooperate to result in poorer survival and why are these genes dysfunctional in OSA? We are uniquely well positioned to exploit our data and extensive Rottweiler cohort and the IWH and Deerhounds to address these two critical research objectives and to expand out from these to compare human and canine cancers and to understand potential mechanisms and functions. To this end we will use a combination of molecular genetics, bioinformatics, and modelling approaches.

Objectives:

1. We will develop a tissue microarray of the samples, which will be used alongside full face osteosarcoma paraffin blocks to undertake analysis of tumour samples using clinical pathology – immunohistochemistry and microscopy alongside H-scoring.
2. Analysis of clinical data vs clinical pathology findings will be conducted.

3. We will undertake pathway analysis and potential pharmaceutical interventions.
4. To determine the molecular mechanisms underlying the dysfunctional expression of the gene network we have established, we will use bioinformatics tools to investigate the promoter and enhancer regions of these genes to identify potential regulatory factors involved in the activation of expression of these genes. We will then use Sanger sequencing and PCR-based SNP assays to test for association between known SNPs (available from the NCBI-dbSNP database and published studies) and OSA incidence, outcome and disease free-survival.
5. We will additionally use bioinformatics analysis of osteosarcoma vs other tumour types and human vs canine osteosarcoma using a One Health approach2,4,5.
6. We will conduct molecular modelling-based structure-activity relation studies of associate polymorphisms using de novo protein structure prediction approaches and/or homology modelling approaches where existing X-ray/NMR structures are available for protein homologs.
7. Writing literature reviews and original research articles for publications on osteosarcoma mechanisms
8. Organising breed health outreach days and communicating feedback to stakeholders

The postgraduate researcher will be supported by a vibrant and skilled supervision team, with the benefit of others in the group (postgraduate researchers, postdoctoral researchers and a senior technician) who know and understand all of the techniques well. We will additionally be working with collaborators in Switzerland (Bern).

Full project location: University Park;Sutton Bonington Campus;

Using computational methods to predict child cognitive development and mental health.

Project Supervisor: Sobana Wijeakumar

School: Psychology

Description: The rotation will encourage learning skills essential for the project i.e. analyzing data from video recordings and brain imaging techniques. There will be two goals. First, the successful candidate will learn to develop and implement computational vision techniques to analyze existing video recordings of interactions between infants/toddlers and their parents during free play sessions. Second, they will learn to apply machine-learning and if applicable, deep-learning techniques to analyze longitudinal brain imaging data from children and their parents.

Location: University Park;Jubilee Campus;

Full project description: The Mental Health of Children and Young People survey conducted in 2022 states that 18% of school-age children have a probable mental disorder, with no reduction in probability rates from 2020 to 2022. These estimates and others emphasize the need to intervene very early in life to ensure that children are set on the path to healthy neurocognitive development without delays. To do so, it is important to understand how cognitive function and social interactions emerge and develop as early as the first few years of life.

To this end, the successful candidate will work with a multidisciplinary group of scientists on data acquired from Project NeuroSync, a three-year longitudinal project conducted at the University of Nottingham examining neurocognitive development in concert with caregiver involvement from infancy through to toddlerhood. The project contains behavioural, brain imaging, home environment, socioeconomic status, and dyadic

interactions data from around 90 families from Nottingham, Lincoln, Sheffield, Leicester, and surrounding areas.

The first goal of the project will be to develop novel computational vision methods to extract looking behaviours in parents and infants as they engage with objects and with each other during naturalistic play interactions. The second goal of the project will be to use machine learning techniques to extract and examine movement data from these dyads during these interactions. Importantly, both aims will focus on developing cutting-edge tools that can be used to evaluate looking behaviours and movement from 6 months to 3 years of age in children. The third goal of the project will be to develop deep-learning techniques to analyze brain imaging data from children and their parents during a range of executive function tasks and during dyadic interactions. The final goal is to use advanced method computational modelling and statistical methods to integrate neurocognitive, home environment, and socioeconomic status data collected during infancy to predict executive functions and mental health and wellbeing outcomes before children transition into schooling. The project invites enthusiastic and motivated candidates who are passionate about using methodological advancements to address and tackle cognitive and mental health inequities in early childhood.

Links: Project website / University website

Twitter: @NeurosyncP Instagram: @neurosyncp

Full project location: University Park;Jubilee Campus;

Do Archaea use Conservative DNA Synthesis in the Absence of Replication Origins?

Project Supervisor: Thorsten Allers

School: Life Sciences

Description: The Allers laboratory works on DNA replication, recombination, and repair in Archaea. We use Haloferax volcanii as a model organism for archaeal genetics. Haloferax volcanii is unusual in that it does not require DNA replication origins, making it ideal to investigate the rules of life.

In the lab rotation project, strains of Haloferax volcanii lacking DNA replication origins will be used to examine the mode of DNA synthesis and the role of DNA polymerase D. Aphidicolin, a drug that targets DNA polymerase B but not DNA polymerase D, will be used to selectively inhibit lagging-strand DNA synthesis. Growth of cells in the presence of aphidicolin will be monitored in real time – we expect that aphidicolin resistance will be proportional to the number of origins deleted.

The student will acquire experimental skills in microbial genetics, molecular biology, and bioinformatics. The project will involve the generation of a range of gene-targeting plasmids, which will be used to manipulate the genome of Haloferax volcanii. Mutant strains will be tested for defects in DNA replication, recombination, and repair, by a range of microbiological techniques including microscopy and flow cytometry, pulsed-field gel electrophoresis, and genomic technologies including Nanopore DNA sequencing.

The student will have the opportunity to take part in the annual UK Archaea Workshop in January 2025, as well as joint lab meetings with the research groups of Ed Bolt and Stephen Gray.

Location: QMC;

Full project description: The project will investigate the role of DNA polymerase D in origin-less DNA replication in Archaea, and implications for the evolution of DNA replication. DNA replication is initiated at chromosomal sites called origins. Replication origins are assumed to be essential, but we have shown that in Haloferax volcanii, a member of the Archaea, life without origins is possible. Archaea are the third domain of life, alongside eukaryotes and bacteria, and the machinery for DNA replication is strikingly similar in archaea and eukaryotes.

We have shown that deletion of all replication origins from the chromosome of Haloferax volcanii is not only possible but leads to accelerated growth. Initiation of DNA replication in the absence of origins involves homologous recombination: it depends on the archaeal recombinase RadA and involves the formation of a three-stranded DNA structure called a D-loop. This mode of DNA replication – in the absence of origins – is termed recombination-dependent replication.

We have obtained data showing that in Haloferax volcanii cells lacking all origins, only one of the two replicative DNA polymerases – DNA polymerase D – is used to carry out DNA synthesis. DNA polymerase D is unique to Archaea and its catalytic core has a 'double-psi β-barrel' architecture that is also seen in RNA polymerase. This homology has led to the proposal that the replicative polymerase of the Last Universal Cellular Ancestor was the ancestor of archaeal DNA polymerase D.

Notably, DNA polymerase D is incapable of lagging-strand DNA synthesis, which suggests that recombination-dependent replication in Haloferax volcanii uses only leading-strand DNA synthesis. Similar observations have been made in yeast, where break-induced replication is used to repair one-ended DNA breaks and involves a migrating D-loop of leading strand DNA synthesis. But unlike DNA replication in Haloferax volcanii lacking origins, break-induced replication in yeast is slow and error-prone. Is this because only Archaea have DNA polymerase D?

The first phase of the project will use genetics in Haloferax volcanii. A modified version of the Meselson Stahl experiment will be used to determine whether replication in the absence of origins is indeed by leading strand DNA synthesis. Aphidicolin, a drug that targets DNA polymerase B but not DNA polymerase D, will be used to selectively inhibit lagging-strand DNA synthesis. Interactions of DNA polymerase D with replication proteins such as primase, and with recombination proteins such as RadA, will be monitored in vivo using a split-GFP fluorescence assay. Genomic techniques will be used to determine the location of replication initiation at D-loops, by ChIP-seq using tagged RadA.

The second phase of the project will use biochemistry with purified proteins, to recapitulate the genetic findings. Synthetic DNA substrates that mimic D-loop recombination intermediates will be generated using the archaeal recombinase RadA, and will be used to initiate DNA synthesis using DNA polymerase D. This reaction will be supplemented with other components of the replication and recombination machinery, depending on results from the initial phase of the project.

This project will test whether the ancestral form of DNA replication – and potentially, RNA replication – was initiated by recombination. It would have involved a migrating D-loop of leading strand DNA synthesis, which was carried out by the ancestor of archaeal DNA polymerase D. Lagging-strand DNA synthesis may have evolved later, eventually giving rise to the semi-conservative model of DNA replication proposed by Meselson and Stahl in 1958. The results that emerge from this project will have profound implications for the evolution of DNA replication.

Full project location: QMC;

Sexual dimorphic impacts of glucocorticoids upon metabolic resilience.

Project Supervisor: Craig L. Doig

School: School of Science and Technology (NTU)

Description: Metabolic plasticity mammals is evolutionarily conserved and responsive to fed-fasted cycles. Driven by a combination of nutrient, hormonal and signals, homeostasis pivots according to metabolic demands. During active phase (daytime for humans) carbohydrates will provide the energy required. During the night-time (resting phase) metabolic will switch to using fats as a fuels source. This plasticity in fuel use is essential to sustain health of the individual. However, in many chronic diseases this adaptive process breaks and an overreliance on carbohydrates dominates. The mechanistic processes underlying this are unclear but excess exposure to the steroid hormone class glucocorticoids manifest profound loss of metabolic plasticity.

This project will use a combination of advanced in vivo mouse physiology and cutting-edge molecular techniques. It will conduct metabolic phenotyping of male and female mice exposed to glucocorticoids and 13C-labelled carbon to understand how excess steroid hormones govern fuel choice decisions made by organisms. In parallel, the project will conduct downstream analysis of metabolically active tissues to delineate the underlying mechanisms dictating the sexual dimorphic impacts of glucocorticoids.

Location: Clifton Campus;

Full project description: Produced by the hypothalamic-pituitary-adrenal (HPA) axis glucocorticoids are essential for life. They are naturally occurring, powerful stress hormones. Responsible for activating the 'fight or flight' response, the glucocorticoid cortisol in humans (corticosterone in mice) is pivotal in determining physiological responses to stimuli. This makes them crucial determinants within any extreme situation (1). Stress in the extreme environment is multi-factorial, and stressors rarely exist in isolation. Initiators of physiological stress comprise of diet, temperature, altitude, health status, and sustained physical activity. These all interact to aggravate glucocorticoid exposure levels that individuals may experience (2). Our studies show that exposure to elevated glucocorticoids negatively impacts whole-body metabolic function and physical performance. Importantly, our recent data show glucocorticoids have a sexually dimorphic impact. In males, sustained exposure to glucocorticoids increases the use of carbohydrates as a source of fuel. However, in females, we see dietary- derived carbohydrates being shuttled toward storage as lipids. This proposal will work to define the sex-specific impacts of the multi-stressor extreme environment. This work will better understand the consequences of operating in challenging environments and generate translatable impacts to mitigate disproportional outcomes.

The PhD proposal will bring together the in vitro, in vivo and analytical techniques involving training at both NTU and UoN. There will also be opportunities for collaborative work in the UK or USA to generate novel samples for downstream analytical measurement.

This work will help reveal some of the most evolutionarily conserved, fundamental pathways responsible for governing tissue physiology and whole-body homeostasis. At present shifts in glucocorticoids are associated with many metabolic pathologies. However, target pathways and driving mechanisms lack definition. Using a combination of genetic mouse tools and leading-edge molecular analysis this project hopes to provide new insight into the basic functions that govern life.

Full project location: Clifton Campus;

Ageing, neurodegeneration and oxygen - does nature have an alternative to haemoglobin for us?

Project Supervisor: Lisa Chakrabarti

School: Veterinary Medicine and Science

Description: The rotation will enable the student to learn techniques in mitochondrial physiology and respirometry. They will spend time culturing continuous cell lines and primary lines, if they wish they can generate some primary cells from tissues. Cells and human tissues from patients with Parkinson's disease and sex and age matched controls will be analysed by western blotting and immunohistochemistry. There will also be the opportunity to use bioinformatics approaches to analyse proteomics datasets. The student will be able to focus on the techniques that interest them the most.

Location: Sutton Bonington Campus;

Full project description: Neurodegenerative disease, and many other diseases of ageing, seem to involve mitochondrial dysfunction. Frequently a decline in cardiovascular function is a feature of the ageing process. This project will focus on the function of intracellular and in particular mitochondrial haemoglobin, to understand what its role is outside the vascular system. The unique approach is to do interaction proteomic and epigenetic studies across species – including some that are exceptionally long-lived - to enable the understanding of the functional range of this protein. Strong collaborations will allow parallel experimental approaches and also cryo-EM to be applied to uniquely haemoglobin-less icefish. Icefish thrive in deep Antarctic waters and are the only vertebrates that have a complete absence of haemoglobin. The student will discover whether the icefish have a mechanism that we could use to improve oxygenation of tissues and cells in people. Instead of fixing the defect or mutation, it may be possible to utilise the biological adaptations relied upon by icefish. The project will explore pathways and mechanisms in human disease samples and datasets. Applications of this research will be valuable in haemoglobinopathies, hypoxic-ischaemia injury, diseases of ageing and even the maintenance of human life within inhospitable environments.

Full project location: Sutton Bonington Campus;

Investigating the influence of the tumour microenvironment in 3D models of Diffuse Midline Gliomas

Project Supervisor: Beth Coyle

School: Medicine

Description: Paediatric diffuse midline gliomas have a dismal survival rate and are very difficult to model in animals. We need to move away from 2D cell culture and grow cells in more realistic representations of the 3D tumour microenvironment.

We have recently established a 3D culture system that will underpin this project, and the rotation period will be used to train the student in this innovative culture system. We will also ensure the student has the skills to complete the molecular and imaging analyses required as the project evolves (such as RNAseq, confocal fluorescence microscopy and immunocytochemistry).

An important aspect of the initial lab rotation will be to give the student the confidence to become a contributing member of our research groups which are co-located within the Biodiscovery Institute. Not only will the student be able to appreciate the range of research activities in our wider research teams, but they will learn to share updates of their day-to-day progress and join in with the discussions around research challenges and successes. We believe it is particularly important to encourage students to discuss and question their own and others findings.

Location: University Park;

Full project description: Diffuse midline gliomas are highly aggressive childhood tumours that occur in the developing brainstem. These tumours typically arise in the pons, but infiltrate and expand throughout the brainstem causing progressively worsening symptoms. They are difficult to access surgically and lack effective therapeutic options, resulting in most patients surviving less than one year. Recent molecular analysis has revealed that these tumours are distinct from adult high grade gliomas and the latest World Health Organisation classification of brain tumours described four distinct primary subtypes, namely H3.3 K27M-mutant, H3.1 K27M-mutant,

H3-wildtype and EGFR-mutant. The H3 K27M-mutant subgroups represents almost 80% of DMGs and exhibits missense mutation in histone 3 with the substitution of lysine (K) for methionine (M) at position 27. The H3K27M mutation leads to the global loss of H3K27 trimethylation and subsequent gain of H3K27 acetylation, which has been linked to oncogenesis through upregulation of proto-oncogenes and suppression of cellular differentiation. Although the exact role of the H3 K27M mutation in tumour development is not fully understood, the mutation is linked to a worse prognosis with those harboring H3.3 having an even shorter survival than those with H3.1 mutations.

The location of these tumours means that not only are they difficult to treat but they are also difficult to model in animals. Similarly, traditional 2D culture methods fail to replicate the 3D nature of these tumours and key features of the tumour microenvironment (other cell types, hypoxia, extracellular matrix factors and mechanical stiffness). The aim here is to use a customizable peptide-based hydrogel to mimic the 3D microenvironment of the developing brainstem. We will then analyse the growth and invasion of different molecular subtypes in order to identify key factors that drive the aggressive behavior of these tumours. Compared to normal brain tissue, DMGs experience much lower levels of oxygen and hypoxia alters their response to therapy and overall methylation levels. Since altered methylation profiles are known drivers of these

tumours, we are particularly interest in how altering oxygen levels affects the behaviour of the different molecular subtypes. The final stage of this project will be to investigate ways in which the aggressive behavior of DMG can inhibited in our representative 3D TME models. These findings will help guide future clinical trials in children affected by this devastating brain tumour.

Full project location: University Park;

Exploring the Potential of Methenamine as a Novel Approach for Urinary Tract Infection Treatment

Project Supervisor: Alasdair Hubbard

School: School of Science and Technology (NTU)

Description: Urinary tract infections (UTIs) are one of the most frequent infections in healthy women and consequently, in the UK, UTIs are the second most common reason for prescribing antimicrobials and account for 1-3% of all medical consultations.

Methenamine hippurate is an antiseptic, first used over 100-years ago as a treatment for recurrent UTIs in Nordic countries. In the UK, the recent multi-centre ALTAR trial found that methenamine hippurate was non-inferior to current prophylaxis treatment of UTIs in women.

In the rotation project we aim to understand whether methenamine hippurate acts synergistically or antagonistically with recommended antibiotics for the treatment of UTIs. We will assess the effect of methenamine hippurate on the activity of trimethoprim, nitrofurantoin, cefalexin, fosfomycin and pivmecillinam. This will be achieved through minimum inhibitory concentration, following EUCAST guidelines, and minimum bactericidal concentration assays in the presence and absence of methenamine hippurate. Synergistic and antagonistic interactions between the antibiotics and methenamine hippurate will be determined with checkboard assays.

This project will guide the best use of the antiseptic by identifying those antibiotics that able or unable to be used in tandem with methenamine hippurate to improve treatment success and patient outcomes.

Location: Clifton Campus;

Full project description: Urinary tract infections (UTIs) are one of the most frequent infections in healthy women and consequently, in the UK, UTIs are the second most common reason for prescribing antimicrobials and account for 1-3% of all medical consultations. The predominant cause of UTIs is Escherichia coli, accounting for 75% of uncomplicated UTIs. Importantly, UTIs caused by E. coli are associated with high levels of recurrence of infection (40%). The incidence of antimicrobial resistance to first-line antibiotics are increasing, especially towards trimethoprim (33.4%), nitrofurantoin (1.5%) and cephalexin (12%). Overuse of antibiotics to treat recurrent UTIs will increase the incidence of resistance.

Methenamine hippurate is an antiseptic, first used over 100-years ago as a treatment for recurrent UTIs in Nordic countries. In the UK, the recent multi-centre ALTAR trial found that methenamine hippurate was non-inferior to current prophylaxis treatment of UTIs in women. Antimicrobial resistance, specifically multidrug resistance, were equal to or lower after treatment with methenamine hippurate compared to traditional antibiotics up to 12 months post treatment. Therefore, methenamine hippurate represents an

attractive alternative to traditional antibiotics for the treatment of recurrent UTIs. However, little is currently known about the mode of action of methenamine hippurate.

In this project we aim to further understand how methenamine hippurate prevents recurrent E. coli infections in the bladder, identify any synergistic interactions with recommended antibiotics for the treatment of UTIs and its role in selection of antimicrobial resistance. First, we will assess the effect of methenamine hippurate on the activity of trimethoprim, nitrofurantoin, cefalexin, fosfomycin and pivmecillinam. This will be achieved through minimum inhibitory concentration, following EUCAST guidelines, and minimum bactericidal concentration assays in the presence and absence of methenamine hippurate. Synergistic and antagonistic interactions between the antibiotics and methenamine hippurate will be determined with checkboard assays. Secondly, we will how methenamine hippurate prevents recurrent of infection in a physiologically relevant environment. Here, we will use 3D cell culture model of the human bladder (urothelial organoid) to determine the effects of methenamine hippurate on the attachment and intracellular lifecycle of E. coli during a UTI, as well as the ability to form biofilms. Third, we determine the effect of methenamine hippurate on the selection of resistance to the above antibiotics. We will use evolutionary ramp experiments to select for and determine frequency of resistance in the presence and absence of methenamine hippurate. Selected resistant mutants will be whole genome sequenced to identify the genetic determinants of resistance. In addition, the mutants will be fully characterised using fitness, population establishment, collateral susceptibility and mutant selection window assays.

This project is expected to further the understanding of the mechanism of action methenamine hippurate in reducing recurrent UTIs and produce an evidence base to increase confidence in its use. Furthermore, data produced within this project will guide the best use of the antiseptic by identifying those antibiotics that able or unable to be used in tandem with methenamine hippurate to improve treatment success and patient outcomes.

Full project location: Clifton Campus;

The effect of physical activity on the immune system in physically inactive older adults.

Project Supervisor: John Hough

School: School of Science and Technology (NTU)

Description: This study aims to examine changes in dendritic cell Toll-Like Receptor function, cytokine release , T- cell and dendritic cell counts, and the cortisol and testosterone changes in response to the cycling stress test before and after a 12 day training period. The novelty of this study is that currently there is no knowledge of the dendritic cell alterations with short duration training in adults. These cells are vital in the response of the immune system to novel infection risks. Understanding this information will support the development of physical activity programmes to support healthy ageing.

Location: Clifton Campus;

Full project description: Worldwide people are living longer, but this extended lifespan is not paralleled with a continuation of good health. There is strong evidence of age-associated changes in our immune systems. These changes increase the risk of morbidity and mortality with an "immune risk phenotype" (IRP) now identified predicting 2- and 4-year mortality in older adults. Characteristics of the IRP include poor blood T-

cell response to foreign particles (antigens), a reduced naïve T-cell population, and the presence of antibodies against a virus in the plasma. The term used to describe the biological ageing of the immune system is "immunosenescence". It is accepted that those who regularly complete moderate-intense exercise experience a lowered infection risk when compared with their sedentary counterparts. This suggests that regular modulate physical activity may help to combat immunosenescence and that sedentary behaviour may advance immunosenescence.

Despite the reduced infection risk when regular physical activity is undertaken in older adults, there is still a lack of understanding surrounding the potential mechanisms by which physical activity might improve or maintain 'immune health' in older adults. Some suggested mechanisms may be an exercise-induced increase in lymphocyte β2 adrenergic receptor sensitivity. Maintenance of receptor sensitivity allows catecholamine mediated immune cells redistribution between blood and tissue with each bout of exercise. This redistribution with regular exercise increases immune surveillance which may reduce latent viral reactivation. In addition, activated skeletal muscle release important T-cell regulating myokines e.g. IL-7 and IL-15. These myokines help to maintain T-cell activity.

Examination of these mechanisms may uncover a direct link between physical the amelioration of immunosenescence and therefore emphasise the importance of the prescription of physical activity to maintain immune health. These mechanisms would highlight the important role that physical activity plays in the prevention of and therapy for chronic low-grade inflammation. Therefore, physical activity could serve as an effective strategy against the development of an increased inflammatory environment associated age-related diseases such as cardiovascular disease and type 2 diabetes.

The main aims of this research are to consider:
- If exercise delays immunosenescense.
- If the introduction of regular exercise to a sedentary individual alters their IRP profile to a non-IRP state.

Full project location: Clifton Campus;

Immunity in the face of diversity and the development of protective vaccines against African trypanosomes.

Project Supervisor: Catarina Gadelha

School: Life Sciences

Description: The lab rotation will carry out the first steps of the PhD project using a smaller set of surface antigens. In the project, invariant surface molecules identified through sensitive, quantitative proteomics will be screened for immunogenicity using introduced tags and a novel assay to test for host immune visibility and antibody-killing efficacy in vitro. Identified immunogens will then be characterised for biological function and parasite viability through the generation of mutants using inducible gene silencing, and mutant phenotype analysis assayed by fluorescence and electron microscopy and biochemical methods. In carrying out this rotation, students will gain training in molecular biology, advanced microscopy, biochemistry and cell culture, all of which should be useful to work in many other laboratories. In doing so, students will identify exposed essential molecules that are potential vulnerabilities for the development of vaccine against a lethal human infectious disease.

Location: QMC;

Full project description: Background:Where possible, disease elimination through vaccination is safe, effective and cheap, and the UK government has a significant interest in the development of new vaccines for infectious diseases. Pre-clinical vaccine testing starts with the identification of unique and exposed pathogen components capable of generating a protective immune response (through long-lived antibodies and memory B-cells), and proceeds to the immunisation of a cohort of animals with a potential immunogen, followed by pathogen challenge and monitoring of disease.

Hypothesis and Research Plan:

This project will use high-throughput proteomics and advanced informatics for the high-confidence identification of surface-exposed antigens of African trypanosomes – human parasites transmitted by tsetse fly bite, that threaten ~60 million people each year (WHO, 2021). Surface-exposed antigens were identified for the in vitro experimental model (Gadelha et al 2015 Mol Cell Proteomics), rendering confidence to the methodology and training proposed here. This project will investigate which antigens are present in in vivo models (host-derived parasites that cause chronic and acute trypanosomiasis in humans), and whether vaccines against them would confer protection. Genome-wide Loss-of-Fitness screens will identify surface-exposed antigens essential for successful establishment and maintenance of infection. The intersection of protein and DNA data will be used to prioritise vaccine candidates on the basis that essential targets are less likely to be amenable to immune escape. Finally, a population analysis using Next Generation DNA Sequencing will measure the geographic distribution of validated surface antigens that are under diversifying selection, such that to pinpoint the ideal vaccine candidates that offer pan-protection across parasite strains encountered in the whole Africa continent.

Expected outcomes and Impact:

Effective candidates will then be taken forward to manufacturing test and pre-clinical analysis. Recombinant antigens will be injected into animal models of disease, which will then be challenged with trypanosomes to test for protective effect in the host. Please note: Home Office project licence is already in place. These data will provide the basis for priming two existing vaccinology pipelines in project collaborators' institution (Wellcome Sanger Institute-Cambridge and Roslin Institute-Edinburgh). It is also expected that antigens identified here will include potential drug targets; any candidates that appear druggable will be quickly communicated to the TDR Targets Database to facilitate drug discovery for neglected disease pathogens.

Training:

This is an inherently interdisciplinary vaccinology project, requiring mastery of high throughput and advanced quantitative science techniques – representing a unique training opportunity within the growing field of digital technologies and informatics for health. Skills will include the interpretation of omic's data and other large datasets, statistical testing, programming, bioinformatics and high throughput image analysis. The student will also receive training in several wet-bench techniques such as molecular biology, gene silencing, cell transfection, handling of pathogens, cell sorting and microscopy. Bioscience research often involves training and application of in vivo techniques; these form a relevant part of the proposed project, and the student will be encouraged to consider the refinement of their experiments, alternatives to the use of animals, and the statistical justification of the number of animals required, in accordance with the 3Rs.

Full project location: QMC;

What is the fate of silver in silver resistant bacteria?

Project Supervisor: David Scott

School: Biosciences

Description: Silver is an excellent antimicrobial: it is more lethal to bacteria than mercury but relatively benign to humans, except in high concentrations. As such it has been used as an unregulated antimicrobial in many applications; consequently due to its overuse and release into the environment, there has been a rise in bacterial silver resistance.

Silver resistant bacteria have been imaged using electron microscopy and silver metal particles are observed on the inside of the cell. It is unclear as to why this is, and how these particles are generated, especially given that there is a well defined set of metal ion efflux systems whose specific purpose is to export silver ions out of the cell.

The project will use the latest electron microscopy techniques to image and then reconstruct a 3D model of silver resistant bacteria so as to locate precisely where the metal particles are in the cell. This is the first step in elucidating the mechanism of metal particle generation by the resistance mechanism, or by cellular processes that interact with the exported metal ions.

Location: Sutton Bonington Campus;Research Complex at Harwell;Diamond Light Source;

Full project description: Life works in three dimensions. We will use a combination of cryo electron microscopy methods and super-resolution microscopy to understand the spatial distribution of the silver particles and the components of the bacterial silver resistance mechanism.

In Gram negative bacteria the resistance mechanism is made up of SilP, an inner membrane ATPase, SilABC an RND+ two membrane spanning efflux pump, SilRS a two component system and SilEFG a periplasmic chaperone system. We have structures for SilC and SilF, and functional information on SilE and SilF.

Using a combination of cryo electron tomography and cryo-FIB-SEM we wish to image the bacterial cell in slices and then use the data to reconstruct a model of the cell. In concert with this, we will produce knockout mutants of the sil components and image these to see where there are functional and spatial differences in silver particle distribution. We will produce fluorescence tagged versions of the Sil proteins which will allow their imaging via super-resolution microscopy, which can be combined with cryo EM data in order to understand the mechanism of silver resistance.

The project will combine:

1. molecular biology techniques to produce suitable gene knockout and tagged proteins training in cryo EM sample preparation and the acquisition of data at local, regional and national resource
2. training in image analysis and reconstruction
3. training in super-resolution microscopy
4. relating structural data to biological function

The project will be based primarily on the Sutton Bonington campus but will use facilities at University Park campus, the University of Leicester and our DTP partners the Research Complex at Harwell and Diamond Light Source.

Full project location: Sutton Bonington Campus;Research Complex at Harwell;Diamond Light Source;

The exploration of brain-penetrant analogues of cannabidiol (CBD) for enhanced targeting of treatment-resistant cancers.

Project Supervisor: Shailesh Mistry

School: Pharmacy

Description: The rotation project offers the opportunity to experience training in a number of disciplines. Whilst fluorinated analogues of CBD have been previously described, their evaluation has focused on the use of behavioural in vivo animal models of psychological disorders. Recent work within our group has identified the anticancer properties of 4'-F-CBD. Others have shown that fluorinated CBD analogues might exhibit increased penetration of the blood brain barrier (BBB). These observations offer the attractive prospect of developing new CBD analogues with improved brain penetration and anticancer activity. During the rotation project the student will synthesise a fluorinated CBD analogue (S. Mistry). The student will also get exposure to the in vivo work currently undergoing for the delivery of CBD to brain its distribution between different anatomical brain regions (P. Gershkovich). The student will also get the opportunity to work on molecular mechanisms of cannabidiol activity (S. Alexander) and on the in vitro efficacy of cannabidiol in various brain cancer models (T. Bradshaw).

Location: University Park;QMC;

Full project description: CBD is the most abundant non-psychoactive cannabinoid isolated from Cannabis sativa, with a vast therapeutic potential. Previous work (Breuer et al, 2016) describes CBD analogues bearing fluorination at either the C4-, C7- or C10-positions. Fluorination at the C4-position (4'-F-CBD, or PECS-101) resulted in significantly increased anxiolytic, antidepressant, anti-compulsive and antipsychotic activity in in vivo animal behavioural models compared to CBD. These effects were observed following intraperitoneal (i.p.) injection at up to 10× lower dose compared to CBD. The differences in in vivo activity between 4'-F-CBD and CBD suggests that either 4'-F-CBD has increased efficacy at a molecular level, or that more 4'-F-CBD is delivered to the active site (i.e. the brain). Increased permeability of other fluorinated compounds across the blood-brain barrier (BBB) and into the brain has also previously been demonstrated.

Moreover, recently we have demonstrated the anti-cancer properties of 4'-F-CBD for the first time (Brookes et al, manuscript in preparation). 4'-F-CBD was found to inhibit glioblastoma multiforme (GBM) cell growth in vitro. 4'-F-CBD could also overcome the two common resistance mechanisms to the standard of care treatment, temozolomide (TMZ): O6-methylguanine-DNA methyltransferase (MGMT) over-expression, and a deficiency of mismatch repair (MMR). Similarly to CBD, 4'-F-CBD also demonstrated synergy with TMZ and derivative T25. 4'-F-CBD (PECS-101) has also recently been reported to prevent chemotherapy-induced neuropathic pain following i.p. injection in mice. Our preliminary findings, along with literature reports of 4'-F-CBD activity in vivo, demonstrate the vast therapeutic potential of 4'-F-CBD. Our results demonstrate similar activity of 4'-F-CBD to CBD in vitro, with no significant differences between the concentrations required to inhibit cell growth by 50% (GI50). This suggests that the increased efficacy seen in vivo is likely a result of increased delivery to the brain.

Our subsequent brain biodistribution studies (Brookes et al, manuscript in preparation) demonstrated key differences between CBD and 4'-F-CBD after oral administration. When the whole brain was studied, there was more consistent delivery of 4'-F-CBD, resulting in higher exposure of the tissue to the fluorinated cannabinoid. This was also true in most anatomical brain regions while the olfactory bulb and striatum were the only regions with higher exposure to CBD than 4'-F-CBD. The higher exposure of the tissue to

4'-F-CBD is in support of reports of preferential delivery of fluorinated molecules across the BBB and into the brain. It is likely that the higher exposure of the brain to the fluorinated cannabidiol is responsible for some of the increased potency over CBD observed in vivo.

The overarching aim of this PhD work is to develop novel CBD analogues (through exploration of fluorination and other approaches) for enhanced uptake into brain tissue, and optimal distribution between anatomical brain regions for better treatment outcomes of the most aggressive brain cancers, such as glioblastoma or diffuse intrinsic pontine glioma. This multidisciplinary project will involve synthetic organic and medicinal chemistry, in vivo pharmacokinetics and biodistribution in rodents, mechanistic molecular pharmacology and cell culture efficacy studies.

Full project location: University Park;QMC;

Using Artificial Intelligence Approaches for the Design of New Bacteria for Synthetic Biology, Health and environmental biology.

Project Supervisor: James McInerney

School: Life Sciences

Description: "Leveraging GPT Transformers for Pangenome Presence/Absence Data Interpretation". Background:Pangenomes are collections of genes that are found within a particular species or group of closely related species. Presence/absence data from pangenomes can provide insights into the genetic diversity and evolutionary history of species. The goal of this project is to use the autoGPT library, a variant of the transformer-based GPT architecture, to analyze pangenome presence/absence data.

Objectives:
- Train a GPT transformer model on presence/absence data from pangenomes using the autoGPT library.
- Evaluate the ability of the trained GPT model to predict and interpret presence/absence patterns.
- Optimise the model hyperparameters to improve prediction accuracy.

Methods:

Data Collection:
- Gather presence/absence data from publicly available pangenome databases.
- Preprocess the data to ensure it is in a format suitable for training with autoGPT.

Model Training:
- Use the autoGPT library to train a GPT transformer model on the presence/absence data.
- Split the dataset into training, validation, and test sets to monitor the model's performance and avoid overfitting.

Model Evaluation:
- Assess the model's ability to predict presence/absence patterns on unseen data (test set).
- Use metrics such as accuracy, F1-score, precision, and recall to evaluate the model's performance.

Hyperparameter Optimisation:

- Employ techniques such as grid search or random search to explore different hyperparameter combinations.
- Use Bayesian optimisation or other advanced hyperparameter tuning methods if feasible.
- Track changes in model performance (using validation set) as hyperparameters are adjusted.

Analysis and Interpretation:
- Investigate patterns learned by the GPT model.
- Use the trained GPT model to generate potential novel presence/absence patterns and analyze their biological significance.
- Compare the performance of the GPT model with traditional machine learning models or other methods used in the analysis of pangenomes.

Expected Outcomes:
- A trained GPT transformer model capable of interpreting pangenome presence/absence data.
- Insights into the potential advantages and limitations of using GPT transformers for bioinformatic analysis.
- A set of optimised hyperparameters that maximize the prediction accuracy of the GPT model on the test set.

Potential Challenges:
- Ensuring the quality and consistency of the presence/absence data.
- Overfitting due to the complexity of the GPT model.
- Interpretability challenges associated with deep learning models.

This project offers students a unique opportunity to combine advanced machine learning techniques with bioinformatics. By the end of the rotation, the student will have gained experience in both fields and will have contributed to the ongoing exploration of innovative methods in pangenome analysis.

Location: University Park;

Full project description: Pangenome Interpretation using GPT Models

1. Introduction: The genetic diversity inherent in prokaryotes offers a treasure trove of information, critical to understanding the mechanisms of adaptation, survival, and environmental interaction. The comprehensive collection of all genes within a species or group of related species, termed the pangenome, serves as a dynamic resource that encapsulates this genetic diversity. This proposal will harness the advanced capabilities of Generative Pre-trained Transformer (GPT) models, similar to chatGPT, to delve into the intricate patterns embedded within prokaryotic pangenomes.
2. Objective: The primary aim of this initiative is to train a GPT AI system on presence-absence data from prokaryotic pangenomes, enabling the model to identify and predict intricate genomic patterns. Subsequently, the AI will be tasked with proposing potential synthetic genomes, representing blueprints for creating viable prokaryotes.
3. Methodology:
    a. Data Collection and Preprocessing: Generate relevant presence-absence data from genomic databases. We will use pangenome preparation programmes, such as ROARY and PANAROO.
    b. GPT Model Training:Using the autoGPT library, a GPT transformer model will be trained on the preprocessed data. The dataset will be partitioned into training,

validation, and test subsets to ensure rigorous model evaluation and to mitigate overfitting.
   c. Synthetic Genome Proposal:Post-training, the AI will generate proposals for synthetic genomic constructs, based on the patterns and relationships it extracts from the training data.
   d. Feasibility Assessment:Laboratory-based experiments will be designed to synthesise these genomic constructs and introduce them into suitable hosts. The primary goal is to evaluate their potential viability as real prokaryotic organisms.
4. Expected Outcomes:This initiative will produce a GPT AI system proficient in pangenome pattern recognition and synthetic genome proposal. If the laboratory assessments indicate positive results, it could signify a pioneering step in microbial engineering and synthetic biology, as well as having profound implications for understanding antibiotic resistance, microbial adaptability to changing climate, and much more.
5. Implications and Future Directions:The intersection of advanced AI with genomic science could revolutionise our methodologies in microbial engineering. If the AI demonstrates proficiency in crafting viable genomes, it could instigate a paradigm shift in how we approach genetic research, microbial adaptability to climatic shifts, and the ever-evolving challenge of antibiotic resistance.
6. 6. Conclusion:The "Pangenome Interpretation with GPT" initiative represents an ambitious endeavour at the confluence of bioinformatics and AI. By leveraging the capabilities of GPT transformer models in the realm of prokaryotic pangenomes, we aspire to uncover novel insights and methodologies that could redefine the horizons of microbial genomic research.

