Biotechnology and Biological Sciences Research Council

Nottingham BBSRC Doctoral Training Partnership – Provisional Project List 2023

Content

Introduction

We are looking forward to meeting you at your interview for the Nottingham BBSRC Doctoral Training Partnership.

This booklet includes a list of the projects you will be able to select from if you are offered a place on the programme. You should look through this booklet and submit your indicative top three project choices using this form by **Friday 17 March 2023.**

Your project choices will be used to inform the interview panel of your research interests and you should be ready to talk about why you are interested in these specific projects in your academic interview. If you are also undertaking an interview for a CASE project, your DTP interviewers will be aware of this as well.

While the projects you submit at this stage will help us to understand your research interests, you will not be recruited to a specific project, and will have the opportunity to meet with supervisors and discuss projects in more detail once you have received an offer of a place, prior to submitting your final lab rotation preferences in the summer.

Using this Booklet

Projects in this booklet are listed by lead School. You can use the index to browse project titles or use the Ctrl-F function to search for keywords or supervisor names.

Biosciences

6 Cannabidiol regulation of maladaptive fear: psychological and neurobiological mechanisms

Lead Supervisor: Carl Stevenson

Lead School: Biosciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: The student will be involved in currently running cannabidiol-related projects in the laboratory of Dr Gershkovich in the School of Pharmacy, University Park Campus, including:

1. Development of oral drug delivery systems of cannabidiol for enhanced uptake into the intestinal lymphatic system for better treatment of autoimmune diseases.

2. Optimization of brain delivery of cannabidiol for better treatment of brain cancers.

The student will be trained for and directly involved in the following techniques and activities:

1. HPLC methodology for the determination of cannabidiol in animal blood and tissues

2. Design, development and characterisation of lipid-based formulations of cannabidiol

3. In vitro lipolysis experiments for rank ordering the performance of lipid-based formulations

4. Direct involvement in in vivo pharmacokinetic experiments in rats

5. Pharmacokinetic analysis and modelling of the obtained in vivo results

Full Project Description: Background

Learning that certain cues predict threat, and adapting to changing environmental contingencies by learning that such cues no longer predict threat, are both adaptive and crucial for survival. These processes can be investigated using fear conditioning and extinction in rodents and understanding how different brain areas and neurotransmitters are involved is an important challenge in biology. This also has clinical implications since anxiety-related disorders are characterised by abnormal threat processing, while extinction forms the theoretical basis for exposure therapy as a psychological treatment for these disorders.

A drawback of these treatments is that their effects are often limited or temporary, resulting in symptom relapse, while medications can be ineffective and have side effects. Promising research is investigating how existing or new medications can boost psychological therapy. Cannabidiol is the main non-psychotropic constituent of cannabis and has a favourable side effect profile. This phytocannabinoid is gaining interest as a treatment for anxiety-related disorders by reducing fear, both directly and indirectly by enhancing extinction. We recently showed that cannabidiol prevents the return of fear over time after extinction and enhances extinction under stress, which normally interferes with extinction [Papagianni et al. (2022) Int J Mol Sci 23:9333].

Aim

This project will follow up on our exciting recent findings by determining the neural and pharmacological basis of cannabidiol regulation of extinction under stress in rats. The student will employ an inter-disciplinary approach combining different in vivo behavioural and pharmacological

methods with ex vivo bioanalytical techniques. Previous evidence indicates that cannabidiol regulation of fear and extinction involve serotonin (5-HT1A) and cannabinoid (CB1) receptor signalling, respectively. Cannabidiol may enhance extinction under stress directly by elevating brain endocannabinoid levels and CB1 receptor signalling. This will be determined by examining (1) the effects of cannabidiol on endocannabinoid levels ex vivo in relevant brain areas after extinction under stress, and (2) if the effects of cannabidiol on extinction under stress are inhibited by blocking CB1 receptors systemically and locally in the brain in vivo. Cannabidiol may also enhance extinction under stress indirectly by reducing fear/stress through activation of 5-HT1A receptors. This will be determined by examining (1) the effects of cannabidiol on stress mediators (e.g. noradrenaline, corticosterone, corticotropin releasing factor) levels ex vivo in the blood and/or relevant brain areas after extinction under stress, and (2) if the effects of cannabidiol on stress mediator levels and extinction under stress are inhibited by blocking 5-HT1A receptors systemically and locally in the brain in vivo.

Fit with BBSRC remit

This project combining animal behavioural, in vivo pharmacology, and ex vivo bioanalytical techniques falls within the 'Frontier Bioscience' and 'Bioscience for Health' remits. Providing in vivo and analytical skills training (e.g. brain cannula implantation surgery, systemic and central drug administration, behavioural testing, LC-MS determination of endocannabinoid and stress mediator levels in tissues) is strategically important for UKRI to develop capacity and capability in the UK life science base. Therefore this project also addresses important biosciences skills gaps highlighted in the 2015 'MRC and BBSRC review of vulnerable skills and capabilities'.

Lab Rotation Location: University Park;

Full Project Location: Sutton Bonington Campus;University Park;

11 How microbes optimise root function? Integrating supramolecular chemistry and 3D-printing to decipher it

Lead Supervisor: Gabriel Castrillo

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: Three main objectives are proposed for the rotation:

1- Protoplast preparation. Seeds will be allowed to germinate with a bacterial synthetic community for 2 weeks. Roots will be collected and protoplasts from roots will be prepared following Birnbaum and co-workers protocol. Collected root tissue will be digested with a mixture of cellulase and pectolyase to release protoplasts, and the protoplasts collected by centrifugation. Protoplast will be quantified in the microscope and their volumes and morphological characteristics determined.

2- Protoplast sorting. For the rest of the rotation the student will use for protoplast preparation the marker lines of root tissues available in our lab. These lines express the GFP in the individual root tissues. GFP-expressing cells will be isolated using FACS at the UoN, School of Medicine with GFP detected at 488 laser/520nm emission.

3- Molecularly designed matrices based on self-assembling peptides. The student will optimise the composition of the matrices to be used to capture the protoplasts and enable recreation of key features of the native tissues.

At the end of the rotation the student will be trained in plant growth, media and plate preparation, bacterial inoculation and synthetic community preparation, root protoplast preparation, sorting, and biomaterials design.

Full Project Description:

Similar to animal guts, plant roots have evolved specialised cell layers to regulate mineral nutrient and water homeostasis. Recently our lab has discovered that, as for animals, this layer in the root collaborates with the resident microbiota to maintain homeostatic integrity. Nevertheless, the molecular players that regulate the establishment of the microbiota across the different root tissues remain unknown.

The plant roots adsorb mineral nutrients and water to distribute them to the rest of tissues and organs. This root function is critical for plant growth and development. These processes are conditioned by the anatomy of the root since the mineral nutrients and water infiltrate the root from the soil through the different tissue layers to reach the central vasculature. In nature, root layers must permit the colonisation of the metabolically active microbiota inhabiting the root and still protect the root functions. In animals, mechanisms controlling the crosstalk between the gut and the microbiota have been characterised and health consequences when this coordination fails described. Unfortunately, in plants, the function of root layers has been primarily studied under axenic conditions, and the mechanisms of how the microbiota influences root function remain ill defined.

Therefore, there is an unmet need to understand how the different root layers work with the microbiota to control root functions and the consequences of these interactions. This is of critical importance to develop microbial-based strategies to improve agricultural outcomes. We will do this through an innovative multidisciplinary approach integrating supramolecular chemistry, living material engineering, 3D printing, biofilm studies, and techniques to study microbiota composition. The integration of these different areas represents a unique opportunity that overcomes challenges that have, until now, prevented understanding these mechanisms.

To define these mechanisms important for plant nutrition we defined three key objectives:

1- Optimising a novel self-assembling living material to recreate the in vivo characteristics of the individual root tissues. We will engineer and validate a new bottom-up biofabrication system based on the co-assembly of peptide amphiphiles (PAs) with protoplasts isolated from root tissues to reproduce single tissue characteristics of the root. The PAs will be designed to recreate key physical and molecular features of the native root environment and enable printing of 3D structures that reproduce geometries, sizes, surface topographies, and root identities.

2- Resolving the spatial distribution and tissue responses to a synthetic microbiota in the different artificial root layers. We will use a 4-members synthetic community of bacteria labelled with different fluorophores to define the spatial distribution of the microbiota in the different 3D printed root tissues. We will collect material to analyse the transcriptomic profiles across the root tissues. These results will be compared to those obtained in roots from intact plants.

3- Understanding microbial mechanisms relevant for the microbiota establishment in the different root tissues. We will generate and use bacterial mutant libraries to identify microbial gene networks relevant for the individual tissue colonisation. Using wild-type and mutant bacterial we will also characterise mechanical and physical properties of the biofilm across root tissues.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;University Park;

14 Discovering new components of biochemical mechanisms of oxygen-sensing in plants

Lead Supervisor: Michael Holdsworth

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: A mechanism of plant oxygen-sensing has been discovered by the host laboratory (Gibbs et al Nature 2011, Nature Communications 2018), but key components of this mechanism still remain to be discovered, and evidence points to the existence of other oxygen sensing systems in plants (Holdsworth, Nature Plants 2017). Using already available resources the student will combine physiology, molecular biology and Mass Spectrometry (MS) approaches to investigate the nature of oxidation changes to key transcription factors and other regulatory proteins that control the plant oxygen sensing system. The rotation will include

1. physiological analysis of the hypoxia (low oxygen) tolerance of plants containing mutations in potentially novel components of the oxygen-sensing system, that will provide new components for biochemical analysis (Holdsworth Biosciences).

2. Biochemical investigation of oxygen sensing by key transcription factors using an in-vitro plant extract system, investigating the influence of inhibitors of oxygen-utilising enzymes on bacterially expressed transcription factors through MS analyses (Oldham Chemistry)

The rotation will equip the student with an interdisciplinary understanding of biochemical mechanisms required for plant response and tolerance to hypoxia, key for developing novel crops with flooding resistance.

Full Project Description: Analogous but mechanistically different oxygen-sensing pathways were adopted in plants and metazoan animals, that include ubiquitin-mediated degradation of transcription factors and direct sensing via dioxygenase enzymes (Holdsworth and Gibbs Current Biology 2020). However, it is clear in plants that undiscovered mechanisms exist, and that the known mechanism is not completely elucidated, with novel components still to be uncovered (Holdsworth Nature Plants 2017). This is an increasingly important area of plant biology, as response to reduced oxygen (hypoxia) during flooding as a key determinant of crop yield.

Hypothesis: The hypothesis underlying this project is that novel genetic components of plant oxygen-sensing exist defining biochemical mechanisms for sensing and survival in response to reduced oxygen.

Four objectives will address this hypothesis, using Arabidopsis thaliana novel genetic components of the known pathway of oxygen-sensing (through oxygen-regulated Ubiquitin-mediated degradation

of ERFVII transcription factors), to identify oxygen-sensors defining new pathways, and to transfer this information to the crop species Brassica rapa.

Objective 1 (0-12 months, Holdsworth, Biosciences): Identifying novel oxygen-sensors: Gene expression data has been used to identify oxygen-requiring enzymes that could potentially act as physiological oxygen-sensors. Mutant lines with disruptions in these genes will be analysed by physiological and molecular biology approaches for changes in tolerance to low oxygen, that will identify novel sensors.

Objective 2 (0-48 months, Holdsworth, Biosciences, Oldham Chemistry): Defining the biochemistry of the known plant oxygen-sensing system: Although the known oxygen-sensing mechanism has been studied in great detail, we have unpublished recent data that shows that there is a key 'missing link' genetic component. This will be investigated biochemically using a plant in vitro extract and using in vivo transgenic material through MS approaches used to identify specific biochemical changes associated with oxygen sensing. Novel genetic components will be derived from bioinformatics approaches. These components will be analysed physiologically as in Obj 1 to define their role(s) in providing biochemical changes associated with MS observations. A major goal of this objective will be to completely reconstitute the plant oxygen-sensing system in vitro using purified components.

Objective 3 (12-48 months, Holdsworth, Biosciences, Oldham Chemistry): Defining the relative contributions of novel and known oxygen-sensing mechanisms to plant hypoxia tolerance. Genetic approaches in Arabidopsis will be used to combine mutants for different novel components identified in Obj 1 & 2. Mutant combinations will then be assayed for tolerance to hypoxia, flooding and waterlogging. The biochemistry of the oxygen-sensing systems will be investigated using MS approaches to define the effect of oxygen sensors on substrates.

Objective 4 (24-40 months, Holdsworth, Biosciences): Transferring genetic information to Brassica rapa, a closely related diploid, easily genetically manipulated and large scale genetic/genomic resources are already available, and it is crop affected by flooding: Orthologous genes for novel oxygen-sensors will be identified and targeted for disruption through CRISPR approaches. The effect of these lesions on tolerance to waterlogging and flooding will be assessed using physiological approaches

Training associated with this project: The student will obtain comprehensive interdisciplinary training in biological chemistry, plant genetics, molecular biology and bioinformatics.

Lab Rotation Location: Sutton Bonington Campus;University Park;

Full Project Location: University Park;Sutton Bonington Campus;

20 Molecular readers of mRNA methylation – co-ordinating gene expression and environmental response.

Lead Supervisor: Rupert Fray

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The methylation of certain adenosines (m6A) in messenger RNA is an ancient process that has been conserved through plant and animal evolution. Plants engineered to

have a lower level of m6A methylation show severe developmental defects and altered responses to environmental cues. During the lab rotation, the student will investigate the role of two RNA binding proteins that specifically bind to methylated RNA. They will be trained in vector design and DNA assembly technologies. They will gain experience in protein production and purification and will carry out protein-RNA binding assays.

Full Project Description:

Please provide a description of the full PhD project (500 words)

Methylation of mRNA is a fundamental process that is conserved across eukaryote Kingdoms. It is required for normal differentiation, development and environmental responses, but many questions remain as to how this post-transcriptionally regulates gene expression. The Fray, Archer and Mongan groups have collaborated extensively over the past 10 years to address the role of adenosine methylation (m6A) in biological systems including yeast, plants, insects and mammalian cells.

The m6A methylation mark is added by a complex of at least six “writer” proteins, and mRNAs tagged with m6A are specifically bound by “reader” proteins containing a motif (YTH domain) that selectively recognises the modification. Different classes of YTH proteins exist in the nucleus and cytoplasm. Those YTH proteins in the nucleus can effect how the mRNA is processed (different splicing outcomes or different transcription termination sites) and this can result in different proteins being made. When the YTH proteins in the cytoplasm bind an mRNA they can influence whether the mRNA is translated, stored or degraded and so control how much of a protein is made.

This project will utilise resources that we have developed in the model plant Arabidopsis thaliana. Plants with reduced levels of mRNA methylation have developmental abnormalities, altered response to hormones, and respond differently to biotic and abiotic stress. Excitingly, the contribution that the cytoplasmic and nuclear YTH proteins make to these different characteristics of the low methylation plants can be investigated using genome editing to mutate and delete members of the two reader families.

The student will use CRISPR/Cas9 genome editing, transcriptomic analysis, plant genetic transformation, phenotypic analysis and con-focal microscopy to study the roles of these m6A readers. They will use this information to dissect the role of the different classes of YTH proteins during normal plant growth and in response to environmental signals such as heat and drought stress and to pathogen attack.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

44 Tissue tectonics during gastrulation in mammalian disc embryos

Lead Supervisor: Ramiro Alberio

Lead School: Biosciences

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

47 Gene regulatory network analysis of human formative embryonic stem cells

Lead Supervisor: Masaki Kinoshita

Lead School: Biosciences

DTP Research Area: Biotechnology

Lab Rotation Description: Our group is interested in how pluripotent stem cells (such as embryonic stem cells) are maintained in vitro. Recently reported formative stem cells are maintained under activating low Tgfb/Activin/Nodal siganlling and inhibition of Wnt and retinoic acid signalling pathways. In this rotation project, we will focus on how other extracellular signals impact gene regulatory network of formative pluripotency. The student will assess the effects of growth factors and small molecules for the self-renewal of human stem cells. We will treat cells with various signalling agonists and antagonist including small molecules and check their impacts on the self-renewal by qRT-PCR and immunostaining of known markers of undifferentiation as well as linage committed genes. This rotation project will be carried out in parallel with the recently awarded BBSRC funded project which studies pluripotency in livestock species, and both projects are complement each other.

Techniques to be learnt: RNA extraction, cDNA synthesis, RT-qPCR, immuno-fluorescence antibody staining, fluorescent microscopy imaging, stem cell culture.

Full Project Description: Pluripotency exists transiently in early mammalian development. In humans, it emerges as “naïve” epiblast cells in the blastocyst. After embryo implants, these cells acquire epithelial morphology and change supporting gene regulatory network (GRN). This early post-implantation pluripotency phase is called “formative” period. The formative epiblast cells still maintain the key pluripotency gene such as OCT4 until gastrulation ends. The formative phase takes several days in human embryo and cells are supposed to prepare for the gastrulation. When the embryo starts gastrulation, cell in the embryo still have a plasticity however, their competence to multi-lineage differentiation starts to be restricted. This end point of pluripotency is called “primed” pluripotency. OCT4 expression is maintained from naïve to primed pluripotency transition however, supporting GRN changes over the time.

These pluripotency phases were mostly studied using mice however, the development of mouse embryos is different from those of humans. 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 phase progression and rewiring of pluripotency gene network. We are interested in the phase represents early post-implantation stage, which is formative stage.

We will perform CRISPR/Cas9 based gene knockout approach to identify genes important for the maintenance of formative pluripotency.

1. We have previously identified Otx2 plays an important role in mouse formative stem (FS) cells. We also have identified a few other genes which is essential for the self-renewal in mouse cells. The student will also analyse the published transcriptome data to list other candidate genes, which is specifically expressed in human formative stem (FS) cells as well as any related human embryo data.

We will assess whether candidates genes are crucial in humans or not by gene knockout individually using CRISPR/Cas9.

2. In parallel to the experiment above, we will perform the genome wide gene knockout screening using CRISPR-gRNA libraries. We will establish the reporter human FS cell lines which we can monitor the gene expression by FACS. The primary candidate of such reporter gene is OCT4 and other candidates will be identified in the expression analysis in 1. We will use lentivirus gRNA libraries and monitor reporter genes expression. After the transfection of virus, we will collect the reporter negative fraction and pool of cells from reporter protein negative fraction(s) will be sequenced by next generation sequencer to identify which gRNAs affect the reporter gene expression.

Screening will produce the good number of candidate genes, so the student will perform experiments to confirm its effect individually. The student will work with the most interesting gene to reveal the genetic correlation and functional mechanisms of the gene in detail by using state-of-art molecular biology techniques.

This project will be closely related to the recently awarded BBSRC funded project which studies pluripotency in livestock species. We will investigate genes identified in this project in large mammals and vice versa.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

49 The perfect storm: how tow problematic processes combined can give a plant an advantage. Abiotic stress tolerance in neo-tetraploids.

Lead Supervisor: Sina Fischer

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: For the 9-week mini project students will work on cloning and further characterisations of neo-tetraploid phenotypes in the model species A. thaliana. Consequently, they will learn/gain confidence in many highly important methods such as general molecular techniques, plant husbandry, experiment planning, data analysis and bioinformatics:

1) Experimentation with Arabidopsis thaliana and Hordeum vulgare in growth rooms alongside SF and GM. The student will learn to work with neo-tetraploid seeds which are already available in the group. He/She will perform pilot experiments to assess germination frequency. These will involve abiotic stress responses.

Training will be provided in the areas of bioinformatics, PCRs, qPCRs, genotyping, and selection of polyploid lines.

2) The data generated will be used as part of the training in statistical analysis (using R, mainly ANOVA and regression analysis), as well as in data presentation (using the dedicated graphics software packages in R).

3) The green gate system will be used to start (possibly finish) the cloning process of one of the genes (MBF1c) identified in previous studies as highly relevant in neo-tetraploids. The goal is to study localization and expression of MBF1c in neo-tetraploids.

4) Potentially, training in tissue culture in barley.

Full Project Description: Neo-tetraploids have some amazing phenotypes. They are tolerant to abiotic stress, accumulate more potassium in their shoots, have larger cells and changes in their gene expression profiles. But how do they manage all of this? And how can we utilize these changes?

Join the project and a young team to study the molecular basis of neo-tetraploid phenotypes. As shown by my group recently (Fischer et al., 2022) we identified a set of genes with altered regulation in neo-tetraploids. Now we want to link this change of expression to the phenotypes we have observed such as the impact on potassium and survival under heat stress. Essentially, we have the ambitious goal to generate a synthetic plant that is diploid but mimics expression patters and phenotypes of a neo-tetraploid. With this poof of principal in the model species A. thaliana we can then start to alter genes in crops with the aim of generating useful phenotypes, such as abiotic stress tolerance, in diploid plants like barley. Why not use neo-tetraploids here? This remains tricky as they often suffer from low yield which we have seen in our neo-tetraploid tomato plants. Neo-tetraploids exhibit a range of phenotypes which could be uniquely useful in a changing environment. Among them tolerance to heat, drought and salinity all of which are processes regulated by the phytohormone ABA. We discovered a very promising ABA-response gene MBF1c which is differentially regulated in neo-tetraploids, suppresses the shoot K phenotype when knocked out and is important for heat tolerance. Now the question is both, how is it regulated and what does it regulate. We also found a set of genes which are very important for the expression patterns observed in neo-tetraploids, among them WRKY9. This transcription factor is one of the hub genes (hub genes influence many other genes) in the ploidy network (Fischer et al., 2022). Thus, understanding its role in neo-tetraploids is of high priority. We expect to explain many of the network changes we have been observing by studying this gene.

The PhD candidate would study the molecular basis of phenotypes in neo-tetraploids by focussing on the characterisation of MBF1c and WRKY9 in a neo-tetraploid background. This would give the student expert knowledge in many common molecular techniques which are used in laboratories all over the world and applied to many different research questions making the student versatile and competitive after their education is concluded. Furthermore, the output from these experiments will be described in high impact publications which will be important not only for the research community but will also enable to student to apply to competitive post doc positions once they finish their PhD project. To mention a few details, the experiments will include cloning a tagged version of the genes to a) visualize their location in diploids vs neo-tetraploids, b) asses the protein stability by Western blotting and through Co-IP assays (immunoprecipitation of protein able to bind to our protein of interest) the binding partners will be study. Further the candidate would also contribute to our goal of generating a synthetic, neo-tetraploid like plant by lowering the expression of WRKY9 by 35% in diploid plants. This will mimic the expression in neo-tetraploids and will be followed by gene expression studies using RNAseq. The final task for the PhD candidate is to start a comparative analysis between A. thaliana neo-tetraploids and barley neo-tetraploids. This will show, which of the mechanisms discovered are generally applicable. The student will both, set up a tissue culture system for the generation of neo-tetraploid barley and work on tetraploid barley lines already present to study effects such as germination under ABA treatment.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

51 Resurrecting Root Hairs in Duckweed – a trait lost for millions of years

Lead Supervisor: Anthony Bishopp

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The nine-week rotation is aimed at introducing the student to as many of the techniques used on this project as possible and provide transferable training.

Bioinformatics

The gene loss described in this project is based on the current collection of six sequenced duckweed genomes. Our collaborator in the USA is currently sequencing and annotating genomes for additional duckweed species. The student will have training in searching these genomes for key transcription factors involved in root hair initiation and elongation.

Microscopy

We commonly visualise root anatomical traits using clearing and confocal microscopy. The student will have training in both these techniques.

Cloning

The lab mostly uses Golden Gate recombination for plasmid construction. During the 9-week rotation the student will have the opportunity to clone simple elements (e.g. promoters) into entry vectors.

Transformation of Spirodela callus

The student will transform existing Spirodela callus with a plasmid containing RUBY using methodology already established in the group. This incorporates a gene regulatory pathway that turns tyrosine into vivid red betalain, and the student will easily be able to recognize regions of the callus that have been transformed due to their dark red pigmentation.

Full Project Description: Context: In both plants and animals, organ loss has occurred frequently during evolution. There are several examples in animal systems, such as the loss of limbs in snakes and vision in cavefish, where the molecular mechanisms underpinning organ loss are well understood. Despite the process being common in plants, we lack a molecular understanding of the underpinning processes.

Duckweeds are the ideal plant system to investigate the molecular mechanisms underpinning organ loss and uncover how downstream gene regulatory networks atrophy. Duckweed is a monocot that has returned to the aquatic environment. In doing so it has undergone phenomenal anatomical simplification in both shoot and root systems. Duckweeds are reduced to a single frond with individual genera being either rooted or rootless. Within the root-bearing genera, all species have lost pericycle cells meaning that roots cannot branch and all duckweed species have lost the ability to form root hairs. Quite simply, we do not know of a single family of plants or animals that has undergone a comparable degree of structural reduction. Advances in genomics and duckweed transformation place duckweeds as ideal models with which to study organ loss and templates in which gene regulatory networks that have been dormant for millions of years can be revived.

Understanding the molecular mechanisms governing the evolution and loss of organs informs us of the rules controlling radical changes in body plans and reverse engineer how complex networks

arise. In this project, the student will investigate the loss of one trait, root hairs. Why root hairs? The molecular network controlling root hair formation is highly conserved. The same group of transcription factors (RSLs) that regulate root hair formation in flowering plants regulate rhizoid formation in liverworts. Liverwort RSL genes can even complement root hair mutants in Arabidopsis. RSL genes are present in every multicellular plant from liverworts to flowering plants except one key group (the Class II RSLs) are missing from duckweeds. In this project, the student will re-introduce these genes to duckweed to test whether this is sufficient to restore a feature that has been absent from duckweeds for millions of years.

Work Plan: The student will firstly investigate components upstream of the Class II RSLs in the greater duckweed Spirodela polyrhiza. This will be done by creating reporters in Spirodela to examine expression patterns and expressing the Spirodela genes in Arabidopsis to test whether they can trans-complement root hair mutants. The student will then create transgenic Spirodela lines in which Class II RSL genes will be expressed. We predict that this will activate a suite of downstream genes that were previously dormant, as many Spirodela genes still contain root hair-specific cis elements within there promoters. The student will combine RNASeq and promoter motif analysis to determine how much of the root hair network is activated, and use confocal/light sheet microscopy to visualise epidermal cells and test for root hairs.

Impact: Beyond duckweed, resurrecting ancestral traits has importance in opening new synthetic biology projects and can be used to restore features present in wild relatives but missing from crop plants, such as secondary metabolites with benefits to health.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

53 Systems biology approach to unravel how wheat roots emerge and grow at specific angle in soil

Lead Supervisor: Rahul Bhosale

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: In cereal crops such as wheat, different root-types (e.g., primary, seminal and crown) emerge and grow at distinct angles to minimise self-competition and improve capture efficiency of water and nutrients from different soil profiles. We recently identified a wheat egt1 (enhanced gravitropism1) mutant defective in this distinct root angle phenotype (Fusi et al., 2022). To pinpoint underlying mechanisms, we are currently mRNA sequencing samples from individual root-types in mutant vs wildtype during and post emergence.

During the lab rotation, student will evaluate this dataset and conduct experiments involving wheat egt1 mutant to gain experience in both computational and experimental techniques that would be used in the main project.

Week 1-4: Analyse transcriptome dataset to find differentially expressed genes in the mutant vs wildtype. Perform GO enrichment to find overrepresented biological processes and construct gene regulatory networks for each root-type. Follow up literature, mutant search and expression analysis for key genes.

Week 5-8: Design and setup gravistimulus experiments for mutant and wildtype. Timeseries root imaging using IR robots and root bending response quantification using RootNav2 or imageJ softwares. Root sampling for biochemical, microscopy and qRT-PCR analyses to measure ROS/cell wall signals controlling root angle.

Week9: Preform review literature and write report.

Full Project Description: Background:

Root angle is an important agronomic trait, which enable plants to capture soil resources from different soil profiles. For instance, steeper roots can capture mobile water and nitrogen from deep soil, whereas shallow roots capture immobile phosphorous from topsoil. Root-types such as primary, seminal and crown emerge and grow at distinct angles, called gravitropic setpoint angles (GSA), which are determined by competing gravitropic and anti-gravitropic offset (AGO) mechanisms. GSA of root-types can be easily visualised and studied in wheat, an important UK and worldwide crop, by subjecting vertically grown roots to gravistimulus. Primary root bends faster than 1st set and much faster than the 2nd set of seminal roots until they reset to their original GSA over time.

Despite our recent discovery of EGT1 controlling root angle in cereal crops (Fusi et al., 2022), we lack fundamental understanding about (i) root-type specific downstream components functioning in competing gravitropic and AGO mechanisms (ii) whether the number or the amount of expression of these components determine the GSA in each root-type and (iii) how (i and ii) are modulated during environmental conditions.

To address this knowledge-gap, we are currently generating transcriptome dataset for individual root-types harvested at different timepoints after gravistimulus (addition to dataset mentioned in rotation project). DTP student will use systems biology approach to build the gene regulatory networks (GRNs), identify predictors of GSA in each root-type and validate them using existing genetic and genomic resources.

Work plan:

Year1-2: Building root-type specific GRNs functioning in gravitropic and AGO mechanisms

Generated transcriptome datasets will be analysed to find differentially expressed genes (DEGs) in each and between root-types. Statistical and machine learning based tools (e.g., ARACNE, GENE3) will be used to build GRNs. These GRNs will be studied in combination with DEGs identified in egt1 mutant vs Wildtype (impaired in AGO) to find gravitropic and AGO specific modules.

Year2-3: Predicting regulators that determine GSA in each root-type.

Implement computational models at cellular and macroscopic scales and use parameters to identify best predictors (e.g., genes/TFs) of root angle in each root-type using sensitivity analysis. This will also help determine whether number of these predictors (operating in gravitropic vs AGO mechanisms) or their amplitude of expression underpins the GSA in each root-type.

Year3-4: Validating GSA predictors using genetic and genomic resources in wheat.

To prioritise key predictors, we will investigate how much natural variation in root-type specific GSA (being generated for >800 Watkins landraces, available through JIC) is explained by the polymorphism within predictor genes. Prioritised predictors will be validated using TILLING mutants, RNAi or CRISPR-Cas9 approaches under optimal/sub-optimal conditions. Hormones such as auxin are

known to influence both gravitropic and AGO mechanisms. If any predictors are involved in hormone signaling pathways or related processes, we will validate them using existing mutants at RRes.

Output and impact:

The identified genetic components and fundamental knowledge will provide innovative genetic solutions to breeders (via the BBSRC Developing Future Wheat Breeder Tool kit) to help expedite improvements in wheat and other related cereal crops.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

59 Exploiting wild relative species to increase global wheat production

Lead Supervisor: Julie King

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The 9 week mini-project will primarily focus on training of key techniques and gaining experience in experimental design and laboratory techniques. This will involve working with three aspects of the wheat pre-breeding programme, namely wide crossing techniques, molecular marker development and cytogenetics, all of which are key to the PhD project.

Weeks 1-3:

Cross bread wheat with the wild relative species to obtain F1 plants. This will give the student key glasshouse and plant management skills in addition to an insight into wheat spike morphology and the techniques of emasculation and pollination.

Weeks 4-6:

a) Use bioinformatic methods to exploit public databases for available sequence information for SNP discovery and development of KASP markers.

b) Validate the KASP markers by testing them on parental species using a high-throughput genotyping platform incorporating the PCR technique.

Weeks 7-9:

a) Extract DNA from the wild relatives and related wheat progenitor species to develop fluorescent probes.

b) Use the probes to characterise the wild relative species using GISH.

Full Project Description: Background

Wheat is one of the world's most important sources of food. However when production needs to increase by circa 50% over its present level to feed the rapidly increasing global population, climate change is driving yield down (in addition political conflicts, e.g., Ukraine also negatively impact production). Thus urgent action is required to increase wheat production if a major humanitarian disaster is to be averted. One way this can be achieved is via the production of new higher yielding wheat varieties.

Conventional breeding methods have made steady progress in increasing production. However, these increases are gradual and insufficient to meet the gains required globally by 2050. Unlike wheat, which has a limited gene pool, its wild relatives provide a vast reservoir of genetic variation that can be exploited to develop superior varieties, i.e., wild relatives result in significant jumps in production. In the past the difficulty in transferring genetic variation from the wild relatives has limited its use. However, the Nottingham Wheat Research Centre (WRC) has overcome all previous bottlenecks. The WRC is now the world leader in the field and the material it generates is distributed worldwide.

Programme

The aim of this project is to exploit genetic variation in the wild relatives to increase global wheat production. This will be achieved in two ways: 1) by analysing and transferring genetic variation that results in significant yield gains (identified at RRES by Malcolm Hawkesford's group) and 2) transferring into wheat genetic variation from wild relatives (identified at the JIC by Paul Nicholson's group) for resistance to wheat blast (WB) and fusarium headblight (FHB). These two diseases, of which little or no resistance exists in wheat, result in yield losses valued in many billions of pounds each year.

1) Increased yield

The lines carrying the gene(s) which increase yield will be crossed to the variety Paragon and self-fertilised. Progeny that are either homozygous for the high yielding gene(s) or lack the gene(s) will be identified and the seed multiplied (UoN). Field plots of these two different genotypes will be grown at RRES and comparisons made for a range of characters including overall yield. These analyses, which will be published, will enable an understanding of the biological nature of the yield increases observed and will also inform breeders on how best to exploit it in breeding programmes (years 1-3).

2) Disease resistance

F1s will be created between wheat and two related species and these backcrossed several times to generate lines which carry single chromosome segments from the wild relative. These lines will be identified via KASP molecular markers and genomic in situ hybridisation (GISH) (UoN years 1-3). Lines homozygous for wheat wild relative chromosome segments will be screened for WB and FHB at the JIC (years 3 and 4).

All of the germplasm generated in this project will be distributed to collaborators in the commercial sector, the USDA for distribution in North America and CIMMYT for global distribution.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;Rothamsted Research;JIC;

68 Enhancing plant protein bioavailability

Lead Supervisor: Molly Muleya

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: Plant proteins are often associated with molecules that inhibit their digestion, including dietary fibre, phenolic compounds, tannins, phytic acid and digestive enzyme inhibitors (e.g. trypsin and chymotrypsin inhibitors). Fermentation is a processing method that has multiple effects on food ingredients and is suggested to reduce many of these so-called anti-nutritional factors (ANFs) and thereby enhance the bioavailability of plant proteins. For the rotation, the solid state fermentation method for tempeh (Rhizopus spp.) will be used to process a range of whole seed legumes, such as soya, and the alternative protein sources bambara ground nut (Vigna subterranea) and winged bean (Psophocarpus tetragonolobus). The impact of tempeh fermentation on the activities of the digestive enzymes, trypsin and chymotrypsin, will be assessed by incubating samples prior to and following fermentation. Samples will then be run on SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to determine whether factors that inhibit these enzymes are reduced by the fermentation. The rotation will allow the establishment of a method that quantitatively and qualitatively determines the extent of proteolysis and the types of protein being degraded. If effective, this method will not only be applicable to the associated DTP project but also other ongoing projects in our labs.

Full Project Description: Digestion inhibitors include dietary fibre and associated anti-nutritional factors (ANFs) which cause poor protein digestibility of plant foods. There are no guidelines describing concentration thresholds for such factors beyond which protein digestibility is impaired. It is important to understand the impact of fibre and associated inhibitors on plant protein digestibility in situ as it is the form in which the foods are consumed. This project will use an innovative approach to determine the threshold limits beyond which digestion inhibitors impair the digestibility of protein/ indispensable amino acid (IAA) without actually removing them from the plant matrix.

Objective 1: Establish the effect of different proportions of a diverse range of plant matrices on the IAA digestibility of a highly bioavailable extrinsic protein.

Highly digestible milk protein that is intrinsically labelled with 15N will be generated at the Centre for Dairy Science Innovation (UoN). These isotope-labelled milk proteins will be blended with different proportions of purified ANFs, as well as several plant matrices, to achieve different ratios of total protein: inhibitor. Both purified proteins and various plant sources will be selected based on their importance to the UK and global food system (through our links with various international collaborators). The different milk protein-plant matrix blends will be digested following the INFOGEST static digestion protocol, which is well established in our labs. Discrimination between the heavy 15N isotope and the normal 14N isotope will be made using a Liquid Chromatography-Tandem Mass Spectrometer (LC-MS/MS) so that digestion kinetics at the intestinal phase can be determined allowing the interactions and mechanisms involved to be established. In addition, SDS-PAGE will be used to determine the impact of the ANFs on whole protein digestion.

Objective 2: Establish the effect of various processing methods on digestion inhibitors (ANFs) and the impact on IAA digestibility.

Processing techniques can remove or reduce many of the ANFs and positively impact on digestion. However, it is not known what protein: inhibitor proportions are needed. For the more complex digestion inhibitors such as fibre and phenolic metabolites (tannins) it is less clear if their reduction leads to improved digestibility, as during processing they can undergo modifications which could actually impair protein and IAA digestion. QTOF-MS will be used to quantify the concentrations of specific phenolic compounds that modify IAA digestibility before and after processing. A range of plant-based ingredients identified in objective 1 will be processed using typical processing methods available in our labs which fit the following criteria:

i. Cause significant modification of the ANFs,

ii. Applicable for household and/or industrial scale,

iii. Environmentally sustainable.

Processed products will be mixed with the isotopically labelled milk protein and analysed as described in objective 1. Overall the project will:

1. Evaluate the inhibition of protein digestion by different plant sources due to the presence of ANFs.

2. Determine the concentration threshold of ANFs above which digestibility is impaired.

3. Determine the impact of processing on digestibility thereby establishing suitable processing methods and ANF target concentrations for plant food formulation.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

73 Manipulating targeted genes to improve barley resilience: characterising novel environmental stress regulators.

Lead Supervisor: Guillermina Mendiondo

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: During the rotation the student will be received general training in molecular biology techniques apply to crops for example: growing plants under control and stress conditions, root sampling, root anatomy using vibratome sectioning and laser ablation tomography, confocal microscopy to image sections and image quantification using rootscan or automated tools based on machine learning algorithms, crops bioinformatics, CRISPR, western Blot, barley transformation (tissue culture). All these techniques will be a snapshot of techniques used in the main project.

Full Project Description: Background: Impact of changing climatic conditions on global crop production. We need to increase the population to feed the world but while reducing the reliance on fertiliser application. One solution is to develop varieties that have improved soil resource capture and thus improved yield under suboptimal conditions. Crop yields are globally affected by abiotic stresses such as drought, salinity and waterlogging. Ubiquitin-mediated proteolysis via the Plant Cysteine Oxidase (PCO) branch of the PRT6 N-Degron pathway controls the plant response by regulating the turnover of proteins involved in sensing and/or conferring tolerance to abiotic stresses. Root cortical senescence (RCS). Importance of cortical senescence towards this especially improving yield while reducing metabolic burden of maintaining cortical tissues under abiotic stresses. RCS is a very important breeding target for edaphic stress tolerance. The fundamental understanding about genes and mechanisms controlling this process is missing. Interestingly, we recently observed that mutants in PRT6 N-degron pathway have increased RCS. This project focuses on newly discovered and uncharacterized N-Degron pathway substrates to shed light on their specific downstream stress regulation functions, offering new opportunities for stress resilience research. We have previously identified two such substrates, BERF1 and RAF; this project will study

their specific roles as key regulators of environmental stress. A recent paper showed RAF and BERF1 were upregulated in a transcriptional analysis, which indicated their important role of these gene in the development of root cortical senescence (RCS). Thus, in this DTP project we aim to dissect this to improve our fundamental understanding about genes controlling this process and identify novel genetic components/solutions that could help us develop varieties tolerant to abiotic stresses.

Work plan:

Year 1: Map the development of root cortical senescence during drought in the mutant and WT. Decide zones and timepoints for sampling roots and perform RNAseq of mutant vs WT under control and drought stress and sample different zones, including RCS formation to understand genes expressed. Prioritise some candidates' genes based on previous work and its relevance in drought, etc.

Year 2-3: Develop CRISPR, RNAi, reporter lines to validate downstream targets of PRT6 N-degron pathway functioning in regulating root cortical senescence.

Year 3-4. Perform drought experiment in soil columns in Hounsfield facility. Harvest roots for Laser ablation tomography and see whether RCS formation is impaired in the mutant and its impact on the cost benefit of forming RCS in the mutant vs WT. Also study shoot physiological parameters using PhenoSpex trait finder in the Hounsfield facility.

Year 4: Deploy novel genetic solutions to breeding companies.

Project output and impact: This project focuses on newly discovered and uncharacterized N-Degron pathway substrates to shed light on their specific downstream stress regulation functions, offering new opportunities for stress resilience research. This project therefore combines fundamental research into understanding the drivers for abiotic stress resilience, with application to generate resilient barley for deployment.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

80 The Root to Net Zero

Lead Supervisor: Sacha Mooney

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The nine-week lab rotation will be based at the University of Nottingham. The student will receive one to one training in the operation, use and application of state-of-the-art X-ray Computed Tomography at the Hounsfield Facility. The student will be trained in sample preparation techniques, instrument test conditions and post scanning image processing.

The student will visit Rothamsted and sample intact soil cores from a multi-site experimental platform (the Large Scale Rotation Experiment (LSRE)) that tests the effect of reduced, tillage, crop diversity and organic amendments on multiple system properties. Cores will be taken from no-till+cover crop plots at the two sites to quantify the soil porous architecture and compared with cores from ploughed plots (conventional tillage). The project will then involve planting wheat into the soil cores for the initial growing phase (c.3-4 weeks) and assess the legacy effect of this soil

management in the response to the wheat roots in comparison to control soil cores (conventionally tilled soil). Specifically, how the wheat roots utilise biopores, reshape the rhizosphere in the no-till+cover crop soil cores, affect soil structure and consequently resource capture.

Full Project Description: Agricultural intensification coupled with a changing climate is causing soils to become increasingly vulnerable to stresses including drought, low nutrient levels, and compaction. Achieving sustainable intensification of agricultural production to feed 10 billion people by 2050, whilst reducing environmental impacts is a significant challenge. Changing diets and reducing food waste are part of the solution. However, as recognised in the UK government's Clean Growth Grand Challenge, significant green growth in the agri-food sector is also necessary to meet this demand without compromising other targets, in particular that of neutrality in carbon emissions by 2050.

Regenerative agriculture (which includes reduced tillage, cover cropping and diversified rotations) seeks to address these global issues and is rapidly expanding across the UK due to it benefits in enhancing soil health, incorporating fewer chemicals and pesticides, and reduced fuel and labour costs. Our recent research (Cooper et al., 2021) suggested duration under no-tillage can significantly enhance both the quantity and quality of soil carbon whilst simultaneously reducing the release of greenhouse gas emissions through a modification in the soil's architecture. However, little research has focussed on the response of roots to changes in soil structure across a gradient of regenerative practices, specifically the longer-term legacy effects throughout the remainder of the rotation. Observations show the importance of the soil-root interface, the rhizosphere, and the capacity of the rhizosphere to retain and/or transport water, nutrients, and as a source of greenhouse gases. However, our knowledge of the interactive effects across a gradient of regenerative practices, and their combined impacts on soil structure and the response of roots (e.g., via root exudation), and as a key mechanism of greenhouse gas release, remains unclear.

This project aims to addresses these key knowledge gaps at the soil aggregate, field and farm scales using the established LSRE at Rothamsted Research (one experimental platform based at Harpenden on a clay loam soil and a duplicated platform located at Brooms Barn on a sandy loam soil). This studentship will seek to address three key objectives:

1) Quantify the changes in soil structural properties and the impact on the transport of greenhouse gas release across a gradient of regenerative practices using state-of-the-art X-ray Computed Tomography facilities (Year 1).

2) Identify the differences in root and root exudation responses to a range of regenerative managements (Year 1 & 2).

3) Assess the interactions between soil structure, root responses and greenhouse gas release to identify key benefits and trade-offs of contrasting regenerative farming practices across spatial scales (Year 2 & 3).

This studentship will directly contribute to improving the sustainability and securing the longevity of UK cropping systems, and ultimately aid decarbonisation of primary production, with findings that are directly applicable to key policy strategies. In addition, the student will be able to expand their knowledge of soil-root interactions through exposure to ongoing relevant BBSRC programmes at Rothamsted Research (AgZero+) and the University of Nottingham (Breakthru).

Lab Rotation Location: Sutton Bonington Campus;Rothamsted Research;

Full Project Location: Sutton Bonington Campus;

85 Investigating the prebiotic potential of dietary fibre and polyphenolic compounds in non-alcoholic beer

Lead Supervisor: Stephen Lawrence

Lead School: Biosciences

DTP Research Area: Biotechnology

Lab Rotation Description: 1. Analysis of beers to determine their dietary fibre content and composition. Students will utilise High Performance Anion Exchange chromatography to separate and quantify the monosaccharides present in the samples.

2. Students will learn how to use the Grainfather brewing equipment to produce wort and then ferment into beer. Non traditional brewing materials that can provide dietary fibre will be incorporated into the wort. Samples collected from this will be analysed for standard beer analysis and dietary fibre determination.

3. In-vitro fermentation systems inoculated with gut microbiota samples will be used to allow laboratory-based analysis of the non-traditional brewing ingredients to provide dietary fibre. The ability of the gut microbiota samples to metabolise the materials will be determined by monitoring short chain fatty acids known to benefit intestinal and systemic health. These metabolites will be measured using GC-FID.

Full Project Description: The inclusion of dietary fibre in the diet has been shown to have numerous health benefits, helped in part by the wide range of structures and compositions of known dietary fibres. There are multiple mechanisms for fibre functionality through the digestive system that are related to their induced health benefits. These relate to the properties of the fibres including bulking, viscosity inducing and, of key interest in this project, fermentability. These fermentable fibres act as a source of energy for microorganisms in the large intestine, contributing to gut microbiota growth and diversity and the release of physiologically important metabolites such as sc-fatty acids (contribute to intestinal/systemic health).

Despite the health benefits, the average daily intake of dietary fibre remains below recommended amounts. Education can help but there is also an opportunity to develop products to fill this “fibre-gap” in modern diets. Of interest to this project is the incorporation of ingredients that contain significant amounts of dietary fibre into fermented beverages (beer). Due to its ABV, beer with an ABV>0.5% cannot support nutritional claims. However, the popularity of non-alcoholic beer is growing, and there is great interest in their potential to contribute to the intake of dietary fibre. Work in my lab has looked at the introduction of dietary fibre using non-traditional ingredients to expand the range of fibre types in non-alcoholic beer. The occurrence of these non-starch polysaccharides in brewed beverages has sparked discussion as to whether they could have physiological impacts.

Many of these non-starch polysaccharides are defined as prebiotics which have the potential to act as substrates for gut microbiota. This community of microbes ferments indigestible fibres, producing by-products of metabolism that are known to benefit intestinal (butyrate) and systemic health (propionate/acetate). However, information on the fermentation of non-starch polysaccharides in the gut is limited. The aim of this project is to investigate the potential of dietary fibres and polyphenols present in non-alcoholic beer to modulate the gut microbiota. This knowledge will benefit the growing health/wellness category present in most leading brewing companies and help with the promotion of non-alcoholic beer as part of a healthier lifestyle.

WP1–Characterisation of dietary fibre/polyphenolic compounds in beer.

WP2–Profiling of gut microbial community using phenotype microarrays. Microbial growth will be assessed in the presence of different fibre/polyphenolic compounds present in non-alcoholic beer.

WP3–In-vitro fermentation assessment of microbial use of dietary fibre/polyphenolic compounds. In-vitro fermentation systems inoculated with gut microbiota samples will be used to allow laboratory-based assessment of the different fibres/polyphenols as functional foods. The response of the microbiota will be assessed using high-throughput molecular-based methods (16S-rRNA gene sequencing/shotgun metagenomics/metatranscriptomics/metaproteomics) that allow characterization of microbial abundance and functional responses to different dietary stimuli.

WP4–Analysis of metabolic products generated during in-vitro fermentation. The breakdown of fibre/polyphenolic compounds will be characterised using untargeted metabolomics. Metabolomic data will be integrated with omics data to allow network-based analyses of microbiome responses to different dietary substrates. Outputs from this work will give mechanistic insights as to how different beer-derived substrates are broken down by gut bacteria.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

99 AI-based image analysis and computational modelling of the axolotl spinal cord during regeneration

Lead Supervisor: Osvaldo Chara

Lead School: Biosciences

DTP Research Area: Biotechnology

Lab Rotation Description: During this lab rotation, the student will be introduced to image analysis, computational modelling and programming languages and, specifically, Python. The student will learn different types of mathematical modelling approaches and will focus on computational models that are suitable to simulate biological tissues: agent-based models, in particular, cell-based models. The student will learn how to perform image analyses of tissues and learn how to simulate state-of-the-art cell-based computational models, in the context of development and regeneration of tissues. The student will also learn different statistical techniques to analyse model simulations and how to compare (qualitatively and quantitatively) the model simulations with experimental data. The student will participate of the Journal Club and Group meetings were she or he will learn how to present and discuss exciting new preprints as well as seminal articles of development and regeneration of tissues from a quantitative perspective.

Full Project Description: Context: In contrast to humans, salamanders like the axolotl can resolve severe and extreme injuries of spinal cord throughout complete and faithful regeneration. Although more than 250 years have passed since the original discovery of salamander tail regeneration after amputation by Spallanzani, the governing mechanisms underlying these unparalleled regeneration capabilities are not yet understood. In this interdisciplinary PhD project, we will investigate how the axolotl regenerates the spinal cord after amputation. This project is part of an international collaboration between the lab of Elly Tanaka, a world leader in regeneration in the axolotl at the Institute of Molecular Pathology in Vienna and our lab, which is the only modelling lab in the UK and

possibly in the world investigating regeneration of this salamander. Recently, our two labs demonstrated that tail amputation leads to a particular spatiotemporal distribution of cycling cells in the axolotl (Rost et al., 2016. eLife.5:e20357; Rodrigo Albors et al., 2015 eLife.4:e10230). By combining a new transgenic axolotl using FUCCI technology (AxFUCCI) with the first cell-based computational model of the regenerative spinal cord, we found that regeneration is orchestrated by a particular spatiotemporal pattern of neural stem cell recruitment along the anterior-posterior (AP) axis (Cura Costa et al., 2021. eLife.10:e55665). We predicted that this recruitment is executed by a hypothetical signal generated at the amputation plane of the spinal cord.

Project work plan: In this PhD project, the student will investigate the signalling mechanisms triggered by salamander tissue injury and how these signals are controlled. The student will pursue this project by combining image analysis and computational modelling, in close collaboration with our experimental colleagues. Since our collaborators are in Vienna, the student will need to visit that city during the course of the PhD. To quantitatively and mechanistically understand the regenerative response, the student will quantitatively analyse confocal images of AxFUCCI obtained by our collaborators and use AI methods to recreate the initial condition of a cell-based computational model of the axolotl spinal cord during regeneration. This image analysis will generate detailed knowledge of cell geometries and growth that will be embedded within the computational multicellular model, enabling accurate simulations of signalling mechanisms.

Significance and outcomes:

- While the immediate goal of this project is to mechanistically address how spinal cord regenerates in salamanders, the ultimate goal is to understand why this trait is absent in humans. Thus, the knowledge gained would pave the way for future therapeutic strategies within the emergent field of regenerative medicine.

- Furthermore, the AI-based image-analysis and modelling methodology developed in this project will be of wide interest for multiscale modellers. The techniques developed are relevant to many contexts where tissue-scale growth depends on cell proliferation, such as embryo development, cancer and plant growth.

- The project is highly interdisciplinary and international (with supervisors from three different Schools and collaboration with a leading experimental group in Vienna) broadening the research within the University and in this field.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;Jubilee Campus;University Park;

103 Characterisation of a novel set of wheat wild relative introgression lines for photosynthetic traits to improve food security and heat tolerance in wheat.

Lead Supervisor: Stella Edwards

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The rotation will focus primarily on the key processes of photosynthetic trait analysis using high throughput screening techniques, wheat husbandry, KASP genotyping and data analysis.

Weeks 1-2

A sub-set of the 400 lines of interest will be grown in the WRC glasshouses and growth rooms. Knowledge will be acquired of how to grow wheat and distinguish growth stages for reproducible measurements. Key molecular techniques such as KASP genotyping analysis (DNA extraction, PCR) will be learnt to characterise the genotypic basis of plant material tested.

Weeks 3-7

Key physiological techniques such as high-throughput Fluorcam to measure chlorophyll fluorescence kinetics and screen for heat tolerance. Infra-red gas analysis to measure photosynthesis will be taught during the screen for photosynthetic traits such as photoprotection and heat tolerance, providing a comprehensive data set for analysis.

Weeks 8-9

Data analysis and use of R programming language to analyse the data obtained. Post-rotation, lines of interest may enter a pre-breeding programme (wide crossing techniques, KASP genotyping, cytogenetic analysis (GISH) and sequence analysis to further characterise the traits of interest during the project.

Training: The student will obtain comprehensive interdisciplinary training in plant genetics, molecular biology, plant physiology and photosynthesis.

Full Project Description: Background

It is recognised that crops such as wheat, which feeds over 2.5 billion people world-wide providing 20-50% of daily calorie intake needs to be improved through finding novel genetic variation for key agronomic traits. One of those key traits is the improvement of photosynthesis, which is a significant challenge in crop research and the key to improvement of food security.

Wheat growth is limited by abiotic factors such as high-temperature stress with processes such as photosynthesis being severely affected, therefore variation for key photosynthetic traits is required. Wheat has insufficient natural genetic variation which stemmed from a genetic selection pressure during domestication, however, novel germplasm sources such as wheat wild relatives is a key source of variation in traits that wheat has either lost or never obtained. Wheat wild relatives were not subjected to this genetic bottle neck, so they still convey many of the traits that wheat requires.