Full project location: University Park;

Characterisation of equine asthma endotypes

Project Supervisor: Sarah Blott

School: Veterinary Medicine and Science

Description: Mild-moderate equine asthma (mEA) is a common problem in horses which negatively affects athletic performance. Diagnosis of mEA is made by integrating the observation of clinical signs with cytology testing carried out on bronchoalveolar lavage (BAL) or tracheal wash (TW) fluid. The relative cell type proportions observed in the fluid are key to diagnosis. During this rotation, the student will be introduced to statistical modelling of longitudinal cell count data from TW fluid and how this can be used to define mEA phenotype. The work will enable the student to understand different cellular responses seen over time in mEA cases and controls, and to develop skills in the use of R for statistical modelling. In the second part of the rotation, the student will gain skills in the analysis of transcriptomics (RNA seq) data. Traditional cytology techniques are limited in accuracy, as it is difficult to obtain absolute cell counts from microscopy samples and subjective scores are used as a summary. Single cell RNA-seq data allows gene marker sets to be identified, which will enable more accurate and objective cell-type abundance estimates to be made. The mEA phenotype will be re-defined, based on objective cell-type abundance estimates and with a wider range of identified cell types.

Location: Sutton Bonington Campus;

Full project description: Asthma is a common inflammatory airway disease in horses; defined by experts as a spectrum of disease, ranging from a mild-moderate form previously known as inflammatory airway disease (IAD) to a severe form, previously

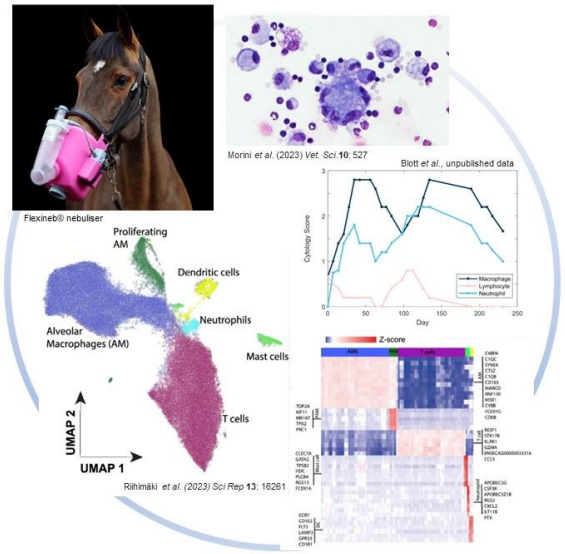
known as 'heaves' or recurrent airway obstruction (RAO) (Couëtil et al., 2016). Clinical signs of mild-moderate equine asthma (mEA) include impaired athletic performance or prolonged respiratory recovery following exercise, and can include cough, the presence of tracheobronchial mucus, airway obstruction and hyperresponsiveness. In comparison with severe equine asthma (sEA), horses with mEA do not have respiratory abnormalities at rest. For mEA, bronchoalveolar lavage (BAL) or tracheal wash (TW) fluid shows mild increases in neutrophils, eosinophils and/or mast cells (Kinnison et al. 2022). At the transcriptome level, both mEA and sEA exhibit downregulation of genes involved in the genesis, length and motility of respiratory epithelium cilia. mEA results in overexpression of genes encoding inflammatory mediators, while the sEA transcriptome shows evidence for upregulation of genes involved in bronchoconstriction, apoptosis and hypoxia, with down-regulation of genes involved in the formation of the protective muco-protein film and enrichment of gene networks activated in human asthma (Padoan et al. 2022).

In humans, the asthma phenotype is heterogeneous and has been divided into endotypes (molecular pathways driving disease) (Lötvall et al. 2011) which can be grouped into two major types: Th2-high (eosinophilic) and Th2-low (non-eosinophilic) (Kuruvilla et al. 2019). Equine asthma shows similar heterogeneity of phenotype, with some studies identifying three subgroups for mEA: mastocytic, neutrophilic and mixed (Karagianni et al. 2021). The mastocytic and mixed subgroups have been shown to have allergen-specific IgE present, suggesting that this form of equine asthma is allergy related (Hansen et al. 2020), while the neutrophilic group may represent asthma triggered through viral or bacterial infection. The proposed project aims to more precisely characterise mild-moderate equine asthma (mEA) endotypes, based on clinical signs, cell cytology, immunology and genetics. The delineation of specific endotypes will be an important step in improving the management and treatment of equine asthma.

Programme of work:

The project will use pre-existing clinical, cytology and genetic data collected on 72 Thoroughbred racehorses in training, while also providing the opportunity to generate new data from archived samples taken from these horses. Endotypes will initially be characterised through the statistical analysis of the cytological and genetic data previously collected on the horses. This will include:

1. Statistical modelling of longitudinal cell count data, to determine disease profile over time. Cytology cell counts taken on each horse at weekly intervals over 12 months will be modelled using mixed non-linear statistical models to obtain longitudinal cell count profiles.
2. Cluster and machine learning analysis of cell count data to identify potential endotype groups, and testing whether identified endotype clusters show correlation with longitudinal profiles identified in objective 1.
3. Genetic analysis (SNP-based heritability, genome-wide association and gene-set analysis) to identify DNA variants and gene pathways associated with endotypes derived in objectives 1 and 2.



Information on the endotypes identified will then be used to select samples for transcriptomic analysis (RNA sequencing), enabling the functional effects of DNA variants associated with asthma endotypes to be elucidated.

Full project location: Sutton Bonington Campus;

Combining C-H functionalization and SuFEx click chemistry for peptide bioconjugation

Project Supervisor: Warren Cross

School: School of Science and Technology (NTU)

Description: New methods for peptide modification are in high demand in a number of biological areas, including tools for chemical biology and in pharmaceutical drug discovery. We have developed new peptide modification reactions that use metal-catalyzed C-H functionalization chemistry. Our C-H functionalization method works on native, "off-the shelf" peptides, and crucially leaves biologically important heteroatoms untouched.

So far, we have demonstrated the C-H functionalization reaction on model peptides. In this lab rotation project, the aim will be to showcase the chemistry in RGD peptides, a biologically important class of peptides that has been used in the synthesis of peptide-drug conjugates for targeted cancer treatment.

This lab rotation project will involve the preparation of RGD peptides using solid-phase peptide synthesis (SPPS), and the application of our palladium-catalyzed C-H functionalization to these RGD peptides. The scope of the modifying group will be investigated and will include groups that enable further chemical manipulation of the modified peptide. Further chemical manipulation by attachment of a fluorophore or a drug candidate molecule, as examples, will demonstrate potential biomedical applications of the modified RGD peptides.

Location: Clifton Campus;

Full project description: Peptide modification has a critical role in drug discovery, the diagnosis of disease, and the understanding of biological mechanism. Most methods of peptide modification rely upon the reactions of heteroatoms, which are often crucial to biological function. To address this shortcoming, there has been much recent interest in metal-catalyzed C H functionalization of peptides; these new methods have the advantage that they leave important heteroatoms untouched and are applicable to "off-the-shelf" native peptides.

At NTU we have used C-H functionalization for the modification of phenylalanine and tryptophan residues in peptides: in the presence of a palladium catalyst, these aromatic residues react with alkenes to give modified peptides.

In further recent work, we discovered that ethene sulfonyl fluoride (ESF) can be used as the alkene. The resulting peptide modification installs a sulfonyl fluoride group in the peptide; this sulfonyl fluoride group can be further manipulated by sulfur-fluoride exchange chemistry (SuFEx), a click reaction of much current interest for biological application.

Our combination of C-H functionalization / SuFEx chemistry enables selective modification of amino acid residues that are difficult to edit chemically: for the first time, targeted manipulation of these residues is possible.

The aim of this project is to demonstrate the biological application of a C-H functionalization / SuFEx strategy for peptide modification in three areas:

Area 1. conformationally constrained peptides for drug discovery: In drug discovery, peptide therapeutics are an attractive alternative to small molecule drugs, often possessing higher target specificity. However, native peptides generally have poor pharmacological properties; cyclic peptides and stapled peptides (in which the peptide is constrained in its bioactive conformation), have been shown to improve these properties. Here we will use C-H functionalization / SuFEx chemistry to synthesise cyclic and stapled peptides of pharmaceutical importance.

Area 2: Area 2. peptide-drug conjugates. Conjugation of a small molecule drug to a peptide has been used to control drug delivery. We will fabricate conjugates of tumour targeting RGD peptides with chemotherapy drugs such as doxorubicin. As one example, the cyclic peptide (c[-RGDf(NMe)V-]), should undergo palladium-catalyzed C-H functionalization with ESF, followed by SuFEx reaction with doxorubicin, giving the peptide-drug conjugate.

Area 3: Area 3. covalent binding peptide probes. Covalent binding of drugs to proteins enables irreversible inhibition, potentially enhancing selectivity and pharmacological duration. Most covalent inhibitors target cysteine residues, and there is a need for new chemistry to select for alternative amino acid residues. SuFEx click chemistry enables the targeting of residues such as tyrosine and lysine. This part of the project will use C-H functionalization / SuFEx chemistry to investigate peptide drugs and probes and their inhibition of appropriate target proteins, for example, the mRNA decapping scavenger enzyme DcpS.

The nature of the synthetic chemistry proposed is extremely versatile, with biological applications in areas beyond those described here. The project will be adapted to the interests of the PGR undertaking the project. Peptide chemistry will be based at NTU; research on catalyst development, and spectroscopic and modelling investigations will take place at the University of Nottingham.

Full project location: Clifton Campus;University Park;

Developing bismuth-based broad-spectrum antivirals for human and animal health

Project Supervisor: Sophie Benjamin

School: School of Science and Technology (NTU)

Description: This is a cutting-edge cross-disciplinary project between NTU and UoN that integrates synthetic chemistry and applied virology to develop broad-spectrum antiviral against major respiratory viruses, including coronaviruses and influenza viruses which affect both humans and animals. The COVID-19 pandemic highlighted the need for effective antivirals to treat active infections, in conjunction with vaccines to prevent infection. Bismuth complexes are known for their low toxicity and antimicrobial properties; recently, it was found that certain bismuth complexes inhibit the replication of the SARS-CoV-2 virus.

The chemical structure and behaviour of bismuth antimicrobials is not well understood despite their clinical use. A functional challenge is their low solubility, often leading to formulation as a colloidal suspension limited to oral treatments. The aim of this project is to synthesise a new, water soluble bismuth complex, and perform initial assessments of its antiviral properties. The PhD candidate, in the synthetic chemistry laboratory (NTU), will explore a range of synthesis and characterisation methods, including crystallographic and spectroscopic techniques to generate a new molecular bismuth complex. They will

also undertake a 3-week work visit to a containment level 2 virus research facility (UoN) for introduction to key virology techniques including cell culture and hemagglutination assays.

Location: Clifton Campus;Sutton Bonington Campus;

Full project description: This cutting-edge cross-disciplinary project integrates synthetic chemistry and applied virology to develop broad-spectrum antivirals against major respiratory viruses which affect humans and animals, including influenza viruses and coronaviruses. The project is flexibly designed to accommodate the interest and training needs of the prospective student to maximise seamless cross-over between disciplines.

The COVID-19 pandemic highlighted the need for effective antivirals to treat active infections, in conjunction with vaccines to prevent infection. Development of antiviral resistance remains an intractable problem of organic antivirals. Metallodrugs, i.e. drugs containing metal elements, may provide a means of overcoming this challenge, as bacterial resistance has been shown to be less common for these types of drugs.

Bismuth complexes are known for their low toxicity and antimicrobial properties; several have been clinically approved for the treatment of gastrointestinal H. pylori infections. It was recently found that some bismuth complexes inhibit the replication of the SARS-CoV-2 virus in in vivo trials with hamsters. Understanding the mode of action of bismuth drugs is a growing subject of current research. It has been proposed that Bi(III) ions displace Zn(II) ions within enzyme active sites, due to their high affinity for cysteine residues, to irreversibly deactivate bacterial or viral enzymes such as helicases. The main class of clinically approved bismuth drugs are bismuth citrates. Despite their clinical use, their chemical structure and behaviour is not well understood. A functional problem is their low solubility, often leading to formulation as a colloidal suspension for oral use only. There is evidence that different bismuth complexes have significantly different clinical efficacy, but no structure-function relationships have yet been developed, largely due to the poor understanding of their mode of action. Often antiviral testing is limited to a small set of existing bismuth compounds.

The overall aim of this project is to develop new Bi-based antiviral agents by rational design. The project has three objectives:

Objective 1: Design and develop a library of new Bi complexes with well-defined bismuth coordination environments, functionalised to target properties such as water solubility and improved antiviral efficacy. Incorporate functional groups within the ligand structure of new complexes. Investigate zwitterionic ligands to improve solubility, and hemilabile ligands employed to develop complexes which are robust in physiological conditions but can easily be partially displaced by enzyme binding sites. Explore different complexation methods including solvothermal and microwave reactions.

Objective 2: Investigate the structure of the new complexes both in solid and solution phases and explore their ligand exchange properties with cysteine. Use analytical techniques such as single crystal X-ray diffraction, powder diffraction, mass spectrometry, NMR and IR spectroscopy.

Objective 3: Undertake antiviral evaluation of new complexes against a number of important respiratory viruses including SARS-CoV-2 and influenza A virus. Investigate properties such as cytotoxicity, antiviral activity and dose response using techniques including cell culture, qPCR, focus forming assays and hemagglutination assays. Identify structure-antiviral function relationships to feed back into drug design.

Full project location: Sutton Bonington Campus;Clifton Campus;

Do ligands with different chemical structures impact conformational changes in GPCRs?

Project Supervisor: Shahida Mallah

School: Life Sciences

Description: To investigate the binding pocket - amino acid residues of prostacyclin (IP)-receptors coupled to G-proteins with therapeutic use in pulmonary arterial hypertension.

Prostacyclin (PGI2) is a potent vasodilator and platelet inhibitor, recent in vitro research carried out on novel analogues of prostacyclin have shown in pharmacological assays such as VASP-P phosphorylation, Tango-Presto Assay, and calcium assays that two isomers of a prostacyclin analogue display a different pharmacological potency. Z isomer is approximately 200-fold more potent than E isomer. PGI2 compounds bind to prostacyclin receptor that is not crystallised yet. Therefore, homology model-based studies were carried out to identify differences at ligand-receptor interface. Novel residues (Cys151, Cys259, The256) along with notable common residues (M99, D60) in human IP receptor are identified which are potentially involved in binding. These residues will be mutated, and pharmacological assays will be used to validate the findings.

- To design primers to mutate specific amino-acid residue in IP/PTGIR plasmid
- To clone IP plasmid via bacterial transformation
- To isolate IP plasmids and express into HEK293/HTLA cell line
- To establish GPCR based PRESTO Tango assay to do G-protein independent drug screening

Site directed Mutagenesis, PCR, Bacterial Transformation, cell culture, western blotting, Luminescence.

Location: QMC;Research Complex at Harwell;Diamond Light Source;

Full project description: Background: Prostacyclin (PGI2) is derived from arachidonic acid and act as a potent vasodilator, inhibits platelet aggregation, and have anti-inflammatory and anti-proliferative actions. Prostacyclin binds with prostacyclin receptors (IP) which couple to G-proteins. IP receptors are mainly expressed on platelets and vascular smooth muscle cells. Although therapeutically prostacyclin mimetics and non-prostanoid drugs are used in the treatment of cardiovascular disease but less emphasis is given to the receptor structure and how prostanoid drugs produce downstream specific effect. In this project, human IP-receptor structure will be explored and how drugs bind at receptor level.

Aims: The major aim of the project is to understand the structural basis for prostanoid action on prostacyclin receptors:

- To understand the molecular interactions between the ligand and receptor binding pocket
- Do ligands with different stereoisomers induce different conformations?
- Endogenous mimetics vs non-endogenous ligands will also be evaluated.
- Downstream signalling mapping will be carried out by using CAMP measurement.
- Do-GPCRS' conformation differ in structure within different sexes.

Methodology: Human IP plasmid will be cloned into a range of insect and mammalian expression vectors. Thermstabilising chaperone proteins such as T4 lysozyme will be incorporated either onto the N-terminus or IC3 for some constructs. Chemical transfection-based methodology or viral transduction will be utilised to express plasmid into human embryonic kidney cells (HEK293) or Spodoptera frugiperda. Receptor expression will be confirmed based upon fluorescently labelled tags.

Receptor isolation

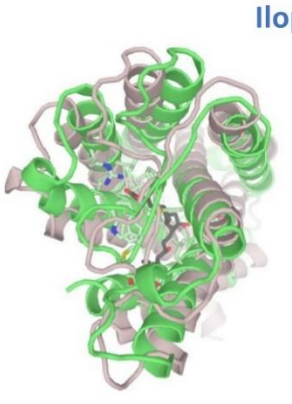
Purification and reporter tags incorporated during cloning (e.g. thermostbilised green fluorescent protein/ deca-His/ twin step/ FLAG) will be used to purify the receptor. The multi construct approach will help to ensure success. Buffer, salts and encapsulation agents (detergents, amphipols, lipid and co-polymer nanodiscs and peptides) will be screened to find optimal conditions for protein purification using the facilities at the Membrane Protein Laboraortory (Diamond/ RCaH). The most promising conditions will be scaled-up to produce IP co-purified with functional modulators (agonists and antagonists) for structural studies.

Receptor characterisation

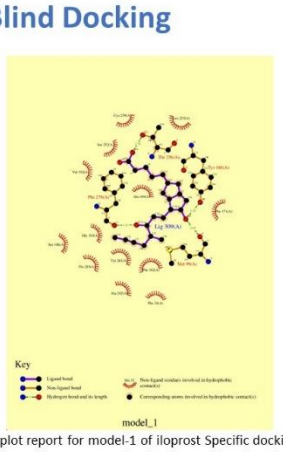
A range of approaches including X-ray crystallography and cryo-electron microscopy as well as complementary tools such as mini- G-proteins . To ensure high-quality proteins samples and understand ligand protein interactions a range of biophysical techniques will be used. These include Surface plasmon resonance, flow-induced dispersion analysis and nano differential scanning fluorimetry.

Potential outcome and Impact of the project: The results from this project will aid into fundamental scientific contribution into structure discovery. This will also enable us to understand receptor preferences in analogues vs non-analogues. Also, it will facilitate potential structure-based drug design.

Full project location: QMC;Diamond Light Source;Research Complex at Harwell;



The binding site of iloprost in prostacyclin receptor homology model



A Ligplot report for model-1 of iloprost Specific docking into Prostacyclin receptors showing key residues involved in binding

Applying RNA Interference Technology to Understand the Role of 1q Amplification in Multiple Myeloma Pathogenesis

Project Supervisor: Zahraa Al-Ahmady

School: School of Science and Technology (NTU)

Description: During the lab rotation project, the student will be trained on several state-of-the-art cell culture techniques and cell viability assays that will be tested on a collection of multiple myeloma cell lines with different levels of chromosome 1q gain such as U266 (3 copies), KMS12BM (3-4 copies), MOLP8 (6-8 copies). Using RNA interference technology the student will test the effect of several candidate genes on chromosome 1q that are implicated in the multiple myeloma progression. The silencing effect of those genes will be studied in isolation as well as combined to decipher any synergistic effect. Silencing will be confirmed by quantification at the RNA level using qRT-PCR post-transfection as well as at the protein level with Western blot or ELISA.

The next steps in the project will be in vitro assays to determine the effect of silencing the candidate genes on multiple myeloma cell lines' growth. Following the lab rotation, the student will replicate the methodology described above on primary patient samples as well as in in vivo NSG mice multiple myeloma models.

Location: Clifton Campus;

Full project description: Multiple myeloma is the second most common haematological cancer. It is a plasma cell tumour that is characterised by a remarkable molecular heterogeneity and remains an incurable malignancy despite the recent expansion of available treatment options. Primary genetic events create the culprit immortalised plasma cells while the accumulation of secondary genetic events and microenvironment changes derive the malignant transformation. There has been a significant increase in the available therapies for multiple myeloma. However, despite their effectiveness, many are associated with limiting side effects and none of those therapies targets the very genetic events that lead to the malignant transformation of the plasma cells.

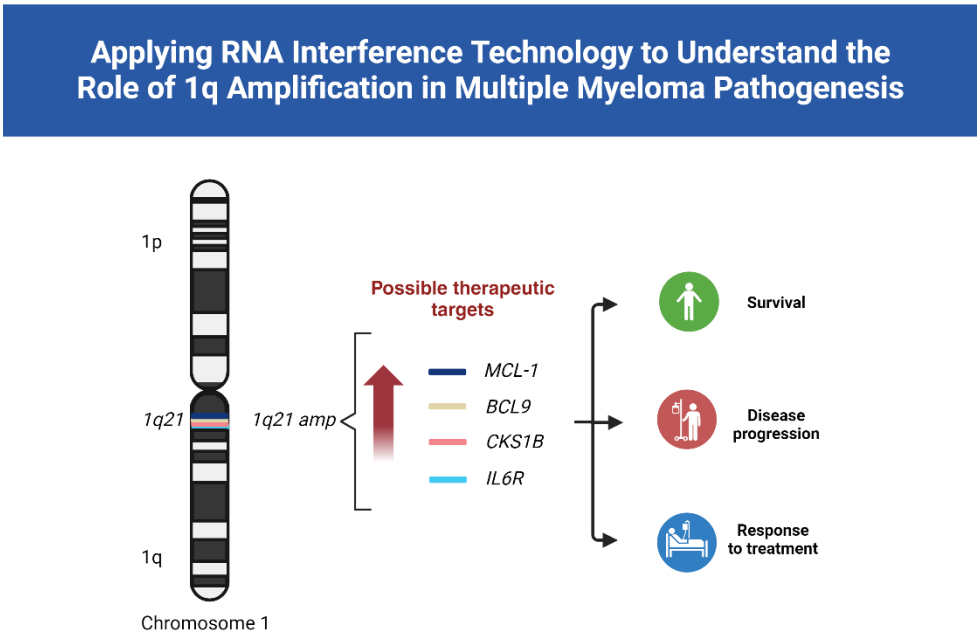
Several molecular aberrations collaborate to bring about the malignant transformation of plasma cells. Primary genetic events include trisomies of odd-numbered chromosomes and/or chromosomal translocations involving the immunoglobulin heavy chain. Those changes are not enough, however, to derive the full malignant transformation and hence transformation to multiple myeloma is characterised by the acquisition of secondary genetic events such as chromosome 1 abnormalities which is the main focus of this project. 1q gain is a clonal secondary copy number change that is observed in around 40% of myelomas. 1q gain (3 copies) and amplification (≥4 copies) are associated with increased cytogenetic risk and poorer survival. 1q gain and amplification were associated with a significant reduction in progression-free survival (53 vs 21.8 months respectively). Furthermore, when 1q gain is combined with other high-risk cytogenetic changes was associated with significantly inferior survival. Several candidate genes on chromosome 1q are implicated in the aforementioned risk. CKS1b (CDC28 Protein Kinase Regulatory Subunit 1B) is located on chromosome 1q21. Its protein product, CKS, has a confirmed proliferative and pro-survival role. MCL-1 is a member of the BCL-2 family and is a negative regulator of apoptosis. Other targets of interest on chromosome 1q are ANP32E (acidic leucine-rich nuclear phosphoprotein 32 family member E) and BCL9 (1q21.2). The latter encodes a transcriptional co-activator of the Wnt signalling pathway. BCL9 overexpression was identified in two-thirds of multiple myelomas and was associated, at least in part, with chromosome 1q amplification with a negative impact on myeloma cell growth.

Despite the clear association between chromosome 1q status and the cytogenetic risk, it is not quite clear whether this risk is related to a specific gene on chromosome 1q or if this effect is imparted by the combined effect of several of those genes.

This project will set out to answer this question using lipid nanoparticles (LNP) RNA interference that targets multiple high-risk genetic targets on chromosome 1q mentioned

above. We plan to explore the impact of the downregulation of single and multiple genes on the growth and viability of myeloma cell lines and primary human samples. In addition, using NanoString™, we plan to identify important downstream RNA expression pattern changes that can be further exploited as therapeutic targets.

Full project location: Clifton Campus;University Park;



Cancer-related cognitive impairment: Investigating the impact of peripheral tumours on neuronal plasticity in the brain

Project Supervisor: Graham Sheridan

School: Life Sciences

Description: We have recently generated several large proteomic datasets that will inform us of the global molecular changes occurring in neuronal synapses of the hippocampus, prefrontal cortex, and hypothalamus in response to the growth of breast tumours. The aim of the PhD lab rotation is to analyse these mass spectrometry-generated data sets in more detail and to pinpoint key proteins of interest to investigate further.

The PhD student will perform Western blot and ELISA analysis of mouse brain tissue and validate changes in the expression of key proteins of interest in the hippocampus, prefrontal cortex, and hypothalamus. They will learn how to prepare protein samples from frozen brain tissue and how to run Western blots and ELISAs to quantify changes in synaptic protein expression. They will also learn how to perform the image analysis and statistical tests required to confirm changes in protein expression.

The PhD student will also have the opportunity to help current members of the lab with the organotypic brain slice culturing method and the generation of tumour spheroids using 3D cell culture techniques. Learning this method will be valuable should the student continue in the lab with their own PhD project.

Location: QMC;

Full project description: Cancer continues to be one of the leading causes of death worldwide, with global estimations of up to 18 million new cases and 10 million deaths each year. One of the most common complaints amongst cancer patients, including patients in remission, are subtle but significant neurocognitive changes, such as memory impairment. It is estimated that up to 75% of cancer patients experience cognitive problems throughout their disease progression. Interestingly, cognitive disturbances are frequently reported in patients with tumours outside the central nervous system (CNS) that have yet to metastasize to the brain, e.g. breast cancer.

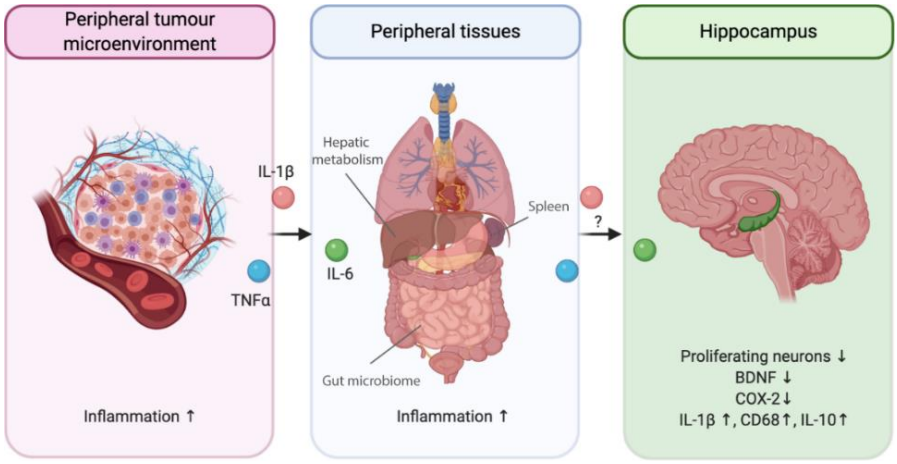
Numerous longitudinal and cross-sectional studies have reported cognitive dysfunction in cancer patients with non-CNS tumours following chemotherapy; a condition termed 'chemo-brain' or 'chemo-fog'. Therefore, most research to date has focused on cancer-related cognitive impairment (CRCI) caused by the side-effects of antineoplastic drugs. Cognitive complaints commonly reported in patients receiving chemotherapy included deficits in memory retention, executive functioning, psychomotor speed, verbal memory, and attention. However, pre-treatment measures of cognitive function are imperative to accurately determine if the observed cognitive deficits were induced by chemotherapy or if they were already present before the administration of the antineoplastic drugs. It is, therefore, vital to know the patients' baseline cognitive function, in order to define the degree of cognitive decline following treatment.

More recent studies have begun to include pre-treatment cognitive assessments of cancer patients and the data suggests that up to 35% of cancer patients with non-CNS tumours experience decreased cognitive functioning prior to receiving any therapy, including surgery, radiotherapy, chemotherapy and hormonal therapy. This PhD project aims to investigate the molecular and cellular dysfunctions induced in the brain by non-CNS tumour growth (e.g. breast cancer).

Using a mouse model of mammary tumour growth, we have evidence to suggest that breast cancer induces significant changes in the proteome of neurons located in key areas of the brain involved in cognition and memory formation, including the hippocampus and prefrontal cortex. Interestingly, tumour-bearing mice also demonstrate memory deficits in the absence of any chemotherapy treatments. Moreover, peripheral tumour growth causes elevated levels of stress hormones which appear to impact synaptic protein expression in stress-responsive brain regions, including the hypothalamus. Therefore, this PhD project will investigate the impact of molecular changes in these key brain areas on neuronal plasticity.

The project will involve analysis of proteomic datasets from the hippocampus and prefrontal cortex to pinpoint key drug targets and signalling pathways to investigate further for their role in the induction of cancer-related cognitive impairment. The successful PhD student will then develop a novel 3-dimensional organotypic slice culture model of the brain which can be co-cultured with tumour spheroids so as to mimic the in vivo environment. This novel ex vivo brain slice culture model will enable the student to interrogate how peripheral tumours impact the structure of the brain (e.g. myelin and blood vessels), the neuroinflammatory state of the brain (e.g. microglia and astrocyte reactivity), and changes in synaptic plasticity in the hippocampus (e.g. via live-cell Calcium Imaging or Electrophysiology techniques).

Full project location: QMC;



Can a bad cell turn good? Investigating metabolic resilience of reactive astrocytes in the immune response to brain cancer.

Project Supervisor: Sebastien Serres

School: Life Sciences

Description: Characterizing a new brain slice culture – (4 weeks): Organotypic brain slices will be obtained from healthy rodents and grown on cell culture inserts with culture medium previously optimised. High resolution fluorescence microscopy and metabolic assays will be used to characterise healthy astrocytes. Level of astrocyte markers (GFAP, S100b and COX1 proteins) will be imaged, whilst cellular metabolism will be monitored non-invasively using resazurin viability assay by sampling the culture medium. Live intracellular calcium imaging will also be recorded in response to physiological stimulus to confirm astrocyte functionality.

Developing a viable tumour model in brain slice culture – (3 weeks): Fluorescently tagged human glioblastoma cells will be micro-injected in the same brain slices using a fine capillary under a microscope, as optimised previously. The same high resolution fluorescence microscopy will be used to image and characterise tumour growth, astrocyte reactivity and metabolic changes. Level of markers of astrocyte reactivity (STAT3 phosphorylation) will be assessed, whilst metabolic remodelling associated with astrocyte reactivity to tumour will be quantified using fluorescently labelled glucose and lipid tracers.

Data analysis and write-up – (2 weeks) :Image processing tools will be used for microscopy analysis (Image J), Excel Solver and GraphPad Prism for calculating metabolic rates using kinetic models.

Location: QMC;

Full project description: Background:

In health - Astrocytes are the most abundant brain cells, outnumbering neurons and constituting 30% of all brain cells. Functioning primarily as metabolic buffers for neurons, they fulfil crucial physiological functions including the production and export of metabolic substrates such as lactate to meet neuronal energy requirements, the prevention of neurotoxicity via synaptic glutamate homeostasis and the control of neurovascular coupling via calcium-dependent pathways. In response to disease,

astrocytes become reactive and undergo phenotypic changes through activation of signal transducer and activator of transcription 3 (STAT3) pathway.

In cancer - STAT3-mediated reactive astrocytes promote tumour growth and immunological evasion in glioblastoma (GBM), a devastating adult brain tumour. This is associated with a significant metabolic remodelling of sugar and fat metabolism in reactive astrocytes. Although STAT3 signalling is a highly attractive therapeutic target, its role in healthy immune function renders it unsuitable in GBM patients. In contrast, targeting fat metabolism could reduce STAT3-mediated astrocyte reactivity with minimal off-target effects, with fat representing a non-crucial substrate in healthy CNS metabolism.

Significance:

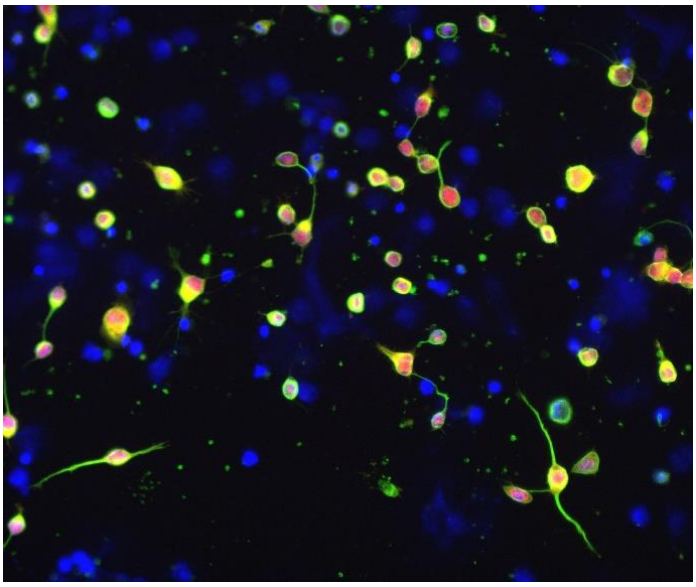
Brain slice culture has great potential in neuro-oncology as it offers the same architecture and cellular complexity of the in vivo brain for infiltrative glioblastoma to growth and interact with healthy brain cells. By using this model and a gene silencing approach, metabolic resilience of reactive astrocytes can be tested as a more suitable therapeutic approach for targeting the brain immune response to GBM.

Hypothesis: "Metabolic resilience is linked to STAT3-mediated astrocyte reactivity and could be targeted instead of STAT3 signalling with minimal off-target effects on healthy brain functions".

Workplan:

- Objective 1 – establishing a molecular link between STAT3 activation and fat metabolism. Adeno-associated viruses (AAV) with a fluorescent GFP tag that drive specific transgene expression only in astrocytes and encoding for murine SOCS3 or murine STAT3 will be microinjected in organotypic brain slices. GFP-labelled astrocytes will be assessed for either inhibition or activation of STAT3 pathway by high resolution fluorescence microscopy. Changes in carbohydrate and fat metabolism in GFP-labelled astrocytes will be correlated to STAT3 expression using fluorescently labelled glucose and lipid tracers and immunofluorescence staining. The impact of STAT3 silencing on astrocyte functionality will be assessed by live intracellular calcium imaging.
- Objective 2 – identifying relevant inhibitors of metabolic resilience in reactive astrocytes. A panel of fatty acid metabolism inhibitors will be tested in organotypic brain slices previously micro-injected with a GFP-labelled AAV that induces STAT3-mediated astrocyte reactivity. The same high resolution fluorescence microscopy will be used to confirm fatty acid metabolic remodelling, reduced astrocyte reactivity and intact astrocyte functionality in terms of calcium dependent pathways.

- Objective 3 – Validation of metabolic inhibitors in relevant GBM model from surgical biopsies. Fluorescently tagged tumour cells from surgically resected GBM will be micro-injected in healthy rodent cortical slices using a fine capillary under a microscope, as optimised in the rotation. The same high resolution fluorescence microscopy will be used to determine the effect of metabolic inhibitors on astrocyte reactivity, tumour growth, calcium-mediated signalling pathways in astrocytes. From this, the best candidates will be selected as in vivo therapeutic targets for future studies.



Full project location: QMC;

Sugar addiction – is it all in your genes or all in your tongue?

Project Supervisor: Peter Aldiss

School: Medicine

Description: The student will have the opportunity to take analyse key metabolites and hormones from initial recruit by genotype studies thus allowing them to understand how genetic variation in the SI gene impact the metabolic fate of sucrose using metabolomics, and its downstream physiological effects on appetite related hormones. Further, they will have the opportunity to work with Dr. Sally Eldeghaidy where they will develop competencies in functional neuroimaging and taste fMRI and trial key sensory science methods for use in the project. Similarly, they will have the opportunity to work with Dr. Tristan Dew and the Food Sciences team to develop competencies in screening compounds with therapeutic potential in cell models to investigate their efficacy.

Location: QMC;University Park;Sutton Bonington Campus;

Full project description: Excess intake of dietary sucrose is a major contributor to obesity and type 2 diabetes and is associated with cardiovascular disease and other comorbidities.

Genetic variants in the sucrase-isomaltase (SI) gene (which catalyzes the hydrolysis of sucrose to glucose and fructose) lower BMI and improve metabolic health whilst simultaneously reducing the intake and liking of sweet foods which is of particular relevance given the expression of this enzyme in the tongue.

The focus of this PhD will be on the common p.Val15Phe (15Phe) rs9290264 single nucleotide polymorphism (SNP) which could reduce enzymatic activity, and by proxy sugar metabolism and sweet taste by ~35% in around 10% of the UK population – here, the student will seek to understand how this enzyme regulates the intake, and preference for sugar at both the molecular, and behavioural level.

Working with Dr. Aldiss the student will perform recruit by genotype studies for individuals homozygous for the p.Val15Phe variant and seek to understand how they

metabolise sucrose and, working with Dr. Sally Eldeghaidy the student will determine how reduced SI activity in the tongue can alter sweet taste, including the intensity of sweet foods and their appeal compared to other tastants i.e. salty and bitter using cutting-edge sensory science techniques in the School of Biosciences. Alongside this, the student will utilise functional MRI methods developed by Dr. Eldeghaidy and the Precision Imaging Beacon to assess the neurological responses to sweet taste and how this is impacted by the variant with the aim of identifying key regions of the central nervous system which may be regulating a taste aversion to sweet foods.

In collaboration with Dr. Tristan Dew, the student will work to identify novel neutraceuticals that can inhibit SI with a view to developing a therapeutic to reduce population wide sugar intake. The initial focus here will be on polyphenols, and other plant bioactives which have been shown to inhibit SI activity, and the development of a polyphenol-rich chewing gum which, when combined with a compound that binds the sweet taste receptor can reduce sweet taste, digestion, and preference in humans. Starting in cellular models the student will test the efficacy of these compounds to inhibit enzymatic activity and, given that SI is co-expressed with the sweet taste receptor T1R3 in sweet taste cells, the student will investigate the interaction between these two key regulators of taste and metabolism at the molecular level including using state-of-the-art imaging methods, multi-omics analyses and the generation of primary taste cells.

Finally, it is expected that the student will work along the food sciences team to develop pilot neutraceuticals which can be tested in pilot studies to assess whether these bioactive compounds regulate the taste and brain responses to sugar in humans.

Full project location: University Park;QMC;Sutton Bonington Campus;

Diminished astrocyte-neuron interactions induces sensory neurodegeneration due to disturbance in metabolic homeostasis

Project Supervisor: Richard Hulse

School: School of Science and Technology (NTU)

Description: A failure to meet neuronal energy demands underpins neurodegenerative disease including long-lasting pain, with adverse health related complications occurring more frequently in an aged and obese population. The nervous system relies upon differing cell types (neurons and astrocytes) to interact to ensure neuronal energy consumption is matched by processes that support neuronal energy production. Our work highlights that metabolic stressors including high fat diet and oxygen deprivation initiate neurodegenerative mechanisms through disturbances in neural tissue energy handling. In addition, we have identified regulators (hormonal mediation) of energy production that are associated with alterations in neuronal function and health.

During this lab rotation project, training will be provided in primary neuronal cell culture by isolating spinal cord neurons and astrocytes to investigate how neurons and astrocytes respond to metabolic stressors. Calcium imaging of neurons and astrocytes will be measured alongside evaluating the astrocyte proteome in differing experimental environments that model metabolic stress including hyperglycaemia and glucocorticoid modulation. Whilst determining cellular expression profiles in spinal cord tissue using immunofluroresence, tissue clearing and confocal microscopy. These experiments will provide insight into how the somatosensory nervous system responds to those underlying stress factors that initiate the onset of neurodegenerative disease and pain.

Location: QMC;Clifton Campus;

Full project description: Neurodegeneration and pain are highly prevalent adverse health related complications that arise due to an ageing population. Increased susceptibility to neurodegeneration arises also due to metabolic disorders such as diabetes and obesity. It is recognised that neurological disease proceeds due to a dysfunction in the integrity of the neuron that impedes normal neurological function. To support normal neural activity there are a number of differing cell types that make up the somatosensory nervous system. This is typified by the microvasculature (or the blood spinal cord barrier) that comprises largely of endothelial cells. However the microvessel wall also comprises of astrocytes, smooth muscle cells and pericytes, which act in harmony with the endothelium to regulate tissue perfusion and provision of energy resource. However, dysfunction in this coordinated communication is implicated in age and metabolic disturbances in neurological disease. Astrocytes are widely regarded as integral in maintaining capillary architecture, neuronal function and tissue health. Furthermore, astrocyte dysfunction (termed astrogliosis) is characteristic of neurodegenerative disease and is a pathological hallmark of damage to the sensory nervous system. Astrocytes are a potent source of inflammatory maladaptation and regulator of metabolic homeostasis (energy production and utilisation) within neural tissues. This ensures sufficient energy provision to support integral neural tissue function such as controlling nociception and pain perception. In rodent models that utilise metabolic challenge ie induction of hypoxia, high glucose or high fat diet, the level of astrogliosis is elevated in the dorsal horn versus age matched sham controls, alongside presentation of chronic pain. Our work has identified in these rodent models, mediators of cellular stress (glucocorticoid) are responsible for metabolic and transcriptome maladaptation, which we hypothesise to impede microvessel, neuronal function and tissue integrity. However, it remains unclear whether alterations in neural tissue metabolic state is mediated by astrogliosis, and whether identified glucocorticoid signalling is fundamental in the causation of sensory neurodegeneration and long-lasting pain.

Project Objectives:

In this research project we propose to manipulate astrocyte activity specifically, through utilising spatiotemporal mouse models (associated adenovirus (AAV) delivered chemogenetic DREADD driven control) to target astrocyte activity and to determine maladaptation to metabolic challenge. Key objectives include [1] to determine impact of dietary insult and glucocorticoid signalling upon astrocyte-neuronal interactions and pain perception. [2] using invitro and invivo model systems to identify novel mediators that initiate the onset of sensory neurodegeneration at the level of the spinal cord and pain. These investigations will incorporate primary cell isolation from rodents for invitro cell modelling such as calcium assays, biochemical assays, as well as invivo studies focussing upon nociceptive behavioural assays, confocal/two photon microscopy and intravital imaging of the spinal cord to evaluate interaction between differing cell types in particular focussing upon astrocyte-neuronal interactions in relation to the development of chronic pain states.

Full project location: QMC;Clifton Campus;

Does carbonation and non-sugar sweeteners in carbonated beverages impact appetite sensation ?

Project Supervisor: Sally Eldeghaidy

School: Biosciences

Description: As a key component of the project will involve development and design of a model carbonated beverage, carbonated beverages on the market will need to be reviewed and have their sensory properties characterised to help in its development.

The lab rotation will therefore be a mini project to review the range of carbonated drinks on the market and characterise the sensory properties particularly carbonation (fizziness) and sweetness intensity. After appropriate health and safety training the student will recruit healthy volunteer set up a descriptive analysis profiling using sensory software and conduct a sensory trial to collect sensory profile data for selected beverages and statistically analyse the resulting data. The anticipated but approximate time span for the mini project can be found below.

- Weeks 1-3: Writing and getting ethics into place and recruitment of healthy volunteers.
- Weeks 3-6: Running the sensory trial where participants characterise the sensory properties on carbonation (fizziness), sweetness intensity of selected carbonated beverages on the market.
- Weeks 6-9: Data analysis.

The mini project has been carefully designed so the student doing the lab rotation learns and develops skills required for the main PhD project that can be developed further and used throughout the PhD.

Location: University Park;Sutton Bonington Campus;

Full project description: The soft drink industry widely adopted production of non-sugar sweetened (NSS) carbonated beverages due to evidence that sugar sweetened (SS) carbonated drink consumption contributes to global increases in obesity. However, the World Health Organisation recently reported that NSS carbonated drinks also increases the risk of obesity. With evidence both SS and NSS carbonated beverages significantly increase obesity risk, a common factor must be a cause.

Carbon dioxide (CO2) is key to both SS and NSS carbonated beverages. It's tasteless and non-caloric. Carbonated beverage impact on appetite, food intake and weight gain has rarely been studied. To date, only one study in rodents explored CO2 effects on appetite regulation and reported increased food intake, weight gain and ghrelin release supported by a small human study where carbonated beverages increased ghrelin release over non-carbonated beverages. Ghrelin is a 'hunger hormone' that stimulates appetite and promotes food intake and is a reward stimulator. If carbonation increases ghrelin release it could be a key factor behind global increases in obesity.

This project will use multidisciplinary approach to integrating brain imaging and sensory measurements to investigate the impact of carbonation alone or with different sugar types (SS and NSS) on brain-related areas associated with appetite sensation, food intake and appetite control in humans.Furthermore, to investigate long-term effects of carbonated beverages on appetite control and obesity risk, an animal trial will be included.

Study 1 (0-12 month- UoN): Development of a model beverage system varying in carbonation and sugar types. A full experimental sample design will be conducted generating samples varying in carbonation level and two sugar types (SS vs NSS).

Study 2 (12-36 month - UoN) : The effect of carbonation and sugar type on appetite control and food intake. Selected subjects will come to our lab to drink the developed beverages (300ml). After 5 mins post-consumption, blood samples will be collected every 15 mins for 2 hours for measurement of Ghrelin, Leptin, PYY and GLP1 by ELISA,

all key to appetite regulation. In addition, brain scans will be collected before and after consuming the drinks.

Study 3 (36-48 month – UoN/UoA): The impact of carbonation and sugar type on long-term obesity risk. Briefly, weanling rats (n=10) in each treatment will have ad libitum access to either flat or carbonated Water, SS beverage or NSS beverage to determine long term impacts on food intake and weight gain using metabolic monitoring systems. Body and fat depot weights following euthanasia at 4 months of age will be recorded and plasma and tissues collected. Primary analyses will be measures of appetite control and include plasma quantification of Ghrelin, Leptin, PYY and GLP1 and relevant quantification of proteins and or genes expressed in the brain to assess correlations with food take.

Full project location: University Park;Sutton Bonington Campus;

How does survivin inhibit apoptosis?

Project Supervisor: Sally Wheatley

School: Life Sciences

Description: Hypothesis: Inhibition of cell death by survivin is VDAC2 dependent. The aim of the rotation is to inhibit VDAC2 and survivin in normal human cells, and assess the effect of their absence(s) on cellular response to two apoptotic stimuli, UV and taxol. VDAC2 and survivin will be depleted using siRNA and, as an alternative strategy, via efresin and YM155 treatment respectively. The first task we will be to optimise the efficacy of these agents in inhibiting their targets using immunoblotting, inspection of mitochondrial morphology using live fluorescence imaging and mitotracker staining and immunostaining for survivin. Next a killing curve will be carried out to determine the IC50 of UV and taxol. Once these parameters have been established, VDAC2/ survivin compromised cells will be subjected to UV or taxol, and the extent of cell death assayed using FACS and fluorescence imaging. Data collected will provide substantial evidence as to whether survivin-VDAC2 interaction is central to survivin-mediated cytoprotection against the intrinsic apoptotic pathway. The student will learn cell culture and live cell fluorescence imaging, siRNA, FACS and apoptosis read outs.

Location: QMC;

Full project description: In multicellular organisms programmed cell death, referred to as "apoptosis" is a carefully regulated process that ensures defective and damaged cells are removed from the body without causing harm or inflammation to the surrounding tissue. In addition to their infamous role in respiration, mitochondria have many other functions, including activation and apoptosis and regulation of calcium signalling.

Voltage dependent anionic channel 2 protein, VDAC2, is a 32kD outer mitochondrial membrane (OMM) porin that regulates traffic of key molecules into (calcium) and out of (cytochrome c) mitochondria. During apoptosis it recruits the pro-apoptotic Bcl2 family member, Bak, to the OMM (Roy et al., 2009). Once recruited it oligomerises with Bak to generate large transmembrane channels which in turn enables cytochrome c to be released from the mitochondria into the cytoplasm where it can interact with the apoptosome and activate caspase 9, ultimately causing caspase 3/7 activation and cell destruction. Interestingly cells deficient in VDAC2 can respire normally, however, they are defective in two pathways that may be linked, calcium signalling and (intrinsic) apoptosis.

Survivin is an anti-apoptotic protein that can prevent cellular destruction instructed by both intrinsic and extrinsic apoptotic pathways. A member of the inhibitor of apoptosis (IAP) family it has some properties that relate to caspase inhibition, but whether it truly acts in this manner, or whether it can prevent cell death in another way(s) is still uncertain (reviewed in Wheatley and Altieri, 2019). Recently the Wheatley lab discovered that survivin can interact directly and specifically with VDAC2, but not VDACs 1 or 3. Survivin is not normally present in the mitochondria, however, under conditions of stress, including cellular transformation (cancer), and hypoxia, it gains access to it. This is partly via its N-terminus which forms an abbreviated, but functional MTS (Dunajova et al., 2016).

The goal of this project is to determine whether the interaction between survivin and VDAC2 is responsible for the ability of survivin to prevent apoptosis in cells experiencing stress, and whether this involves the cytosolic or mitochondrial pool of survivin. We will assay apoptotic response using a variety of techniques including advanced fluorescence imaging and fluorescent activated cell sorting (FACS) analysis in both live and fixed cells, as well as with conventional biochemical analyses. Initially we will work in transformed (cancerous) and normal cultured human cells. We will manipulate survivin levels and localisation, inhibit VDAC2 expression/ activity and stress cells with hypoxia, UV and using a variety of drugs known to initiate apoptosis (DNA damaging agents, and the microtubule stabliliser, taxol). The project will involve molecular cloning, cell culture, fluorescence and calcium imaging, apoptotic assays, and biochemical analyses including immunoblotting, protein expression in vitro, and immunoprecipitation. With assistance from the second supervisor there is also the opportunity to test our hypothesis that survivin inhibits apoptosis and calcium trafficking by inhibiting VDAC2 in flies, as both proteins and mechanisms are conserved between these organisms.

Collectively these data will determine whether survivin-VDAC2 signalling is worth pursuing in future therapeutic strategies.

Full project location: QMC;

"Receptor Activity-Modifying proteins (RAMPs) as novel pharmacological intervention to target Vasopressin receptor nanodomain "

Project Supervisor: Isabella Maiellaro

School: Life Sciences

Description: The student will study Vasopressin receptor (VR) -associated cAMP nanodomain in HEK. Different receptors have been described to have functional cAMP-associated nanodomains. These cAMP nanodomains are responsible for the receptor-specific functions.

In this rotation, the student will focus on studying the cAMP domain associated with the VR receptor. To achieve this, the student will utilize FRET -based biosensors available in the lab that are targeted at the VR receptor and positioned at 0 ,20 and 60 nm below the receptor using "rulers."

The student will express this construct in cells and activate the receptor while monitoring real-time cAMP accumulation within the cells. Additionally, the student will investigate whether the expression of RAMPs can alter the spatiotemporal dynamics of the V2R-associated nanodomain. This analysis will determine the impact of RAMPs on modulating receptor function.

During this rotation, the student will gain experience in cell culture and transfection techniques, conduct live video imaging experiments, and analyse the results. The student will also profile the dynamics of V2R-induced signals in the presence and absence of RAMPs within the range of 0 to 60 nm from the receptor.

Location: QMC;

Full project description: Cell surface receptors allow cells to detect and respond to signals from the external environment. The binding of an extracellular ligand to a cell surface receptor initiates a cascade of signals that begins at the plasma membrane. Several accessory proteins can modulate receptors in their functions. In this project we will focus on one emerging important class of receptor modulators: Receptor-activity–modifying proteins (RAMPs). RAMPs are single-pass transmembrane protein family with three distinct members: RAMP1, RAMP2, and RAMP3. RAMPs are globally coevolved and coexpressed with GPCRs, currently described to have more than 40 interacting partners. GPCR-RAMP complex facilitates transport of receptors to the cell surface, alter ligand specificity, GPCR activation, G protein coupling and affect their downstream signalling cascade. The consequences of these interactions on GPCR function and physiology lays the foundation for new molecular therapeutic targets. So far interaction between RAMPs and receptors have been studied at the plasma membrane. However upon activation, many receptors enter the endosomal system, a large, dynamic tubulovesicular network extending throughout the cytoplasm. Although the endocytic system has traditionally been viewed as a conduit that transports receptors to a degradative or recycling fate, endosomes are also a site at which receptor signalling can be initiated, sustained, and terminated.

Recently we discovered the existence of Receptor Associated Independent cAMP Nanodomain (RAIN) which translate receptor activation into intracellular effect. This nandomains represent a novel physiological mechanism and potential pharmacological target. Whether RAMPs are able to modulate receptor RAINs it is not known. In particular we are interested in understanding if RAMPs can modulate Vasopressin receptor (VR) cAMP domain. Vasopressin is a peptide hormone produced in the hypothalamus and released from the posterior pituitary. Secretion of vasopressin is followed by activation of its receptors throughout the body mediating osmoregulation, cardiovascular stability, and homeostasis but also serves as a corticotropin secretagogue and influences cognition, learning, and memory. Vasopressin receptor and RAMPs are co-express in several tissues. It has been shown that the vasopressin function are mediated by compartmentalised cAMP however , the dimension of such domain and the regulation is still elusive. Additionally, vasopressin receptors can signal from both the plasma membrane and from the endosome and which of this compartment is modulated by RAMPs is not known.

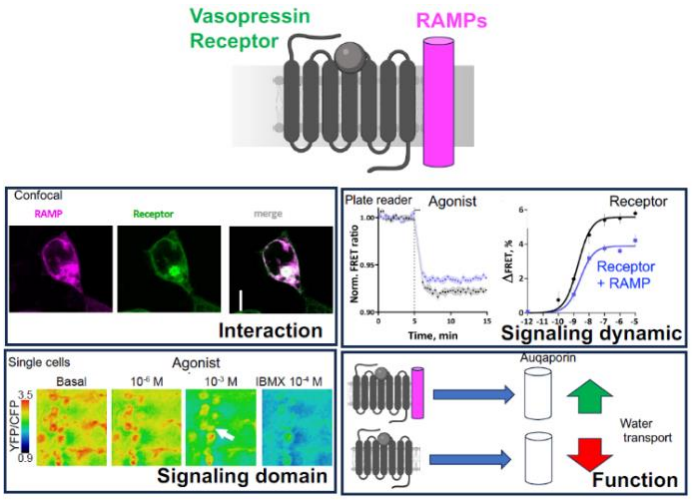
Here we hypothesis that RAMPs modulate vasopressin function by shaping receptor-associated cAMP signals.

To test this hypothesis we aim 1) to describe with spatio-temporal accuracy the vasopressin receptor associated cAMP signals using FRET-based nanorulers 2) to study how overexpression of RAMPs can modulate VR cAMP domain 3) to investigate the role of RAMPs in modulating endosomal signalling by analysing the dynamics of endosomal signalling in presence and absence of RAMPs, and by following tagged RAMPs-VR

complexes from the plasma membrane to the endosomes using specific markers 4) to determine the physiological function of such interaction having as read out the translocation of aquaporin.

Understanding this modulation and dynamics can open new avenue for pharmacological targeting VR – related disease.

Full project location: QMC;



Investigating the Anti-Inflammatory Effects of Celecoxib and Loxoprofen in Glioblastoma (GBM) with and without Bio-Nanoantennae Electrical Stimulation.

Project Supervisor: Frankie Rawson

School: Pharmacy

Description: Glioblastoma (GBM) is a highly aggressive and inflammatory brain cancer with limited treatment options. Recent advancements in the field of bioelectronic medicine have opened new avenues for exploring innovative therapies. This 9-week PhD training project aims to investigate the potential anti-inflammatory effects of two non-steroidal anti-inflammatory drugs (NSAIDs), Celecoxib and Loxoprofen, in the context of GBM, both in the presence and absence of electrical stimulation.

Objectives: To assess the anti-inflammatory properties of Celecoxib and Loxoprofen on GBM cells. To evaluate the synergistic effects of bio-nanoantennae electrical stimulation when combined with these NSAIDs. To explore the underlying molecular pathways involved in the observed effects.

Location: University Park;QMC;

Full project description: Cancer is a leading cause of death worldwide. Conventional cancer treatments, such as chemotherapy and radiation therapy, are often ineffective and can have severe side effects. A pressing need exists for innovative cancer therapies. Our recent breakthrough utilised a ground-breaking bioelectronic medicine approach to influence cancer cell behaviour. We harnessed bio-nanoantennae to transport a protein called cytochrome c (Cyt c) into cancer cells derived from the malignant brain cancer, 'glioblastoma' (GBM). Cyt C is a key regulator of apoptosis, and induced cancer cell death in primary GBM cells. Importantly, the method precisely targeted cancer cells, sparing astrocyte (healthy brain) cells from any adverse effects, as we reported in a recent study published in Nature Nanotechnology (2023). Biological processes that are not significantly altered in cortical astrocytes (such as inflammation pathways) warrants further interrogation experimentally, to determine why astrocytes are relatively resistant to electrical stimulation as a specific extracellular stress source.

To gain a deeper understanding of this phenomenon's biological mechanisms, we aim to expand our research by applying this novel bio-actuation tool to other types of hard to

treat cancers such as lung, pancreatic and oesophageal. We will seek to investigate whether the approach's selectivity is governed by a consistent pathway, potentially offering a ground-breaking, universally effective cancer treatment. This will be achieved by employing transcriptomics to sequence and analyse the complete set of RNA molecules expressed by these cells, and functional bioassays to assess the biological activity of identified genes and their resulting proteins, including ion channels.

Full project location: University Park;QMC;

How does Fusobacterium nucleatum cause gum disease? The contribution of nucleotide second messengers to virulence pathways

Project Supervisor: Dr Sarah Kuehne

School: School of Science and Technology (NTU)

Description: During the nine-week lab rotation, the student will have the opportunity to familiarise themselves with working under anaerobic conditions. They will use either the set of c-di-AMP or ppGpp mutants (already available in teh lab of the primary supervisor) and compare these to the wildtype (parental strain) grown planktonically (OD and colony forming unit (CFU) measurements) and as biofilms (crystal violet staining to quantify biomass, live/dead staining and confocal microscopy). This will provide the student with a first data set and importantly critical skills, required for the PhD. They will gain an inside into some of the fundamental techniques and will also experience the lab of the primary supervisor.

Location: Clifton Campus;

Full project description: The aim of this PhD project is to understand how signalling pathways (of c-di-AMP and ppGpp) contribute to the lifestyle and survival of Fusobacterium nucleatum.

F. nucleatum is one of the most abundant species in the oral microbiome, where it is found as dental plaque biofilms growing anaerobically in periodontal lesions, and in saliva. It is pivotal in the shift from a healthy oral microbiome to a disease-associated one, resulting in a range of debilitating and costly-to-treat infections. Furthermore, F. nucleatum has been associated with a plethora of extraoral conditions, including colorectal cancer and preterm birth.

Whilst the composition of the oral microbiota has been studied in great detail, virulence pathway mechanisms are largely unexplored. Biofilm formation, coaggregation with multiple other bacterial species, adherence and invasion of host-tissues are major virulence factors of F. nucleatum. Our preliminary data indicates that the c-di-AMP and (p)ppGpp second messengers are vital to establishing fusobacterial biofilms, required for infection.

- Objective 1: Elucidating the role of c-di-AMP and (p)ppGpp in fusobacterial physiology. Growth patterns of previously generated mutants in the c-di-AMP and ppGpp pathway will be compared to the parental strain under a variety of physiologically relevant conditions. This will include biofilms. These will also be analysed for antimicrobial susceptibility. The mutants will further be characterised by determining the amount of nucleotides produced.
- Objective 2: Identification of cellular pathways controlled by c-di-AMP and (p)ppGpp. RNAseq of wildtype and mutant strains grown as monospecies biofilms (time points will be chosen in line with nucleotide production, measured in objective 1) will be

conducted to reveal regulated pathways, allowing a comprehensive understanding of these signalling systems in F. nucleatum. To confirm identified targets, qPCR will be conducted. Following this, deletion mutants of up to 5 key representatives with altered transcription will be created to confirm their importance for growth, biofilm formation and antibiotic resistance.

- Objective 3: Establishing the role of c-di-AMP and (p)ppGpp in F. nucleatum - host interactions. Adhesion to and invasion of host tissues have been recognised as pivotal virulence components of F. nucleatum. Whilst some mechanistic data exist, the importance of F. nucleatum's adhesins and regulatory pathways in the process has not been elucidated. F. nucleatum is an invasive organism, which has previously been linked to adhesins like FadA and Fap2. The invasion of two adhesin mutants (FNNΔfadA and FNNΔfap2) were tested in our laboratory recently, showing that FNNΔfadA invaded significantly less than the wildtype. Additionally, the impact of the second messengers on the inflammatory host response is unknown. This objective will explore the role of c-di-AMP and (p)ppGpp in adherence and invasion of cell cultures. Host responses, including viability, proliferation, chemokine and cytokine levels will be measured. The data will provide mechanistic insights into the role the second messengers play in F. nucleatum's pathogenesis.

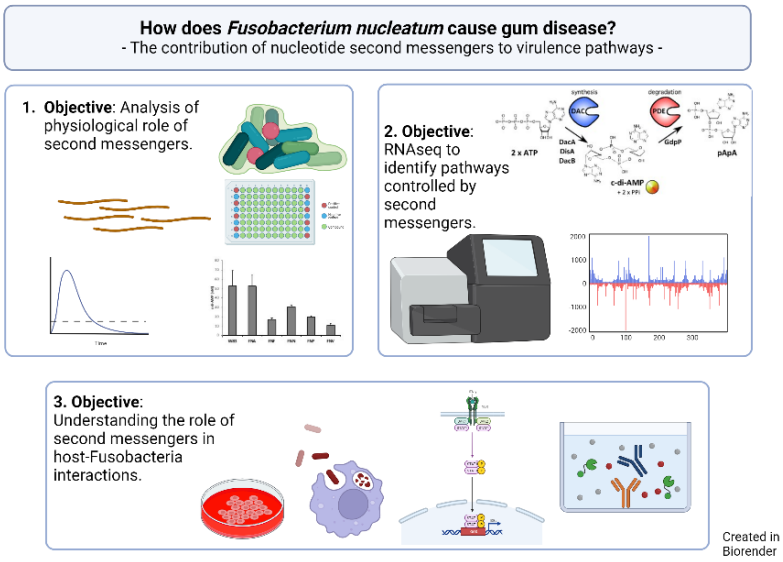
These key objectives will provide fundamental insights into the infection biology of F. nucleatum driven by second messenger signalling and the potential of disrupting this pathway for therapeutic gain.

References:

Muchova M, Balacco DL, Grant MM, Chapple ILC, Kuehne SA, Hirschfeld J. Fusobacterium nucleatum Subspecies Differ in Biofilm Forming Ability in vitro. Front Oral Health. 2022 Mar 15;3:853618. doi: 10.3389/froh.2022.853618. PMID: 35368312; PMCID: PMC8967363.

Irving SE, Choudhury NR, Corrigan RM. The stringent response and physiological roles of (pp)pGpp in bacteria. Nat Rev Microbiol. 2021 Apr;19(4):256-271. doi: 10.1038/s41579-020-00470-y. Epub 2020 Nov 4. PMID: 33149273.

Full project location:
University Park;Clifton Campus;



Deubiquitinase structure determination for drug design

Project Supervisor: Jonas Emsley

School: Pharmacy

Description:

Ubiquitin specific proteases (USPs) can rescue proteins from destruction by the proteasome by reversing ubiquitination. This way, USPs influence processes such as proliferation and apoptosis. At present, drugs targeting the ubiquitin system are generally proteasome or ubiquitin E3 ligase inhibitors. However, from a structural perspective USPs are expected to be better and more selective drug targets and agents targeting USPs are expected to have fewer side effects. A molecular understanding of the structure and interactions of a protein drug target are important for rational drug design for healthcare applications. In the lab rotation you will receive training in contemporary protein biochemistry and structural biology approaches and learn how to apply these to the characterisation of a ubiquitin specific protease. The rotation will consist of the following parts:

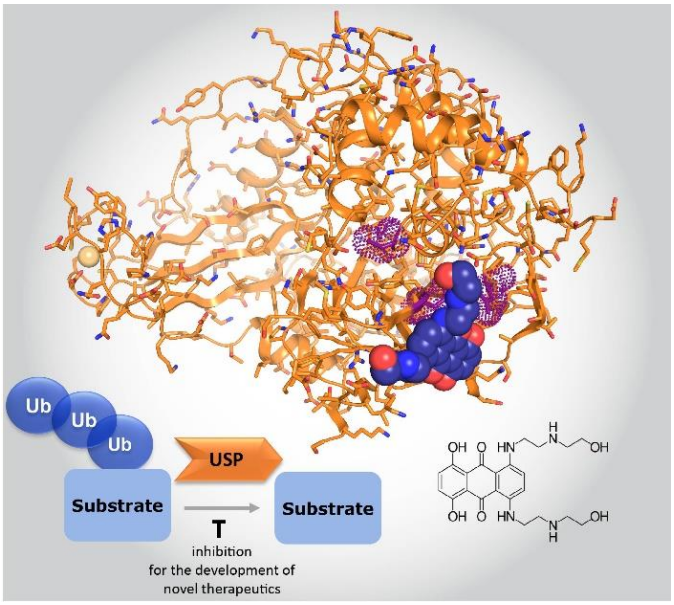
- Reading of scientific literature on the topic and general lab induction
- Mutagenesis to inactivate USP15 for substrate trapping
- Generate a USP15-substrate complex with the mutated protein using protein expression and purification protocols available in the lab
- Perform preliminary structural characterisation of the USP15-substrate complex using crystallisations and predict structures and complexes using AlphaFold.
- Data analysis

Location: University Park;

Full project description: Rational drug design relies on the availability of detailed structure information and the molecular properties of key binding sites that can be targeted by small molecular inhibitors or biologics. With the emergence of artificial intelligence structure prediction methods (AlphaFold) and key advances in cryo-electron microscopy and X-ray crystallography, we have access to unprecedented tools to facilitate rational drug design.

In this project, we will make use of these advanced technique toolkit to characterise the structure and substrate interactions of a ubiquitin specific protease (USP) for drug design. There is growing evidence that the multi-functional USP15 is highly relevant for biomedical research. USP15 has been implicated in TGF-β and NFkappaB signalling, mRNA processing and the innate immune response. USP15 depletion results in DNA double strand repair defects. Moreover, USP15 was shown to regulate the ligase MDM2 with effects on the stability of p53 in cancer cells. In line with these findings, USP15 is dysregulated in many cancers. For example, the USP15 gene is found amplified in human breast and ovarian tumours and in glioblastoma and USP15 is involved in the innate immune response to viral infection and tumours. Furthermore, knockdown of USP15 rescues the mitophagy defect of Parkinson disease (PD) patient fibroblasts. Together, these findings render USP15 a promising drug target in a range of human disorders, but no selective inhibitor is available to date. We previously solved the crystal structure of the USP15 N-terminal and protease domains. Hence, structure determination of USP15 in complex with substrates and inhibitors is feasible so that we can better understand the substrate specificity and promising binding pockets that can be targeted for drug discovery. Expression protocols for active full-length USP15 are also established in the lab.

This project's aim is to better understand the structure, function and specificity of ubiquitin specific protease 15 in order to design specific inhibitors for this protease. The project will consist of the following parts: (a) Generation and biophysical characterisation (ITC, SPR) of USP15-substrate complex (b) Elucidation of molecular basis of USP15-substrate interactions using complementary state-of-the-art structural biology techniques including X-ray crystallography or single particle cryo-electron microscopy, AlphaFold (c) Mutagenesis of key residues responsible for recognition and proof-of concept studies to evaluate the impact of these in cellular assays. The project builds on existing expertise, access to the latest state-of-the-arts facilities at synchrotrons (Diamond Light Source at Oxford or the European Synchrotron Radiation Facility at Grenoble; Midlands Regional Cryo-Electron Microscope Facility at Leicester) Research and exciting preliminary data available in the lab. These structural insights will shed light onto fundamental principles of USP15's ubiquitin deconjugation mechanism, the molecular basis of substrate recognition and will provide a platform for the development of novel therapeutic agents targeting USP15 and closely related homologues USP11 and USP4.



Full project location: University Park;

Design and development of allosteric modulators for human beta-adrenoceptors

Project Supervisor: Barrie Kellam

School: Pharmacy

Description: β-Adrenoceptors (β-ARs) are G Protein-Coupled Receptors (GPCRs) and long-established therapeutic targets (e.g. in asthma/cardiovascular disorders). Surprisingly, we still don't fully understand how these complex proteins function and interact with small molecule ligands. Recently, x-ray crystal structures of allosteric ligands bound to the β2-AR have been published. Allosteric ligands bind at distinct sites to the orthosteric binding site (where endogenous agonists such as epinephrine / norepinephrine bind), offering an exciting and alternative approach to modulating receptor function. Furthermore, they potentially have several advantages over orthosteric ligands, through their non-competitive mode of action.

Our group has further developed one of these ligands ('cmpd-6') into a fluorescent tool, enabling pharmacologically labelling of/screening against this allosteric binding site.

Project aims:

- Develop a library of fluorescent 'cmpd-6' analogues, exploring a range of different fluorophores.
- Pharmacologically characterise the library of ligands at the β1-AR and β2-ARs using bioluminescence resonance energy transfer (BRET) and confocal imaging

The knowledge gained on this multidisciplinary project will advance our understanding of allosteric binding sites for β-ARs. Furthermore, these tools are critical to establish a screening platform, enabling the discovery of new ligands which can bind to this allosteric site, which is the starting point of the main PhD project.

Location: University Park;QMC;

Full project description: GPCRs are important signal transduction proteins residing in the cell membrane and are essential regulators of many homeostatic processes and targeted by >30% of drugs. The β-ARs (family A GPCRs), are key regulators of the cardiovascular and respiratory systems and well-established drug targets. β1, β2, and β3 subtypes have distinct tissue distributions and pharmacological activity.

Despite drugs targeting the β1-AR/β2-AR being in clinical use for over 60 years, new ways of targeting these receptors, are now emerging. Endogenous β-AR agonists (e.g. epinephrine, norephinephrine) and existing therapies target the orthosteric binding site (OBS) of these receptors, which is extracellularly exposed. Recently, with a combination of x-ray crystallography and pharmacological studies, a limited number of allosteric binding sites (ABS) have been identified for the β2-AR, which are topographically distinct from the OBS. As allosteric ligands don't compete for the OBS, they can modulate the receptor and a bound orthosteric ligand in a distinct manner offering a range of potential therapeutic advantages. One such ligand – 'cmpd 6' has successfully undergone further chemical modification by our group to create a novel fluorescent ligand, enabling further exploration of this ABS. Furthermore, as the 'cmpd 6' ABS is situated at the interface between the cell membrane and cytosolic face of the protein, this facilitates the development of proteolysis targeting chimeras (PROTACs). PROTACs targeting this ABS, would combine a β2-AR allosteric site-targeting moiety, and an E3-ligase targeting moiety, separated by a suitable linker, thus promoting ubiquitination of the receptor, ultimately leading to its degradation by the existing cell machinery. Such an approach has been recently reported for another GPCR - the chemokine CCR9 receptor (Huber et al, 2022).

Thus, the PhD project provides an opportunity to explore two exciting complementary multidisciplinary areas:

1. Discovery of new ligands targeting the 'cmpd 6' ABS:
    a. Use the fluorescent compounds developed in the rotation project to screen our in-house medicinal chemistry compound collection (over 80K compounds) to identify new allosteric ligands for the 'cmpd 6' site.
    b. b. Pharmacologically characterise any new ligands to determine affinity for β1-AR/β2-ARs and any modulatory effect on co-bound orthosteric ligands.
2. Development of novel β2-AR PROTACs:
    a. Use computational molecular docking studies to predict the ligand-target interactions of new ligands with the β2-AR and identify suitable attachment points for linkers
    b. Design and synthesise a library of PROTACs based on 'cmpd 6' and newly identified ligands that have suitable physicochemical properties for cell penetration
    c. Pharmacologically characterise the library of PROTACs for affinity towards the β2-AR, selectivity over other β-ARs/GPCRs and ability to degrade β2-AR concentrations.

This chemical biology-focused project will span the disciplines of synthetic chemistry, modelling and pharmacology to increase our understanding of β2-AR biology. The approach of using PROTACs to target therapeutically important GPCRs is relatively

unexplored and means this project will be of direct relevance to future drug discovery efforts

Full project location: University Park;QMC;

Dissecting quality control of bacterial outer membrane biogenesis

Project Supervisor: Jack Bryant

School: Life Sciences

Description: Drug-resistant bacterial infections cause 1.27 million deaths annually. Gram-negative bacteria are of particular concern because the outer membrane (OM) envelops the organism, providing resistance to many antibiotics and stresses. Central to biogenesis of the OM is the BAM complex, which is responsible for folding of proteins into the OM. The essential BAM complex components are highly conserved and reduction of BAM activity leads to increased susceptibility to drugs to which Gram-negatives are normally resistant. Despite inhibitors of the complex being recently identified, these inhibitors remain poorly characterised and discovery of drugs that target this complex is significantly under-exploited.

We recently developed a high-throughput antimicrobial screening assay for the BAM complex. We have identified two antimicrobial peptides that inhibit BAM activity. However, use of antimicrobial peptides is hampered in part due to the expense of peptide synthesis and optimisation. This rotation project will test and refine a cheap and reliable mechanism for producing and optimising antimicrobial peptides in E. coli.

Skills developed: Cloning (Gibson assembly etc), protein overexpression and purification, protein interaction analysis (methods: AUC/ITC/MST/SEC), bacterial growth inhibition assays and morphology analysis by microscopy. Students will also be trained in literature review, data analysis and presentation skills at group meetings.

Location: University Park;

Full project description: The Gram-negative bacterial outer membrane (OM) acts as a permeability barrier to antibiotics and environmental stresses. As such, faithful biogenesis of this membrane is key to bacterial survival. Central to this is the BAM complex, which is responsible for folding of OM proteins (OMPs). Despite intensive study, we do not understand the mechanisms that ensure faithful activity of the BAM complex in folding OMPs. We recently characterised the structure of BepA, a periplasmic protease that functions as a chaperone and quality control protease to ensure faithful folding of the OMP LptD by the BAM complex. BepA is one of four M48 family proteases in Escherichia coli that are thought to be part of a surveillance mechanism that roots out incorrectly folded OMPs. However, the roles of the remaining proteases (YcaL, LoiP and HtpX) and BepA remains poorly understood.

This PhD project will use a high-throughput genetics tool (Transposon Directed Insertion site Sequencing-Xpress – TraDIS-Xpress) to identify all of the genes in E. coli that either become essential to survival in the absence of the proteases, or increase survival of these mutants. This will be complemented by purification of each protease and pull-down assays to identify the genetic and physical interaction networks of the M48 proteases.