From these wheat wild relatives through a pre-breeding programme undertaken at the BBSRC Nottingham Wheat Research Centre (WRC), over 400 homozygous lines of wheat containing novel genetic variation for many if not all traits of importance from distant wild relative introgressions have been produced (King et al 2022). Previously, it has been shown at the WRC that there is a vast array of genetic variation in the wheat wild relatives for photosynthetic traits (McAusland et al 2020) and in some of the early 23 introgression lines previously produced. Therefore, the opportunity to further screen over 400 novel homozygous introgression lines for major photosynthetic traits such as 1) heat stress, 2) night-time temperature tolerance, and 3) photoprotection through the high-throughput screening methods are available at UoN. Further genetic analysis in our pre-breeding programmes can lead to promising genetic variation in photosynthetic traits which have been previously unseen.

Aims and Objectives:

1. Physiological analysis. This project will take over 400 lines produced at the WRC with novel genetic variation from key wheat wild relatives and will be screened for major photosynthetic traits, including for heat-stress, night-time temperature tolerance and photoprotection in collaboration with Professor Erik Murchie and the WRC using high-throughput phenotyping, infra-red gas analysis and hyperspectral analysis.

2. Molecular characterisation and pre-breeding of lines of interest. Photosynthetic traits in lines of interest can be characterised further through a pre-breeding programme, to target the genes of interest using methods such as crossing, KASP genotyping, molecular cytogenetic and sequence analysis. Lines produced can be incorporated into wheat pre-breeding programmes world-wide, boosting genetic variation for photosynthetic traits in wheat.

Objectives Years 1 and 2

Screening 400 homozygous wheat wild-relative introgression lines produced at the WRC for photosynthetic traits. Techniques including high-throughput Fluorcam screening for fluorescence kinetics and heat stress. Infra-red gas analysis (Li-Cor6800) photosynthetic measurements, and hyperspectral analysis in the WRC glasshouses and growth rooms.

Objectives Years 3 and 4

Any lines of interest can be further developed through a pre-breeding programme to produce smaller introgressions without deleterious genes using techniques such as crossing, KASP genotyping, molecular cytogenetic analysis (GISH) and sequencing.

References:

King, J., (2022). Introgression of the Triticum timpoheevii Genome into Wheat Detected by Chromosome-Specific Kompetitive Alllele Specific PCR Markers

Front Plant Sci 13:919519 https://doi.org/10.3389%2Ffpls.2022.919519

McAusland, L., (2020) Variation in key leaf photosynthetic traits across wheat wild relatives is accession dependent not species dependent. New Phytol, 228: 1767-1780. https://doi-org.nottingham.idm.oclc.org/10.1111/nph.16832

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

105 RNA processing in the malaria parasite

Lead Supervisor: Ellen Nisbet

Lead School: Biosciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: Malaria kills about 400,000 children each year in sub-Saharan Africa. It is caused by the single-celled eukaryote Plasmodium, a member of the apicomplexa group of parasites which also include Toxoplasma. These parasites have an extraordinary evolutionary history, for they were once photosynthetic, and contain a remnant chloroplast, known as an apicoplast. Many commonly-used anti-malarial drugs work by inhibiting RNA transcription and protein synthesis in the apicoplast. However, there is rising drug resistance so new drugs are urgently required.

This project aims to discover how RNA processing occurs in the apicoplast. We have identified a number of essential proteins that control apicoplast RNA processing. One of these proteins is PPR1, which we have shown is essential for cellular survival. It binds to specific regions of RNA, preventing RNA cleavage from occurring. This protein has the potential to be a target for a novel anti-malarial drug.

In the rotation project, the apicoplast PPR1 protein will be expressed in E. coli, using previously published techniques. We will then take the protein to our collaborators at Leicester, to undergo preliminary CryoEM to obtain a structure. In parallel, PPR1:RNA binding assays will be carried out, to determine how it protects RNA from degradation.

For info, see

Hicks et al 2019 'An essential pentatricopeptide repeat protein in the apicomplexan remnant chloroplast': https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6899631/

Nisbet and Hicks 2016 'Transcription of the apicoplast genome' https://www.sciencedirect.com/science/article/pii/S0166685116300937?via%3Dihub

Full Project Description: Malaria kills about 400,000 children each year in sub-Saharan Africa. It is caused by the single-celled eukaryote Plasmodium, a member of the apicomplexa group of parasites which also include Toxoplasma. These parasites have an extraordinary evolutionary history, for they were once photosynthetic, and contain a remnant chloroplast, known as an apicoplast. Many commonly-used anti-malarial drugs work by inhibiting RNA transcription and protein synthesis in the apicoplast. However, there is rising drug resistance so new drugs are urgently required.

This project aims to discover how RNA processing occurs in the apicoplast. It brings together parasitology, molecular biology, proteomics and structural biology in the first step of the development of a new class of anti-malarial drugs.

Our research has shown that long, polycistronic RNA transcripts are synthesized in the apicoplast. These primary transcripts and are rapidly cleaved to mature RNA molecules. We have identified a key proteins involved in this process, an RNA binding protein (PPR1), a member of the pentapeptide repeat protein family. We have shown that the protein is essential, and have established a conditional knock-down cell line. In addition, we have established a heterologous expression system for it, and have shown that PPR1 is functional in vitro.

We have identified a number of essential proteins that control apicoplast RNA processing, including PPR1 (Hicks, eLife, 2019) and an RNAase. We now wish to see how these proteins work together to control RNA processing.

Depending on the interests of the PhD student, the options are:

- To examine how a PPR1 knock-down affects cellular morphology and life-stage progression (This is in conjunction with collaborator Prof Ross Waller, University of Cambridge)

- To obtain a structure of PPR1

- To characterize the newly-identified RNAse, and to determine if it interacts with the PPR1 protein.

- To identify further RNA-binding proteins in the apicoplast using the OOPs protocol (Queiroz et al, 2019: https://www.nature.com/articles/s41587-018-0001-2). Newly identified proteins can then be purified, and the genes encoding them knocked out.

For info, see

Hicks et al 2019 ‘An essential pentatricopeptide repeat protein in the apicomplexan remnant chloroplast‘: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6899631/

Nisbet and Hicks 2016 ‘Transcription of the apicoplast genome’ https://www.sciencedirect.com/science/article/pii/S0166685116300937?via%3Dihub

Lab Rotation Location: Sutton Bonington Campus;with trips to Leicester;

Full Project Location: Sutton Bonington Campus;with trips to Leicester and Cambridge;

108 The honeybee protein Royalactin: A key to keep mammalian stem cells pluripotent?

Lead Supervisor: Reinhard Stoger

Lead School: Biosciences

DTP Research Area: Biotechnology

Lab Rotation Description: cDNAs encoding Royalactin and its mammalian structural analogue, Regina, have been cloned into a piggyBacTM vector system and been successfully introduced into Embryonic Disc Stem Cells (EDSCs) of the pig. This has currently been confirmed by the presence of green fluorescence of cells (the piggyBacTM vector expresses a GFP reporter gene). The effect(s) of Royalactin and Regina on transfected EDSCs must now be evaluated. The rotation-project will involve 1) Propagation of Royalactin/Regina transfected EDSCs, by 2) Immunohistochemistry to determine the cellular localisation of these two proteins; 3) Use PCR-based gene expression profiling to determine the precise pluripotency state of the EDSCs. This will allow the student to become familiar with the laboratory and basic procedures, some of which will likely be used throughout duration of the PhD work.

Full Project Description: The fields of regenerative medicine and animal biotechnology offer exciting opportunities which are driven by advances in stem cell biology. Our knowledge of embryonic stem cells largely rests 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 an 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 aim of this PhD project is to explore the biology of the proteins Royalactin and Regina and their potential as universal ‘stem-cell-maintenance factors’, operative in all mammalian stem cells.

1. Sequence-verified vectors have been constructed using the piggyBac vector system, which is highly effective for inserting foreign DNA into the host genome of mammalian cells. These vectors

express either the gene encoding the honey bee 'Royalactin' or the pig (Sus scrofa domesticus) 'Regina' protein. Currently, pig Embryonic Disc Stem Cells (EDSCs) have been transfected. The effect(s) of Royalactin and Regina on the 'stemness' of pig EDSCs will be evaluated using a multitude of approaches and techniques. This includes assessment of morphology and replication time of transgenic EDSCs. The presence of markers specific to pluripotent cell states, as well as linage committed genes/proteins and self-renewal will be monitored by qRT-PCR and immunostaining.

2. To explore if the Royalactin/Regina mediated pathway(s) of pig EDSCs self-renewal is similar to the one described in mouse embryonic stem cells, genome-wide transcriptome levels will be established by RNA-seq from transfected and non-transfected pig EDSCs. Discovery of conserved gene-networks will motivate assessment of Royalactin/Regina self-renewal effectiveness in stem cells of other mammals, including human.

3. The project will also start to dissect the mechanistic action(s) of the proteins Royalactin and Regina: Their cellular location(s) will be monitored using immunofluorescence in cell lines expressing the genes encoding these potential pluripotency factors.

4. Structurally, both Royalactin and Regina are members of the β-propeller superfamily of proteins, and also form part of the subfamily of 6-bladed β-propeller proteins. Although the two proteins derive from different taxonomic groups, with no obvious amino-acid sequence homology, they share marked similarity in 3D structure, suggesting that this may play a functional role in maintenance of pluripotency. This part of the PhD project will aim to gain insight between structure and function of the two 6-bladed β-propeller proteins. Using state of the are molecular-biology techniques, hybrid proteins will be generated, containing propeller-structures found in Royalactin and Regina.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

115 THE ROLE OF HUMAN PROLYL OLIGOPEPDITIDASE (hupop) IN INFLAMMATION AND PARASITE INVASION

Lead Supervisor: Ivan Campeotto

Lead School: Biosciences

DTP Research Area: Biotechnology

Lab Rotation 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. Human POP has been shown to be involved in the regulation of many neurodegenerative disorders and in several inflammatory conditions. Thus, there is an increasing interest in targeting POP for the development of novel therapeutic agents.

HuPOP will be targeted for diagnostic and therapeutic applications using two strategies:

1. Co-crystallisation of new natural inhibitors with HuPOP, which are available in Dr Campeotto's group, and of fragment-based screening from compound libraries available at the Diamond Light Source

2. Characterization of anti-HuPOP monoclonal antibodies (mAbs) being isolated in Spring 2023 in Campeotto's group

During the lab rotation, HuPOP will be produced recombinantly in Dr Campeotto's group. Crystallisation screening will be performed at VMXi at Diamond Light Source with Dr Mikolaje to identify the best crystal-growth conditions. In addition, mAbs will be purified, screened, and ranked for binding affinity using Surface Plasmon Resonance in Campeotto's group.

Full Project Description: Importance

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 concentration is higher in the brain, compared to the rest of the body, and its activity has also been recently linked to inflammatory conditions and immune stimulation. However, its roles in these processes remain elusive.

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 to understand its role in inflammation.

Research background

HuPOP activity has been detected in human plasma and its sequence is 44% identical to Prolyl Oligopeptidases from the parasite Trypanosoma cruzi (TcPOP), which is also secreted in the blood and allows parasite invasion by degrading components of the extracellular matrix such as collagen and fibronectin.

TcPOP has been extensively characterised in Campeotto's group, it is essential for parasite invasion and a panel of new inhibitors are available from the collaboration with Prof Christian Gruber at the University of Vienna (Austria).

Interesting, preliminary data show that those compounds inhibit HuPOP and TcPOP with similar affinities, therefore, offering the possibility of targeting both enzymes at the same time with a potential synergic effect on both, parasite invasion and parasite-induced inflammation.

Additionally, anti-HuPOP mAbs, 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: Determine the crystal structures of HuPOP apo- and 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 (DLS) with Dr Mikolajek. 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.

Lab Rotation Location: Sutton Bonington Campus;Diamond Light Source;

Full Project Location: Sutton Bonington Campus;

132 Evaluating impacts of elevated CO2 and temperature on yield and grain quality in Barley genotypes

Lead Supervisor: Sofie Sjogersten

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: During the rotation the student will be received general training in growing plants under control and stress conditions, measurements in light-saturated net CO2 assimilation rate, stomatal conductance and transpiration rate (by Licor) in flag leaves, leaf relative water-content, grain yield and yield components, biomass, plant establishment, leaf conductivity and chlorophyll content. The student will gain experience in experimental design in laboratory and field techniques as well as in research best practices and conduct in the working place. The student will also gain experience in data analysis and bioinformatics. The student will develop research at two different levels simulation of field conditions alongside laboratory. The data generated will be used as part of the training in statistical analysis (using Genstat, mainly ANOVA and regression analysis), as well as in data presentation (using the dedicated graphics software SIGMA plot). All these techniques will be a snapshot of techniques used in the main project.

Full Project Description: Background: Rising atmospheric CO2 concentration and temperature are two main components of global climate change. Future food production might benefit from increasing levels of atmospheric CO2 if it can capitalize from the additional CO2. Since phenology, physiological responses, biomass accumulation, yield and grain quality are dependent on genetic factors, environmental variations and their interactions, identifying CO2-responsive traits would provide plant breeders with information to target traits to maximize the positive effects of elevated CO2, such as yield increases, and to minimize the negative impacts. Barley breeding will require good quality of seed under the new challenges to supply the industry. Targeting specific physiological pathways that increase grain quality would be highly desirable. We will screen commercial barley genotypes and barley lines manipulated genetically in the target pathway the response to the grain quality parameters of plants growing under high CO2 and high temperatures. We will grow a selection of commercial barley genotypes, barley landraces and barley mutants non-GMO (novel genetic pathways well characterized) under ambient and simulating climate change conditions (+Temperature and + CO2) in controlled environment growth rooms at UoN. Barley grain

parameters, plant physiological traits, including below ground carbon investments into the root system and the net carbon balance of the plant soil system traits will be quantified. The student will gain training in a wide range of state-of-the art plant genetical and phycological measurements as well as techniques for assessing greenhouse gas fluxes and carbon sequestration. This project will give the student the skills needed for developing climate resilient agricultural system that contributes to the UK's target of reducing the carbon footprint of agriculture.

Work plan:

Year 1-2: Screening newly developed genetic resources in barley (mutant lines non-GMO), landraces and commercial elite lines in the UK to evaluate their performance under ambient and simulating climate change conditions in controlled environment growth rooms. Physiological traits to be evaluated include light-saturated net CO2 assimilation rate, stomatal conductance and transpiration rate in flag leaves, leaf relative water-content, grain yield and yield components, biomass, plant establishment, leaf conductivity and chlorophyll content.

Year 3: Using the data from year 1-2 a subset of barley lines will be selected for assessment of the net ecosystem carbon balance, greenhouse gas emissions, and soil carbon sequestration and how they are impacted by +Temperature and +CO2. This will be tested using the controlled environmental growth rooms. In parallel, field trials of the selected barley lines will be used to determine the variation in the same parameters in field growing conditions. Seed quality parameters will be analysed

Year 3-4: Decide zones and timepoints for sampling barley grains and perform RNAseq in barley lines with contrasting response to high CO2 and temperature. Writing papers.

Project output and impact:

This project focuses on developing climate resilient agricultural system that contributes to the UK's target of reducing the carbon footprint of agriculture. The project is aimed at producing 2 to 3 high impact publications.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

135 Understanding crop traits in a complex environment: partitioning the G×E interaction by spatial scale.

Lead Supervisor: Murray Lark

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The lab rotation will give students experience of combined field and laboratory work integrated by a strong spatial statistical. Students will investigate the spatial scales at which physico-chemical properties (soil moisture, texture) of the soil exert an effect on the microbiome (assessed with MIR spectra) and the expression of plant physiology (e.g. stomatal conductance). This will use a spatially nested sampling design. Students will be introduced to nested designs for spatial sampling, and the analysis of data with the linear mixed model. This will be introduced with the R package integrated on the GitHub platform, a valuable research

environment regardless of their final project selection. They will use these methods to plan spatially nested sampling schemes across the UoN Farm.

Students will collect field data with proximal sensors and soil/plant samples for chemical and spectral analysis. Standard microbiology methods like PFLA and microbiome analysis will also be introduced. These data will then be analysed collectively on the Rotation GitHub site. The aim will be to characterise the spatial scales at which most variation in the observed plant trait is accounted for by the soil property. The final step will be drafting and writing a paper describing the exercise.

Full Project Description: A key step in taking biological innovations from the laboratory to scale is the discovery of gene•environment interactions (G•E) which control the expression of traits across the landscape. Environmental variation in the landscape is complex and occurs at multiple scales. At the broadest scales, for example, latitude constrains intercepted solar radiation over the growing season. At intermediate scales are variations in soil parent material and land use history, while at the finest scales microtopography and effects of soil macrofauna are commonly expressed. Some of these factors may interact with genetics in important ways, while other effects may just be additive, or insignificant overall. An effective methodology to study G•E must identify the interactions which matter for decision making (for example, can this new variety be recommended across all agroecological zones (AEZ), or only for soils of larger pH in certain AEZ?)

This project will develop such a G•E methodology and will trial it in tests of the effects of the rhizosphere microbiome on nutrient uptake by crops across the environmental variation of Malawi. The expression of traits will be captured in a linear mixed model in which environmental variation can be analysed into components of different spatial scale across the landscape. This will be based on methods of multivariate geostatistics and empirical basis functions which supervisor RML has developed and applied in other settings.

Recent research has shown that G•E interactions influence agricultural productivity. More specifically, we hypothesise that the interactions of germplasm and the microbiome of the rhizosphere are critical for plant nutrient uptake at different spatial scales. This project will entail the analysis of the bulk soil and root microbiome for selected soils, which then can be upscaled to the full variability of soils included using midinfrared (MIR) spectral analysis. Soil and microbiome characteristics can then be related to the nutrient uptake of closely related but contrasting crop genotypes through the spatial model.

Glasshouse (GH) trials will use sample soils from 150 – 200 sites on a loose transect N-S from the Nyika Plateau to the Shire Valley. In the GH trial we will measure the nutrient uptake response on contrasting varieties. Model so as to identify (i) the key spatial scale at which G•E is expressed (ii) any evidence of non-homogeneous variance between AEZ/soil types (iii) evidence for particular environmental properties associated with the important G•E effects.

On the basis of GH trials a limited number of sites will be selected for on-farm trials to examine the effects in situ and to provide data for analysis in a model with an interaction between genotype and a spatial environmental term.

In both sets of trials soil analysis will include the determination of standard soil characteristics with wet chemistry, the scanning with MIR sensors, and the characterization of the microbiome for selected soils (PLFAs, Amplicon sequencing). The latter can be used to establish calibration functions for the microbiome based on MIR scans which allows to characterize aspects of the microbiome for all soils.

Lab Rotation Location: Sutton Bonington Campus;Rothamsted Research;

Full Project Location: Sutton Bonington Campus;Rothamsted Research;Malawi;

136 Enhancing plant protein extrudate quality

Lead Supervisor: Jo Gould

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: Inclusion of ingredients that are high in protein typically reduce the expansion quality of the extrudate and thus reduce consumer acceptance – however scientific principles of the reduction are unknown and vary by ingredient. This lab rotation will evaluate the relationship between plant protein quantity (as measured by nitrogen analysis) and extrudate quality (as measured by density and sectional expansion ratio) of blends of commercial plant protein isolates and maize, at varying ratios. Utilisation of a range of plant protein sources will enable the selection of exemplars for further PhD study. Due to the time limit of the lab rotation and requirement for ethical approval for sensory testing, the lab rotation will not include sensory testing of the extrudates. However, the student will be able to be part of ongoing sensory research tests during the rotation.

Full Project Description: Extruded snacks or ready- to- eat (RTE) products are a desired format with an enhanced shelf life generally manufactured from maize, wheat, potato or rice. The processing of these products converts the starch into a readily digestible format. Growing demands to diversify crops usage and enhance protein consumption has promoted the use of legumes in formulations. Extrusion processing is also an ideal candidate to enhance the quality of plant protein through changes in digestibility as well as blending with cereals to enhance essential amino acids content.

Inclusion of ingredients that are higher in protein for example legume flours (20-40% protein) than cereals, typically reduces the expansion quality of the extrudate. However, preliminary work has shown that legume flours from different botanical sources have very different expansion properties despite having similar bulk composition, identifying the need for further study to evaluate microstructural factors. In addition, introduction of plant protein has also been shown to introduce off flavours and taints which in combination with a reduced expansion reduces consumer acceptance.

This PhD project will investigate the blending of plant protein with cereals on extrudate quality with the aim of identifying the molecular and microstructural transformations during extrusion and the impact of these on the consumer acceptability and digestibility of the extrudates.

Objective 1: Elucidate feed composition and microstructure requirements to enhance expansion properties of plant protein extrudates.

Commercially available milled raw materials, air classified protein fractions and protein isolates will be blended with cereal bases at differing ratios and extruded using our ThermoPrism twin-screw extruder using temperature, shear and water content as major variables. Two different screw configurations will also be used. Samples, post die and along the barrel, will be assessed for protein and starch transformations and interactions including the thermorheological properties. Stained confocal images along with microCT will be used to track changes in the microstructures and

correlate these with measurements of extrudate quality (density, sectional expansion index, texture analysis).

Objective 2: Evaluate the sensorial properties and protein digestibility of the extrudates.

The sensory characteristics of a selected number of samples will be evaluated by trained sensory panel, at the same time, protein digestibility, ant nutritional content will be evaluated. These data will be correlated with instrumental analysis data to identify an optimum microstructure for production of a plant protein extrudate. Identification of an optimum microstructure for production of a plant protein extrudate will be used to reverse engineer those microstructures in a range of materials using different hydration, milling, thermal and pressure processing parameters.

Objective 3: Masking off-flavours produced from the extrudates. Many of the plant proteins are known to have an astringent/drying sensation or have a strong flavour. One protein source will be selected based on the digestibility and sensory outcome from objective 2 to further explore the effectiveness of how other ingredients (e.g. flavours) and processing parameters can be used to mask the off flavours.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

144 Improving light interception and photosynthesis in wheat by exploiting newly available phytohormone architecture mutants

Lead Supervisor: Alexandra Burgess

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: Using available wheat accessions at Sutton Bonington campus, this lab rotation will help form the foundation of the skills required in plant phenotyping, covering techniques spanning plant science and computer vision. Candidates will have access to training for in silico canopy analysis including robot-aided image capture, 3D reconstruction and an introduction to deep learning with access to state-of-the-art machines, with the option, should the student desire, more in depth training in Python, in particular PyTorch. In addition, candidates will be trained in plant physiological techniques including gas exchange and chlorophyll fluorescence. Other techniques include plant propagation and husbandry in both the glasshouse and the field, growth assessments of wheat, principles of photosynthesis analysis. In addition, remote sensing using spectroradiometry will also be accessible if desired.

An initial preliminary experiment will be set up prior to the rotation to give candidates access to the latter stages of wheat growth covering full canopy closure and yield production. Dependent upon rotation timing, it is likely this will predominantly focus on glasshouse grown plants although field work may be feasible. Opportunities will be available to meet the full supervisory team, including, where possible, a trip to Rothamsted.

Full Project Description: Canopy architecture, the arrangement of leaf and stem material in 3-dimensions (3D), was a property of green revolution plants that transformed global food production, staving global hunger. In cereals, this predominantly arose from the introduction of dwarfing genes,

targeting phytohormone pathways to reduce overall plant height but increase harvest index and yield. The group at Nottingham produced some unique theories regarding the effects dwarfism on function, for example that it has hampered movement (particularly in response to wind), restricting light distribution to lower canopy layers and thus inadvertently reducing photosynthetic potential. Further, recent research has shown that crop structure has not been optimised for photosynthesis which is currently limiting yield (e.g. Murchie, 2022; Long, 2022). Wheat presents a model plant in which to assess this.

This project combines the work at Rothamsted on hormonal control of architecture and the expertise in Nottingham in 3D imaging of canopies and photosynthesis analysis.

Using a selection of wheat phytohormone mutants developed at Rothamsted this project allows us to directly test the hypothesis that dwarfing genes have created a less favourable canopy structure for photosynthesis. It will uniquely combine analysis of architectural variants with a known genetic origin and thus directly link canopy architecture and function. Mutants have been developed that are known to differ in plant height or leaf angle but high-resolution analysis of these lines has not yet occurred. Combining plant physiology with state-of-the-art image analysis approaches, this project will uncover the links between structural traits, light patterning and photosynthetic function. Dependent on the skillset of the candidate student, opportunities are also available to assess the molecular and signalling pathways underlying phytohormone-induced changes in architecture.

Following a detailed structural analysis of the existing phytohormone mutants, the highest performing lines will be identified using traits such as total light interception, radiation use efficiency and canopy carbon gain. The project is predicted to identify traits relating to plant height and/or leaf angle that facilitate a more efficient canopy light environment for use in future breeding programmes.

Specific objectives of the project are:

1) To capture detailed physiological traits of existing phytohormone mutants including structural information, canopy light interception and photosynthesis. This will make use of a bespoke robot-aided image capture system combined with in silico modelling and image analysis techniques including deep learning. Plant physiological techniques will include chlorophyll fluorescence and gas exchange to capture photosynthetic productivity.

2) Using field trials at both Rothamsted and UoN combined with new video analysis techniques, test the hypothesis that the introduction of dwarfing genes in the green revolution created a less favourable light environment

3) Identify beneficial structural traits from phytohormone mutants to facilitate greater light interception. This will combine both physical plant measurements as well as in silico modelling, including ray tracing and empirical modelling of photosynthesis to predict optimal canopy structure.

4) Optional: determine the underlying molecular and signalling pathways that result in the altered phenotype using expertise and techniques available with the Thomas Lab at Rothamsted.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;Rothamsted Research;

148 Superfood Quinoa: unravelling the underlying mechanism of the high nutritional quality of quinoa

Lead Supervisor: Tristan Dew

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: Background: During this rotation, the student will explore the effect of a range of post-harvest processing operations on the nutritional value of several quinoa varieties. This will include several common forms of cooking, as well as operations intended to improve protein content such as a controlled germination process. During this time, the student will develop key skills in proximate food analysis, applicable to a wide range of plant, food and nutrition research activities. The student will also receive advanced training in the operation and maintenance of LCMS equipment, and the interpretation of associated chromatographic and spectral data. The latter represents an opportunity to acquire specialist knowledge applicable to a wide range of life science related research disciplines.

Week 1: Health and Safety and Lab inductions, generation of risk assessments

Week 2: Advanced LCMS training

Week 3: Generation of post-harvest processing samples (inc. germination, boiling, steaming, microwaving)

Week 4-6: Proximate analysis of quinoa samples (total lipids, carbohydrates, protein)

Week 6-8: Amino acid profiling of quinoa samples

Week 9: Write-up and data interpretation

Full Project Description: Quinoa (Chenopodium quinoa) is an excellent source of protein (12-16%) which, unlike traditional cereals and most plant foods, is nutritionally complete thanks to its provision of all essential amino acids. The lipid content of quinoa is mostly unsaturated, and it is a superior source of carotenoids and b vitamins compared to most cereal crops. Quinoa also contains phytosterols, displaying hypocholesterolaemic capacities in human intervention studies (Farinazzi-Machado et al., 2012; de Carvalho et al., 2013), plus a wide range of phenolic acids, polyphenols and betalaines with antioxidant and anti-inflammatory properties.

Quinoa is predominantly grown in the Andes at high altitude, with 92% of 2008 world production taking place in Peru and Bolivia (FA0, 2013). Quinoa is genetically diverse and highly adaptable with over 250 assessions (Piñuel et al., 2019) and is grown in more than 70 countries worldwide (FAO 2022). The nutritional value of quinoa has been shown to differ between geographical zones (Covarruvias et al., 2020) however, it is unclear whether this relates specifically to strain, soil quality or farming practice (such as irrigation; Walters et al., 2016). In addition, post-harvest operations such as germination, have been shown to significantly increase the protein and polyphenol content of quinoa grains (Choque-Quispe et al., 2021).

Unlike animals, whose body plans are set during embryogenesis, plants maintain the ability to initiate new organs throughout their life cycle. This developmental plasticity allows the plant's root system to optimise uptake of available nutrients and thereby significantly contributes to the nutritional quality of the seed. Root architecture development is shaped by plant hormones, the most important ones being auxin and cytokinin. We will assemble quinoa strains representing a

range of geographical locations and growing conditions, grown under standardised conditions. We will establish LCMS methods to quantify plant hormones (of considerable value to stakeholders across School of Biosciences) and correlate nutritional quality to assession specific differences such as root hormone levels and/or root architecture. In this way we will be able to determine which factors regulate nutritional quality. This research will aid farmers / policymakers to maximise the nutritional benefits of quinoa to local populations.

AIM 1: Investigate the nutritional profile of quinoa strains, before and after processing (e.g. boiling, germination)

- Proximate analysis (total lipids via Soxhlet fat extraction, total protein via elemental nitrogen analysis, carbohydrates via combination of total fibre, reducing sugars assay, total starch via amyloglucosidase / a-amylase)
- Profiling of amino acids, vitamins and polyphenols via LC-MS
- Analysis of antinutritional factors, including phytates and tannins via LC-MS

AIM 2: Investigate the phenotypic differences between quinoa strains grown under standardised conditions

- Establish auxin and cytokinin quantification in plant tissues
- Assess auxin and cytokinin profiles of root tissues
- Traditional phenotyping of root traits

AIM3: Establish why is quinoa more nutritious than other cereal crops

- Correlate datasets from AIM1&2
- Draw conclusions if quinoa nutrient uptake can be optimised by hormone regulated modulation of root architecture and / or through processing
- Explore which other factors add to improved nutritional content of individual quinoa assessions

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

155 Defensive mutualism between plants and an insect pathogenic fungus and its applications in plant pest and disease control

Lead Supervisor: Almudena Ortiz-Urquiza

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The insect pathogenic fungus Beuveria bassiana can establish mutualistic interactions with varied plant species as an endophyte. B. bassiana-colonised plants show increased resistance to pests and fungal diseases. However, the underpinning biochemical and genetic mechanisms are understudied. B. bassiana may protect plants against herbivore pests and diseases directly (e.g., production of toxins and antimicrobials, direct parasitism of insects, or competition for

space and nutrients) or indirectly via activation of the Jasmonic acid (JA) and the salicylic acid (SA) pathways, which boost plant immunity. The rotation will probe some of these hypotheses. From this rotation, the student will gain experience in i) growing and monitoring plants in a controlled environment, ii) handling fungal cultures and plant inoculation, iii) basic molecular techniques (e.g., primer designing, RNA extraction, cDNA production, and qPCR), and iv)) microscopy.

Weeks 1-4: Examining the expression of B. bassiana putative insecticidal toxins during plant colonisation via qRT-PCR.

Weeks 5-8: Identifying changes in the JA and SA pathways upon fungal colonisation. The student will examine gene expression of relevant genes in both pathways via qRT-PCR, using B. bassiana wild-type and SA mutants and Arabidopsis mutants and reporter lines.

Week 9: Reviewing literature and report writing.

Full Project Description: Background

The insect pathogenic fungus B. bassiana can establish intimate associations with many plants. The acquisition of insect pathogenicity in B. bassiana arose last in the multitrophic association among B. bassiana, its plant partners and insect hosts as an opportunistic adaptation allowing the fungus to barter nutrients with its host plants. B. bassiana negatively impacts arthropod pests feeding on colonised plants and safeguards plants from pathogens. Nevertheless, most studies reporting on such benefits for the plant are purely descriptive or show little mechanisms. Increased plant immunity upon fungal communication is common “side effect” of mutualistic fungi, including mycorrhizal fungi. However, genetic transformation is unattainable for most mycorrhizal fungi, and traditional mycorrhizal associations are scarce to non-existing in tractable model plants like Arabidopsis thaliana. The unique lifestyle of B. bassiana (saprophyte, parasite, and mutualist) and its genetic tractability provide an ideal system to examine different aspects of defensive mutualism (protection against predators and parasites in exchange for shelter and food) between endophytic fungi and plants.

Aims

This project aims at understanding and exploiting the molecular mechanisms driving mutualistic fungus-mediated enhanced plant immunity.

1. Interrogating the mechanisms underlying B. bassiana’s protective effect(s) against insect pests.

2. Dissecting the antagonistic molecular mechanisms of endophytic B. bassiana against phytopathogenic fungi.

Rationale

Endophytic B. bassiana confers its host plants with systemic protection against insect pests. Such protection against insects might be orchestrated directly (e.g., direct parasitism through the acquisition of spores from the plant surface or production of toxins within the plant) or indirectly by activating the antiherbivore response (jasmonic acid (JA) pathway) in colonised plants. Similarly, indirect and direct antagonisms have been proposed to explain endophyte-mediated pathogen resistance. Direct antagonism includes antibiosis and competition. Indirect antagonism refers to the activation of the host response, namely systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is salicylic acid (SA)-dependent, while ISR is triggered by jasmonic acid and ethylene (ET).

Work plan

Year 1-2. Characterisation of the indirect B. bassiana-mediated resistance against phytopathogenic fungi in Arabidopsis and wheat via i) RT-qPCR analyses of specific SA, JA and ET marker genes in B. bassiana inoculated plants with increased or impaired production/perception of SA, JA and ET. ii) In planta phenotypic characterisation of wildtype and fungal mutants against phytopathogenic fungi.

Year 2-3. Assessment of the ability of endophytic B. bassiana to control aphids through direct parasitism and/or production of toxins. This includes insect bioassays and behavioural studies using inoculated and non-inoculated plants and dissecting the putative insect toxicity of B. bassiana cry-like toxins and heat-labile enterotoxins through RT-qPCR, construction of targeted-gene knockouts, recombinant expression, and protein purification.

Year 3-4. Examining plant signalling via volatile organic compound (VOC) production upon endophytic colonisation. Endophyte-derived/induced VOCs could enable herbivore recognition, recruiting of insect predators and parasitoids, and plant-to-plant communication through VOCs. We will examine the mode of action of B. bassiana VOCs through VOC collection, GC-MS analysis, wind-tunnel assay on aphids and their natural enemies (e.g., lacewing and parasitoid wasps), and characterisation of the antiherbivore response (e.g., JA pathway) in receiver plants (non-colonised plants exposed to VOCs).

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

156 Nottingham-Adelaide joint PhD Exploring the impact of carbonation and sugar type on appetite control and risk of obesity

Lead Supervisor: Qian Yang

Lead School: Biosciences

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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 (FI) and weight gain (WG) has rarely been studied. One study in rodents explored CO2 effects on appetite regulation and reported increased FI, WG and ghrelin release and supported by a small human study where carbonated beverages increased ghrelin release over non-carbonated beverages. Ghrelin a 'hunger hormone', stimulates appetite and promotes FI and is a reward stimulator. If carbonation increases ghrelin release it could be a key factor behind global increases in obesity.

This project will therefore investigate the impact of carbonation alone or with different sugar types (SS and NSS) on appetite sensation, FI 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.

Professor Amanda Page is an internationally recognised neuroscientist with expertise in animal modelling research allowing access and training to metabolic monitoring systems key to the project. The Sensory Science Centre has renowned facilities and expertise in sensory and consumer research. The student will work with a multi-disciplinary UoN and UoA team.

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 (12-30 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.

Study 3 (30-48 month – 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 FI and WG 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 FI and WG.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;University of Adelaide ;

157 Delivering win-wins for conservation and production in oil palm plantations: Determining links between habitat restoration, biodiversity, and yield

Lead Supervisor: Sarah Luke

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: Using existing ecosystem function data collected from the Biodiversity and Ecosystem Function in Tropical Agriculture (BEFTA) Programme experimental field sites in Riau, Indonesia, the student will conduct an analysis of how rates of ecosystem function vary in relation to oil palm plantation management. Specifically, this will consider whether areas that are managed for greater habitat complexity and biodiversity – including restored riparian buffer areas, and areas with greater levels of understory vegetation – have varying rates of ecosystem functioning compared to plantation areas that are managed using standard practices. For students wishing to continue to the full PhD, this will provide an introduction to the research context for the full project, including developing understanding of the experimental set-up, and learning skills in statistical analysis that will be applied throughout the project. For a student not wishing to continue to the full PhD, the statistical and experimental design training will provide valuable background to a range of other PhD projects and future work. This rotation project will provide the basis for a chapter of the full PhD, as well as a highly publishable stand-alone paper, boosting the research track record of either a continuing or non-continuing student.

Full Project Description: Oil palm is currently grown across more than 18 million hectares of the tropics. It has the highest yield per hectare of all vegetable oil crops, and therefore forms a critical component of global food security. However, the expansion of the industry has driven large-scale loss of tropical forest, and associated declines in biodiversity and increases in carbon emissions. To minimise the environmental impacts and maximise the food security benefits of palm oil production, plantation management needs to maximise yield per hectare, but minimise negative impacts on the environment. An essential requirement for long term sustainability is to find options for ecological intensification - supporting biodiversity that delivers yield benefits within plantations. Within this, finding ways to manage plantations to maximise delivery of pest control, pollination, and healthy soil dynamics is likely to be key. Although agronomic approaches for delivering these functions are well developed by the oil palm industry, there has been little investigation of the role that biodiversity plays in these, or ways that oil palm landscapes can be managed to support ecosystem services and yield.

Based within an established research programme in industrial oil palm plantations in Riau, Indonesia (the Biodiversity and Ecosystem Function in Tropical Agriculture (BEFTA) Programme), this project will consider the effect of plantation management practices on delivery of key yield-supporting ecosystem functions. Working in close collaboration with oil palm industry scientists from the Sinar Mas Agro Resources and Technology Research Institute (SMARTRI), the student will travel to Indonesia to collect field-based data from a series of well-established experimental sites in which large-scale manipulations of understory vegetation complexity and tree planting have been conducted. Data collection by the student could include a range of management options, taxa, and ecosystem functions, depending on the interests of the student. As well as offering a variety of options for new field-based data collection, the well-established field sites also offer an exciting opportunity to link with >8 years of existing data sets. Available data include information on

environmental conditions, biodiversity, rates of ecosystem functions, and yield. Through this highly collaborative, fieldwork- and data-based approach, the student will develop skills in tropical ecology, agronomy, experimental design, data collection, and statistical analyses, as well as developing awareness and understanding of the oil palm industry.

Results from this project will give valuable new insights into landscape-scale management options for delivery of ecosystem functions and maximising yield in oil palm systems. This will not only provide novel insights into the ecological dynamics of human-modified systems, but also bring substantial sustainability and food security benefits. Through our strong existing collaborations with SMARTRI and IPB University, and oil palm sustainability organisations such as the Roundtable on Sustainable Palm Oil (RSPO), findings from the project will be shared directly with growers and policy makers, to deliver important bioscience evidence for increased sustainability of agriculture and food security.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

160 Nottingham-Adelaide joint PhD: Does pregnancy alter sweet taste perception and sweet food cravings?

Lead Supervisor: Sally Eldeghaidy

Lead School: Biosciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: A key component of this PhD is to assess food cravings 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 6-9: Data analysis and further survey optimisation. 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.

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 is world leading in human brain imaging research and the UoA team (Page and Young) internationally renowned 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 UoN, Year 2 UoA): 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. 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 3 and Year 4 UoN): 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.

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

Lab Rotation Location: Sutton Bonington Campus;Online ;

Full Project Location: University Park;Sutton Bonington Campus;

162 Investigating spike and leaf hormone traits for improved abiotic and biotic stress tolerance in wheat

Lead Supervisor: John Foulkes

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: This rotation project will be lab/glasshouse based and will use eight genotypes from the wheat DFW Breeders' Toolkit panel which will be exposed to Zymoseptoria tritici causing Septoria tritici blotch (STB) on wheat as the biotic stressor, or drought stress. Plants will be grown in 2 L pots with four biological replicates (and technical replicates as required for growth-analysis samplings). Plants will be maintained with a day/night temperature of 22/15 °C (16/8 h) with photoperiod extended to 16 h by low-intensity quartz-iodide lights. Physiological traits associated with abiotic and biotic stress tolerance (leaf SPAD, leaf photosynthesis rate (using Licor 6400 gas exchange analyser) will be assessed in addition to disease, and plant biomass, spike DM partitioning and floret fertility (Waddington scale) at mid booting and anthesis. The student will test the hypothesis that genetic variation in leaf photosynthesis rate, plant biomass and spike DM partitioning determines disease and drought tolerance consistent with responses of floret fertility to abiotic or biotic stress.

Full Project Description: Plant hormones regulate the plant response to both abiotic and biotic stresses and identifying key regulatory networks that cooperate to increase the tolerance of plants in multi stress environments can lead to significant crop improvement. Strategies to enhance drought and disease stress tolerance in cereals should include traits that maintain grain number during stressful conditions (DaCosta et al., 2021). There is increasing evidence that grain number is regulated by spike cytokinin levels both under optimal (Ashikari, 2005; Li et al., 2018) and abiotic or biotic stress conditions in wheat. Cytokinins play a key role in the stimulation of cell division and are known to be associated with grain number and leaf senescence. Rapid spike growth occurs from booting to anthesis, and is a key trait which determines final grain number (Slafer et al., 2015). In our recent research we have shown that grain number was correlated with spike cytokinin levels at booting and anthesis CIMMYT spring wheat lines under well irrigated conditions (Love, 2022). Further research is now required under to validate spike hormone traits and identify regulatory networks with other phytohormones for their deployment in wheat breeding to increase abiotic and biotic stress tolerance.

Drought and disease stress can directly affect grain number due to enzyme impairment and photosystem damage leading to reductions in spike fertility and seed set. Variation in sensitivity to environmental stress between booting (meiosis) and anthesis impacts upon grain yield through reduced grain number. Environmental stress affects metabolic regulation via perturbation of spike hormones (cytokinins) homeostasis affecting fruiting efficiency and grain number.

The objectives of the project are to :

(i) Develop an LCMS/MS assay for phytohormones (including zeatin, zeatin riboside, IPA (isopentenyl-adenosine) and 2iP (isopentenyl-adenine) in wheat)

(ii) Identify and validate associations between spike hormone levels, and grain number and grain yield under drought and/or STB disease stress with a focus on cytokinin responses

(iii) Define regulatory phytohormone networks in response to multi stresses and relate these to physiological and tolerance responses

In year 1 Targeted metabolomic assays for wheat leaf and spike samples will be developed to quantify multiple phytohormones including cytokinins (CK) classes of CK (zeatin, zeatin riboside, IPA (isopentenyl-adenosine), 2iP (isopentenyl-adenine) simultaneously using ultrahigh-performance liquid chromatography coupled to mass spectrometry.

In years 2 and 3, a specific subset of genotypes of BBSRC DFW Breeders Toolkit panel will be evaluated under different environmental stress conditions. Measurements will include disease assessments, photosynthetic and photoprotective traits, biomass, spike partitioning index at anthesis, SPAD flag-leaf senescence kinetics, grain yield, HI, grain number and yield components. Leaves and spikes will be sampled at booting and anthesis for assessment of phytohormones, spike cytokinins (zeatin, zeatin riboside, IPA). Results identifying the most promising leaf and spike hormone traits and establishing glasshouse and field protocols for their measurement will lay the groundwork for future genetic studies and marker development.

Ashikari et al. (2005) Science, 309(5735), 741-745. . doi: 10.1126/science.1113373.

Da Costa et al. (2021). Rice Science, 28, I233-242. doi.org/10.1016/j.rsci.2021.04.003.

Li et al. (2018). Hereditas 155, 33. https://doi.org/10.1186/s41065-018-0071-7

Love, B. (2022) PhD thesis. Investigating effects of plat hormones on wheat grain partitioning and yield potential in wheat, University of Nottingham. 257 pp.

Slafer et al. (2015). Food and Energy Security 4, 92-109.

Zavaleta-Mancera et al. (2007). J. Plant Physiol. 164, 1572–1582. doi: 10.1016/j.jplph.2007.02.003

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

170 The impact of nutritional insecurity on health outcomes. How might this be mitigated?

Lead Supervisor: Simon Welham

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The lab rotation will be conducted in two parts. The first will involve participation with ongoing work which aims to enable identification of individuals in need of nutritional support from survey and biological data. Data from live or recently completed food insecurity studies will be analysed to try and determine biological markers (urinary, salivary or hair analysis) and behavioural indicators of progressive food insecurity in an attempt to identify those in need of support who slip through the administrative net. The second part will be conducted in the N/Lab and will join with an ongoing project looking at significant dietary change and how this impacts nutritional intakes from purchasing records. For this second component guided analysis of massive (4 million customer) shopping datasets will be employed. All procedures will be carefully supported and appropriate training will be provided.

Full Project Description: Food insecurity is a worsening problem in the UK. Many of the most economically disadvantaged in society resort to diets composed of cheaper, often processed foods rich in saturated fat, sugar and salt and lacking in fruit and vegetables. Such diets strongly associate

with chronic disease and impaired growth and development in children. For those at the lowest income scales there is a growing incidence of targeted fasting, often to enable food provision for children, as well as frank starvation. Among adults, these exposures are physically and psychologically damaging, whilst for children, they are often worse and more long lived. Exposure to maternal nutrient deprivation during gestation adds additional complications in that offspring are faced with lifelong, programmed, health consequences. The nature of the health outcomes are varied and dependent on context, but in all cases, whilst they may be potentially resolved in the short term, there is permanent damage that will impact later life. It is only in recent years that food insecurity has been seen as a significant UK relevant issue, but there is now an urgent need to determine the extent of the dietary deprivation and the level of its impact on health.

In this project we will look at the relationships between deprivation, diet diversity and diet quality reduction alongside indices of poor health and explore the effectiveness of national and local interventions in mitigating the worst outcomes.

Key research questions:

How are nutrient intakes, particularly micronutrients, impacted in poorer communities?

Is there an identifiable association between chronically low micronutrient intakes and ill health currently apparent in the UK?

Can specific support mechanisms be identified that would enable cost effective assistance to be provided to those most in need and are those already in place, effective?

Methodology.

This project will make use of nationwide and local surveys similar to previous work by our group (Thomas et al. https://doi.org/10.3390/nu14235078) in which dietary intake data alongside markers of food insecurity and health will be collected. Within this work we will aim to access as broad a range of people with limited resources as possible to enable inclusion of those on low incomes but with earnings which exceed the limits for government support.

Local populations will be studied in greater detail to assess clinical markers of nutritional status and consequent health outcomes. Within this group, it will be possible to explore in detail the effectiveness of locally applied interventions both from independent social groups and via Nottingham City Council's Food Charter pledges.

Work using proprietary datasets will also be carried out via the N/Lab in which profiles of nutrient purchases will be mapped nationally against indices of health outcomes. We have unprecedented availability of loyalty card transactional data relating to over 4 million households from a major UK supermarket, which through previously funded research has already been paired with nutritional information, thus supporting massive summarisation of dietary intake for customers (both temporally, over a period of 3 years; and spatially, across the UK), which can now be paired with localised health outcome data. These data will be used to assess regions of food insecurity and associated poor health at fine scale across the country. It is anticipated that the outcomes of this section will be informative for local and national government to help in the targeting of resources for intervention.

Lab Rotation Location: Sutton Bonington Campus;Jubilee Campus;

Full Project Location: Sutton Bonington Campus;Jubilee Campus;

181 Vegans and seaweed: enabling seaweed to become an effective dietary source of iodine by targeted processing to diminish its abundance.

Lead Supervisor: Simon Welham

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The laboratory rotation will involve pilot testing of some of the processing techniques that will be employed in the main project to reduce the seaweed iodine content. Frozen and dried seaweed of different species will be subject to microwave treatment and "popping" for varying durations and temperatures. Processed samples will be assessed using inductively coupled plasma mass spectrometry (ICP-MS) to determine iodine content. This will allow preliminary data regarding efficacy of treatment to be assessed. Broader mineral content will additionally be assessed to determine if effective iodine loss is associated with loss of other nutritionally valuable minerals. Macronutrient analyses will additionally be carried out using proximate analysis to determine loss of carbohydrate, protein and fat. The data generated in the rotation will provide an early indication of the level of treatment required to diminish the iodine content of seaweed and help to establish whether there are species differences in the response to such treatments.

Full Project Description: Vegan and vegetarian diets have gained popularity over the past decade. Currently, well-planned vegan and vegetarian diets are regarded by the British Dietetic Association and other organizations in industrialised countries to be suitable throughout the lifespan, inclusive of infancy and pregnancy. However, concerns have been raised regarding the ability of these diets to adequately provide essential micronutrients, such as iodine.

Iodine is an essential micronutrient, required in trace quantities for the synthesis of thyroid hormones—triiodothyronine (T3) and thyroxine (T4). The thyroid hormones are crucial for the regulation of metabolism, growth, and neurological development. Iodine deficiency presents as a spectrum of clinical disorders termed 'iodine deficiency disorders' (IDD's) which occur when recommended intakes are not achieved (150 μg day−1). These include hypothyroidism, goitre and abnormal thyroid nodular pathology in adults. In infants maternal iodine deficiency impairs fetal growth, in particular neurological development which at its most severe can result in cretinism. Excessive iodine intake (>1000 μg day−1) on the other hand may lead to hyperthyroidism in individuals with preexisting thyroid disease or prior iodine deficiency.

In industrialised countries where people consume a 'Western diet', the key dietary sources of iodine are cow's milk, and dairy products. Seafood, eggs, and seaweed are also iodine rich but are not regularly consumed. Water and salt iodination strategies are present in most states in the US and select countries in Europe, but countries such as the UK have yet to establish a mandatory salt fortification program and so it is not widely available for public purchase. For this reason, individuals who consume diets excluding iodine-rich food, principally dairy, eggs, and/or fish, have increased risk of iodine deficiency. Further complicating this issue is the growing availability and acceptance of plant-based meat and dairy 'alternatives', regularly consumed by vegans and vegetarians, that have negligible iodine content and are not regularly fortified.

Alternative sources of dietary iodine are therefore necessary and some people select consumption of seaweed as a means of achieving adequacy. However, seaweed iodine concentrations are typically extremely high and as such represent a significant risk to excessive intake which itself may have severe health consequences. Seaweed is considered to be a sustainable and carbon fixing crop

and so finding ways of reducing seaweed iodine content to sufficiently low levels to permit consumption would allow much more widespread adoption of seaweed as a sustainable food.

In this project we aim to assess a range of processing steps to determine their capacity to deplete seaweed iodine concentrations. We will additionally explore the consequences of these processes on the retention of other important nutrients as well as their potential impact on product flavour. We will use standard heating protocols, alongside more specialised processes such as extrusion and 'popping' to reduce iodine concentration. Nutritional analyses will include inductively coupled plasma mass spectrometry (minerals), HPLC-mass spectrometry (vitamins) and proximate analysis for macronutrient content.

Eveleigh et al - Br J Nutr. 2022 Jan 21:1-46. doi: 10.1017/S0007114522000113.

Eveleigh et al - Nutrients. 2020 May 29;12(6):1606.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

190 Molecular and biochemical mechanisms of plant-host rhizosphere manipulation by Rhizoctonia solani for increased virulence to Brassica crop species

Lead Supervisor: Rumiana Ray

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The rotation project will be lab/controlled environment based and will aim to characterise root and disease phenotypes caused by R. solani on mutants of Arabidopsis thaliana defective in genes involved in auxin transport and flavonoid biosynthesis. The student will learn microbiology, microscopy, and plant pathology techniques, to perform inoculation bioassays and root/disease phenotyping. He or she will develop biostatistical skills to analyse their data and will learn how to carry out bioinformatics search using specialised software to identity candidate genes for virulence in R. solani. The student will also learn to genotype Arabidopsis mutants prior to inoculation studies. The student will carry RT PCR on existing plant resources for key molecular markers involved in plant defence. In addition, as part of the rotation the student will also be exposed to a number of other routine and some specialised techniques such as: In vitro plant culture , Reporter gene studies, Confocal Microscopy, Image processing packages including ImageJ.

Full Project Description: Rhizoctonia solani is the most aggressive soil-borne pathogen to oilseed rape (OSR, Brassica napus) during the seedling developmental stage of the crop. The pathogen can reduce crop establishment causing losses of more than £80m in the UK alone. Isolates of R. solani are classified in genetically and biologically diverse anastomosis groups (AGs) based on their ability to fuse and reproduce. Isolates of AG2-1 predominate in UK soils and are most virulent to OSR. The pathogen produces auxins, which contribute to decreased defence response and modify root growth and architecture of the plant host for the benefit of the pathogen.

The aims of this project will be to i) define molecularly, biochemically, and genetically the function of auxins produced by R. solani for virulence, disease susceptibility and root trait modification ii) identify auxin candidate genes in R. solani for further functional validation. The overall hypothesis is that specific auxins produced by the pathogen function either as virulence factors or as factors for

root growth manipulation and colonisation by R. solani. It is expected that through this project novel functional genetic information will be gained which can be translated from Arabidopsis to OSR to decrease disease susceptibility and/or identify future targets for control of R. solani by chemical or biological means. The project will investigate the following:

1. What is the function of different auxins produced by R. solani for disease susceptibility and root trait modification? To address this question the student will functionally characterise plant-pathogen interactions between several isolates of R. solani AG2-1 that produce different auxins using Arabidopsis mutants defective in auxin synthesis, transport and signalling plus mutants defective in flavonoids accumulation and production of reactive oxygen species.

2. What is the genetic or molecular basis of the defence response to R. solani? The student will carry out artificial inoculations with mutants with virulent and avirulent disease phenotypes from 1. Transcriptomic, metabolomic and pharmacological approaches will be used to identify key candidate genes and signals mediating host defence during plant pathogen interactions. Candidate genes and signals identified using these approaches will then be further validated by molecular genetic approaches (eg qRT-PCR; hormone profiling) using a number of B. napus genotypes showing varying degrees of resistance to R. solani.

3. Identification and functional validation of candidate genes in R. solani. Selected isolates which produce different quantities and types of auxins in vitro and in planta will be used to investigate, using pharmacological and molecular approaches, the auxin biosynthesis pathway in R. solani. Orthologue candidate genes of interest will be searched for in sequenced fungal genomes. The genome of sequenced AGs of R. solani will also be interrogated. CRISPR-CAS9 based genome editing will be used to generate mutants for a few candidate genes in AG2-1 for functional validation.

The student will receive training in several key aspects of molecular biology, plant pathology, functional genomics, bioinformatics, metabolomics and fungal biology, and will acquire specific technical and transferable skills set from their PhD studies to enhance their future scientific career.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

192 Bio-inspired wax-based edible packaging for food waste and environmental footprint reduction

Lead Supervisor: Vincenzo di Bari

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The student will join the Food Structure and Biomaterials research group, within the Division of Food, Nutrition and Dietetics, School of Biosciences.

Vincenzo di Bari will be the primary supervisor and will provide the initial training on how to characterise the physicochemical properties of plant waxes (e.g., chemical composition through gas chromatography, melting/crystallisation behaviour using DSC, self-structuring and thin-film spreading using microscopy).

The student will learn the key principles for edible wax-based packaging preparation and characterisation using a range of techniques (polarised light microscopy, rheology, XRD, DSC).

The student will also acquire the fundamental knowledge for fungi isolation and characterisation. This will done in collaboration with A. Ortiz-Urquiza in Plant Sciences.

The project will include laboratory work (up to approx. 65% of the time) with data analysis and reporting (approx. 35%). As part of the lab rotation, the student will learn how to collect and analyse data applying the basic statistic tests, critically interpret and compare data with literature findings to explain findings. This lab rotation will give the student the possibility to learn all the skills and foundation necessary to carry out the experimental PhD project independently.

Full Project Description: Context: About 2.37 billion people face food insecurity world-wide. The UN estimates 30% of produced food is wasted, corresponding to about 1.3 billion tons/year, costing about $2.6 trillion. This is mostly due to fungi spoilage on post-harvest and transportation. Whilst plastics extend products shelf-life, their use comes with high environmental costs. Edible packaging is an emerging technological approach to reduce decay where the material “wrapping” the food is a biodegradable, thin (below 0.3 mm) edible film.

Edible packaging is the most promising way to improve food security without plastics to reduce environmental footprint.

Vision: A bio-inspired design approach for plant waxes-based edible packaging modulating food physicochemical and microbiological properties to reduce the fungal threat to food spoilage.

Rationale and Aim: Waxes form the natural outermost water barrier in aerial plants and vegetables enabling life by reducing water loss, nutrients preservation and modulating microbial colonisation. We aim to mimic this functionality by designing an easy-to-apply plant wax-based edible packaging to coat products and host desirable microorganisms reducing decay and spoilage caused by fungi.

Hypothesis: Given its hydrophobic and inert nature, a plant wax-based edible packaging will (1) reduce water loss and gas exchange; (2) host desirable microorganisms and (3) prevent fungi attachment decreasing spoilage.

Project program and objectives: The student will define a new bio-inspired approach for edible packaging mimicking how plant waxes form films on leaves/fruit surfaces and how are colonised by microorganisms.

This is a truly interdisciplinary project as reflected in its four objectives:

1: Defining bio-formulations to develop plant-based wax edible packaging to simulate the architecture of plants leaf-waxes. This will require tailoring plant waxes physicochemical properties and approaches for film deposition on selected high-value products: cucumbers and strawberries.

Products will be grown in glass-houses to ensure full traceable history and controlled conditions. Physicochemical characteristics to assess include pH and titratable acidity, composition, gas exchange, mechanical properties. Rice-bran, sunflower and wheat wax plant waxes will be tested.

2: (a) Identify symbiotic bacteria colonising the surface of selected products and formulation and deposition strategies for bacteria incorporation within the wax-based edible packaging. This is crucial to ensure bacteria survive within the casted films and can compete with fungi to prevent spoilage on storage. (b) Characterise the wetting behaviour of formulated packaging to evaluate its ability to prevent surface water accumulation to inhibit fungi growth.

3: (a) Isolate the fungi causing spoilage evaluating the kinetics and mode of product infection and (b) assessment of formulated wax-films with and without incorporated bacteria to reduce fungi activity from post-harvest throughout shelf-life during storage under supermarket shelf simulated conditions.

4: Map levels, and determinants of, public acceptance of edible food packaging in the UK.

Commercial application and project strategic value

Plant waxes are edible by-products of the food industry with low-value applications. Validating waxes use for edible packaging will reduce food waste, single plastic usage and provide a feasible route for waxes upgrade into the food supply chain. Identifying bacteria strains to prevent fungi spoilage will strengthen a bio-based approach replacing synthetic chemicals.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

195 Molecular and functional charecterisation of rice axr4 mutants

Lead Supervisor: Ranjan Swarup

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: As part of the rotation students' will be involved in analysis of rice axr4 crisps mutants and will be exposed to a number of routine and some specialised techniques in Molecular Cell Biology, plant phenotyping and plant physiology such as:

Reporter gene studies

In situ immunolocalisation

Confocal Microscopy

Lightsheet Microscopy

In vitro plant culture

Microtome sectioning

PCR and RT-PCR

Image processing packages including ImageJ and WinRHIZO

Full Project Description: The auxin influx carrier AUX1 is a membrane protein which is localised to the plasma membrane (PM) and plays a key role in root gravitropic responses. AUX1 belongs to a small gene family (AUX/LAX) comprising four members-AUX1, LAX1, LAX2 and LAX3. We have previously shown that the correct targeting of AUX1 to the PM is mediated by AXR4 and in the absence of AXR4, AUX1 protein mainly accumulate in the endoplasmic reticulum (ER) (Dharmasiri and Swarup et al, Science). Cloning and characterisation of AXR4 revealed that AXR4 is localised to the ER. We now show that AXR4 regulates trafficking of LAX2 and LAX3 and interacts with AUX1. AXR4 appears to be a plant specific protein and may function as an ER accessory protein (ER

accessory proteins are a special class of ER proteins that facilitate folding of their cognate target proteins).

Though AXR4 like sequences have been identified in several plants including rice, poplar and barley their role in root development and root gravitropism remains unclear.

In rice there is one AXR4 like sequence but this sequence shows only about 30% similarity at amino acid level with Arabidopsis AXR4. Functional complementation approach shows that rice AXR4 is a true homologue of Arabidopsis AXR4 and can complement Arabidopsis axr4 mutant. Furthermore, rice AXR4 is localised in the ER and can restore targeting defects of LAX2 and presumably AUX1 and LAX3.

Recently, using CRISPR-Cas9 based genome editing, a rice axr4 mutant has been obtained. Similar to Arabidopsis knock outs, rice AXR4 mutant is agravitropic. In addition, we have recently shown that root hair elongation under low P requires AUX1 (Bhosale et al 2018, Nature Communication; Giri et al, 2018, Nature Communication) and the axr4 crispr mutant is also defective in root hair elongation response under low P. This further supports the view that rice AXR4 behaves similar to AtAXR4 in regulating trafficking of auxin influx carrier.

Interestingly, preliminary results show that rice axr4 mutants show some interesting above ground phenotypes including shorter stature, fewer tiller numbers and defects in grain filling.

This project proposal will characterise AXR4 crispr mutant to investigate the molecular basis of above ground defects seen in AXR4 crispr mutants.

Aims and objectives

1. Detailed characterisation of axr4 mutant

a. Root defects

b. Shoot defects

c. Seed filling defects

2. Expression studies

Reporter lines will be created (AXR4Pro::AXR4-GFP and AXR4Pro::GUS) to investigate expression and localisation of AXR4 at organ, tissue, cellular and sub-cellular level.

3. Identification of AXR4 targets

A cross linking and proteomics based approach will be used to identify potential targets of AXR4.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

201 Food insecurity and obesity: What are the underling factors of an evident paradox

Lead Supervisor: Andreia Moura

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The candidate will have the opportunity to analyse cross-sectional data from the UK National Diet and Nutrition Survey (NDNS). The NDNS holds information on socio-economic and dietary status of a sample of 1000 UK adult residents. Statistical analysis of the data will allow identifying possible associations and correlations between the following variables: socio-economic status, BMI, Type 2 diabetes, metformin use, and vitamin deficiencies.

From week 1 to week 4 the candidate will get acquainted with the data and receive training on the statistical tests to be performed (Pearson's r, Chi-Square, t-Test, ANOVA and non-parametrical tests).

From week 5 to week 9 the candidate will write a report describing the results of the analysis and present those to our research group. The possibility of publishing the results will be discussed.

Full Project Description: Obesity, and food insecurity, are well-established consequences of social disadvantage. In fact, among the several determinants of obesity, social inequality is the biggest risk factor. The co-existence of food insecurity and obesity is known as the 'food insecurity-obesity paradox'. Attempts to elucidate the reasoning behind this paradox, especially in the US have identified the relatively high affordability of energy-dense, processed products (comfort foods), as the key cause of obesity under food insecure circumstances. Foods that are highly palatable and intensively advertised can be a source of comfort during hardship.

Poor dietary patterns partially explain why vitamin deficiency is common among people who are both food insecure and overweight or obese. Increased adiposity is associated with micronutrient deficiencies, particularly folic acid, vitamin B12 and vitamin C. There is strong evidence that vitamin B12 deficiency is highly prevalent among obese and food-insecure individuals. This is highly relevant as folic acid and vitamin B12 deficiency cause hyperhomocysteinemia, which is associated with insulin resistance and diabetes. This indicates that food insecurity combined with a high BMI can predispose individuals to disturbances in glucose metabolism. Dysregulation in the metabolism of micronutrients seems to worsen when individuals are treated with metformin, a drug commonly prescribed for type 2 diabetes and pre-diabetes. Although vitamin B12 deficiency as a consequence of long-term treatment with metformin is very common, the mechanism behind this association remains to be elucidated.

Considering the above, this project will investigate the intricate associations between food insecurity, obesity, vitamin deficiencies and insulin resistance. Research exploring the food insecurity-obesity paradox in the UK population remains scarce. This proposal sets out to identify the key factors behind the obesity-food insecurity phenomenon in the East Midlands region in the UK. The overall aim is to investigate underlying factors and determinants of obesity in adults living in food-insecure households and potential vitamin deficiencies caused by food insecurity and obesity. The association of the same variables with insulin dysregulation will also be explored. The research will help to identify multifactorial causes and consequences of obesity and food insecurity and prevent micronutrient deficiencies in vulnerable populations.

This 4-year PhD program will start with qualitative methodologies (interviews and focus groups), recognized as appropriate to investigate social and behavioural issues with in-depth results. Adult individuals living with obesity and under food insecure circumstances will be interviewed over a period of two years to help to answer: What are the underlying factors explaining the food insecurity-obesity paradox in the UK population? How those factors can be addressed in nutrition interventions?

In years 3 and 4, the correlation between obesity, food insecurity, vitamin deficiencies, and insulin resistance will be investigated using data from the UK National Diet and Nutrition Survey (NDNS).

The NDNS survey provides information on the socio-economic status, anthropometry, food intake and micronutrient status of around 1000 UK residents. The PhD candidate will perform statistical analysis to determine possible associations and correlations between obesity, food insecurity, vitamin deficiencies, and insulin resistance among the UK population.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

202 Development of analytical technique for measurement vitamin D and its metabolites to assess

Lead Supervisor: Preeti Jethwa

Lead School: Biosciences

DTP Research Area: Biotechnology

Lab Rotation Description: Background: Vitamin D is available in a variety of formulations (e.g. tablets, capsules, chewables). Several food supplements (e.g. fish oils, mushroom extracts) are also sold on the basis of their high Vitamin D content. Unlike medical preparations, there is no legal requirement to register / authorise food supplements for UK sale.

During this rotation, the student will explore the impact of formulation, price, and purchase origin on the vitamin D content of a wide variety of food and vitamin supplements. The student will develop and validate a new LCMS method to quantify vitamins D2 and D3 – vital bioactives that are highly sensitive to UV and moisture-induced damage during storage. The student will receive training in LCMS method development and analysis, which represents an opportunity to acquire specialist knowledge applicable to a wide range of life science related research disciplines.

Week 1: Health and Safety and Lab inductions, generation of risk assessments

Week 2: Acquisition of food supplements. Advanced LCMS training

Week 3-6: Development of targeted LCMS methodology to quantify vitamin D2 and D3 in food supplements

Week 6-8: Analysis of food and vitamin supplements

Week 9: Write-up and data interpretation

Full Project Description: Vitamin D was discovered more than 80 years ago with its ability to cure rickets in children. Vitamin D is a secosteroid and comes in two distinct forms, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol; Figure 1). The significance of vitamin D in calcium and phosphorus homeostasis is well appreciated. However, recent epidemiological data have indicated that it has several extra-skeletal physiologic actions including protection against obesity, depression, covid-19 and many more. This has increased the need to find an alternative way of measuring vitamin D and its metabolites, that not only is quick and cheap but can be used to measure vitamin D and its metabolites across different material (food, supplements, biological fluids (blood, serum, plasma, urine, breast milk) .

Project aim: Develop and validate a new LCMS method to quantify vitamin D metabolites particularly D2 and D3 as that are highly sensitive to UV and moisture-induced damage during storage, you will utilise this method to investigate the following:

- The use of vitamin D has risen by 13% particularly since evidence suggested it could protect against COVID-19, with the vitamin and supplement market forecast to rise to £559 million. Here you will use the validated methodology to measure the vitamin D content in a variety of food and vitamin supplements and rank them with regards to formulation, price and purchase origin.

IMPACT: Identification of the best supplements and food in UK

2. You may have heard about circadian (daily) rhythms with regards to sleep/wake patterns, but they are involved in much more as the entire body is controlled by daily oscillations including when you should eat a meal or exercise, take medication, and take nutritional supplements. Masood et al (2015) found in healthy participants had their highest levels of vitamin D at noon, while their lowest levels were at 6 AM. However it is well known that consumption of high fat diet can alter circadian patterns; therefore in this study you will validate the analytical methodology for blood/plasma and urine and

- determine whether changes in vitamin D across the day is associated with body composition – therefore could changes be associated with predisposition to obesity

- determine whether there is an optimum time for the consumption of vitamin D supplements to help prevent metabolic diseases.

IMPACT – identification of the optimum time for consuming vitamin D supplements for maximum health benefits.

3. Prolactin is important for the production of breastmilk, it peaks in the early morning and lowest in late afternoon or early evening. Interestingly increased levels of prolactin had increase vitamin D in the plasma, however it is unknown if there is a relationship between vitamin D and prolatin levels in breastmilk. Vitamin D is important for growth of bone and muscles as well as the developing brain, currently women who exclusively breastfeed are told to take vitamin D supplements however which are the best and when is the best time to take this. Therefore, in this study you will validate the analytical methodology for breastmilk and assess the relationship between prolactin and vitamin D levels in breastmilk obtained at different times of the day and determine if there is a difference in those taking vitamin D supplements.

IMPACT - identification of optimum time to breasfeed for optimum vitamin D levels and determine whether prolactin plays a role.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

206 Elucidating the gene regulatory control on pollen development associated with heat and light stress.

Lead Supervisor: Zoe Wilson

Lead School: Biosciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: Pollen formation is critical for plant reproduction and food production and is thus essential for plant breeding and food security. Nevertheless, pollen development is highly susceptible to environmental stress, which can result in a lack of viable pollen and sterility; variation in pollen production and fertility is frequently seen between species and in different environments. This poses a significant problem for maintaining future crop yields with increasingly volatile environmental conditions. Control of pollen wall formation, and thus viable pollen development, is regulated through a series of transcription factors, which act in the tapetum cell layer in the anther. These transcription factors have been shown to respond differently to altered temperature and light, and to be modified by SUMOylation. SUMOylation has been shown to coordinate growth control under changing environmental conditions by directly modifying the activity of major transcriptional regulators in plants. This rotation will involve the analysis of mutants that are modified in their ability to SUMOylate key transcription factors linked with pollen development. These will be analysed under different environmental stress conditions to establish the link between environmental sensing and pollen development.

Full Project Description: Pollen formation is critical for plant reproduction and breeding, and thus for the production of crops and food. Ensuring successful reproduction is therefore essential for food security. However, pollen development is highly sensitive to environmental stress, which can result in a lack of viable pollen and sterility under different stressful environments. This poses a significant problem for maintaining future crop yields due to increasingly volatile environmental conditions and climate change. Pollen formation is controlled by a maternal cell layer within the anther called the tapetum, which encloses the developing pollen. This cell layer functions both as a coordinator and as a factory, controlling pollen development, but also the manufacture and secretion/release of pollen wall materials.

A number of transcription factors (TF), which are expressed within the tapetum regulate this process, in particular 5 bHLH TFs are involved. These bHLH TF proteins interact with each other and also regulate the expression of each other, as well as distinct sets of downstream genes. This means there are a series of complex, potentially competitive, interactions resulting in feed-forward and feed-back regulatory loops, which ensure that the correct levels of gene expression occurs at the right time and place. This is critical to ensure the formation of the intricate and resilient pollen wall, and viable pollen. We have shown that three of these bHLH TF are labelled by Post- Translational Modifications via Small Ubiquitin-like Modifier (SUMO) tags. SUMOylation is a reversible process known to modify protein activity, localisation or stability, particularly in response to stress. It is currently not known how these modifications impact tapetum function and pollen viability, however we have also found that these TF respond to growth under different environmental conditions (heat and light). This has identified a mechanism whereby plants can respond to changing environments and abiotic stress during flowering to ensure that pollen formation and fertility is maintained, under natural, variable environmental conditions. Such adaptation will be extremely valuable to maintain crop yield in volatile environments and climate change.

This project will investigate the control and impact of these TF protein-protein competitive interactions and establish the role of SUMOylation on their activity and function. This will be conducted through a combination of molecular genetic and biochemical analyses of the network, and the impact of SUMO tagging of these proteins on TF activity and interactions, and the downstream regulatory network will be investigated. This will enable characterisation of how plants ensure that pollen formation and fertility is maintained under changing environments and abiotic stress. This project will focus on the model plant Arabidopsis to capitalise upon available molecular genetic tools to rapidly test this network. However, we have also been working on the equivalent

genes in barley which show a high level of conservation; this project will build on these data to help establish the role of SUMOylation in the barley tapetum gene network to enable the rapid deployment of this knowledge to target environmental resilience in pollen development in crops.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

Chemistry

10 A metabolic-driven signalling system controls DNA replication

Lead Supervisor: Panos Soultanas

Lead School: Chemistry

DTP Research Area: Bioscience for Health

Lab Rotation Description: The rotation student during the 9 week rotation will be over-expressing and purifying the main proteins required for this work, Pyruvate Kinase (PykA), the EI protein that is responsible for the uptake of glucose to fuel glycolysis, the transcription factor for gluconeogenesis CcpN and the CcpN regulator YqfL.

Experience will be gained in transformations of expression vectors in E. coli expression strains, the IPTG-induced over-expression of proteins, collection of bacterial pellets, protein purification strategies and techniques, SDS-PAGE to assess the expression levels, as well protein quantification methods.

Full Project Description: DNA replication is regulated in a wide range of growth conditions to achieve timely and accurate genome duplication prior to cell division. Failures in this regulation cause DNA double-stranded breaks with potentially disastrous consequences for genome stability, cell viability and human health including cancer. To cope with these threats, cells tightly control replication initiation using well-known mechanisms. They also couple DNA synthesis to nutrient richness and growth rate by modulating the initiation and elongation phases of replication. The underlying mechanism of this metabolic control of replication, its interface with classical initiation control functions and its net contribution to the overall replication control and genetic stability are unknown.

Emerging data suggest that the metabolic control of DNA replication involves enzymes of central carbon metabolism (CCM) and on CCM metabolites. In a recent (2022) study supported by the BBSRC (BB/R013357/1) (Horemans et al, 2022) we showed for the first time that the metabolic control of replication in B. subtilis depends on the pyruvate kinase (PykA). Our current unpublished data suggest that the metabolic control of replication in B. subtilis depends on a system connecting replication to glycolysis and gluconeogenesis, and that this system responds to carbon sources and involves sensors (PykA, YqfL, EI and CcpN) of sentinel metabolites (ATP, ADP, AMP, PEP and Pi) and a complex cascade of phosphorylation events. Our aim is to reveal the molecular mechanism that underpins this signalling system.

Hypothesis and objectives: The central hypothesis of our grant application is that the metabolic control of replication is orchestrated by a signalling system. According to our preliminary data, this system is proposed to use energy-containing small molecules (ATP, ADP, AMP, PEP) as sentinels of cellular metabolism and protein Enzyme I (EI, the enzyme that uptakes glucose to fuel glycolysis), CcpN (the transcription factor of gluconeogenesis), YqfL (a CcpN regulator) and PykA as sentinel sensors. Our unpublished data also suggest that these sensors convey the metabolic signal to the replication machinery by modulating the activity of enzymes essential for replication initiation and elongation (namely DnaA, DnaE, DnaC and DnaG) through protein-protein interactions and a cascade of protein phosphorylation. Our objectives are:

i. To characterize in vivo the impact of PykA, YqfL, CcpN and EI on replication,

ii. To characterize in vivo the regulatory circuits geared by these sensors,

iii. To demonstrate in vivo that these circuits are travelled by phosphorylation events,

iv. To confirm/characterize the protein interactions between sensors (PykA, YqfL, CcpN and EI) and between sensors and replication enzymes (DnaE, DnaG, DnaC and DnaA) in vitro,

v. To demonstrate the kinase/phosphatase activities of purified sensor proteins, validate the phosphorylation cascades, and identify the phosphorylated residues,

vi. To characterize the effect of protein interactions and phosphorylation on replication enzymes activities in initiation and elongation assays.

Our work falls firmly within the major BBSRC strategic priority of 'Advancing the Frontiers of Bioscience Discovery' and in particular 'Understanding the Rules of Life'. Understanding the mechanism of the metabolic control of replication will impact across a wider spectrum of the BBSRC portfolio.

Lab Rotation Location: University Park;

Full Project Location: University Park;

26 Machine learning and molecular simulation of trinucleotide repeats for drug discovery

Lead Supervisor: Jonathan hirst

Lead School: Chemistry

DTP Research Area: Bioscience for Health

Lab Rotation Description: Predicting RNA-small molecule binding poses

RNA is a potential target for new small molecule drugs. Designing active compounds can be facilitated by computational modelling. Most of the available tools developed for these prediction purposes, such as molecular docking or scoring functions, are parametrized for protein targets. The performance of these methods, when applied to RNA-ligand systems, is insufficient.

This rotation project will survey the docking methods currently tailored specifically for RNA and will benchmark them, using data from the literature. We will consider the programs rDock (with two desolvation potentials: dock and dock_solv) and AnnapuRNA. The testing set will comprise 33 RNA-small molecule complex structures, which have been studied previously in the literature. The assessment will evaluate three main factors that may influence the structure prediction, namely, the starting conformer of a ligand, the docking program, and the scoring function used.

Skills to be developed during the rotation include: familiarity with the Linux operating system, python programming, scripting, familiarity with the docking software, broader skills in data management and data analysis.

The rotation student will be embedded in computational chemistry suite, with plenty expert PhD students and postdocs on hand to provide day-to-day assistance.

Full Project Description: Nucleotide expansion disorders are a group of neurological diseases that result in the expansion of repetitive DNA sequences in both coding and non-coding regions of genes.

The most common is the trinucleotide repeat disorder (TRED), which is likely caused by slippages during DNA replication. These are then transcribed into RNA hairpins that can be translated into a toxic protein or sequester proteins. Overexpression of the TRED r(CUG) RNA repeats r(CUG) in the 3' untranslated region of the DMPK gene induces the neurological disease myotonic dystrophy 1 (DM1). Once r(CUG) is transcribed, it folds into a double stranded RNA (dsRNA) hairpin structures and sequesters proteins such as muscleblind-like factor 1 (MBNL1), resulting in the loss of MBNL1 function and the unwinding of r(CUG).

Once MBNL1 is sequestered within the nucleolus, it leads to mis-regulation of genes such as muscle specific chloride channel 1 (CLCN1) and 27 additional genes that cause the symptoms of myotonia and heart arrythmia. The zinc fingers (ZnF1-4) play a role in recognizing and binding to pyridine-pyridine (C-C and U-U) mismatches of r(CUG). The mismatches are highly dynamic. This results in the dynamics properties of base flipping and potential unwinding of hairpin r(CUG). Base flipping provides an anchoring site for MBNL1, in which the MBNL1 proteins can unwind the double helix into a single stranded molecule. Unwinding of r(CUG) is facilitated by weak interactions between U-U base pairs, that leads to an overall destabilisation of dsRNA.

Several small molecules that bind to U-U mismatches in order to stabilize r(CUG) as well as inhibit MBNL1 have been explored. One such an example is pentadiamine which rescued splicing defects in DM1 cell culture model, and Hoechst 3342. Conversely, a single molecule study showed that r(CUG) is still able to bind to MBNL1 when U-U mismatches were bound by a small molecule, but when a bivalent ligand is used, inhibition had increased by 50-fold. The increase in affinity of bivalent ligands arises from the thermodynamic advantage of co-operative binding, and upon binding of the first ligand the overall entropy of the ligand-CUG should reduce by having the second ligand in near vicinity to the U-U mismatch. Once bound the base flipping and base fraying should be reduced.

However, currently there are no models that address the atomistic details of binding bivalent ligands to r(CUG) hairpins. This is a concern, since the design of drugs requires understanding of binding poses and the dynamic properties of the target structure. There is numerous data on the conformations of duplex r(CUG) but not on the hairpin r(CUG). This project will explore full atomistic modelling of (CUG)12 hairpins and the binding of various bivalent ligands. The bivalent ligands have the same binding modules triazine-acridine that are known to form Janus-Wedges between the two U-U mismatches. This project will focus on the binding of the tetrazine unit to the mismatches, and the overall conformational changes of the r(CUG) structure, as this will inform the design of future small molecules.

Lab Rotation Location: University Park;

Full Project Location: University Park;

38. New Hybrid Spider Silk and Peptide Amphiphile Derived Materials for Healthcare Applications

Lead Supervisor: Neil Thomas

Lead School: Chemistry

Lab Rotation Description: Peptides of both natural and synthetic origin are increasingly being used as scaffolds and supports for biological materials involving living cells, ranging from tumour spheroids for evaluation of new cancer treatments, to biodegradable hydrogels to provide meshes that accelerate wound healing in regenerative medicine. We will bring together two different types

of peptide-based materials: recombinant spider silk (RSS) (which has been shown to be both non-pyrogenic/non-immunogenic to mammalian tissues, whilst having exceptional elasticity and toughness) with peptide amphiphiles (PAs) (hydrogen-bonding peptides with polar amino acid head groups and hydrophobic alkyl tails capable of self-assembling into well-defined nanofibres) in order to create new biocompatible supramolecular structures with superior properties to existing products such as the extracellular-matrix (ECM) substitute Matrigel. By integrating both peptide platforms, we will develop a new kind of tuneable hydrogel-based material with unique properties. Preliminary studies have generated hybrid materials possessing new topographies.

The Mata and Thomas groups have independently used a variety of chemical and biological methods including 'click' chemistry and un-natural amino acid mutagenesis to functionalize PAs and RSS respectively. In the rotation project the researcher will express a silk protein and combine this with different PAs to self-assemble new materials, which they will then characterize.

Full Project Description: The PhD will be focused on the creation, characterisation and exploitation of new hybrid self-assembling biomaterials composed of combinations of recombinant spider silk proteins (SSPs) and peptide amphiphiles (PAs) that have been selected to form new architectures and to display biologically active ligands that promote mammalian cell growth and differentiation. This project will bring together the distinct but complementary expertise of three groups: The Goodacre group undertakes research into the evolution and adaptation of spiders to different environmental niches using bioinformatics and experimental biology, in part through looking at the properties of the proteins that make up the different silks they produce. In collaboration with the Thomas group, they have cloned and expressed in E. coli, silk genes from several different spider species found in harsh environments to generate new recombinant materials with properties desirable in regenerative medicine; The Thomas group has extensive experience in protein engineering and the modification of proteins using site directed mutagenesis including the introduction of un-natural biorthogonal amino acids such a L-azidohomoalanine, together with enzyme-mediated and chemical conjugation of bioactive peptides and antibiotics. Together, the Goodacre and Thomas groups have produced both broad-spectrum antibacterial silk threads and matrices and ones that promote human cell growth; The Mata group has extensive experience working with PAs and has developed new methodologies where PAs can be used to co-assemble with larger macromolecules to guide hierarchical assembly into functional macroscopic structures that are robust, bioactive, and responsive. The group has demonstrated the capacity to create complex in vitro models and regenerative materials using this approach.

Techniques

- The PhD researcher will gain experience in the sustainable generation of recombinant spider silk in E. coli and the modification of SSPs and PAs using copper-catalyzed and strain-promoted 'click' reactions as well as site-specific enzyme mediated modifications. The Mata and Thomas groups have both used azide-alkyne 'click' chemistry in order to site specifically modify PAs and SSPs respectively and therefore have a range of bioactive ligands (RGD and IKVAV peptides as cellular cues; fluorophores for imaging of self-assembly processes; antifouling agents and controlled release antibiotics and other drugs) that will be used to functionalize the new PA/SSP hybrid scaffolds.

- The researcher will have an opportunity to use bioinformatic and next-generation sequencing approaches to study gene sequences encoding silks that possess desirable new physical or mechanical properties and to make recombinant versions of these.

- A key element of the PhD will be characterization of the new materials generated. This will include looking at the mechanical and rheological properties with structural characterisation using a manifold of techniques including SEM, TEM, CD and NMR.

- The researcher involved in the project will also be trained in mammalian cell culture in order to evaluate the behaviour of cells in the presence of a range of differently functionalized PA/SSP scaffolds to determine if they offer superior performance as extracellular matrix mimics, meshes to accelerate wound healing or as tools in drug discovery such as creating new tumour spheroid models that will reduce the requirements for animal testing (3Rs).

Lab Rotation Location: University Park;

Full Project Location: University Park;

120 LPMO-inspired artificial metalloenzymes for selective oxidations

Lead Supervisor: Luisa Ciano

Lead School: Chemistry

DTP Research Area: Biotechnology

Lab Rotation Description: During the nine-week lab rotation, the student will acquire the basic skills to carry out the PhD project (please refer to project description).

The student will synthesise a simple ligand that could be used for Cu-coordination and characterise it by NMR, MS and, if possible, X-ray crystallography. The Cu-coordination will be investigated by EPR spectroscopy, providing the basics of this technique. Skills obtained: organic synthesis procedures; coordination chemistry; spectroscopy (mostly NMR and EPR).

The student will then produce and purify a small selection of proteins that could be used as the scaffold for the artificial metalloenzymes that will be developed during the PhD project. The sequences of interests will be expressed in standard expression systems (E. coli) and purified by affinity chromatography, followed by initial characterisation. Skills obtained: standard protein expression protocols, protein handling and purification, basic protein characterisation (e.g. gel electrophoresis).

Full Project Description: Biocatalysis is rapidly emerging as a powerful and widespread tool for a range of chemical industries - from the synthesis of complex and highly functionalised pharmaceuticals to the bulk production of biofuels. The popularity of biocatalysis stems from an ever-increasing ability to design and manufacture bespoke catalysts which are able to carry out a range of desirable transformations with reactivity and selectivity which are unrivalled by existing chemical methods.

One such transformation is the selective oxidation of organic molecules - a reaction which is used to produce millions of tons of alcohols, carbonyl- and epoxide precursors each year in all areas of chemical industry, but is still far from optimised. As a consequence of growing environmental concerns, the need for a catalytic methodology has never been greater and biocatalysis may present an optimal solution. Chemical methods are evolving but still require harsh conditions, high temperatures and often present the problem of over-oxidised impurities, whereas enzymes can perform the same reactions in water, under ambient conditions and with complete selectivity to the desired compound.

Lytic polysaccharide monooxygenases (LPMOs) are a class of Cu-based metalloenzymes, discovered in 2010-2011, which are able to perform the selective oxidation of C-H bond found in polysaccharides. Their unique active site is formed by a copper ion coordinated by two histidine residues, one of which being the amino-terminus of the protein, in an arrangement commonly referred to as the "Histidine brace". These enzymes have not only revolutionised the field of biofuel production, but also transformed the notion of how Cu-catalysed oxidations occur in Nature. The copper active site is found on a very solvent exposed surface of the protein, which is perfect for polysaccharide activity, but prevents these enzymes to be effective in the selective oxidation of small molecules.

The objectives of this project will be to produce and characterise LPMO-inspired artificial metalloenzymes, and to evaluate their oxidative abilities towards small molecule substrates. Ligands able to mimic the His brace arrangement will be synthesised, characterised and coupled to an engineered globular protein, before or after addition of Cu ions. The so-formed artificial metalloprotein will be characterised by a variety of techniques, such as X-ray crystallography, EPR spectroscopy and mass spectrometry. The enzymatic activity and oxidative abilities will be evaluated by assays on an array of possible substrates. Further cycles of engineering and characterisation will then be performed to tune the properties and the substrate scope of the metalloenzyme.

The successful candidate will gain skills in protein production, purification and engineering, spectroscopy (e.g. EPR, NMR), structural biology and biochemical assays.

Candidates for this project will have a Chemistry, Biochemistry or Natural Sciences degree with a strong interest in bioinorganic/biological chemistry and will be working on a multidisciplinary project, gaining experience in a wide variety of Chemistry and Biochemistry techniques.

Lab Rotation Location: University Park;

Full Project Location: University Park;

128 Targeting disease-relevant RNA sequences using cyclic peptides

Lead Supervisor: Nicholas Mitchell

Lead School: Chemistry

DTP Research Area: Bioscience for Health

Lab Rotation Description: This rotation project will suit a student with a degree in Chemistry, Molecular Biology, or a related subject, who is interested in designing next-generation therapeutics to 'drug' disease-relevant RNA sequences.

The lab rotation will involve the preparation of a series of cyclic peptides followed by binding studies with a model RNA sequence using isothermal calorimetry (ITC) and fluorescence anisotropy. The student will have the opportunity to learn the basics of computational modelling to gain insight into the peptide-RNA complex.

No prior experience in synthetic chemistry or computational modelling is required for this project, full training will be provided. The rotation will allow the student to gain expertise in all of the techniques relevant for the full PhD project, which include solid-phase peptide synthesis, HPLC, MS, NMR, ITC, UV-Vis, bioinformatics, and modelling.

Full Project Description: RNA is involved in the majority of cellular processes and is therefore a potential therapeutic target of a broad range of diseases. However, due to the inherent flexibility of this biopolymer, it was long considered as 'undruggable.' While progress has been made using small molecules to target RNA sequences, such agents require a stable tertiary fold for successful binding. Cyclic peptides provide a more flexible and versatile motif with which to bind selectively, with high affinity, to RNA structures and thus represent a potential next-generation drug to modulate disease-relevant RNAs.

The PhD project will initially employ bioinformatics to identify the interacting regions of RNA-binding proteins. Cyclic peptides will be designed based on the identified sequences and synthesised to rigidify the geometry of the binding residues. The Mitchell lab (School of Chemistry) has recently developed a novel peptide cyclisation technique using visible-light-mediated chemistry. This method enables us to prepare 'stapled' peptides cyclised with a hydrocarbon linker using unprotected peptides in aqueous solution.

Biophysical characterisation of peptide binding to model RNA sequences will be conducted in the Dreveny lab (School of Pharmacy) using a variety of techniques such as ITC and fluorescence anisotropy. Computational modelling of the peptide-RNA complex will be explored in the Hori lab (School of Pharmacy) to gain additional insight into the peptide-RNA complex. Once these proof-of-concept studies have been completed, a library of cyclic peptides will be prepared, either synthetically using peptide array technology or via phage display, to screen against disease-relevant RNA sequences, in vitro, to identify potential therapeutic leads.

Lab Rotation Location: University Park;

Full Project Location: University Park;

129 Cascade reactions combining enzyme and organometallic catalysis for renewable energy

Lead Supervisor: Deborah Kays

Lead School: Chemistry

DTP Research Area: Biotechnology

Lab Rotation Description: The nine-week lab rotation will involve the investigation of the compatibility of thermostable bovine carbonic anhydrase with ionic liquids. Initially the student will be trained in making up the necessary buffer solutions and using these to undertake enzymatic assays using the bovine carbonic anhydrase. Alongside this, the student will synthesise some relevant ionic liquids, chosen for potential biocompatibility, such as cholinium glycinate and cholinium geranate. The ionic liquids will be characterised by techniques such as NMR spectroscopy, ICP and Karl Fischer titrations to determine purity and water content. Following this, analogous enzymatic assays in the presence of varying concentrations of the ionic liquids will be performed. This mini-project, while self-contained, will provide a valuable starting point for the full PhD project into the baseline techniques, and the potential compatible ionic liquids to use in the follow-on enzymatic cascade reactions.

Full Project Description: Cascade reactions are of significant interest in synthetic organic chemistry as they enable high atom economy and reduction of waste generated by the chemical processes, in addition to cutting the overall work involved in the process. Systems where different enzymes and homogeneous organometallic complexes are combined successively in a one-pot or tandem

processes, can be combined as catalysts in a range of different chemical applications. The main advantage of the use of organometallic reagents in such cascade processes will be that a metal catalyst presents the potential to provide a functionality that is absent from enzymes. It may also avoid the complexity associated with the use of a second enzyme.

However, a challenge with such approaches is the potential incompatibility of the highly reactive metal complexes with coordinating functionalities present in the mixture, such as accessible amine or thiol groups present in enzymes. We will thus encapsulate this organometallic catalyst into a protein (producing an artificial metalloenzyme or in an ionogel), to protect it from the normal enzyme. In this way, our technique will overcome mass transfer limitations by keeping the catalyst and the reagents in homogeneous phase.

The overall aim of this project is to investigate chemoenzymatic cascades for the hydrogenation of CO2 in (homogeneous) liquid phase, using earth abundant organometallic catalysts based on cheap starting materials. This will use thermostable carbonic anhydrases for CO2 hydration to bicarbonate, coupled with transition metal catalysts for this process. The enzyme will be separated from these first-row transition metals catalysts (iron and cobalt catalysts will be investigated) during the catalytic cascade by incorporating the latter into either a protein, or an ionogel. The overall objectives will be to:

1. Synthesise catalytic systems containing an enzyme that is separated from an organometallic catalyst during the catalytic cascade (using incorporation of the latter into proteins or ionogels)

2. Characterise these catalyst systems using spectroscopic methods such as ICP-MS, UV/vis, IR and EPR spectroscopy and spectroelectrochemistry, underpinned by theoretical modelling

3. Investigate these systems in the catalysis CO2 hydrogenation, determination and optimisation of reaction conditions (aim is in presence of water / alcohol mixtures)

4. Investigate the reactions using kinetic methods, using data in-situ spectroscopic measurements and the isolation/trapping of intermediates, allowing us to postulate reaction mechanisms

This will form a challenging PhD project as the student will combine enzyme and organometallic chemistry go beyond the current state-of-the-art in enzyme cascade reactions. However, this project will also establish the use of such organometallics in metalloenzymes and bespoke, protein-compatible ionogel systems, a step change that will allow other researchers to build upon our findings. Furthermore, moving away from the platinum group metals towards base metals in catalysis is highly desirable in terms of toxicity, cost and environmental sustainability.

This is an exciting multidisciplinary PhD project will provide training in the development and characterisation of new metalloenzymes, organometallic chemistry, catalysis, statistical methods, molecular modelling and reaction mechanisms.

Lab Rotation Location: University Park;

Full Project Location: University Park;

168 Self-assembled theranostics: combing PARASHIFT MRI with drug delivery

Lead Supervisor: Ben Pilgrim

Lead School: Chemistry

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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.

Lab Rotation Location: University Park;

Full Project Location: University Park;

Engineering

18 Biotechnological recovery of critical metals from wastes for a circular economy

Lead Supervisor: Helena Gomes

Lead School: Engineering

DTP Research Area: Biotechnology

Lab Rotation Description: The lab rotation will introduce the student to processing waste materials, bioleaching and microbiology work, cultivating Cupriavidus metallidurans and assessing critical metal recovery from wastes.

The candidate will initially explore spent car catalyst wastes to recover platinum group elements (e.g., Pt, Pd) in batch bench scale experiments. The team have developed a unique and rapid (solvent free) process for converting waste (inorganic) materials into microspheres, which not only increases the materials surface area but also enhances materials amorphous content (and hence ion leaching rates).

The candidate will initially assess the ability of the wild type Cupriavidus metallidurans CH34 strain to recover the platinum group elements using the processed and unprocessed waste material, followed by biological engineering of the host for improved metal recovery. This will allow the student to become familiar with the growth and manipulation of the microbial host, and the use of the various gene tools (ie. CRISPR) available. Specifically, they will: (1) learn to design and build synthetic genetic parts, modules and assemble them into vectors. (2) learn to genetically manipulate the host, creating and characterising insertion and deletion mutants via a range of molecular biology techniques, microscopy, flow cytometry and elemental component analysis.

Full Project Description: The demand for critical metals is increasing exponentially as technologies needed to achieve net-zero by 2050 – including renewable energy, hydrogen, and carbon capture – require more metals than their fossil-fuel-based counterparts. To achieve improved resource efficiency, decreased reliance on raw materials, and increased recycling to reduce carbon emissions, we urgently need new efficient, low-cost, low-energy solutions to recover precious metal resources from waste streams. Bioleaching (i.e. microbial leaching and recovery) is an environmentally friendly technology being explored for metal recovery from anthropogenic waste streams (e.g., metalliferous ores and wastes, including fly and bottom ashes of municipal solid waste incineration, slags, and mineral mining waste ore stockpiles). Using microorganisms isolated from natural settings (e.g. extreme environments, acid mine drainage etc.) to generate keys minerals (metals) or organic acid (as metabolites) and improve metal solubility by enzymatic reactions has huge potential to address the major environmental issues with current traditional energy-intensive technologies like pyrometallurgy. However, bioleaching is in its infancy, and current issues to be resolved concern the slow dissolution kinetics and low metal leaching rates from waste streams. In this multidisciplinary project, we aim to enhance bioleaching and metal recovery from wastes by (1) using a novel flame spheroidisation process (developed at UoN) to amorphise the waste streams and (2) develop biological engineering of metal resistant microorganisms to enhance and increase their biomineralisation capability to produce precious metals. Flame spheroidisation is a solvent free process which can be used as a rapid, low-cost pre-treatment to amorphise the waste (ie making the materials glassy to increase ion leaching rates), as well as increase the materials surface area, by generating microspheres. Further, post bioleaching, the micro spherical morphology of the

materials will enable them to enter the Circular Economy for application in alternate uses where spherical particles are desired (i.e. Fillers, Sintering, Coating Materials, etc). Life Cycle Assessment of the processes involved will allow to quantify and optimise the environmental footprint of this solution for metal recovery.

Larger surface area will also allow for greater abioti-biotic interface and is expected to allow for greater resource recovery. Cupriavidus metallidurans CH34 has shown to exhibit extraordinary metabolic versatility, including chemolithoautotrophic growth; degradation of BTEX (benzene, toluene, ethylbenzene, xylene); high resistance to numerous metals; biomineralisation of gold, platinum, silver, and uranium; and accumulation of polyhydroxybutyrate (PHB). These qualities make this organism a highly valuable host for biotechnological applications such as bioremediation, bioprocessing, and for potential generation of bioelectricity in microbial fuel cells (MFCs). We are developing an extensive synthetic biology tool-box (Turco et al., 2022) to which these project outcomes will significantly contribute to further engineer C. metallidurans CH34 for enhanced biomineralisation of key metals from complex hard to process waste materials (such as spent car catalysts and other mineral waste streams). We will also investigate how their interaction with the metals affects their physiology and electrochemical behaviour in MFCs.

Lab Rotation Location: University Park;

Full Project Location: University Park;

23 Using Artificial Intelligence to bridge the gap between Mass Spectrometry Imaging and Multispectral Optical Imaging for Mapping Disease in Tissue

Lead Supervisor: George Gordon

Lead School: Engineering

DTP Research Area: Bioscience for Health

Lab Rotation Description: The first 9-week rotation will spent in the labs of Dr. Rian Griffiths, learning the latest techniques in Mass Spectrometry Imaging that produce high accuracy maps of biomarkers, metabolites, peptides and proteins. This will be applied to samples of tissue. The second 9-week rotation will be spent in the lab of Dr. George Gordon learning how to collect multispectral images (up to 16 wavelength bands) of tissue samples and then using convolutional neural networks, a cutting-edge AI technique, to identify chemical signatures.

Full Project Description: Background:

Multispectral imaging is becoming an increasingly important tool for producing real-time maps of chemical abundance of endogenous and exogenous components in tissue, with important in vivo applications in disease diagnosis. In contrast to conventional three-colour (red/green/blue) imaging, in multispectral imaging many wavelengths of light are measured at each pixel: an optical spectrum representing the combined reflectance of the many constituent chemical substances. The challenge is then to separate these components to estimate the true abundance of each substance at a particular pixel. Complex algorithms to achieve this separation abound, but so far Artificial Intelligence algorithms have shown only limited utility for this task, due to the lack of accurate ‘ground truth’ images on which to train algorithm outputs.

Mass spectrometry imaging (MSI) can provide accurate ground-truths because it provides high-resolution macroscopic (20□m) and microscopic (2□m) imaging and high accurate-mass chemical maps. Aquisition is achieved by rastering an ion beam or laser across a tissue section, or sampling via a liquid junction, extracting then detecting ionised biomolecules, endogenous and exogenous compounds can be detected in a single experiment.

In this project, murine brain tissue will be analysed via MSI to develop ground truth data that will inform multispectral imaging of endogenous compounds. Control animals will be compared with those dosed with drugs to assess therapeutic success.

Aim and Objectives

The aim of this project is to develop and train an Artificial Intelligence system (e.g. convolutional neural network) that takes as input a multispectral image from a low-cost compact camera and accurately estimates chemical abundances, in effect producing an estimated mass spectrometry image. This will be achieved through specific objectives:

1. Build a low-cost multispectral imaging platform using optical filters and a camera

2. Collect data from murine brain tissue samples using multispectral imaging system

3. Image the same tissue samples using MSI approaches

4. Build and train an AI system to map between the two datasets

Impact

In the near term multispectral imaging could enable real-time visualisation of tumour tissue during brain surgery, allowing surgeons to accurately excise tumour tissue while maximally preserving normal brain function. Further, because multispectral imaging can be implemented using low-cost miniaturised components, it suitable for use in next-generation medical endoscopes that will improve early detection of cancer in the gastrointestinal tract. Dr. Gordon is working closely with clinical partners and a start-up company to commercialise new endoscope technology. Longer term, this project will establish a pipeline for validating multispectral imaging with MSI ground-truths, allowing the technique to be applied to detect many different diseases in other organs of the body.

This project would be suitable for you if:

- You are interested in doing a mixture of hands-on experimental work and coding
- You want to learn about the latest AI techniques
- You can code (e.g. MATLAB, python)
- You want to work on something with an important medical application

Lab Rotation Location: University Park;

Full Project Location: University Park;

78 Biocatalytic valorisation of flower waste: Towards sustainable feedstock for fine chemical industry

Lead Supervisor: Samantha Bryan

Lead School: Engineering

DTP Research Area: Biotechnology

Lab Rotation Description: Immobilisation of Streptomyces sp. on wool

This project involves developing a robust protocol for immobilising Streptomyces sp. onto wool for the first time. Biocatalysts provide a benign alternative to traditional metal catalysts for chemical transformations; however, their recovery and re-use limits their application for commercial scale applications. Immobilising biocatalysts on to a support can alleviate this challenge and enable retention of biocatalytic activity. Wool is a natural fibre with rich surface functional groups, which allows for easy immobilisation of catalysts and has successfully been used as a catalyst support for both metal and enzyme catalysts.

The immobilisation of Streptomyces sp. on wool will be achieved through a series of pre-treatment techniques on wool and the protocol will be optimised for to achieve catalyst loading. The wool-Streptomyces sp. catalyst will be characterised using EDX, SEM and FTIR. The immobilised catalyst system will be tested for catalytic activity and re-usability for geraniol esterification reaction and the performance will be benchmarked against the catalytic activity of free Streptomyces sp. in a batch reactor. The reaction conversion will be measured by collecting samples and analysing them using gas chromatography. The immobilisation protocol developed in this project will be used to prepare catalyst cloths for application in continuous reactor configurations like the spinning mesh disc reactor for reaction scale-up.

Full Project Description: Did you know countries like India produce more than 300 kilotons of flower waste every day? Imagine re-using the flower waste to make high value starting materials for the chemical industry!

With an increasing awareness about resource use and government regulation, chemical industries are fast realising the need for sustainable manufacturing. Identifying sustainable alternatives for petrochemical feedstock (like biomass) and using resource efficient technologies (to replace the conventional batch reactor) are two strategies which are fast gaining research and industry attention to enable sustainable production of chemicals.

In this two-fold objective project we will: (i) demonstrate for the first time the potential of Streptomyces sp. as a biocatalyst for valorisation of flower waste into high value chemicals and (ii) investigate process scale-up in continuous reactors.

Flowers are an abundant source of aromatic and biologically active compounds, which are important raw materials for the food, pharmaceutical and the cosmetic industry. Currently, these compounds are either obtained from fresh flowers or produced using synthetic routes, both of which are not sustainable solutions in the long run. This project will focus on the conversion of terpene alcohols (commonly present in flower biomass) into terpene esters and methacrylate, which are important starting materials for food, fragrance and polymer industries.

The limitations of current state-of-the-art to produce these compounds are: (i) long reaction times due to mass transfer limitations, (ii) loss in product yield due to product inhibition and (iii) limited reusability of expensive enzymes. Microbes like Streptomyces sp. are an attractive solution to

challenges associated with metal and metal organic catalysts (toxic, expensive and susceptible to catalyst poisoning) and provide a benign pathway to carry out chemical transformations. Streptomyces sp. are ubiquitous in the natural environment and excrete a large number of extracellular enzymes for the solubilisation of plant-derived polymers.

The first part of this project will first focus on investigating the reaction mechanism for biocatalytic conversion of terpene alcohols derived from flower waste. This involves identifying the right reaction conditions to maximise the product yield on the bench scale. The reaction mechanism will also be modelled using molecular dynamics and will be validated using experimental kinetics results. The second part of the project involves applying the reaction protocol developed in the first stage to demonstrate scale-up in micro-channel (MC) and spinning disc reactors (SDR). These reactor configurations are fast gaining industry attention for their ability to achieve fast reactions due to increased mass transfer, small chemical inventory, scalability and inherent safety. This project for the first time will test the potential of the MC and SDR for whole cell catalysed reaction systems

Lab Rotation Location: University Park;

Full Project Location: University Park;

93 Proteins in Alien Environments

Lead Supervisor: Anna Croft

Lead School: Engineering

DTP Research Area: Biotechnology

Lab Rotation Description: Environments with high charge can impact significantly on protein structure and function, while maintaining activity in these environments is crucial for exciting new applications, such as in electrically active bioimplants and sensors, inducible pharmaceutical release devices, bio-based batteries, and industrial biotechnology. This project will explore the impact of highly charged organic cations and anions (in the form of the myriad of ionic liquids available) on structuring of proteins and enzymes and the impact on function, particularly with reference to the incorporation of active and functional biopolymers such as silk, or enzymes into 3D-printed ionogels. The rotation project thus has scope, depending on student interest, to take this either down a purely experimental route looking at structuring and solubility impacts under high-throughput conditions with concomitant statistical analysis; examining and characterising specific formulations for 3D printing processes; computational analysis through molecular dynamics simulations on the impact of ionic liquids; or a combination of experimental and computational approaches to provide feedback to one another.

Full Project Description: Proteins are remarkable and adaptive biomaterials that can be modified to fit and function in a hugely diverse range of environments, from anaerobic conditions, to high salt, and dramatic changes in solvent properties, alongside the environments induced by protein-protein interactions or shear stress. Understanding the diversity in environment-induced structure can help in targeted redesign of proteins to be resistant to such conditions, or to adapt to generate new functions and application scope. One particular question is how proteins may structure differently in extreme environments, and potentially access new functionality not yet discovered.

This PhD project will explore these impacts of extreme conditions through the exemplar of 3-D printing of ionogel-based structures. Ionogels are exciting materials that have already found

application in a range of areas, including drug delivery, biosensors, and batteries. Our approach specifically targets achieving this through the use of biorenewable and biosourced materials to maximise sustainability, and also potentially enhance biocompatibility for applications in regenerative medicine and industrial biotechnology. Ionogels are formed by gelation of ionic liquids – liquids below 100 °C which are constituted solely of ions and which can impart electrical properties to the final gel product. Gelation can be induced readily in the presence of amino-acid based materials such as proteins and other peptide derivatives, and the formulation aspects here will be studied in detail. This includes examining a range of potential ionic liquids – there are over 10^6 known single ion combinations – through emerging high-throughput approaches and using the robotics systems in the centre for additive manufacture. At all stages of the project, there exists scope for enhancing the knowledge gained through computational analysis, primarily through molecular dynamics simulations of the protein-ionic liquid mixtures. A focus here will also be in understanding new protein landscapes that can be accessed in the ionogel matrix. Gel formulations will be characterised closely, narrowing these down to printable formulations, with additional characterisation of the electrical and biocompatibility properties, impact of shear, as well as kinetic parameters where enzymes are incorporated. An exemplar application looking at how to electrically interface with cells, entering the exciting new field of bioelectrochemistry, will be based on the combined properties derived for printing and from the testing of electrochemical properties.

Lab Rotation Location: University Park;

Full Project Location: University Park;

100 Engineering improved carbon capture and conversion in Cyanobacteria utilising metal organic frameworks (MOFs)

Lead Supervisor: Andrea Laybourn

Lead School: Engineering

DTP Research Area: Biotechnology

Lab Rotation Description: During the lab rotation the student will learn to grow and manipulate Cyanobacteria in a photobioreactor. The student will utilise three different cyanobacterial strains, Synechocystis sp. PCC 6803, Synechococcus sp. PCC 7942 and Synechococcus sp. PCC 7002, each cyanobacterial strain will be grown at 300C with a light regime of 60 and 100 µE m−2 s−1. The student will assess the growth rate of each strain over a 10-day period utilizing OD measurements. Furthermore, the student will utilize PAM measurements and chlorophyll content to access cellular fitness. The CO2 and O2 concentration in the media will be monitored with an off-gas analyser. The student will run 3 different experiments in triplicate in BG-II media at 60 and 100 µE m−2 s−1 at 300C. (1) air only (control), (2) flue gas (waste gas from Tata Steel) (3) 0.4% CO2 (control).