This in vivo work will be complemented by an in vitro assay for BAM-mediated folding of OMPs to analyse the effects of E. coli M48 proteases on BAM activity in a purified system. In addition, X-ray crystallography will be used to provide structural insight into their mechanism of auto-regulation. We will initially take one of the proteases through

the pipeline, with the others following if time permits. Together, the genetics, biochemistry and structural biology approaches will provide a unique training opportunity in diverse molecular biology techniques and enable us to elucidate the roles of these proteins in the cell and their repertoire of substrates.

The project will be divided into three key objectives:
Objective 1: Identify genetic and physical interaction networks of BAM quality control proteases

Task 1.1 Determine the genetic interaction network of M48 proteases by TraDIS-Xpress.
- Creation of TraDIS-Xpress libraries, sequencing and data analysis.
- Hit validation by CRISPRi knockdown
- Follow up studies based on hits.

Task 1.2 Identification of the regulatory protease physical interaction network.
- Affinity chromatography of protease interaction partners/substrates.
- Mass spec ID of interaction partners/substrates.
- Validation studies of hits.

Objective 2: Determine the effect of quality control proteases on BAM activity

Task 2.1 Analyse the impact of BepA, YcaL and LoiP on BAM activity in vitro.
- Overexpression and purification of proteases and derivatives.
- Assay the effect of proteases on the BAM assay.

Objective 3: Elucidate the structure of quality control proteases

Task 3.1 Structure determination of YcaL, LoiP and HtpX.
- Overexpression and purification of HtpX.
- Crystallisation and structure determination of YcaL, LoiP and HtpX.

Task 3.2: Analyse complex formation between each protease and the BAM complex.
- Complex formation and analysis by pull downs, AUC/SEC/ITC.

Full project location: University Park;

Developing induced pluripotent stem cell-derived human skeletal muscle models to investigate healthy and pathological ageing

Project Supervisor: Matthew Brook

School: Life Sciences

Description: The project aims to establish the culture of induced pluripotent stem cell (iPSC) derived skeletal muscle, to understand fundamental and pathological processes relevant to ageing and diseases of the neuromuscular unit.

The goal of the rotation is to calibrate our iPSC-derived skeletal muscle cultured in monolayer against established alternatives including immortalised (C2C12) and primary human/mouse skeletal muscle, by completing a detailed characterisation. Accordingly, as part of this rotation the prospective student will acquire broad expertise in cell culture as well as the molecular (Western blotting, RT-PCR, fluorescence microscopy) and functional (assaying muscle contraction) approaches highly relevant to the project going forward.

Location: QMC;

Full project description: One of the fundamental roles of skeletal muscle is to maintain skeletal structure and locomotion enabling completion of essential daily activities. In constituting ~40% of body weight, skeletal muscle is the largest organ in the body and acts as a major control hub over whole-body metabolic health. Therefore, maintenance of skeletal muscle throughout life not only preserves physical independence, but also confers protection from a host of metabolic morbidities (Brook et al 2015 Acta Physiol (Oxf) Jan;216(1):15-41). However, many individuals undergo muscle mass loss because of inactivity, ageing, and as a result of muscular diseases. As such, understanding the regulation of muscle across a range of conditions, yet the exact molecular mechanisms that control muscle homeostasis remain unclear, in part due to lack of experimental models to explore and manipulate.

The emergence of induced pluripotent stem cells (iPSC) culture and differentiation towards theoretically any lineage including skeletal muscle, in parallel with CRISPR gene editing approaches, presents new opportunities to explore skeletal muscle (dys)function – which we propose to do as part of this project.

Experimental objectives

1. Calibrating iPSC-derived skeletal muscle model: Following on from the rotation, and after validating the successful differentiation of iPS cells towards skeletal muscle, we will further calibrate the cellular model by subjecting the cells to a range of stimuli. Muscle contraction and nutritional provision are two of the most powerful stimulators of intramuscular signalling, regulating muscle size. Accordingly, we will investigate the acute changes in myotube signalling in response to a range of nutritional interventions and electrical pulse stimulation contractions. The activation of key signal transduction pathways will be investigated using western blotting and RT-PCR, with morphological changes assayed using fluorescence microscopy. This will confirm if iPSC-derived skeletal muscle respond as anticipated and present a baseline for future investigations.
2. How do gene variants associated with neuromuscular diseases impact muscle formation and function? We will then explore how gene variants associated with neuromuscular diseases including myotonic dystrophy and motor neurone disease impact muscle formation and function. To do this we will exploit a library of iPS cells established in the supervisor's labs. We will differentiate these patient derived iPS cells into skeletal muscle and assess these lines using measures established in 1.
3. Understanding neuromuscular communication: Finally, we will address mechanisms of muscle-neuron cross talk, including extracellular vesicles, addressing how this maybe perturbed by pathological variants probed in 2. To do this we will isolate conditioned media from iPSC-skeletal muscle (control/disease) and add this to cultured iPSC-derived motor neurons (protocols well established in the supervisors' labs), to determine (dys)functional responses. Secretions will further be probed by quantitative 'omics approaches.

Collectively this planned programme of research will deliver a significant training opportunity in cutting-edge cell culture and related molecular and functional approaches, and deliver new models to better define mechanisms that regulate skeletal muscle homeostasis in health and disease.

Full project location: QMC;

Does pregnancy alter sweet taste perception and sweet food cravings?

Project Supervisor: Matthew Elmes

School: Biosciences

Description: A key component of this PhD is to assess food cravings and sweet taste perception during pregnancy, therefore an effective questionnaire/survey needs to be developed and will be a key aim of the lab rotation project.

This mini project will review the different food craving and dietary intake questionnaires already published within the literature to allow the student to develop an effective survey to assess the association between food cravings and dietary intakes. Once developed the student will pilot the survey with a small participant group (n=20) to get feedback to optimise the survey further and to gain skills in data collection and statistical analysis.

- Weeks 1-3: Literature searching and development of food craving and dietary intake survey. Writing and getting ethics into place and recruitment of participants.
- Weeks 3-6: Pilot the survey with the recruited participants and data collection and feedback.
- Weeks 6-8: Data analysis and further survey optimisation.
- Weeks 8-9: exposure to sensory measurements related to sweet sensing.

The mini project has been carefully designed so the student doing the lab rotation learns and develops skills required for the main PhD project that can be developed further and used throughout the PhD

Location: University Park;Sutton Bonington Campus;

Full project description: Background and aims: Sweet food cravings during pregnancy are very common and could increase gestational weight gain and significant risk of gestational diabetes. Food cravings typically arise in the first trimester, peak during the second then diminish towards term. Interestingly, unlike healthy pregnancy, women with gestational diabetes demonstrate sweet taste preference late in pregnancy.

Pregnant women also report changes in taste perception, particularly in the first trimester. However, previous research is limited to single time points during pregnancy and based on retrospective surveys or self-reports. The mechanism of altered taste during pregnancy is not fully understood. It could be driven by hormonal alterations, but recent studies suggest the central nervous system may be involved. Animal studies have demonstrated food cravings during pregnancy to be associated with reorganised brain connectivity affecting reward circuitry. However, there is limited research on understanding the neurophysiological mechanism underpinning food craving and taste alterations during human pregnancy.

Research on the effects of BMI on taste perception during pregnancy is also surprisingly lacking. This omission is important as 60% of UK women are currently overweight or obese. The reduced ability to detect sweet taste could lead to increased cravings for sweet foods during pregnancy, directly increasing the risk of gestational diabetes that is 4-fold higher in obese pregnancies.

The PhD student will work with a multidisciplinary team from the University of Nottingham (UoN) and University of Adelaide (UoA) to explore the neurophysiological mechanisms underpinning food craving and sweet taste alterations during pregnancy across UK and Australian cohorts. The UoN and the UoA are internationally renound for their nutritional research. Thus, this PhD project will offer the student an exciting training opportunity, with a combination of training not offered elsewhere. In addition, it

will offer the opportunities for a multidisciplinary team to work together via this PhD training opportunity.

Study 1 (Year 1): To identify patterns of sweet food craving during pregnancy and the impact of BMI on sweet taste perception, craving and consumption in UK and Australian participants. Online food frequency questionnaires, dietary intake and craving surveys will be collected from the same cohort of women at each trimester of pregnancy and postnatally.

Study 2 (Year 2 and Year 3): To explore the neurophysiological mechanism underpinning food cravings and sweet taste alterations during pregnancy. Participants will be invited each trimester of pregnancy, and again postnatally to the Sir Peter Mansfield Imaging Centre (SPMIC) for brain imaging scans to measure brain activity at the resting state and in response to food cravings and sweet taste perception.

Study 3 (Year 4): To carry out a small animal study to determine the potential mechanism and impacts of altered taste perception during pregnancy.

Outcomes: Research expertise will be gained on eating behaviour, sensory perception and brain imaging but further skills include:
• Human intervention studies across two countries
• Quantitative and qualitative data analysis and methodologies
• Sensory perception evaluation techniques.
• Tools to assess brain responses to food perception

Full project location: Sutton Bonington Campus;University Park;

Self-assembled theranostics: combing PARASHIFT MRI with drug delivery

Project Supervisor: Ben Pilgrim

School: Chemistry

Description: The student would first undertake a short synthetic route (2-3 steps) to prepare a novel coordinating motif for lanthanide ions and then self-assemble these with different lanthanides, based within the School of Chemistry. The aim is to test new coordinating motifs on smaller complexes of a single lanthanide to gauge stability, optimise potential effects on chemical shift and relaxation properties, before incorporating these motifs into larger structures during the full PhD project. After the synthetic work is finished (should be achievable in four weeks), the student will analyse the prepared complexes first by conventional 1H NMR spectroscopy and then attempt some hands-on preliminary in vitro magnetic resonance imaging (MRI) and spectroscopy (MRS) on model systems within the latter part of the rotation, with our collaborator Dr Pete Harvey.

These MR studies will include monitoring variation in interactions with model biomolecules and biorelevant media (e.g. albumin, serum). This rotation hence would allow the student to develop several new skillsets, including synthetic chemistry, analytical techniques, imaging, and data processing/analysis. The student will gain an understanding and appreciation of the interconnected aspects of the full project while acclimatising themselves within the Schools of both Chemistry and Pharmacy.

Location: University Park;

Full project description: Biological processes underlying diseases ranging from cancers through to neurodegeneration are complex and difficult to evaluate in real time by

current methods. Furthermore, many therapies to address these diseases can only be evaluated post-treatment, meaning the biology under investigation must be inferred rather than measured. This project sets out a new method to track therapeutic processes in the body by following drug delivery and function in real-time, providing hitherto inaccessible information on disease bioscience. We propose to utilise self-assembled metal-ligand complexes to generate unparalleled contrast in magnetic resonance imaging (MRI), giving real-time molecular information in complex biological environments. Simultaneous encapsulation of drugs within the metal-ligand complexes will result in theranostic (therapeutic + diagnostic) platforms, with the goal of correlating MR signal to drug release and biological outcome.

MRI is a powerful tool for non-invasive imaging, with unparalleled imaging depth and an ideal combination of spatial/temporal resolution, though typical 1H water-based imaging is limited by background signal and restricted to single-channel imaging. Focus has turned to multichannel heteronuclear imaging and magnetic resonance spectroscopy (MRS), but probes suffer from poor signal intensity.

Paramagnetic shift (PARASHIFT) imaging, through incorporation of lanthanide(III) ions, boosts signal. Existing probes exploit local symmetry to place nearby nuclei in equivalent environments so their signals come at one frequency (e.g., trifluoromethyl's three 19F or tertbutyl's nine 1H). However, despite this local symmetry, most probes have low overall symmetry.

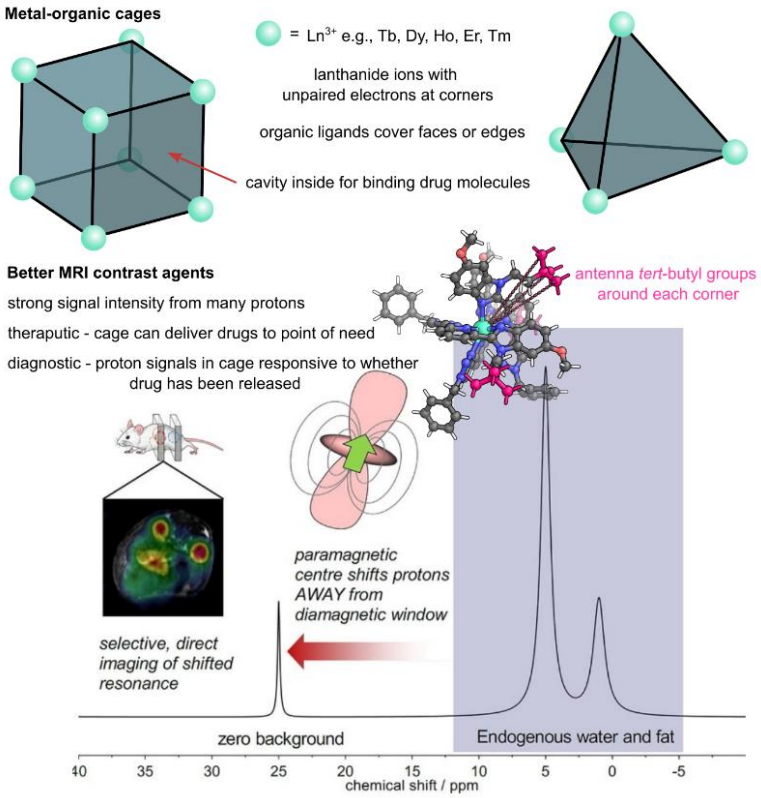
Metal-ligand self-assembly is an unrivalled strategy for constructing complex molecular architectures from simple building blocks, allowing facile access to metal-organic cage structures of the highest symmetries. Common polyhedral shapes (tetrahedra/cubes/octahedra) can all be constructed, with metal ions for the corners, ligands covering the edges/faces, and a well-defined inner cavity for guest binding. However, such structures have yet to be exploited for MRI.

This project will harness these unique metal-organic cages to provide new insights into biological phenomena in real-time. We envisage target complexes could have signals an order of magnitude more intense than existing agents (a cage synthesised by Dr Pilgrim had 216 1H nuclei in the same environment vs 18 in current state-of-the-art). The beauty of self-assembly means a library of different candidates should be accessible with minimal synthesis.

Metal-organic cages have already shown promising biomedical applications, with examples of anti-cancer agents and drug delivery capsules, but this field is still in its infancy. We will develop dual purpose MRI probes that encapsulate therapeutics for drug delivery, with the cage cavity customisable for a range of existing therapeutics. Ultimately, we will pursue methods to correlate the cage's PARASHIFT signal to drug release, resulting in a unique system to monitor at ultra-high resolution how cells and tissues respond to disease-localised drug release.

The student will benefit from supervisors with multidisciplinary teams spanning schools, Chemistry and Pharmacy, which span multiple areas of the biosciences. Dr Pilgrim has an extensive background in biomimetic materials self-assembly, and Prof Alexander is experienced in drug delivery applications and student mentorship. The student will also benefit from the collaborative expertise of Dr Pete Harvey in preclinical MRI.

Full project location: University Park;



Effect of the extracellular matrix on metabolite regulation and distribution in primary human macrophages

Project Supervisor: Anna Piccinini

School: Pharmacy

Description: Metabolic reprogramming is directly linked to immunity. Changes in the major metabolic pathways affect immune cell responses. Moreover, immune cell subsets, including macrophages, appear to rely on distinct metabolic pathways to promote survival, differentiation and function. However, most of these findings have been made in mice or using cell lines cultured in standard, 2D plastic culture systems, and there are several outstanding questions. Notably, macrophages constantly sample their surroundings and can detect small changes in their 3D microenvironment that, in turn, affect their phenotype and behaviour. This raises the question as to whether the extracellular matrix (ECM), which is the major components of the extracellular microenvironment, actively influences macrophage metabolism. We have established a physiological, 3D culture system which recapitulates the healthy ECM and allows the culture of primary human macrophages. Moreover, our work suggests that depletion of specific ECM proteins causes metabolic changes in cells.

In this rotation, you will learn how to generate the 3D culture system and apply it to investigate how the ECM affects macrophage metabolism. You will undertake metabolomics and pathway analysis to define the metabolic profile of primary human macrophages housed within an ECM compared to that of cells in standard, 2D plastic cultures.

Location: University Park;

Full project description: The field of immunometabolism, emerged in the past ten years or so, shows that metabolic reprogramming is directly linked to immunity. Precise

changes in the metabolites of the major metabolic pathways affect immune cell responses. Moreover, immune cell subsets, including macrophages in different activation states, appear to rely on distinct metabolic pathways to promote cell survival, differentiation and function. For example, "inflammatory macrophages" use glycolysis, the TCA cycle, the pentose phosphate pathway, fatty acid synthesis and amino acid metabolism to support phagocytosis and the production of inflammatory cytokines. Whereas "reparative macrophages" use the TCA cycle, fatty acid oxidation and amino acid metabolism to support oxidative phosphorylation and inhibit inflammatory signals.

However, most of these discoveries have been made in mice or with cells cultured in standard, 2D plastic culture systems, and there are several outstanding questions. Notably, macrophages constantly sample their surroundings and can detect even small changes in their 3D microenvironment that, in turn, affect their phenotype and behaviour.

This raises the question as to whether the extracellular matrix (ECM) and its constituent proteins, which are the major components of the extracellular microenvironment, actively influence macrophage metabolism, integrating complex signals in space and time. We have established a physiological, 3D culture system which recapitulates the healthy ECM and allows to culture and study primary human macrophages. Moreover, our work suggests that deletion of specific ECM proteins, whose expression is induced during inflammation, cause metabolic changes in cells.

In this PhD project, you will investigate the effect of the ECM on macrophage metabolism and immune function. You will isolate primary monocytes from peripheral human blood and differentiate them into macrophages in our 3D culture system or in standard 2D, plastic cultures. There will be the possibility to conduct CRISPR/Cas9 genome editing to generate ECM lacking individual proteins with a known function in inflammation and immunity. You will employ molecular biology techniques, including RNA extraction, cDNA synthesis and qPCR, and immunological methods, including ELISA, FACS and western blotting, to extensively characterise their phenotype. You will determine their metabolic profile by performing 'untargeted' liquid chromatography-mass spectrometry (LC-MS)-based metabolite profiling, followed by multi- and univariate statistical analysis to identify key metabolic changes and pathways. You will use biochemical assays to validate key findings. In addition, you are likely to perform functional assays such as phagocytosis, cell adhesion or migration that will allow you to link any metabolic changes to cell function. Finally, the project will involve bioinformatics analysis of large metabolomics data sets and meta-analysis of published datasets.

Full project location: University Park;

Pluripotent stem cell research related to human development and regenerative medicine

Project Supervisor: Masaki Kinoshita

School: Biosciences

Description: Our group is interested in how pluripotent stem cells (such as embryonic stem cells) are maintained in culture. We recently reported establishment of formative stem (FS) cells, which are maintained under low Tgfb/Activin/Nodal siganlling and inhibition of Wnt and retinoic acid pathways. In this rotation project, we will test how other extracellular signals impact gene regulatory network of formative pluripotency. The student will assess the impacts of growth factors and small molecules for the self-

renewal of human stem cells. We will treat cells with various signalling agonists and antagonist and check their impact on the self-renewal by qRT-PCR and immunostaining. This knowledge helps to develop the better culture medium for the human pluripotent stem cells. This rotation project will be closely related to our current BBSRC funded project, which we develop the novel stem cell lines from pig and sheep.

Techniques and skills that the student will learn: RNA extraction, cDNA synthesis, RT-qPCR, immuno-fluorescence antibody staining, fluorescent microscopy imaging, stem cell culture.

Location: Sutton Bonington Campus;

Full project description: Pluripotency exists transiently in early mammalian development. It is a very short period and it consists of three continuous phases called naïve, formative and primed. Naïve phase represents pre-implantation stage, formative phase represents early post-implantation and primed phase represents gastrulation stage, when cells start to commit their fate. These pluripotency phases were mostly studied using mice however, the development of mouse embryos is different from those of humans in timing and morphology. More crucially, unlike model animals, it is impossible to examine post-implantation stage embryos directly in humans. Therefore, stem cell culture acts as an ideal model to understand post-implantation stage human development and gene regulatory network. We use recently developed formative stem (FS) cells to understand post-implantation stage development.

1. We have previously identified Otx2 plays an important role in mouse FS cells. We also have identified a few other genes which is essential for the self-renewal in mouse cells. Firstly the student will test such mouse genes in human cells. Also the student will learn how to analyse transcriptome (RNA-seq) data and list up candidate genes to test, which is specifically expressed in human formative stem (FS) cells as well as any related human embryo data.
2. In parallel to the experiment above, our lab will perform the genome-wide gene knockout screening using CRISPR/Cas9-gRNA libraries. We will establish the reporter human FS cell lines which we can monitor the gene expression by FACS. The candidate of such reporter gene is OCT4 and OTX2 and also genes identified in the expression analysis in 1. We will use gRNA libraries and monitor such reporter gene expression. We will collect the cells using reporter expression pattern and perform next generation sequencing experiment to identify which gRNAs affect the reporter gene expression.

The outcome from 1 and 2 will produce the good number of candidate genes, so the student will perform experiments to confirm its effect individually. We will assess them by gene knockout individually using CRISPR/Cas9. Our primary interest are transcription factors and we will reveal the genetic correlation and functional mechanisms of the gene in detail by using state-of-art molecular biology techniques including next generation sequencing experiments.

We previously found the FS cells have enhanced differentiation than conventional ES cells. If we identify epigenetic modifiers in 1 and 2, we will address why these cells have better differentiation focusing on epigenetic changes. We will perform histone mark ChIP-seq (active or repressive marks), ATAC-seq (chromatin accessibility), whole-genome Bisulfite sequencing (DNA methylation) and identify the critical modification which makes FS cells superior in differentiation.

All these analyses enhance the understanding of human pluripotent stem cells, which can be applicable to basic and clinical research as well as the regenerative medicine.

This project will be closely related to the BBSRC funded project which studies pluripotency in livestock species. We will investigate genes identified in this project in large mammals and vice versa, so the student is expected to closely work together with a post-doc researcher on this project.

Full project location: Sutton Bonington Campus;

Mammalian stem cells: an experimental platform to study epigenetic changes in development and neurodegenerative disorders

Project Supervisor: Reinhard Stoger

School: Biosciences

Description: We have previously identified elevated levels of the epigenetic mark 5-Hydroxymethylcytosine (5hmC) in the cerebellum of patients with Parkinson's disease (Stöger et al. npj Parkinson's Disease 3, 6 (2017). https://doi.org/10.1038). The rotation-project will involve immunofluorescence detection to determine possible differences in 5hmC levels among cell types in brain tissue sections of Parkinson's patients. Of particular interest will be to check if 5hmC variations coincide with the potential presence of stem/progenitor cells in the human cerebellum; expression of the neural lineage markers PAX6 and SOX2 will be used to explore this. The antibody-based immunofluorescence approach for the detection of 5hmC, PAX6 and SOX2 will first be optimised on pluripotent stem cells from pigs. This will allow the student to become familiar with the laboratories at the School of Biosciences and at the School of Veterinary Medicine and Science and procedures, some of which will likely be used throughout duration of the PhD work.

Location: Sutton Bonington Campus;

Full project description: The fields of regenerative medicine and animal biotechnology offer exciting opportunities, which are driven by advances in stem cell biology. Stem cells can be maintained in culture; they can be differentiated into particular cell types, such as neurons and they can be manipulated to test drugs and their effects on the biochemical, energetic and epigenetic states.

Our knowledge of embryonic stem cells rests largely on work with embryonic stem cells derived from the mouse (Mus musclulus), a classic mammalian model organism. Conditions for long-term maintenance of mouse embryonic stem cells in their undifferentiated, pluripotent state have been established. For embryonic stem cells of other species, including human, these culture conditions do not work. Finding universal culture conditions that maintain all mammalian embryonic stem cells in a distinctive, undifferentiated state of pluripotency, without affecting their genetic and epigenetic integrity will be a considerable progress.

Remarkably, 'Royalactin', a protein from the honey bee (Apis mellifera) can maintain pluripotency by activating a ground-state pluripotency-like gene network (https://doi.org/10.1038/s41467-018-06256-4); 'Regina' a mammalian structural analogue of Royalactin also induces a naïve-like cell state. The limitation of the above mentioned study is: only mouse embryonic stem cells were used to demonstrate puzzling characteristics of Royalactin and Regina, respectively.

The aims of this PhD project are to:

Optimise the maintenance of mammalian stem cells using Royalactin and/or Regina. Sequence-verified vectors expressing either Royalactin or Regina have already been used to transfect pig Embryonic Disc Stem Cells (EDSCs). These 'transgenic' EDSCs will be studied to evaluate the effects of Royalactin and Regina on maintaining the 'stemness' (the undifferentiated state of cells) employing multitude of approaches and techniques. This includes assessment of morphology and the presence of markers specific to pluripotent cell states. Linage committed genes/proteins and self-renewal will be monitored by qRT-PCR and immunostaining.

Differentiate in vitro uncommitted stem cells towards differentiated neurons. This system will be used to manipulate cells to mimic neurodegenerative conditions such as Parkinson's and Alzheimer's disease. It is therefore necessary to establish a robust protocol which allows reproducible experimentation.

Explore the interplay between mitochondrial function and epigenetic regulation of the nuclear genome. Altered mitochondrial energy production is a hallmark of many neurodegenerative syndromes and diseases. Metabolites derived from mitochondria influence the nuclear epigenetic landscape; our understanding how the epigenetic states become deregulated in certain neurodegenerative syndromes is rudimentary. The 'stem cell – neuronal differentiation' system will be used to analyse mitochondrial activity by High Resolution Respirometry in cells mimicking normal and diseased conditions. The effects on the epigenome, DNA modifications in particular, will be measured using a variety of techniques, including, Nanopore sequencing, Array-based methylation studies and immunodetection.

Full project location: Sutton Bonington Campus;

Facilitating protein degradation to impair cell survival

Project Supervisor: Cristina Montiel-Duarte

School: School of Science and Technology (NTU)

Description: The nine weeks lab rotation will be used as a 'mini' project where the student will design primers for subcloning and mutagenesis and prepare relevant plasmids. The student will also transfect human cell lines with successful constructs and/or siRNA to study their effects on proliferation and viability using the IncuCyte life cells analysis system and fluorescence microscopy.

The overall goal will be the assessment of potential targets to later study modifications and interactions through immunoprecipitations and proximity ligation assays and to develop novel interaction assays suitable for high throughput screening.

Location: Clifton Campus;

Full project description: Molecular glues are small molecules, part of the broader small molecule medicines field, that can facilitate associations between proteins, either reinforcing known interactions or creating novel ones. As such, they are a tool that has been used to facilitate protein degradation by engaging a target with the ubiquitin-proteasome system.

The design of new molecular glues can be facilitated by the availability of (predicted) protein structures. However, when the ultimate goal is treatment, it is still necessary to ensure an appropriate selection of the protein target.

DNA methyl transferases (DNMTs) are enzymes that transfer a methyl group from the donor S-Adenosyl methionine (SAM) to a cytosine, a modification generally associated with gene repression. Distinct changes in DNA methylation patterns occur during both aging and cancer and are associated with abnormal DNMTs expression and mutations. Consequently, DNMT inhibitors have been developed and are in use in the clinic: decitabine and azacytidine are approved for haematological cancers but they have poor pharmacokinetic properties and furthermore, resistances are arising.

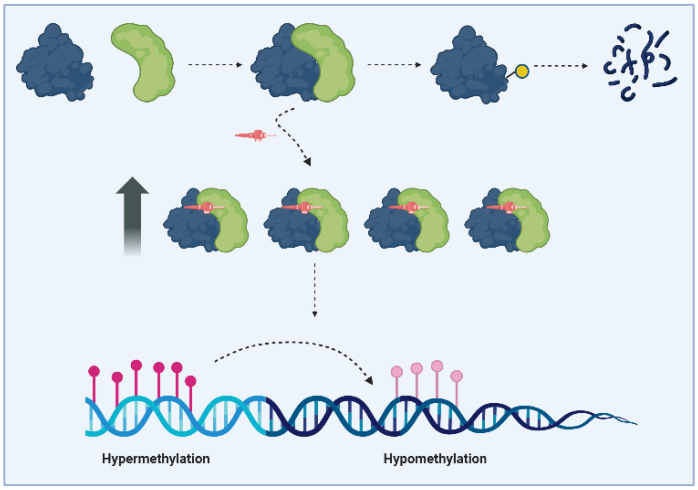
In spite of DNA methylation relevance in gene expression, very little is known about DNMTs regulation through post-translational modifications (phosphorylation, acetylation, methylation etc.) and most of the information published refers to the DNMT1 isoform, whilst both DNMT3A and DNMT3B isoforms play an active role in malignancies.

Study aim

Our unpublish data (from immunoprecipitation and proximity ligation assays) suggests that DNMT3A and DNMT3B levels and activity can be decreased by novel post-translational modifications and this project will aim to identify the enzymes involved in these modifications. The project will also explore the identification of molecular glues that might enhance those interactions and modifications to induce a reduction in the methylase activity.

The project supports a new collaboration between Dr Montiel-Duarte's group at NTU and Prof Heery's group at the U. of Nottingham. The student will develop methodologies to express different DNMTs mutants and identify interacting partners in the context of human cell lines, studying the role of post-translational modifications in the activity and levels of the enzyme as well as in cancer cells proliferation and survival.

The studentship provides a unique opportunity to develop core experimental skills in biochemistry, structural biology and bioinformatics, facilitated by the supervisory team and the excellent research facilities involved.



Full project location: The Nottingham Trent University (the John van Geest Cancer Research Centre) will be the main location, complemented by short stays at the University of Nottingham Gene Regulation & RNA Biology Group (GRRB).

Assessment of a Genomic Informational Field Theory (GIFT) for genetic analysis of complex traits in high performing athletes

Project Supervisor: Ian Varley

School: School of Science and Technology (NTU)

Description: The rotation will be tailored to the student's academic background. It will use genotypic and phenotypic data related to high performing athletes obtained from the supervisory team.

a) If the student has little experience in quantitative genetics, the period will be divided in two parts. Part I (5 weeks), the student will be trained in the field of quantitative genetics and our method (Genomic Informational Field Theory; GIFT). Part II (4 weeks), the student will undertake a pilot study to compare GIFT for genetic associations to traditional methods using selected phenotypic information, such as bone mineral density and muscle characteristics.
b) If the student is already familiar with current quantitative genetic methods the 9 weeks will be undertaken as a single block during which the student will be trained to perform genome-wide analysis using GIFT and to compare this to candidate gene analysis for selected phenotypes (e.g. muscle characteristic, bone mineral density). There would also be follow-up analyses to predict the functional significance of genetic associations.

Location: Sutton Bonington Campus;Clifton Campus;

Full project description: Genetic research involving athletes is conducted to identify genetic traits and variations that may influence an individual's athletic performance, injury susceptibility and potential, in turn informing health and wellness in the broader population. However, genetic studies conducted in high performing athletes usually have relatively low sample sizes which reduces the statistical power of Genome-wide association studies (GWAS) and can lead to an increased risk of false positives and/or negatives.

Members of the supervisory team at the University of Nottingham (UoN) have developed a new method (Genomic Informational Theory: GIFT), specifically designed to determine genotype-phenotype associations where the sample size (i.e., number of athletes) is small. The main conceptual advantage of GIFT is the way information previously considered as 'noise' (i.e., unexplained variation in the data) is included in the model. The new method integrates that information into the model by analysing the mixing of genotypic states on a single scale defined by the phenotypic values ranked as a function of their magnitude. This new method contrasts with classic methods that consider each genotypic state on a separate phenotypic scale, with the associations between genotype and phenotype defined simply by the differences between phenotypic means. GIFT has unprecedented potential to detect small genes effects even in relatively small sample sizes. As the members of the team at Nottingham Trent University (NTU) and Manchester Metropolitan University have a vast experience in conducting studies assessing genetic associations with physical characteristics of athletes. Combined, the supervisory team possess probably the largest genotype-phenotype dataset for an athletic population (~2000 samples, with ~800 from individual sports), which is considered small for GWAS but should be amenable to analysis using GIFT. The collaboration ideal to identify genetic traits and variations that may influence an individual's athletic performance in turn informing health and wellness in the broader population.

Project: Interindividual genetic variability in humans can arise as a consequence of single- nucleotide polymorphisms (SNPs). The project will use GIFT to identify functionally significant SNPs in a range of phenotypes. To do so, we will use recently genotyped information on athletes held by the supervisory team. The objectives of this DTP are to undertake association analyses to identify functionally significant genetic variants and to compare the efficiencies of GIFT with previously conducted candidate gene studies. This will extend the analyses already undertaken to determine which method can best identify functionally significant SNPs.

Work plan

Phase-1: Advanced training with GIFT such as to explain its conceptual difference with classic GWAS and candidate gene studies. This will allow the student to redefine key notions from genetics used by traditional methods within the framework of GIFT.

Phase-2: Will involve generating simulated data enabling the student to compare GIFT under different scenarios linked to variation in genotypes and/or phenotypes.

Phase-3: Will involve running GIFT to identify functionally significant genetic variants in phenotypes.

Phase-4: Will extend these analyses to include other datasets in the UK, and will assess the extent to which variant allele frequencies differ between populations.

Full project location: Sutton Bonington Campus;Clifton Campus;

Exploring the role of Endoplasmic Reticulum (ER)-targeting pathway changes in cancer

Project Supervisor: Natalie Mack

School: Biosciences

Description: The SRP receptor is a key component of SRP-dependent cotranslational Endoplasmic Reticulum (ER)-targeting. Through loss of function analyses in Drosophila melanogaster and immunostaining of human breast tumours, we have produced several lines of evidence to suggest that SRP receptor changes may contribute to tumour progression (Canales Coutino et al. 2020; and Mack et al. manuscript in preparation). However, we currently lack data from cancer cell lines to support these initial findings and are yet to analyse the effects of their overexpression; this is the focus of the rotation project. Briefly, the student will analyse expression levels of SRPRA and B in an array of cancer cell lines (likely Breast and/or Brain cancers as available) and manipulate their function (loss or gain) by overexpression, knockdown or mutation, to examine the effects on tumour-relevant phenotypes such as cell proliferation, migration, and invasion. The rotation student will culture relevant cell lines using aseptic techniques and perform western blot analyses for SRP receptor protein levels. They will also undertake molecular biology techniques to clone the human SRPRA and B genes into appropriate mammalian expression plasmids which can then be used to perform overexpression in the cell lines and analyse resultant phenotypes.

Location: Sutton Bonington Campus;

Full project description: The full project aims to better understand the role of ER-targeting in cancer development and progression. This will be done through a combination of in vitro and in vivo experiments, using mammalian cell culture and the fruit fly, Drosophila melanogaster. This is a collaborative project led by supervisors in the Schools of Biosciences and Life Sciences.

Firstly, the role of the SRP receptor needs to be further explored to build on existing data and work out exactly how and when higher or lower SRP receptor expression or activity may be contributing to tumourigenesis. Data from online cancer databases indicates that SRP receptor expression changes, including increased and decreased levels, correlate with poorer survival in several cancer types. Overexpression correlates in Breast and Brain cancers, however, conversely, our data suggests decreased SRPRA levels in advanced Breast cancers correlates with poorer survival. Our current hypothesis is that the SRP receptor has dual oncogenic and tumour suppressive functions which are context-dependent and this project will involve analyses that interrogate this. Specifically, the student will investigate the effects of SRP receptor overexpression in several Drosophila tumour models to add and compare to our existing loss of function data. In addition, the effects of increasing or decreasing SRP receptor levels on tumour relevant phenotypes (growth, migration, and invasion) will be explored in vitro in relevant cell lines and utilising a variety of assays. Moreover, our existing data shows that Notch signalling is perturbed by SRP receptor changes, but other signalling pathways affected now need identifying to fully elucidate the molecular mechanisms behind the observed phenotypes. This will involve unbiased analyses of cellular gene and protein expression levels.

Secondly, the role of other ER-targeting pathway components in tumourigenesis will be investigated, aiming to develop knowledge of how these pathways in general contribute to cancer and therefore establish whether they may be useful as biomarkers or therapeutic targets. There is potential compensation between different ER-targeting mechanisms and the impact of this on tumourigenesis is currently not known. The student will utilise our established Drosophila tumour models to assess the impact of decreased or increased expression of the various ER-targeting pathway components. Using sophisticated genetic techniques, tumours can be generated in the dorsal thorax epithelium by driving knockout of the tumour suppressor gene Lgl or by overexpression of oncogenic Ras (RasV12), and the tumours labelled with GFP to enable subsequent analysis by live confocal imaging. Concomitant knockdown or over-expression of individual ER-targeting pathway genes will be performed to determine if they affect tumour development or progression. The ER-targeting pathways are highly conserved between Drosophila and humans, making this organism an ideal model system for testing our hypotheses. Moreover, any genes that show changes in the Drosophila tumour models could be further investigated using in vitro cell culture techniques as proposed above for the SRP receptor. Finally, immunostaining of tissue arrays from relevant human cancer types could be performed to further validate any data obtained from the Drosophila and in vitro experiments and data found on the cancer databases.

Full project location: Sutton Bonington Campus;QMC;

Understanding the hierarchy of cell division in trypanosome parasites

Project Supervisor: Bill Wickstead

School: Life Sciences

Description: The lab rotation will give an introduction to the PhD project using a subset of genes. In the project, we will grow and genetically modify trypanosomes to tag specific components of the chromosome segregation machinery. These will be followed through cell division by fluorescence microscopy to analyse the timing and location of their recruitment. We will then knockdown other parts of the system and also perturb the system with known inhibitors to understand the dependency relationships between different essential parts of the system. This work provides an introduction to some of the

techniques in molecular cell biology, genetics and quantitative microscopy employed by the lab that will be part of the PhD project.

Location: QMC;

Full project description: Correct cell division is essential to life. However, the way in which division is achieved differs greatly between organisms. This is particularly the case for many important parasites, the division of which is often very different from the cells of their host. Understanding this cell division machinery is important to understanding the fundamental biology of the parasite, but also because differences between parasite and host present opportunities for the development of new treatments.

African trypanosomes are single-celled parasites of the blood. They cause a deadly disease in humans in sub-Saharan Africa and a wasting disease of cattle that kills ~3 million cattle per year and creates an estimated loss of ~$4 billion from African economies. African trypanosomes have an unusual genome structure that is linked to their pathology and encompasses ~120 chromosomes all of which are moved by a cell division machinery that is very different from most other eukaryotes. How this system works is an important outstanding question in parasitology and will tell us how the system evolved as well as which parts are potential targets.

The lab has developed a genetic method that allows us to quickly decode the fitness costs of removing specific components of the cell division machinery. This means we now know the essential components of cell division in 3 important trypanosome species. In this project, a student will use these essential components to decode the dependency relationships between the components (e.g. which parts recruit which) and also study how they affect spindle assembly and cytokinesis in the different disease-relevant trypanosome species. A small number of these components are being developed as targets for small molecule inhibitors and the student will also use their lines to understand how specific drugs perturb the process of cell division. The student will work closely with post-doctoral researchers in the lab who have helped developed the genetic technology and are screening libraries of 100,000s of mutants to identify genes involved in specific aspects of the process. By combining these genome-wide approaches with specific mutants, we are seeking to decode the hierarchy behind the essential parts of the chromosome segregation machinery and look for ways to inhibit the parasite-specific parts of the process.

Full project location: QMC;

Defining the role of fucosyltransferase 2 (FUT2) genetic variants in airway mucocilliary function and inflammatory crosstalk

Project Supervisor: Ian Sayers

School: Medicine

Description:

i. Human bronchial epithelial cell culture at air liquid interface and quality control (epithelial markers e.g. ECad, CK14 using immunofluorescence) (Lead Sayers)
ii. An introduction to genome editing via CRISPR/Cas9 (Lead Sayers).
iii. An introduction to rheological, biophysical and spectroscopic method used to study mucins (Lead Yakubov).
iv. An introduction to key inflammatory cells of relevance in the lung including macrophages (Lead Martínez-Pomares)

Location: University Park;Sutton Bonington Campus;QMC;

Full project description: We recently identified genetic variants spanning fucosyltransferase 2 (FUT2) as associated with chronic sputum production, cough and importantly reduced lung function suggesting a significant role for this gene in mucocilliary function. Fucosyltransferases catalyse the attachment of fucose to glycan chains and fucosylation (and glycosylation) are important processes that make proteins more active and can change the stability of proteins. FUT2 has been implicated in several biological processes including modifying mucins (e.g. glycosylation of MUC5AC, modifying gel-forming capabilities), virus-host interactions and cell-cell interactions. We hypothesise that alterations in FUT2 expression and activity that are driven by genetic variants significantly impacts i) the properties of lung mucins MUC5AC and MUC5B and subsequent mucocilliary clearance in the airway epithelium and ii) epithelial cell – inflammatory cell crosstalk in the context of viral infection in the lung.

This proposal brings together a significant team of researchers with expertise in respiratory genetics/cell models (Sayers), mucins, biophysical analyses (Yakubov), and immunology (Martínez-Pomares). We will use physiologically relevant models of the human airway epithelium (air liquid interface, precision cut lung slices) combined with molecular biology approaches to mechanistically determine the key regulatory processes determining FUT2 gene expression and activity. This will include CRISPRi to systematically silence regions of chromosome 19 implicated by the set of genetic variants as enhancers and identify key regulatory regions and pathways mediating FUT2 gene expression. Similarly we will use CRISPR/Cas9 to delete FUT2. Importantly, we will study effects in the context the epithelial cell model and then during viral infection including using an epithelial cell – macrophage model to investigate the downstream inflammatory response. Finally , in each of these context we will determine the functional consequences of altered FUT2 gene regulation on mucus levels (particularly MUC5AC and MUC5B), composition, organization and properties (e.g. measurement of mucin glycoforms, rheology, supramolecular network organization using atomic force microscopy).

This project will provide i) mechanistic insight into the genetic, molecular and cellular regulation of mucin production and mucus properties by FUT2 of relevance for mucociliary dysfunction and viral induced inflammation, ii) target identification for pre-clinical screening of interventions to restore homeostasis.

Full project location: University Park;Sutton Bonington Campus;QMC;

Genomic characterization of Staphylococcus haemolyticus and associated bacteriophage

Project Supervisor: Jonathan Thomas

School: School of Science and Technology (NTU)

Description: During the nine-week rotation we will begin to isolate lytic phage from environmental samples and skin swabs that can be characterized more comprehensively as part of the full PhD project. You will gain experience of next-generation sequencing of these bacteriophage and testing of host range. You will also start to screen a collection of commensal staphylococci, isolated from skin swabs from undergraduate students, for S. haemolyticus isolates. Over the nine-week lab rotation you will phenotypically and genotypically characterise these strains, starting with 16S rRNA sequencing to determine

species, followed by disk diffusion tests to determine antimicrobial resistance and biofilm assays.

Location: Clifton Campus;

Full project description: Staphylococcus haemolyticus is a common cause of clinical infections and after S. epidermidis is the second most frequently isolated coagulase-negative Staphylococcus (CoNS) from clinical cases, notably from blood infections, including sepsis, as well as meningitis and endocarditis. S. haemolyticus isolates are highly antimicrobial-resistant, with some reports finding it to exhibit the highest level of resistance amongst members of CoNS, with resistance to antibiotics of last resort such as vancomycin and teicoplanin having been reported. Phage therapy utilises lytic phage that infect the bacterial cell, replicate and then kills the bacterial cell by bursting out from within. While there has been much research into bacteriophage that affect the superbug S. aureus and could potentially be used therapeutically, little work has been done on S. haemolyticus bacteriophage.

This project will be a mix of lab work and bioinformatics. In this project you will characterize a collection of S. haemolyticus isolates obtained from the QMC by whole genome sequencing and through a series of phenotypic tests (antimicrobial susceptibility tests, biofilm assays, etc.). You will bioinformatically screen the genome sequences for signatures of positive selection to identify genes that have proved advantageous over the evolutionary history of the species. Finally you will screen environmental samples and skin swabs for lytic bacteriophage that infect S. haemolyticus from both human and veterinary populations. These will be sequenced and characterized to identify phage that could potentially be used as a therapeutic alternative to antibiotics. Electron microscopy will be used to examine phage morphology, while host range tests will determine which lineages of S. haemolyticus the phage can infect.

Overall, this project should provide the opportunity to gain experience with cutting-edge molecular biology techniques, while developing tools that could be used as an alternative to antimicrobial compounds in an important nosocomial pathogen.

Full project location: Clifton Campus;

The contribution of glycation signalling to unhealthy aging and neurodegeneration

Project Supervisor: Joern Steinert

School: Life Sciences

Description: The Steinert lab is interested in the physiological and pathological effects mediated by nitric oxide (NO) and associated post-translational modifications, such as glycation, with particular focus on target proteins involved in synaptic release mechanisms. The student will predominantly learn about the physiology of synaptic transmission at the Drosophila neuromuscular junction (NMJ) and central nervous system. This includes electrophysiological but also behavioural, learning and memory function and imaging studies to characterise neurotransmitter release and neuronal function in larvae, young and aged flies. We perform studies to identify proteins which are modulated by glycation using immunoblotting and immunocytochemistry.

- Week 1-2: Behavioural studies will cover learning and memory, climbing activity (negative geotaxis).
- Week 3-5: Immunohistochemistry on adult fly brains to assess protein expression and morphological phenotypes including confocal imaging.

- Week 6-9: Induction to larval neuromuscular junction and adult fly brain electrophysiology of Kenyon cells (if time allows, combination with Calcium imaging).

Location: QMC;

Full project description: Background: During aging, several functions of cellular metabolism become compromised. This includes changes in energy metabolism and the generation of free radicals in conjunction with reduced antioxidant capacity which leads to cellular stress and neuronal dysfunction. These changes predispose the brain to neurodegeneration. Of particular interest to this project is the nitric oxide-associated increase in neuroinflammation with resulting aberrant post-translational protein modifications. These modifications directly promote an irreversible process called glycation which increases with aging and promotes neurodegeneration cause by enhanced accumulation of protein aggregates.

The aim of this project is to identify the mechanisms and protein targets undergo post-translational modifications and assess impacts on neuronal function. This characterisation will be done on several levels:

i. from ultrastructural measurements of synaptic vesicles,
ii. assessment of neuronal functions by electrophysiology/live imaging,
iii. protein biochemistry and confocal imaging and
iv. characterisation of changes in behaviour, such as deficits in learning and memory, locomotor activity and life span. We will modify pathways involved in redox, nitric oxide and glycation signalling by genetic manipulations and pharmacology.

Experiment plan:
A: Survival: The lifespan of wild type and mutant flies will be measured using longevity assays and survival curve analyses.

B: Negative geotaxis: This robust assay, widely used in the neurodegeneration field, measures the effects on motor functions. The negative geotaxis performance of flies will be measured, and the climbing index will be calculated.

C: Locomotor, sleep and circadian rhythmicity: Using the Trikinetics DAM system we propose to monitor and analyse the daily locomotor activity and sleep flies will be monitored in 12 hours light/dark cycle (collaboration with University of Leicester, Breda, Giorgini).

D: Learning and memory: Memory will be then tested using a T-maze for odour-associative learning and a preference index will be calculated as measure of learning.

E: Physiological and morphological properties of mushroom body CNS neurons and the neuromuscular junction (NMJ). Using standard immunocytochemistry techniques, we aim to analyse the morphology of CNS structures. We will investigate possible functional changes of these neurons upon treatments and induction of glycation in various genetic mutants. Brains and synapses will be used to assess: i) electrophysiological characterisation of Kenyon cells (KC) by whole-cell patch clamp and NMJs by two-electrode voltage-clamp; and ii) Ca2+ signalling using genetically encoded Ca2+ reporter (GCaMP6f). In visually identified Kenyon cells, basal activities, current-induced action potential firing, passive cell parameters will be recorded. In a different subset of experiments following expression of GCaMP6f, intracellular Ca2+ responses in KCs will be recorded.

F: Oxidative stress, mitochondria respiration and blood-brain-barrier: We aim to investigate the level of oxidative stress markers by measuring Superoxide Dismutase, glutathione peroxidase activity and cholesterol levels. Immunocytochemistry experiments followed by confocal analyses (SLIM Facility, Nottingham) and immunoblotting will confirm protein expression in adult fly brains. The level of lipid peroxidation and protein carbonylation will be tested. Using an Oroboros O2K high resolution respirometer, this part of the project aims to study the mitochondrial respiration.

G: Protein biochemistry: We will identify protein targets which undergo post-translational modifications (nitric oxide and glycation-mediated) using Western blot analysis.

Full project location: QMC;

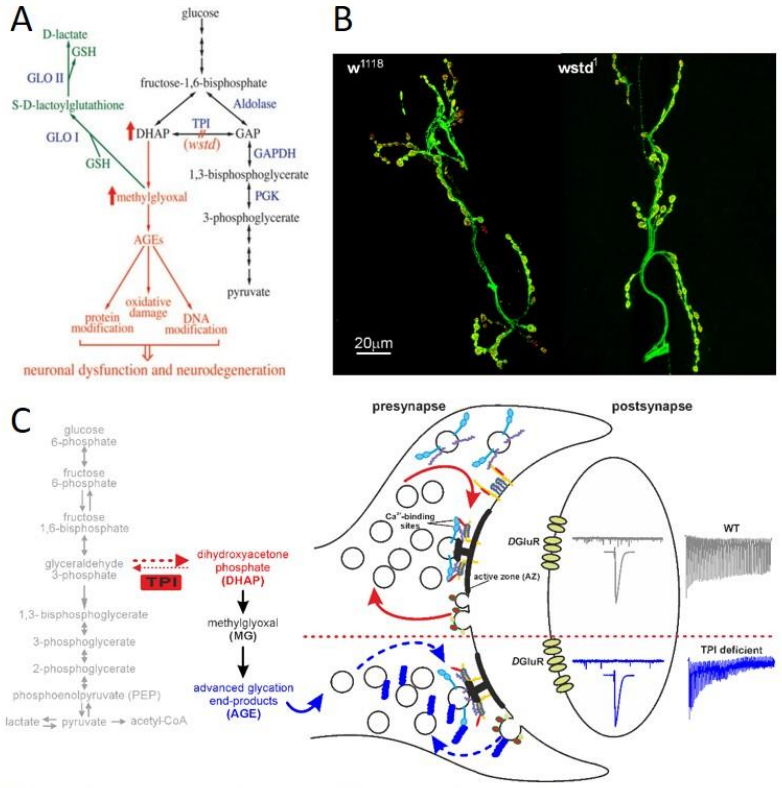


Illustration of Glycation signalling and its functional assessment. A, signalling cascade showing the metabolism involving TPI function and AGE formation. B, Immunocytochemistry of the Drosophila neuromuscular junction from a wild type and TPI mutant larva. C, proposed synaptic signalling in response to TPI dysfunction and AGE formation.

Defining the biophysical properties of chromosomes

Project Supervisor: Daniel Booth

School: Medicine

Description: This rotation will provide the student with an appropriate knowledge-base and skills-set to prepare for the full PhD project. This will include training in cell division assays and methods to isolate mitotic chromosomes from cancer cells. If successful, the student will be trained to "capture" these chromosomes in an optical trap using either our newly awarded Lumicks C Trap system or two home-built optical trapping microscopes in Engineering (Wright lab). Here the chromosomes will undergo stretch and resistance tests (pulling and pushing) to determine chromosome compliance across several cancer cell lines.

The cell division and chromosome work will be performed in the brand new Biodiscovery Institute, an endeavour that houses ~350, academics, clinicians, researchers and PhD students across five floors of state-of-the-art laboratories and research space. The optical trapping work will take place in the Optics and Photonics Group Faculty of Engineering and in the Boots Science Building. They will be part of a vibrant and friendly multidisciplinary team and benefit from the hands-on support of not only the PI but also

several PhD (including other BBSRC) students and Postdocs, across the laboratories of each supervisor.

Location: University Park;

Full project description: Research Aim: Determine how Ki67 and the mitotic chromosome periphery contributes to the biophysical properties of chromosomes in cancer cells

Why: Ki67 is a famous cancer proliferation marker – but what is it doing at the surface of mitotic chromosomes? Addressing this will lead to new insights into fundamental chromosome biology but also how Ki67, a therapeutic target, functions in cell division fidelity.

Since their discovery by Walther Fleming in 1882, chromosomes have been one of the most actively studied components of the cell. The condensation, congression and segregation of chromosomes are key events of cell division and by extension, life. Chromosomes consist of six major compartments. Five of these—nucleosomes, telomeres, the kinetochore, the centromere and the chromosome scaffold—have been studied extensively for decades and are all linked with disease states if functionally compromised. The sixth and least understood compartment is the mitotic chromosome periphery (MCP), a sheath that covers the entire outer surface of chromosomes, like a thick winter glove (the MCP) covering a hand (the chromosome).

The MCP was first described over 130 years ago, but in the interim has remained stubbornly mysterious. As recently as 2014 its functions were still unknown. Our lab then reported that Ki67 was the main organiser of the MCP, showing that the depletion of Ki67 resulted in the mis-localisation of all other chromosome periphery proteins (cPerPs) and loss of the entire compartment. This breakthrough provided an opportunity to perform an initial characterisation of the MCP by dissecting its function(s) for the first time. One hypothesis is that the MCP supports chromosome dispersion (preventing chromatin "tangling"), with Ki67 acting like an electrostatic "brush" that recruits a "phase-separated" coating to the chromosome surface.

The focus of this project is to determine if Ki67 and the MCP contributes to the biophysical and biomechanical properties of mitotic chromosomes – allowing chromosomes to "bounce", similar to dodgem cars – in what would be an entirely new mechanism towards chromosome segregation fidelity.

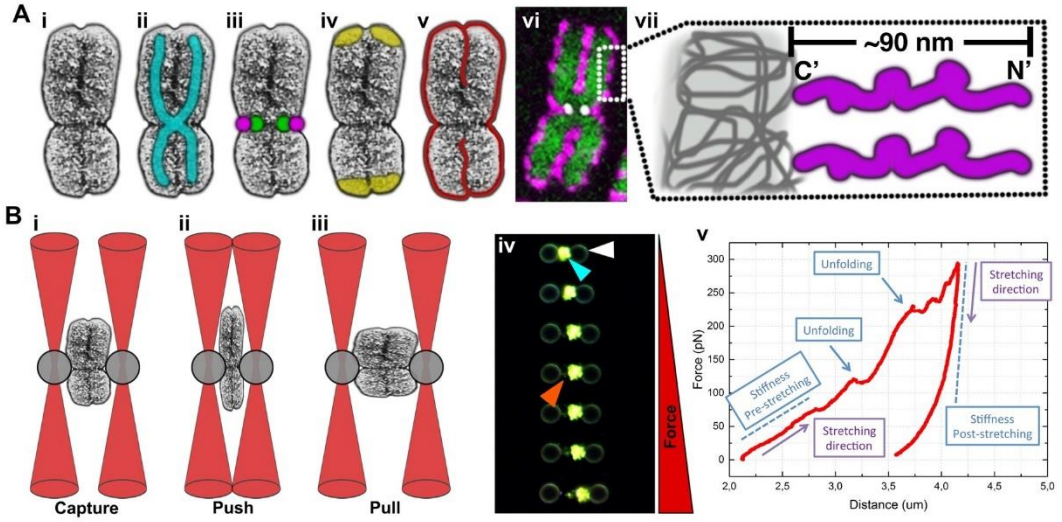
This project will exploit our 2023 award of a Lumicks optical trapping system – providing hands on experience with state-of-the-art technology that few others have access to.

- Objective 1 – Use CRISPR engineering to establish cell lines with tuneable levels of Ki67
- Objective 2 – Validate the cell lines microscopically and biochemically
- Objective 3 – Isolate and then analyse chromosomes using optical trapping to determine chromosome biophysics relative to Ki67 protein levels.
- Objective 4 – Investigate compounds to chemically disrupt Ki67 and explore how this impacts chromosome biophysics – a natural entry into exploring the therapeutic potential of the MCP

Teams Booth and Wright are well funded, offer hands-on guidance and importantly, provide a supportive environment/network of PhD students and post-docs (and the PI's of course!). Supervisory support from Tania Mendonca will provide hands-on training for wet lab work – isolation of chromosomes and training for the Lumicks system. This is a truly multidisciplinary PhD and the student will be exposed to and learn from researchers

from a wide range of backgrounds working at the interface between the biomedical sciences and optical engineering.

Full project location: University Park;



A) Chromosomes consists of six major chromosome compartments. i) chromatin, ii) scaffold, iii) kinetochore & centromere, iv) telomeres and finally, v) the poorly understood mitotic chromosome periphery (MCP). The MCP covers the entire outer surface of all mitotic chromosomes, but its biophysical properties are unclear. This project will begin to address this. vi) Confocal image of a human metaphase chromosome probed for DNA (green), Ki67 (magenta) and CENP-C (white), clearly showing Ki67 nicely decorating the surface of the chromosome. Vii) One model proposes that Ki67 is a "brush" at the surface of chromosomes – potentially providing biophysical properties. Bi-iii) Schematic showing one of our proposed Super-C-Trap experiments to test chromosome compliance. Purified chromosomes will be captured between two beads (grey) held in the optical trap (red). The captured chromosome will undergo stretch/resistance manipulation by forcing beads closer together (Aii) or pulling them apart (Aiii). This will be performed in the presence and absence of Ki67 (and the MCP). Biv-v) Proof of principle experiments – i.e. we already have part of the system up and running! A purified chromosome (blue arrow) was captured between two beads (white arrow). Bead retraction forced chromatin to unfold (orange arrow). V) The force data retrieved from iv was plotted.

Cardiovascular regulation by insulin-like growth factor receptor 1

Project Supervisor: Samantha Cooper

School: Life Sciences

Description: Unravelling the underlying mechanisms involved in normal cardiac physiology is crucial for understanding disease aetiology and identifying drug targets that may be modulated by drugs to treat patients. Cardiovascular diseases are the leading cause of death worldwide, where ageing significantly increases the risk of developing cardiovascular issues. The insulin-like growth factor receptor 1 (IGF-1R) has a protective role upon the cardiovascular system. Unfortunately, upon ageing, the level of IGF-1R produced in our body decreases and leaves us susceptible to cardiovascular complications. For example, lack of IGF-1R can lead to impaired angiogenesis which reduces wound healing abilities.

This lab rotation will determine (i) where the IGF-1R receptors localize in primary endothelial cells, and then (ii) measure the impact of IGF-1R modulation (activation or inhibition) on the formation of new blood vessels. To monitor receptor location in live cells (i), fluorescent antibodies in conjunction with confocal imaging will be used. Blood vessel formation will then be investigated by an angiogenesis assay (ii), whereby endothelial cells will be cultured on a Matrigel to monitor proliferation and formation of vessel-like tubes. The effect of IGF-1R signalling on tube formation will be quantified in real-time using a label free phase microscope (LiveCyte PhaseFocus) .

Location: QMC;

Full project description: Insulin-like growth factor 1 (IGF-1) has an important role in maintaining cardiovascular health. IGF-1 is the endogenous ligand of the insulin-like

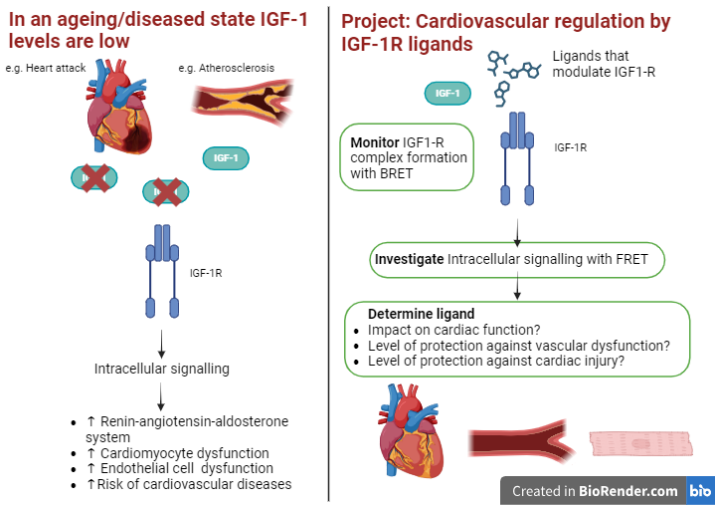
growth factor receptor 1 (IGF-1R), a receptor tyrosine kinase (RTK) expressed throughout the cardiovascular system (CVS). IGF-1R activation leads to cardiomyocyte and endothelial cell hypertrophy, proliferation and differentiation. IGF-1 also offers protection against oxidative stress and apoptosis in these cell types. However, levels of IGF-1 decline with age, proposed to subsequently enhance renin-angiotensin-aldosterone system activation, as well as cardiomyocyte and endothelial cell dysfunction. Unsurprisingly, IGF-1 has therefore been linked to cardiovascular diseases (CVDs) such as hypertension, atherosclerosis and heart failure. However, the signalling involved in the development of these diseases is not fully understood. This project aims to explore the signalling of IGF-1R in the context of CVDs.

Firstly, pressure myography of small resistance arteries (ex vivo) will probe the effect of commercially available inhibitors (e.g. linsitinib) and activators that target IGF-1R signalling. Since small resistance arteries significantly contribute to blood flow and blood pressure regulation, the level of vascular resistance, and changes to vessel wall strain and stress induced by IGFR-1-targeting ligands, will provide insight into their impact on blood pressure.

Secondly, we will explore the effect of IGF-1R ligands on cardiomyocyte survival, contractility and calcium signalling using the IonOptix cardiomyocyte contractility system. Primary cardiomyocytes will be isolated simultaneously with small resistance arteries to minimise animal use. As cardiomyocyte dysfunction contributes to heart failure (HF), these investigations could indicate whether IGF-1R inhibition/activation increases risk of HF.

Thirdly, IGF-1R signalling pathways will be quantified in real-time (in vitro) to probe the mechanisms of IGF-1R regulation in the CVS. 'Upstream' RTK signalling will be monitored using bioluminescence resonance energy transfer (BRET) to measure complex formation (dimerization) of IGF-1R monomers. Briefly, this sensitive technique uses a NanoLuciferase-tagged 'donor' receptor and fluorescent-tagged 'acceptor' receptor. As many IGF-1R ligands also act via the insulin receptor (IR), heterodimerization between IGF-1R and IR will also be investigated. 'Downstream' intracellular signalling will also be monitored using fluorescence resonance energy transfer (FRET), with conformational biosensors containing a substrate sensitive to phosphorylation by ERK or AKT. Finally, split Nanoluciferase assays (NanoBiT), with luminescence isolated defined IGFR-1/IR complexes, will allow the kinetics of homodimer- or heterodimer-specific signalling complexes to be detected in response to IGF-1 ligands using BRET. All in vitro approaches will be monitored in cells overexpressing the proteins of interest, quantifying the effect of both agonists and inhibitors on IGF-1Rs. Future plans will include using CRISPR/Cas9 gene editing to investigate complex formations at endogenous levels in physiologically relevant endothelial cell lines.

The outcome of this project will explore IGF-1R-mediated regulation of the CVS and provide mechanistic insight into how modulation of intracellular pathways activated by IGF-1R impact cardiovascular phenotypes, exploring prospective new drug targets for the treatment of CVDs. This project will enable training in ex vivo methodologies, primary cell culture, confocal imaging, BRET, and FRET.



This project also exposes the student to wider mentorship and Team Science initiatives within the Centre of Membranes Proteins and Receptors.

Full project location: QMC;

Exploring the interplay of miRNA, circular RNA and long non coding RNA with the transcriptional regulators KAT6A and KAT6B

Project Supervisor: Hilary Collins

School: Pharmacy

Description: KAT6A and KAT6B are histone acetyl transferases which control numerous pathways involved in development and stem cell function and have been implicated in human health and disease.

There is increasing evidence that these transcription factors control the expression of microRNAs but also that their own function can be moderated by the interplay of miRNAs, long non coding RNAs and circular RNAs.

A model CRISPR CAS9-edited KAT6A knockout cell line (HEK293) is already available in the lab. The rotation project will design CRISPR plasmids for the development of a KAT6B knockout line and validate the cell line if time allows. This part of the project would involve CRISPR guide design, subcloning, cell culture transfection and genomic DNA PCR analysis to validate the line.

Location: University Park;

Full project description: The full project will aim to determine the role KAT6A plays in the expression of microRNAs. The model KAT6A knockout cell line (HEK293) will be used to study alterations in expression of microRNAs in wild type versus KO cells. This part of the project would involve cell culture, microRNA extraction, RNAseq and bioinformatics analysis. This would be extended to the KAT6B and double KO cell lines once these knockouts are validated.

Validation of KAT6A miRNA or lncRNA targets would be achieved using RTqPCR.

Given the evidence that KAT6 proteins are important in cell metabolism, the analysis could be extended to looking at RNAs changes in different cellular compartments. e.g. is mitochondrial RNA expression affected by Kat6 knockdown?

It has also been proposed that KAT6A/B transcriptional function is controlled by circular (circRNAs) and long non coding RNAs but the extent of these interactions is unknown. Circular RNAs (circRNAs) are covalently closed loops of RNA that can arise in cells through backsplicing events, they are now known to encode functional proteins, in addition to acting as microRNA sponges and decoys for RNA binding proteins. Long non-coding (lnc) RNAs are non-coding RNAs longer than 200 nt., they can interact directly with proteins and miRNAs and can regulate gene expression at the epigenetic and transcriptional level and affect expression in a variety of ways.

Therefore we propose to look at the interaction of the transcriptional regulators KAT6A and KAT6B with circ and lnc RNAs via pull downs and sequencing. This would be achieved by the pull down of KAT6A and KAT6B in wild type cells, cross linking and the determination of novel circ or long non coding RNAs interacting partners.

KAT6A circRNA has also been identified in a number of cancers including prostate cancer, oral squamous cell carcinoma and more recently as a prognostic indicator in

Chronic Lymphocytic Leukaemia. We would aim to clone and characterise this circRNA. The questions we would like to answer are: how prevalent are these circRNAs and do circKAT6B RNAs exist? What effect does expression of these circRNAs have on cell growth and behaviour?

Three questions
1. What miRNAs are affected by KAT6A and KAT6B?
2. Do lncRNA and circ RNAs interact with KAT6A and KAT6B proteins and modulate their function?
3. How prevalent are KAT6A and KAT6B circ RNAs and are they functional?

Full project location: University Park;

Investigating the macrophage- mast cells cross-talk and its role in cancer.

Project Supervisor: Anna Malecka

School: Veterinary Medicine and Science

Description: Macrophages and mast cells play undisputed role in cancer development and metastasis by influencing immune responses, angiogenesis, and cell proliferation. Recent data suggest that both cell types are interacting together enhancing or inhibiting each other functions. However, the mechanisms of these interactions and their effect on cancer growth and resistance to therapies are poorly understood and need further investigation. The lab rotation will supply preliminary data regarding co-localisation of mast cells and macrophages in cancer tissue and the cytokines expressed by macrophage phenotypes.

The student will use multiplex fluorescence immunohistochemistry to determine the co-localisation of mast cells and macrophages in colorectal cancer tissues. They will also learn how to use AI to train the computer to identify tissue regions and specific cells. The student will also gain experience in cell culture and will generate primary human macrophages from healthy volunteers. Macrophage quality and phenotypes will be assessed using flow cytometry, microscopy, and ELISA.

The rotation will provide the student with training in immunohistochemistry, cell culture, flow cytometry, data analysis and statistics. The student will also take part in lab meetings with the opportunity to present their data in a friendly, supportive environment and will participate in relevant seminars and presentations.

Location: University Park;

Full project description: It is well known that immune cells do not act on their own but are in constant crosstalk with the environment which influences immune functions. It is also widely recognised that adaptive immune responses are directed and regulated by innate immune cells such as macrophages and mast cells. However, even though various innate immune cells often co-inhabit the same niches and demonstrate overlapping functions they are usually investigated in separation leaving their mutual interactions, and the consequences of these interactions poorly understood.

Mast cells and macrophages are innate immune cells which act as first line of defence in response to infection or danger signal (e.g., from damaged cells). They play key roles in fighting infections and maintaining homeostasis, wound healing, and angiogenesis. Mast cells and macrophages are present in cancers and have proven role in cancer growth and resistance to therapy. Recent research demonstrated that mast cells and macrophages interact with each other in a variety of disorders in both synergistic and antagonistic

manner. Mast cells can influence the distribution and function of macrophages by promoting macrophage influx into the tissue and strengthening their signal by secreting the same or complementary cytokines. For example, both cell types were found located in proximity at the tumour front in colorectal cancer and were shown to cooperate to promote tumour growth by releasing pro-inflammatory and pro-angiogenic factors. On the other hand, mast cells can inhibit macrophage phagocytosis in early stages of infections. These recent results show the importance of mast cell-macrophage axis. This project will investigate the role and mechanisms of this axis in cancers in aim to understand its importance for therapeutic approaches.

Initially the student will compare the co-localization of mast-cells and macrophages in various cancers including brain and colorectal cancer. Based on these findings the student will further investigate the effect of mast cells and macrophages co-localisation on their activation status by looking at the expression of multiple pro- and anti-inflammatory markers. This work will be done by doing spatial analysis of paraffin embedded cancer tissues from patients using multiplex fluorescence immunohistochemistry. The data will be analysed using AI machine learning and bioinformatics.

Based on the initial findings, the student will investigate the macrophage - mast cell interactions in a multi-cellular in vitro model using cell lines and primary immune cells generated from healthy donors. The student will focus on the effect of co-culture on activation of intracellular pathways in macrophages and mast cells leading to secretion of pro- and anti-inflammatory cytokines, extracellular vesicles and expression of activation markers. This will be done using a variety of laboratory methods including ELISA, western blotting, microscopy and flow cytometry. Finally, the student will validate the data using primary cells isolated from fresh biopsies of cancer patients.

Results from this project will provide the basic understanding of macrophages-mast cells interactions in cancer setting. This data will be helpful in informing strategies to develop novel therapeutics. The student will benefit from training in a wide variety of skills and working in a friendly team.

Full project location: University Park;Sutton Bonington Campus;

Ladybird alkaloid, Harmonine, and Analogues for Inhibition of NMDA Receptors and Acetylcholinesterase in Alzheimer's Disease

Project Supervisor: Ian Mellor

School: Life Sciences

Description: The initial few weeks of the rotation will involve the synthesis of the mixture of harmonine isomers using the synthesis already developed within the Stockman labs.

The student will assess the activity of the mixture of harmonine stereoisomers against NMDA receptors (Mellor lab). This will be important in determining whether both or one of the isomers are active. NMDA receptors containing GluN1-1A and GluN2B receptors will be expressed in Xenopus laevis oocytes through injection of cDNA and subjected to voltage-clamp analysis. Glutamate or NMDA activated ionic currents will be assessed in the absence and presence of harmonine to measure its inhibitory potency; we already have evidence that an alkaloid extract from Harmonia axyridis (harlequin ladybird) containing predominantly (ca. 90%) harmonine is a strong inhibitor of NMDA receptors.

Location: University Park;

Full project description: Dementia is a growing human problem and has a massive financial impact due to cost of caring for sufferers. Alzheimer's disease accounts for the majority of dementia cases and at present is lacking suitable treatments. The only options are memantine that is an inhibitor of NMDA receptors and is thought to reduce glutamate mediated excitotoxic cell death, or several acetylcholinesterase inhibitors that improve cognitive function through elevation of acetylcholine levels to combat the deficit of cholinergic neurons. The success of these treatments is quite variable and not always long-lasting.

Our preliminary work using extracts from Harmonia axyridis ladybirds where the major component is harmonine, have indicated strong inhibition of NMDA receptors and this has been confirmed using an almost pure fraction containing harmonine. However, purification of harmonine has proved particularly difficult and produced only very small quantities of the compound, hence the need for synthetic production of the natural compound and the possibility of studying structural analogues. It is known that harmonine is also an acetylcholinesterase inhibitor, thus combining two beneficial therapeutic properties in a single compound. The aim of this project will be to devise an efficient synthesis of harmonine in a way that will also allow the generation of structural analogues and to investigate their mechanism and site of inhibition.

The Stockman group has previously developed a synthesis of a mixture of harmonine stereoisomers. The project will build on this prior work, and investigate cis-selective olefin metathesis (using recent work by Hoyveda), and also investigate other approaches – e.g. ring-closing alkyne metathesis followed by Lindlar reduction. The synthetic approaches will then be used to probe the SAR of harmonine. The synthesis is inherently flexible, thus many alterations can be made, including chain lengths, position and stereochemistry of the alkene, and range of groups around the amines. The total synthesis of harmonine and analogues will provide a thorough training in synthetic chemistry. (Two previous collaborations on total synthesis / biological evaluation between Stockman and Mellor have proven their track record for producing high quality publications)

We will use an electrophysiological approach to assess the potency of harmonine and its analogues against NMDA receptor functioning and to determine their mode and site of action, essential for therapeutic development of these compounds. Human NMDA receptors will be expressed in the Xenopus oocyte subjected to voltage-clamp analysis to measure ionic current in response to glutamate or NMDA in the absence and presence of harmonine and analogues. We will focus our study on GluN1-1a/GluN2B subunit-containing receptors that are considered to be important in mediating excitotoxic cell death. This approach will also enable us to examine several subtypes of NMDA receptors as well as mutants of these subtypes. We will also assess activity at other ionotropic receptors such as AMPARs, nAChRs and GABAARs, and voltage-gated ion channels, all of which are essential components in neurotransmission; this will inform us about selectivity of the compounds. This part of the project will provide the student with extensive training in electrophysiological techniques.

Full project location: University Park;

Reconstitution of mRNA deadenylation by components of the microRNA repression machinery

Project Supervisor: Sebastiaan Winkler

School: Pharmacy

Description: You will focus on protein assemblies involved in human mRNA degradation, which play a pivotal role in microRNA-mediated repression. Bacterial co-expression systems will be employed as an enabling technology to obtain protein complexes. The purified protein complexes will subsequently be used for the reconstitution of RNA degradation processes.

Specifically, active sub-modules of the Ccr4-Not deadenylase as well as the complete protein complex (2-5 proteins) will be reconstituted following methodology established in the lab. Following initial evaluation of purity and homogeneity of the purified proteins, it may be possible to initiate the structural characterisation using gel filtration coupled to multi-angle light scattering and small angle x-ray scattering available at the Research Complex at Harwell and the Diamond Light Source.

The training includes transferable skills focussing on DNA cloning and protein techniques, such as (restriction enzyme-free) DNA cloning, protein expression and purification, gel electrophoresis and western blotting, as well as the characterisation of biochemical samples using biophysical techniques. The project offers the opportunity to work at world-class facilities at the Research Complex at Harwell in Oxfordshire.

Location: University Park;

Full project description: Background - Cytoplasmic mRNA in eukaryotes is characterised by a long poly(A) tail, which has a typical length of 50-100 nucleotides in human cells. The tail is important for efficient translation and prevents unregulated degradation of mRNA. Particularly since the discovery of microRNAs, it has become increasingly evident that control of mRNA degradation is a critical step in eukaryotic gene regulation.