The student will be trained up on advanced analytical instrumentation including PAM and Oxygen electrode. They will also learn how to run photobioreactors. The student will also learn how to synthesise metal-organic frameworks and characterise them using a variety of analytical techniques (X-ray diffraction, electron microscopy, thermogravimetric analysis, IR spectroscopy etc).

Full Project Description: The need to develop sustainable, renewable Industrial processes is crucial and one of the foremost global challenges facing humanity. Traditional manufacturing processes are unsustainable, utilising non-renewable feedstocks, and releasing large quantities of greenhouse gases and toxic side streams and waste products into the environment. The financial cost of emitting

CO_2 is set to increase with many countries implementing carbon taxes on companies that burn fossil fuels. Thus, there is a drive towards Carbon Capture and Storage (CCS) technologies. It is very clear that utility scale breakthroughs, will need to be fast and cheap. There is therefore an opportunity to exploit technologies which use CO_2 as a cheap, potentially cost-negative, feedstock for the manufacture of key industrial chemicals, thereby creating a 'circular economy', which adds value, maximises efficiency and builds flexibility and security into the supply chain. Numerous carbon capture and conversion techniques have been proposed to ameliorate the CO_2 challenge. This project aims to develop a carbon sequestration and conversion platform utilizing metal organic frameworks (MOFs) to capture, concentrate and release CO_2 directly to cyanobacteria, which will then convert the CO_2 to high value products. Metal organic frameworks (MOFs) are adsorbent materials that have already been utilised for the selective capture of CO_2 from industrial waste streams. MOFs are formed through pervasive coordination bonds between organic ligands and metal cations. MOFs are distinguished by their ultrahigh porosity and surface area, tuneable pore size, geometry and their versatility making them excellent vehicles for carbon capture. Cyanobacteria are extant examples of the first microbes capable of oxygenic photosynthesis that tapped into an unlimited supply of electrons (by splitting water), enabling evolution of complex life. They are ideal cell factories requiring only CO_2 and light as the sole carbon and energy source, furthermore they can be utilised to generate a sustainable array of high value products from waste CO_2, fixing 1.83 kg of CO_2 per 1 kg of biomass. Therefore, they are excellent chassis for carbon capture, sequestering CO_2 into high value by products.

The primary objectives of the PhD project are;

1. Design and evaluate several different MOFs to assess CO_2 capture from waste flue gas and its subsequent release to cyanobacteria.

2. Assess the toxicity of the MOF in BG-II media to cyanobacteria.

3. Design, construct and validate a MOF/protein hybrid to increase CO_2 fixation in cyanobacteria.

4. Engineer a cyanobacterial chassis capable of the conversion of carbon monoxide to carbon dioxide and the fixation of NOX to Nitrogen.

The student will be part of a multi-interdisciplinary project encompassing separation technologies, materials chemistry, porous materials for applications in gas storage and separation, metabolic engineering and fermentation. The student will receive dedicated mentoring from the supervisory team and will benefit from their substantial expertise. The project will promote skills acquisition in a unique multidisciplinary environment working across Advanced Materials Research and Sustainable Process Technologies groups; providing the student with an array of transferable skills, highly prized by employers in the growing bioeconomy.

Lab Rotation Location: University Park;

Full Project Location: University Park;

153 Novel biocement formulations based on polyhydroxyalkanoate (PHA) producing microorganisms

Lead Supervisor: Konstantina Kourmentza

Lead School: Engineering

DTP Research Area: Biotechnology

Lab Rotation Description: The lab rotation will introduce the student to microbiology and fermentation work, cultivating polyhydroxyalkanoate (PHA) producing bacteria in the lab and extracting the PHA biopolymers from the cellular biomass. The candidate will initially explore basal media supplemented with high purity carbon sources (glucose, fatty acids) in batch bench scale experiments, with PHA producing bacteria available in-house, able to produce PHA biopolymers of different chemical composition. A training on relevant analytical techniques and methods on the chemical analysis of PHA This will allow the student to become familiar with the growth and downstream processing techniques available.

Moreover, the student will follow a methodology to develop PHA-based biocement formulations and conduct preliminary tests regarding its mechanical characteristics (i.e., compressive and flexural tests).

The above activities will give the candidate with a real taste of the research tasks, techniques and methodologies that will be followed while pursuing this PhD topic. Specifically, they will: (1) learn to design and conduct fermentation experiments, (2) learn and implement downstream protocols for the extraction and purification of PHA, (3) bind biocement constituents using the extracted PHA and test their compressive strength via a range of interdisciplinary analytical methodologies.

Full Project Description: Climate change and depletion of non-renewable resources have driven decarbonisation strategy plans to promote the transition towards a circular economy. Biomass-based construction materials have been identified as low-carbon materials used to produce products for civil and geotechnical applications. Research has focused on the feasibility of eco-efficient materials in engineering practice mainly to produce building materials. However, there is limited evidence of successful practical applications in the field of construction materials for other applications.

The most used binder in the construction sector is cement due to its ability to bind aggregates and produce concrete structures characterized by durability and high strength. Annual cement production reached 4.3 billion tons in 2021, while around 0.59 tons of CO2 are emitted per ton of cement produced, accounting for up to 7% of the global greenhouse gas emissions. The global cement market size is projected to grow, exhibiting a CAGR of 5.1% during the forecast period between 2022-2029. Subsequently, CO2 emissions are expected to increase. However, investment in net-zero construction materials is necessary to meet the COP26 climate goals, as the aim is to decrease direct emissions intensity of cement production from 0.59 tons in 2021 to 0.43 tons of CO2 emitted per ton of cement produced, by 2030.

To address the above challenges, this PhD project will aim to develop novel formulations of biocement to reduce associated carbon footprint and improve the mechanical properties, compared to traditional cement. Currently, research on biocement is focusing on the production of calcium carbonate from microbes via a process called microbial induced calcite precipitation (MICP). However, MICP-biocement is characterized by lower compressive strength of around 3MPa compared to 25MPa of cement, thus limiting its application. In addition, during the MICP process,

ammonia occurs as a by-product, which is toxic, corrosive and harmful to the environment. Finally, MICP-biocement precipitation heavily relies on calcium sources and urea which makes the overall process far more costly compared to traditional cement.

An alternative worth pursuing here is the application of natural biopolymers as bio-binders, as they are characterized by high tensile strength and high ductility, which make them ideal binder substitutes for cement. Polyhydroxyalkanoate (PHA) biopolymers are accumulated within bacterial cells by a vast majority of microbes, able to naturally produce PHAs under unbalanced growth conditions. PHA biopolymers are non-toxic, biodegradable and can be sustainably produced from renewable resources, thus contributing towards the development of circular bioeconomy while providing an alternative to oil-derived plastics.

This PhD will aim towards the development of novel biocement formulations based on PHA. The research activities will include the fermentative production of different PHA structures and the assessment of these structures as novel construction binders. Biocement formulations based on PHA will be tested regarding their physical and mechanical properties, while a numerical model will be developed to simulate biocements' response. Moreover, AI (artificial intelligence) will be used to optimise the mix design for target mechanical characteristic properties.**Lab Rotation Location:** University Park;

Full Project Location: University Park;

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

Lead Supervisor: Kevin Webb

Lead School: Engineering

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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.

Lab Rotation Location: QMC;

Full Project Location: University Park;QMC;

186 Metalloenzymes for the biodegradation of rubbers and polyolefins

Lead Supervisor: Anca Pordea

Lead School: Engineering

DTP Research Area: Biotechnology

Lab Rotation Description:

The project aim is to discover new latex clearing proteins (Lcp's) for the degradation of rubber and to engineer these, in order to diversify their substrate scope towards different synthetic rubbers.

During the rotation, the student will be trained in fundamental techniques required in the project, such as established procedures for the expression of Lcp in E. coli, activity determination using an oxygen consumption assay, and analytical techniques for the characterisation of the mixture of oligomer products (NMR, HPLC and gel permeation chromatography, GPC). The student will develop a colorimetric assay for the activity of Lcp from crude extracts towards synthetic rubbers. The student will also perform bioinformatic analyses of sequenced genomes of environmental isolates, to identify novel promising Lcp candidates, to be further explored during the PhD.

Full Project Description: Waste derived from polymer-based material is not easy to decompose, thus creating a serious environmental concern once the material reaches end of life. Amongst approaches for the recycling and repurposing of these materials, the emergence of enzymes that can degrade industrial polymers promises to revolutionise our approach to circular economy. Enzymes are renewable and they catalyse reactions in a specific manner, thereby offering the potential to control the outcome of degradation.

The vision of this research is to develop metalloenzymes for the cleavage of carbon-carbon bonds in synthetic polymers. Much exciting research has been published recently, regarding the enzymatic degradation of hydrolysable polymers such as PET. However, the most problematic and abundant plastic types are polymers with carbon-carbon backbones (polyolefins such as polyethylene, polypropylene and rubbers such as polyisoprene, polybutadiene); these plastics are much more difficult to degrade and recycle than PET. Very few enzymes have been suggested to biodegrade PE and PS by oxidation, but the mechanistic pathways remain unknown.

We propose to discover and engineer metalloenzymes to break down synthetic hydrocarbon polymers, such as synthetic rubbers (polymers with C=C bonds in main chain). Latex clearing proteins (Lcp's) have been shown to degrade natural rubber (latex) into oligomers. Data also suggests that synthetic rubbers can also be enzymatically degraded. Although a few Lcp's have been characterised so far, the substrate and product spectrum of these enzymes remain largely underexplored. In our laboratory (Pordea group), we are working with Lcp from Streptomyces sp. K30, which is one of the best characterised Lcp's. We are currently investigating the effect of mutations in the substrate recognition tunnel on the promiscuity of Lcp towards different synthetic rubbers. We implemented a range of analytical techniques to characterise the rubber degradation, using substrates in emulsion, powder and film form. A recent report demonstrated the increased ability of Lcp K30 to degrade synthetic polyisoprene by using a biphasic system with water, polymer and a hydrophobic solvent. In our hands, the enzyme maintained activity with up to 1% (wt) hydrophobic solvents, however, using higher solvent ratios had a detrimental effect on enzyme activity. Furthermore, our lab (Bryan group) has access to a range of organisms isolated from the environment, which have the ability to break various synthetic polymers (plastics), including hydrocarbon polymers such as PE, PP and PVC.

In this project, the aim is to discover novel rubber degrading enzymes within organisms isolated from the environment and to engineer these enzymes, to expand their substrate scope to synthetic polymers. In a first approach, we will develop a medium-high throughput method to engineer Lcp's, including a rapid screen to assess the activities of large libraries of mutants towards different substrates. We will apply this approach to engineer Lcp's with increased activity and stability in the presence of hydrocarbon solvents, because we hypothesise that the presence of these solvents would facilitate the degradation of hydrophobic synthetic rubbers. Active and stable Lcp mutants will be tested for their ability to degrade a range of synthetic rubbers, starting with isoprene rubber

(cis-polyisoprene). Secondly, we will assess the ability of a set of environmental isolates to degrade synthetic rubber, sequence the genomes of promising candidates and perform bioinformatic analyses to identify promising rubber degrading enzymes. Enzymes will be expressed in E. coli and characterised and the most promising enzymes will be further engineered towards rubber degradation.

Lab Rotation Location: University Park;

Full Project Location: University Park;

205 Engineering High Value Products from Plastic Waste

Lead Supervisor: Rachel Louise Gomes

Lead School: Engineering

DTP Research Area: Biotechnology

Lab Rotation Description: During the lab rotation the student will learn how to grow the selected bacterial strains on poly (ethylene terephthalate) PET. The strains provided have already been shown to utilise plastic. Samples (cell extracts and spent media) will be collected and they will be analysed using advanced metabolomics methods. The student will utilise several different techniques including small scale fermentation, growth rates, sample extraction using organic solvents and liquid chromatography-mass spectrometry (LC-MS/MS) analysis. The student will be trained in LC-MS/MS analysis and this will involve the optimisation of the existing LC-MS-based metabolite profiling. They will also participate in the weekly (with supervisors) and monthly group meetings (research group / analytical).

Full Project Description: The Problem: Plastic is essential to modern society, driven primarily by its incredible versatility as a material coupled with its low production costs and energy requirement1. As a result, global production exceeds 330 million tons of oil-based plastics per annum and the enormity of the multi-tiered environmental plastic problem is now very apparent. Only 9% of plastic gets recycled and even then, only a limited number of times due to thermal degradation. The remaining plastic pollutes the environment or sits in landfill sites, where it can take up to 500 years to decompose, leaching toxic chemicals into the ground. Traditional plastics such as the polyester poly (ethylene terephthalate) (PET) are made from oil-based raw materials. PET makes up almost one sixth of the world's annual plastic production of 311 million tons. Despite being one of the more commonly recycled plastics, only half is ever collected and recycled, considerably less actually ends up being reused.

The Solution: We propose to upcycle polyesters into highly valuable products (e.g. antibiotics, therapeutic peptides) both of which are of considerable interest possessing several highly sought-after properties including protease resistance and rigid scaffolds that can be almost infinitely diversified and for healthcare. Their size and molecular complexity means that they can target protein–protein interactions, a task that current small molecule drugs struggle to achieve. The PhD project will target poly (ethylene terephthalate) (PET), the most abundant, mechanically recyclable polyester, produced at a market demand of circa 30 million tons per annum.

Hypothesis: The overarching hypothesis of this project is that plastic waste can act as a stepping stone to a circular plastics economy by creating high value chemical products.

Aim: The overall project aims are to engineer a host platform for the conversion of PET to high value peptides/antibiotics.

Objectives: The primary objective being to develop a microbial cell factory capable of converting PET to peptides: the student will utilize a combination of gene knockouts/knock-ins coupled with overexpression of target enzymes to enable maximised PET degradation and the efficient conversion of the PET breakdown products, terephthalic acid (TPA) and ethylene glycol. Conventional liquid chromatography (LC)-mass spectrometry (MS)-based metabolomics and stable isotope-assisted metabolic pathway analysis methods, coupled with 13C flux will be utilised to predict in vivo enzyme reaction rates, unravelling metabolism, and providing exemplar kinetic data, allowing for the development of designer strain with improved plastic degradation and TPA conversion.

References to learn more about the challenge and project:

AUSTIN H ET AL, (2018) PNAS 115 (19) E4350-E4357.

DANSO D ET AL, (2019) Appl Environ Microbiol. 85(19)

JACQUIN J ET AL, (2019) Front. Microbiol., 10:865.

MCGLADE J ET AL, (2021). From pollution to solution: A global assessment of marine litter and plastic pollution. United Nations Environment Programme. October 2021, Pg 151. ISBN: 978-92-807-3881-0

RU J ET AL, (2020) Front Microbiol 11:442.

Lab Rotation Location: University Park;

Full Project Location: University Park;

Maths and Biosciences

127 Modelling auxin response dynamics within Marchantia polymorpha

Lead Supervisor: Leah Band

Lead School: Joint appointment between School of Biosciences and School of Mathematical Sciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: This interdisciplinary PhD project aims to create and test the first ever model of auxin transport and signalling within the liverwort Marchantia polymorpha, enabling us to study auxin dynamics within a whole organism for the first time and providing a platform for investigating the role of feedbacks within the auxin pathway. The project will involve mathematical modelling, as well as lab work to generate the necessary experimental data to parameterise and test the model predictions.

During the lab rotation, students will learn about current auxin models (both from our groups and others) and get hands-on experience of creating and programming mathematical models using the python-based modelling framework developed by the supervisory team. They will also learn more about the lab work through shadowing existing PhD students within Dr Bishopp's group. They will attend weekly 'root group' meetings and relevant seminars with both mathematics and plant science. During the rotation, students will create a prototype Marchantia auxin model using idealised cell geometries to represent the 2D multicellular geometry. Embedding these geometries into the established modelling framework, they will create ODE models, integrating key transport and signalling components, to gain some initial predictions about the auxin response distribution.

Full Project Description:

Context:

The plant hormone auxin controls many aspects of plant development. Key to this control is the auxin distribution, and how this leads to a distributed auxin response via the auxin signalling network. In this project, we will study auxin transport using a simple model organism, the liverwort, Marchantia polymorpha. Focussing on Marchantia has several advantages: its body plan can be represented by a 2D array of cells, and both for the auxin transporters (PINs) and signalling network (ClassA ARFs and Aux/IAAs) there is only one member of the key protein families. This greatly contrasts with Arabidopsis, where most auxin research is conducted, which has a complex architecture, 8 PINs, 23 ARFs and 29 Aux/IAAs. This project will develop a mathematical model of auxin transport and signalling in Marchantia, revealing how these processes combine to control auxin-regulated development.

PhD work plan:

The first aim is to create a multicellular model of auxin transport and signalling in Marchantia using real cell geometries and PIN distributions, obtained by generating and imaging reporter lines to characterise the geometries and MpPIN1 distribution (the only PIN in Marchantia). These data will be integrated into our python-based modelling framework, together with ODEs representing auxin transport and signalling.

The model will be tested via chemical treatments that perturb specific transport components, and imaging reporter lines. The modelling will address the long-standing question of how auxin signalling

feeds back on auxin transport via regulation of PINs. It also provides a platform to test the transport capacity of individual PINs from different species. This is especially relevant to PIN1 orthologues from monocots: these have been proposed to have sub-functionalised and canalise differently in response to auxin; however, currently there is no suitable environment to test this. Studying potential mechanisms by modelling these processes within this simpler organism and comparing with data will give novel insights into these important questions.

Furthermore, we will investigate how the auxin-signalling-network components control the dynamics of the auxin response. These protein families have greatly expanded in other plant species. The model will help understand why these expanded families were beneficial to angiosperms and how manipulating these proteins could modify the auxin response dynamics within specific tissues, providing a targeted method to manipulate downstream developmental responses in crop species.

Significance and deliverables:

- This project will create the first model of auxin transport and signalling in Marchantia.
- Focussing on a species with a simple body plan, we will create a virtual organism model and achieve a sought-after goal within the multiscale biology community.
- The project will lay foundations for using Marchantia as a model organism for plant systems biology, with huge potential for future projects.
- Revealing precisely how ARF and Aux/IAA properties determine the auxin response would enable future efforts to manipulate developmental responses in crop species.
- The tools developed would enable Marchantia to be used as a chassis whereby proteins from crop species could be introduced into a living 2D tissue, enabling detailed quantification of their properties, providing knowledge that could underpin crop breeding.

Lab Rotation Location: University Park;

Full Project Location: University Park;

Life Sciences

1 Developing the next generation of immune-modulatory molecular materials to modulate the fate of macrophages

Lead Supervisor: Babatunde Okesola

Lead School: Life Sciences

DTP Research Area: Biotechnology

Lab Rotation Description: Week 1 – 2: Introduction to essential pieces of equipment (peptide synthesizer, mass spectrometer, HPLC, Rotatory evaporator, Rheometer, NMR, FTIR, Circular dichroism spectrophotometer, etc) for the material synthesis and characterization. Student will work with Dr Okesola across the School of Life Sciences, School of Chemistry and BDI to learn the use of important pieces of equipment, which they will need to carry out their project. This will enable them to know what piece of equipment they need for various aspects of the project, where they are located across the University and the risk assessments involved.

Week 2 – 3: Student will work with Dr Okesola and Prof. Ghammaeghami's team to isolate monocytes from human whole blood using magnetic labelling

Week 4 – 5: Student will work with Dr Okesola to learn hydrogels preparation using various molecular building blocks including alginate, hyaluronic acid, agarose, etc.

Week 6 – 9: Student will independently isolate monocytes from human whole blood and will work with Dr Okesola and Prof. Ghammaeghami's team to learn macrophage differentiation and characterisation using flow cytometry and fluorescent microscopy.

Full Project Description: Project overview:

This is a multidisciplinary PhD project focused on development of novel molecular biomaterials with immune modulatory properties with potential applications in immune therapy and vaccination.

Background and aims:

The immune microenvironment is a complex system and plays a critical role in biological processes of some of the most pressing healthcare challenges such as chronic inflammation, wound healing, cancer, and implant integration. Macrophages are a heterogeneous group of immune cells that play important roles in resolution of injury, infection and tumor growth. Like other types of immune cells, macrophages respond to different environmental cues including extracellular matrix (ECM) stiffness, redox state and cytokines and are thereby polarized into specialized functional subsets. The possibility to control macrophage polarization toward either pro-inflammatory (M1) or anti-inflammatory (M2) phenotype using matrix stiffness has been demonstrated, however, molecular mechanisms that drive such phenotypical changes have remained elusive [1]. Hypoxia (oxygen shortage) is another important tissue environmental factor, its impact on macrophage polarization and subsequent modification of the inflammatory microenvironment have not been fully established. Hypoxia can promote macrophages accumulation, polarization and modify the inflammatory microenvironment in most solid tumours, which are generally oxygen-deficient, leading to poor prognosis. Modification of such microenvironment by molecular oxygen supply can switch macrophage phenotype between the tumour-associated M2 and the tumour-killing M1 phenotypes.

Lab Rotation Location: University Park;QMC;

Full Project Location: University Park;QMC;

2 The contribution of a high fat diet to sporadic Alzheimer's Disease mediated via the gut-brain axis communication

Lead Supervisor: JOERN STEINERT

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: During the rotation, the student will be exposed to all available and planned approaches. Ongoing MRes projects established all methods which include testing for animal behaviour (learning and memory, negative geotaxis [climbing activity], imaging studies). Furthermore, an introduction to confocal imaging with protein immunohistochemistry will be offered as well as shadowing of electrophysiological studies.

Specific timings:

Week 1-2: Behavioural studies will cover learning and memory, climbing activity.

Week 3-5: Immunohistochemistry on adult fly brains to assess protein expression and morphological phenotypes including confocal imaging.

Week 6-9: Induction to adult fly brain electrophysiology of Kenyon cells (if time allows, combination with Ca imaging).

Full Project Description: Background: Alzheimer's disease (AD) is the most common form of dementia and its neuropathology is characterised by the presence of amyloid beta (Aβ) plaques and neurofibrillary tangles, loss of neurons followed by broad neurodegeneration. Autosomal dominant mutations in amyloid precursor protein (APP), presenilin and tau are associated with a minority of AD cases, the majority of cases develops sporadically in the aging population. While their causes are largely unknown, genetic predisposition and potentially modifiable environmental factors may contribute to the progression of the disease.

Overall, using several well-established and robust AD models, the project will investigate the contribution of diet (high-fat diet [HFD]) and gut microbiome on the progression of AD during the aging process to identify conditions that may exacerbate or reduce the risk of the pathology progression.

Key aims are to:

1. identify the mechanisms by which a HFD causes a cholesterol- and ApoE4-mediated Aß42 production via altering APP and secretase interactions.

2. test if a HFD exacerbates AD pathology in genetically predisposed animals.

3. test if administration of probiotics ameliorates the dietary-induced pathology thereby alleviating AD hallmarks.

Experiment plan:

A: Survival: At present the impact of a HFD on AD mutant flies has not been conducted in fruit flies. The lifespan of AD-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 following the expression of toxic proteins. 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.

D: Learning and memory: Memory will be then tested using a T-maze for odour-associative learning and a preference index will be calculated.

E: Physiological and morphological properties of mushroom body neurons. Using standard immunocytochemistry techniques, we aim to analyse the effects of HFD and probiotic supplementations on the morphology of these structures to evaluate possible exacerbations or ameliorations conferred by the dietary treatments. We will investigate possible functional changes of these neurons upon treatments. Brains will be used to assess: i) electrophysiological properties of

Kenyon cells (KC) by whole-cell patch 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: Upon dietary treatments, 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. As diet impacts on the host's metabolome, we will identify metabolomic changes associated with changes in the diet (HFD/probiotics supplementation).

Lab Rotation Location: QMC;

Full Project Location: QMC;

12 Understanding age and sex related brain connectome changes

Lead Supervisor: Tracy Farr

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: The student will undertake training surrounding visualisation and analysis of magnetic resonance imaging (MRI) data. This will begin with tutorials surrounding data visualisation with various software platforms including those used routinely in our laboratories such as FSLEyes and ITK-SNAP. Once the student is proficient, we will provide training with different aspects of data pre-processing. Starting with functional (fMRI) data, the student will be shown how to judge image quality, and will be able to work through some basic scripts to correct for motion, filter, and smooth the data. We encourage the student to tweak some of the parameters to assist with understanding how the manipulations change the data. Finally, we will explore different types of image registrations. The student will learn when and how to apply a linear and a non-linear approach, and will gain experience using standard templates and atlases. Our labs routinely use FSL and ANTS for such manipulations, which means the student will obtain experience with bash via a Unix shell (a Linux command line tool). This will provide an essential foundation to image processing that would benefit any project that may employ image analysis, but also many students are interested in learning these skills.

Full Project Description: The lead supervisor has a strong background in preclinical magnetic resonance imaging (MRI) and has recently developed an image processing pipeline for mouse functional MRI (fMRI) data (Hall, et al., 2022 Stroke. 53(5): 1735-45). This type of imaging is sensitive to changes in oxygen metabolism that correlate with brain activity patterns. Thus, the pipeline is able to characterise the functional mouse brain connectome. Via collaboration with the University of Adelaide in Australia, we would like to propose a studentship that firstly aims to adapt the pipeline for use in rats. Our collaborators in Adelaide have collected fMRI data from aged male and female rats, and there are additional groups that have experienced traumatic brain injury. The student would be trained to use specialist software to test and optimise different pre-processing strategies for this rat data. This would also involve working with a new rat brain MRI atlas and template

obtained through collaboration with the Preclinical Imaging Team at King's College. The second goal of the project will be to train the student to use a computational approach to interrogate connectome changes in response to age and sex, and to use statistical and mathematical modelling to determine neuroimaging predictors of healthy ageing. The secondary supervisor has a wealth of experience with the analysis of human fMRI data, and a particularly strong knowledge of programming as well as statistical approaches to interrogate the fMRI data. Our collaborators in Adelaide have also acquired diffusion MRI data in the rats. This technique is sensitive to random motion of water and is often used to visualise white matter due to fact that water diffuses faster parallel to structures. It can be used in a similar manner to the functional data to examine structural connectivity changes in the brain. Structural information will be correlated with the functional data, and ultimately, with ex vivo measures of biological mechanisms that are proposed to underpin healthy ageing and those associated with chronic neuroinflammation in response to brain injury.

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 ongoing research of the lead supervisor. In addition to my own imaging work, I have a variety of collaborations in which I provide neuroimaging expertise to other groups that use rat models. There is also opportunity to extend this work into new neuroscience areas. In summary, this project is at the interface of neuroscience, medical physics, and computational neuroscience and offers a variety of collaborative opportunities.

Lab Rotation Location: QMC;

Full Project Location: QMC;

19 Isolating monoclonal antibodies by B cell cloning in important livestock and companion animals to understand disease immunology leading to novel therapeutics and vaccines

Lead Supervisor: JK Ball

Lead School: Life Sciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The candidate will learn the tools used to stain and sort antigen-specific B cells and subsequent cloning of monoclonal antibodies from sorted B cells. If time allows the student will also express and characterise antibody hits.

Full Project Description: The project will focus on further development of our monoclonal antibody discovery pipeline and its application to key livestock (bovine) and companion (dog) animals health - the final focus of the project will be decided by the interests of the student. Bovine targets will be blue tongue virus (BTV) - we have archived PBMCs that contain memory B cells clonally activated following BTV immunisation. Canine mAb discovery will focus on diseases where mAbs have been shown to provide therapeutic benefit (ie cancer (PD-1) and pain (Trka/NGF-1). The bovine platform is well-established. The canine platform will harness our understanding gained from the development of the bovine platform, but apply it to canine B cell responses. Cancer and pain targets will be pursued in collaboration with Prof Nick Bexfield (Cambridge) and Prof Sarah Hamer (Texas) who have well established study cohorts for provision of requisite samples.

Lab Rotation Location: QMC;

Full Project Location: QMC;

24 How does GPCR/RAMP signalling co-ordinate blood-brain barrier development?

Lead Supervisor: Rob Wilkinson

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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 hyperpermeability 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.

Lab Rotation Location: QMC;

Full Project Location: QMC;

31 Comprehensive analysis of immune responses using a refined intranasal cigarette smoke exposure model of COPD in mice

Lead Supervisor: Lucy Fairclough

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: Under this lab rotation project, the student will experience all the skills and techniques necessary to enable them to undertake a comprehensive analysis of immunological mechanisms in a murine model of COPD.

Specifically, the student will learn how to generate a defined cigarette smoke extract to be used in the intranasal model (week 1). Following this, the student will practice sterile tissue culture techniques, isolating cells from freshly collected mouse spleen (weeks 2-3), prior to gaining experience in staining isolated cells using a panel of fluorescently conjugated antibodies against cellular markers of interest and testing optimal concentrations of antibodies to be used in the panel (weeks 3-7). Finally, they will then learn how to analyse the multicolour flow cytometry data using both Kalusa and FlowSOM (I am Academic Lead for Flow Cytometry at UoN) (weeks 8-9).

In order to achieve these goals, the student will make use of the substantial bank of mouse offspring tissues collected from Dr Watkins' previous (Watkins et al., 2018.), and ongoing (under a BBSRC

funded grant) studies. From this experimental basis, the student will have the fundamental skill sets to undertake the planned broad screen of immune function within our murine smoke-exposed model.

Full Project Description: Background: We have carried out extensive research examining the role of the immune system in Chronic Obstructive Pulmonary Disease (COPD). COPD remains a growing but neglected global epidemic. Our research, utilising human samples, has been key in identifying the important role for cytotoxic cells, NK cells, NKT cells and B cells in disease. Using this knowledge, we have identified novel compounds that may be used for treatment of COPD (MRC P2D funding). However, using human samples does not give us the ability to examine whole body immune and physiological responses in individuals with COPD. The development of a murine model would overcome this knowledge barrier.

Presently there are limited UK established murine models that accurately reflect COPD phenotype. COPD in current models is induced using a variety of methods, including either whole-body or nose or head only cigarette smoke exposure, porcine pancreatic elastase instillation, genetic manipulations or infection models. However, the insight gained from such models is limited as they

do not enable accurate quantification of cigarette smoke delivery, do not consider responses in both males and females, and do not look at long term immune responses. We have a Home Office Project License (awarded to Dr Watkins) in place to develop a novel murine model of intranasal cigarette smoke extract exposure, with which we can address these issues.

Aims: The aim of this PhD project is to build on our initial preliminary studies, to obtain a detailed, mechanistic understanding of the biological processes through which COPD develops and progresses. Furthermore, this project will explore the aetiology and disease progression in both males and females, allowing for novel insight into COPD differences between the sexes. Data from this proposal will enable development of new treatments for COPD patients.

Objectives: The specific objectives of this proposal are:-

1, Optimise the intranasal mouse model in terms of cigarette smoke extract exposure (duration and concentration) that induces a phenotype (lung damage, pro-inflammatory mediator status, tissue immune cell infiltration) representative of mild/moderate COPD phenotype as seen in humans.

2, Define the progression and pathophysiological/immunological mechanisms of COPD using established experimental and physiological endpoints including remodelling of the lung smooth muscle (histology), lung alveolar damage (histology, RT-qPCR) and lung immune cell infiltration (flow cytometry), reduction in blood oxygen saturation (pulse oximeter) and elevated profiles of tissue and peripheral immune cells (flow cytometry, ELISA, qPCR, Western Blotting).

3, Establish the sex-specific differences in COPD development and severity. Here, the project will establish whether the onset, underlying mechanisms and magnitude of COPD pathophysiological changes differ between males and females.

4, Establish whether CSE-induced changes are reversible by maintaining the mice for an additional period of time after their final exposure to the cigarette smoke extract. During this time, parameters such as weight, blood oxygen saturation, circulating profiles of immune cells while alive and tissue morphology and molecular profiles after culling will be assessed. These studies will inform on tissue and immune system recovery following exposure cessation.

Lab Rotation Location: University Park;

Full Project Location: University Park;

34 High-value chemicals from renewable one-carbon feedstock

Lead Supervisor: Ying Zhang

Lead School: Life Sciences

DTP Research Area: Biotechnology

Lab Rotation Description: The 9 weeks rotation will serve as an introduction to the available gene tools. The student will:

Week 1-2: Training in microbiological methods and techniques such as the use of gaseous feedstock for growth and handling of oxygen sensitive bacteria, and generation of competent cells.

Week 2-6: You will be given a predesigned Transposon plasmid to generate a set of random mutants. Generated mutants will be confirmed by Inverse PCR and their genomic DNA isolated. CRISPR technology in methanotrophs will also be trained and knock out plasmids will be designed and cloned.

Week 6-9: Mutants will be phenotypically characterised. You will be trained to monitor growth by determining optical density and colony-forming-units. You will monitor the feedstock consumption by gas chromatography, and determine the concentration of fermentation products by LC-MS or HPLC.

Through all these procedures, the student will learn the basics techniques required to undertake the project. This will include, how to electro-transform and conjugate from E.coli into methanotrophs, how to screen putative mutants for transposon integration by Inverse PCR and agarose gel electrophoresis, and how to derive nucleotide sequence data covering the transposon insertion site. They will also be training in the use of the required DNA analysis software, including DNASTAR, CLC Bio workbench.

Full Project Description:

Ectoine is a high value chemical (£1500/kg) with a significant global demand (~15,000 Mt per annum.), especially in cosmetic industry and biomedical sectors. It is used as moisturizer, UV protector and anti-aging ingredient in cosmetic product formulation, and as enzyme stabilizer, cell, protein and nucleic acid protectant (from pH and/or heat/cold shock) in biomedical research. Ectoine can easily be synthesized chemically. However, large-scale chemical synthesis of ectoine is not competitive with biotechnological production due to the need for high cost precursors such as diaminobutyric acid. The current process for the biotechnical production of ectoine is an expensive two-step batch fermentation using pricey sugars and fossil fuels as feedstock by Bitop and Merck. The working horse in this procedure is halophilic bacteria such as Halomonas elongate. The bacterial milking process exploits the ability of H. elongata to release ectoine in response to dilution stress to the medium. Given the very high ectoine production costs (~£1-4 million/ton), there is a demand to develop alternative low-cost and renewable strategies.

Methane and methanol fixing bacteria isolated from saline and alkaline environments such as Methylomicrobium spp, Methylobacter spp, Methylarcula spp accumulate high levels of ectoine (up to 11 g/L) to survive the harsh conditions, which is almost doubled the current yields from commercial production using H elongate (6 g/L). For the biological production of ectoine, a

significant increase in productivity and reduction in production cost could be achieved by replacing the fed-batch process with a continuous fermentation using cheaper feedstocks such as methanol. Carbon emission generated during the process can be further reduced by utilising green methanol and waste methanol.

AIM: In this project, we will develop an ectoine production bioprocess from methanol fermentation, aiming to tackle the following challenges: 1). The basic research on methanol consuming ectoine producing bacteria is lacking, likely due to the existing time and labour intensive genetical manipulation methods. 2). The untamed wild type haloalkaliphilic methanotrophic strains are not robust enough to be used in harsh industrial process. 3). Ectoine produced by haloalkaliphilic methanotrophs can also be degraded as a source of carbon and energy. In short, we need to develop strains of bacteria that will work more efficiently in the process – growing quicker and producing desired products more consistently.

STRATEGY: Our group recently established an arsenal of genetic tools in methane and methanol fixing bacteria, including CRISPR/Cas9, CRISPRi, Tn5 transposon, which will allow us to investigate and enhance the basic knowledge in haloalkaliphilic methanotrophs and manipulate the ectoine pathway of bacteria to improve their yield and productivity.

The main project tasks will be: (i) identify Tn5 transposon mutants with modified ectoine yield and/or growth profile, investigate the gene and/or pathway involved in the modification using our newly developed CRISPR technology; (ii) characterize chosen mutants for its performance in lab scale gas fermentation bioreactors, using the wildtype haloalkaliphilic methanotrophic strains as benchmarks; (iii) design and implement modifications to pathways strategically to improve yield and productivity using a combined approach of system and synthetic biology.

Lab Rotation Location: University Park;

Full Project Location: University Park;

40 Visualizing dynamic cross-talk between neurons and astrocytes in vivo to advance understanding of neuroplasticity

Lead Supervisor: Kim Chisholm

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: The goal of this rotation will be to develop an understanding of astrocyte biology (in vitro through cell culture and through immunohistochemistry of spinal cord slices). In addition, the student will learn relevant microscopy skills (confocal and epifluorescence microscopy) to study calcium transients in cultured cells (astrocytes and neurons) and to image spinal cord slices stained through immunohistochemistry. This will provide essential laboratory experience and transferrable skills while also providing a solid foundation for the proposed studentship. The student will also be exposed to vivo imaging methods, shadowing ongoing work and supporting design, setup and data analysis.

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, VC and TB to optimise exposure to a wide range of skills and research approaches.

Full Project Description: Visualizing dynamic cross-talk between neurons and astrocytes in vivo to advance understanding of neuroplasticity

This studentship will explore the temporal and spatial pattern of astrocyte activation and their interaction with neuronal networks in different states of neuroplasticity.

Plasticity of the nervous system underpins many important physiological and pathological processes and involves complex changes in signaling between neuronal and non-neuronal networks. Blocking astrocyte function can block or reverse neuroplasticity in vivo, confirming their pivotal role. Traditional techniques could not simultaneously study neuronal and astroglial cellular network in physiologically representative systems, limiting mechanistic understanding of the dynamic interactions between astrocytes and neurons and how these lead to neuronal plasticity.

This studentship will use recently developed in vivo fluorescence microscopy methods to probe the dynamic interactions between neurons and astrocytes in different models of neuroplasticity in the intact rodent. The types of questions that will be addressed include how patterns and frequencies of neuronal activity influence the activity of astrocytes in vitro and in vivo, and how interfering with astrocyte function alters neuronal calcium signaling and neuroplasticity. The knowledge gained by this studentship will advance our understanding of why astrocytes have a pivotal role in neuronal plasticity in vivo.

This studentship will suit individuals interested in the plasticity of the nervous system and the role of different cellular compartments, which is relevant to many neurobiological processes, including spinal plasticity associated with chronic pain states. This studentship 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: To develop skills in genetic tools, such as AAVs and transgenic mouse lines, to express calcium indicators in neurones and astrocytes 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 models of spinal cord plasticity. These models are already established, including models of inflammatory induced and nerve injury induced spinal cord plasticity.

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

With these skills the student will develop and investigate research questions related to astrocyte/neuron interaction in states of neural plasticity. These may include:

1) Is astrocyte calcium signaling linked to neuronal activation in naïve states and states of plasticity?

2) How does inhibition of neuronal subsets in models of spinal cord plasticity alter astrocyte activation patterns?

3) What is the role of other neuroimmune cells, such as microglia, in the dynamic interactions between astrocytes and neurons?

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.

Lab Rotation Location: QMC;

Full Project Location: QMC;

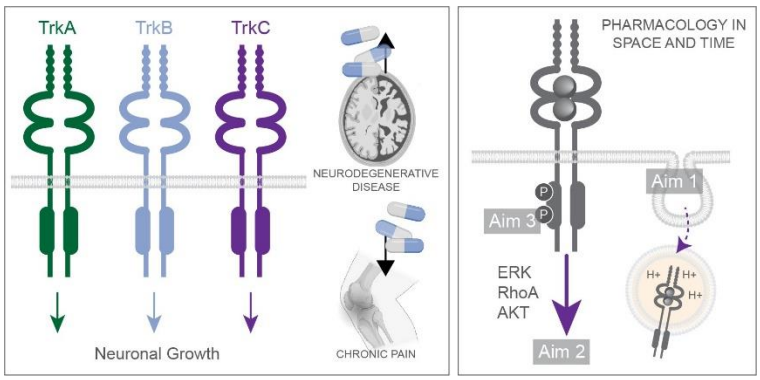
42 Spatiotemporal dynamics of growth factor signaling via neurotrophin receptors

Lead Supervisor: Chloe J Peach

Lead School: Life Sciences

DTP Research Area: Biotechnology

Lab Rotation Description: Pharmacological understanding of how drugs work – and how future drugs could work – are instrumental for patients across diseases. Existing therapeutics only act on 3% of human proteins, of which the majority target receptors. Receptors are fundamental membrane proteins often considered 'druggable' due to their ability to detect extracellular stimuli and evoke an intracellular response. Receptor Tyrosine Kinases (RTKs) are a family of 59 receptors that respond to growth factors. They induce cell proliferation, migration and survival in embryonic development and adulthood. Anti-cancer RTK inhibitors were developed to block proliferation via multiple kinases, however RTKs are central to many other pathologies. Neurotrophins are a family of growth factors (e.g., Nerve Growth Factor (NGF)) that stimulate neuronal growth. Their receptors (TrkA, TrkB, TrkC) are promising targets for chronic pain (antagonists) or neurodegenerative disease (agonists), however they lack selective, efficacious drugs without side effects. This mini-project aims to quantify the pharmacology of agonists and antagonists at TrkA, TrkB and TrkC. The student will use a 96-well format to monitor signalling using luminescent reporter genes in model cell systems to quantify transcription downstream of ERK, RhoA or PKC. Training will be provided in molecular pharmacology, cell culture, transfection and in vitro signalling assays.



Full Project Description: Neurotrophins are a family of growth factors including Nerve Growth Factor (NGF), Brain-Derived Growth Factor (BDNF) and Neurotrophins -3 and -4. They bind to the extracellular domains of respective Trk receptors and induce RTK dimerization. These conformational changes enable the auto- and trans-phosphorylation of key tyrosine residues in their intracellular kinase domain. Recruitment of adaptor proteins triggers various signaling cascades, such as ERK (proliferation), RhoA (migration) or AKT (survival), however there is limited understanding of Trk signaling kinetics over time.

Receptors are often considered as static membrane proteins. Upon activation, however, Trk receptors move from the cell surface into subcellular vesicles. These endosomes are mobile to move around intracellular compartments, each with unique biochemical properties (e.g., acidic pH). Seminal findings demonstrated that – despite removing receptors from extracellular stimuli – TrkA and TrkB continue signaling from endosomes. Limited studies have investigated TrkC. Many disease states, such as cancer, involve elevated levels of growth factors. As such, many receptors will already have been internalized. In the context of neuronal morphology, Trk therefore signals from subcellular endosomes that travel considerable distances along axons for transcriptional responses in the nucleus. This spatial modulation of signaling has also been overlooked by drug discovery efforts, where signaling endosomes are no longer accessible to membrane-impermeant drugs designed to target cell surface receptors. It is unknown how Trk localization affects their real-time pharmacology.

Rationale: This project aims to understand when and where neurotrophin receptors are moving (trafficking), how this implicates their functional responses (signaling) and what receptors can interact with in their distinct microenvironments (protein-protein interactions). Studies will test the hypothesis that despite structural similarities, there are distinctions between the pharmacology of TrkA, TrkB and TrkC. In order to investigate their biology with spatial (where) and temporal (when) resolution, this project will apply state-of-the-art approaches from the field of G protein-coupled receptors to the RTK family. This includes established techniques to probe the dynamics of neurotrophin receptor biology, as well as developing novel methods to unveil signaling complexes.

Project Approach: Firstly, neurotrophin receptors will be visualized moving around living cells in real-time. Fluorescent tags (e.g, HaloTag) enable the localization of receptors to be monitored using advanced imaging approaches available in the Centre of Membrane Proteins and Receptors (COMPARE). This will use a neuronal cell line with axonal projections. Secondly, kinetics of growth factor-induced signalling will be investigated using resonance energy transfer (RET). Fluorescence-based RET (FRET) sensors localized to specified subcellular regions will measure ERK signaling over time. We will also study the effect disease-modifying mutations on spatiotemporal signaling. Thirdly, studies will investigate protein-protein interactions with neurotrophin receptors. Novel bioluminescence-based RET (BRET) approaches will be developed to measure recruitment of adaptor proteins to Trk receptors. Mechanisms regulating RTK interactions will be investigated using gene silencing to identify regulatory molecules involved in Trk signaling. This project will enable training in cell culture of specialized cells, imaging, BRET, FRET and DNA cloning. This project also exposes the student to wider mentorship and Team Science initiatives within COMPARE.

Lab Rotation Location: QMC;

Full Project Location: QMC;

48 Evolutionary genomics of trematode parasites and their intermediate snail vectors

Lead Supervisor: Angus Davison

Lead School: Life Sciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The rotation will include training in molecular biological laboratory techniques, including an initial phase on DNA extraction methods for snails, and PCR-based methods to detect parasites. DNA samples generated during this first phase will undergo whole genome sequencing.

In the second part, the student will receive training in basic bioinformatics, then apply these methods to the whole genome sequences generated from the parasite and snail data.

The data generated during this rotation will contribute to the first part of the student's thesis.

Note that the precise make-up and balance of the rotation project will vary depending upon the existing skills and requirements of the student, whether bioinformatics or wet-lab, or both.

Full Project Description: Molluscs such as slugs and snails are intermediate vectors of important pathogens of humans and their livestock. In an agricultural context, snail-transmitted parasites have a high economic cost, because snails transmit trematodes to sheep and cattle, costing the industry hundreds of millions per annum. There are also direct impacts to human health. For example, schistosomiasis, a major neglected tropical disease of humans, is frequently transmitted to persons from snails when undertaking agricultural work in or around water bodies. In Africa, where the problem is most acute, about 200 million people are infected with schistosom parasites, causing fever, fatigue and an increased risk of bladder cancer, with tens of thousands dying from schistosomiasis worldwide each year (WHO estimates 200,000 globally), and incurring the widespread use of toxic molluscicides, which then enter the food chain.

To date, the majority of research on trematode parasites in Africa has focussed on the schistosome/human interaction, neglecting both the variety of snail intermediate vector species, and also other trematode parasites that infect livestock. Yet, strategies for both control of agricultural disease and also elimination of schistosomes should consider the intermediate host snail.

Of the limited prior work on these snail vectors, molecular genetic research has mainly used species-specific methods to identify parasites in snails, thus limiting the findings to species known to be present. More recently, metabarcoding methods have proved useful in providing a comprehensive catalogue of trematode parasites within a snail. However, while useful, both of these approaches neglect the snails, usually requiring further costly but low-power PCR-based methods to identify the species.

In this study, we will take advantage of advancing genome sequencing technologies and an existing resource of field-collected snails. Population genomic methods will be applied to the snails and the parasites, to take a first step towards understanding interactions between the snails, the schistosomes in the natural environment.

Specifically, whole genome sequences of the snails will be used to 1) identify and quantify parasites present within the snail 2) assemble whole snail genomes from fragmentary information 3) describe the phylogenomic structure and species status of the snails, and 4) synthesise this information, relating parasite presence/absence to underlying genetics and geography of the snail host.

Data from whole genome sequencing will be used to understand whether infection by a particular species of trematode makes snails recalcitrant to infection by other parasites, a finding that would have direct implications for surveillance and control. In a pilot study, we have shown that this approach works, detecting pre-patent trematode infection in snail hosts. A more comprehensive PhD study will prepare the ground for future studies that are directly aimed at controlling these diseases of humans and livestock.

Lab Rotation Location: University Park;

Full Project Location: University Park;

50 Testing an outer membrane vesicle (OMV) vaccine against Clostridioides difficile

Lead Supervisor: Ruth Griffin

Lead School: Life Sciences

DTP Research Area: Biotechnology

Lab Rotation Description: Deploying the pETDuet-1 vector designed for co-expression, the coding sequence of a colonisation factor and the receptor binding domain of Toxin B of Clostridioides difficile will each be fused to the coding sequence of a signal peptide and linker that we established directs surface localisation in Escherichia coli. The construct will be used to transform an E. coli expression strain in which we previously deleted two genes. Following induction of expression of these genes, outer membrane vesicles (OMVs) will be harvested from a late log culture and analysed for size distribution and concentration by ZetaView NTA (at NTU) and for charge by Dynamic Light Scattering (DLS), abundance of heterologous antigen and surface localisation by Western blotting and immuno-dot blotting. Stability of the OMVs will be assessed following lyophilisation, room temperature storage and reconstitution in PBS by repeating the above characterisations. The student will be trained in cloning, inducing cultures, isolating OMVs by centrifugation of culture filtrates, ammonium sulphate precipitation and ultracentrifugation as well as in analysing their physical properties. Students will be encouraged to engage with the literature throughout the project and will be expected to present at group meetings to troubleshoot together and practice scientific communication and team-working.

Full Project Description: Background

Ninety percent of infections occur in our mucosal tracts including gastrointestinal, respiratory and urogenital tracts, where secretory IgA antibodies (sIgA) are our first line of defence. sIgA coats bacteria/viruses trapping them in mucous preventing colonisation. These clumps are then cleared from the body. Vaccines aim to mimic natural immune protection, but injected vaccines induce little or no mucosal sIgA. Two Phase III trials of injected toxoid vaccines against the gut bacterium Clostridioides difficile failed to promote any protection in the gut. This reinforces the need to develop new vaccines administered via mucosal routes such as oral/nasal that induce sIgA at the target site of infection.

Injected vaccines have progressed from the traditional approach of deploying weakened whole pathogens to safer approaches of using defined parts of the pathogen with adjuvant (immune enhancer). However, mucosal vaccines require strong potency to overcome tolerance (due to their continuous exposure to allergens) and thus rely on the traditional live attenuated approach which is unsafe for the immunocompromised. Novel mucosal vaccines are urgently needed, however due to

two major bottlenecks, only five infectious diseases are targeted by mucosal vaccines to date including polio, rotavirus, typhoid and cholera (oral) and influenza (nasal).

The first bottleneck for developing mucosal vaccines is identifying adjuvants that are sufficiently potent yet safe. The second important bottleneck is identifying a suitable delivery vehicle with good bioavailability (readily taken up by host cells). Outer membrane vesicles (OMV) offer an attractive solution since they are self-adjuvanting (being rich in lipids that act as danger signals for the innate immune system) and being particulate, more readily taken up by host cells than soluble antigen. Furthermore, OMVs can be lyophilised, packed into gelatin capsules and coated in enteric polymer for targeted delivery to the ileum where sIgA is released, protecting the colon.

Objective

With the focus of my group on developing novel mucosal vaccine platforms, the aim of this project is to apply the OMV prototype developed by BBSRC DTP student, Barbora Martinkova, to C. difficile. From the work of another PhD student, Cansu Karyal, we have seen encouraging intestinal sIgA and serum IgG responses to a colonisation factor of C. difficile given orally, with even greater responses when presented on liposomes. This project will exploit our established OMV delivery system along with our promising vaccine antigens to create OMVs co-expressing the colonisation factor and immunogenic domains of the C. difficile toxins. After characterisation of the physical properties of OMV formulations and confirmation of surface display of the antigens, the vaccine will be tested after oral delivery to hamsters (established C. difficile animal model). Antibody titres in intestinal fluid and serum will be compared to naïve control animals and the functionality of these antibodies in blocking the adhesion of C. difficile to Caco-2 cells, and in neutralising C. difficile toxins in Vero cells, determined. The immunogenicity data will be benchmarked against our previous data and a protective efficacy study will be conducted in the hamster model.

Lab Rotation Location: University Park;

Full Project Location: University Park;

60 Understanding multisystem proteinopathy gene (dys)function in immune cells through multi-omic analyses of human stem cell models.

Lead Supervisor: Daniel Scott

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: The aim of the rotation will be to generate key tools to be used in the full project, namely a series of induced pluripotent stem cell (iPSC) lines in which key multisystem proteinopathy (MSP) genes are deleted. To achieve this the prospective student will receive training in human iPSC culture, CRISPR gene editing and conversion of iPSC to iPSC-macrophages.

Full Project Description: Multisystem proteinopathies (MSP) represent a collection of dominantly inherited disorders that can manifest as amyotrophic lateral sclerosis (ALS) (affecting motor neurons), frontotemporal dementia (FTD) (affecting cells of the brain), inclusion body myopathy (IBM) (affecting muscle), Paget's disease of bone (PDB) (affecting the bone), or in combinations, where multiple cell types/tissues are affected. Genome-wide association studies have identified

MSP-associated variants in genes including; VCP, PFN1, HNRNPA1, HNRNPA2B1, MATR3, OPTN, ANXA11, TIA1 and SQSTM1. Defining the biology of why patients with identical mutations in the same gene develop distinct clinical phenotypes, affecting different tissues, even sometimes within the same families remains a priority. The existence of modifier genes is an obvious possibility, but the high prevalence of pleiotropy even among closely related family members argues that other stochastic factors, perhaps at the cellular level, may be at work. In this regard, it is notable that many of these 'MSP genes' are highly expressed in macrophages – tissue-specific immune sentinels that act as effectors of the innate immune response. This is particularly pertinent given there is an emerging inflammatory component common to all MSP disorders. Despite this, the study of how MSP genes influence macrophage function has been overlooked, in part due to the difficulties of genetically manipulating primary human macrophages.

In work leading up to this project, the supervisory team has established methods for CRISPR gene editing of iPSCs, and the conversion to of iPSCs to a host of MSP-relevant cells including macrophages of the blood, brain and bone, and motor neurons. In this studentship, we propose to further utilise these cutting-edge tools (CRISPR editing, stem cell culture and conversion) to understand how MSP gene deregulation modifies macrophage function. We hypothesise that this will deliver new insights into the etiological relationship between seemingly distinct age-related degenerative diseases of muscle, bone, and brain

Experimental plan

1. CRISPR gene editing to knock-out all nine MSP genes that are shared across the disease spectrum in control iPSC lines.

2. Conversion of iPS cells to myeloid factories, that can subsequently undergo differentiation to highly relevant cell types, including a pan-macrophage (iMac) and tissue-specific residents of the bone (osteoclast) and brain (microglia).

3. Molecular and phenotypic screening of iPSC-derived models. Primary screening of MSP gene-mediated dysfunction will take place in iMacs. We will first assess whether MSP gene knock-out lines induce the protein pathology that typifies MSP (the cytoplasmic accumulation of TDP-43). Concurrently we will complete quantitative proteomics to define protein deregulation in MSP iMacs. We will stage protein network deregulation against macrophage (dys)function, screening phagocytic capacity of MSP mutant lines (uptake of yeast and bacterial particles) using standard and imaging flow cytometry and by quantifying cytokine production (ELISA) in response to activating stimuli (i.e bacterial cell wall mimics).

4. Establishing tissue-specific iPS macrophage culture. We will then explore the conversion of MSP gene knock-out lines to osteoclasts and microglia, to validate common and distinct molecular dysregulation, established in 3.

5. Development of advanced stem cell models. Given the motor neuron component to a number of the MSP disorders, we will investigate the co-culture of iPSC-derived macrophage models with iPSC-motor neurons, to understand how macrophage deregulation may influence motor neuron homeostasis/function.

Lab Rotation Location: QMC;

Full Project Location: QMC;

65 Identifying peptide neurotoxins from centipede venoms with therapeutic or pesticidal potential

Lead Supervisor: Ian Mellor

Lead School: Life Sciences

DTP Research Area: Biotechnology

Lab Rotation Description: The object of the lab rotation will be to use patch-clamp analysis of the NaV1.7 subtype of voltage-gated sodium channels to investigate its modulation by a known NaV peptide neurotoxin.

Objectives of the rotation will be:

1. Synthesis and characterisation of a chosen peptide with NaV activity.

2. Culture the TE671 cell line, which expresses the NaV1.7 subtype of voltage-gated sodium channels. Cells will be plated onto coverslips for transfer to the stage of an inverted microscope for patch-clamp analysis.

3. Training in patch-clamp recording. Once successful, the student will develop experimental protocols for the stimulation of NaV1.7 responses and these will be characterised in the absence and presence of the synthesised peptide.

4. Analysis of the patch-clamp data comparing various functional parameters of NaV1.7 in the absence and presence of the peptide neurotoxin. Analysed data will be interpreted to propose the mode of action of the peptide.

This will expose the student to the most important techniques associated with the main project and provide experience of both partner labs. It will provide essential training for the main project. Objective 1 will take place in the Chemistry Building, 2-4 in the Life Sciences Building, both at University Park Campus.

Full Project Description: Venomous animals use their venom to paralyse their prey in order to rapidly subdue it. To achieve this, many venom components are directed at ion channel proteins in the nervous system to disrupt rapid signalling and, thus, cause rigid or flaccid paralysis. Centipedes are one such group of predatory animals that can subdue prey animals often larger than themselves but have received very little attention in terms of researching their venom.

Recently we have shown that the application of venom from the centipede, Scolopendra hardwickei, to both human and insect voltage-gated sodium (NaV) channels has a major impact on their normal functioning, generally causing a gain of function in these channels. We also noted an antagonistic effect against nicotinic acetylcholine receptors (nAChRs). Venoms from other closely related species have also indicated the presence of peptides targeting voltage-gated calcium (CaV) channels, voltage-gated potassium (KV) channels and TRPV1 channels, and so it is likely that other neuroactive components are present in S. hardwickei venom. Following this, we generated a transcriptome from the venom glands of S. hardwickei and identified 415 separate peptide and protein components within numerous families having well-conserved sequences. The majority of smaller peptide components were rich in cysteine residues that form multiple disulphide bridges, a hallmark of peptide neurotoxins.

The overall aim of this project is to identify peptide neurotoxins from the S. hardwickei venom gland transcriptome and to determine their ion channel targets and modes of action. Proposed objectives

of the project are to: 1. Conduct bioinformatic analyses to group peptides into their families; to search for related peptides from other species for hints on potential targets and to determine signal and propeptide sequences. 2. Assess the activities of individual peptide toxins at a range of ion channel targets, including NaV, CaV, KV, TRPV1, TRPA1 and nAChR, paying attention to subtype (important for therapeutic potential) and species (important for pesticidal potential) selectivity. 3. Determine the mode and site of action of selected peptide neurotoxins. 4. Design and synthesise mutant peptides to investigate which residues are essential for their activity. 5. Develop methods for the rapid and reproducible folding of the peptides into their native conformation. 6. Explore the replacement of disulfide bridges with stable, non-native linkages to enhance stability.

The project will employ a wide range of techniques including a variety of bioinformatic approaches; solid-phase peptide synthesis; HPLC purification, and mass spectrometric (ESI/MALDI) analyses of products; electrophysiological techniques such as voltage-clamp and patch-clamp coupled to protein expression in Xenopus oocytes and cell culture for pharmacological characterisation of venom peptides; molecular biology techniques to engineer target ion channel cDNAs and cRNAs for expression; molecular modelling of peptides.

The wide range of techniques will equip the student well for employment in the drug and pesticide industries, but will also be useful for a wide range of other biotechnology careers. A long term goal for this type of research is to identify new compounds that have potential as leads for new drug therapies or as pesticides.

Lab Rotation Location: University Park;

Full Project Location: University Park;

66 Understanding common mechanisms of repeat expansion RNA toxicity using primary and patient-derived stem cell models

Lead Supervisor: Rebecca Trueman

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: In the 9 week rotation, the student will learn primary cell culture techniques and establish primary cultures of neurons from both wild-type and repeat expansion mouse lines. This will involve training in dissection and aseptic cell culture techniques. Using in situ hybridisation, immunocytochemistry, imaging, and image analysis you will establish how RNA repeat expansion mutations alter neuronal structure, branching, and axonal growth, and how the mutation affects cell viability in culture. This will set you up with fundamental cell culture techniques required for the full PhD project.

Full Project Description: Dozens of incurable neurological diseases, for which advanced age is amongst the biggest risk factors, are typified by the erroneous expansion of short repeat sequences in coding and non-coding regions of the transcriptome. In these repeat expansion disorders, expanded mutant RNA aggregates together and sequesters other biomolecules (proteins/RNA), to form microscopic RNA foci in distinct subcellular compartments. How such RNA foci contribute to neurotoxicity and whether such mechanisms are conserved across repeat expansion disorders remains poorly defined.

In work leading up to this project, the supervisory team have established models of Myotonic dystrophy (DM) and motor neuron disease (MND) – which represent two archetypal examples of RNA repeat expansion disorders. Both diseases are characterised pathologically by neuronal vulnerability. We hypothesise that this arises from as yet undescribed, but conserved mechanisms of deregulation resulting from RNA foci expression in neurons. To test this hypothesis we will define the subcellular RNA foci interactome using quantitative proteomics in mouse models of DM1 (wherein we can turn foci expression off and on) and patient-stem cell derived neurons (DM1 and MND). This will enable us to robustly define a common (and unique) foci interactome and deliver new insights into the molecular mechanisms that contribute to repeat expansion disease pathogenesis.

Experimental plan

1. Models (Year 1)

This project will establish neuronal models of DM and MND - representative of broader RNA repeat expansion disorders. As indicated the student will first explore primary cell culture of TRED960i mice (a model of DM). Concurrently, we will stage findings in mice cell lines to patient-derived iPSC models. We will differentiate DM1 and MND iPSC cells already in house, to neurons and complete a phenotypic screen of patient-derived neurons (axon growth, electrophysiology).

2. Mechanisms (Year 2)

With the models established, we will then apply a battery of molecular and imaging approaches to resolve subcellular localisation of RNA foci in neurons, before utilising quantitative proteomics to identify proteins sequestered to them – to define a common (and unique) foci interactome. Following identification we will utilise bioinformatic tools to understand shared pathway deregulation.

Recently, it has been proposed that in addition to RNA foci acting to sequester biomolecules, toxicity may also arise from the translation of RNA expansion repeats into dipeptide repeat proteins (DPRs) by repeat-associated non-AUG (RAN) translation. To address whether such RAN translation products are toxics to neurons, we will assess protein expression by quantitative western blotting and immunocytochemistry. Furthermore, using our cellular models, we will manipulate RAN translation products using mimics/inhibitors to understand the molecular consequences of DPR expression to gain a greater understanding of RAN translation role in these repeat expansion disorders.

3. Targeting RNA foci (Year 3)

We have compounds that instigate the dissolution of RNA foci in DM muscle cells. We will apply these to the neuronal models of DM1 and MND to confirm efficacy. Success in these models would unlock new opportunities to target repeat expansion diseases more broadly.

Lab Rotation Location: QMC;

Full Project Location: QMC;

87 High-throughput decoding of mitosis in trypanosome parasites

Lead supervisor: Bill Wickstead

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: The lab rotation will give an introduction to the PhD project using a smaller set of mutants. In the project, we will grow and genetically modify trypanosomes to tag newly identified components of the chromosome segregation machinery. These will then be followed through cell division by fluorescence microscopy to analyse their interactions. We will then knockdown these components and test against known mutants in chromosome segregation and for synthetic interactions with drugs that interfere with mitosis. These data will provide the basis for some of the larger work that will make up the PhD project and also introduce some of the most important techniques of modern molecular and cellular parasitology.

Full Project Description: Correct cell division is essential to life. The way in which this is achieved, however, differs greatly between organisms. This is particularly the case for many important parasite species, the division of which is often very different from host cells. Understanding this cell division machinery is important to understanding the basic 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 researchers to generate and follow 100,000s of mutants to assess the function of genes in a specific process. This PhD project will use this new genetic method, along with biochemical and cell biological approaches to understand the cell division machinery of African trypanosomes and related parasites. By combining the genome-wide approach with specific mutants, we will define the essential parts of the chromosome segregation machinery, identify new components and decode how the system is put together.

Lab Rotation Location: QMC

Full Project Location: QMC

96 Splitting STAT Dimers to Understand Interferon Balance: A Strategy to Dissociate Beneficial and Detrimental Interferon Effects in Infection?

Lead Supervisor: U Vinkemeier

Lead School: Life Sciences

DTP Research Area: Biotechnology

Lab Rotation Description:

The lab rotation project will introduce students to the foundations of interferon signal processing. They will learn about the biochemistry and cell biology of interferons and some striking examples in which they can alter the behaviour of cells. The focus will be on the signal transducer and activator of transcription (STAT) proteins, a family of ancient transcription factors that are indispensable for the transmission and interpretation of interferon and cytokine signals. Successful students will work

with mammalian cell lines to study cellular and molecular responses to interferon treatment of cells. The experiments will demonstrate interferon's growth inhibiting effects and show how induction of the so-called antiviral state protects from infection with a pathogenic virus. These striking phenomena are also the rationale for using interferon-modulating immunotherapies in the clinic to treat cancers, viral infections, and autoimmune diseases such as multiple sclerosis. In addition to cell biological methods, we will use biochemical experiments to analyse the posttranslational modification and inducible DNA binding of STAT transcription factors and use fluorescence microscopy to track changes in their subcellular distribution in interferon-treated cells. The hosting lab moreover provides access to world leading facilities and a supportive environment conducive to exciting research.

Full Project Description: Background: Interferon signalling is critical for health, but knowledge of key aspects remains opaque. Interferons (IFNs) constitute a large family of proteins that alter the behaviour of cells in important ways through changes in gene transcription. Discovered more than 60 years ago for their role in the first line of defence against virus infections, these proteins have now more than reached the potential envisioned by the early discovering virologists. Interferons are commonly used in anti-hepatitis B virus therapy, and they have found additional therapeutic applications for oncology and multiple sclerosis. It has been recognized that interferons enhance innate and acquired immune responses and modulate normal and tumour cell survival and death. Studies of interferons have resulted in fundamental insights into the mechanisms of cellular signalling, gene transcription and the workings of the innate and adaptive immune systems. Mutations in STAT proteins and other interferon pathway constituents give rise to a spectrum of severe inherited immune system diseases characterised by susceptibility to infections, autoimmunity, and cancers, unambiguously demonstrating the vital importance of IFNs to the health of animals and humans.

Hypothesis and aims: We postulate that preformed STAT dimers are crucial determinants of interferon signalling and cytokine biology at large. To test this hypothesis, naturally occurring STAT protein mutations will be used to split STAT dimers in vitro and in vivo. This is done to ascertain if STAT dimer spitting changes the nature of interferon signalling. Specifically, it is the aim of the research project to determine if the splitting of STAT1/STAT2 heterodimers shifts the IFN-I-induced transcriptome towards IFN-II, and if this in turn boosts cell-autonomous antimicrobial defences. This PhD project will deliver fundamental insight in the assembly of preformed STAT dimers to understand the balance between Interferon-I and Interferon-II pathways and determine if dimer splitting can offer new ways forward to harness interferon's microbicidal potential. The project supports the BBSRC's long-term aims to deliver discovery-led frontier bioscience research that will underpin strategies for innovation in tackling infections and improving health.

Methods: There are particular hurdles in the study of dynamic processes such as IFN signalling that involve transient protein interactions and the rapid translocation of large protein complexes from the cell membrane to the nucleus. The project thus needs access to a diverse range of tools and methods to achieve its goals. These include live cell fluorescence imaging and FACS imaging to track the intracellular movements of STAT proteins. Studies of natural dimer-splitting STAT mutations will use gene-edited immune cell lines generated by CRISPR/Cas9 technology. Recombinant proteins will be produced in bacteria and insect cells for structural studies of STAT dimerization by analytical ultracentrifugation or protein crystallography. Next generation sequencing and quantitative reverse transcriptase PCR will be used to trace transcriptome changes caused by STAT dimer splitting mutations. Consequences for antimicrobial protection are assessed with cell-based infection experiments and various pathogens. Finally, good science thrives where cutting-edge technology

operates in a collegial, intellectually stimulating setting. Our labs in the QMC and the Research Complex at Harwell provide such an environment.

Lab Rotation Location: QMC;Research Complex at Harwell;

Full Project Location: QMC;Research Complex at Harwell;

101 Investigating the regulation of skeletal muscle mass using an in-vitro model of exercise.

Lead Supervisor: Matthew Brook

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: The student will undertake training in C2C12 cell culture techniques and establishing cell lines. This will take place at the QMC medical school cell culture facilities that are fully equipped with all required instrumentation. The project will use a C-Pace EP (IonOptix, MA, USA) to implement various electrical pulse stimulation contraction regimes, alongside nutrient treatments. Using fluorescence microscopy, cells will be imaged to investigate morphological changes in myotube size and myonuclei number. Further, cells will be collected and processed for analyses via western blotting and RT-PCR. This will be completed in the molecular biology labs of the QMC medical school under the guidance of the lead supervisor.

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 such as insulin resistance (Brook et al 2015 Acta Physiol (Oxf) Jan;216(1):15-41)

Typically, muscle mass remains stable during early life, being in a continual balance between muscle protein synthesis and muscle protein breakdown. However, throughout life, many individuals undergo muscle mass loss because of inactivity, illness, injury and the gradual muscle mass loss with ageing. As such, understanding the regulation of muscle mass is of great importance. Resistance exercise training (RET) combined with supportive nutrition is an effective strategy to promote muscle growth, yet the exact molecular mechanisms that control muscle homeostasis remain unclear (Brook et al 2019 Eur J Sport Sci. Aug;19(7):952-963)

Many signal transduction pathways are activated in contracting muscles depending on exercise intensity and duration. Insights into how the activation of these pathways regulate muscle mass can be gained using C2C12's- a muscle stem cell model that can be differentiated to form muscle myotubes in vitro. Applying electrical pulse stimulation to these myotubes results in contraction, enabling the investigation of exercise paradigms in vitro (Nikolic 2017 Acta Physiol (Oxf) Jul;220(3):310-331)

This project will investigate the acute changes in C2C12 myotube signalling in response to electrical pulse stimulation. As part of the project, students will be involved in the establishment of cell culture studies and collection of samples. The activation of key signal transduction pathways will be investigated using western blotting and RT-PCR. The morphological changes in C2C12's will be investigated using fluorescence microscopy. This project will allow students to investigate the molecular regulation of skeletal muscle homeostasis using an in vitro model of exercise.

Lab Rotation Location: QMC;

Full Project Location: QMC;

102 Role of cancer cell derived extracellular vesicles in neuron development and connectivity

Lead Supervisor: Federico Dajas-Bailador

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: Investigate the capacity for cancer line derived EVs to modulate axonal development and function (FDB/GH/BC) A crucial part of our proposal will aim to determine the key cellular processes behind the regulation of neuron development during cancer. As cortical neurons have a key role in brain's connectivity, those cellular mechanisms that can impact local plasticity in the axon terminals can lead to long term developmental problems. Thus, the cortical axons that constitute neuronal connections in the brain are a primary site of interest in the mechanistic understanding of long-term developmental problems associated with early life cancer survivors.

During your rotation with us you will be fully embedded within our research team. You will get the chance to explore the wide range of in vitro, ex vivo and in vivo approaches we utilise in our labs to probe the way the central nervous system is modulated by EVs. You will take part in studies, learn common techniques, undertake and observe the kind of measurements you will utilise in your PhD project. We will introduce you to our team as well as the wider neuroscience and cancer research communities in Nottingham.

Full Project Description: Childhood cancer survivors experience significant challenges beyond successful treatment. Most commonly they experience on-going, debilitating pain which can lead to anxiety, depression and social withdrawal as well as other profound negative impacts on their lives.

Extracellular vesicles (EVs) are lipid-based particles considered multifunctional molecular complexes controlling cell function. The term EV captures different kinds of vesicles, macrovesicles, exosomes and apoptotic bodies. These sub-types are distinguished by their size, origin, content, function and mechanism of release. Highly heterogeneous and complex in their composition, EVs are loaded with a variety of molecular cargos, including nucleic acids, lipids and proteins, both in their surface and lumen. EVs can facilitate/mediate cell-cell communication to modulate homeostatic cell properties, and have also been linked to some neurological pathologies, such as glioblastoma and neurodegenerative disease.

EVs are released from almost all cell types in the body, including cancer cells before, during and after exposure to chemotherapeutic agents. Although there has been increasing understanding of the EV-mediated mechanisms driving cancer development, reports on the role that tumour-derived EVs can have on neuronal processes at key stages of maturation have been largely missing.

EVs in the CNS can target cells in their vicinity and those located at distance from the site of origin via the cerebrospinal fluid and also following passage into the blood stream. EVs secreted from tumour cells support tumour growth and survival as well as acting to modify the tumour microenvironment locally and more distally through the creation of pre-metastatic sites. The application of chemotherapy is known to alter both the rate of EV release as well as the cargo of biological materials they contain for intercellular communication.

The cargo which EVs contain originates from the cell which produced them. Within the nervous system a large body of evidence points to micro RNAs (miRNA) and small non- coding RNAs (sncRNAs) as an important cargo within EVs. Although there are plentiful studies which investigate the role of EVs in cancer and neurodegenerative disorders there are very few which address their role in neurodevelopment or in pathologies of early life. A thorough review of this area has been performed by Sangani et al (2021) in which the role of EVs in CNS development is reported. Recent work in the FDB lab with cortical primary neurons also demonstrates how EVs can regulate neurodevelopmental processes via snc-RNAs. What is less understood is how EVs play a role in pathological conditions of the nervous system in early life, and how EVs released by tumour cells can impact these processes, both before, during and after chemotherapy.

You will gain a unique skill set spanning bioinformatics, primary and cell-line culture, cancer and neuron cell biology and in vivo neuroscience. The PhD Student will be embedded in labs which have significant external funding from charities and research councils. The supervisory team have significant success in PhD supervision and are dynamic researchers who embed inclusivity into their lab ethos and encourage their students to help shape project direction as part of a research team.

Lab Rotation Location: QMC;

Full Project Location: QMC;Biodiscovery Institute;

106 Developing a new assay for Congenital Heart Disease research

Lead Supervisor: Andrew Renault

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: Overall project aim: To develop a novel Drosophila model to accelerate the translation of genetic findings to a new mechanistic understanding of congenital heart disease (CHD). We will focus on the Notch pathway because the pathway is highly conserved and there are a large number of CHD associated rare variants.

Rotation: The student will develop and test the genetic tools that will later be used for assaying the functionality of Human Notch1 variants using the fly. To express these we will use the UAS/Gal4 mis-expression system. We have a recently generated Notch-Gal4 Drosophila line which is inserted into the Notch endogenous locus. The student will test this line to verify that it can drive expression in the heart and test if is a Notch null mutant by comparing defects in the heart to existing Notch mutants. The student will also create UAS Drosophila Notch and UAS human Notch1 constructs that can be later used to test if the over-expression of these wild type versions are able to rescue Notch loss of function.

Scientific outcome: A validated over-expression system and set of phenotypic analyses that can be used to test the functionality of HsNotch1 disease variants.

Full Project Description: Background:

Congenital heart disease (CHD) is the most common congenital defect affecting approximately 1% of live births contributing to a significance incidence of birth-defect related mortality. CHD includes a wide range of cardiovascular phenotypes spanning localized anatomical defects to more complex

abnormalities with common forms including bicuspid aortic valve (BAV) and Tetralogy of Fallot (TOF).

A significant underlying cause of CHD is genetic. Although many of the genetic variants identified disrupt genes that play a role in normal cardiac development the mechanisms are not fully understood. In addition, large scale whole exome sequencing studies are identifying increasing numbers of rare variants in these genes, however translating the position and nature of these rare variants into new biological understanding remains a challenge with a heavy reliance on relatively slow mammalian studies in which variants are studied one by one.

One of the major pathways underlying CHD, contributing to both BAV and TOF, is the Notch pathway. This fits with a well-established role of Notch signalling in heart development. In vertebrates, Notch has an early restrictive effect on cardiogenic mesoderm specification and a later role in patterning, valve morphogenesis and ventricular chamber development. The situation is similar in Drosophila where Notch signalling negatively regulates the specification of the cardiogenic mesoderm and later is required for correct cell specification between cardioblasts and pericardial cells.

Notch is a single-pass transmembrane receptor that is activated upon binding of one of a number of ligands (Delta or Jagged) in a cell-contact dependent manner. In humans and other vertebrates, there are multiple paralogues of both the Notch receptor (Notch1-4) and ligands (Delta1,3,4, Jagged1-4). The situation in Drosophila is much simpler with a single Notch receptor, just two ligands (Delta and Serrate) all of which show high degrees of similarity with their mammalian counterparts (eg. DmNotch & HsNotch1, Drosophila Delta & Jagged and Drosophila Serrate & Jagged2 show almost identical domain organisations and 61%, 61% and 45% overall similarity respectively).

Aim:

The goal of this studentship is to develop a novel Drosophila model to accelerate the translation of genetic findings to a new mechanistic understanding of CHD and to speed up the initial screening of variants for further study. We will use the Notch pathway because of the highly conserved nature of the pathway and the large number of CHD associated rare variants identified.

Scientific outcomes:

1) The grouping of rare HsNotch1 disease associated variants into functional classes such that representatives can be selected for further study. This will provide a cheaper, faster and higher throughput alternative to their characterisation in mouse.

2) Proof of concept that this approach could be used for the study of rare variants of other disease associated genes that have a Drosophila homologue. This approach could be applied to other human genes with a highly conserved Drosophila homologue. It is estimated that 65% of human disease-causing genes have a functional homologue in flies and a significant number are expressed in Drosophila tissues that perform the equivalent function in humans.

Lab Rotation Location: QMC;

Full Project Location: QMC;

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

Lead supervisor: Catarina Gadelha

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation 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 gene silencing, and mutant phenotype analysis assayed by 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.

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 (www.gov.uk/government/groups/uk-vaccines-network). 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 Africa.

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 (www.tdrtargets.org) 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 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.
Lab Rotation Location: QMC

Full Project Location: QMC

111 Epigenetic regulation of blood stem and progenitor cell formation during embryogenesis

Lead Supervisor: Martin Gering

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: In the lab rotation project, you will perform the first two steps of a single cell RNA-sequencing experiment that aims to compare the gene expression profiles of haematopoietic stem and progenitor cells (HSPCs) of wild-type (wt) and tet2/tet3 double mutant zebrafish embryos. In step 1, you will determine how many embryos you will need to isolate 20,000 HSPCs from zebrafish embryos that carry the gene trap allele qmc551 of gfi1aa. This gene trap allele expresses GFP under the control of the gfi1aa promoter in red blood cells (RBCs) and in HSPCs. To eliminate the RBCs, the embryos will also carry a transgene that drives RBC-specific dsRed expression. Such embryos will be dissociated, and fluorescence-activated cell sorting will be used to first enrich and then purify GFP single positive HSPCs. Based on the number of GFP+ HSPCs in the purification step, you will calculate how many wt embryos are needed to isolate 20,000 HSPCs. Getting the same number of tet2/tet3 double mutant embryos from crosses of double heterozygous carriers will be challenging, which is why in step 2, you will test CRISPR/Cas9 technology to generate generation zero tet3 mutants in a tet2 mutant background.

Full Project Description: Haematopoietic (blood) stem cells (HSCs) are rare bone marrow cells that maintain our blood system throughout life. In the clinic, they are used to treat patients with haematologic malignancies and inherited blood disorders. Their use is limited by our inability to expand them in vitro. Variable engraftment and graft-versus-host disease in allogeneic transplantation settings (using cells from unrelated donors) also pose considerable challenges that could be overcome with patient-specific HSCs derived in vitro from abundant cell sources. Currently, this approach is hampered by our incomplete knowledge of the molecular programming of HSCs during embryogenesis. In vertebrate embryos, HSCs first develop from haemogenic (blood forming) endothelial cells (HECs) that lose their endothelial characteristics and turn into blood cells. This endothelial-to-haematopoietic transition (EHT) is accompanied by changes in the cells' gene expression profiles, the details of which are incompletely understood. We have generated lines of zebrafish that carry mutations that interfere with this process, causing a reduction in the number of HSCs and their more mature progeny. One of these lines carries a gene trap transposon in the gfi1aa gene and expresses a GFP reporter under the control of the gfi1aa promoter. The transposon abrogates Gfi1aa expression in HECs but leaves the formation of definitive blood cells intact as the

gene's paralogue, gfi1ab, is upregulated in qmc551 homozygotes. Loss of both genes reduces the number of HSCs and their progeny. A similar phenotype is also observed in embryos that are depleted for Gfi1aa/ab's interaction partner and histone demethylase Lsd1 or are double mutant for the ten-eleven translocation (Tet) methylcytosine dioxygenases Tet2 and Tet3, suggesting that all three epigenetic regulator families play overlapping roles in the same cellular process. Making use of the GFP expression in HECs, HSCs and their progeny in qmc551 carriers, we aim to study EHT in live embryos and to isolate the cells to examine their gene expression profiles in both wt and mutant zebrafish. These experiments will involve high resolution fluorescence microscopy to image live cells, fluorescence-activated cell sorting to separate cell populations and single-cell RNA sequencing to determine the transcriptomes of individual cells. To increase the availability of some of the mutant embryos for cell isolation, CRISPR/Cas9 technology will be used to create biallelic mutations in generation zero (G0) embryos. Our lab has successfully used this method to generate gfi1aa/1ab/1b triple and lsd1 single mutant embryos to phenocopy the respective mutant embryos derived from incrosses of heterozygous carriers. Once generated, RNA-sequencing data will be analysed using appropriate bioinformatics tools to identify subpopulations of cells and examine differential gene expression in related subpopulations in wt versus gfi1aa/ab, lsd1 and tet2/3 mutant cells. Differential gene expression will be verified in RNA in situ hybridisation experiments. CRISPR/Cas9 technology may be used to target genes that we consider to be novel players in the process. The data generated in this project promise to improve our understanding of the epigenetic changes that drive HSC development in the vertebrate embryo, underpinning efforts to generate patient-specific HSCs from more abundant cell sources in vitro.

Lab Rotation Location: QMC;

Full Project Location: QMC;

122 Influence of microbiome-derived metabolites on mental health and stress resilience

Lead Supervisor: Madeleine (Maddy) King

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: The mammalian gastrointestinal tract contains trillions of microbes that are intricately linked to host brain function and mental health via the gut-brain axis (GBA). Microbiome-derived metabolites (MDMs) influence this axis. This PhD will examine how manipulation of MDM levels and associated microbiome changes during critical neurodevelopmental windows enhances stress-resilience to improve mental health across the lifespan, and the rotation will demonstrate the principal techniques associated with the main project.

Controlled studies in animals are invaluable for investigating mechanisms of host communication and establishing causality with respect to the GBA. Most studies are carried out in mice, which process some MDMs in very different ways to humans. The rat offers a more appropriate model for research with translational potential, but its gut microbiome (i.e. species present and their function) has not been studied in detail. The student will learn how to characterize and isolate bacterial communities associated with the rat gastrointestinal tract, using shotgun metagenomics and cultivation work, respectively. They will acquire the skills (microbiology, bioinformatics, statistics) necessary to analyse these data, receive introductions to cell culture and rodent behavioural studies, and attend lab meetings to integrate within both research groups and share findings.**Full Project**

Description: Composition and function of the gut microbiome alters throughout the lifespan and is further influenced by environmental factors like diet, stress and medication. This has profound implications for host brain function and mental health. For example, animals reared in germ-free environments (to prevent microbial colonisation) or treated with non-absorbable antibiotics (to selectively or completely suppress gut microbes) show changes in anxiety and memory, plus differences in synaptic connectivity and serotonergic neurotransmission within the hippocampus and amygdala. Furthermore, we have shown that transfer of intestinal microbiota between individuals can also transfer behavioural phenotypes, with positive or negative consequences for the host (D'Amato et al. 2020 Microbiome 8:140).

Bidirectional communication across the gut-brain axis involves neuronal, immune and neuroendocrine mechanisms, and accumulating evidence demonstrates that microbiome-derived metabolites such as short-chain fatty acids (SCFAs) are also key players. We are interested in how differences in microbiome composition and metabolism influence whether an individual is resilient to chronic psychosocial stress or placed at increased risk of maladaptive responses and psychiatric illness. This exciting PhD will combine in vitro and in vivo neuroscience, microbiology and bioinformatics approaches to address this fascinating question.

Chronic stress increases permeability of the blood-brain barrier (BBB) (leading to cytokine infiltration and microglial activation), whereas our research shows the SCFA propionate (produced along with acetate and butyrate by bacterial fermentation of plant-based polysaccharides in the large intestine) helps maintain BBB integrity (Hoyles et al. 2018 Microbiome 6:55). Initial in vitro studies will provide further insight into the way physiologically relevant SCFA concentrations regulate brain-derived neurotrophic factor (BDNF) signalling in hippocampal neuronal and microglial cell lines exposed to corticosterone and inflammatory cytokines (which are elevated by chronic stress). The student will then complete an accredited training course and obtain a Home Office personal license. This will enable them to mimic early-life adversity in rats via translationally relevant interventions (like post-weaning social isolation and chronic unpredictable mild stress) that impact upon gut microbiome composition (Dunphy-Doherty et al. 2018 Brain Behav Immun. 68:261-273) and induce dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. The student will manipulate SCFA levels during critical neurodevelopmental windows (by dietary alterations and direct systemic or intracerebral administration) to examine whether this protects against well-characterised negative behavioural consequences of chronic stress exposure (deficits in social interaction and pro-social ultrasonic vocalisations, impairments across a broad array of cognitive domains). This work has clear potential to identify novel strategies that promote healthy ageing by enhancing stress resilience. Mechanistic insight will be provided by comprehensive evaluation of potential neurobiological correlates of resilience; HPA axis function, BDNF signalling, microglial activation and hippocampal neurogenesis.

The project will provide excellent training in a range of highly desirable skills, strengthened by a Professional Internship at the interface of microbiome and neuroscience research. The student will be encouraged to undergo additional career development via scientific societies that provide specialised training for Early Career Researchers (https://www.bap.org.uk/nonclinical, https://www.ecnp.eu/early-career-scientists/workshops) and to disseminate findings though conference presentations, journal publications and public engagement activities.**Lab Rotation Location:** Medical School;Clifton Campus;

Full Project Location: Clifton Campus;Medical School;

130 The Benefits of Fungal Sex - Applications to Food and Medical Mycology

Lead Supervisor: Paul S Dyer

Lead School: Life Sciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The lab rotation will introduce candidates to working with filamentous fungi. This will involve training in sterile working, elements of molecular biology and classical genetics, and use of biochemical assays. Project work will relate to Penicillium and Aspergillus species studied in the laboratory for exploitation of sexual stages, which have important applications in food and medical mycology. Rotation work will involve two complementary aspects.

Week 1-3; 8-9: Training in methods to induce sexual reproduction and collect sexual progeny. Will include the design and development of a novel molecular diagnostic as a specific rotation project aim to identify sexually compatible partners with increased efficiency.

Weeks 3-8: Screening of a fungal isolate collection for desired traits of interest. Classical and biochemical tests will be tested and optimised and used to screen for aspects such as the following: (a) Protease activity. This can have an important impact on food flavour and quality in mould-ripened foods, but the genetic basis is poorly understood in key species; (b) Hyphal compatibility. Ongoing work suggests that certain factors influence ability of hyphae to fuse and trigger sexual reproduction. But these are little understood, and candidate strains need to be identified prior to possible further PhD studies.

Full Project Description: Fungi have beneficial but also detrimental properties. Beneficial fungal species include Saccharomyces cerevisiae, used in alcohol production and baking, and Penicillium roqueforti and Penicillium camemberti used in production of mould-ripened blue and white cheeses. Additionally, fungi have been used for the production of valuable metabolites including penicillin and statins. However, fungi can also cause severe animal and crop plant diseases. This includes notably the opportunistic pathogenic fungus Aspergillus fumigatus, which causes life-threatening aspergillosis disease in humans. Disease management of A. fumigatus has recently been complicated by the development of antifungal drug resistance.

The present project has the overall theme of exploiting knowledge of fungal sexual reproduction to lead to improved food quality/security and better understanding and management of aspergillosis disease. Within the Nottingham fungal group we have identified for the first time methods to induce sexual reproduction of Aspergillus and Penicillium species of major importance in the food and medical sectors. This project aims to exploit these breakthroughs and improve understanding in the following areas.

(1) What is the genetic basis of variation in sexual fertility? Although sexual cycles have now been induced in key species of food and medical importance, a large variation in sexual fertility has been observed. This has proved an obstacle to crossing certain fungal strains with traits of interest. Classical crossing and genetic analyses, together with newly developed bioinformatic/molecular screens will be employed to dissect out QTL/BSA markers linked to variation in sexual fertility. This will involve use of both the blue cheese fungus P. roqueforti and the pathogen A. fumigatus, for which isolates of varying fertility are available together with genome resources.

(2) Genetic manipulation for sexual fertility. Follow up genetic manipulation using candidate genes identified from area (1) will be undertaken using a combination of classical crosses, classic molecular transformations, and recently developed CRISP-CAS methods for gene editing. This should validate

candidate genes and provide a very valuable tool for further exploitation of the sexual cycle in strains of interest.

(3) Understanding the genetic basis of specific traits of interest. Certain traits are very important for the biology of Aspergillus and Penicillium species of economic importance, but remain poorly understood. Two specific traits will be investigated via a combination of classical and molecular genetic approaches, linked to work in the initial lab rotation: (a) Protease activity. This can have an important impact on food flavour and quality in mould-ripened foods, but the genetic basis is poorly understood in key species; (b) Hyphal compatibility. Factors influencing hyphal fusion to allow gene flow are little understood in Aspergillus and Penicillium species. We aim to determine the mono- or polygenic basis of such traits and identify causal genes via sexual crossing and QTL analysis.

The PhD will offer training in classical microbiology and biochemistry, bioinformatic/genomic and molecular-genetic work, and statistical data analysis. The overall research has potential applications in the food, medical and biotechnology sectors. There will be the possibility of some industrial work with a University start-up company 'Myconeos.com'.

Lab Rotation Location: University Park;

Full Project Location: University Park;

133 "Partners in crime: Understanding how Receptor Activity-Modifying proteins (RAMPs) modulate receptors signalling"

Lead Supervisor: Isabella Maiellaro

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: Please describe the nine week lab rotation project associated with the project (200 words)

The student will characterize the role of the regulatory protein RAMP2 in modulating Parathyroid Hormone 1 Receptor (PTH1R) endosomal signaling.

Activation of PTH1R by its ligand PTH triggers a sustained cAMP signal produced at the plasma membrane as well as at the endosomes. We know that RAMP2 can modulate PTH1R signaling at the plasma membrane, in the rotation the student will study the impact of RAMP2 on PTH-induced endosomal signaling. The student will analyze if expression of RAMP2 can alter the spatio temporal dynamics of PTH-induced cAMP signals in HEK cells. cAMP signals will be recorded in living cells using a FRET based fluorescent biosensor in which PTH1R will be expressed alone or together with RAMP2. The student will learn cell culture and transfection, perform live video imaging experiments and analyze them. The student will profile the dynamics of PTH-induced signals in presence and absence of RAMP2.

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.

We recently studied the interaction of RAMP2 with Parathyroid Hormone 1 Receptor (PTH1R), a class B GPCR, important regulator of mineral ion homeostasis and bone metabolism. PTH1R can signal from both the plasma membrane and from the endosome. Specifically, the ligand Parathyroid hormone (PTH) is able to induce prolonged signalling, which originates from the membrane and also from the endosomes.

Using fluorescent biosensor to monitor PTHR1 activation and intracellular signalling we discovered that RAMP2 modulates the kinetics of activation of PTH1R and its signalling at the plasma membrane.

Here we aim 1) to investigate the role of RAMP2 in modulating endosomal signalling by analysing the dynamics of endosomal signalling in presence and absence of RAMP2, and by following tagged RAMP2-PTHR1 complexes from the plasma membrane to the endosomes using specific markers. Additionally, we aim 2) to determine the mechanism by which RAMP2-PTH1R complex modulate β-arrestin2-mediated signalling. β-arrestins are cytosolic adaptor proteins that trigger endocytosis and kinase activation leading to specific signalling pathways that can be localized on endosomes. To this goal we will used established technology to monitor β-arrestin2 recruitment and change in conformation.

There are several tissues in which both PTH1R and RAMP2 are highly coexpressed (i.e., lungs, kidneys, and placenta). Since PTH1R/RAMP2 complex is not obligate but tissue-dependent, the knowledge gained in this project might be pharmacologically attractive and might represent a source of unique, tissue-specific biased signalling patterns.

Lab Rotation Location: QMC;

Full Project Location: QMC;

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

Lead Supervisor: Fiona J Whelan

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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.

Lab Rotation Location: University Park;

Full Project Location: University Park;

142 Unravelling the role of transporter proteins in paediatric medulloblastoma

Lead Supervisor: Ian Kerr

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: Medulloblastoma is the commonest malignant childhood brain tumour. Under standard therapy, relapse occurs in 30% of patients and is almost universally fatal, accounting for 10% of all childhood cancer deaths. Identification of membrane transport proteins which might be therapeutic targets is at the heart of this project. This will be achieved by a combination of wet laboratory work and bioinformatics analysis of big data sets.

In the rotation you would receive training in bioinformatics analysis and medulloblastoma cell culture as well as receiving a grounding in the work the supervisors have done previously with several joint PhD students. We have our own large-scale gene expression datasets as well as access to public datasets that will let us screen for possible transporter genes of interest; for example by looking for transporters where differences in gene expression are associated with survival. We will refine the choice of targets where functional assay is possible (e.g. via chemical inhibition or genetic knockdown). You will develop your growing body of bioinformatics analysis to identify, with the supervisors, a number of transporter targets that will be investigated in the subsequent main PhD. In other words, in the rotation you start to co-design the final project.

Full Project Description: Medulloblastoma is the commonest malignant childhood brain tumour. Under standard therapy, relapse occurs in 30% of patients and is almost universally fatal, accounting for 10% of all childhood cancer deaths. There is a genuine need to better understand the biological mechanisms that underpin two major and negative indicators of disease progression: i) drug resistance and ii) tumour invasion.

Membrane transporters play an extraordinary repertoire of roles in cell biology; the human genome encodes for well over 400 different transporter proteins responsible for the regulated movement across the plasma membrane and organelle membranes of the vast majority of metabolites. This regulated transport in turn impacts cell signalling, metabolism and survival amongst other processes. The central hypotheses of this project is that transporters may be both vital in the invasion and chemoresistance of medulloblastomas, and that druggable transporter proteins could be future therapeutic targets.

The rotation above will form part of an initial screen of current databases (and we are generating further gene expression, protein LC/MS-MS datasets in on-going projects) to identify candidate transporters. This “big data” analysis is a vital skill for the modern day bioscientist and will continue through in to the main project and form the first results chapter of the final thesis.

Having identified putative transporters of interest we will investigate their function and effect of modulating their activity in models of medulloblastoma. We have enormous experience in cellular (i.e. in vitro) models of medulloblastoma both in traditional 2D culture systems but more recently in relevant 3D model systems. The advantages of 3D culture systems are increased similarity to the “real” tumour environment and the ability to increase complexity of the model by including multiple cell types within it (e.g. tumour immune cells). 2D culture techniques still have some advantage in terms of scalability of certain assays (e.g. viability assays, transport assays), and both culture types have their role in this project. In the remainder of the PhD we would use the most appropriate 2D and 3D culture methods to investigate the effects of modulating transporter activity on both medulloblastoma response to current chemotherapies and to invasion of the surrounding environment. Where we cannot achieve chemical inhibition of a defined transporter target we will explore the feasibility of CRISPR knockdown – which we have successfully performed in several other medulloblastoma projects.

The correlation of patient data (i.e. bioinformatics analysis primarily, but also potentially analysis of new patient tissue microarray samples) with functional data will provide us with better understanding of the role of particular transporters in tumour cell biology, but will also help identify possible druggable targets in this devastating disease.

We expect the student would present their data at UK and international meetings relevant to transporter biology and neuro-oncology, and that the student would work with wider members of the two research groups to enhance their appreciation of the contribution of team science to understanding disease.

Lab Rotation Location: QMC;University Park;

Full Project Location: QMC;University Park;

149 Development and application of an advanced glycan production platform using synthetic biology / engineering biology

Lead Supervisor: John Heap

Lead School: Life Sciences

DTP Research Area: Biotechnology

Lab Rotation Description: BACKGROUND: Glycoconjugates make excellent vaccines but are expensive to produce, glycoengineering of bacterial cells promises a cost-effective alternative.

CHALLENGE: Glycan biosynthesis genes have typically been cloned whole (‘en bloc’) from the native organism into E. coli, with original regulatory sequences intact. However, gene expression within these pathways has been optimised by natural selection for synthesis in the native host and is unlikely to be optimal for heterologous production in an unrelated organism, where it may cause toxicity, metabolic burden, mutations and failure to express.

SOLUTION: We recently developed and validated a platform for combinatorial construction and optimisation of glycan biosynthesis and polymerisation genes. Instead of constructing individual pathway designs, a large ‘library’ of many millions of variants is constructed, varying the expression of each enzyme combinatorially.

AIM: During the rotation the student will apply cutting edge synthetic biology and glycoengineering tools and techniques to assemble a novel glycan synthesis locus using our established platform, and utilise established screening techniques to identify the most promising candidates. To fit in the nine-week timeframe, a pre-identified glycan cluster will be targeted, for which the supervisors will design and source synthetic DNA ready for the start of the project.

Full Project Description: SUMMARY: This exciting synthetic biology studentship uses our new combinatorial glycan assembly platform to produce novel synthetic glycans in E. coli, both developing a rapid, flexible and future-proof platform and making useful vaccine candidates identified by the student.

The project benefits greatly from Dr Faulds-Pain's and Dr Heap's BBSRC funded project 'Development and application of an advanced glycan production platform' (BB/W005816/1).

BACKGROUND: Glycans are ubiquitous biological structures, and many are important in health and disease. Bacterial glycans are often associated with cell surface structures and often elicit a memory T cell response, making them excellent antigens for vaccines. However, the production of glycoconjugates currently involves expensive chemical methods, preventing their use in low-income countries and veterinary medicine. Glycoengineering in bacterial cells promises a cost-effective alternative for vaccine production.

POTENTIAL AND CHALLENGE: We can build designer E. coli strains, repurposing native cellular machinery for safe production of glycans as vaccines - which are greatly needed worldwide. However, this is more complex than the simple way DNA sequences specify the sequence of amino acids in proteins: The variety of glycans is much greater, polymerisation can be linear or branched, and chirality of the sugars can be crucial to an effective immune response. Consequently, many more enzymes (and large gene clusters) are required for the formation of specific glycan structures, and we have to learn to ‘speak glycan’ like a new language.

Conventionally, glycan gene clusters have been cloned whole (‘en bloc’) from the native organism into E. coli, with original regulatory sequences intact. However, this approach has often failed, because expression of these clusters has been optimised by natural selection for synthesis in the native host and is unlikely to be well-suited to production in an unrelated organism, where it may cause toxicity, metabolic burden, mutations and failure to express.

SOLUTION: REFACTORING AND COMBINATORIAL OPTIMISATION OF HETEROLOGOUS CLUSTERS. Now, modern DNA synthesis and assembly allows us to synthetically redesign and rebuild gene clusters from parts, constructing large libraries of many variants varying the expression of each enzyme. High-performance variants can be identified by screening. We have used this approach to successfully express the glycan synthesis loci of Campylobacter and the eukaryotic core oligosaccharide, surpassing conventional methods. Deep sequencing and rapid screening lets us understand and refine these libraries further, applying a design-build-test-learn approach.

AIM: The student will use the new platform technology to build novel synthetic glycans in E. coli, both advancing the underlying platform capability and making useful vaccine candidates.

APPROACH:

DESIGN: The student will increasingly decide which glycans to target and will be encouraged to respond to emerging pathogens. BUILD: Glycan synthesis genes will be synthesised in our Start-Stop Assembly format. Hierarchical multi-part DNA assembly will generate libraries of millions of variants. TEST: Specific gold-standard and new high-throughput screening techniques will be developed including immunoblots, pull-down assays and FACS; and structures analysed by mass spectrometry. LEARN: Libraries will be analysed via our deep sequencing pipeline, and results used to optimise vaccine candidates.

Lab Rotation Location: University Park;

Full Project Location: University Park;

152 Streamlining synthetic genomes for designer organisms

Lead Supervisor: Benjamin Blount

Lead School: Life Sciences

DTP Research Area: Biotechnology

Lab Rotation Description: We can now build whole synthetic genomes with designer features not found in nature including SCRaMbLE, a revolutionary system that allows us to rapidly shuffle and delete sections of chromosomes. This has a massive range of applications in biotechnology and fundamental biology.

A major goal of the field is to be able to design synthetic genomes containing only genes required for a cell to perform a certain task with maximum efficiency, safety and predictability. SCRaMbLE would be an ideal method for determining which genes would populate such designer genomes, but a design quirk means that a third of genes cannot be deleted by SCRaMbLE. We have designed and synthesised a next-generation yeast synthetic chromosome to bypass this issue.

In the rotation project, the student will use CRISPR to show that essential genes can be deleted when our new synthetic chromosome is present. They will then show that the SCRaMbLE system can now be used to delete previously protected genes.

The rotation project will introduce key concepts and techniques in synthetic biology, engineering biology, synthetic genomics and molecular biology. These will include DNA design, assembly and transformation; CRISPR gene editing; genome reduction in yeast by SCRaMbLE; PCR genotyping; and nanopore sequencing.

Full Project Description: The Sc2.0 International Synthetic Yeast Genome Project is finalising the construction of the first ever synthetic eukaryotic genome. Synthetic genomes allow the genetic content of a cell to be fully designed in silico and then built in vivo, allowing widespread changes and new functions not feasible using existing physical gene editing techniques. This technical capability to create bespoke genomes programming organisms for specific tasks would have a wide range of applications - particularly in developing disease models, developing new therapeutics and converting industrial processes to sustainable alternatives. Unfortunately, our understanding of which genes would be needed to program desired cellular behaviour under different growth conditions is lacking. The current methods of determining what these minimal gene sets might be are slow, laborious and inadequate.

To overcome this, we have designed and synthesised a next-generation synthetic chromosome to allows us to randomly and indiscriminately delete different combinations of genes on a massive scale. During this project, the student will generate these deletions and sequence the resultant populations of genetically diverse cells en masse. We will then apply pangenomic bioinformatics techniques to this data and determine the contribution of each gene to cell viability in a range of conditions. The student will then test promising minimal gene sets to determine improved efficiency of growth and bioproduction. The project will not only greatly enhance our understanding of the relationship between gene content and cellular function, but will also lay down the foundation for bespoke synthetic genomes to be created for more efficient, predictable and safe biotechnology.

Supervision Team:

Benjamin Blount is an expert on synthetic genomics and part of the Sc2.0 consortium. He built synthetic chromosome XI and contributed to the tRNA neochromosome of the synthetic yeast genome project. He was the first to show that the SCRaMbLE genome rearrangement system encoded in Sc2.0 chromosomes could be used to optimise strains for bioproduction using synthetic pathways.

Fiona Whelan is an expert on the use of bioinformatic and microbiological approaches to study microbial pangenomes and gene associations within populations.

John Heap is an expert in combinatorial approaches to metabolic engineering and the application of synthetic biology techniques to the improvement of strains for biotechnology.

Lab Rotation Location: University Park;

Full Project Location: University Park;

164 Creating sustainable biofertilisers: optimising endophytic bacteria as nitrogen providers for global plant crops

Lead Supervisor: Klaus Winzer

Lead School: Life Sciences

DTP Research Area: Biotechnology

Lab Rotation Description: The rotation project will serve as an introduction into the handling and genetic manipulation of nitrogen-fixing endophytic bacteria. Its scientific objective will be the generation of fluorescent reporter protein-labelled Acetobacter strains, i.e. strains that can be observed using confocal fluorescence microscopy. A range of different fluorescent reporter proteins will be used allow parallel labelling of cells (via constitutive expression) and quantification of nitrogenase expression (when linked to the nitrogenase operon promoter). The reporters will first be generated in Escherichia coli, then introduced into Acetobacter and tested for expression under various conditions.

The generated reporter constructs will be used in the main PhD project to investigate the location of Acetobacter cells within plants and expression of nitrogen fixation genes in vitro and in planta.

The rotation student will:

1. Receive training in general microbiological methods, modern genetic approaches including HiFi gene assembly, state-of-the-art microbioreactor systems, and the use of high-throughput cultivation facilities

2. Generate and test a range of reporter plasmids carrying e.g. green and turquoise fluorescing GFP protein derivatives.

3. Transfer the generated reporters into two different Acetobacter strains and validate their performance by monitoring for constitutive and induced expression under various conditions including nitrogen and oxygen limitation.

Full Project Description: Background and aims:

Global agriculture is responsible for emissions of 9.2 billion tonnes CO2eq of greenhouse gases. Of these, around 1.1 billion tonnes are generated by the production and use of synthetic nitrogen fertilizers, with additional negative impacts on ecosystem health and drinking water quality. There is urgent need but also promising opportunity to strongly reduce fertilizer use by providing farmers with a highly effective, cheaper and biological alternative.

Of particular interest are endophytic bacteria capable of fixing atmospheric nitrogen in symbiotic relationship with crop plants. Similar to the Rhizobia, these bacteria provide fixed nitrogen in return for sugars, a crucial difference being their ability to establish symbiotic relationships with a wide range of plant families. Indeed, these bacteria can improve growth of key food crops, including wheat, rice, maize and soy. To maximise performance of these "biofertilisers" it is necessary to first understand and then optimise their nitrogen fixing capacities as well as other plant growth promoting functions.

This proposal seeks to investigate the regulation of nitrogen fixation and plant hormone production in a promising broad-host-range Acetobacter endophyte, with the aim of optimising these functions to achieve maximum host growth promotion. The work is supported NetZeroNitrogen Ltd., who will provide promising lead strains, provided access to their facilities and equipment as in kind-support, and make a donation towards the envisaged transcriptomics studies (£2,000).

Experimental approach:

1. Regulation of nitrogen fixation

Nitrogen fixation via the nitrogenase enzyme is usually negatively regulated by the availability of fixed nitrogen such as ammonium, but also other species-dependent factors such as oxygen availability. To identify the genes involved in nitrogen fixation, ammonium assimilation and their regulation, a combination of bioinformatics and experimental approaches will be employed. Suspected regulatory and established enzymatic functions will be identified in Acetobacter genomes by bioinfomatic means. The global response to nitrogen starvation will be investigated by RNAseq, providing transcriptomic profiles of strains grown under nitrogen limitation. Nitrogenase expression in particular will investigated using fluorescent reporters to establish influencing factors and conditions of maximal expression. Fluorescent reporters and qPCR will also be employed to study their expression in planta (rice/tomato). Through confocal microscopy the location of fluorescently tagged strains and relative expression of nitrogenase in different plant tissues will be studied, e.g. in nitrogen starved plants.

2. Promoting nitrogen fixation

Attempts will be made to increase nitrogen fixation by placing nitrogenase and other contributing genes under the control of stronger promoters, both constitutive and inducible (e.g. via light-dependent control systems to allow for in planta induction). The effect of overexpression on bacterial growth, net nitrogen fixation and plant growth promotion will be evaluated using wild type and nitrogenase mutants for comparison.

3. Plant hormones

Acetobacter produces various plant hormones including indole-3-acetic acid. As a fallback option, bacterial hormone synthesis and contribution to plant growth promotion may be investigated. Biosynthetic pathway genes will be identified bioinformatically and their expression within plant tissues studied using fluorescent reporters and qPCR. The effects of pathway mutation and overexpression on plant growth will be assessed.

Lab Rotation Location: University Park;

Full Project Location: University Park;Sutton Bonington Campus;

177 Immune mechanisms linked to age-related cognitive decline and cerebral amyloidosis

Lead Supervisor: Marie-Christine Pardon

Lead School: Life Sciences

DTP Research Area: Bioscience for Health

Lab Rotation Description: Characterisation of LPS-induced inflammation and amyloidosis in tissue and organs

The objective of the rotation is to establish a methodology to quantify the level of pro-inflammatory cytokines in the brain and liver in response to LPS, reflecting the intensity of the immune central and peripheral response, as well as the level of aggregated amyloid, reflecting amyloidosis.