The project will focus on the multi-subunit Ccr4-Not deadenylase complex, which is involved in the shortening and removal of the mRNA poly(A) tail (deadenylation), the initial and often rate-limiting step in mRNA degradation. The complex also regulates translation by direct binding of translation factors and the ribosome.

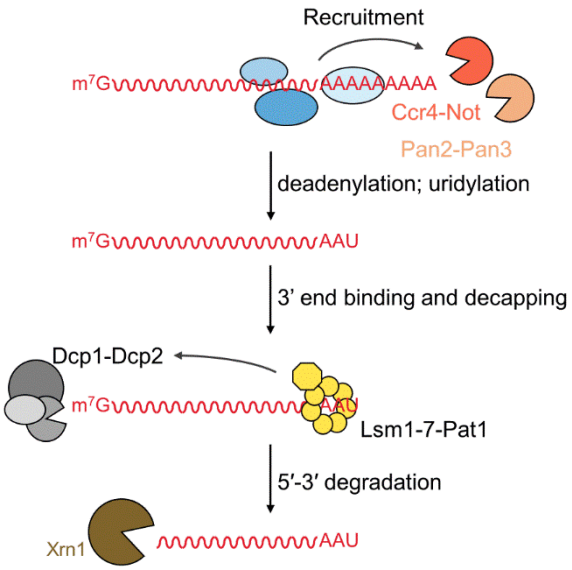
The Ccr4-Not complex can be recruited to target mRNAs by binding directly to RNA-binding proteins. These include proteins such as GW182 (TRNC6), a component of the microRNA repression complex, proteins containing the YTH domain, which recognises RNA containing the covalently modified N6-methyladenosine base, and proteins that are known to regulate mRNA stability such as tristetraprolin (TTP/ZFP36), PUM2 and Nanos, which recognise specific sequences in the 3’ end of the mRNA.

Aim - The aim of this project is to reconstitute deadenylation of mRNA in a test tube using defined components, purified proteins and synthetic RNA molecules, as well as their structural characterisation. This approach will refine current models of mRNA degradation and is designed to reveal unexpected behaviour and characteristics of the proteins involved.

Experimental plan - The project will involve the expression and purification of recombinant proteins, including sequence-specific RNA binding proteins (such as the protein TTP and the silencing domain of GW182) and a minimal Ccr4-Not deadenylase module using bacterial and/or baculovirus co-expression systems. In addition, a

synthetic RNA substrate containing a long poly(A) tail (50-75 bases) will be prepared. Using structure-guided site-directed mutagenesis, the mechanism of deadenylase recruitment will be investigated using biochemical methods based on fluorescence-based detection of the RNA substrate.

Part of the experimental work may be completed at the Research Complex at Harwell (RCaH), which has excellent facilities for large-scale protein expression and purification. In addition, a full suite of biophysical instruments is available for sample characterisation. All facilities are staffed by expert technical staff with post-doctoral qualifications who are available for training and experimental design during the course of this studentship. The RCaH is adjacent to both the Diamond Light Source (protein crystallography, small angle X-ray scattering), which opens up opportunities to apply these techniques for the structural characterisation of human Ccr4-Not complexes.



The experimental data will provide insight into the mechanism of RNA degradation and the structural dynamics of the Ccr4-Not complex. In addition to a detailed theoretical understanding of regulated mRNA degradation and gene regulation, this project will provide in-depth training in recombinant DNA techniques, protein expression and affinity purification using various expression systems, and quantitative analysis of enzymes. The results obtained will be a significant step towards the understanding of regulated mRNA degradation at the molecular level.

Full project location: University Park;

Synthesis of fluorescently labelling ligands for the atypical chemokine receptor 3 (ACKR3)

Project Supervisor: Luke Steven Schembri

School: Pharmacy

Description: This project will focus on the atypical chemokine receptor 3 (ACRK3) protein in humans. Students will gain skills in the key techniques in medicinal chemistry of molecular modelling and chemical synthesis. It will involve the design and conformational analysis of ligands for the ACKR3 receptor based off a ligand previously reported by Wijtmans and coworkers (VUF11207, Wijtmans, M. et al. Euro JOC, 51, 184–192) and an in-house homology model. Two sites on the model ligand will be examined to design new ligands and the lowest-energy conformation of such analogues will be examined as this can influence binding affinity. These ligands will then be synthesised and characterised in the chemistry lab. Time permitting, such ligands could be evaluated for their binding affinity in cells overexpressing the ACKR3 receptor. To carry out the project, the student will be trained in the use of programs such as Maestro (Schrödinger), Mestre Nova and chemdraw. They will gain skills in molecular modelling, design, synthesis, purification and characterisation of molecules and will segue well into the main project.

Location: University Park;

Full project description: The atypical chemokine receptor 3/CXC-chemokine receptor 7 (ACKR3/CXCR7) is a chemokine GPCR, which cycles rapidly via binding to beta-arrestin but does not activate G-proteins. Interestingly, it scavenges a variety of ligands, such as chemokines from the bloodstream, by binding to them and undergoing endocytosis, thereby regulating the function of it's sister receptor CXC-chemokine receptor 4 (CXCR4). It plays a role in cancer metastasis and inflammation, but these mechanisms are not completely understood. Ligand-directed labelling ligands (LDLLs) can covalently tag a receptor with a fluorophore (e.g. via a nucleophilic attack of a lysine residue) and provide a much-needed fluorescence-based method of studying receptor localisation in such pathophysiological settings, hence filling this knowledge gap.

This project aims to gain a better understanding of how ACKR3 is involved in such diseases. The student will design, synthesise and evaluate a small library of ligands for this receptor. The best ligand of this set will be used to synthesise LDLLs, which consist of a ligand, reactive spacer, and fluorophore, and can covalently label ACKR3 with a fluorophore for use in fluorescence-based localisation assays.

Previous attempts to use existing homology models for ACKR3 to design modified versions of the known high-affinity ligand VUF11207 (Wijtmans, M. et al. Euro JOC, 51, 184–192) that bore additional linkers and fluorophores were unsuccessful, suggesting the protein model is inaccurate. Phase one of the project will therefore involve the development of an improved model using new cryoEM data (PDB code 7SK9, released July 2022). Due to its limited resolution (3.7 Angstroms), considerable modelling-based remediation may be required to generate a validated high-resolution model. With this in place, a focussed virtual library of analogues will be designed and screened in silico. The highest ranked ligands will be synthesised and pharmacologically evaluated for their binding affinity using luminescence based binding fluorescence microscopy approaches in cell lines expressing tagged variants of ACKR3.

Phase two will begin with molecular modelling of the strongest binding ligand from phase 1 to model the spacer length between the ligand and fluorophore, and where to place the reactive electrophilic site within the spacer so it is close to a lysine residue. Two viable lysine residues have been identified, which are close enough to the active site for labelling but will not block the active site upon labelling. This is important as fluorescence-based assays with the labelled receptor will involve, for example, examining the downstream signalling effects upon binding of other ligands to the receptor. The LDLL congener (ie. ligand and spacer only) from the molecular modelling studies will then be synthesised and evaluated for their binding affinity at ACKR3 to ensure the spacer does not hinder binding. Finally, the fluorophore can be linked to the congener to synthesise the final LDLL. Compounds will be tested for their ability to label the ACKR3 in vitro using biophysical and pharmacological approaches. Successful compounds will be tested for their ability to label endogenous ACKR3 in relevant cancer lines and monitor it's membrane dynamics and distribution using confocal and advanced microscopy approaches.

Full project location: University Park;QMC;

How does GPCR/RAMP signalling co-ordinate blood-brain barrier development?

Project Supervisor: Rob Wilkinson

School: Life Sciences

Description: The aim of this project will be to knock out and knock down a gene within zebrafish embryos and characterise the effect of its loss on the formation and function of blood vessels. Genes will be selected from a list of potential Calcrl/Ramp2 targets generated previously by RNA sequencing zebrafish calcrl/ramp2 mutants. Using genome editing approaches, the student will generate a zebrafish G0 mosaic mutant by CRISPR/Cas9 and also knock the same gene down by CRISPR interference (CRISPRi), which uses a catalytically inactive form of Cas9 (dCas9). The extent of knockout/knockdown will be quantified by qPCR and/or in situ hybridisation. The consequence of loss of gene function on developing blood vessels will be determined using confocal and/or lightsheet fluorescence microscopy of transgenic zebrafish embryos with fluorescently labelled vessels. The cardiovascular phenotype generated by each approach will be directly compared.

During the project the student will learn how to perform microinjection of zebrafish embryos to facilitate gene knockout by Crispr/Cas9 and gene knockdown by CRISPRi, quantification of gene expression by qPCR and/or in situ hybridisation, confocal and/or lightsheet fluorescence microscopy of transgenic zebrafish embryos and associated molecular biology techniques. The student will also learn how to interrogate genomic databases including Ensembl.

Location: University Park;

Full project description: To ensure tissue homeostasis, the central nervous system must be protected from hormones, neurotransmitters or pathogens circulating in the blood, while still allowing vital nutrients to reach the brain. To achieve this, blood vessels which vascularize the central nervous system (CNS) display unique properties, termed the blood-brain barrier (BBB). The BBB heavily restricts vessel permeability and protects the brain from injury and disease. Loss of barrier properties during diseases including stroke, diabetes and vascular dementia contribute to underlying pathology and worsen disease. Conversely, the restrictive permeability of the BBB poses challenges for drug delivery to the CNS. The genetic mechanisms which regulate permeability of the BBB are poorly understood but are potential therapeutic targets where abnormal vascular barrier function contributes to disease. We have identified a G-Protein Coupled Receptor (GPCR) complex, Calcrl/Ramp2, essential for normal BBB permeability. How Calcrl/Ramp2 achieves this function remains unknown.

We use zebrafish to study how vascular permeability is controlled because zebrafish embryos are optically translucent and develop outside of the parent. This allows us to label blood vessels fluorescently and directly observe leaky blood vessels in zebrafish embryos using a microscope. In zebrafish, the BBB is quickly established by 72 hours post fertilisation and importantly, mechanisms which regulate blood vessel formation and function in zebrafish are highly conserved with humans.

Using CRISPR/Cas9 genome editing, we have generated zebrafish mutants of Calcrl and Ramp2. Calcrl and Ramp2 comprise the Adrenomedullin receptor and are dysregulated in diseases including diabetes, where vascular hyperpermeability is a problem. Zebrafish calcrl and ramp2 mutants possess a leaky BBB. By employing transgenic and mutant zebrafish embryos with fluorescently labelled blood vessels, this project will examine the role of Calcrl and Ramp2 in regulating vessel permeability and BBB function.

Zebrafish calcrl/ramp2 mutants display leakage of large fluorescent dyes within the developing brain vasculature at stages where these vessels are normally intact and the BBB is normally functional. Interestingly, leakage of fluorescent dye was contained within large numbers of microvesicles, suggesting vascular hypermerpeability is an active process in these mutants.

In zebrafish and mice, the BBB develops in a graded fashion, where the BBB is first established in the hindbrain, then the midbrain, and finally the forebrain. How this graded development of the BBB is established remains unknown, but we have evidence it fails to establish in our zebrafish calcrl/ramp2 mutants.

We hypothesise that calcrl/ramp2 mutants display an activation of transcellular permeability pathways leading to increased permeability of the BBB and that Calcrl/Ramp2 signalling controls development of the BBB gradient.

To test these hypotheses, you will use spatial RNA sequencing to identify molecular mechanisms underlying calcrl/ramp2-mediated vascular permeability. To test candidate genes, you will use cutting edge CRISPR/Cas9 and CRISPR interference technologies developed within our group. To determine how Calcrl/Ramp2 control BBB permeability, you will perform live imaging of blood vessel formation and function within zebrafish embryos using confocal and lightsheet fluorescence microscopy. This project will identify novel molecular mechanisms which control vessel permeability and which may be candidates for therapeutic manipulation during disease.

Full project location: University Park;

Studies of GPCR signalling using single molecule protein-protein interaction quantum biosensors

Project Supervisor: Dmitry Veprintsev

School: Life Sciences

Description: Quantum biosensing techniques promise a significantly improved single molecule sensitivity and a possibility to identify rare signalling events that are biologically important among a large excess of receptor molecules that are "silent". However, the receptors and their signalling partners have to be engineered to include paramagnetic tags, as well as surface immobilisation tags. The rotation project will focus of designing the constructs, cloning them, expressing them in mammalian cell culture, following by labelling them with paramagnetic tags and attaching to biosensor surfaces, verified by fluorescent microscopy. You will learn protein engineering and molecular cloning techniques, mammalian cell culture, membrane protein biochemistry, chemical biology methods and confocal microscopy or flow cytometry.

Location: QMC;

Full project description: G protein coupled receptor coordinate functions of our body by transmitting extracellular hormonal to the inside of the cell. Upon hormone binding, they activate G proteins. Subsequently, they are phosphorylated and recruit another

class of signalling molecules, arrestins. Ultimately, signalling events in cell are protein-protein interactions modulated by post-translational modifications. Many existing drugs, natural compounds and animal venoms mimic hormone action or prevent receptor signalling.

Because GPCRs exist in multiple conformations in the absence of the ligands, and there are multiple G proteins, receptor kinases and arrestins, the signalling process is very heterogeneous. Biologically and pharmacologically relevant events may represent only a small fraction of all observed protein-protein interactions, making it sometimes difficult to observe the important events as they are "masked" by other, more prevalent but less important, binding interactions. Single molecule techniques can advance our understanding of signalling events, leading to better understanding the signalling cascades and ultimately contributing to drug discovery.

Quantum sensing can detect fundamental parameters, including electric and magnetic fields, important to protein structure and function, with unprecedented sensitivity. Such sensing methods promise superior sensitivity compared to existing single molecule fluorescence techniques.

The goal of this project is to develop quantum biosensors to study GPCR signalling.

The student will engineer GPCRs (β2-adrenoceptor and CB2 cannabinoid receptor) to incorporate paramagnetic tags that will translate protein-protein interactions into observable changes in atomic scale defects in diamond, Nitrogen Vacancies (NV). These changes can be optically detected. Nanosized diamonds rich in NVs will be attached to the proteins and act as quantum biosensors. This part of the project will be done in the Veprintsev lab. The quantum sensing experiments will be performed using a bespoke microscope developed within the Prof Mather group and augmented with metalenses (Kenney) for enhanced sensitivity.

The project combines multiple technical developments of both GPCR expression and protein engineering and chemical biology, as well as progress in quantum biosensing, with the unique combination of expertise provided supervisors. In addition to learning various techniques, the student will be embedded in the Centre of Membrane Proteins and Receptors, one of internationally leading centres for GPCR research and a vibrant community of over 50 PhD students and postdocs, and within the Optics and Photonics group at the Faculty of Engineering.

Full project location: QMC;University Park;

Mesoporous metal surfaces as novel platforms for SERS-based biomedical diagnostics

Project Supervisor: Anna Vikulina

School: School of Science and Technology (NTU)

Description: The aim of the lab rotation is to familiarise the candidate with the design and formulation of hybrid biomaterials, which have various biomedical applications ranging from drug delivery and regenerative medicine (further used in linked project) to biomedical diagnostics (which is the scope of this project).

The following objectives are set (corresponding experimental skills and specific learning outcomes are given for each objective below):

- synthesize hybrid functional microparticles by means of co-synthesis of vaterite, (bio)polymers, and biomacromolecules in aqueous and biofriendly environment on

plastic surfaces. Skills & Knowledge: (micro)particle formulation, mesoporous materials, adsorption phenomena, and the use of software for particle analysis;
- characterise synthesized hybrids in terms of their morphology, internal structure, and encapsulation capacity. Skills & Knowledge: get acquainted with optical and fluorescence microscopy, scanning electron microscopy, and absorption spectroscopy, aspects of Physical Chemistry of adsorption, imaging and image analysis;
- investigate the influence of selected parameters (e.g., temperature, pH, ionic strength, concentration, surface chemistry) on in situ growth of hybrid functional microparticles on the surface. Skills & Knowledge: understanding of crystal growth, surface analysis, material engineering.

Location: Clifton Campus;

Full project description: Background and state-of-the-art.

Surface-enhanced Raman spectroscopy (SERS) is a very powerful analytical technique with capacity to detect a range of biomarkers and drug metabolites in complex biological probes down to single molecule detection. Modern market of SERS instrumentation offers miniaturised user-friendly SERS devices suitable for point-of-care in vitro diagnostics, which opens broad horizons for both, clinical diagnostics and personalised medicine. However, there are inherent challenges of SERS technology which obstacle its further lab-to-clinics transition [1].

SERS requires the enhancement of the Raman signal by plasmonic materials, which are typically metal nanoparticles (NPs) organised to so-called SERS substrates of variable and often sophisticated dimensions and shapes with the architecture controlled at the nanoscale [1]. Moreover, if NPs are decorated with target ligands and Raman reporters to generate fingerprint signal, SERS detection might become superior over all alternative methods.

However, fabrication of such hybrid SERS substrates requires nanoscale control over their structure while co-assembling multiple components - plasmonic NPs, Raman reporters, ligands. Production of such complex nanoarchitectures represents one of the key modern SERS challenges.

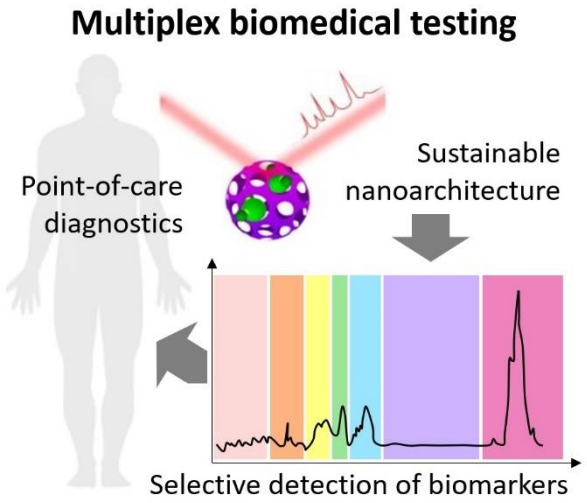
Aim and originality.

This project aims to develop new approach for the formulation of nanostructured surface-supported SERS substrates and verify its analytical performance for biomedical applications. The originality of this project is in the use of hybrid organic/inorganic mesoporous crystals as the templates for the fabrication of nanostructured surface-supported SERS tags. This will allow to: i) control the structure of SERS substrates; ii) incorporate the ligands and Raman reporters for selective detection; and iii) stabilise SERS substrates.

Objectives and methodology:
- Formulation of SERS microparticles: mesoporous metal (e.g., Au, Ag) particles will be fabricated via hard templating onto decomposable vaterite templates [2]. Vaterite is sustainable “green” material, with tailor-made physical-chemical properties (size, tuned porosity and shape) and can host various molecules of interest including fragile target molecules, e.g., antibodies. The following approaches will be probed for encapsulation and stabilisation of Raman reporters and target ligands with/without chemical crosslinking: co-synthesis and adsorption onto pre-formed crystals.
- Integration of SERS microparticles into the substrate material: SERS microparticles will be structured on the surfaces of various chemistry with/without particle

elimination via: i) the growth of vaterite crystals on the surface and metal coating, ii) deposition of pre-formed SERS microparticles, iii) integration of SERS microparticles into the sintered/melted composition.

- Verification of analytical performance: SERS detection performance (selectivity, sensitivity, reproducibility, etc.) will be assessed for quantification of model biomarkers, e.g., carcinoembryonic antigen (present in over 50% of lung cancer cells); troponin I - marker of acute myocardial infarction; and drugs (heroin, opiates, codeine). Different compositions of the tags will be probed and methods optimised. A model product of the SERS substrates will be designed for further prototyping.
- Dissemination of knowledge: project results will be published in peer-reviewed journals, presented at conferences, relevant stakeholders (companies producing SERS devices) will be contacted for future technology development beyond the project.



References. [1]. L. Ouyang et al. Rev Anal Chem, 2017, 36(1), 20160027. [2]. A. Vikulina et al. Biosensors, 2021, 11 (10), 380.

Full project location: University Park;Clifton Campus;

Mapping adenosine receptor interactomes in human cells utilising NanoBRET protein-protein interaction & CRISPR/Cas9 mediated proximity proteomics.

Project Supervisor: Laura Kilpatrick

School: Pharmacy

Description: The rotation project will focus on adenosine receptor biology in human cells. The student will be trained in the key techniques of mammalian cell culture, molecular biology and CRISPR/Cas9 gene editing. The project will involve studies of adenosine receptor localisation in human cells in response to diverse stimuli (adenosine receptor agonists, hypoxia, inflammatory mediators). The student will undertake CRISPR/Cas9 editing experiments appending a 11 amino acid luminescent tag to adenosine receptors in human cell lines, which will provide experience of the strategy that will be used to introduce and validate Bio-ID tags during the PhD studentship as well as being used as a protein tag in its own right. This work will involve the design of CRISPR reagents, delivery to cells (via electroporation), and downstream screening for edited cells via PCR, bioluminescence assays, and confocal microscopy. The student will also be trained in NanoLuc bioluminescence resonance energy transfer (NanoBRET) assays such as ligand binding and intracellular adapter protein techniques that are key assays in the Kilpatrick and Hill labs.

All of these techniques are integral to the programme of work proposed in the main studentship with the experience gained in the rotation facilitating a smooth transition to the main project.

Location: QMC;University Park;

Full project description: Adenosine receptors (ARs) are a family of G-protein coupled receptors, with four subtypes (A1, A2A, A2B and A3), that are expressed throughout the body in a broad range of cell types. ARs represent attractive drug targets, with drugs in clinic or trial for a range of areas including cancer, cardiovascular, and respiratory diseases.

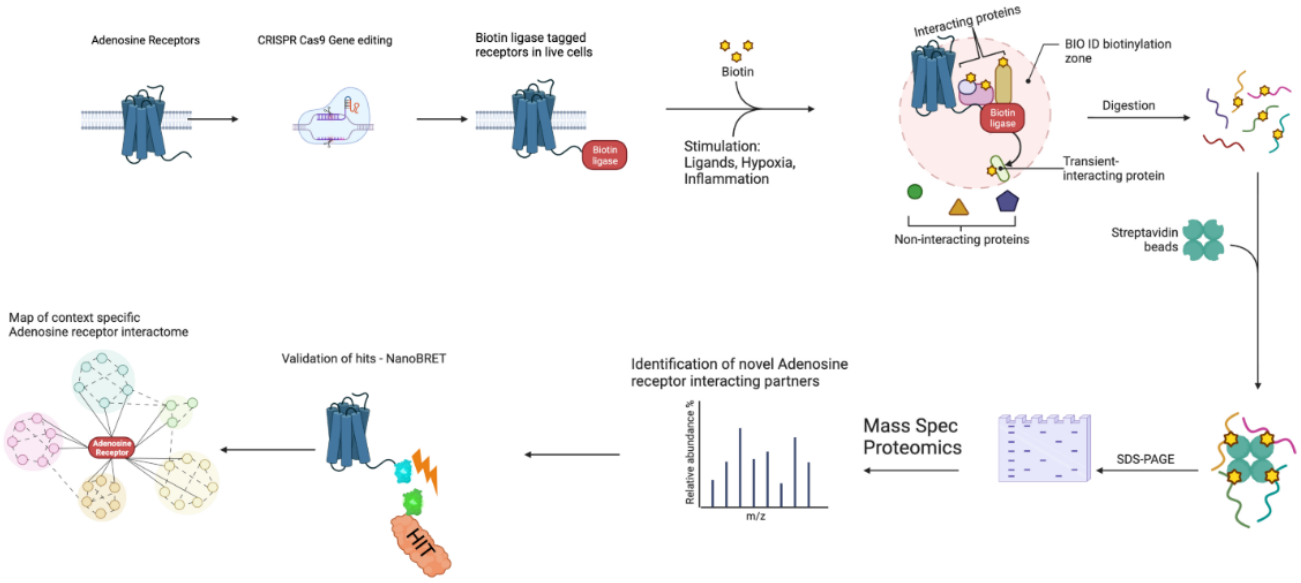
The key goals for this project are to identify the proteins that A2AAR and A2BAR interact with in the cellular environment. This will be done in human cell models at endogenous expression levels (and timing) of all proteins by utilising CRISPR/Cas9 mediated tagging of the C-terminus of the A2AAR or A2BAR with biotin ligases. These ligases biotinylate interacting proteins within a ~ 30 nm radius, with interactors subsequently identified via mass spectrometry and bioinformatic analysis to profile the context specific interactomes for these signalling receptors (eg. at specific cellular locations using complementary fragments of split biotin ligases). These protein 'hits' will be validated in real time in living cells using a combination of CRISPR/Cas9 gene editing and luminescence-based proximity assays (NanoBRET). This will be aided by tools we have already developed and validated in our group (eg. AR subtype specific fluorescent ligands, cell organelle localised BRET based biosensors).

Proteomic investigations of AR interacting proteins will be performed in a range of human primary cell types, and in response to a range of diverse (patho)physiological stimuli such as adenosine receptor agonists, inflammatory mediators, and hypoxia. Furthermore, the formation and interaction of endogenous heteromeric complexes between different AR subtypes will be investigated, by utilising split biotin ligases and split luminescent proteins (NanoLuc Binary Technology), with each component expressed on a different AR subtype. This will allow the determination of heteromeric specific interacting partners potentially highlighting new avenues for co-targeting receptors therapeutically.

The final phase of the project will involve the manipulation of AR interacting proteins that have been identified and validated in earlier phases. The expression levels of proteins found to interact with ARs will be manipulated (CRISPR knock out/repression, siRNA knock down, or overexpression) and the affect upon AR signalling studied. This will provide evidence as to the potential of these newly discovered interacting partners to be targeted for clinical benefit, and importantly the patho-physiological context in which this would be relevant.

This project will further our fundamental understanding of AR signalling and regulation in response to a wide range of disease relevant stimuli. Mapping of the AR interactome has the potential to identify novel drug targets and inform drug discovery efforts across a broad range of diseases. By undertaking this project, the student will be supported by and welcomed into a team of researchers from a range of disciplines (molecular pharmacology, cardiovascular physiology, medicinal chemistry) who are focused on the interplay of all aspects of AR biology as part of a large, well-funded MRC Programme grant. As part of the Centre of Membrane proteins and Receptors (COMPARE), the student will have access to mentorship schemes and 'Team Science' career development initiatives championed within COMPARE.

Full project location: University Park;QMC;



CRISPR generated models of Human KAT6 Syndromes : Can we restore function?

Project Supervisor: David Heery

School: Pharmacy

Description: I am happy to adapt the rotation project to the skill level/ previous experience of the student. We can provide training in a range of molecular and cellular biology techniques including PCR, subcloning, expression analysis, protein purification, Y2H and confocal microscopy. CRISPR targeting constructs are ready to use for transfection into cell lines with cell sorting of GFP+ cells. These can be analysed for expression of KAT6A in western blots and by PCR genotyping. In parallel, we can explore the co-expression of FLAG and HA tagged KAT6A and BRPF1 proteins using confocal microscopy, including mutants we previously generated which disrupt the interaction of these proteins. This would provide data to support a new hypothesis that BRPF1 proteins may target to both mitochondria and nucleus, explaining the functional link between gene expression and metabolism.

Location: University Park;

Full project description: KAT6A and KAT6B are unique human lysine acetyltransferases that regulate gene expression in stem cells and during development. These proteins can bind DNA and acetylate histones and thus modify chromatin to regulate genes (Dreveny et al, Nuc Acids Res 2014; Costello-Heaven et al, in preparation). Mutations in the genes encoding KAT6A and KAT6B are rare and are associated with neurodevelopmental disorders in children. These patients have a range of symptoms including speech deficit and metabolic disturbances (https://kat6a.org) but the underlying mechanisms are poorly understood. Anecdotal evidence suggests that symptoms in some children can improve when they are treated with dietary metabolic supplements.

To understand the disease, we have used CRISPR gene editing in cell lines to knock out KAT6A or introduce mutations mimicking those found in patients. The most common mutations are stop codons, which result in loss of the C-terminal half of KAT6 proteins. RNA Seq and metabolomics analysis of KAT6A KnockOut cell lines has revealed

alterations in expression of genes that regulate development and metabolism consistent with the symptoms seen in patients. The consequences of expressing truncated KAT6 proteins lacking the C-terminus (as found in patients) is unknown.

This project will explore the functions of KAT6A and KAT6B in disease using CRISPR CAS9 to knock-in tags and generate clinical mutations (stop codons) in these genes. As it is known that certain drugs such as aminoglycoside antibiotics can enable the ribosome to translate through premature stop signals, we will test the effects of these drugs to rescue full or partial functionality in the cell models. We have established a whole range of downstream assays we can use that will involve western blots, confocal microscopy, chromatin binding, gene expression (RTqPCR or RNA Seq) and other assays of KAT6A/B function.

You would join a team in the Gene Regulation & RNA Biology Group involving two other DTP students (Yr1 and Yr2) working on other aspects of the structure and function of KAT6 proteins in collaboration with Diamond Light Source.

Full project location: University Park;

The effect of the menopause on cognitive function and visual attention

Project Supervisor: Laurence Warren-Westgate

School: Life Sciences

Description: During the nine-week lab rotation associated with the project, the student will validate the new test system in healthy young females (aged 18-35) and correlate the outcomes with habitual dietary and physical activity patterns.

As part of the novel multidisciplinary project utilising behavioural, psychological, and physiological measures, the student will undertake training in cognitive function, visual attention, physical activity, and dietary analysis. This will take place at the QMC Medical School under the guidance of the lead supervisor. Habitual levels of physical activity will be assessed via a combination of questionnaire (IPAC) and triaxial accelerometery (GeniActiv, Activ insights, UK). Habitual dietary patterns will be measured using food diaries, which will then be analysed using Nutritics food data management software (Nutritics, Ireland) and anthropometric measures (height, weight, BMI) will be recorded. The student will receive training in how to measure cognitive function and visual attention using a screen-based eye-tracking device (Tobii Pro, Tobii AB, Sweden) to investigate associations between cognitive function and visual strategies, by analysing saccades (rapid eye movements) and fixations (periods where the eyes are relatively still, focused on a particular point).

Location: QMC;

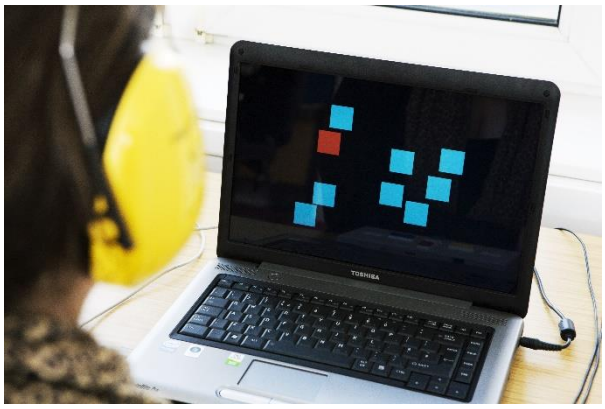
Full project description: It is believed that as many as two-thirds of women may encounter varying degrees of cognitive impairment related to menopause. This condition, often described as 'brain fog,' is broadly characterised by challenges in decision-making, difficulty retaining new information, loss of concentration and mental clarity, and an increase in forgetfulness. Recent literature highlights the rise in subjective cognitive complaints across menopause transition, providing impetus for further investigation into the menopause-cognitive decline association, with a need to better characterise the specific areas of impairment (Reuben et al 2021 Climateric. Mar;24(4):321-332). Furthermore, it underscores the significance of this research area,

as mild cognitive impairment has been associated with a nine-fold increase in the risk of developing dementia (Mitchell et al 2014 Acta Psychiatr Scand. Dec;130(6):2–265).

It is suggested that cognitive decline can impair selective attention, sustained attention and visual search. Indeed, recent studies have demonstrated that changes in visual attention can serve as early markers of cognitive decline, due to observable alterations in visual gaze patterns (Wolf et al 2023 Front Psychol. 2023 Jul 20;14:1197567). However, this is yet to be explored in relation to menopause transition.

The aim of this project is to better characterise the decline in cognitive function during menopause transition. As part of the novel multidisciplinary project utilising behavioural, psychological, and physiological measures, the student will undertake training in cognitive function, visual attention, and insulin sensitivity analysis. This will take place at the QMC medical school under the guidance of the lead supervisor. Menopausal symptoms will be measured using validated questionnaires, including the Greene Climacteric Scale (GSC) and Menopause Rating Scale (MRS). Habitual levels of physical activity will be assessed via a combination of questionnaire (IPAC) and triaxial accelerometery (GENEActiv, Activ insights, UK). Habitual dietary patterns will also be assessed using food diaries and analysed using Nutritics software (Nutritics, Ireland). To measure and analyse cognitive function, the project will use a battery of cognitive tests (MOT, RTI, RVP, PAL, SWM) available through CANTAB digital cognitive assessment (Cambridge Cognition, UK). During these tests, visual gaze data in the form of saccades (rapid eye movements) and fixations (periods where the eyes are relatively still, focused on a particular point) will be recorded via a Tobii Pro screen-based eye tracker (Tobii AB, Sweden), to investigate associations between cognitive function and attentional strategies. Further, anthropometric measures (height, weight, BMI) and insulin sensitivity will be assessed using fasting blood glucose and insulin levels. All measures will be processed for statistical analyses. These measures will be repeated after a 6-week period of resistance training to assess whether regular exercise can restore cognitive function and visual attention.

The final phase of the PhD will recruit a separate cohort of post-menopausal women. With this cohort, we will assess the efficacy of screen-based attention-enhancing exercises for restoring cognitive function. The focus of the training tasks will be derived from the characteristics of the cognitive decline identified during the first phase of the project, therefore allowing us to target the areas of cognitive functioning impaired by menopause transition.



Full project location: QMC;

Hybrid Multifunctional Scaffolds for Guided Tissue Regeneration: combating Periodontitis and Associated Infections

Project Supervisor: Dmitry Volodkin

School: School of Science and Technology (NTU)

Description: Title: "Characterization and optimization of delivery vehicles for pH-triggered release in periodontal microenvironment".

This research project aims at evaluation of the potential of vaterite CaCO3 microcrystals to serve as smart delivery vehicles for controlled release of bioactives into the oral cavity and tooth-supporting tissues. The crystals are cost-effective, biocompatible and biodegradable inorganic containers with tremendous loading capacity. Recent studies conducted by our PhD students revealed that the release of the payload from the crystals is governed by both recrystallization and pH-mediated dissolution (doi10.1021/acsami.5b05848) and can be modulated via inclusion of polymer matrices (doi10.1016/j.matdes.2019.108020).

In this project, hybrids of CaCO3 crystals and diverse biopolymers will be tailored to possess various modes of release in simulated oral biological fluids under pH of health and disease conditions. Hybrid crystals will be characterised using cutting-edge techniques such as SEM, X-ray diffraction, CLSM. Current group members will provide continuous support in the lab "Active-Bio-Coatings" led by Prof. Assoc. Dmitry Volodkin.

The project will quickly acquaint the candidate with modern technologies vital for biomaterial characterisation and controlled release strategies. Research skills gained will be versatile and valuable for the linked PhD project and other research related to Biomaterials.

Location: Clifton Campus;

Full project description: State-of-the-art and aims: Periodontitis is a serious gum infection caused by bacteria (affect 10-15% of the adults around the world) and considered as the main cause of the tooth loss. The main treatment of advanced periodontitis is surgery followed by deposition of a barrier membrane that further guides tissue regeneration (so-called GTR strategy). An ideal GTR membrane should: i) emulate the extracellular matrix (ECM) and promote tissue formation, ii) supress secondary bacterial infection and development of antimicrobial resistance, iii) provide proper mechanical support and biodegradation rate. Although GTR is widely used in clinics, majority of them are "passive" barriers that don't demonstrate all functions above. Therefore, novel "active" membranes fulfilling the criteria above are required. They will be developed in this project and named Hybrid Multifunctional Scaffolds (HyMuSc).

Methodology:

HyMuSc will be composed of nanofibers produced using biodegradable polymers and high-throughput electrospinning technology, which engineers a biomimicking ECM. Mesoporous CaCO3 microcrystals will be integrated into HyMuSc to provide multiple functions that include: i) endowing HyMuSc with desired mechanical properties; ii) Ca2+ dope which is essential for cementum and bone repair; iii) hosting, protecting and releasing fragile bioagents in controlled manner, e.g. growth factors and antimicrobial peptides that will release on demand with well-defined release profile to promote tissue growth and overcome bacterial resistance, respectively. pH-sensitivity of CaCO3 vectors will permit triggered release of antimicrobials in response to saliva pH, as a first condition for periodontal bacterial contamination. Fluorescent nanosensors will be integrated into HyMuSc to probe pH, oxygen and calcium level within the 3D tissue in

real time for understanding and tuning HyMuSc properties to optimise performance. Finally, the regeneration of hard tissues will be controlled by novel biomimetic supramolecular matrices impregnated into the HyMuSc, e.g. elastin-like biopolymers that hierarchically guide mineralization.

Supervisory team will complementary support the candidate in design of Biomaterials ($CaCO_3$ microcrystals and hybrid materials, lead supervisor), Biosensing (nanosensors for tissue mapping, supervisor#2), Bioengineering (electrospinning, supervisor#3) and Biomineralisation (hard tissue regeneration, supervisor#4).

Novelty and impact:

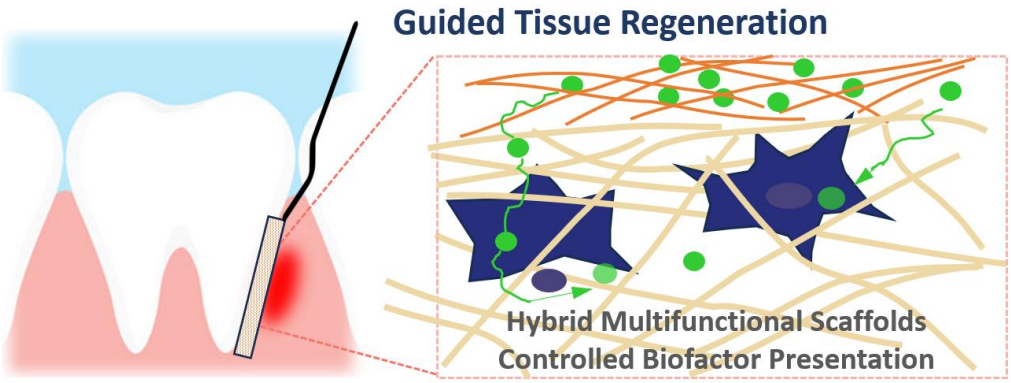
Newly designed HyMuSc integrates high level of bio-mimics with controlled presentation of biofactors representing a truly informed and guided tissue regeneration membrane that will optimise effective periodontal tissue growth for optimal therapeutic benefit. 3D mapping utilising fluorescent nanosensors will allow deeper fundamental understanding of biological processes that underpin tissue regeneration.

This highly interdisciplinary project will equip the PhD candidate with a broad spectrum of technologies at forefront of Material Science including electrospinning, fluorescent nanosensing and biomineralization. This will provide vital skills that are readily translatable into academia, industry and clinics. This project will also provide many opportunities to collect new data important for PhD thesis production, publication of highly impactful research articles and production of technologies subject to commercialisation opportunities.

Fitting into global research themes:

This project is complimentary to strategic topics "Medical Technologies and Advanced Materials" at NTU and "Transformative technologies" at UoN and is tightly connected with "Health and wellbeing" theme targeted by both universities. Electrospinning will occur MTIF using ISO13485 Standards to enable faster commercialisation.

Full project location: University Park;Clifton Campus;



Developing a model of the ageing neuroimmune system

Project Supervisor: Andrew Bennett

School: Life Sciences

Description: The project will involve isolation of peripheral blood mononuclear cells from human blood, and their subsequent differentiation into microglia - the immune cells

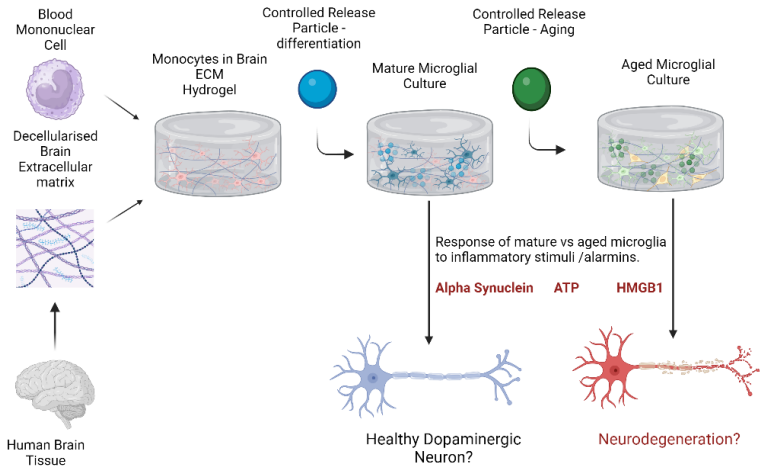
of the brain. Cells will be isolated from human blood using immunopanning, and then grown on brain-derived extracellular matrix hydrogels. The response of the cells to the hydrogels, and to inflammatory stimuli will be assessed by Taqman QRTPCR and western blotting to asses the response at the mRNA and protein levels. The project will provide training in primary human cell culture, followed by isolation of mRNA and protein from the cells. The project will then focus on the changes in inflammatory proteins and mRNA levels in response to stimuli such as ATP and bacterial LPS. The project will also look at the expression of mature/ageing microglial markers to asses the effect of hydrogels on cell differentiation/senescence.

Location: QMC;

Full project description: Microglia are the immune cells of the brain. As we age, the response of the neuroimmune system changes and many individuals develop low grade inflammation which is thought to be a major cause of neurodegeneration. The way in which older microglia behave, their response to inflammatory stimuli, and their subsequent effects upon neuronal cells are not currently well understood. Models of microglia currently used to study age related changes in neuroinflammation are either derived from the brains of rat pups or iPSC-derived microglia. Both of these models display immature microglial phenotypes and as such are not suitable to study microglial behaviour over the lifespan. This project will use human blood mononuclear cells that we have been able to differentiate into microglia as our model. They will be grown on human brain-derived extracellular matrix hydrogels to create a physiologically relevant culture environment that will improve upon existing 2 and 3-D culture matrices and will allow us to culture these cells alone and in co-culture with neuronal cells over a longer time period.

The cellular responses to hydrogels of different chemical and protein constituents will be assed using gene expression and protein assays to look at changes in microglial activity and maturation. We will integrate controlled release particles into the hydrogels to allow consistent delivery of cytokines and chemicals necessary to drive microglial maturation. Once we have established an optimal hydrogel and differentiation protocol, we will use chemical senolytic agents, also delivered via controlled release, to generate a model of aged microglia. We will then compare the responses of aged microglia to those with an adult but not senescent phenotype. Specifically we will look at responses to low grade inflammatory stimuli, and the subsequent effects upon microglial interaction with neuronal cells in co-culture. The project will involve the isolation and differentiation of primary human cells, the production, characterization and development of novel brain-derived hydrogels; the use of controlled release technology to deliver cellar cues for differentiation and ageing and the use of RNAseq, QPCR, western blotting, ELISA and fluorescent microscopy to determine cellular responses to inflammation and ageing. This will provide us with a human, physiologically relevant model of microglial biology as we age, and the subsequent effects upon neuronal physiology and function.

Full project location: QMC;



Building a model of the ageing neuro-immune sytem in vitro to study the role of microglia in Parkinsons Disease

The role of astrocyte-secreted extracellular vesicles transglutaminase-2 in regulating early synaptic dysfunction in Alzheimer's disease

Project Supervisor: Elisabetta Verderio Edwards

School: School of Science and Technology (NTU)

Description: During the 9 week lab rotation there will be the opportunity for the prospective student to interact with both teams at NTU and UoN.

NTU will offer essential training in mammalian cell culture and extracellular vesicles (EV) isolation and EV characterisation, using state of the art technology including single EV measurement of antigens (Exoview, Nanoview) and Nanoparticle Tracking Analysis (Zetaview). UoN will offer an introduction to iNPC protocols: how to generate a pure population of functional stem cell-derived astrocytes from induced neuronal progenitor cells, directly reprogrammed from skin biopsies of living people.

The prospective student will join established teams of PhD students and post-docs and will be invited to attend laboratory meetings and journal clubs and join in any aspect of the "lab life".

Location: Clifton Campus;University Park;

Full project description: Synaptic dysfunction is a well-established early mechanism of Alzheimer's disease (AD) and contributes to pathology spreading in the brain over time, in part due to propagation of amyloid peptides along connected neurons (1). Dysregulation of neuronal activity is also attributed to negative effects from non-neuronal cells of the brain, like microglia and astrocytes (2). Astrocytes are essential in regulating the function and survival of neurons, especially through secretion of molecules contained in extracellular vesicles (EVs). Defects in astrocyte function have been shown to contribute to AD. Importantly, EVs from reactive astrocytes exert detrimental effects on synapse stability, neurite differentiation and neuronal firing (3).

Our recent studies in rodent models have shown that EVs secreted by reactive astrocytes can control intraneuronal Ca2+ concentration in neurons (4). We demonstrated that the protein transglutaminase-2 (TG2), present on the surface of astrocytes-derived EVs, make them more stably attached to axons projections. TG2 is a calcium-dependent enzyme with a protein crosslinking activity involved in neurodegeneration. We identified TG2 as a surface-cargo of EVs derived from reactive astrocytes which is responsible for intraneuronal Ca2+ rise in neurons (4). We also identified that a subunit of neuronal Na+/K+-ATPase is inhibited by extracellular TG2 and that Na+/K+-ATPase is an interactive TG2 partner (4). Furthermore, a trend increase in TG2 activity in AD-derived skin biopsies was observed in pilot data on a small number of control and sporadic AD. These data suggest that TG2 plays a novel important role in synaptic dysfunction and in neurodegeneration through calcium overload. Building on these fundamental advances, this project aims to understand the role played by the astrocyte-secreted transglutaminase-2 (TG2) on synaptic function and neuronal survival in Alzheimer's disease (AD).

The specific objectives will be to:

1. Investigate how astrocyte-secretion of TG2 in EVs is affected in AD. We will measure EVs-TG2 of control and AD human astrocytes (iNPCs-differentiated astrocytes), at the single EVs level. We will also test into the role of AD-associated stresses (oxidative stress, inflammation, amyloid plaques) in astrocytes reactivity/production of EVs-TG2.

2. Explore how changes in extracellular TG2 contributes to propagation of AD.We will dissect how changes in TG2 levels/activity may affect synaptic function by calcium imaging and electrophysiology.
3. (iii) Explore how dysregulated TG2 interaction with synaptic proteins in neurons contributes to synaptic dysfunction in AD.TG2 binds proteins involved in synaptic function. We expect that increased binding of TG2 to these neuronal proteins negatively impacts synaptic function. Blocking this interaction could be of interest in building a novel strategy for AD treatment.

1. Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci. 2010;13(7):812-8.

2. Gabrielli M, et al. Microglial large extracellular vesicles propagate early synaptic dysfunction in Alzheimer's disease. Brain. 2022;145(8):2849-68.

3. Prada I, et al. Glia-to-neuron transfer of miRNAs via extracellular vesicles: a new mechanism underlying inflammation-induced synaptic alterations. Acta Neuropathol. 2018;135(4):529-50.

4. Tonoli E, et al. Extracellular transglutaminase-2, nude or associated with astrocytic extracellular vesicles, modulates neuronal calcium homeostasis. Prog Neurobiol. 2022;216:102313.

Full project location: Clifton Campus;University Park;

Mechanistic Springboard for Healthcare Driven Portable Sodium Sensors

Project Supervisor: Galina Pavlovskaya

School: Medicine

Description: The student will learn ultra highf-field microimaging and sodium spectroscopy in the Sir Peter Mansfield Imaging Centre using samples of skin-containing GAGs, namely hyaluronan, dermatan sulfate, chondroitin sulfate, heparan sulfate and keratan sulfate. The student will learn on how to work with microscopic quantities of MRI samples using state of the art cell/tissue inserts (Annaida Technologies, Switzerland) in this unique to Nottingham microimaging setting. Sodium spectroscopy will identify characteristic time cues that are associated with the strength of sodium-GAG interactions. The samples can then be embedded for comparative histochemical GAG analysis in the Biodiscovery Institute.

Location: University Park;Sir Peter Mansfield Imaging Centre, BDI;

Full project description: Project Vision. The human dermis is a sophisticated natural hydrogel with a 4.5 liters average volume. Approximately 7.2g of sodium, or 18.2g of salt (3x daily recommended adult intake), are stored by ionic interactions with the macromolecules (proteins, proteoglycans (PGs) and glycosaminoglycans (GAGs)) comprising the dermis. Our preliminary data [1] has shown that the non-osmotic sodium storage is localized within the human dermis and its capacity to store sodium is diminished in the type 2 diabetes (T2D). This paradigm changing observation could also be relevant in chronic kidney disease (CKD), hyponatremia (HNa) and chronic heart failure (CHF) and in acute patients. If clinicians can measure sodium storage in the skin using potable sodium sensors, this could have enormous clinical utility regarding diagnostics and management strategies.

Aims and objectives. The project aims to obtain key mechanistic clinical data to provide a springboard for rapid development of portable sodium sensors. Namely, the student will focus on ex vivo characterisation of the mechanism of sodium interaction with glycosaminoglycans (GAGs) in the dermis using our state-of-the-art ultra-high magnetic field inserts (Annabella, Switzerland) for cells and tissues. Skin punch biopsies and cells will be obtained from consented patients and controls. GAGs in these samples will be visualised by sodium MRI, and the strength of sodium interaction with the GAGs will be probed by sodium magnetic resonance spectroscopy (MRS). In addition, complete MR metabolomics will be performed in these samples.

GAG sodium binding depends on GAG type, composition, length, and total concentration within the localised microenvironment. For GAG analysis, quantitative fluorescent tagging is technically challenging for intact extracellular matrix due to steric exclusion, the quality and availability of antibodies, and sulphation motif dependence. We therefore propose to use both traditional optical and electron analyses, and our novel imaging mass spectrometry method whilst pairing the sample with sodium status to fingerprint molecular changes to dynamic sodium skin deposition as in [1]. Sodium free and bound fractions will be determined for each biopsy. Following ex vivo MR imaging, each skin biopsy will be passed on to the BDI team for GAG identification and localisation, overall sodium content, ultrastructural analyses and axiom free compositional analysis based upon unique to Nottingham GAG imaging mass spectrometry technique on tissue sections.

Endpoint. Determined ex vivo sodium skin storage capacity compared to the outcome of ex vivo imaging and correlated to the mechanism of sodium GAG interaction in each sample obtained from T2D, CKD (sodium overload), HNa, CHF (sodium underload) and controls (normal sodium load). A detailed understanding of the GAG complement of the dermis, how this alters in disease cases and how that correlates with sodium storage determined by sodium MRI ex vivo.

[1] https://doi.org/10.1172/jci.insight.145470

Full project location: University Park;Sir Peter Mansfield Imaging Centre, BDI;

Characterising a novel chemogenetic method for inducing aberrant adult hippocampal neurogenesis

Project Supervisor: Edward Beamer

School: School of Science and Technology (NTU)

Description: Aberrant hippocampal neurogenesis occurs following an insult to the brain, such as a seizure, stroke or traumatic injury. It is thought that this this aberrant hippocampal neurogenesis then contributes both to cognitive impairment and the development of chronic epilepsy. There are, however, no studies demonstrating the effect of aberrant hippocampal neurogenesis in the absence of wider pathologies associated with these acute insults. We have demonstrated that aberrant hippocampal neurogenesis can be induced, in mice, with a novel chemogenetic approach. During the nine-week rotation, students will focus on characterising this chemogenetically induced aberrant hippocampal neurogenesis in mice, using a range of immunohistochemical approaches. They will compare the time course of changes to the rate of neurogenesis with vehicle-treated mice as well as mouse models of acute insult, including kainic acid-induced status epilepticus and a model of haemorrhagic stroke. Maturation of neurons will be investigated using a number of endogenous markers, such as Nestin and

Doublecortin. Migration of newly formed neurons will be investigated using tracking molecules, such as IdU and BrdU. Dendritic morphology of newly formed neurons will be investigated using virally delivered fluorescent markers and analysed using specialist software.

Location: Clifton Campus;

Full project description: Aberrant hippocampal neurogenesis occurs following an acute brain insult, such as a seizure, stroke or traumatic injury. While aberrant hippocampal neurogenesis is associated with both cognitive impairment and the development of chronic epilepsy, it has proven difficult to establish a causal relationship. We have developed a novel, chemogenetic approach to inducing aberrant hippocampal neurogenesis, without the broader pathological process associated with severe brain injuries. This project aims to characterise the changes to hippocampal neurogenesis using this approach, compare to two mouse models of acute insult, and investigate whether chemogenetically-induced aberrant hippocampal neurogenesis impacts on cognition, seizure threshold and markers of epileptogenesis. This will help develop an understanding of whether aberrant hippocampal neurogenesis is an important contributor to the development of chronic epilepsy and whether it is a potentially fruitful target for early pharmacological intervention following an acute insult.

We have demonstrated that aberrant hippocampal neurogenesis can be induced chemogenetically, by delivering viral vectors expressing designer receptors exclusively activated by designer drugs (DREADDs) directly into the dentate gyrus. This leads to the expression of DREADDs and the fluorescent protein mCherry in cells which express GFAP. In the dentate gyrus, this includes both astrocytes and neural progenitor cells. Successful transfection of cells can be visualised histologically, through the expression of mCherry. Systemic injection of CNO then induces Ca2+-influx into cells expressing the DREADD. We have shown that this leads to a spike in hippocampal neurogenesis which appears similar to that seen in mouse models of acute insult, such as status epilepticus, stroke and traumatic brain injury.

This PhD project has a number of aims, building on this pilot data. The first aim of the project is to characterise the rate of neurogenesis, biochemic maturation, morphological development and migration of newly generated neurons and compare with mouse models of acute insult (haemorrhagic stroke and kainic acid-induced status epilepticus). Maturation of neurons will be investigated using a number of endogenous markers, such as Nestin and Doublecortin. Migration of newly formed neurons will be investigated using tracking molecules, such as IdU and BrdU. Dendritic morphology of newly formed neurons will be investigated using virally delivered fluorescent markers and analysed using specialist software. Biochemical maturation of neurons may be investigated using digital spatial profiling technology (NanoString).

The second aim of the project will be to use a second vector, which expresses the DREADD on the Nestin promotor, which has a higher level of specificity for neural progenitor cells in the hippocampus. The student will develop skills in stereotaxic injection while delivering the viral constructs to the dentate gyrus.

The third aim of this PhD project will be to investigate the contribution of aberrant hippocampal neurogenesis, induced through the two different chemogenetic approaches, to cognitive impairment and epileptogenesis. Cognitive impairment will be investigated using a number of behavioural assays, such as the Barnes maze. Epileptogenesis will be investigated both through mapping histological markers associated with epilepsy (e.g. hippocampal sclerosis, mossy fibre sprouting), and through investigating the seizure threshold using infusion of chemoconvulsants or electrical kindling.

Full project location: Clifton Campus;

TARGETING HUMAN PROLYL OLIGOPEPDITIDASE (HuPOP) IN INFLAMMATION

Project Supervisor: Ivan Campeotto

School: Biosciences

Description: Human Prolyl Oligopeptidase (HuPOP) is a member of the serine-protease enzyme family, which recognises specific peptides in biological active molecules and cleaves them after a proline residue. HuPOP has been shown to be involved in the brain in the regulation of many neurodegenerative disorders, whilst its role in inflammation and modulation of the innate immunity has been elucidated outside the brain only recently (Payne et al., 2021). Thus, there is the urgent need of targeting HuPOP outside the brain to develop novel therapeutic agents, which can modulate inflammation and immune response in several human diseases.

Novel HuPOP inhibitors are available from plants and from medicinal chemistry, respectively from our collaboration with Prof. Christian Gruber (Medical University of Vienna, Austria) (Retzl et al., 2020) and with Prof. Timo Myöhänen (University of Helsinki,Finland) (Etelainen et al., 2023).

During the rotation the student will express and purify HuPOP following an established protocol (unpublished) using state-of-art fermentation equipment in Campeotto's group to:

- Screen inhibitors in enzymatic assays using fluorescence
- Determine binding affinity of the inhibitors using Isothermal Titration Calorimetry or Biolayer interferometry
- Set-up crystallization trays of HuPOP in complex with the best inhibitors identified from step 1 and 2

Location: Sutton Bonington Campus;

Full project description: Prolyl Oligopeptidases are members of the serine-protease family, which cleave peptides smaller than 30 amino acids at the C-terminal side of proline residues. Cumulation of peptides in the brain causes a variety of disorders including: Alzheimer's disease, Huntington's disease, mania, clinical depression, dementia and autism. HuPOP activity has also been recently linked to inflammatory conditions and immune stimulation. However, its roles in these processes needs to be elucidated.

There are no structural data of HuPOP, nor monoclonal antibodies (mAbs) against it, so further research is required to discover new HuPOP inhibitors and new anti-HuPOP mAbs, respectively for therapeutic applications and as diagnostic to use HuPOP as biomarker.

Research background:

HuPOP has been produced in E. coli expression system via fermentation in Campeotto's group in milligram amounts, as required for biophysical and structural studies.

A panel of new inhibitors from plants are available from the collaboration with Prof Christian Gruber at the University of Vienna (Austria) and from Prof. Timo Myöhänen at the University of Helsinki (Finland)

Preliminary data show that those compounds inhibit HuPOP and can also inhibit parasite protein homologues from Trypanosoma species, which can also cause inflammation.

This also offers the possibility of targeting Human and parasite enzymes at the same time with a potential synergic effect on both, parasite invasion and parasite-induced inflammation.

Additionally, anti-HuPOP mAbs, are currently being produced in Campeotto's group will be tested as research and diagnostic tools to detect HuPOP levels in the blood and to pave the way to understand its role in inflammation.

PhD objectives:

- Objective 1: Characterization of anti-HuPOP mAbs for diagnostic applications. Monoclonal antibodies (mAbs) against HuPOP will be purified and screened. The student will use biophysical techniques to quantify individual binding affinities and kinetic properties, whilst in parallel he/she will set-up a cell-based assay with HEK293 cells to detect HuPOP levels. These studies will be in collaboration with Prof. Lisa Chakrabarti (UoN) and pave a way to develop a serological test to detect HuPOP-induced inflammation.
- Objective 2: Determine the crystal structure of HuPOP in complex with anti-HuPOP mAbs. The student will purify anti-HuPOP mAbs to co-crystallise them in complex with HuPOP. This will highlight the immunogenic portion/portions (epitopes) of HuPOP. At the same time the mAbs will be tested against the parasite homologue oligopeptidase Tc80, to identify any cross-reactivity, which may indicate underlying immune-suppression.
- Objective 3: Biophysical studies and determination of the crystal structures of HuPOP in complex with novel inhibitors.

Recombinant HuPOP expression has been achieved in Campeotto's lab from E. coli. The student will express and purify the protein for crystallization screening using VMXi beamline at the Diamond Light Source. Successful conditions will be reproduced in-house to co-crystallise HuPOP bound to the inhibitory peptides as well as to screen a library of fragment compounds at DLS. All inhibitory compounds, peptides and small molecules, will be screened and ranked using Isothermal Titration Calorimetry (ITC) in Campeotto's group before co-crystallisation experiments.

The student will have the opportunities to spend some time at Diamond Light Source, if desired.

Full project location: Sutton Bonington Campus;

Unravelling the role of mitochondrial sulfide oxidation in diabetic neurodegeneration

Project Supervisor: Nicholas (Nik) Morton

School: School of Science and Technology (NTU)

Description: The student will perform cutting-edge mitochondrial physiology and energetics studies on primary brain tissue from a model of diet-induced diabetes and genetically engineered to lack the mitochondrial enzyme thiosulfate sulfurtransferase. This includes mitochondrial respiration in the Oroboros T2k and cellular respiration/glycolysis in the Agilent Seahorse. The student will also have access to training in animal physiology and disease modelling with cutting-edge in vivo analysis of metabolic health and energy expenditure using a Promethion indirect calorimetry system and non-invasive TD-NMR assessment of longitudinal body composition

Location: Clifton Campus;Sutton Bonington Campus;

Full project description: Ageing and obesity-associated type 2 diabetes may accelerate neurodegeneration and are intimately linked to oxidative stress and mitochondrial dysfunction. Sulfide, a gaseous molecule produced principally from the endogenous metabolism of sulfur containing amino acids, modulates oxidative stress through the cytosolic NRF2/KEAP signalling pathway and by modulating mitochondrial respiration and redox function. Sulfide is oxidised, to maintain homeostasis and prevent toxicity, through the enzymatic sulfide oxidation pathway (SOP), including the sulfide:quinone oxidoreductase (SQOR), persulfide dioxygenase (aka ETHE1) and thiosulfate sulfur transferase (TST). Severe or fatal sulfide toxicity occurs if damaging mutations occur in the upstream SOP enzymes SQOR or ETHE1. However, we have described a high sulfide-resistant phenotype in mice lacking TST, that exhibit mild hepatic metabolic dysfunction, but protection from atherosclerosis, another common age– and obesity-related cardiometabolic disease. This suggests that downstream SOP inhibition may confer some benefits on specific organ systems through tissue-specific sulfide elevation, consistent with the beneficial effects ascribed to putative sulfide donor therapeutics in pre-clinical models of metabolic, vascular, inflammatory and neurodegenerative disease. We have developed tissue-specific genetic models of TST manipulation to test the hypothesis that tissue-specific sulfide elevation through SOP inhibition protects against organ dysfunction.

In this PhD project the student will focus on neurodegenerative disease processes induced by type 2 diabetes using the established Tst gene deficient model (Tst–/– mice) where diabetes has been exacerbated by exposure to high fat high sugar diets, or chemical impairment of pancreatic beta cell insulin secretion (multiple low dose streptozotocin). Furthermore, the student will generate a new model of brain-specific Tst deficiency and overexpression using bespoke, validated, flanked by loxP (floxed) Tst knock-down and cre-inducible human TST overexpression alleles. The student will investigate the impact of TST-mediated sulfide modulation on in vivo whole animal energetics, mitochondrial physiology and molecular (mass spectrometry-based proteomics/persulfidomics) neuropathological, and behavioural phenotypes to comprehensively test whether TST inhibition or activation represents a therapeutic strategy against metabolic disease-associated neurodegeneration, often referred to as the growing global crisis of "type 3 diabetes".

Full project location: Clifton Campus;Sutton Bonington Campus;

Robustness of the blood cell development during vertebrate embryogenesis

Project Supervisor: Martin Gering

School: Life Sciences

Description: In the lab rotation project, you will use the CRISPR/Cas9 technology to generate a stable Lsd1 mutant zebrafish line. First, you will design oligonucleotides to suitable Cas9 target sites in the zebrafish lsd1 locus. With the help of these oligonucleotides you will generate an in vitro expression construct, a plasmid on which you will transcribe a guide RNA in vitro. This guide RNA will then be incubated with the Cas9 enzyme and injected into one-cell stage zebrafish embryos. A subset of injected embryos will be genotyped to see whether your guide RNA was able to target the lsd1 locus efficiently. These experiments will involve genomic DNA extraction from embryos, polymerase chain reaction on the genomic DNA, agarose electrophoresis and sequencing of the PCR products. Siblings of embryos in which the gene was targeted successfully will be grown up to adulthood. The aim is to generate generation 0 fish that generate gametes that carry the lsd1 mutation.

Location: QMC;

Full project description: the vertebrate embryo, blood cells develop from the mesodermal germ layer next to endothelial, pronephric duct and mesothelial cells. During their differentiation, the cells not only activate blood cell-specific genes, but they also repress genes that are characteristic for immature mesodermal cells and for alternative differentiation paths. Recent data from a current PhD student have shown that Gfi1/1b transcriptional repressors play a key role in stabilising red blood cell development from the early mesoderm. Comparing gene expression in wild-type versus gfi1aa, gfi1ab and gfi1b triple mutant cells in a single-cell RNA sequencing project, the student could show that mesodermal cells specified as red blood cell progenitors are not able to undergo differentiation into mature red blood cells in the absence of the 3 Gfi1a/1b proteins. Instead, they become endothelial and mesothelial cells. To which extent single and double mutant cells are also converted remains unknown and shall be analysed in your project.

Your project will also investigate the role the Gfi1/1b interaction partner Lsd1, a corepressor that demethylates histone H3 lysine 4 to inactivate active promoters. Interestingly, zebrafish lsd1 mutants do not appear to have the same drastic red blood cell defect. Using single-cell RNA sequencing on lsd1 mutant red blood cell progenitors, you will be unravelling the gene expression profiles of lsd1 mutant red blood cell progenitors. You will use bioinformatic tools to compare the molecular trajectory of gfi1aa/1ab/1b triple mutant and the lsd1 single mutant progenitor cells. The current student has already worked out a protocol that will help you to generate complete biallelic knockouts in CRISPR/Cas9-injected embryos using the latest double guide ribonucleoprotein technology. These generation zero mutant embryos will help you boost the number of mutant embryos available for cell isolation and subsequent single-cell RNA-sequencing. These biallelic mutants are, however, not suitable for generating a stable line as these embryos are not viable. Therefore, the rotation project is used to generate heterozygous mutant carriers by using a less efficient older CRISPR/Cas9 technology. Some of the injected embryos will carry the mutant lsd1 allele in the germ line and pass it on to the next generation. This will allow you to generate a stable lsd1 mutant that will be extremely useful at the point when you want to verify the single-cell RNA sequencing data in homozygous mutant embryos. Homozygous mutant embryos will be derived from the stable line of heterozygous mutant allele carriers and used in RNA in situ hybridisation experiments in which you will confirm the findings of the single-cell RNA-sequencing experiment. This project promises to provide insights into the mechanisms that confer robustness to the process of blood cell differentiation during embryogenesis. A better understanding of the molecular trajectories during cell differentiation is a prerequisite for the development of in vitro protocols aimed at generating blood cells for regenerative medicine from pluripotent stem cells.

Full project location: QMC;

Investigating the causal role of brain rhythms for cognitive flexibility in the ageing brain

Project Supervisor: Nicholas Myers

School: Psychology

Description: Working memory, our ability to hold information briefly in mind, depends on the interplay of a distributed network of brain areas. How these different brain areas communicate for successful memory is still under debate. One proposal is that they

synchronize especially when information in working memory needs to be updated, re-evaluated, or transferred so it can control behaviour.

This 9-week lab rotation will begin to examine this question by testing the causal role of brain rhythms in the controlled access of working memory contents. Recent research has found that neural synchronization in particular frequencies (delta and theta bands from ~2-6Hz) predicts both successful updating of working memory contents, successful representation of those contents at a neural level, and successful behaviour. In this project you will examine whether interrupting or inducing these rhythms through transcranial alternating current stimulation (tACS) can similarly affect our ability to update our working memory. You will pilot a new working memory updating task and learn to use neurostimulation techniques in humans.

- Weeks 1-3: Piloting the new working memory task, training in tACS, literature review
- Weeks 4-7: Data acquisition, further training in tACS, and behavioural analysis
- Weeks 8-9: Final analysis of data, begin write-up of findings

Location: University Park;

Full project description: This PhD project will examine the neural basis of the flexible use of working memory, the role of brain oscillations in this process, and whether working memory decline in ageing can be reduced through novel approaches to brain training and neurostimulation.

Working memory is the ability to hold important information in mind for short periods of time. It is an essential part of cognition and is linked to success in education and in adult life. However, working memory capacity is strictly limited, allowing us only to maintain about three or four items at a time, and this limit further declines as we age. Mitigating age-related working memory decline is therefore a crucial goal in cognitive neuroscience, particularly in an ageing society.

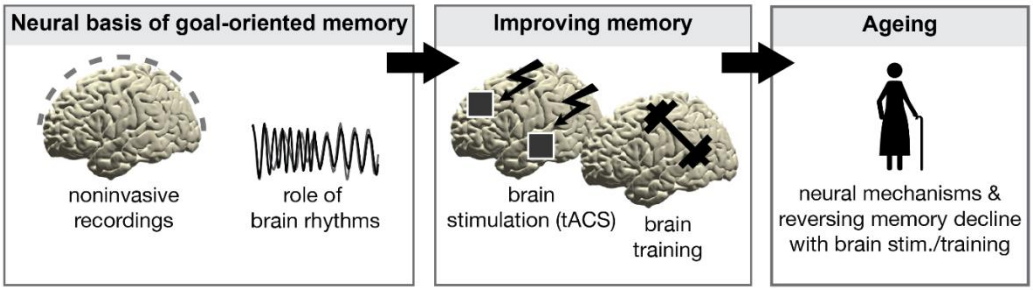
Previous efforts to improve working memory capacity through training have generally fallen short, typically showing improvements only on a core task that does not generalize to other working memory-dependent abilities. These studies have typically focused on improving capacity itself, rather than focusing on how we use that capacity to best support behaviour. This PhD project will take a different angle, focusing on the brain basis of how we are able to select the most important information to store in working memory, an ability that seems relatively spared in ageing.

Recent studies suggest that the controlled and flexible use of limited working memory resources is orchestrated by neural oscillations that synchronize different brain areas to allow for the efficient transfer of information from memory-related brain areas to brain areas guiding behaviour. The PhD project will systematically build on this empirical basis by (1) examining brain rhythms in successful working memory management via electroencephalography (EEG), (2) investigating the causal underpinnings of these rhythms through brain stimulation (e.g., transcranial alternating current stimulation, tACS), and (3) examine whether the ability to flexibly control access to working memory in older age can be either trained or improved through neurostimulation. It will therefore span from basic discovery science to contributing to healthy ageing by developing both behavioural training strategies and testing neural stimulation interventions to improve memory.

This project will employ state-of-the-art neuroimaging recording and analysis as well as neurostimulation techniques via (simultaneous) EEG and tACS. It will also involve the development of new cognitive paradigms for precision neuropsychology, which will allow

for more accurate diagnosis and targeted interventions in memory. Students will learn to use brain stimulation and neuroimaging methods, focusing on the analysis of brain-wide synchronization through oscillations and multivariate pattern analysis.

Full project location: University Park;



Multimodal brain networks in cognition

Project Supervisor: Roni Tibon

School: Psychology

Description: In the lab rotation, the student will evaluate data obtained from an open data archive NIMH. Out of the multiple datasets in the archive, the rotation will focus on data obtained with magnetoencephalography (MEG), a method for direct measurement of neural activation with high temporal resolution. Following a literature review, the rotation will compare methods and parameters of MEG connectivity analysis to establish resting-state networks (de Pasquale et al. PNAS 2010; Brookes et al. PNAS 2011; Hillebrand et al. Neuroimage 2012; Colclough et al. Neuroimage 2016). The rotation will include comprehensive training in Matlab or Python programming skills, applied to connectivity analysis of MEG data.

Location: University Park;

Full project description: The ability of different brain regions to coordinate and interact together critically supports high-level cognition. Such brain networks were observed across various imaging techniques, but the correspondence between these techniques is largely unknown. The PhD project will implement recent advances in the analysis of neuroimaging data, in order to combine information across multiple neuroimaging modalities and to relate these multimodal brain networks to cognition, such as memory or emotional processing. The project will explore open datasets (such as data obtained from CamCAN, NIMH, and HCP: https://www.humanconnectome.org/study/hcp-young-adult/document/500-subjects-datarelease) which contain multimodal functional neuroimaging data (namely, fMRI and MEG).

To combine these data, several approaches will be explored, including methods based onmultilayer networks that can be used to describe multiple interacting networks simultaneously (e.g., Tewarie et al., 2016; Mandke et al., 2018). Multivariate approaches and machine learning methods (including neural networks and relevance vector machines) will be used to determine how these multimodal brain networks relate to cognitive performance across a variety of tasks. For the PhD candidate, basic Matlab or Python programming skills are desirable. The project will be supervised by a team of researchers from School of Psychology whose combined expertise extends into neuroimaging, cognitive neuroscience, open-access databases, high-performance

computing, graph theory for multilayer networks and machine learning for neuroimaging data analysis.

Full project location: University Park;

The role of melanopsin-based photoreception in visual analysis

Project Supervisor: Paul McGraw

School: Psychology

Description: Light energy excites retinal photoreceptors (cones and rods). These photoreceptors have different photopigments, and are connected to retinal ganglion cells (RGCs), which form the neural pathway from the eye to the brain. Recently, a subpopulation of RGCs, called intrinsically photosensitive retinal ganglion cells (ipRGC), have been discovered that are themselves light sensitive due to the presence of an additional photopigment called melanopsin. IpRGCs are thought to support non-image forming behaviours, such as regulating pupil size. The pupillary light reflex (PLR) decreases the diameter of the pupil as light intensity increases, to help protect the retina. When light levels are low, the pupil is enlarged to increase photon capture. However, the relative contribution of light sensitive receptors to the pupil response remains largely unknown.

In this lab rotation we will use high-resolution pupillometry to track the dynamic response to light stimulation as a function of its duration, intensity and wavelength. Crucially, we will use a method (silent substitution) that will allow us to isolate and modulate each receptor class, while the activity of others are held constant, thus delineating the contribution of distinct cell types, with their characteristic photopigments, to the pupil response, prior to investigating the role of melanopsin in visual analysis.

Location: University Park;

Full project description: During daylight hours, light perception starts with the activation of three distinct cone classes found in the primate retina, each of which is sensitive to a distinct region of the visible spectrum. When the light level is low, visibility is governed instead by the activity of rod photoreceptors, which contain a more light sensitive photopigment (called rhodopsin). The output from rods and cones form the input to all subsequent stages of visual analysis - projecting via the LGN to visual cortex - that serve to encode the spatial and temporal properties of objects in our environment.

Recently, a sub-population of retinal ganglion cells has been identified in the primate retina that are also photosensitive and signal via the photopigment melanopsin [1]. These neurons were initially thought to be involved in non-image forming activities, such as entrainment of the circadian cycle and controlling pupil size. However, there is now good evidence that they form a new parallel pathway that projects directly to the LGN and can signal large-scale changes in retinal irradiance [1]. Despite the direct projection of melanopsin-based ganglion cells to the LGN, their role in image formation remains largely unknown. Using visual stimulation techniques that independently control the stimulation of rods, cones and melanopsin-based ganglion cells, work on mice has revealed that melanopsin ganglion cells have spatially large receptive fields and a sluggish temporal response [2]. Recent behavioural measurements in humans using melanopsin-isolating stimuli, show this class of receptor responds best to low spatial and temporal frequencies, when presented in the peripheral retina [3]. Furthermore, when melanopsin stimulation is delivered at higher contrast levels, it perceptually modulates the appearance of coarse gratings [3].