The rotation project first involves preparation of tissue from mice previously treated with LPS and their untreated control to extract insoluble amyloid beta, the main constituent of aggregates as well as soluble proteins that includes the pro-inflammatory cytokine Tumor-Necrosis-alpha (TNFα), amyloid precursor protein (APP), whose proteolysis generates amyloid, and soluble amyloid. Quantification of the concentration of these molecules will be carried out using Enzyme-linked immunosorbent assays (ELISAs). The first step will involve optimising the assay to determine the optimal dilution of samples need to maximise the efficiency of the ELISAs. In addition to experimental skills, the rotation project will also involve the acquisition of computer and analytical skills to quantify the protein concentration in tissues and statistically analyse the impact of LPS.

Full Project Description: “Inflammageing” is a state of low-grade chronic systemic inflammation associated with dysfunctional immunity which frequently occurs with ageing in the absence of overt infection. It is a risk factor for accelerated age-related cognitive decline and cerebral amyloidosis, with the potential to also increase the likelihood of later developing senile dementia. This is part due to excess levels of Lipopolysaccharide (LPS), a toxic gram-negative bacteria produced by the gut in response to systemic infections and inflammation. The mechanisms whereby LPS can exacerbate brain ageing are far from being understood but involves abnormal function of immune effector cells of the central nervous system. Under healthy conditions, microglia, the resident macrophage of the brain, and astrocytes play a critical role in maintaining the structural integrity of the brain, regulating

immune responses and preventing the build-up of toxic amyloid aggregates. They become dysfunctional under chronic inflammation as seen with ageing, promoting neuroinflammation, neurodegeneration and cerebral amyloidosis.

Our recent work has led to the identification of two major anti-inflammatory molecules that are downregulated in the brain at onset of LPS-induced systemic inflammation (Pardon et al. 2016mScientific Reports, 6, 19880 ; Agostini et al. 2020, Brain Behaviour & Immunity, 83, 87-111), leading to the hypothesis that their deficiency contributes to the detrimental effects of LPS. The proposed PhD project aims to test this hypothesis in models of brain ageing and cerebral amyloidosis. This project is interdisciplinary and will combine pharmacological, histological, molecular and behavioural approaches to establish how these molecules affect the immune responses of microglia and astrocytes to LPS, their ability to clear amyloid deposits and ultimately, cell death and cognitive function.

Immune responses and amyloid clearance will first be investigated in in vitro models, using cell lines, primary microglia and astrocytes collected from preclinical models of amyloidosis, and human stem cells-derived microglia and astrocytes. They will be characterised using a range of methods including Enzyme-linked immunoassays, western immunoblotting, immunohistochemistry and flow cytometry. This involves determining if the molecules are effective in reducing LPS-induced inflammation, assessed via quantification of pro-and anti-inflammatory cytokines, cell viability, and amyloid phagocytosis. Dose responses experiments will be carried out to determine the effective doses against increasing levels of inflammation and to select the most effective molecule for follow-up in vivo testing.

Further evaluation of the potential of the most effective of these molecules in reversing LPS-induced cognitive decline and amyloidosis will be conducted in aging mice and a mouse model of cerebral amyloidosis using cognitive and behavioural assessment, histological and functional analysis of brain markers of microglial and astrocytic function, immune mediators and amyloidosis following treatment with LPS.

This interdisciplinary PhD project offers broad scientific training covering ageing, immunology and neurobehavioural research, histology, physiology, molecular biology, cell culture and protein expression analyses. It offers candidate s multiple experimental and transferable skills to investigate a major public health issue using cutting-edge technologies.

Lab Rotation Location: QMC;

Full Project Location: QMC;

182 Convergent evolution in the Anthropocene: investigating how human activity has shaped molecular adaptation to new environments

Lead Supervisor: Mark Ravinet

Lead School: Life Sciences

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: Previous work in the lab has shown that adaptation to human environments can result in clear genomic signatures of selection. This rotation will focus on examining selection in the Iago sparrow (Passer iagoensis), a species endemic to the Cape Verde

Islands. This species is widespread across the archipelago and is closely associated with humans on some islands but not on others. We have recently assembled and annotated the Iago sparrow reference genome and have previously resequenced over 60 individuals from human-associated and non-human associated populations. The lab rotation project will map this resequence data to the new reference genome, investigate population structure, recent demographic history and perform genome scans in order to identify signatures of selection. The student will then compare the candidate genes and gene pathways identified in this analysis with a previously identified set in the house sparrow (P. domesticus) in order to quantify the overlap between genes under selection. This rotation will provide an insight into the extent of convergence between these closely related species and will act as an introduction to the wider themes of the PhD project. Training will be provided in genomic data analysis, bioinformatics, HPC use, population genomics and population genetic theory.

Full Project Description: Human activity can drive extinction, perturbation and displacement of other species. Yet in some cases, organisms have adapted to and even thrive in human environments. Human activity is widespread and increasing; i.e. another 2.4 billion people will live in urban settlements by 2050. Pervasive anthropogenic change is impacting more species than ever, with evidence of more rapid phenotypic evolution in human vs natural environments across multiple taxa. Despite its ubiquity, large-scale human activity is a relatively new phenomenon in evolutionary terms. Can it act to shape convergent molecular adaptation over short evolutionary timescales?

Species adapting to anthropogenic niches have access to new resources but also face new challenges such as increased population densities and greater disease risk. Therefore, rapid adaptation to human environments might drive convergent evolution in genes and gene pathways related to metabolism and immunity. This project seeks to test this hypothesis across a range of different evolutionary scales, i.e., within a genus and across orders. To achieve this, it will use birds as a model system; there are multiple examples of adaptation to human environments across the avian tree of life and a wealth of genomic and phenotypic data available.

The project will first focus on comparative evolutionary genomics of Passer sparrows. Adaptation to humans has occurred independently at least four times in this genus. We have assembled the genomes of all four species, plus two non-human associated species as well as collating whole-genome resequencing and transcriptomic data. The project will use these data to quantify whether there is convergent selection on candidate genes across the genus. Comparing transcriptomic and population genomic data, we will evaluate the importance of protein-coding versus regulatory changes for rapid adaptation to human environments. For two Passer species that have recently invaded anthropogenic niches we will compare population level bisulfite sequencing, transcriptomic data and immune response between urban and rural populations. This will allow us to quantify the role that epigenetic changes via DNA methylation may play a role in rapid adaptation to the challenges presented by human environments.

The project will next identify general patterns of convergence underlying adaptation to human environments across the avian tree of life. We will use the association between avian population and human densities as a metric to quantify the extent of adaptation to human environments. We will then compare genomes of human-adapted to non-human adapted species for convergent signatures of positive selection across genes and gene pathways involved in metabolism and immune response. Finally, we will quantify rates of evolution in non-coding regions in order to examine convergent changes in regulatory regions.

Our ability to shape the evolution of other species also has profound effects on our own society and stability. For example, increased human-animal contact has recently been identified as a major risk

factor in the emergence of zoonotic disease. Understanding how human activity acts as a general rule for shaping evolution at the molecular level therefore plays an important role in safeguarding future biosecurity.

Lab Rotation Location: University Park;

Full Project Location: University Park;

Mathematics

167 How a gaseous hormone is made, mobilized and mediates stress responses in plant tissues.

Lead Supervisor: John King

Lead School: Mathematics

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The rotation will comprise confocal imaging of (i) ACO-GFP (transcriptional) reporters, to observe dose-dependent ethylene production in different cell types of Arabidopsis root tips, helping both to reveal the ethylene responsive nature of ACO genes and to discover which of the five ACO genes play prominent roles in ethylene biosynthesis; (ii) ethylene reporter EIN3-GFP/ein3eil1, with different doses of ethylene at different time points, helping establish how fast ethylene accumulation responds to external ethylene level. These observations will inform model parametrisation to understand how and where ethylene is synthesised and moves within or across the cells. Thus as part of the rotation the student will be exposed to a number of routine and some specialised techniques in Molecular Cell Biology, plant phenotyping and plant physiology such as:

Reporter gene studies

In situ immunolocalisation

Confocal Microscopy

Lightsheet Microscopy

In vitro plant culture

Microtome sectioning

PCR and RT-PCR

Image processing packages including ImageJ and WinRHIZO.

This experience would be of particular value to a mathematically trained student.

Full Project Description: Ethylene is a vital gaseous signal regulating critical plant developmental and stress responses. A recent publication by the co-host lab reported roots use ethylene to sense soil compaction [Pandey et al, 2021, Science]. They discovered diffusion of ethylene released by roots is suppressed in compacted soils as the proportion of inter-connected air spaces are significantly reduced. In compacted soils, high ethylene concentrations build up around and in root tip tissues,

triggering growth inhibition. However, after genetically disrupting the ethylene response machinery, mutant roots were able to penetrate compacted soil (unlike the wildtype). Breeding crops resilient to soil compaction stress is vital for global food security efforts. The co-hosts recent work revealing a role for ethylene provides an exciting means to attain this. However, to facilitate this goal, fundamental knowledge about how ethylene is made, mobilized and mediates compaction stress responses in plant roots is required.

The DTP project will exploit a mathematical-modelling-based systems biology approach to determine how ethylene moves between its sub-cellular site(s) of synthesis and is eventually release from root tissues into the soil. The student will initially adapt an anatomically accurate vertex model of the Arabidopsis root that captures key sub-cellular, cellular and tissue scale features of the root tip site of ethylene synthesis. This hydrophobic gaseous molecule favours diffusion across ER networks (via plasmodesmata (PD) connecting one cell to another) rather than hydrophilic cytosolic and apoplastic routes. To reveal the pathway of ethylene movement at a subcellular scale, the student will exploit an ethylene biosensor to reveal if the signal is moving between and within cells via PD and ER, respectively. Based on the distance, membranes, and other compartments that ethylene needs to traverse, the student will be able to precisely model, with the help of lead supervisor John King and collaborator Olivier Martin (Paris), how ethylene reaches the root surface. Collectively, these innovative systems biology approaches promise to unveil entirely new routes of ethylene movement across root tissue types and bridging from subcellular to root-rhizosphere scales.

The DTP will involve cutting edge imaging technologies such as Light Sheet microscopy and hormone biosensors in addition to mathematical modelling and plant cell biology. This represents an ambitious, novel, yet realistic project as it relies on well-established techniques and state-of-the-art equipment in the co-host lab. The main supervisor's mathematical modelling expertise and knowledge of the topic area will ensure that the project produces high-quality results and that the student will receive exemplary guidance as they embrace new techniques in a highly interdisciplinary environment.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

Medicine & Chemistry

194 Deuterium metabolic imaging for precision imaging studies in oncology

Lead Supervisor: Peter Harvey

Lead School: Medicine & Chemistry (50/50 split)

DTP Research Area: Bioscience for Health

Lab Rotation Description: Changes in metabolic pathways are well known markers of disease. However, we lack sufficient tools for monitoring metabolic pathways in vivo. Deuterium metabolic imaging has strong potential for addressing this shortfall and we aim to demonstrate its potential in this project.

In the lab rotation, the student will become familiar with the highly interdisciplinary work that would form the core of the full project. The student would begin by screening known deuterated

compounds in the MRI scanner, to become familiar with data acquisition, processing, and interpretation. The student would work with existing PhD students on cell culture to demonstrate the potential of deuterium metabolic imaging to monitor metabolism of key deuterated metabolites in a well-defined setting. Comparative HLPC, mass spectrometry, and metabolomics would be applied to validate the deuterium imaging. If of interest to the student, there would also be the opportunity to develop skills in synthetic chemistry to create relevant deuterated compounds for further study.

The cross-faculty supervisory team possess lab and research groups within the Schools of Medicine, Chemistry, and Physics and the student would have the opportunity to engage across the schools and learn from the broad experience of current researchers across the connected research groups.

Full Project Description: Metabolism plays a crucial role in the origin and development of multiple diseases, including neurodegeneration, cardiovascular disease, diabetes, and cancer. Cancer mutations that result in metabolic reprogramming of the tissue microenvironment have been associated with tumor therapy resistance, relapse, and metastasis. Currently, the only available techniques to routinely monitor glucose metabolism are positron emission tomography using radioactive tracers and hyperpolarized magnetic resonance imaging. Although these techniques are quite powerful, they do not show metabolism downstream of glucose uptake or provide quantitative information about metabolism and possess limited lifetimes post-injection.

Deuterium metabolic imaging (DMI) is a novel approach that provides quantitative measures of local tissue metabolism using safe 2H-labeled substrates. DMI has enormous clinical potential, and its implementation in Nottingham and Tübingen will open opportunities for the development of new, image-guided tumour therapies. DMI particularly benefits from ultra-high magnetic field as this provides greatly increased sensitivity. Nottingham has recently acquired the hardware needed for 2H measurements with phantoms, cells, rodents, and humans. Our facilities are complemented by our partners in Tübingen and between us we possess a broad range of ultra-high-field (UHF) preclinical/clinical MR scanners (3T, 7T, 9.4T, 14.6T).

In this PhD fellowship project, we will work together to demonstrate the benefits of the UHF for DMI to demonstrate precision imaging studies in oncology, with glioblastoma as a key model in Nottingham and lymphoma in Tübingen. The specific aims for this work:

- Optimise three-dimensional chemical shift imaging sequences for 2H measurement at high field. We will use the already acquired knowledge with DMI in both Nottingham and Tübingen to develop new micro-coils to image cell metabolism with 2H/1H at the cellular level with high precision.

- Study cell metabolism with DMI. The student will identify the metabolic genetic drivers in several relevant cell lines in vitro. We will perform a full metabolic study with these cells, including oxygen consumption rate (OCR), extracellular acidification rate (ECAR), metabolomics, transcriptomics, and immunocytotoxicity essays.

- Monitor therapy with DMI. The student will address an essential metabolic question about therapy approaches in cancer cell lines using several standard chemotherapeutics. We are also currently developing deuterated chemotherapeutics that could be investigated alongside these studies.

- In vivo DMI with an orthotopic Hodgkin lymphoma mouse model. We will move towards preclinical applications of DMI in the selected mouse models. Studies will be performed using 7T MRI at Nottingham and the hybrid 7T PET/MRI and UHF 14.6 T MRI in Tübingen.

- Assess capability of translation to human imaging. More info: Cocking/Auer/Bowtell et al, Magnetic Resonance in Medicine, 2022, in press.

The student will be based within the renown Sir Peter Mansfield Imaging Centre and Precision Imaging Beacon at Nottingham and have access to the world-leading Werner Siemens Imaging Centre at Tübingen. We have established a networking program in precision imaging (seedcorn funding UT-UoN) and the student will participate closely in these collaborative activities. These cross-institutional centres provide include excellent training in medical imaging, immunology, oncology, molecular biology, synthetic chemistry, and translational sciences.

Lab Rotation Location: University Park;

Full Project Location: University Park;QMC;

Medicine

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

Lead Supervisor: Bethan Phillips

Lead School: Medicine

DTP Research Area: Bioscience for Health

Lab Rotation 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 MG (atrophy susceptible) and 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.

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) and strengthens collaborative links for a University of Nottingham ECR (Bass), 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 relevant by Autumn 2023.

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, honing 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.

Lab Rotation Location: University of Nottingham Medical School at Derby, Royal Derby Hospital Centre, Derby

Full Project Location:

13 The role of glycosaminoglycans in Alport Syndrome

Lead Supervisor: Kenton Arkill

Lead School: Medicine

DTP Research Area: Bioscience for Health

Lab Rotation Description: The student will have access to tissue blocks from Alport Mice Kidneys. The student will learn to quantify glycosaminoglycans (GAGs) by traditional fluorescence methods.

GAGs are polysaccharide chains up to ≈150nm long and are an intrinsic part of the filtration barrier within kidneys. However, in pathology there is compensation, and in Alport Syndrome the basement

membrane defects we believe are compensated for by alterations in the GAG content. Understanding this compensation in general and in Alport Syndrome in particular is vital to mitigating the genetic symptoms and early onset Kidney disease.

Full Project Description: The vascular glycosaminoglycan (GAG) composition is predominately heparan sulphate,

chondroitin sulphate and (unsulphated) hyaluronan which partly define the permeability of blood vessels. People with Alport Syndrome have a disrupted basement membrane, itself rich in GAG, that overtime gives rise to vascular complications, including too much protein escaping the kidney.

Interestingly animal models of this disease have an increased depth of glomerular endothelial GAG, which implies a compensation mechanism. Further, in pregnancy the sufferers have a 10-fold increase in protein in their urine which returns after birth. This project is to determine the compensation and the structural/compositional changes in pregnancy such that a future method of treatment can be devised. The difficulty with GAG research is that antibodies are poor and depend on specific sulphation motifs that can alter in pathology. We have therefore been developing imaging mass spectrometry (ToF/Orbi-SIMS) to determine the difference between the GAGs and their sulphation motifs. We have had success on the cellular scale using mice diabetic kidneys (would have figure 1!), but need to develop multiscale workflows to achieve sub-optical resolution across capillary walls. Once achieved we have a one-stop-shop for sub optical scales with an entire mass spectrum of compositional changes.

General Hypothesis: That Imaging mass spectrometry can detect GAG changes on a scale suitable for analysis of capillary (including glomerular) walls.

Exemplar Hypothesis: The GAG compensation observed in Alport Syndrome glomerular capillaries is to reduce albumin escaping the vessel lumen, but in pregnancy this compensation is disrupted.

Aim 1: Determine an imaging mass spectrometry regime to detect generic and specific GAG composition across the glomerular filtration barrier. This includes separating endothelial, basement membrane and podocyte GAG contents.

Aim 2: Spatially determine any differences in GAG composition through the glomerular filtration barrier and predict the difference it would make to the albumin transport.

The project is highly multi-skilled and suits a range scientific background and can be directed accordingly. As an example: A biomolecular scientist may wish to concentrate on how the compensation occurs, but a physical/computation scientist may wish to focus to deconvolute imaging regimes to better define what the compensation is.

We expect the student will enjoy learning the sample preparation, fluorescent staining, multiple imaging methods, 3D mapping and corelative technics to transfer between multiple instruments. The student would be supported by the wider vascular laboratories, the advisors' own groups but also from the regional Centre for Kidney Research and Innovation, local clinical collaborators, and further collaborations in Manchester and the USA.

Lab Rotation Location: University Park;

Full Project Location: University Park;

33 Transcriptional control of EC heterogeneity in health and disease

Lead Supervisor: Andrew Benest

Lead School: Medicine

DTP Research Area: Bioscience for Health

Lab Rotation Description: Key skills related to downloading, curating and analysis sc and bulk RNAseq datasets will be established

Full Project Description: The endothelium comprises endothelial cells (EC) which line all blood and lymphatic vessels. Conceptually speaking the endothelium can exist as a (albeit physiologically active) quiescent tissue, which forms the interface between the circulation and interstitium. Over recent years its importance to underpinning all disease conditions has been increasingly recognised resulting in the endothelium being the determinant of health and disease conditions. Endothelial heterogeneity can be assessed at different levels; anatomical, organ specific or during dynamic remodelling; allowing vascular biologists to determine vessel heterogeneity such as arteries, veins, sinusoidal EC but cutting edge transcriptomic and proteomic techniques have revealed even greater heterogeneity at even the capillary level1. Such heterogeneity is dynamic – capillary ECs can switch their phenotype from quiescent to an activated one – resulting in altered permeability, adhesion, migration and proliferation– in response to conditions such as ischaemia, infection, diabetes and ageing – these diverse pathologies all result in some level of EC activation or dysfunction and are key focus areas of the BHF. EC plasticity is in part controlled by transcription factor (TF) activation, resulting in seminal work highlighting how TFs such as FOXO1/cMYC control EC metabolism and therefore activation state

1) What is the lymphEndoMT signature during developmental and pathological lymphangiogenesis?

Using publicly available and peer reviewed datasets, including endothelial repostitories such as endoDB, CRESCendo and datasets associated with publications

2) how are TF networks interlinked: using a combination of RNAi and ChipSeq analysis detailed transcriptomic analysis will be performed

3) Can we mimic EC phenotypes using in silico KO techniques.

Lab Rotation Location: University Park;

Full Project Location: University Park;

46 The metabolic basis of how brain cancer hijacks the healthy brain

Lead Supervisor: Ruman Rahman

Lead School: Medicine (if a supervisor is based in Medicine, they should complete the additional form for match-funding approval from the School)

DTP Research Area: Bioscience for Health

Lab Rotation Description: Biodiscovery Institute, School of Medicine, University of Nottingham (5 weeks):

Fluorescently tagged human cells derived from the malignant brain cancer, glioblastoma (GBM), will be co-cultured with healthy rodent cortical neurons using 2-dimensional monolayers. High resolution fluorescence microscopy will be used to image and characterise neurons, GBM cells and the formation of synaptic connections which recapitulate neuronal-glial electrical communication.

School of Life Sciences, University of Nottingham (4 weeks):

Live intracellular calcium and nitric oxide imaging will be applied to co-cultured monolayers to characterise neuronal and synaptic activities. In addition, electrophysiological recordings will be performed from neurons to illustrate to the student, the potential power of these approaches in elucidating neurophysiological changes in the context of glioblastoma-neuron interactions. Functional assays involving uptake of fluorescently labelled glucose and lipid tracers will be used to measure metabolic changes in neurons in presence and/or absence of GBM cells. This will allow us to characterise the potential of synaptic connectivity between neurons and brain tumour cells.

Full Project Description: Background:

A new paradigm in brain health and disease describes how cancer cells can plug into — and feed off — the brain's complex network of neurons. Evidence from neuroscientific studies have revealed that biochemical and electrochemical signalling from healthy neural cells in the brain, can foster a microenvironment which promotes dysregulated growth of glial cells [1,2]. Glioblastoma, a currently incurable grade 4 malignant tumour arising in astrocytes, can form synapses that hijack electrical signals from healthy neurons to drive their own growth. Such enigmatic neuron-glial synapses have been identified in rodents with human brain tumour xenografts, and from primary tissue derived from surgical theatre. However, there is a paucity of laboratory models which recreate these neuronal-glial interactions.

Importance of study:

This project will recapitulate the molecular snapshot immediately post-surgery, where healthy brain cells provide a permissive ecosystem for micro-deposits of residual, infiltrative glioblastoma cells, to survive, and regenerate the recurrent tumour. By doing so, critical metabolic pathways which underly aberrant electrically active glioblastoma tissue will be identified, opening a new therapeutic avenue.

Hypothesis:

Aberrant electrochemical signalling at neuronal-glioblastoma synapses is associated with therapeutically actionable metabolic pathways.

Workplan:

Objective 1 – Generation and characterisation of electrically-active tumour-neuronal 3-dimensional co-cultures

A panel of fluorescently tagged primary patient-derived cell lines isolated from the glioblastoma and infiltrative margin (proxy of residual disease spared by surgery), will be co-cultured with rodent cortical neurons to generate 3D spheroids. Neurophysiological methodologies will be applied to study neuronal and synaptic plasticity including measurements of intracellular calcium and nitric oxide, and adenosine triphosphate (ATP) dynamics (via ATeam1.03YEMK fluorescence resonance energy transfer (FRET) sensor). In addition, fluorescently labelled glucose and lipid metabolic tracers will be used to identify metabolic changes associated with aberrant electrochemical signalling at neuronal-glioblastoma synapses.

Objective 2 – Elucidation of cellular metabolism associated with electrically active glioblastoma tissue

Ex vivo measurement of cellular metabolism within co-cultured neuronal-glioblastoma spheroids will be determined by magnetic resonance spectroscopy (1H, 13C and 31P), complemented by advanced imaging analysis, data processing and metabolic modelling. Radiolabelled markers of glucose and lipid metabolism will be used including 13C glucose, 13C lactate and 13C acetate, to characterize metabolic interactions associated with electrically active glioblastoma tissue. One part of the project

will be to determine the role of the glycolysis end-product, lactate, as a preferred energy substrate source to fulfil the energy needs of electrochemical signalling at neuronal-glioblastoma synapses.

Objective 3 – Validation using surgical biopsies of the glioblastoma infiltrative microenvironment

Ex vivo slice cultures will be derived from primary tumour tissue from the infiltration margin of glioblastoma. Electrophysiological and metabolic assays conducted on brain slice preparations will validate candidate neuronal and metabolic signalling pathways, offering novel therapeutically actionable signatures.

Outputs:

- Doctoral graduate immersed with inter-disciplinary cellular neuro-oncology and electrophysiology expertise.
- Two high-impact publications.
- Presentation at a global neurobiology conference.

Lab Rotation Location: University Park;Medical School ;

Full Project Location: University Park;Medical School;

57 Airway Epithelial-Myeloid cell crosstalk as a key mechanism in the pathogenesis of Coronaviruses

Lead Supervisor: Ian Sayers

Lead School: Medicine (if a supervisor is based in Medicine, they should complete the additional form for match-funding approval from the School)

DTP Research Area: Bioscience for Health

Lab Rotation Description: The 9 week lab rotation will provide a brief introduction to the essential techniques that will underpin the project including;

i) Human bronchial epithelial cell culture and quality control (epithelial markers e.g. ECad, CK14 using immunofluorescence) (Lead Sayers)

ii) The generation and characterisation of macrophages (Lead Martinez-Pomares)

iii) The characterisation of coronaviruses (Lead Coleman).

iv) Initial handling of transcriptomic datasets from pilot data (Lead Sayers).

Full Project Description: Human coronaviruses (hCOV) are important pathogens. Less pathogenic hCoVs – e.g. hCoV-229E are a broad group of CoVs that infect humans but cause only a mild ‘common-cold-like’ disease, whereas two highly pathogenic hCoVs: severe acute respiratory syndrome (SARS)-CoV-1 and MERS-CoV have become a threat to human health. In 2018, the World Health Organization (WHO) designated MERS-CoV and SARS-CoV-1 as ‘Blueprint Priority Diseases’,

meaning there is an urgent need to develop vaccines and better understanding of how CoVs establish infection in susceptible hosts.

SARS-CoV-2 recently emerged and has subsequently become pandemic, with significant effects on the health and socioeconomic outlook of almost all nations. SARS-CoV-2 causes mild to severe respiratory illness, named COVID-19, which is exacerbated by aging and comorbidities. Some patients can develop acute respiratory distress syndrome (ARDS) or multi-organ injuries, associated with elevated levels of pro-inflammatory cytokines, including IL-6 and TNF-α, alongside minimal amounts of type I IFNs. Reduced Type I IFN production is likely caused by viral antagonism of innate immune responses hampering induction of a robust anti-viral state in the airway epithelium and surrounding tissues and facilitating increased SARS-CoV-2 titres.

We hypothesise that crosstalk between infected lung epithelial cells, targeted by SARS-CoV-2 but poor cytokine producers, and myeloid cells, not susceptible to SARS-CoV-2 infection but major producers of cytokines, will contribute to the cytokine imbalance and storm characteristic of COVID-19.

The main objective of this study is to establish the role of monocytes/macrophages as amplifiers of inflammatory responses during SARS-CoV-2 infection including evaluating the differential effect(s) of: SARS-CoV-2 variants, existing or new drugs and donor characteristics. We will also compare SARS-CoV-2 to different coronaviruses to provide additional insight into the pathogenesis of hCoV infections more generally.

Key stages for the project:

1. Creation and use of primary human airway epithelial cultures to model SARS-CoV-2 pathogenesis. Using our combined expertise, we will extend our initial human cell models to study virus:host interactions in the airway.

2. Investigate if and how the presence of human macrophages influences SARS-CoV-2 infection of differentiated human lung epithelial cells.

3. Characterise the capacity of different SARS-CoV-2 variants e.g. Alpha, Delta, Omicron to initiate responses in the model.

Based on progress we will extend the study to:

4. Characterise the capacity of different coronaviruses including SARS-CoV-1, MERS-CoV and less pathogenic hCoVs to initiate responses in the in vitro model.

5. Determine the effects of existing e.g. dexamethasone and more novel drugs on 1-4.

6. Investigate the impact of donor characteristics e.g. age, ancestry on 1-4.

Main readouts will include: hCoV replication, cytotoxicity, cytokine production and changes in global gene expression signature in both airway epithelial and macrophage cell types (RNA-seq).

This project has the potential to identify novel targets for anti-coronavirus therapeutics for use in current and future outbreaks.

This proposal combines the expertise of investigators across schools and disciplines including airway epithelial cell models/respiratory medicine (Prof. Sayers), macrophages/immunology (Dr Martinez-Pomares) and coronaviruses (Dr Coleman).

Lab Rotation Location: University Park;

Full Project Location: University Park;

69 Exploring the key genes and signalling pathways associated with taste receptors and sensory reception in the bowel

Lead Supervisor: Abdolrahman Shams Nateri

Lead School: Medicine (if a supervisor is based in Medicine, they should complete the additional form for match-funding approval from the School)

DTP Research Area: Biotechnology

Lab Rotation Description: During the project rotation, the student will develop practical research skills in 2D and 3D tissue culture methods and transferable skills such as the ability to evaluate others' work critically.

Practical skills in culturing taste stem/progenitor cells and 3D taste organoids will be developed by spending 7-8 hours per day in the laboratory for over five weeks. Once these skills are mastered, we will examine the differences in gene expression and signalling pathways in their responses to tastants. In this training period, the student will be mainly supervised by Dr Nateri (Medicine) and supported by the Cancer Genetics and Stem Cells Group (BioDiscovery Institute).

A limited number of researchers are working in this area internationally; thus, a depth and breadth of comprehensive knowledge of the literature are required before working on the proposed research project. Therefore, the student will spend 2- weeks focussing purely on the literature review, mainly supervised by Dr Ford (Biosciences) and supported by the Sensory Science Group.

The lab rotation aims to provide both knowledge and confidence to embed the skills required for the linked PhD effectively.

Full Project Description: Background & Rationale:

This multidisciplinary PhD project presents an exciting opportunity to work across three schools (Medicine, Biosciences and Veterinary) in Nottingham while collaborating with Monell Chemical Senses Centre, US., taking current expertise in signalling and bowel disease (Nateri), transcription and RNA-sequencing (Nigel), sensory perception (Ford), and chemosensory biology (Ozdener), to investigate cell signalling pathways associated with sensory reception in the bowel. This collaboration aims to uncover the mechanisms behind nutrient-sensing phenotype, and sensory optimisation, with the implications on consumer food choice behaviour and nutrition.

Recent progress in unravelling the tongue's taste buds' nutrient-sensing mechanisms has triggered studies on the existence and role of chemosensory cells in the gut. Indeed, the gastrointestinal tract is the crucial interface between food and the human body and can sense basic tastes in much the same way as the tongue through possible similar G-protein-coupled taste receptors. These receptors' taste' the luminal content and transmit signals that regulate nutrient transporter expression and nutrient uptake and release gut hormones and neurotransmitters involved in regulating energy and glucose homeostasis. Chemotherapy drugs could change the taste receptor cells in the mouth and bowel. Hence, they play a prominent role in the communication between the lumen, epithelium, smooth muscle cells, afferent nerve fibres and the brain to trigger adaptive responses that affect gastrointestinal function, food-intake and glucose metabolism.

Gut hormones produced by gastrointestinal enteroendocrine cells modulate vital physiological processes, including glucose homeostasis and food intake, making them potential therapeutic candidates to treat obesity and diabetes. Advances in long-term culturing human mini-gut (aka Organoid) that resemble in-vivo architecture include enteroendocrine cells while maintaining native signalling pathways to tastants. An organoid culture will allow this novel investigation to explore signalling pathways associated with the expression of taste receptors in different endocrine cells along the gut that control hormones' released in response to nutrients.

Hypothesis: Bowel hormone-secreting enteroendocrine cell taste-specific receptors modulate nutrient sensing in health and disease

More specifically, we aim to:

1) Identify the transcriptional profiles of intestinal enteroendocrine cells isolated by fluorescence-activated cell sorting (FACS) from mouse ChgA-hrGFP organoids underlying bitter, sweet and salty tastes. We will use mice expressing a genetically encoded fluorescent protein (hrGFP) under the control of the chromogranin A (ChgA) promoter to selectively label enteroendocrine cells for single-cell characterisation.

2) Phenotypically characterise intestinal cells sensitivity (bitter, sweet and salty), by generating knockout ChgA-hrGFP organoids using CRISPR/Cas9 strategy (the loss-of-function study) for the most highly expressed sensory receptor and by overexpressing of the lowest expressed sensory receptor using lentiviral vectors (gain-of-function study) in intestinal organoids cells.

These observations may identify unknown transducer elements underlying thermal, sour, salt, and other taste qualities. Ultimately, this collaboration aims to uncover the mechanisms behind nutrient-sensing phenotype, and sensory optimisation, with implications on consumer food choice behaviour and nutrition.

A limited number of researchers work in this area nationally and internationally; thus, our student(s) will learn cutting-edge approaches and experience essential for developing academic careers and collaborate with scientists from other disciplines to improve the healthy digestive system and patients' care and achieve high-impact publications.

Lab Rotation Location: University Park;BDI3;

Full Project Location: University Park;BDI3;

72 Investigate the importance of stromal signalling in colorectal cancer using 3D cancer models

Lead Supervisor: Alan McIntyre

Lead School: Medicine (if a supervisor is based in Medicine, they should complete the additional form for match-funding approval from the School)

DTP Research Area: Bioscience for Health

Lab Rotation Description: The student will:

1. Interrogate the RNA seq data sets to identify key pathways and targets.

2. Investigate key target genes/proteins identified from the RNA-SEQ using a variety of cell and molecular biology techniques to develop skill that will support the full PhD project described below.

Full Project Description: BACKGROUND:

Tumours aren't just made up of tumour cells. Stromal cells infiltrate tumours and make up a large proportion of tumour mass impacting tumour phenotypes including proliferation, drug resistance and metastasis. Patient-derived xenograft (PDX) models have been widely adopted in order to provide better representation of patient tumour heterogeneity. However, our laboratory and others have shown that human stroma is rapidly lost following transplantation of patient tissue into mice, and replaced by mouse stroma. Stromal signalling to cancer cells is known to drive important aspects of tumour biology including proliferation, invasion and drug resistance. While mouse stromal cells can provide some paracrine signals to human cancer cells, key signalling pathways are missing in xenograft models. For example, mouse HGF (hepatocyte growth factor) is unable to bind to the human c-Met receptor, and so paracrine signalling through this receptor is lost in the PDXs. Of course, it is also missing in simple in vitro models, including 3D models in which cancer cells are grown as monocultures. As a result, drugs targeting such signalling pathways which are important drivers of tumour progression, cannot readily be tested in these models.

AIMS:

1. We will use RNAseq analysis of paired patient and PDX samples to identify a set paracrine signalling pathways between the stroma and cancer cells, that are lost.

2. Use 3D co-culture models, incorporating human stromal as well as cancer cells, to investigate the role of these signalling pathways.

AIM1. Using the RNA seq data we will identify signalling pathways that are activated in the presence of human but not mouse stromal cells. Pathways to be investigated will be selected from those demonstrated to be lost in PDX models in vivo, based on the RNAseq data described above, and using HGF-cMet signalling as an exemplar. To understand the involvement of these pathways on phenotypic effects of co-culturing cancer cells with stromal cells, the project will use knockdown or specific inhibition of selected pathways.

AIM2. Using direct and indirect 3D co-culture models of colorectal cancer PDX-derived cells and fibroblasts [1, 2], we will investigate the influence of the fibroblasts on cancer phenotype (e.g.growth, invasive behaviour, drug response), through modulation of identified signalling. To do this we will use appropriate molecular targeted therapy drugs, growth factors and genetic manipulation.

IMPACT:

Understanding how the normal stromal cells within the tumour influence tumour behaviour will allow us to modulate these interactions as an approach to cancer therapy.

Ultimately, the project will develop and characterise new models of colorectal cancer that incorporate key signalling pathways activated in cancer but missing from models that lack important aspects of the tumour microenvironment. These will provide more clinically-relevant models that can be used in drug development, enabling more relevant readouts of likely clinical response and investigation of drugs targeting the tumour microenvironment. Such models may also provide opportunities to replace or at least reduce the use of animals during drug development programmes.

1. Pal, A., et al., A 3D Heterotypic Breast Cancer Model Demonstrates a Role for Mesenchymal Stem Cells in Driving a Proliferative and Invasive Phenotype. Cancers, 2020. 12(8): p. 2290.

2. Saunders, J.H., et al., Individual patient oesophageal cancer 3D models for tailored treatment. Oncotarget, 2017. 8(15): p. 24224-24236.**Lab Rotation Location:** University Park;

Full Project Location: University Park;

75 Electric field targeting and understanding of bioelectricity in cancer

Lead Supervisor: PS Jayaraman

Lead School: Medicine (if a supervisor is based in Medicine, they should complete the additional form for match-funding approval from the School)

DTP Research Area: Bioscience for Health

Lab Rotation Description: Investigating the expression levels of Vimentin and Notch3 receptor in a range of bile duct cancer and immortalised bile duct cells

Multiple bile duct cancer cell lines from different etiologies will be investigated for expression levels of Vimentin, citrullinated Vimentin, Notch3 receptor and the PRH transcription factor that controls their expression - using Western blotting and using flow cytometry. Different antibodies will be used to determine the antibody that is most specific to the proteins on the cell surface.

Full Project Description: BACKGROUND

The vision of the research is to develop our biological understanding to facilitate new treatment modalities for difficult to treat cancers such as bile duct cancer. We have reported that intracellular bioelectrical changes underpin cancer but our ability to sense single cell bioelectrical events is limited. We have developed new functional impedimetric nano-antenna which will be applied to address this. We will investigate bioelectrical targeting in bile duct cancer cells. This will be used in conjunction with more traditional electrophysiological measurements of ion currents to correlate electrical behaviours. Moreover, unlike normal bile duct cells, we have shown that bile duct cancer cells can express both high levels of Vimentin and also express high levels of the Notch3 Receptor protein. We will use nano-particle technology to target and report the bioelectrical activity of cells expressing these proteins in response to an electric field. Chemically inert nanoparticles functionalised with porphyrin rings and inactive cytochrome oxidase (we term Nanoantenna) and antibodies that specifically recognise citrulinated Vimentin and/or Notch3 Receptor will be targeted to tumour cells by the antibody. We will investigate the applications of external electric field across the liver region and establish reporting effects of the nanoatennae within the tumour cells. These will undergo polarisation and which enables us to sense electrical changes inside of cells. This will also result in activation of cytochrome C and fluorescence through a change in redox state and, ultimately, apoptotic death of the tumour cells. In addition whole-cell recordings will allow us to measure changes in cell membrane potential, voltage-dependence, single channel activity to determine the role of bioelectricity in cancer cells.

HYPOTHESIS

Inert nanoparticles with latent apoptotic activity can be targeted to bile duct cancer cells that express high levels of Notch3 Receptor and Citrulinated Vimentin on the cell surface. Bioelectrical activity in the target cells will be induced by the application of a localised electric field thus allowing

wireless targeted sensing and elimination of cancer cells. Alongside, the application of patch clamp electrophysiology will determine changes in underlying electrical activity involved in apoptosis of cancer cells.

EXPERIMENTAL METHODS AND RESEARCH PLAN

Gold nanoparticles will be functionalised with Citrullinated Vimentin antibody or Notch 3 antibody or both and also functionalised with cytochrome using EDC/NHS coupling chemistry to covalently tether proteins to the particle surface. Surface analysis using, TEM, EDX, XPS, AFM, SPR will be used to optimise surface chemistry for apoptotic killing ability. Electrodes will be printed using 3D printing technology. Optimisation of EF stimulation and switching control of modified nanoparticles. Expected Result: Antibody and cytochrome c modified conductive gold nanoparticles (year 0-1.5).

Assessment of Notch3 and citrullinated vimentin cell surface expression using flow cytometry with specific antibodies in cancer and non-cancer cell lines. Delivery and cytotoxicity of functional nanoparticles to CCA cells in vitro (cell lines with low and high Citrullinated Vimentin and Notch 3 expression (AKN1 and AKN1-PRH and AKN1-Vimentin cells). Measurement of fluorescence for the activity of nanoparticles. Measurement of apoptosis using cleaved caspase 3 assays and annexin V staining. Comparison of effect in tumour cells and primary liver and bile duct cells from untransformed liver. Electrophysiological assessment of individual cancer cell electrical characteristics in cells treated with nanoparticles to identify downstream signalling pathways.This will include changes in resting membrane potential, uion current activity linked to cell death and delineated using arrange of ion channel modulators. Expected Result: Wireless killing of cancer cell lines in vitro (years 2-3)

Lab Rotation Location: University Park;BDI C/D Floor;

Full Project Location: University Park;BDI C/D Floor;

84 Mapping microtubules: using proximity proteomics to reveal novel therapeutic targets

Lead Supervisor: Daniel Booth

Lead School: Medicine (if a supervisor is based in Medicine, they should complete the additional form for match-funding approval from the School)

DTP Research Area: Bioscience for Health

Lab Rotation 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 in cancer cells to define the interactome of the mitotic spindle – arguably the most important of all subcellular machinery. The student will become familiar with the interactome (BioID) pipeline using techniques including; light microscopy, cell culture (both neural and brain tumour), biochemistry, mass spectrometry, bioinformatics.

This 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. They will be part of a vibrant and friendly 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.

Full Project Description: Research Aim: Determine the compositional differences between microtubules of the mitotic spindle and microtubules of axons.

Why: To identify novel, cell type specific therapeutic targets.

Microtubules are an integral part of the cytoskeleton, with critical yet diverse roles in; maintenance of cell shape/structure, cell motility and intracellular transport of cargo. Microtubules also form the basis of the mitotic spindle – the machinery responsible for the equal division of chromosomes into two daughter cells. As a result of the latter function, microtubules have long-been a key target for cancer therapy - as stopping microtubules, stops cells dividing. However, since the most commonly used anti-microtubule agents, such as Taxanes, indiscriminately target all microtubules (including those in neurones), then painful side effects, such as peripheral neuropathy, often develops in parallel.

One alternative approach is to instead target proteins that bind specifically to microtubules (Microtubule Associated Proteins). Indeed, there are examples known, albeit few, of cell type specific MAPs, i.e those expressed in neurones but not present in dividing cells. However, there is yet to be a comprehensive study to define the "microtubulome" of different cell types.

This project will use tubulin (the building blocks of microtubules) as a bait for fusion to a BioID tag. BioID is an advanced proteomics tool that permits the protein neighbourhood of desired targets to be mapped. The microtubule 'interactome' will be defined in multiple cell types, including dividing cells and terminally differentiated cells, such as cultured neurones and astrocytes. A comparative analysis of spindle interactome versus the axonal interactome, is expected to reveal novel cell-type specific MAPs to be further characterised for future use as therapeutic targets (with reduced side effects).

This exciting project will provide the student with multiple useful cell biology skills, including; cell culture, microscopy, biochemistry, mass spectrometry and bioinformatics. The student will benefit from a collaboration (and placement) at the Wellcome Trust Centre for Cell Biology Proteomics facility, in Edinburgh.

Objective 1 – Use CRISPR/cas9 to genetically engineer cell lines to express BioID-Tubulin

Objective 2 – Validate the cell lines microscopically and biochemically

Objective 3 – Expand the cell lines for mass-spectrometry analyses

Objective 4 – Use bioinformatics to enrich for candidates considered as "unique" to specific cell lines

Objective 5 – Perform initial characterisation of 'hits' to reveal utility as tractable therapeutic targets, using patient tumour samples.

Team Booth is well funded, offers hands-on guidance and importantly, provides a supportive environment/network of PhD students and post-docs (and the PI of course!). Supervisory support from Chisholm lab will provide access to in vivo tools, including in vivo microscopy and behavioural assessment which can be used for further characterisation of MAPs. Supervisory support from Rahman lab will provide training in patient-derived brain tumour and neural culture both as 2D monolayers and 3D spheroids, access to primary tumour surgical biopsies (for validation of in vitro experimental data) from brain tumour patients.

Lab Rotation Location: University Park;

Full Project Location: University Park;

92 Understanding uPAR isoform biology and interactions in airway relevant models to support therapeutic targeting

Lead Supervisor: Michael Portelli

Lead School: Medicine (if a supervisor is based in Medicine, they should complete the additional form for match-funding approval from the School)

DTP Research Area: Bioscience for Health

Lab Rotation Description: The 9 week lab rotation will provide a brief introduction to the essential techniques that will underpin the project including;

i) Primary cell culture of human bronchial epithelial cells at 2D and 3D differentiated (air liquid interface) models with quality control of models using epithelial markers e.g., Immunofluorescence of ECad, CK14 (Lead Portelli)

ii) An introduction to genome editing via an established vector-free CRISPR/Cas9 system (Lead Sayers).

iv) Investigation uPAR protein structure and relevant interactions through protein modelling (Lead Emsley).

Full Project Description: The Urokinase plasminogen activator receptor (uPAR) regulates activation of the urokinase plasminogen activator (uPA), regulating the plasminogen/plasmin activation cycle and its extracellular proteolytic cascade. In addition to this role in plasminogen activation and fibrinolysis, uPAR presents with functionality through non-proteolytic activity via interactions with a multiple different cell bound co-factors, e.g., integrins and G-protein–coupled receptors. This non-proteolytic function allows uPAR to regulate signaling cascades and ergo various diverse functions in different cells/tissues, including cytoskeletal dynamics, cellular adhesion, and cellular migration. UPAR's proteolytic and nonproteolytic cascades allow it to play an active role in pro-airway remodeling processes, e.g., extracellular matrix remodeling, cell migration, coagulation, cellular proliferation, release of inflammatory cytokines, and growth factor activation. We and others have linked altered uPAR expression and activity to lung disease [PMID: 19443020, PMID: 19878584, PMID: 26869673, PMID: 27624865].

uPAR is a complex molecule which exists as a membrane-bound form bound through a glycosylphosphatidylinositol (GPI)-anchor, carrying out its non-proteolytic functions through multiple co-receptors interactions. Through proteolytic cleavage of or splice-variation driven lack of expression of the GPI anchor results in a soluble cleaved (scuPAR) and soluble spliced (ssuPAR) form of the receptor respectively, each of which have potential proteolytic and mnon-proteolytic function.

uPAR represents an appealing therapeutic target for the treatment of multiple respiratory diseases and blocking selected functions of uPAR may be beneficial in halting/reversing airway remodeling changes.

This project aims to significantly extend our understanding of the structure/function of different forms of uPAR to provide new understanding and opportunities. The project has four aims:

1. Use genome editing to produce an uPAR null bronchial epithelial cell and compare the effect of reintroducing full length vs scuPAR vs ssuPAR vs cleavage-resistant uPAR on epithelial functions. This will help us to understand functional role of uPAR isoforms.

2. Use the same technology to knock-in a tagged/labelled form of uPAR for use in experiments to identify the key co-receptors in the airway epithelium.

3. Define the role of identified uPAR co-receptors in mediating epithelial functions outlined in 1. This will provide support for specific uPAR/co-receptor targeting.

4. Use molecular modelling and structure determination (x-ray crystallography) to examine the structure of different uPAR isoforms in isolation and bound to uPA and co-receptors and enzymes-cofactors from the contact system. [PMID: 29619369]

This project is ideally placed for a PhD student as it provides excellent training in functional genomics/cell biology/respiratory medicine (Portelli/Sayers) and protein structure/function/modelling (Emsley). The programme of work is multidisciplinary and spans two Schools at the University of Nottingham; Medicine and Pharmacy.

Lab Rotation Location: University Park;Biodiscovery Institute;

Full Project Location: University Park;Biodiscovery Institute;

114 Proteomic and electrophysiological features of the aged human neuromuscular junction

Lead Supervisor: Mathew Piasecki

Lead School: Medicine (if a supervisor is based in Medicine, they should complete the additional form for match-funding approval from the School)

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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.

Lab Rotation Location: Derby Royal Infirmary;

Full Project Location: Derby Royal Infirmary;

134 Development of an in vitro 3D model to test environmental factors that influence chronic inflammation diseases

Lead Supervisor: Paloma Ordóñez Morán

Lead School: Medicine (if a supervisor is based in Medicine, they should complete the additional form for match-funding approval from the School)

DTP Research Area: Bioscience for Health

Lab Rotation Description: The student will learn how to isolate 3D organoids from patient's biopsies. We will first validate our approach treating the cells with the recent described agent herbicide propyzamide present on weed control during the production of vegetables, fruits, and ornamental plants, and also in golf courses and sport fields. It is known that this factor increases inflammation in the small and large intestine (Sanmarco et al., Nature 2022). We will then monitor by imaging its effect in the regeneration of epithelium (LGR5-GFP stem cells) when exposed to an inflammatory cocktail (TNFalpha, IL6 and IL24) that mimic inflammation in vitro due to the immune system contribution.

Full Project Description: Background

Inflammatory bowel disease (IBD) is a complex chronic inflammatory disorder of the gastrointestinal tract. After induced-damage due to constant inflammation exposure, the regeneration of intestinal tissue from IBD patients is impaired largely because inflammation reduces the stem cell population which is fundamental for tissue repair. Indeed, mucosal healing achieved by induced regeneration of intestinal epithelial cells achieved by stem cell activation is associated with a more favourable prognosis, including low risk of surgery, reduced cancer risk, and indicators of quality of life (Sartor et al., Nat Clin Gastroenterol Hepatol; Alatab et al., Lancet, 2020). Many studies have identified genetic factors as causes of IBD but in the last years, the evidence shows that environmental factors such as dietary emulsifiers and oxazoles are also key determinants of IBD susceptibility and severity. Unfortunately, the identification of environmental factors relevant to IBD and the mechanisms by which they cause, or influence disease has been hampered by the lack of platforms for their investigation (Sanmarco et al., Nature 2022). For this reason, our project aims to establish an in vitro model combining bioengineering, biology, and molecular medicine to specifically help identify the mechanisms by which these environmental factors affect the regeneration of epithelial stem cells.

Objectives and Research Plan

We have optimized a culture model that supports 3D human intestinal organoids derived from patients' biopsies by using a versatile self-assembling platform where peptide amphiphiles (PAs) can organize key ECM components into nanofibrous matrixes (PA-ECM) (Figure 1). Using this matrix, we will expand fresh human IBD patients' cells as 3D-patient-derived organoids, which maintain disease specific features and their stem cell properties. These organoids are derived from UC mucosal samples from inflamed (I) and non-inflamed (NI) colonic areas that are collected from the QMC in collaboration with Dr. Gordon Moran's team. Our ethics approval is: 17/EM/0126. These organoids will be lentiviral transduced to express LGR5-GFP to monitor stem cell pool number and localization.

Our aim is to use our 3D model to study the impact of specific environmental chemicals (detailed below) in IBD organoids derived from healthy, inflamed, and non-inflamed biopsies (Figure 2). We will test how these factors affect the ability of stem cells to regenerate the intestinal epithelium.

We will study the EPA ToxCast database, which collects data on the activity of chemicals used in industry, agriculture, and consumer products in high-throughput biochemical and cell-based assays to select candidates that can damage intestinal epithelium. From this database, only 111 chemicals have been linked to intestinal inflammation associated in bioassays associated with tumour necrosis factor (TNF), interferon (IFN), interleukin-1β (IL-1β), JAK/STAT, peroxisome proliferator-activated

receptor (PPAR), and AHR signalling. These candidates would be tested in our in vitro approach to monitor LGR5-GFP stem cells implication in regeneration. We will be able to detect specific chemicals that can affect epithelial regeneration and would help to understand the mechanism behind.

Conclusion

In this multi-disciplinary project, we aim to develop a model for testing how environmental factors (i.e., chemicals that the populations is commonly exposed to) affect epithelial stem cells in the intestinal epithelium, which can cause or influence chronic inflammation diseases. Despite ongoing efforts in mouse models, clinical paradigms in IBD are still imprecise so our approach based on adult human 3D organoids technology will help to understand basic mechanisms driving the disease forward and test treatments effect on epithelial cells. We envision a high number of potential applications of these results for therapeutic purposes based on our 3D in vitro model.

Lab Rotation Location: University Park;

Full Project Location: University Park;

Pharmacy

17 Mass deployable self-administered DNA vaccines

Lead Supervisor: James E. Dixon

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: The student will learn the process of transfection of cells (DC cells and 293T cells), undertake ELISAs to confirm gene expression of the vaccine pDNA. They will generate MAPs and use those to transfect cells and measure efficiency and cell viability. The student will learn several techniques for sizing of the formulation particles (DLS, Zeta) and explore DNA encapsulation and integrity after formulation. This will generate the basis of them to move forward in a PhD project to generate MAPs for animal testing.

Full Project Description: Although mRNA vaccine strategies have provided a rapid response to the COVID19 pandemic, issues of production scale-up and cold-chain requirements restrict their suitability for developing countries and veterinary use. With the increasing emergence and re-emergence of viral diseases, most of which are zoonotic, there is increased focus on developing technologies that increase the speed and cost-effectiveness of mass vaccination. Plasmid DNA (pDNA) is a more stable and amenable vector for nucleic acid vaccines but innovative strategies to improve immunogenicity and delivery are required.

The studentship will focus on the key development needed of globally rapidly deployable vaccines employing pDNA strategies we are presently translating for COVID19 (Innovate UK) and Zika (SBRI). Marrying effective vector engineering and antigen-presentation strategies with easy skin-based delivery would be transformative as a mass vaccination strategy. Microarray patches (MAPs) allow the pain-free, self-administration of vaccines intradermally. MAPs (a patch base with fine micro-projections <1 mm) would remove cold-chain and healthcare professional-dependence. Furthermore, skin has high antigen presenting cell (APC) density including Langerhans' cells (epidermis and dermis), which would subsequently enhance trafficking of vaccines to draining lymph nodes to elicit enhanced, dose-sparing, adaptive immunity.

The swine-origin H1N1 subtype influenza A virus that was responsible for the 2009 global pandemic (pH1N1) will be used as an exemplar. For decades, the most widely used seasonal influenza vaccine has consisted of an inactivated reassortant virus that has core proteins of a virus isolated in 1934 ('PR8') that can be manufactured to high titre with surface glycoproteins (haemagglutinin, HA, and neuraminidase, NA) of current viruses. The composition of human seasonal influenza vaccines is reviewed annually to keep pace with arising antigenic variants; the four viruses currently included in vaccines include a pH1N1 variant. These vaccines offer poor efficacy when compared to nucleic-acid counterparts.