In this project we plan to explore the role of melanopsin-based receptors in human spatial and temporal vision. We will ask whether the melanopsin channel supports motion perception. We will address this using a combination of stimulus manipulation known to isolate the melanopsin channel (silent substitution) and the measurement of adaptation effects using visual psychophysics. Aftereffects have a long history in vision science and have been fundamental in establishing the presence of visual mechanisms that encode basic properties of objects such as its size, orientation, position and shape. We will exploit a form of temporal adaptation that arises when humans adapt to a visual field that changes gradually in intensity. Following adaptation to a stimulus that increases in intensity, a steady test light appears to perceptually dim [4]. Superimposing this temporal afterimage on a luminance gradient causes the test stimulus to appear to move [4]. Measuring this effect, and other related aftereffects, will allow us to define the image-based spatial, temporal and chromatic operating range of melanopsin-based cells in human vision and elucidate their role in motion vision.

1. Dacey, D.M., Liao, H.W., Peterson, B.B., Robinson, F.R., Smith, V.C., Pokorny, J., Yau, K.W. and Gamlin, P.D., 2005. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. Nature, 433(7027), pp.749-754.

2. Allen, A.E., Storchi, R., Martial, F.P., Bedford, R.A. and Lucas, R.J., 2017. Melanopsin contributions to the representation of images in the early visual system. Current Biology, 27(11), pp.1623-1632.

3. Allen, A.E., Martial, F.P. and Lucas, R.J., 2019. Form vision from melanopsin in humans. Nature Communications, 10(1), pp.1-10.

4. Anstis, S.M., 1967 Visual adaptation to a gradual change of intensity. Science, 155(3763), pp. 710-712.

Full project location: University Park;

Perturb and record: a multimodal approach to the study of brain connectivity in the attentional network.

Project Supervisor: Domenica Veniero

School: Psychology

Description: The planned PhD project requires combining different neuroscience methods, therefore, the rotation will include training on basic and advanced electrophysiological (EEG/MEG) data analysis (including data cleaning and analysis in time- and frequency-domains) and TMS data collection, a non-invasive brain stimulation method. This will include how to use individual magnetic resonance brain images to target specific brain areas. Since the project requires the acquisition of EEG data during brain stimulation, part of the rotation will also focus on how to combine the 2 techniques and how to deal with TMS interference on EEG data. Additional skills acquired during this time will include familiarising with different software such as Matlab toolboxes used to analyse EEG data (EEGlab and Fieldtrip).

Location: University Park;

Full project description: When meeting a friend in a busy square, you will look around to spot them. You'll probably scrutinise a small region and, if you don't see them, you will move to the next one. In other words, you will pay attention to small parts of the scene. This happens because our brain has limited resources and can only deal with a

small amount of information at any given time. Deploying attention and processing visual information seem effortless but require communication across many brain areas. In this project, we will investigate how this communication happens and how it relates to people's behaviour. To this end, we will use various techniques to perturb the natural brain activity and record how the perturbation we are introducing affects brain signals and participants' performance.

How will we perturb the brain activity? We will activate brain areas that are responsible for attentional deployment using a non-invasive brain stimulation technique known as Transcranial Magnetic Stimulation (TMS). The exciting part is that activating an area will be quickly followed by activating a second area, mimicking what happens when the two communicate in a natural context. This will be repeated over time to reinforce the connections between the two areas. Before and after TMS, participants will perform a task to measure how fast they can process and memorise information. This will allow us to evaluate if and how reinforcing the connections between areas with TMS improves humans' cognitive abilities.

How will we record this? We will also record brain activity to understand how changes in signals exchanged between areas caused by the TMS perturbation relate to behavioural modifications. To do so we will use EEG or MEG, which are both able to detect changes in brain activity at a millisecond timescale. We will also trace changes in neuronal fibre tracts to gather anatomical information.

Using this new approach with TMS, we will establish how communication across brain areas takes place and how it relates to cognition. In addition, thanks to the combination of different techniques, we will be able to identify which specific signal relates to specific aspects of people's behavioural performance (for example memory or processing speed).

Full project location: University Park;

Influences of anxiety on learning and memory

Project Supervisor: Christopher Madan

School: Psychology

Description: During the lab rotation project, the student will conduct research on the effects of anxiety on learning and memory in humans. This rotation is designed to provide hands-on experience in experimental methodologies commonly employed in the topic. The first portion of the rotation will be dedicated to understanding and applying psychophysiological techniques (measurement of skin conductance) and correlate these with memory performance. These methods offer insights into the physiological responses associated with cognitive processes, providing a deeper understanding of how anxiety levels can relate to and influence learning and memory processes.

The student will run behavioural experiments, which will allow them to observe and measure the direct effects of anxiety on learning and memory. Next, the student will have the opportunity to work with pre-existing datasets. These datasets encompass results from various experimental methods, and there's potential for them to include data from advanced brain imaging techniques such as functional magnetic resonance imaging (fMRI) or electroencephalography (EEG). Such exposure will equip the student with skills in data analysis and interpretation. Lastly, the rotation may also introduce the student to computational approaches, depending on the mutual agreement between the student and their supervisors. This will enable the student to integrate theoretical

knowledge with practical application, ensuring a balanced coverage of what the full PhD would entail.

Location: University Park;

Full project description: While the analogy of the brain as a computer is prevalent, the reality is that cognitive and behavioural processes are multifaceted and understanding how anxiety and cognition (i.e., basic learning and memory processes), particularly when these involve biologically relevant events that elicit physiological responses and learning – which will ultimately provide insights into the biological basis of cognition. One of the most captivating areas within psychology and neuroscience is the exploration of how emotional states, particularly anxiety, influence learning and memory. This goal of this PhD project is to delve into this complex relationship, studying the biological foundations of cognition and the nuanced interplay between emotions and memory.

Central to this PhD will be the design and implementation of research studies focusing on the effects of anxiety on the transfer of valence between different events. We have recently documented remarkable transfer of valence (i.e., positive or negative emotions) between events experienced together—such that otherwise emotionally neutral events become imbued with emotional properties, and this can be a putative mechanism that underlies the features observed in anxiety disorders. Towards this goal, a general aim of this project is to understand the phenomenon of transfer. The mechanism for this effect relies on an interaction between memory systems, namely episodic memories along with conditioning mechanisms--in memories that involve biologically relevant events that elicit physiological responses. These studies will employ a diverse range of methodologies, centered around psychophysiology and behaviour. Beyond this central focus, a range of other research approaches are available, to be discussed by the PhD candidate and supervisory team. Options include examining aging, brain imaging, computational modelling, and potentially studies in non-human animals. Brain imaging methods include the use of fMRI or EEG to characterise the involvement of specific brain regions in avoidance behaviour. Computational modeling will involve simulate underlying learning and memory processes and predict the potential impact of anxiety on cognition and physiology.

Given the complexity of the associated data, the PhD candidate will be expected to possess basic programming skills, such as in Matlab or Python. These skills will be critical for data analysis, especially when navigating the complexities of datasets from brain imaging or computational models. However, the supervisory team is committed to supporting further skill development.

Full project location: University Park;

Neural underpinnings of driving behaviour

Project Supervisor: Matias Ison

School: Psychology

Description: In this lab rotation you will have a unique opportunity to learn about OPM-MEG scanning, a transformative new technology that allows scanning in real-world tasks that include head movements. You will also be trained on how to collect and perform basic analysis of eye movements data, and implement driving scenarios using specialised software, which will be fully exploited during the PhD project. This project brings together an interdisciplinary team based at the School of Psychology and the

SPMIC/School of Physics and it is expected that you will also be supported by other lab members, including PhD students and postdocs.

Location: University Park;

Full project description: Efficient visual search is an essential requirement for interacting safely with our physical environment and losses in search performance have dramatic consequences for a broad range of visually-guided behaviours. By way of example, Department for Transport Statistics reveal that drivers failing to search properly ('looked but fail to see') was a contributory factor that accounted for 26% of the fatal road accidents in the UK in 2018 (DfT, 2018). Visual search is often impaired in specific populations, such as the elderly, and Alzheimer's patients. This PhD project will seek, for the first time, to elucidate the neural underpinnings of visual search in a naturalistic setting.

The supervisory team has very recently developed a unique driving system that combines a MEG compatible driving unit (with a steering wheel and pedals), with eye tracking and 'wearable' MEG - which exploits quantum technology (Optically Pumped Magnetometers -OPMS) to enable free head movement during scanning. This is the only combined system of this type in the world that allows simultaneous recordings of high-resolution brain activity and eye movements during simulated driving. The data analysis pipeline for this system will also exploit new state of the art techniques pioneered by Dr Ison.

The PhD project will study the relationship between behaviour, gaze control, and brain activity in a driving task. Participants will drive along several scenarios and face different types of hazards, such as a car from an offside road failing to give way at a crossroad junction. Previous studies have shown that robust visual evoked responses emerge preceding the driver's intention to perform emergency braking. However, unlike previous studies, the new system will be able to localise the neural generators of these responses. Prior to participating in the experiment, subjects will have a full visual assessment to quantify any differences in basic visual capacity known to affect driving performance. Depending on the student's interests, the PhD student will be involved in co-designing the main experiment. This will focus on the experimental manipulation of a relevant psychological variable, such as active vs passive driving for autonomous vehicles, distraction processing while driving, or ageing.

Within the PhD project there is the scope to interact with industrial partners EyeGym, who have developed platforms to deliver training to improve visual search. This will follow a between-subjects design, where a group of subjects will undergo training between driving tasks. In the trained group, it is anticipated that the improved performance will be coupled to reliable changes in the associated brain responses (e.g. signal amplitude or neural latency) measured using OPM MEG. If the neural signature of search performance can be established, it offers a route to future development of an objective system for tracking changes in visual search performance over time and in different groups of subjects.

Full project location: University Park;

The neurochemical dynamics of human semantic cognition

Project Supervisor: JeYoung Jung

School: Psychology

Description: Neurotransmitters (GABA and glutamate) have been linked to clinical and cognitive outcomes. Alterations of neurotransmitter levels are seen in neurodevelopmental/neurodegenerative/neurological/psychiatric disorders. GABA and glutamate have been associated with various cognitive functions, including memory and learning. Given the functional relevance of neurotransmitters in the context of both pathological and healthy cohorts (especially in the context of development and aging), understanding how GABA and glutamate change with age in a healthy cohort is important to understand the typical and atypical progression of human cognition.

In this rotation, a student will learn about cognitive testing to quantify human semantic memory function and in vivo neuroimaging techniques to measure GABA and glutamate concentrations and neural activity in brain regions related to human semantic memory function.

- 1-3 weeks: You will acquire knowledge about semantic memory and healthy aging.
- 2-8 weeks: You will learn to measure various human cognition using cognitive test battery (e.g., ACE-II), evaluate semantic memory function, and analyse behavioural data. You will collect data from healthy young and old adults using MR spectroscopy (MRS) and functional magnetic resonance imaging (fMRI) during semantic processing. You will learn the basic MRS and fMRI data analysis.
- 7-9 weeks: You will analyse MRS and fMRI data by comparing young and old adults.

Location: University Park;

Full project description: The overall objective of the PhD project is to derive novel insights into the neurochemical basis of semantic cognition in healthy ageing and to extend the current understanding of the neural mechanisms underpinning human semantic memory from the neurotransmitters to behaviours. This will be achieved using a range of neuropsychological testing, neuroimaging, and brain stimulation in a multi-modal integrated approach.

As the primary neurotransmitters in the brain, GABA and glutamate play a critical role in regulating responsiveness and excitability within neural networks and in synchronizing cortical neuronal signaling activity. They are involved in a wide range of physiological and biochemical processes, including the regulation of cognition, memory and learning, neural development, and adult neurogenesis. Thus, GABA and glutamate play a significant role in aging and in neurodegenerative disorders, including dementia.

Semantic cognition refers to our ability to use, manipulate, and generalize knowledge in order to interact with the world, by producing time- and context-appropriate behaviors. Therefore, impairments in semantic memory can have a severe impact on quality of life (e.g., dementia, stroke). Few neuroimaging studies of cognitive ageing have been concerned with semantic cognition, demonstrating specific regional patterns of age-related differences in neural systems. They are frequently interpreted in terms of compensatory shifts, which help to support performance or degeneration, which reflects reduced specificity of neuronal responses rather than compensation. However, it remains unclear how these functional changes in the semantic system are linked to GABAergic and glutamatergic activity in ageing.

This PhD project investigates the GABA and glutamate systems underpinning human semantic cognition in healthy ageing. You will study healthy young and old participants,

who will have various brain scans, including structural, functional, and metabolic neuroimaging and perform experimental tasks measuring key cognitive properties of semantic processing. A sub-group of this cohort will also participate in brain stimulation experiments in combination with neuroimaging to enhance semantic memory and to measure its effects at the neurochemical and behavioral levels. This project will answer two key questions in cognitive neuroscience: (a) How do neurotransmitter systems shape human higher cognition? (b) How does neuromodulation mediate the neural activity leads to neuroplasticity?

To answer them, you will deploy a range of cutting-edge methodologies - multimodal imaging. Specifically, you will employ biochemical imaging (MRS): (a) to measure GABA and glutamate activity in key regions of semantic cognition; (b) to combine this methodology with the use of other neuroimaging (fMRI) and psychological experimental paradigms to link neurotransmitter activity to semantic processing in the healthy brain (young and old); (c) to combine multimodal imaging and brain stimulation for exploring neuroplasticity, driven by neuromodulation (e.g., TMS, tACS, FUS).

This project will advance our understanding of the neurotransmitter systems underpinning semantic cognition and their role in ageing. This work will contribute to the knowledge of pharmacological treatments acting on the neurotransmitter system and the application of brain stimulation as a potential therapeutic tool.

Analysis and training will involve the use of neuroimaging (e.g., MRS and fMRI), brain stimulation, and software packages such as PsychoPy, SPSS, and/or Matlab. A basic understanding of neuroanatomy and cognitive neuroscience is expected.

Full project location: University Park;

Mapping the brain circuits integrating vision and touch with high resolution imaging

Project Supervisor: Dr Elisa Zamboni

School: Psychology

Description: During the nine-week lab rotation, the student will train in analysing high resolution fMRI data. We also aim to introduce the student to standard (Gradient Echo BOLD) and advanced (Vascular Space Occupancy) acquisition techniques. The latter will involve MR safety training and operator training of the 7T Philips scanner at the Sir Peter Mansfield Imaging Centre. Here, the opportunity to learn setup of stimulation kit (screen and vibrotactile stimulation for digits), data transfer and organisation will be provided. the data acquired will have high spatial resolution (laminar level; sub-mm), thus requiring training in advanced image analysis techniques using multiple tools (e.g., FSL; LAYNII) and programming languages (e.g., Python/Unix and Matlab). Particular emphasis will be given to the importance of obtaining high quality cortical laminar segmentations of the MRI data. From here, it will be possible to explore modelling of haemodynamic response functions across the cortical layers and compare the spatial specificity of the signals between different acquisition techniques (linked to understanding the interplay between acquisition sequence and vasculature organisation of the brain).

During lab rotation, analysis techniques to obtain touchmaps (i.e., topographic maps indicating how individual fingers are represented in somatosensory cortex) will be provided, together with exploring layer-specific functional connectivity analysis techniques.

Location: University Park;

Full project description: Multisensory integration is a core brain function known to involve cortical/subcortical brain regions that combine information from multiple sensory sources into a new output signal that differs from each individual input. Moreover, the cortex is anatomically divided into layers. Processing input and output information is well characterised across the layers and can be used to distinguished feedforward and feedback connections linking different cortical areas. Another fundamental organisational principle of the brain is that different portions of the cortex are dedicated to different functions (e.g., somatosensory area organised into body representation). This project will capitalise on recent advances in functional brain imaging (magnetic resonance imaging) to investigate laminar-specific processing mechanisms in the brain non-invasively. Ultra-High Field (UHF) MRI (MRI scanners of 7Tesla and above) facilitates submillimetre spatial resolution, allowing to quantify measurements of modulations of feedforward/feedback connections across cortical layers. Using visual and somatotopic stimulation in the scanner, the project proposes to first map how individual fingers are represented in the brain of individual volunteers. Using high resolution imaging here is key as it offers the ability to determine the connectivity between visual and somatosensory areas at layer-resolved resolution, thus teasing apart bottom-up and top-down information flow during sensory processing. Once the baseline circuitry integrating vision and touch is identified, the project proposes to use visuo-tactile illusions to tap into mechanisms of sensory plasticity: while we can trick our brains into believing that a phantom limb belongs to ourselves, what are the brain mechanisms underpinning this illusion? This will require implementation of a Virtual Reality-based digit-stretching illusion where the cameras on a VR headset track the digits of volunteers and as they start interacting with their own fingers, as slightly pulling the digits, the VR rendering generates a stretching illusion, which results in perceiving one own's finger as elongated. This type of altered sensory stimulation will be part of the manipulation to assess changes in the neurorepresentation of digits in somatosensory cortex. A key advantage of using UHF functional MRI here will be the ability to characterise individual differences in these mechanisms and relate such differences to behavioural measures of body perception (e.g., biases in perceived body shape), efficacy of visuo-tactile illusion (e.g., comparison between rubber-hand, computer-, and VR-based illusions).

The project provides training and development of skills in brain imaging at high-resolution, using standardised acquisition protocols, and development of alternative techniques (Vascular Space Occupancy) that provide more localised and less biased measurements. Training here will include operating the 7T scanner at the Sir Peter Mansfield Imaging Centre. Dissemination of research outputs (academic conferences and participation at outreach events) is also planned.

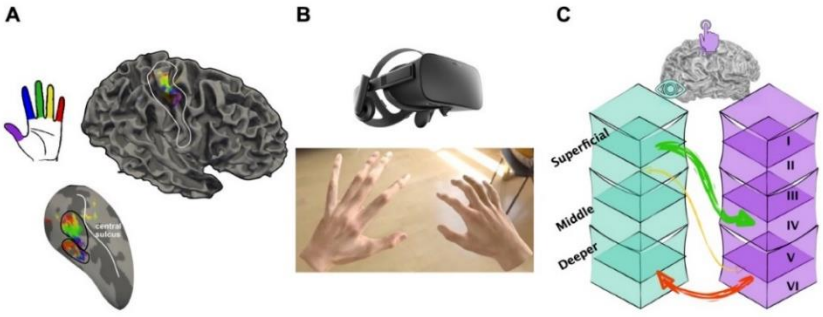


***Figure 1** – Pilot data showing mapping of somatosensory information relative changes based on altered visuo-tactile inputs. (A) Somatotopic map of the left-hand digits acquired at 1.5mm3 on Siemens MAGNETOM Vida system at the Institute of Sport (Manchester Metropolitan University). Each finger was stimulated for 4s in a block-design fashion and resulting maps are displayed on an inflated 3D model of the right hemisphere. The posterior bank of the central sulcus (white line) shows orderly representation of the digits. Insert at the bottom highlights the digit-topography. (B) Virtual Reality manipulation achieved on accessible systems for home use. The cameras on the VR-headset (top) track the hands and digits of volunteers and as they interact with the digits, the VR rendering generates a stretching illusion (hand on the left), which results in participants to perceive their fingers as elongated. (C) Schematic showing the processes to be investigated. Following the visuo-tactile, illusion-based VR manipulation, changes in the circuitry integrating visuo-tactile information (red and green arrows between visual in cyan and somatosensory in purple, areas) as well as reorganisation of the digit-topography (A) can be quantified.*

Full project location:
University Park;

Neurochemical changes in response to human errors and shifts in attention

Project Supervisor: Claudia Danielmeier

School: Psychology

Description: During the lab rotation, the student will work with a larger team of psychologists and physicists involved in a study that investigates neurochemical changes associated with working memory functions in healthy individuals and individuals with early psychosis.

The student will have the opportunity to join our ongoing functional magnetic resonance spectroscopy (fMRS) project looking at working memory functions. fMRS is being used to quantify changes in neurometabolite levels (like glutamate and GABA) while individuals are engaged in a cognitive task. There will the opportunity to work at the Sir Peter Mansfield Imaging Centre and in the School of Psychology to help with data acquisition and learn how to analyse functional magnetic resonance imaging (fMRI) and fMRS data acquired at a high field strength (7T) scanner. The student will have the opportunity to learn more about different aspects of working memory functions and associated changes in glutamate and GABA levels in the medial frontal cortex. We will then start planning how these methods could best be used in the context of detecting own errors and shifting attention which will be the main focus of the following PhD project.

Location: University Park;

Full project description: Shifting attention to relevant information is crucial for many different everyday life tasks. However, attentional processes are often impaired in various mental health disorders, for instance in ADHD or schizophrenia, or in neurological patients. These attentional impairments will influence other cognitive processes as well, e.g. adapting behaviour flexibly to changing circumstances. One example, where we need to shift our attention quickly, is after having committed an error. Often, we commit errors because we got distracted by irrelevant information and then need to focus again on what is relevant for the task. Previous functional magnetic resonance imaging (fMRI) studies (e.g. Danielmeier et al., 2011, 2015) have shown that error processing activity in the medial frontal cortex can trigger attentional adjustments in task-relevant brain areas. In the proposed PhD project, we will focus on the underlying neurochemical processes related to error detection and the associated signal that triggers shifts in attention. From past research it is known that there are glutamatergic connections between medial frontal brain areas, involved in error detection, and the basal forebrain that could play a central role in these shifts of attention.

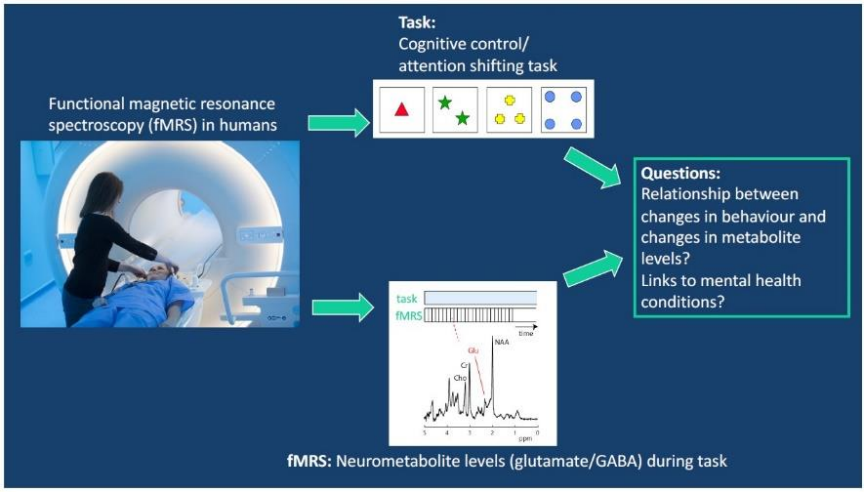
Concentrations of metabolites, including the major neurotransmitters glutamate and GABA, can be measured in the brain using magnetic resonance spectroscopy (MRS). MRS studies have typically been performed while the brain is at rest. However, recent research (including at Nottingham) has used MRS to measure functionally-relevant changes in neurometabolites over time (functional MRS or fMRS). Observed changes in glutamate and GABA are linked to changes in task demands and, therefore, provide a means to probe neuronal activation at a more direct level than that of fMRI.

We will work together with experts in MR physics at the Sir Peter Mansfield Imaging Centre and will make use of the ultra-high field strength MR system (7 T) to perform sensitive measurements of both GABA and glutamate.

In the current PhD project, we propose to use fMRS to measure glutamate and GABA changes related to errors in task responses as a first step. We will test the assumption that glutamate levels increase when an error has been committed. This study could be

combined with fMRI to identify whole brain functional networks associated with attentional shifts after errors. There are several options to develop this work further after the initial study (e.g. focus on basal forebrain or on neurochemical changes in task-relevant visual areas or application in different attentional tasks) which will be co-designed with the PhD student. The planned experiments will be conducted in healthy individuals to gain a better understanding of the underlying neurochemical changes. However, a longer-term goal would be to apply this knowledge to patient populations with a view to contribute to improving therapies for attentional impairments.

The PhD candidate will receive training in functional magnetic resonance imaging (fMRI), functional magnetic resonance spectroscopy (fMRS) and cognitive task design (if required). This is an exciting opportunity to contribute new studies to the emerging field of fMRS research in the area of error processing and shifts in attention.



Full project location:
University Park;

The role of oestrogen in mediating muscle stem cell function in women

Project Supervisor: Sophie Joanisse

School: Life Sciences

Description: In this lab rotation students will complete an in vitro mini-project using immortalised human muscle cells to begin to understand the effect of female sex hormones (oestrogen and progesterone) on aspects of the muscle repair process. Muscle repair/regeneration is a complex process and involves several distinct but essential phases, one of which is the activation of resident muscle stem cells (satellite cells).

Students will first determine if hormone levels of oestrogen and progesterone mimicking that of women in pre- peri- and post-menopause affect the activation and proliferation of myoblasts. In addition to learning standard cell culture methods, students will complete an MTT assay (cell viability and proliferation) and a scratch assay (cell migration). Students will also differentiate cells to determine if varying female sex-hormone levels protects myotubes from 'damage' and whether myotube size is affected. In completing this part of the project students will build on their cell culture techniques and use immunofluorescence microscopy to determine myotubes size and use electric pulse stimulation to induce myotube 'damage' and the ELISA assay to determine the concentration of creatine kinase in cell media as an indirect measure of muscle damage. Students will learn how to analyse and interpret all of the results generated in this mini-project.

Location: QMC;

Full project description: Skeletal muscle possesses a remarkable capacity for adaptation and repair, processes essential in the maintenance of skeletal muscle health.

The ability of skeletal muscle to respond to such stimuli, is in large part, due to muscle resident stem cells, commonly referred to as satellite cells (SC). The regulation of SC is a highly regulated process and involves signalling from adjacent structures and other cell types within the skeletal muscle (capillaries – lined with endothelial cells, inflammatory cells etc.) as well as circulating factors[1]. Importantly, SC, endothelial cells and mitochondria express oestrogen receptors (ER) in humans implying a role for oestrogen in the direct regulation of SC[2]. Currently, the majority of muscle physiology research is biased towards males, in fact a recent editorial highlighted that between 1993-2021 only 11% of papers published in the journal of Applied Physiology, Nutrition, and Metabolism identified females/women as the primary experimental group. In agreement with this, although the SC response following exercise induced muscle damage (EIMD) has been a topic of intense research, most of this work has focused on men. Therefore, this PhD project will focus on describing sex-hormone, specifically oestrogen, signalling in human skeletal muscle.

Our current knowledge surrounding the role of oestrogen signalling in the regulation of SC and the muscle repair process in humans is limited. Menopause is a naturally occurring process that occurs in all women, characterized by sudden oestrogen-deficiency and is an ideal model to study the role of oestrogen in skeletal muscle. Interestingly, there is an accelerated loss of muscle mass during the transition into menopause[2]. SC are essential in repair and play a key role in the growth and maintenance of skeletal muscle health. More recently, the interaction of SC and capillaries has also been described and is important in the muscle repair process[1]. Therefore, defining the role of oestrogen in SC function will enhance our ability to understand the mechanisms underpinning muscle loss during the menopause.

An in vitro model will be used to determine if (a) intrinsic differences exist in primary myoblasts from age-matched pre- and post-menopausal women and (b) if exposure to higher sex-hormone levels impact myoblast function of post-menopausal women.

An in vivo model will be used to determine if oestrogen levels influence (a) SC content/activation following EIMD; (b) the muscle repair process and, (c) capillary content.

To answer these questions, the PhD project will study age-matched (middle aged) pre- and (early)post-menopausal women (not currently taking oral contraceptives/hormone replacement), a naturally occurring model of oestrogen-deficiency. The PhD will be completed at the University of Nottingham within the world-leading David Greenfield Human Physiology Unit, where all testing and sample collection involving participants will be completed and overseen by a team of medical professionals. The PhD student will be supervised by core members of the MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research and the Nottingham NIHR Biomedical Research Centre and will have the opportunity to collaborate on an MRC funded project by co-supervisor Dr. Matthew Brook examining protein turnover in pre- and post-menopausal women.

1. PMID: 27760421
2. PMID: 35907119
Full project location: QMC;

What makes endothelial cells start growing? interactions between transcription factors and microtubules

Project Supervisor: Andrew Benest

School: Medicine

Description: This research project will investigate the regulation of ZEB1 expression and function of the inn cultured vascular cells and how this is relevant to endothelial cell proliferation. The project will involve cells in culture and basic molecular techniques including qPCR analysis, protein biochemistry, western blotting, qPCR analysis, mRNA isolation quantification and analysis and bionformatic approaches to analysing transcriptomic and proteomic data. The essentials of primary cell culture, and how to manipulate gene expression with siRNA cell phenotyping using immunofluorescence, confocal imaging and deconvolution technology OR key skills related to downloading, curating and analysis sc and bulk RNAseq datasets will be established.

Location: University Park;

Full project description: Endothelial cells line all blood and lymphatic vessels in the body, where their normal physiological role is to regulate the transit of cells and nutrients between the tissue and the vascular systems. During times of disease, including cancer, diabetes, neurodegeneration and inflammation the endothelium is primed to adapt. One such adaptation is to begin proliferating, as a way to increase vascularity or resolve inflammation. We have previsouly identified a transcription factor that is dynamically regulated during vessel growth, and we have recently found it has key roles in regulating the mitotic machinery within the cell.

Our work will contribute to our understanding of these pathways through investigation of Zinc finger E- box-binding homeobox 1 (Zeb1) a transcription factor that appears to 'moonlight' as regulator of cell division fidelity. We have recently discovered a novel function for the transcription factor Zeb1, during mitosis. Mitosis marks a period of the cell cycle with residual transcription activity, raising the interesting possibility that the mitotic role of Zeb1 is a discrete function. We hypothesise that Zeb1 has a critical role in chromosome segregation fidelity via a yet-to-be-defined mechanism linked to the spindle apparatus, potentially due to microtubule binding.

Zeb1 has a well characterised role in epithelial to mesenchymal transition (EMT), where it functions as a transcription factor to promote a highly motile and invasive phenotype. This is supported by our recent RNAseq analyses which revealed that Zeb1 RNAi caused mis-regulation 636 of genes of which (49 of 636) genes areheavily associated with the cytoskeleton and cell division. Particularly striking was the enrichment of multiple genes associated with the mitotic spindle, including MAP7 and HOOK1.

Preliminary experiments confirmed that Zeb1, which is nuclear during interphase, is redistributed to spindle microtubules following nuclear envelope breakdown. This resulst in metaphase arrest but also a clear aberrant mitotic phenotype noteworthy was an increase in spindle defects and multipolar spindles – phenotypes more readily attributed to mis-regulation of MAP7 or HOOK1, but not for Zeb1. Collectively, this is compelling evidence for a novel Zeb1 function during mitosis, but also raises several important questions:

1.How does Zeb1 contribute to cell division outcome?
a.Determine the functional consequences of Zeb1 RNAi and ectopic overexpression on mitosis
b.What is the recruitment mechanism for Zeb1?

2.What is the Zeb1 interactome?
a.Determine Zeb1 to MT binding mechanisms
We hypothesise that Zeb1 has a uniquely adapted 'interactome' to perform discrete functions.

Full project location: University Park;

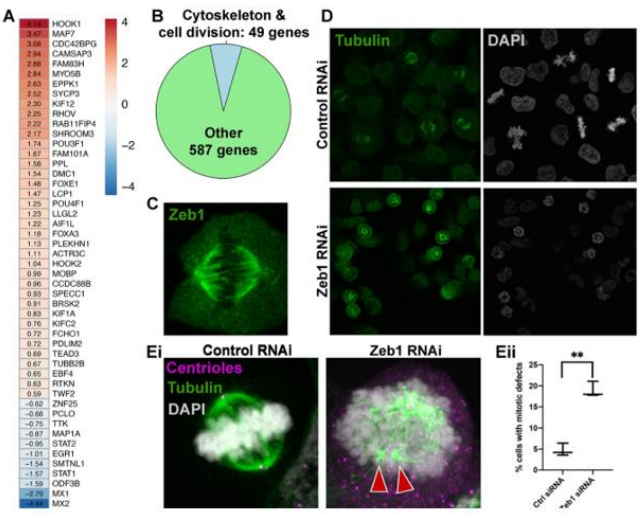


Figure 1 – Zeb1 is an important regulator of mitosis. A) Heatmap list of the 49 mis-regulated cytoskeleton and cell division genes, shown in B. B) Pie chart showing core result of a transcriptomics analysis in endothelial cells. 636 genes were identified as significantly mis-regulated following depletion of Zeb1. Panther analysis revealed that 49 of these were categorised as 'cytoskeleton' or 'cell division'. C) Zeb localisation to spindle microtubules of a metaphase cell. D) Representative images showing the impact of Zeb1 RNAi on cell cycle arrest. Ei) Representative example of spindle pole defects (red arrows) following Zeb1 RNAi. Eii) Quantification of mitotic defects.

Differentiation of human amniotic epithelial stem cells into beta islet cells using conditional cellular reprogramming.

Project Supervisor: Abdolrahman Shams Nateri

School: Medicine

Description: During the project rotation, students will develop practical research skills in 2D and 3D tissue culture methods, as well as transferable skills such as the ability to critically evaluate others' work.

Practical skills in culturing epithelial cell lines in both 2D and 3D cultures will be honed through daily laboratory work, dedicating 7-8 hours per day for 3 weeks. Once proficiency in these skills is attained, the focus will shift to the isolation of human amniotic epithelial cells for 4 weeks. During this training period, supervision will primarily be provided by Dr. Nateri and Dr. Jones (Medicine), with support from the Cancer Genetics and Stem Cells Group within the BioDiscovery Institute.

Given the limited number of researchers working in this specialized field internationally, a comprehensive understanding of the existing literature is essential before embarking on the proposed research project. Consequently, students will spend two weeks exclusively conducting a literature review under the guidance of Dr. Nateri and Dr. Nia Jones (Medicine).

The laboratory rotation is designed to equip students with the knowledge and confidence necessary to incorporate the skills required for their connected PhD studies effectively.

Location: University Park;

Full project description: Background: Pancreatic islet isolation and engraftment are currently offered to patients with difficult-to-control type 1 diabetes (T1D) as a substitute for inactive or destroyed beta cells, aiming to prevent glycemic instability. The remarkable progress in successful islet transplants over the past three decades is a result of continuous research that has led to improvements in islet cell processing,

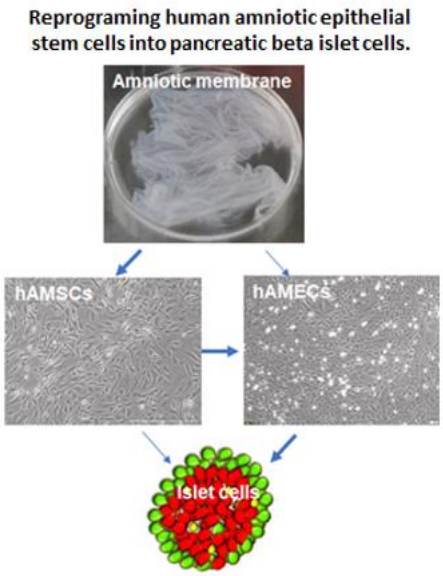
transplantation technology, immunosuppression regimens, and patient selection and management [1]. Presently, the available options for sources of islets for islet transplant surgery encompass the patient's own pancreas (autotransplant), islets from brain-dead cadavers, or the utilization of biopsied pancreatic islets from living related donors (allogeneic transplant).

Challenges such as inflammatory and noninflammatory damage to post-transplanted islet cells and the vascular deployment of islet cells in the liver further complicate the process. Consequently, the broader implementation of islet transplantation hinges on overcoming technical limitations, including an insufficient supply of islet cells and the necessity for chronic systemic immunosuppression, which entails risks for both the islet graft and the patient [2].

Human amniotic epithelial cells (hAECs), derived from pluripotent placental epiblasts, possess immune-privileged properties and the ability to maintain multilineage differentiation potential [3]. They also demonstrate the capability to modulate the local immune response, suggesting exciting prospects for therapeutic applications in tissue regeneration [4]. Unlike induced pluripotent stem cells (iPSCs), hAECs do not give rise to tumours or teratomas and exhibit immunomodulatory and anti-inflammatory characteristics. This presents notable ethical, technical, and scientific advantages over other stem cell resources. Studies involving mouse models have exhibited the potential of hAECs to differentiate into surfactant-producing alveolar epithelial cells and hepatocytes [5].

The limited availability and survival of islet cells, along with the prolonged immunosuppression regimens required for allogeneic transplantation, pose significant constraints on utilizing islet transplantation to treat patients with T1D. Moreover, the viability of transplanted islet cells is impeded by immune-mediated and inflammatory reactions. hAECs derived from the placenta present a potential solution, as they exhibit tolerogenic, non-immunogenic, and non-tumorigenic and stem cell characteristics. Preliminary evidence suggests that these cells can differentiate into islet beta-like cells capable of insulin production, offering a promising source for islet replacement.

Nevertheless, a key hurdle in employing stem cells for islet transplantation has been the challenge of scalability. The therapeutic application demands a substantial quantity of cells, necessitating the use of seven-term placentas to treat a single patient. The controlled replication (CR) method emerges as a promising strategy to expand hAEC cultures substantially, all the while preserving their immune-privileged attributes and their potential to differentiate into insulin-producing beta cells.



Hypothesis:

hAECs isolated from the placenta can be propagated using the humanized CR methodology, all the while retaining their primary phenotype, which includes pluripotency. They also exhibit promising characteristics for development into endoderm lineages, including pancreatic beta cells.

Specific Aims:
1. Isolation of human amniotic epithelial cells (hAECs) from the placenta using the Gramignoli protocol.
2. Cultivation, Expansion, and Characterization of hAECs.

3. Differentiation of hAEC-CRs into Insulin-Producing Beta Cells.
4. Validation of Functional Activity of hAEC-CRs through Co-transplantation with Human Islet Cells in a Diabetes Mouse Model.
Full project location: University Park;