Aims and objectives:

The PhD aims to develop intradermal DNA delivery platforms to exploit UoN and Nemaura expertise in drug delivery. Using MAPs, peptide pDNA complexes and virology/ vaccinology knowledge will generate the most effective vectors to generate immunity against influenza variants. Activities are planned as work packages (WPs) (GANTT).

Lab Rotation Location: University Park;

Full Project Location: University Park;Sutton Bonington Campus;

28 ENHANCING NEXT GENERATION THERAPEUTICS – Development of a rapid in vivo screen for a library novel polymeric formulation and their excipients with nematode viability

Lead Supervisor: Veeren Chauhan

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: This exciting 9-week project will provide a flavour for the PhD project. This training project will build understating on novel biodegradable polymer synthesis and their application in the development of new drug delivery technologies, in preparation for in vivo screen in C. elegans, a free-living nematode that is simple model system for complex human biology. The project will take place in the School of Chemistry & Pharmacy as detailed below.

TASK 1 POLYMER PRODUCTION - CHEMISTRY

(WEEKS 1-4) 1.1 Synthesis of PGA polymer and derivatives

(WEEKS 1-4) 1.2 Purification

(WEEKS 2-5) 1.3 Physicochemical characterisation

TASK 2 NANO FORMULATION – CHEMISTRY & PHARMACY

(WEEKS 4-7) 2.1 Formulation method optimisation

(WEEKS 5-7) 2.2 Nanoparticle size & stability analysis

(WEEKS 6-7) 2.3 Model Drug Loading (coumarin)

TASK 3 NEMATODE CHALLENGE - PHARMACY

(WEEKS 3-9) 3.1 Nematode growth and maintenance

(WEEKS 4-9) 3.2 Nematode life-cycle characterisations

(WEEKS 4-9) 3.3 Nematode and Nano-formulation biocompatibility assay

(WEEKS 7-9) 3.4 Nano-formulation and nematode bioactivity

Full Project Description: CHALLENGE: The major obstacles when translating newly identified active pharmaceutical ingredients (APIs) into next generation therapeutics are their solubility in aqueous environments and in vivo bioavailability.

This is primarily due to the use of high throughput, combinatorial screening approaches during drug discovery, where over 40% of APIs result in poorly water soluble high permeable candidates. Furthermore, humans are large complex animals, which are not completely understood and can be both scientifically and socio-economically challenging to characterise. Therefore, there is a strong international drive to replace, reduce and refine the use of animals in research so that this precious resource is reserved.

PROJECT: This exciting project will directly address this challenge by investigating the development of a platform screening technology that enables APIs with polymeric biodegradable scaffolds and

assess their bioavailability in line using the most completely understood animal on the planet, Caenorhabditis elegans.

METHOD. The first phase (Years 1-2, quarters 1-8) of this project will be built upon novel chemistry demonstrated by the supervisory team that have recently shown that novel polymers can be synthesised by exploiting the chemo- and regioselectivity of enzymes to create linear glycerol-based polyesters. These polyesters will be readily conjugated or used to encapsulate APIs. This will produce a library of novel biologically active polymeric- drug delivery systems which will form the basis of next generation therapeutics.

The second phase (Years 2-4, quarters 9-14) of this project will be to assess in vivo biocompatibility, bioavailability and bioactivity in C. elegans populations using the development of high-content imaging. Biocompatibility will be assessed by monitoring motility and nematode lifecycles (progeny production) through the application of time dependent brightfield microscopy analysis methods [Journal of Material Chemistry B. in press]. Bioavailability will be mapped using model fluorescent drugs (e.g. doxorubicin) across the anatomy of larval and adult stages nematodes, with particular emphasis on tracking distribution of therapeutic delivery. Bioactivity will be determined by studying global shifts omic nematode profiles in the nematodes though the application of liquid chromatography mass spectrometry methods (LC-MS).

TRAINING: This project will provide the PhD student with expertise in polymer synthesis, pharmaceutical formulation, whole organism culture and big data analysis. The project will also permit multiple national and international outreach and communication opportunities (Journal of Controlled Release Conference, Las Vegas, C. elegans International Conference, Glasgow) to showcase research and build the foundations for a successful research or industrial career trajectory.

IMPACT: This project has the potential to streamline and optimise formulation pathways for new APIs that will accelerate the development of next generation therapeutics, that could benefit hundreds of millions of people globally. This project has the potential to produce high impact peer reviewed articles for novel polymer synthesis (ACS Macro Letters 7.02, or Biomacromolecules IF 6.99) as well as transformative therapeutic delivery (Journal of Controlled Release IF or 11.47 and Biomaterials IF 15.3). Furthermore, the new materials developed as part of this PhD could contribute the develop of University intellectual property which could form the basis of a patent or licencing opportunity.

Lab Rotation Location: University Park;

Full Project Location: University Park;

62 Identifying the molecular mechanisms of bioactive natural products using high throughput data collection and artificial intelligence

Lead Supervisor: Cornelia de Moor

Lead School: Pharmacy

DTP Research Area: Biotechnology

Lab Rotation Description: Cordycepin is a product of the insect-infecting fungus Cordyceps militaris. We have shown that cordycepin affects the insect immune system, allowing more efficient infection. Remarkably, Cordyceps is famous as a traditional Chinese medicine, and many publications,

including ours, demonstrate that it has anti-inflammatory and anti-cancer properties. Using high throughput methods, we have collected information on the changes in protein, mRNA and metabolite levels, as well as on mutations that convey resistance to cordycepin. In this rotation, you will experimentally validate some of the candidates that have come out of these individual datasets (using siRNA knockdown, quantitative PCR, western blot and/or ELISA), reanalyse some of our data using new bioinformatic methods and combine two of different datasets to extract additional information using machine learning.

Full Project Description: When novel drugs are designed by the pharmaceutical industry, they tend to separately tackle the target engagement and the delivery of the compound to the tissue. In contrast, natural products made by plants and fungi have been selected by evolution and many have been shown to have potential as medicines, biotechnology tools and in sustainable pest control. Unlike designed drugs, we often do not understand what the mechanism of action of natural compounds is and their biodistribution through the body frequently does not follow the rules derived for synthetic products. Understanding their mechanism of action and delivery is therefore not only beneficial for developing the compound itself, but also is highly likely to teach us other ways of designing drugs and as well as safe biochemicals. One such product is cordycepin, isolated from the caterpillar infecting fungus, Cordyceps militaris, a widely used health food in the Far East. We have shown that the biological function of cordycepin is to repress the insect immune system, highlighting its potential in biological pest control. In addition, cordycepin has a been shown to be effective in a large number of animal models of disease and consistently has anti-inflammatory and anti-proliferative signalling. It inhibits PI3K/AKT/mTOR signalling and activates AMPK, consistent with the reputed life-prolonging effects of the fungus. Moreover, we have shown that cordycepin has a very unusual metabolism, which is likely to target it to specific tissues in vivo.

To understand how cordycepin works, we have collected over 10 sets of high throughput data, including RNA-seq data, proteomics, metabolomics and genetic screening data. In this project you will transform these data to allow them to be compared to each other using machine learning and use them to predict key players in the mechanism of action and delivery of cordycepin. You will then return to the bench and test your predictions on the metabolism and/or mechanism of action of cordycepin. This probably will involve modifying genes using CRISPR/Cas9 and conducting an RNA-seq, proteomics or genetic screen yourself. You are also likely to test some of your predictions in primary cells.

This project is the culmination of many years of work of a large collaborative team and you are likely to be involved in its most exciting stages, as we hone in on the molecular mechanism of cordycepin in your research and move forward to clinical application in other projects. There may be opportunities to analyse human samples in the later stages of the project and this work is very likely to lead to a high impact publication.

Lab Rotation Location: University Park;Sutton Bonington Campus;

Full Project Location: University Park;Sutton Bonington Campus;

64 Exploring the impact of circulating endothelial glycocalyx fragments on sepsis outcomes

Lead Supervisor: Andrew Hook

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: The aim of the rotation is to analyse reference samples and introduce the multi-disciplinary nature of the project, with an emphasis on analytical sciences but exposure to the microbiological, physiological and clinical aspects. The rotation will provide training in:

- sample handling including using the automated piezo-dispensing system to produce arrays of materials, enabling high throughput screening.

- Time-of-flight secondary ion mass spectrometry (ToF-SIMS)

- 3D-OrbiSIMS. This state-of-the-art instrument is one of only four academic instruments in the world and allows for high mass resolution SIMS analysis. This technique will be crucial for correctly assigning ions through utilising the MS-MS capabilities, which will underpin structural analysis.

- Multivariate analysis required to analyse and interpret the complex datasets.

During the rotation the student will spike artificial urine and plasma samples with purified glycosaminoglycans (GAG). The samples will be deposited as microarrays using automated liquid handling systems and analysed by both ToF-SIMS and 3D-OrbiSIMS. This will enable the analysis of GAGs to be compared between the two bodily fluids to determine which is more suitable for identifying GAGs associated with sepsis.

Full Project Description: Sepsis is a dysregulated host response to infection that leads to life-threatening organ dysfunction, killing up to 30% of the millions it affects each year and costing the NHS £2.5 billion annually.[1] Although early identification and treatment can improve clinical outcomes, there is currently no dependable biomarker for sepsis.

During sepsis, the glycocalyx (a mesh-like physical barrier present at all endothelial interfaces composed largely of glycosaminoglycans (GAGs)) is degraded leading to an increase in circulating GAG fragments.[2] Detection and serial monitoring of these circulating GAG fragments using high performance liquid chromatography tandem mass spectrometry (HPLC MS/MS) has been found to predict risk of organ dysfunction, hospital length-of-stay, mortality, and long-term disability. Moreover, the composition of the glycocalyx changes with age, which may contribute to clinical outcome from sepsis. An improved understanding of changes to glycocalyx structure and associated endothelial function through rapid and sensitive detection of circulating fragments could facilitate highly personalized approaches to care and identify novel therapeutic strategies. Unfortunately, HPLC MS/MS methods for detection of circulating GAGs are expensive (£200 per sample) and slow (i.e., days to weeks), limiting its clinical utility.

Recently, ToF-SIMS has been used by our group to enable rapid, inexpensive mass spectrometry assessment of pharmaceutical GAGs, showing high sensitivity differentiation between the same GAGs from different animal sources whilst requiring only nanogram quantities of material.

The project will explore the following hypotheses:

1. ToF-SIMS analysis of GAGS within patient blood and/or urine samples can be used to identify sepsis cases from non-sepsis cases

2. The GAG fragment profile can be assessed by ToF-SIMS over time to monitor sepsis progression

3. Glycocalyx composition varies with age, which can be assessed by ToF-SIMS analysis of shed GAG, and this leads to varied sepsis clinical outcomes.

To explore these hypotheses the project has 4 work packages (WP)

WP1 – Collection of animal samples

The student will work with project partner Joseph Hippensteel at the University of Colorado to acquire blood and urine samples from mice with multiple simulated sepsis conditions. This aim will also explore age-dependent changes in circulating GAG fragments in these models. This will include short (1-3 month) placements at the University of Colorado.

WP2 – Collection of human samples

The student will work with the clinical project partners Marc Chikhani (Nottingham University Hospitals) and Joseph Hippensteel (University of Colorado) to acquire relevant human samples.

WP3 – Analysis of biological samples

Acquired samples will be analysed by ToF-SIMS in a high throughput fashion. Relevant reference samples will be analysed using the 3D-OrbiSIMS instrument to enable the SIMS spectra to be interpreted in regard to specific GAG structures.

The SIMS analysis will be supported by microbiological analysis of samples to determine if there is a detectable bacterial species within sepsis-positive patients and characterise any virulence factors. This will be compared with circulating GAG composition to assess any relationship. This will be supported by project partner Marc Chikhani.

WP4 – Multivariate analysis

A suite of multivariate analysis approaches will be utilised to enable the robust analysis of the SIMS data.

1. Sanderson, M., et al., Predicting 30-day mortality in patients with sepsis: An exploratory analysis of process of care and patient characteristics. Journal of the Intensive Care Society, 2018. 19(4): p. 299-304.

2. Sullivan, R.C., et al., Endothelial glycocalyx degradation during sepsis: Causes and consequences. Matrix Biology Plus, 2021. 12: p. 100094.

Lab Rotation Location: University Park;

Full Project Location: University Park;

86 Programming cell function through understanding and decoding Intracellular bioelectrical networks

Lead Supervisor: Frankie Rawson

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: Combinatorial switching of bioelectrical transistors of Patient-derived glioma cells and breast cancer cell lines will be used to study the effect of altering bioelectrical switches at the membrane. Using more than one cell type will enable us to identify more general phenomena. We are selecting cancer cells as a model to study bioelectrical currents as they have evolved mechanisms to cope with cellular induced stress due to their nature, being highly

proliferative, decreasing pH in solid tumours and higher ROS generation. Modulation of bioelectrical signals will be performed in a combinatorial approach allowing us to assess cell phenotype effects with bioelectrical alterations. This will be achieved by instructing several naturally occurring cues e.g., modulation of NDBT, dcytB and Glut transporter, oxygen levels (InvivO2 hypoxia workstation, Baker Ruskinn), and other electrolyte levels in the medium (Ca2+, H+, and Na+) will be altered as previously. This will be performed by using chemical electron transport inhibitors, and siRNA and knocking out key membrane electron transporters.**Full Project Description:**

All cells modulate their function through the control of ion and electrical currents and are essential to life. For example, voltage gradients enable proton motive gradients to convert electrical energy in adenosine triphosphate a high-energy molecular store.1 Bioelectricity also provides network-coordinated response as seen through neuronal relays by controlling organism movement, pain, touch, hearing and sensing of our environment. These neuronal network communications are underpinned by action potentials, defined as the alteration of membrane potentials and ion charge movement along an axon. These are ultimately governed by voltage-sensitive ion channel which allows the transport of key ions such as sodium and potassium on opening, determined by voltage structural changes to membrane electron transport proteins. These ion channels act as molecular current switches, analogous to a transistor found within microchips which are used to programme software within the hardware of a computer. There is an abundance of different molecular switches that control bioelectrical circuits and we have recently reviewed some of these 2. Importantly we have identified how some of the transistor-like systems modulate bioelectricity and are altered in times of cellular stress. For example, faradaic and ionic currents are increased through the gating of transistor-like switches including dcytB and Glut transporters. We infer that the currents enabled bioenergetic reprogramming allowing cells to deal with external stress 3. However, to fully understand how bioelectrical programming signals modulate cell function through these bioelectrical circuits the biochemical and genetic signalling pathways associated with these bioelectrical alterations need to be elucidated. Moreover, work has also been performed identifying the sodium-driven bicarbonate transporters which involved sodium currents and alteration in proton current to enable cells to manage hypoxia. The bioelectrical circuits in that work were not measured. We assert that the identified systems all enable cell reprogramming through careful gating of membrane like-transistor that control currents.

The hypothesis to be tested is that multiple interconnected bioelectrical circuits modulate cellular function via transistor-like behaviour to modulate cell stress. This stands to revolutionise our ability to then use bioelectrical switches to programme cell behaviour which has far-reaching consequences for health and disease; but more broadly in sustainable energy and chemical production, as the cell will then become a bio-computer that we can programme on demand for a given application.

2.1. Aims and Objectives

Therefore, the overall aim is to elucidate how membrane bioelectrical transporters, tune bioelectrical current networks, by acting as cellular-like transistors that sense bioelectrical signals and guide cell phenotypes through regulation of transcription via modulation of intracellular signalling cascades, transcription factors and global RNA profiles. Towards this aim, we have 2 objectives that represent individual work packages:

(WP 1): Obj 1. Modulate bioelectric signals and identify changes in cellular phenotypes in normal, hypoxic and metabolic stresses environments.

Whilst individual electron transporters have been looked at in modulating the transport of ions and electrons in cell stress to date. We will elucidate the interplay between underlying bioelectrical circuits including those modulated through Dcytb, Glut, and NBDT.

(WP 2): Obj 2. Modulate bioelectric signals and characterise changes in cellular signalling.

For the first time, the resulting modulation of cell protein-like transistors will be correlated with signalling pathways. This facilitates the decoding of bioelectrical circuits on signalling transduction pathways.

Lab Rotation Location: University Park;

Full Project Location: University Park;

95 Investigate the role of biomolecules at the interface between biofilm and substrate (substratum) e.g. Pseudomonas quorum sensing molecules and Staphylococcus guanine pentaphosphate in biofilms using surface mass spectrometry

Lead Supervisor: Rian Griffiths

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: Bacterial infection by microbes such as Pseudomonas aeruginosa and Staphylococcus aureus costs the NHS an estimated £2 billion per year. Bacterial signalling molecules are secreted by bacteria to communicate with one another to colonise a surface, creating a biofilm. Infection from fungi such as Candida albicans can also be life-threatening, with a mortality rate between 46-75%. In reality, biofilms infections are not just one microbe, they are a mixture of different microbes (e.g. bacteria in combination with fungi). The project will focus on understanding the signatures of bacterial communication molecules in biofilms of Pseudomonas aeruginosa cultured on top of Staphylococcus aureus (modelling skin wound infection), using surface sampling mass spectrometry data.

Skills include data handling of large datasets, data analysis using specialised software for mass spectrometry data and interpretation. Students will also be guided in literature review skills and be expected to present at progress meetings and a group meeting, enabling practice of presentation skills, scientific communication and working within a team.

Full Project Description: Aim: Investigate the role of biomolecules at the interface between biofilm and substrate (substratum) e.g. Pseudomonas quorum sensing molecules and Staphylococcus guanine pentaphosphate in biofilms using surface mass spectrometry

Background:

Bacteria attach to surfaces forming a biofilm. Across Europe, ~6% of hospital inpatients suffer from healthcare-associated infections. Biofilms formed on implanted medical devices and in skin wounds cause chronic infections that are both highly resistant to host immune systems and highly tolerant of antibiotics. Antimicrobial resistance threatens to rival cancer in mortality and financial burden by 2050. Biomaterials coatings on medical devices, that prevent biofilm formation, reduce healthcare related infection. The Alexander group previously described polymer biomaterials that promote or prevent biofilm formation; determined via high through-put analysis of an array of candidate

polymer structures. In wound infections, Staphylococcus aureus co-exists with Pseudomonas aeruginosa. Investigating the biomolecules secreted sub-stratum and at the interface specifically will benefit mechanistic understanding, aiding future polymer design and informing appropriate antibiotics.

Liquid extraction surface analysis (LESA) offers direct analysis; coupling to high resolution (trapping) mass analysers (HRMS), allows high accurate mass analysis, aiding identification. A novel sample format specifically designed for the analysis of secreted compounds e.g. quorum sensing metabolites, DNA and lipids (the secretome); biofilms grown on 0.2 µm pore polycarbonate discs (allowing diffusion of small biomolecules) on media, before removal and analysis of the media via LESA.

Objectives:

- Investigate differences in the secretome from polymicrobial biofilms of Pseudomonas grown on Staphylococcus aureus for 24, 48 and 72 hours
- Investigate differences in the secretome from polymicrobial biofilms of Pseudomonas grown on Staphylococcus aureus treated with biocide (triclosan) or an inhibitor
- Prepare polymer coated polycarbonate discs
- Investigate differences in the secretome from Pseudomonas biofilms grown on polymer coated polycarbonate discs

Methodology:

WP1 (months 1-12) timepoints: Pseudomonas aeruginosa quorum sensing molecules and Staphylococcus aureus guanine pentaphosphate, for example, will be analysed in the secretome of mono- and inter-species biofilms grown on polycarbonate discs via LESA-MS.

WP2 (months 12-36) understanding mechanisms: Differences in Pseudomonas aeruginosa quorum sensing molecules will be analysed in the secretome of PA-SA inter-species biofilms grown on polycarbonate discs under different conditions such as drug (triclosan) treatment or inhibition via LESA-MS. A bank of PA mutants will be utilised to investigate the mechanisms affected by these treatment.

WP3 (months 1-12) Preparation of polymer coated polycarbonate discs: The polycarbonate discs (pore size 200nm) will be coated with a ~2nm layer of a polymer that either promotes (NGPDA) or resists (EdGPEA) biofilm formation. Feasibility of this sample format for understanding the Pseudomonas secretome will be assessed by comparison with uncoated discs.

WP4 (months 18-36) Understanding biofilm formation on polymer biomaterials: Discs prepared via methods optimised in WP3 will be studied in interspecies systems to investigate the substratum secretome of Pseudomonas aeruginosa bacterial biofilm development at different time points, ranging from the early stage of bacterial attachment to established biofilms (4-48 hours). A library of polymers will be studied.

Lab Rotation Location: University Park;

Full Project Location: University Park;

110 Controlling miR-122: a master regulator of liver health and disease

Lead Supervisor: Catherine Jopling

Lead School: Pharmacy

DTP Research Area: Biotechnology

Lab Rotation Description: MicroRNAs are tiny RNA molecules that bind to target mRNAs and regulate protein production, with important consequences for development and the maintenance of a healthy organism. MicroRNA-122 (miR-122) is crucially important in the liver, where it is required for hepatitis C virus infection, but also has an essential role in preventing liver cancer. Moreover, miR-122 functions to coordinate cellular senescence and is thus important in healthy ageing.

miR-122 is produced in liver cells at an exceptionally high level. The factors controlling this very high, tissue-specific expression are poorly understood. Interestingly, miR-122 is produced as part of a long noncoding (lnc)RNA. LncRNAs are a recently discovered class of RNA molecules which are an emerging area of research for biotechnology due to their gene regulation potential.

In the rotation project, you will investigate the role of promoter elements in driving the very high expression of miR-122 using CRISPR/Cas9. Using reagents that have been prepared for you, you will generate and characterise a CRISPR-modified cell line to test the effects of targeted promoter modification on miR-122 production. In parallel, you will use a range of molecular biology techniques to investigate the effect of specific genetic elements on miR-122 transcription and processing.**Full Project Description:** The full PhD project brings together the expertise of the Jopling lab in microRNA biology and the Heery lab in transcriptional control and chromatin. You will carry out an in depth investigation of the factors that drive high miR-122 expression in liver, informed by bioinformatic analysis of publicly available datasets and genome-wide data on transcription and RNA processing in liver cells generated in the Jopling lab. You will use CRISPR modification to manipulate defined elements in the miR-122 promoter, enhancer and gene and use RNA and molecular biology approaches, including 4-thio-uridine labelling of newly synthesised RNA and quantitative real time PCR, to investigate the consequences for miR-122 transcription and processing. The role of chromatin modifications will also be investigated by chromatin immunoprecipitation (ChIP). You will also apply some of the same techniques to investigate the production of other biologically and medically important microRNAs that are generated from lncRNAs. This is also likely to include genome-wide analysis by RNAseq.

In the longer term, the results of this project may be important in providing new avenues for therapeutic manipulation of miRNA expression, with potential relevance to cancer, viral infection and healthy ageing.

Both the Jopling and Heery groups form part of the Gene Regulation and RNA Biology labs within the School of Pharmacy, a supportive and collaborative environment with a number of research groups working in related fields. Your project will be supported by Angela Downie as Assistant Supervisor. She is a BBSRC-funded postdoc who has been carrying out closely related work and will provide training and support in the lab to ensure your project is successful and to enhance your skills development. You will have the opportunity to attend and present your data at national and/or international conferences.

Lab Rotation Location: University Park;

Full Project Location: University Park;

112 Development of a next generation tissue metabolite profiling method for biological research

Lead Supervisor: Dong-Hyun Kim

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: During the lab rotation, the student will learn how to perform cell culture, liquid chromatography-mass spectrometry (LC-MS)-based metabolite profiling and data processing and analysis.

In this mini project, patient derived brain tumour cells (tumour core, invasive margin) and non-cancerous neural cells, will be cultured and metabolites will be extracted using organic solvents. The cell extracts will then be analysed using LC-MS-based metabolite profiling to determine metabolic differences between cells from tumour core, invasive margin and non-cancerous regions.

Training involved for this mini project is:

- Metabolite profiling using mass spectrometry: 1) Hand on training in advanced analytical instrumentation including liquid chromatography, mass spectrometry, data processing and chemometric analysis for large metabolomics datasets, 2) Training in metabolic pathway analysis and chemical structural databases for metabolite identification

- Cell culture: Training in basic aseptic 2D and 3D cell culture techniques and the practical and safety aspects of cell culture work.

There will be opportunity for the student to attend laboratory meetings across the research groups and related seminars.

Full Project Description: Background:

This project offers an improved way to analyse the surfaces of biologically relevant materials using the power of mass spectrometry (MS) and multivariate analysis. Liquid extraction surface analysis (LESA)-nano-liquid chromatography (LC)-MS (LESA-nanoLC-MS) is a unique and new cutting-edge technique which has yet to be fully developed, but offers outstanding potential to solve many problems relating to biological surfaces particularly where only a small amount of biological sample is available, and where there is a need to be able to profile a wide range of small biomolecules simultaneously. Since LESA-nanoLC-MS can be used on any surface with only minimal requirements for sample preparation, and provides exceptionally high sensitivity, it has tremendous flexibility of application.

Recent brain cancer research has focused predominantly on genome-wide gene expression and mutation analysis across the genome but a functional genomic approach (i.e. metabolomics) has been lacking due to technical limitations since the size of surgically-resected brain tissues is often relatively small and available material is sparse. Hence, LESA-nanoLC-MS, which can preserve the native state of surface molecules with minimal sample preparation and provide a rapid snapshot of the cell physiology, is a promising analytical tool to obtain a true physiological in situ representation of the concentration of metabolites and information of spatially resolved metabolic changes from architecturally-intact tissue surfaces directly.

Aims:

The overarching aim of the proposed project is to provide a step change in mass spectrometry-based surface analysis capabilities, leading to a better understanding of the underlying mechanisms of brain tumours as an example disease.

1) Develop a 'next generation' tissue surface metabolite profiling technique using LESA-nanoLC-MS.

2) Apply the novel technique to multiple intra tumour regions of paediatric brain tumour to identify biomarkers (key metabolites) and associated metabolic enzymes.

Plan of work:

(Months 1-18): Develop an innovative untargeted tissue metabolite profiling method using LESA-nanoLC-MS.

A selected authentic standard mixture will be employed to develop a novel nano-LC method using a ZIC-HILIC nano column using a LESA instrument. The standard mixture will consist of biologically relevant compounds such as amino acids, carbohydrates, nucleotides and organic acids. A variety of mobile phases and solvent gradient systems will be tested and LESA method will be also optimised by modifying various parameters such as solvent composition, extraction length and different substrates.

The developed LESA-nanoLC-MS method will be applied to, and further optimised using rat brain tissue sections. Reproducibility and quantitative aspects will be assessed between the groups using coefficient of variation (CV%) and various statistical analyses such as uni- and multivariate analyses. Unambiguous identification will then be performed through high resolution QExactive Orbitrap MS for more accurate fragmentation studies.

(Months 19-36): Application of the LESA-nanoLC-MS-based metabolite profiling method to brain tumour tissues.

The novel surface metabolite profiling will be applied to clinically-distinct intra-tumour regions from adult glioblastoma (a grade 4 malignant tumour arising from astrocytes) and normal brain temporal tissue microarrays.

(Months 37-42): Validation of the biomarkers in patient-derived cells and tissues using the conventional LC-MS method.

Lab Rotation Location: University Park;

Full Project Location: University Park;

113 Defining the interplay between genetics, mucus properties and inhaled drug efficacy in the airways

Lead Supervisor: Cynthia Bosquillon

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: The 9 week lab rotation will provide a brief introduction to the essential techniques that will underpin the project including;

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 drug permeability measurements in epithelial cell layers (Lead Bosquillon)

iv) An introduction to rheological, biophysical and spectroscopic method used to study mucins (Lead Yakubov).

Full Project Description: The airway epithelium acts as the critical interface between tissues and the external environment. The critical element of the protection mechanism against inhaled pathogens and allergen particles is gel-like mucus lining. Unique flow and barrier properties of mucus are key for mucociliary clearance of pathogens and drug absorption. In the airways, the key components of mucus are secreted, gel-forming mucins: MUC5AC and MUC5B. We have completed the largest genetic study of moderate-severe asthma and identified genetic variants in a region encoding MUC2, MUC6, MUC5B and MUC5AC genes are associated with greater risk of developing more severe disease (Sayers, PMID: 30552067). Importantly, we showed that these genetic variants drive increased expression of mucin MUC5AC and a decrease in MUC5B in patient airways, which could modify the flow and barrier properties of the mucus

In related work, we have begun identifying the link between composition and biophysical properties of mucus, which is central for uncovering the physiological role of the discovered genetic variants. (Yakubov, PMID: 29643478). Similarly, we have shown the impact of airway mucus on limiting the absorption of inhaled drugs with certain physico-chemical properties (Bosquillon, PMID: 31154066).

This PhD is set to explore how i) genetics and gene regulation of mucins in epithelial cells change the composition and biophysical properties of airway mucus; ii) how these changes influence the permeability of aerosolised drugs and thus, their efficacy.

This studentship will address three main questions:

1.How do genetic variant regulate the expression of mucin gene(s)?

These analyses will be completed using our human bronchial epithelial air liquid interface in vitro model that mimics the lining of the lung in the laboratory. We will examine mucin production e.g. MUC5B, MUC2, MUC5AC at the mRNA and protein levels in primary human cells (already collected) carrying/not carrying the risk genotype in the presence and absence of relevant stimuli that induce mucins e.g. IL13, Rhinovirus. We will also use CRISPR/Cas9 to perturb the mucin genes and alter mucin gene expression and mucus composition.

2. What are functional effects of carrying specific genetic variants and perturbing mucin genes to barrier properties and drug absorption?

We will assess the permeability of inhaled drugs across mucus of altered compositions produced by the two types of bronchial epithelial cells studied in (1) as well as across the cells perturbed using CRISPR/Cas9. These investigations will make use of an aerosolised drug deposition system and advanced bioanalytical techniques.

3. Do carriers of genetic variants have altered mucus flow and biomechanical properties?

We will use a range of macro-/micro- and nano-rheology approaches (including using the BioFluidic Microscope, recently funded through BBSRC 21 ALERT) to investigate the link between composition and biophysical properties of mucus collected from in vitro cell models (1 and 2) and patient airways

e.g. determine the role of mucin glycoproteins, non-mucin proteins, and Ca2+ in mucin assembly. In addition, Raman and NMR spectroscopies will be used to investigate the structure and organisation of glycoproteins and the complex matrices they form.

This project is ideally placed for a PhD student as it provides excellent training in functional genomics/cell biology/respiratory medicine (Sayers), biochemical, spectroscopy, biophysical methodologies (Yakubov) and drug pharmacology (Bosquillon). The programme of work is highly multidisciplinary and spans three Schools at the University of Nottingham; Medicine, Biosciences and Pharmacy.

Lab Rotation Location: University Park;

Full Project Location: University Park;Sutton Bonington Campus;

125 Novel approaches to targeting beta-adrenoceptors

Lead Supervisor: Shailesh Mistry

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: Beta-Adrenoceptors (B-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 B2-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:

1. Develop a library of fluorescent 'cmpd-6' analogues, exploring a range of different fluorophores.

2. Pharmacologically characterise the library of ligands at the B1-AR and B2-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 B-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.

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 B-ARs (family A GPCRs), are key regulators of the cardiovascular and respiratory systems

and well-established drug targets. B1, B2, and B3 subtypes have distinct tissue distributions and pharmacological activity.

Despite drugs targeting the B1-AR/B2-AR being in clinical use for over 60 years, new ways of targeting these receptors, are now emerging. Endogenous B-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, increasing numbers of allosteric binding sites (ABS) have been identified for the B2-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 B2-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) Pharmacologically characterise any new ligands to determine affinity for B1-AR/B2-ARs and any modulatory effect on co-bound orthosteric ligands.

2. Development of novel B2-AR PROTACs:

a) Use computational molecular docking studies to predict the ligand-target interactions of new ligands with the B2-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 B2-AR, selectivity over other B-ARs/GPCRs and ability to degrade B2-AR concentrations.

This chemical biology-focused project will span the disciplines of synthetic chemistry, modelling and pharmacology to increase our understanding of B2-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.

Lab Rotation Location: University Park;

Full Project Location: University Park;

138 Advanced plastid engineering for therapeutic protein synthesis

Lead Supervisor: Katalin Kovacs

Lead School: Pharmacy

DTP Research Area: Biotechnology

Lab Rotation Description: During the rotation project the students will have the opportunity to develop team science skills (teamwork, critical thinking, time and resource management, record keeping) in addition to speciality skills such as growth and manipulation of the microbial and plant host, and the use of the various gene tools previously developed by our group. Specifically, they will:

1. Learn to design and build synthetic genetic parts and modules to be introduced in plant host.

2. Learn to genetically manipulate the host, creating new and characterizing existing transgenic and transplastomic plants.

3. Student will be introduced to tissue culture and use of biolistics gene gun.

4. Student will learn to use DNA analysis software.

The student will be involved in the in silico design of nuclear and chloroplast targeted DNA constructs. They will receive training in synthetic DNA vector assembly and in bacterial and plant transformation methods including tissue culture and use of biolistics gene gun.

Full Project Description: Chloroplasts carry out many functions beyond light-driven CO2 fixation, they synthesise and store starches, carotenoids and some oils, and most of the plant's amino acids are made within chloroplasts (plastids). Plastids are the site of synthesis and sequestration of many complex and high value secondary metabolites. Transgenes placed on the chloroplast genome can produce proteins at levels up to 300 times that of nuclear transgenes, and this has made them attractive targets for genetic engineering. In addition, chloroplasts are excluded from pollen and the chloroplast DNA is only inherited from the pollinated and not the pollinating crop plant. This has made chloroplasts very attractive as "green factories" for producing novel high value proteins, metabolites, where high levels of gene expression are required. Plants have been developed as green cell factories to produce high value molecules such as vaccines, antimicrobials and other biopharmaceuticals. The technology has now reached the point where some of these are in commercial production in glasshouses in several European facilities. Other high value products such as designer "fish oils" are currently being evaluated in UK field trials of genetically modified oilseed crops.

Chloroplast engineering has the potential to dramatically increase yields of these products and to extend to include therapeutic proteins that can be synthesised on demand in resource limited environments. The proposed technology will utilise direct chloroplast engineering to allow efficient, safe and sustainable production of high value molecules. We have recently designed and implemented a CRISPR-Cas9 based gene-drive system, together with plants engineered to contain a single giant chloroplast, for plastid engineering and we have successfully generated transplsatomic plants. Here, we aim to build on this plastid engineering success by applying these tools to introduce genes of interest for therapeutic protein such as reptelase, teriparatide and filgrastim in model plant systems (such as tobacco) and to expand to include species previously not amenable to plastid engineering, particularly to establish protocols for crop species (such as foxtail millet).**Lab Rotation Location:** University Park;Sutton Bonington Campus;

Full Project Location: University Park;Sutton Bonington Campus;

141 Single molecule biophysical Investigations of expanded nucleotide repeat RNAs

Lead Supervisor: Stephanie Allen

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: The main PhD project will explore the potential of single molecule biophysical approaches to provide new molecular insight into the properties of expanded nucleotide repeat RNAs. This will involve the application a combination of molecular biology/genetic approaches and biophysical techniques, including atomic force microscopy (AFM). As time is limited in the rotation, the focus will be to provide preliminary training in the AFM imaging related aspect of this work. Initially the student will be provided with training on the basics of AFM imaging and experience in RNA handling and sample preparation; to gain experience, these initial studies will focus on commercially available model RNAs (weeks 1-3). In the remaining time, the student will then generate images of expanded repeat RNAs and their protein complexes, utilizing molecules already available through on-going projects. For example, a current MRes project (Dr Borkar's lab), has optimized the lab-scale production of the protein muscleblind (MBNL) and r(CUG)n=136 trancripts, providing an excellent range of materials ready to be characterized. In addition to providing essential experience in preparing, handling and imaging the molecules required, this training project will thus provide important early characterization data of the biomolecular complexes to be further explored in the main PhD.

Full Project Description: Repeat expansion diseases are inherited conditions caused by expanded numbers of simple repeat sequences in the human genome. Expanded trinucleotide repeats are associated with over 50 different human diseases, including myotonic dystrophy (DM). Although the application of genetic and cell biology approaches has improved the understanding of such conditions, relatively little is still known about the fundamental molecular properties of expanded nucleotide repeat transcripts, and consequently how these may contribute to disease states. This project thus seeks to utilize single molecule biophysical techniques, such as atomic force microscopy (AFM), to reveal new fundamental data upon the structure, stabilities and protein interactions of expanded nucleotide repeat RNA transcripts. Specifically, by addressing key issues in DM research we will aim to not only improve the understanding of the molecular basis of DM, but also to impact on the understanding of other disorders associated with nucleotide repeats.

Myotonic Dystrophy type 1 (DM1), is caused by an expansion of CTG repeats in the DMPK gene. If a critical repeat length is exceeded (e.g. > CUG100) the transcripts are retained in the nucleus as distinct foci where they sequester nuclear proteins, such as muscleblind (MBNL), and perturb cellular functions. Importantly, the Brook lab have identified that preventing MBNL protein binding to the CUG expansion can eliminate the formation of such foci, presenting a potential avenue for therapeutic intervention. Whilst promising, the lack of information on the properties and interaction of such long transcripts, is currently hindering progress beyond this point. The proposed work programme therefore seeks to address this gap by using AFM, in combination with molecular genetic approaches, to investigate the structure, stabilities and protein interactions of long RNA molecules containing expanded trinucleotide repeats.

To achieve this, the specific objectives of our research programme will be to:

1. Utilize molecular biology/genetic approaches to generate relevant RNA transcripts (e.g. of a range of lengths and sequences) and MBNL proteins for subsequent characterization (months 1-12)

2. Utilize AFM imaging to visualize the size and structural morphologies of expanded repeat RNAs and their complexes with proteins (months 3 – 15). Note that this aspect builds on a previous PhD project between Profs Allen and Brook (Meullenet (2009)), where we demonstrated the feasibility of utilizing AFM to image expanded repeat RNAs and their complexes with MBNL. Here, we will further develop these findings through the application of newer imaging technologies (to improve image resolution) and by imaging in more biologically relevant liquid environments (e.g controlled buffers).

3. Utilize AFM based force measurements, supported by complementary biophysical methods (e.g. surface plasmon resonance (SPR) and SEC-MALLS), to fully characterize the stabilities, complex heterogeneities and binding dynamics (e.g. kinetics, affinities) of the protein complexes (months 12 – 24)

4. Utilize the approaches developed in 2&3 to investigate the influence of agents known to disrupt the expanded repeat transcript-protein interactions (months 20 – 33)

The proposed programme of research is thus highly multidisciplinary, providing excellent training in both state-of-the-art single molecule biophysical methods, complementary biophysical analytical techniques and molecular genetic approaches.

Lab Rotation Location: University Park;

Full Project Location: University Park;

147 Understanding uPAR receptor structure and interactions to support therapeutic targeting

Lead Supervisor: Jonas emsley

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: Protein crystal structure determination/ protein interactions / modelling of uPar receptor complexes (Lead Emsley).

The uPAR polypeptide is 283 amino acids in length and is linked to the cell surface through a carboxy-terminal glycosylphosphatidylinositol (GPI) anchor. It has been shown that in resting conditions, both uPAR and gC1qR are expressed on cell surfaces at low levels. Following cell activation, uPAR and gC1qR are released and both proteins locate to lipid rafts, previously shown to be critical for uPAR dimerization and cell signalling. The interaction of FXII and HK with uPAR has been previously characterised biochemically and direct binding was mapped at the N-terminal fibronectin type II (FNII) domain of FXII and Domain 5 (D5) of HK.

uPAR is well established as an important regulator of extracellular matrix (ECM) proteolysis, cell–ECM interactions and cell signalling. Though several studies have investigated the role of urokinase (uPA) and uPAR in lung function and explored their role as therapeutic targets, no studies to date have investigated the role of gC1qR-FXII-uPAR in mediating these effects or in respiratory disease progression in general. This represents the first time the gC1qR-FXII-uPAR-HK orchestrated crosstalk and cell signalling in lung epithelial cells will be thoroughly investigated.

Full Project Description:

Urokinase plasminogen activator receptor (uPAR) regulates activation of urokinase plasminogen activator (uPA), triggering the plasminogen/plasmin activation cycle and its extracellular proteolytic cascade. uPAR also exhibits functionality through non-proteolytic activity and interactions with co-factors such as Factor XII (FXII), kininogen (HK). This allows uPAR to stimulate signaling cascades and regulate diverse functions in different cells/tissues, including cytoskeletal dynamics, cellular adhesion, and cellular migration. These proteolytic and nonproteolytic cascades allow uPAR to play an active role in a number of pro-airway remodeling processes, such as extracellular matrix remodeling, cell migration, coagulation, cellular proliferation, release of inflammatory cytokines, and growth factor activation. We have described a complex involving uPar interactions with Factor XII (FXII), kininogen (HK) and gC1qR [PMID:29376892, PMID:29619369] and we have described part of this complex and determined the first crystal structure from this complex for gC1qR-FXII. We now aim to expand these studies to higher order complexes formed by the full-length proteins utilising protein crystallography ,alpha fold.

The uPAR polypeptide is 283 amino acids in length and is linked to the cell surface through a carboxy-terminal glycosylphosphatidylinositol (GPI) anchor. It has been shown that in resting conditions, both uPAR and gC1qR are expressed on cell surfaces at low levels. The interaction of FXII and HK with uPAR has been previously characterised biochemically and direct binding was mapped at the N-terminal fibronectin type II (FNII) domain of FXII and Domain 5 (D5) of HK. The primary goal is to determine the structure of the uPAR-FXII-HK complex using protein crystallography. The results from these structural studies on uPar will be linked to functional studies in the Sayers group with the aim of understanding uPAR in a variety of biological functions and disease processes [PMID: 19443020, PMID: 19878584, PMID: 26869673, PMID: 27624865].

This project aims to significantly extend our understanding of the structure/function of different complexes of uPAR to provide new understanding and oportunities. The project has three aims:

1. Use molecular modelling and structure determination (x-ray crystallography) to examine the structure of different complexes of uPAR bound to co-factor HK and FXII .

2. Use knockin of a tagged/labelled form of uPAR for use in experiments to identify the key co-receptors. (Sayers, Portelli)

3. Define the role of identified uPAR co-receptors in mediating epithelial functions outlined in 1. This will provide support for specific uPAR/co-receptor targeting. (Sayers, Portelli)

This project is ideally placed for a PhD student as it provides excellent training in functional genomics/cell biology/respiratory medicine (Portelli/Sayers) and protein structure/function/modelling (Emsley). The programme of work is multidisciplinary and spans two Schools at the University of Nottingham; Medicine and Pharmacy.

Lab Rotation Location: University Park;

Full Project Location: University Park;

150 Investigating the roles of T lymphocytes in foreign body response to biomaterials

Lead Supervisor: Jing Yang

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: The lab rotation project will allow students to experience various aspects related to the PhD project including learning transferrable skills. Students will carry out the following tasks:

1) Isolation of macrophages and T lymphocytes from human blood. Cultivation of macrophages and differentiation of these cells to functionally different subtypes. Students will learn aseptic in vitro cell culture.

2) Preparation of polymeric and hydrogel biomaterials and material characterisation. This task includes the use of 3D printing to fabricate biomaterials with different topographies and geometries.

3) Characterisation of cellular responses by using various cell and molecular biology techniques, such as ELISA, immunostaining, PCR, and imaging by optical microscopy.

Weekly meetings will be held with students to discuss their progress. Student will give presentations in weekly meetings on literature review and experimental results. Student will learn to formulate hypothesis in the context of existing literature, design and plan experiments to test the hypothesis. Various other transferal skills including data processing and statistics will be included in the rotation project.

Full Project Description: Giant strides have been made in healthcare by employing biomaterials in medicines, implants and medical devices. Advances in novel biomaterials have played critical roles in various biomedical applications ranging from lipid nanoparticles for mRNA vaccine delivery for Covid-19 to implants for total hip or knee replacement. However, how biomaterials interact with cells and other body components is still not fully understood. In particular, immune cells are first responders to foreign materials, and a myriad of activities at the molecular and cellular level happen immediately after implantation or internalisation of a foreign material. For biomaterials that have sizes significantly bigger than phagocytes, elimination of the biomaterials is impossible. Instead, a set of inflammatory and wound healing processes is induced by the presence of the biomaterial. At the end of these processes, macrophages and foreign body giant cells are present on the biomaterials, and a fibrous capsule is usually also formed around the biomaterials. This end stage of the inflammatory and wound healing processes associated with biomaterials is called Foreign Body Response (FBR). FBR is usually undesirable and can compromise or destroy the intended functions of the implanted biomaterials. For example, FBR can cause implanted glucose sensors being encapsulated by a fibrous capsule so that it can no longer correctly measure the blood glucose level. A tissue engineering scaffold that is intended for tissue regeneration and function restoration could be filled by fibrous scar tissues instead. Fibrotic response can separate bone from prostheses in total hip or keen replacement, which compromises implant integration and stability.

Research on the foreign body response to biomaterial implants has been focused on the roles of the innate immune system. Macrophages in particular have been found to be essential for FBR as demonstrated by various experiments including gene knockout animals that are deficient of macrophages. However, the role of lymphocytes in FBR is still largely obscure. In contrast to our ample knowledge in how immune cells in both the innate and adaptive immune systems respond to microbes (viruses, bacteria and fungi) in an integrated manor, our understanding on the orchestration between the two immune systems in response to implanted biomaterials is far less. Lymphocytes appear together with monocyte-derived macrophages in the chronic phase of the inflammatory and wound healing responses after biomaterial implantation. Therefore, their

interactions with and influences on macrophages as well as the effects of biomaterials on lymphocytes themselves must be considered in our efforts to understand FBR.

The aim of this project is to investigate how macrophages and T lymphocytes interact on different biomaterials using an in vitro co-culture system. The effects of the chemical and physical properties of biomaterials on immune cells will be systemically investigated. The underlying molecular mechanisms in immune cell-biomaterial interactions will be studied. Knowledge generated in this project will potentially lead to novel therapeutic targets for mitigating FBR.

Methods used in the project include isolation of monocytes and lymphocytes from human blood, in vitro cell culture, cell and molecular biology techniques for cellular characterisation, preparation and characterisation of biomaterials.

Lab Rotation Location: University Park;

Full Project Location: University Park;

154 Translated Circular RNAs in Health and Disease

Lead Supervisor: Keith Spriggs

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: Circular RNAs (circRNAs) are covalently closed loops of RNA that can arise in cells through backsplicing events. They were once assumed to be artefacts of splicing, but are now known to encode functional proteins, in addition to acting as microRNA sponges and decoys for RNA binding proteins. circRNA research is a rapidly growing field as the importance of these molecules becomes better understood. The stability of circular RNA forms in comparison with linear RNA also makes these molecules valuable to the biotech industry.

Recent analysis in our lab has identified a small number of novel candidate circular mRNAs where the peptide encoded by the circRNA is predicted to have a different function as that from the linear mRNA. The rotation project would clone and characterise these putative circRNAs. The questions we would like to answer are: how abundant are these circRNAs, and what is the effect of their expression on cell growth and behaviour? Students will develop skills in molecular biological and bioinformatic techniques, including cloning, in vitro expression, qPCR, mammalian cell culture, homology and ontology analysis.

Full Project Description: The PhD project will involve several parallel strands, with the intention of maximising the likelihood of success and allowing flexibility to adapt as data are acquired. Currently, the protein coding potential of circular RNAs has been somewhat neglected, despite good evidence that some circRNAs give rise to peptide products. The first part of the project will address this by characterising all the protein coding circRNAs in a transcriptome, involving the optimisation of a novel methodology. This will involve polysome profiling of cultured human cells, fractionation into polysomal (i.e. actively translated) and sub-polysomal (i.e. untranslated), treatment with RNase R to remove linear RNA, followed by RNA seq and/or quantitative rt-PCR. In this way only circRNAs will be sequenced, and those that are translated will be sequenced separately from those that aren't. The circular transcriptome will be characterised and the translation status of each circRNA determined. When the methodology has been optimised, a comparison between healthy cells and those exposed to oxidative stress can be made – several circRNAs have been shown to have roles in

the response to oxidative stress, and oxidative stress is a feature of several pathologies, including cancers.

The nature of the data generated means that a significant part of the project will require bioinformatic approaches, including RNAseq analysis, ontology analysis, comparison with other species, motif identification etc. This will involve the use of the command line in a linux-based environment, and, ideally, the application of python scripting (or similar). Some familiarity with these approaches would be helpful, no detailed knowledge is expected and training will be provided.

In parallel with the transcriptome-level approaches described above, there is also an opportunity to investigate individual circRNAs, with an emphasis on their roles in diseases and their treatments. In this part of the project, a small number of circRNAs will be selected from the data generated in the rotation projects, and from the literature. The sequences for these will be cloned into expression constructs for transfection into cultured human cells and the effects on cell viability, behaviour and morphology determined. Mutational analysis will allow a more detailed investigation of the functions of the circRNAs and their products.

The project will combine laboratory and computational approaches, and the student will gain expertise in key skills in both areas. The research will be conducted in a friendly, collegial laboratory in which six research groups share space and expertise – support is always at hand if necessary. We have experience in all the methods described, although this project represents the first time they have been combined in this way. There is a combination of safe and more risky approaches to balance the dual requirements of data to support a PhD thesis and the excitement of exploring genuinely new areas of the field. Although a project has been described above, there will be flexibility to deviate from this in response to the data as they are collected – input from the student is encouraged (and expected) in experimental design and in shaping the direction of the project.

Lab Rotation Location: University Park;

Full Project Location: University Park;

165 To cleave or not to cleave: ubiquitin signal editing in cancer

Lead Supervisor: Ingrid Dreveny

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: Rotation project:

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
- Generate a novel ubiquitinated substrate mimetic according to protein expression and purification protocols available in the lab
- Conduct binding assays between recombinant USP17 with the generated substrate

- Perform initial structural characterisation of the USP17-substrate complex using crystallisations.
- Data analysis

Full Project Description: Ubiquitination is a key modification that regulates the function and levels of proteins in eukaryotes thereby controlling virtually all cellular processes such as DNA damage repair, cell cycle regulation, and the immune response. Ubiquitin specific proteases (USPs) can rescue proteins from destruction by the proteasome by reversing ubiquitination. The human genome encodes 56 USPs and dysregulated de-ubiquitination is associated with cancer, neurodegenerative disorders, and the host's response to infection. However, we still do not understand how these proteases specifically recognize their substrates: Thousands of human proteins have been shown to be ubiquitinated and the largest binding pocket in USPs accommodates the ubiquitin marker common to most substrates. This not only has major implications for our fundamental understanding of how these proteases select their target but also for drug discovery efforts. Modulation of USP function offers valuable avenues for therapeutic intervention and biotechnological applications.

The main aims of this project are: (1) to test the substrate scope of closely related USPs using substrate mimetics (2) to determine key structural features that are responsible for substrate recognition and (3) to confirm their importance by mutagenesis to ultimately test how they modulate USP function in cancer cells. We will focus on USP17, a cytokine-inducible ubiquitin specific protease that plays a key role in the carcinogenesis and progression of different types of cancers due to its regulation of transcription factors such as c-Myc and Elk-1. The project will consist of the following parts: (a) Production of available and novel ubiquitin fusion substrates relevant to cancer that will be used to probe the specificity of USP17 and related enzymes in binding and enzymatic assays using established protocols. (b) Elucidation of molecular basis of substrate-USP interactions using complementary state-of-the-art structural biology techniques including NMR, X-ray crystallography or cryo-electron microscopy. (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 and exciting preliminary data available in the lab.

Together, the project will offer skill development in an interdisciplinary setting including biochemistry, structural and biophysical techniques and will deliver novel insights into ubiquitin specific protease structure, function in cellular pathways relevant to cancer and potential for selective inhibition.

Lab Rotation Location: University Park;

Full Project Location: University Park;

173 KAT6 Proteins : Unique Gene Regulators and Metabolic Sensors

Lead Supervisor: David M Heery

Lead School: Pharmacy

DTP Research Area: Biotechnology

Lab Rotation Description: You will join an active project in which we are conducting expression purification of N-terminal KAT6A and KAT6B domains involved in DNA and chromatin binding for structural analysis. This is an opportunity to work with a PhD task supervisor to learn protein

purification via FPLC affinity tag and size selection, EMSA assays, subcloning and site-directed mutagenesis. Alternatively (or in parallel) you would also have an opportunity to work with our Senior Research Officer to use confocal microscopy to study YFP-tagged KAT6A proteins in cells, and their response to metabolic challenge, thus learning cell culture and transfection techniques.

Full Project Description: KAT6A and KAT6B are human lysine acetyltransferases that regulate gene expression programs in stem cells and during development. These proteins acetylate histones and thus modify chromatin to regulate gene expression. Mutations in the KAT6A/B encoding genes are associated with neurodevelopmental disorders in children. These patients have a range of symptoms including metabolic disturbances (https://kat6a.org) but the underlying mechanisms are poorly understood. Treatment with dietary metabolic supplements has been reported to some improve symptoms, but larger studies are needed.

We used CRISPR Cas9 to delete Kat6A in cell lines or to generate mutations mimicking those found in patients. RNA Seq and metabolomics analysie of these cell lines revealed alterations in expression of cell growth and metabolism genes and changes in cellular metabolites, consistent with clinical studies.

Our group also investigates the structure and function of KAT6A/B proteins. We have shown that the N-terminal half of KAT6A/B contains gene regulatory functions including DNA binding, histone binding and histone acetylation domains (Deeves et al., 2014; Costello-Heaven et al, in preparation). However, almost nothing is known about the function of the C-terminus, which is deleted in most patients.

This project will investigate our novel hypothesis that KAT6A/B proteins act as metabolic sensors to alter gene expression in response to metabolic stress. We suspect this involves a sequence in the C-terminus we call the Met domain, which causes KAT6A/B to undergo soild-liquid phase transitions. This is a recently discovered property of some proteins that enables them to aggregate and dissociate in response to metabolic signals, controlling function. A similar domain was reported in a yeast protein, but not yet in humans.

The project will undertake structure/ function studies of KAT6A/B Met Domains, by subcloning them into a range of expression vectors, in collaboration with scientists at Diamond Light Source (DLS). We will perform in vitro assays with purified proteins to study aggregation properties of the Met domains in response to metabolic stresses, e.g. treatment with H2O2. We will use Met domain fusions to other proteins as a biotechnological tool to test if we can regulate their activity in cells. If sufficient quantities of pure Met domain are produced, DLS will assist with structural or biophysical analyses (crystallisation or small angle scattering SAX). Site-directed mutagenesis of the Met domain in YFP-tagged KAT6A/B proteins will enable functional studies in cells e.g. RTqPCR of known target genes, confocal microscopy. CRISPR editing of the endogenous KAT6A/B genes to delete the Met domain may also be performed to assist functional studies.

If we confirm the Met domain promotes phase transitions, we will explore its biotechnological applications as a metabolic sensor. Met fused to fluorescent markers (GFP, Halo, NanoLuc) will aid proof of concept of studies for phase transition in response to metabolic stress (H2O2, nutrient starvation, hypoxia etc).

We would be happy to welcome a smart and strongly motivated student preferably with some experience in molecular and cell biology, or any of the techniques described above to join our team to participate in these exciting projects.

Lab Rotation Location: University Park;

Full Project Location: University Park;

175 Investigating heterogeneous structures of RNA and RNA-binding proteins using AFM-guided molecular simulations

Lead Supervisor: Naoto Hori

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation Description: Atomic force microscopy (AFM) is a powerful tool for investigating the structures and properties of biomolecules. However, the measurements still have limitations in time and space resolution. Molecular simulation is a valuable technique to complement such experiments and gain a comprehensive view of molecular structures and dynamics.

In this lab rotation, the student will first learn molecular simulation techniques using a recently developed coarse-grained RNA model and then attempt to evaluate several methodologies by which AFM data can be incorporated into simulations.

Plan:

Weeks 1-4. After learning the basic theory of molecular simulations, the student will conduct simulations of several (CUG) repeat RNA constructs and analyse the results to see the structural diversity. Based on the simulation data, pseudo-AFM images will be generated and compared to actual AFM data previously obtained in Prof Allen's lab.

Weeks 5-9. Further work will be done to optimise appropriate modelling of virtual AFM stages and tips in molecular simulations. These set-ups allow the RNA molecule to be actively guided during the simulation to structures that fits the AFM image.

The rotation student will be supported by experienced postgraduate and postdoctoral fellows in addition to the supervisors.

Full Project Description: Background

RNA molecules and their complex with RNA-binding proteins (RBPs) can aggregate or condense intracellularly, causing a variety of functions and toxicities. RNA molecules are flexible and capable of forming various structures by complementary base pairs, acting as intermolecular glue with other RBPs.

Myotonic dystrophy (DM) is a trinucleotide repeat expansion disease; DM patients have very long CTG repeat mutations in their genes. The long transcript RNAs (CUG repeats) remain in the nucleus and sequester some essential proteins, such as MBNL1, which is suspected to be associated with the disease. However, the details of the pathogenetic mechanism are unknown due to a lack of structural information and understanding of RNA-protein interactions at the molecular level.

Prof Allen and Prof Brook's groups had a previous PhD project on AFM structural observations of CUG repeat RNAs (Meullenet (2009)). The preliminary analysis of AFM images indicated that CUG repeat RNAs form a variety of secondary and tertiary structures. However, due to the limited resolution, only the rough shapes of the RNA molecule could be revealed. It was also not clear how

many MBLN1s were bound and where. In addition, the AFM images were obtained in air, so might not be biologically representative. They, therefore, did not provide a complete understanding of how the structure changes and how it is dynamically conjugated with protein binding.

Aim:

This PhD project will elucidate the diverse structures formed by repeat RNAs in the absence and presence of the MBNL1 protein and how the number of RNA repeats, a critical variable that separates patients from healthy individuals, affects this complex structure. To this end, the project will combine the state-of-the-art RNA molecular simulation model recently developed by Dr Hori and colleagues, Prof Allen's AFM data and expertise, and Prof Brook's long-standing knowledge of CUG repeat RNA and disease association studies.

Plan:

1. The project will initially attempt to verify whether simulated structures of the CUG repeat RNA are consistent with experiments. Structural sampling simulations will be conducted at various repeat numbers and RNA concentrations, and the structural characteristics and degree of aggregation will be verified by comparing them to AFM images.

2. The interactions between the MBNL1 protein and CUG repeat RNA will be modelled, and the protein will be introduced into the simulation system. Data from surface plasmon resonance experiments may be used to design the strength of the interaction. A key question to be answered will be how the presence or absence of the protein affects the ability to form aggregates, their shape, and physical properties. The critical repeat number will be investigated.

3. Small molecules and oligonucleotides that inhibit CUG repeat RNA secondary structure formation and consequently prevent MBNL1 sequestration have been proposed as therapeutic agents for DM. The project will be further developed to see how these drug candidates could inhibit RNA loci formations, by introducing the drug effects implicitly and explicitly into the simulation model.

Lab Rotation Location: University Park;

Full Project Location: University Park;

183 Exploring the interplay of micro, circular and long non coding RNA with the transcriptional regulators KAT6a and KAT6b

Lead Supervisor: Hilary Collins

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation 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. In the rotation project we 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.

Full Project Description: KAT6a and KAT6b are histone acetyl transferases which control numerous pathways involved in development and stem cell function and have been implicated in 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 (lncRNAs) and circular RNAs (circRNAs).

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 circRNAs and lncRNAs but the extent of these interactions is unknown. 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. LncRNAs 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 lnc RNA interacting partners.

KAT6acircRNA has also been identified in prostate cancer. We would also aim to clone and characterise this circRNA. The questions we would like to answer are: how prevalent are these circRNAs and do circKATb RNAs exist? What effect does expression of these circRNAs have on cell growth and behaviour?

Three questions

The expression of which miRNAs are affected by KAT6a and Kat6b?

Do lncRNA and circ RNAs interact with KAT6a and Kat6b proteins and modulate their function?

How prevalent are KAT6a and Kat6b circ RNAs and are they functional.

Lab Rotation Location: University Park;

Full Project Location: University Park;

193 Deciphering the structure-activity profile of darobactin that selectively targets Gram-negative pathogens

Lead Supervisor: Weng Chan

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation 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 colistin.

Colistin is the antibiotic of last resort for treating Gram-negative infections, especially those caused by Pseudomonas aeruginosa and Acinetobacter baumannii. This is a focused study to determine the antimicrobial activity of colistin against several pathogenic P. aeruginosa strains, including colistin-resistant strains, using both MIC assays and our recently established dose-response studies.

Aim 3 (week 4-9): To establish total chemical synthesis of the linear precursor (H-Trp-Asn-ß-hydroxyTrp-Ser-Lys-Ser-Phe-OH) of darobactin.

Full Project Description: Antimicrobial resistance (AMR) poses a global threat to human health. In fact, many of the bacterial pathogens are now resistant to several classes of antibiotics. In a recent analysis, it was estimated that over 1.2 million deaths are directly attributable to bacterial AMR in 2019. Infections caused by the Gram-negative pathogens, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii and Pseudomonas aeruginosa were the major contributors to AMR-associated deaths. Hence, novel antimicrobial agents with unique modes of action are desperately needed to treat infections caused by multidrug-resistant (MDR) Gram-negative pathogens.

Darobactin, a structurally complex antibiotic isolated from a nematode symbiont Photorhabdus khaini, displays uniquely potent antimicrobial activity against many Gram-negative pathogens, including P. aeruginosa, E. coli, K. pneumoniae, as well as their corresponding colistin-resistant strains. By targeting BamA, the mode-of-action (MOA) of darobactin is novel – BamA is an outer membrane (OM) ß-barrel protein responsible for the catalytic folding and insertion of new proteins into the bacterial OM. Given the unique MOA, it is not surprising that darobactin is inactive against Gram-positive organisms.

Darobactin is biosynthetically derived from a heptapeptide, H-Trp-Asn-ß-hydroxyTrp-Ser-Lys-Ser-Phe-OH, which is post-translationally modified to afford the side-chain:side-chain-mediated bicyclic structure. This unique modification is facilitated by radical SAM enzyme(s) that catalyse(s) the formation of both the Trp-Lys C-C bond and the Trp-ß-hydroxy-Trp C-O-C ether bond.

The objective of this studentship proposal is to apply chemical biology approaches to decipher the structure-activity profile of darobactin. This would be achieved by utilising solid-phase peptide synthesis approaches and asymmetric chemical synthesis of amino acid building blocks. Chemically re-engineered analogues of darobactin will be utilised to test structure-activity models, which could lead to the identification of novel pre-clinical lead compound(s) for the treatment of infections caused by MDR Gram-negative pathogens. The overall objectives would be achieved by prosecuting the following work packages:

WP1: To establish synthetic methods for the manufacture of darobactin

Two approaches will be explored: (a) assembly of appropriate functionalised linear precursor heptapeptide followed by catalytic C-C and C-O-C bonds formation, and (b) asymmetric synthesis of

pre-formed (and appropriately protected) amino acid building blocks, followed by peptide assembly. Both chemical and biological catalysts will be explored. We have previously established robust and scalable chemical methods for the synthesis of key building blocks and diverse argyrin analogues, including novel Trp analogues. The experimental data from this study will enable the SAR profiling and the design novel darobactin analogues outlined in WP2.

WP2: To design and chemical synthesis of novel darobactin analogues

The chemical architecture of darobactin is novel and hence lends itself to extensive and unique modifications. Initially, we will focus on structural/chemical changes in the ß-hydroxyTrp and Lys residues.

WP3: To evaluate the antimicrobial potency and scope of selectivity

A detailed antimicrobial activity of darobactin and analogues thereof against several pathogenic P. aeruginosa, including colistin-resistant strains, will be determined using both MIC assays and our recently established dose-response method. The antimicrobial activity against a wide range of both Gram-negatives and Gram-positives will also be evaluated to determine the scope of specificity.

Lab Rotation Location: University Park;

Full Project Location: University Park;

196 Combining CRISPR/Cas9 gene editing with proximity proteomics (Bio-ID) to map adenosine receptor interactomes in human cells

Lead Supervisor: Laura Kilpatrick

Lead School: Pharmacy

DTP Research Area: Bioscience for Health

Lab Rotation 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, CRISPR gene editing, and confocal microscopy. 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 editing experiments appending a short 11 amino acid luminescent tag to the C-terminus of 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. This work will involve the design of CRISPR reagents, delivery to cultured cells (via electroporation), and downstream screening for edited cells via PCR, bioluminescence assays, and confocal microscopy. The student will also be trained in microscopy image analysis and relevant statistical methods. All of these techniques will be 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.

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 will biotinylate interacting proteins within a ~ 30 nm radius, which can be subsequently identified via mass spectrometry and bioinformatic analysis to profile the context specific interactomes for these signalling receptors. These protein 'hits' will be validated in living cells using a combination of CRISPR/Cas9 gene editing and luminescence-based proximity assays. This will be aided by AR subtype specific fluorescent ligands and covalent ligand labelling strategies we have developed in our group.

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, with each component expressed on a different AR subtype. This will allow the determination of heteromeric specific interacting partners and comparison between monomeric signalling 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 in this manner has the potential to identify novel drug targets and therefore 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 project.

Lab Rotation Location: QMC;University Park;

Full Project Location: University Park;QMC;

Psychology

9 Evolution and plasticity of human cognition

Lead Supervisor: Deborah Serrien

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation 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

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.

Lab Rotation Location: University Park;

Full Project Location: University Park;

15 Hippocampo-prefrontal-subcortical circuit in cognition and behaviour

Lead Supervisor: Tobias Bast

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation 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, MedAssociates, 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.

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 functions integrated within this circuit. Consistent with this, dysfunction within this circuit has been implicated in key cognitive and behavioural impairments characterizing neuropsychiatric disorders (Bast, 2011, Curr Opin Neurobiol; Bast et al., 2017, Brit 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 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 and schizophrenia (Bast et al., 2017, Br J Pharmacol). How do such imbalances affect distinct cognitive and behavioural functions? 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 pharmacogenetic methods for neuron-type specific manipulations (which we are currently setting up).

Lab Rotation Location: University Park;

Full Project Location: University Park;

21 Cognitive contributions to interbrain synchrony

Lead Supervisor: Sobana Wijeakumar

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation Description: The rotation will focus on activities necessary to develop skills for the PhD project: (1) to learn how to re-create previously published executive function experimental tasks in PsychoPy, (2) to learn how to collect EEG data in single-participant and dual-participant studies using portable EEG systems, and (3) to learn how to analyze single-participant and dual-participant EEG data using relevant softwares (Fieldtrip and Matlab).

Full Project Description: How, why, and when do two individuals sometimes 'fall into sync' or 'out of sync' when they interact with each other? To answer this, we need to understand what 'synchrony' means. Within the context of social interactions, synchrony is defined as the spatial and/or temporal coordination of biological and behavioural systems between individuals. In simple terms, it refers to the phenomenon of a process working in a similar manner in two individuals interacting with each other. For example, previous work has shown alignment of heart-rate rhythms between mothers and their babies. More recently, neuroimaging techniques have demonstrated that some brain areas synchronize in individuals engaging in joint social interactions (referred from here on as interbrain synchrony). For example, co-operative behaviour between adults or adults and children when playing a computer game elicits interbrain synchrony. Interbrain synchrony is also linked to longer gaze, positive affect and greater engagement and learning in individuals interacting with each other.

A few computational accounts have been proposed to explain interbrain synchrony. One view proposes that synchronization can be explained by reducing prediction error between the 'actual' state/representation in one brain, and the other brain's 'predicted' state. Another view proposes that synchrony emerges through alignment, transformation, and synergy of information between a 'receiver' and a 'sender'. These accounts are informative; however, they adopt a simplified view of interbrain synchrony and do not shed sufficient insight on contributions from key cognitive processes that impact the emergence and adaptability of this phenomenon.

This field has been met with criticisms (2 references provided below) because most previous work has not developed experimental designs with appropriately controlled conditions. Critically, several questions that stem from a cognitive basis of inquiry remain unanswered in the field. First, does greater synchrony during social interactions simply represent common and efficient coordination of brain regions important for executive functions such as working memory to keep common tasks, goals, rules in mind, inhibitory control for supressing irrelevant information and cognitive flexibility to shift between goals? Second, are the spatial and temporal properties of interbrain synchrony dependent on the type of joint interaction tasks? Third, does interbrain synchrony only materialize in the presence of multimodal engagement (similar gaze, speech and tactile information between individuals)? Fourth, are there different types of synchrony i.e., moment-to-moment temporal coordination of brain processes between partners, creation of a unique network of brain regions to process a specific goal between partners; similar alignment between behaviours and then, brain processes between partners. By answering questions such as

these and others, the proposed project aims to develop a cognitive model of interbrain synchrony. To achieve this aim, this project will employ multiple carefully designed experimental paradigms with controls alongside using portable dual-EEG systems in adults during social interactions.**Lab Rotation Location:** University Park;

Full Project Location: University Park;

30 The aging GABAergic system and human semantic memory

Lead Supervisor: JeYoung Jung

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation Description: GABA as measured with MRS has been linked to clinical and cognitive outcomes. Alterations of GABA levels are seen in neurodevelopmental/neurodegenerative/neurological/psychiatric disorders. GABA has been associated with various cognitive function including memory and learning. Given the functional relevance of GABA in the context of both pathological and healthy cohorts (especially in the context of development and aging), understanding how GABA changes with age in a healthy cohort is important to understand 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 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 ageing.

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.

Full Project Description: The overall objective of the PhD project is to derive novel insights into the neurochemical basis of semantic memory in healthy ageing and to extend 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 inhibitory neurotransmitter in the brain, GABA plays a critical role in regulating responsiveness and excitability within neural networks and in synchronizing cortical neuronal signaling activity. GABA is 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 plays a significant role in aging and in neurodegenerative disorders, including dementia.

Semantic memory, of the meanings of words and properties of objects, shapes our understanding of the world and guides our behaviour. 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 memory 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 remain unclear how these functional changes in semantic system are linked to the GABAergic activity in ageing.

This PhD project investigates the GABAergic systems underpinning human semantic memory 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 neurochemical and behavioural level. This project will answer two key questions in cognitive neuroscience: (a) How does 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 activity in key regions of semantic memory; (b) to combine this methodology with the use of other neuroimaging (fMRI) and psychological experimental paradigms to link GABAergic 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 (transcranial magnetic stimulation).

This project will advance understanding of neurotransmitter systems underpinning semantic memory and their role in ageing. This work will contribute to 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) and software packages such as PsychoPy, SPSS, and/or Matlab. A basic understanding of neuroanatomy and cognitive neuroscience is expected.

Lab Rotation Location: University Park;

Full Project Location: University Park;

41 Investigating the causal role of brain rhythms for cognitive flexibility in the ageing brain

Lead Supervisor: Nicholas Myers

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation 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

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 aging 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 behavior. 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 aging. By training participants

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 aging 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 precise 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.

References

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2. Hodkinson, D., Jackson, S., & Jung, J. (2022) Task-dependent plasticity in distributed neural circuits after transcranial direct current stimulation of the human motor cortex: A proof-of-concept study. Front. Pain Res., Sec. Non-Pharmacological Treatment of Pain https://doi.org/10.3389/fpain.2022.1005634

Lab Rotation Location: University Park;

Full Project Location: University Park;

54 Investigating how modulating brain networks can be used to improve mental/brain health

Lead Supervisor: Stephen Jackson

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation Description: Mental health disorders are the single largest cause of disability in the UK, affecting 1 in 4 people with an estimated cost to the economy of £105bn per year. Demand for mental healthcare currently exceeds available NHS resources, particularly for children and young adults. Innovation is needed to transform healthcare delivery for mental/brain health conditions and to widen access to safe and effective treatments.

Neuromodulation has proven efficacy for mental/brain health conditions and is often more effective that drug or behavioural treatments. Biotechnology can offer more effective, accessible, personalised interventions, including novel treatments based upon the next generation of neuromodulation approaches.

In this rotation, students will receive hands-on training in current neuromodulation approaches based upon non-invasive brain stimulation, including neuronavigation-guided transcranial magnetic stimulation, transcranial electrical stimulation, and peripheral nerve stimulation. There is also an opportunity to carry out an industrial placement in a MedTech company developing wearable neuromodulation devices.

Weeks 1-3: Training in the use of neuronavigated transcranial magnetic stimulation techniques.

Weeks 4-5: Training in transcranial electrical stimulation techniques.

Week 6: Industrial placement with Medtech company, Neurotherapeutics Ltd

Week 7-9: Training in peripheral nerve stimulation techniques.

Full Project Description: The pathophysiology of mental health conditions are very often associated with alterations in brain network dynamics, including cortical-subcortical brain circuits such as the cortical-striatal-thalamic-cortical (CSTC) brain circuits implicated in conditions such as Parkinson's disease [PD], Obsessive compulsive disorder [OCD], and Tourette syndrome [TS]. Furthermore, modulation of these CSTC networks using deep brain stimulation [DBS] has been shown to be highly effective in treating all of these conditions. However, DBS is an invasive surgical procedure that

carries a number of risks and not available to the majority of patients, and is not suitable for use with children and adolescents. Furthermore, we do not have a mechanistic understanding of how DBS alters pathophysiology. DBS may alleviate clinical symptoms, (a) during stimulation - by disrupting established abnormal network dynamics, and (b) post-stimulation - by triggering brain plasticity mechanisms.

Non-invasive brain stimulation offers an attractive alternative to DBS and to pharmacological or psychological clinical therapies. Repetitive trains of non-invasive transcranial magnetic stimulation [rTMS] have been shown previously to: entrain brain oscillations during stimulation; alter brain excitability for sustained periods after stimulation; enhance learning and plasticity; and, alter local concentrations of the main excitatory (glutamate) and inhibitory (GABA) neurotransmitters. However, the effects of rTMS can be variable across individuals and our mechanistic understanding of precisely how rTMS can be used to achieve clinical benefits is poor.

Rhythmic electrical stimulation of the peripheral nervous system can also be used to entrain cortical oscillations, and we have demonstrated that rhythmic electrical stimulation of the median nerve can be used to entrain cortical sensorimotor neural oscillations associated with the initiation of movements, and is highly effective in reducing motor and vocal tic frequency, tic intensity and the urge-to-tic in individuals with TS. There remains however a considerable gap in our understanding of the precise mechanisms that underpin successful neuromodulation. In addition, it is highly likely that a better mechanistic understanding of precisely how brain stimulation can alter brain network function will lead to more effective, personalised, therapeutic approaches.

The objectives of this PhD are to: (a) develop a clearer mechanistic understanding of how peripheral nerve stimulation (PNS) can be used to modulate brain network dynamics; and (b) determine more specifically whether the potential clinical effectiveness of PNS depends solely upon rhythmic stimulation, and whether the effectiveness of this type of stimulation can be improved if the timing of the delivery of stimulation is optimised for each individual, relative to ongoing physiological events within their brain. Specifically, current clinical neuromodulation approaches typically apply an open-loop, one-size-fits-all approach, in which stimulation parameters are common across all participants and are not dependent upon the physiological events occurring in the brain. One consequence of this type of approach is that there can be substantial variability in the effects of stimulation across individuals. We will investigate whether the effectiveness of PNS can be improved, and its variability diminished, if stimulation is instead time-locked to physiological events (e.g., the phase of targeted EEG oscillations) within the brain (i.e., closed-loop triggering of stimulation).

Lab Rotation Location: University Park;Jubilee Campus;

Full Project Location: University Park;

56 The striatum as a recurrent neural network

Lead Supervisor: Mark Humphries

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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.

Lab Rotation Location: University Park;

Full Project Location: University Park;

90 Prelude to an error: investigating neural and behavioural signatures to predict and prevent errors

Lead Supervisor: Domenica Veniero

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation Description: As the planned PhD project requires combining different neuroscience methods, the rotation will include training on basic and advanced electrophysiological (EEG) 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).

Full Project Description: Everyone commits errors in everyday life, ranging from typing the incorrect keys on a keyboard or pressing the wrong button on a machine to making the wrong decisions with far ranging consequences. In some workplaces (for instance, in air travel or train traffic control), committing a mistake can even have fatal consequences. Therefore, people spend a lot of effort and resources in preventing errors, but this is not always possible with technical solutions. Thus, it would be extremely beneficial if human errors could be predicted, and thereby prevented before they happen. However, while many studies have investigated the neural and behavioural changes following an error, indicators that could help to predict errors are not very well investigated yet. The aim of this project is twofold: 1- to identify the brain activity that predicts an error using EEG (error-predicting signal) and 2- to interfere with this brain activity using brain stimulation in an attempt to prevent the error from happening.

The project is organised in two phases.

Phase 1 – identify. The first step will be to develop and administer a behavioural task that will allow us to study errors in a lab environment while we record EEG. The aim is to identify the error-predicting signal, an EEG activity that can predict people's performance, and that can differentiate correct and incorrect responses. We will look for this EEG signature in two different time windows: the time that immediately precedes the response and the time that precedes the presentation of the stimulus to which participants are asked to respond. This will allow us to understand if the error can be predicted by how we perform the task itself or by the internal status of the brain just before we start the task. We will also investigate if and how the error-predicting signal is associated with error awareness, i.e., the conscious realisation that an error has been committed.

Phase 2- interfere. At this stage, we will use Transcranial Magnetic Stimulation (TMS), a non-invasive brain stimulation technique, to interfere with brain activity. TMS can be used to briefly and transiently interact with brain activity. The idea is to monitor the EEG signal during the same task used in phase 1 and to trigger the TMS every time that the error-predicting signal emerges. Depending on phase 1 results, the aim will be to either disrupt the activity that leads to error commission or to push the brain activity towards the state that is associated with a correct response. We will be able to investigate what happens when the TMS is applied in terms of changes in behavioural performance but also in the EEG signal itself.

Lab Rotation Location: University Park;

Full Project Location: University Park;

94 Linking visual field maps in the human brain to performance on visual task.

Lead Supervisor: Denis Schluppeck

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation Description: Developing perceptual tasks that probe the functional maps of the visual system

It has been proposed that anatomically distinct and functionally independent processing streams exist in primate cortex (e.g. Goodale & Milner, 1992; Milner & Goodale, 1995; Mishkin et al., 1983; Ungerleider & Mishkin, 1982) for encoding different types of visual information. For example, the dorsal stream originates in primary visual cortex (V1), passes through V5/MT and terminates in parietal cortex. It is thought to play a major role in processing the overall (global) motion of objects, spatial cognition and visual motor planning. The ventral stream also originates in V1 but passes through V4 before terminating in the temporal lobes. Tasks mediated by the ventral stream include global shape perception, visual memory and object recognition. However, much uncertainty remains concerning the most appropriate stimuli to use to probe the properties of these processing streams and constituent visual areas in human vision.

In this rotation we will develop a battery of visual tasks that probe the functional maps of the visual system. The rotation will provide experience in calibrating displays for human vision experiments, experimental design, programming psychophysical tasks (e.g. PsychoPy, Matlab, Psychtoolbox), statistical analysis and visualising behavioural data.

Full Project Description: Functional magnetic resonance imaging (fMRI) provides a powerful tool for measuring how the human brain represents visual space. Throughout the visual pathway, there is an orderly representation of the external world on the cortical surface – forming maps of space. For example, in the primary visual cortex (V1), there is a retinotopic map of the position of elementary visual features, e.g. edges. Downstream areas of V1 utilise this information to form maps of more complex attributes of visual input, e.g. global shape of objects, their motion, colour, and so on.

A recent, large cohort-study (Himmelberg et al, 2021) reported that the visual field maps in different areas of the brain, obtained with brain imaging, are remarkably consistent between individuals, but only when individual differences in anatomy are taken into account. We will use this normative dataset to investigate if the perceptual performance of a new sample of participants varies in a

systematic and predictable manner based on their individual functional maps and anatomy. In the PhD project, we will design a battery of behavioural experiments that rely on different visual areas.

We will use psychophysical methods to accurately quantify individual perceptual performance which can be used to link to individual variations in brain maps. To probe the functional organisation of the visual system, we will use functional magnetic resonance imaging (fMRI) at 3T in combination with anatomical and diffusion-weighted scans. This will allow us to match fMRI maps of visual cortex to behavioural phenotypes to capture the inherent variation that exists in the normal population.

This approach will provide an individual signature that will help us understand the functional consequences of map differences, which then may be applied to cases where maps in distinct brain areas are disordered. A common example would be children and adolescents who have suffered brain damage early in their development. This type of vision loss, called cerebral visual impairment (CVI), is the leading cause of childhood sight loss in the developed world.

Himmelberg MM, Kurzawski JW, Benson NC, Pelli DG, Carrasco M, Winawer J. Cross-dataset reproducibility of human retinotopic maps. Neuroimage. 2021 Dec 1;244:118609. doi: 10.1016/j.neuroimage.2021.118609. Epub 2021 Sep 25. PMID: 34582948; PMCID: PMC8560578.

Wandell BA, Dumoulin SO, Brewer AA. Visual field maps in human cortex. Neuron. 2007 Oct 25;56(2):366-83. doi: 10.1016/j.neuron.2007.10.012. PMID: 17964252.

Lab Rotation Location: University Park;

Full Project Location: University Park;

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

Lead Supervisor: Claire Gibson

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation 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.**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.

Lab Rotation Location: QMC;University Park;

Full Project Location: QMC;University Park;

146 Brain structure and function in patient populations

Lead Supervisor: Christopher Madan

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation Description: In this rotation you will learn more about computational methods for analysing brain structure and function. With a small sample of data for healthy and patient individuals you will use these some of these methods and learn to interpret the findings. The specific patient population can be chosen by the student based on existing data, including Alzheimer's disease, Parkinson's disease, major depression disorder, schizophrenia, autism, and ADHD, among other options.

Full Project Description: While everyone's brain structure is unique, consistent group differences can be found when comparing patient samples to healthy controls. In this project we will use existing MRI data to examine differences in brain structure between groups. Target patient group will be determined based on data availability and students' interest. Options include Alzheimer's disease, Parkinson's disease, major depression disorder, schizophrenia, autism, and ADHD, among others. In addition to conventional measures of brain structure (e.g., cortical thickness, subcortical volume), novel approaches such as fractal dimensionality and sulcal morphology will also be compared.

Analayses and training will involve Matlab and/or Python and software packages such as FreeSurfer and SPM. Many analyses will use high-performance computing cluster facilities. An aptitude for programming will be a strong asset. A basic understanding of neuroanatomy and cognitive neuroscience is expected.

Lab Rotation Location: University Park;

Full Project Location: University Park;

171 Multimodal brain networks in cognition

Lead Supervisor: Roni Tibon

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation Description: In the lab rotation, the student will evaluate data obtained from an open data archive (NIMH: https://openneuro.org/datasets/ds004215/). 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.

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: http://www.cam-can.org/, NIMH: https://openneuro.org/datasets/ds004215/, and HCP: https://www.humanconnectome.org/study/hcp-young-adult/document/500-subjects-data-release) which contain multimodal functional neuroimaging data (namely, fMRI and MEG). To combine these data, several approaches will be explored, including methods based on multilayer 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.

Lab Rotation Location: University Park;

Full Project Location: University Park;

172 The role of melanopsin-based photoreception in visual analysis

Lead Supervisor: Paul McGraw

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation Description: Melanopsin-based spatiotemporal visual integration

Bloch's Law and Ricco's Law describe fundamental physiological properties of the human visual system; capturing the ability of different classes of retinal receptor to integrate light over time and space. Bloch's law (1885) describes the relationship between the duration of a light and its perceived intensity. Bloch showed that the ability to detect a light is reached when the product of its duration and intensity is constant, revealing a time-intensity reciprocity. This relationship has never been measured using melanopsin-isolating stimuli.

Ricco's law (1877) - a spatial analogue of Bloch's law - established the relationship between the area of a stimulus and its perceived intensity. That is, it defines the ability of the visual system to integrate signals over space. The size of this integration zone (Ricco's areas) changes depending on the lighting conditions and the receptors types that are stimulated. We will measure Ricco's areas for the melanopsin-based visual pathway.

These experiments will produce novel data sets and provide experience of the following:

• The physiology of the visual system

• Visual psychophysical methods

• Mathematical modelling for silent substitution

• Radiometric calibration of multi-projector system for generating stimuli

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 photo-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. After-effects have a long history in vision science and have been fundamental in establishing the presence of visual mechanisms that encode basic properties of an 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 after-effects, 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 pattern and motion vision. [words 455]

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Lab Rotation Location: University Park;

Full Project Location: University Park;

179 Substrates of Recognition Memory in Mice

Lead Supervisor: Charlotte Bonardi

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation Description: Animal models are central to research into the neural basis of recognition memory. This project comprises a short series of experiments using the spontaneous object recognition (SOR) procedure, in which mice preferentially explore a novel object rather than one encountered before. SOR is routinely used to evaluate effects of brain manipulations on recognition; yet interpretation of performance is based heavily on human theorising, making translation of results problematic. Thus we argue for a different approach (e.g. Robinson & Bonardi, 2015), applying a well established theory of associative learning based on research on animals (Wagner's SOP model: Wagner, 1981) to performance on SOR tasks. This approach makes novel predictions about the mechanisms underlying performance on a number of variants of the SOR procedure, several of which have been confirmed. This PhD would involve conducting further tests of the model, and evaluating the underlying assumptions of this account of recognition memory performance. The thesis would employ mice in a series of experiments all involving variants of this standard SOR procedure.

This rotation involves behavioural training techniques, in vivo work with mice, use of experimental software to analyse behaviour, process and analyse data, and theoretical interpretation of results.

Full Project Description: Recognition memory is a fundamental cognitive process that declines with age. Animal models of recognition memory are thus central to research into the neural basis of recognition and its age-related decline. Such work typically uses the novel object recognition (NOR) task, in which a previously exposed item elicits less exploration than a novel one. Although NOR is widely used, theoretical interpretation of NOR performance is controversial. Translational theories of recognition are typically based on the human distinction between recollection and familiarity. This approach is not only relatively loose in its predictions, but is also being increasingly questioned on theoretical and methodological grounds. Thus we have argued (e.g. Robinson & Bonardi, 2015) for a different approach. We adopt an associative account of recognition memory based on animal work (SOP: Sometimes Opponent Process; Wagner, 1981) to explain performance on the NOR task. This model makes some clear and novel predictions about the mechanisms underlying performance on a number of variants of the NOR task, several of which have already been successfully tested. The aim of this PhD is to conduct a systematic test of this associative account of recognition memory in a series of experiments with mice.

Lab Rotation Location: QMC;University Park;

Full Project Location: QMC;University Park;

180 Neural underpinnings of driving behaviour

Lead Supervisor: Matias J. Ison

Lead School: Psychology

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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.

A key area of interest is related to the fact that visual search can be improved via training. 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.

Lab Rotation Location: University Park;

Full Project Location: University Park;

Veterinary Medicine & Science

7 Coronaviruses of UK carnivores

Lead Supervisor: Rachael Tarlinton

Lead School: Veterinary Medicine and Science

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The project will provide an introduction to PCR based detection of viral sequences from field samples and bio-informatics processing of sequencing data to retrieve viral genomes from metagenomics/transcriptomics data sets. Prior coding experience not necessary (we can get you started with the basics) but there will be both computer based and lab based work. If the candidate is interested

Full Project Description: Coronaviruses of wild animals display frequent species jumps into new species, most recently causing the COVID-19 pandemic. Our recent work monitoring UK carnivores and mustelids (weasels, stoats, badgers, foxes, otters) for spillover of SARS-2 will be continued and extended in this project. While so far SARS-2 has not been found in UK wildlife we have sequenced novel viruses in stoats but don't know much about these viruses apart from their genetic sequence. This group of viruses is prone to forming recombinant viruses in domestic animals (cats and dogs) with some of these crossing the species barrier sporadically into people (potential spill over into production animals is unknown). It likely that dog and cat viruses also infect many of these species but this has been little studied. This project will focus on screening existing sample banks (and extending sample collection with existing collaborators) for related alphacoronaviruses in UK carnivores and mustelids. The project will characterise the epidemiology of the viruses in their host species and interactions with other pathogens of concern such as bovine tuberculosis in badgers or avian influenza in otters and foxes. The project will also examinethe recombination and cross species transmission potential of these viruses. Further work may include cell culture experiments to characterise how recombination of canine and feline alphacoronaviruses occurs and expression of proteins from alphacoronaviruses to assess cross reactivity with SARS-2 in serology assays (which may complicate screening of wildlife for SARS-2)

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

22 Gene switches and treatment resistance in cancer

Lead Supervisor: Nigel Mongan

Lead School: Veterinary Medicine and Science

DTP Research Area: Bioscience for Health

Lab Rotation Description: You will join a friendly, welcoming, collegial, international, multidisciplinary team of scientists and clinical researchers to learn the skills required for a career in translational research in academia, pharma, clinical genomic diagnostics, government or consultancy.

We have identified a molecular mechanism involving covalent modification of RNA that enables cancer cells to evade existing cancer therapies. This mechanism has also provided insights into fundamental processes of gene regulation, transcription and translation. Therefore our team is using molecular approaches to target this mechanism to dissect out how covalent modifications of RNA, termed epitranscriptomic modifications, can change the behaviour of cells by altering how genes are regulated, and in turn how the transcripts are interpreted in the cell.

During your rotation you will gain experience of key skills including bioinformatics, molecular biology, and functional studies using 2D and 3D cell line models systems and patient derived organoids. You will work alongside team members to examine the expression of components of this cellular machinery in patient specimens using immunohistochemistry. You will gain experience in relating protein expression to clinical data, and using this knowledge to understand the biology of disease. You will gain experience of molecular and pharmacological tools to better understand the potential therapeutic relevance of this mechanism.

Full Project Description: It is over 60 years since Francis Crick defined the central dogma of life, that DNA is transcribed to RNA, which in turn is translated into protein. This overlooks those complex processes that regulate selective exon utilisation during transcription and translational regulation and that are essential for both normal cellular function and are often deregulated in many diseases, notably cancer. Indeed the last two decades have revealed the complexity of epigenetic (how DNA is packaged in chromatin) and epitranscriptomics (how RNAs are covalently modified) mechanisms that underpin Crick's central dogma.

This project will investigate the convergence of epigenetic and epitranscriptomic mechanisms in treatment resistance in breast and prostate cancer. These cancers are the most common cancers affecting women and men respectively and are driven by the estrogen and androgen steroid hormones. Treatments which block estrogen and androgen signalling remain an important therapeutic strategy for these patients. While these treatments are initially very effective, treatment resistance often emerges rapidly in too many patients. For this reason there is an urgent need for new approaches to prevent, reverse or delay the emergence of treatment resistance.

Your project will focus on the dynamic regulation of RNA methylation. We have evidence that the RNA methylation machinery is hijacked by cancer cells to enable them to become resistant to current treatments. Our team of outstanding DTP students since 2016 (Drs. Veronika Metzler, Daisy Haigh, Corinne Woodcock, Jennifer Lothion-Roy, Anna Harris) have identified how cancers hijack RNA methylation to enable treatment resistance. You will investigate the precise pro-oncogenic epigenetic and epitranscriptomic events that occur during treatment resistance, and their functional consequences on gene regulation. This knowledge will enable the development of future treatments targeting these mechanisms to benefit patients with advanced disease.

During this project you will be part of a friendly and welcoming international multidisciplinary team of scientists and clinical researchers in the UK, USA and Sweden. You will learn to use bioinformatics, molecular biology tools and functional assays in cell line models systems and near-to-patient organoids models. You will work alongside team members to examine the expression of components

of this cellular machinery in large cohorts of patient specimens using immunohistochemistry and will relate this protein expression to clinical data. This knowledge will underpin our understanding the biology of disease. You will then use molecular (siRNA or CRISPR-Cas9) and pharmacological tools to better understand the potential therapeutic relevance of this mechanism by treating cell lines and patient derived organoids. Finally you will become proficient in bioinformatic analysis of your datasets.

Your PIP placement will be informed by your career goals. Current and former DTP students in our group have completed placements in academia (USA, UK, India); in large pharma; in a technology transfer office; a patent law firm; a healthcare consultancy and healthcare charity sector (Australia). We will support you career aspirations in anyway that we can.

References

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(* PhD students indicated)

Lab Rotation Location: University Park;

Full Project Location: University Park;

36 Understanding the role of non-coding RNAs in intercellular communication and how its potential for the detection and inhibition of pre-metastatic cancer

Lead Supervisor: Vicky James

Lead School: Veterinary Medicine and Science

DTP Research Area: Biotechnology

Lab Rotation Description: The rotation project will investigate a novel pathway identified by the team to validate how extracellular RNAs are influence the phenotype of target tissue cells. Skills developed within the rotation will be the use of Ingenuity Pathway Software (and/or similar resources) to build hypothesised regulatory signalling loops for subsequent molecular investigation. Validation of elements of the hypothesised pathway will be via quantitative PCR on existing sample sets.

Key skills – big data and molecular biology

Full Project Description: The PhD builds upon the successful work of the group in identifying the mechanisms by which extracellular vesicles influence the metastasis of cancer to bone. 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 (e.g., cancer and dysfunctional immune cells).

The project will explore the non-coding RNA elements of these EVs to determine the potential regulatory mechanisms they control. The project will be a mixture of data analysis to build experimental hypothesises of the types of signalling and regulatory mechanisms that are altered by these groups of non-coding RNAs, combined with molecular biology to validate these hypotheses within in vitro models of prostate cancer.

The project will require the development of molecular biology techniques alongside the ability to analyse large datasets and create experimental hypothesise based on existing data sources. These skills provide an excellent multidisciplinary basis and fit within the BBSRCs remit of approaches.

Lab Rotation Location: Sutton Bonington Campus;University Park;

Full Project Location: University Park;Sutton Bonington Campus;

52 Molecular mechanism of TRIM25 and ZAP mediated antiviral inhibition of arenavirus replication

Lead Supervisor: Toshana Foster

Lead School: Veterinary Medicine and Science

DTP Research Area: Bioscience for Health

Lab Rotation Description: During the rotation, the student will perform arenavirus infection assays using the CL2 Mopeia (MOPV) arenavirus demonstrating the restriction activity of TRIM25 and ZAP against arenavirus replication. The student will use cloning techniques to add to a panel of TRIM25 and ZAP mutants already available in the lab to demonstrate by immunoprecipitation (IP) assays the determinants of the NP interaction. The student will also incorporate RNA-IPs of NP-assocated vRNA from infected cells in which TRIM25 or ZAP are overexpressed or knocked out by CRISPR/Cas9 to demonstrate what promotes disassembly of arenavirus vRNP complexes. Overall, the student will gain experience in cell culture, viral infection assays, the use of CRISPR/Cas9 knockout cell lines and gain skills in biochemistry and molecular biology techniques, such as immunoprecipitation assays.

Key methodologies/timeline:

Weeks 1-2: Cell culture techniques, including transfection and MOPV infection assays.

Weeks 3-4: qPCR set up and data analysis.

Weeks 5-7: Infection of TRIM25 and ZAP WT and mutant overexpression and CRISPR-Cas9 knockout cell lines for protein IP and RNA-IPs

Weeks 8-9: Final data analysis and write-up

All relevant lab training will be provided and there will be the opportunity to present the project at lab meetings and to the wider One virology group.

Full Project Description: This joint project bridges across the virology and structural biology research programs in the Foster, Borkar and Dunham groups, focussing on the cellular antiviral mechanisms that that inhibit viral infection and the virus -host interactions that govern these. The Foster group focusses on the largest family of haemorrhagic fever causing viruses known as arenaviruses. These

zoonotic viruses are rapidly expanding in their genetic diversity leading to increased annual outbreaks in endemic regions and to sporadic imported outbreaks in globally, including recent cases in the UK (1). Understanding how host antiviral proteins block arenavirus replication is imperative to addressing current knowledge gaps on the viral lifecycle processes that are key for the development of effective vaccines and treatments (1).

Recent proteomics studies in the Foster lab, identified the interaction between the arenavirus nucleoprotein, NP and the antiviral E3 ligase tripartite motif-containing protein 25 (TRIM25), a key component of the innate immune response that inhibits the replication of a diverse range of pathogenic viruses, but previously unknown for arenaviruses (2). NP is the most abundantly expressed arenavirus protein, is a major orchestrator of host immunosuppression during virus infection and encapsidates the viral RNA, forming the viral ribonucleocomplexes (vRNPs) needed for infection initiation (1). Using arenavirus infection and CRISPR/Cas9 knockout studies, we have shown that TRIM25 significantly restricts arenavirus replication, that TRIM25 re-localises to NP-containing viral replication sites and that NP- overexpression is sufficient to induce this re-localisation.

TRIM25 was recently identified as key cofactor of the zinc-antiviral protein, ZAP, that targets viral RNAs containing CpG dinucleotides leading to promotion of viral RNA degradation and/or inhibition of viral RNA translation (2). We have demonstrated that ZAP also potently inhibits arenavirus replication and as TRIM25 is also an RNA binding protein, and it remains to be determined how TRIM25 and ZAP activity is co-modulated by their RNA interactions. The molecular structure of TRIM25 is an anti-parallel dimer formed through its coiled-coil domain, that can further multimerise through dimerisation of its N-terminal RING domain, containing E3-ubiquitin ligase activity. The ZAP and other ligand-binding C-terminal PRYSPRY domain is located on either side of the coiled-coil (2). We hypothesise that TRIM25 may multimerise around the helical vRNP complexes, driving disassembly and exposing RNA sites for ZAP recruitment and subsequent RNA degradation.

Thus, this project aims to use a combination of structural biology (expertise in the Borkar group), biochemistry and molecular virology techniques to:

- Characterise how TRIM25 interacts with arenavirus NP protein and with arenavirus viral RNAs with and without ZAP through mutagenesis and arenavirus replication experiments
- Recombinantly express and purify TRIM25 and ZAP proteins alone, in complex and in the presence of arenaviral RNA
- Visualise by electron microscopy and/or X-ray crystallography apo-structures and TRIM25-NP and TRIM25-NP-RNA-ZAP complexes, incorporating specific mutations that modify RNA and co-factor binding.

Given the molecular tractability of this antiviral mechanism across diverse RNA and DNA viruses, findings from this work will provide key evidence that could transform our understanding of innate immune mechanisms and influence therapeutic design.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

55 Catch me if you can: Characterising structure and dynamics of viral RNAs for rational drug discovery

Lead Supervisor: Aditi N Borkar

Lead School: Veterinary Medicine and Science

DTP Research Area: Bioscience for Health

Lab Rotation Description:

RNAs are one of the most challenging systems to study and visualise as single molecules. They require proficiency to synthesise; they constantly change shape, structure and binding partners in solution; and, they are difficult to image via conventional techniques.

Through this lab rotation, the DTP student will be trained in methods for RNA synthesis and characterisation, which will be used in the main project. Beyond RNA handling, this training will also 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.

Summary of lab rotation project:

Weeks 1–4: Synthesise the 5' untranslated region of HIV mRNA using in vitro transcription kits and test the sample quality using Agilent TapeStation at Nottingham DeepSeq facility.

Weeks 2–8: Characterise structural integrity of the synthesized RNA using reverse transcription PCR, gel electrophoresis and analytical gel filtration.

Weeks 7–9: Analyse results and report writing.

Full Project Description:

Context

Viruses, such as HIV, are obligate parasites. They hijack the host machinery to propagate their own genomes and to make their own proteins. Specific regions within the viral genomic and/or messenger RNAs mediate such interactions with the host. For example, the untranslated region (UTR) in the 5'-end of HIV mRNA recruits human protein translation factors, which leads to a 100-fold increase in rate of viral replication in the cell. Thus, RNA UTRs are key targets for development of therapeutic strategies. However, RNAs are also one of the most difficult systems to study and visualise as single molecules – a property that has significantly impeded their use in vaccines and antiviral drugs, particularly compared to proteins.

To address this challenge, our group has been involved in development of novel methods for synthesising and visualising RNAs using a combination of multidisciplinary, experimental and computational approaches. During this DTP project, the student will use these methods and unravel the molecular structure of the HIV UTR. The student will also use state-of-art computational approaches to characterise the conformational dynamics of the UTR and finally determine how this structure and dynamics influence key HIV interactions within the infected cells.

Outcomes and Impact:

This project will determine, for the first time, the structure and dynamics of HIV UTR at high-resolution and highlight novel hot-spots for HIV:human interactions. Such information will have direct implications for development of new antiviral strategies. Moreover, the experimental design could be extrapolated to other RNA viruses of high disease burden, such as coronaviruses, arenaviruses, flaviviruses, etc. Thus, along with high impact publications, the project will also open avenues for novel IP and clinical translation.

Training opportunities

Core competencies gained from the project will include RNA biochemistry, cell and molecular biology, structural biology, molecular dynamics simulations, data analysis, data presentation and writing skills. The project will also include student placement in our collaborator's lab at CIRI, France to learn working with HIV models in biosafety level 3 laboratories.

Research Plan:

Year 1: Optimise in vitro transcription of the HIV mRNA UTR.

Year 2: Characterise the structure of the UTR at residue level using newly established methods of solid-state mass spectrometry (3D-OrbiSIMS) and transmission electron microscopy (TEM) in the Borkar lab. This analysis will be repeated in the presence of human factors that interact with the UTR and regulate protein translation in HIV to highlight hotspots of UTR mediated HIV:human interactions.

Year 3: Use 3D-OrbiSIMS and TEM data as restraints in atomistic molecular dynamics simulations. This experiment will provide accurate information on UTR dynamics and help to characterise its structure - function relationships.

Year 4: Validate the results by rationally designing UTR mutants and assessing wild-type and mutant UTRs function in cell-based, viral replication assay.

Risk mitigation

Where definitive structure and dynamics characterisation of UTRs proves challenging, computational strategies such as modelling and simulations will be employed. Where residue level characterisation of UTR interactions proves challenging for rational design of mutants, random mutations that disrupt conserved structures in the UTR will be used.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;University Park;

58 Novel discovery, pathology and mechanisms of development of cardiovascular structures and functions.

Lead Supervisor: Catrin Rutland

Lead School: Veterinary Medicine and Science

DTP Research Area: Bioscience for Health

Lab Rotation Description: You will be joining a vibrant and successful research team. 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, PCR, and interpreting microCT images and anatomy. You will also learn bioinformatics and comparative and evolutionary biology skills to unravel the links between species which have os cordis and/or cartilage cordis. You will also undertake background reading into os cordis and cartilago cordis, and into heart development and epithelial to mesenchymal transition. 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). 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.

Full Project Description: The os cordis, or heart bone, has been discovered in less than fifteen species (including four that Dr Rutlands group have discovered). Our recently published investigations not only describe os cordis and cartilago cordis and their associations with myocardial fibrosis but also highlights the potential link with humans as they also suffer from the same disorders. Our ground-breaking paper was published in 2020 in Scientific Reports (https://www.nature.com/articles/s41598-020-66345-7) the 11th most cited journal in the world.

Little is known about the os cordis, the heart bone, why it develops or how it develops. Our Scientific Reports paper and other research has already shed some light on the process in relation to endochondral ossification and indeed other os cordis bones have cartilage within or adjacent to them, whilst other species have a cartilago cordis (cartilage in the heart). Age and heart disease are thought to contribute to the presence/absence of these structures, with the most likely contributor towards os cordis development being epithelial to mesenchymal transition (EMT). Within the developing heart, endocardial and epicardial EMTs produce most of the non-cardiomyocyte lineages of the mature heart. There is also a body of work which indicates that cardiac fibrosis and myocardial responses to ischemic injury deploy these developmental mechanisms. EMT has been linked with both fibrosis and ischemic injury which makes this an essential process to investigate. It has also been indicated that neural crest cells become lodged in the atrial wall and eventually differentiate into cartilage which may later undergo ossification but understanding the full mechanisms and their relations to disease states is important.

Objectives and techniques

1) Our group have discovered os cordis/cartilage cordis in four new species. This project will undertake further anatomical and cellular analysis, immunohistochemical staining and microcopy and imaging on these novel discoveries and in other heart conditions.

2) The student will undertake a systematic literature reviews into the areas of os cordis, cartilago cordis, and EMT in heart development and disease.

3) The basic mechanisms of development, and relation to age and disease states and the processes by which os cordis/cartilage cordis formation occurs is not well understood. This project aims to understand the ossification process and look at epithelial to mesenchymal transition within hearts. Histological stains/immunohistochemistry will be undertaken in several species in order to locate and identify the cell and tissue types in addition to cell and tissue morphology in the os cordis, cartilago cordis and the surrounding cardiac tissue. In addition next generation sequencing (RNAseq), qRT-PCR, immunohistochemistry and western blots will be undertaken on os

cordis/cartilago cordis, adjacent tissue and other cardiac tissue from species/animals with and without the structures in order to understand the processes and pathways being activated in the bone tissue.

4) Our os cordis research has drawn international media interest, so one aim will be to undertake widening participation/outreach activities in order to raise awareness of the research and ensure training for the student, in addition to producing published papers.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

63 Processing of dry complete foods for domestic felids detrimentally alters lipid composition for obligate carnivores

Lead Supervisor: David Gardner

Lead School: Veterinary Medicine and Science

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: Lab rotation: the project is cross-disciplinary. We would hope to attract a veterinary student into academic research who would then learn to appreciate the multi-disciplinary nature of scientific investigation. For example, in this project, scientific principles of food processing will be applied to companion animals, underpinned by training into the basic science of lipidomics, as applied to food processing. The data will be translated across to the veterinary world in the hope of understanding why 30% of older cats (>10yrs) get chronic kidney disease as opposed to <5% of omnivores. During the nine-weeks, the student will be introduced to the background to the project and will conduct a number of the techniques to be used routinely throughout the three weeks: collection and preparation of veterinary tissue post-mortem, subsequent extraction of lipids from tissue and wet/dry pet foods and lipidomics of that extract. The latter will incorporate thin-later chromatography to separate lipids according to polarity, followed by LC-MS/MS to gain detailed information about composition. In parallel, we will run GC-MS(-MS) for fatty acid profile together with standard techniques such as total lipid (Soxhlet) and total nitrogen (Dumass) determination, and a megazyme kit for total carbohydrate determination. We would also expect the student to demonstrate that they have a grasp of other key research techniques such as data presentation and analysis (e.g. using Prism, Genstat and SIMCA) and how to read and critique the scientific literature. They will also have to have rudimentary histology and histochemistry techniques, including TEM to characterise the lipids in situ in organs or foods.

Full Project Description: Domestic felids, even when young, have a preponderance of cytoplasmic lipid droplets of unknown origin in their kidneys. Comparable other species such as domestic dogs or feral species such as wildcats are largely free from such ectopic lipid, as are humans, particularly in their kidneys. The domestic cat is unusual in being an obligate carnivore, but living in a domesticated environment eating highly processed, refined foods. Refining of foods, particularly lipids can alter their characteristics such that the body recognises them as foreign, mounting a sterile inflammatory response – the pathogen associated molecular patterns. In this project, we will investigate the proposition that refining of dry pet food for domestic felids alters their lipid composition provoking a PAMP in domestic cat kidney, that could contribute to organ interstitial fibrosis.

Liver and kidney tissue will be obtained from participating veterinary practices from which full ethical approval has already been obtained i.e. the animals are euthanised by a vet for reasons unrelated to this project. Pet foods will be bought from commercial suppliers. Lipid will be extracted and profiled using a number of techniques: thin-later chromatography to separate lipids according to polarity; LC-MS/MS to identify accumulated lipids, GC-MS(-MS) for detailed and in-depth fatty acid profiling (i.e. fatty acid methyl esters), total lipid quantification by Soxhlet method and total nitrogenby the Dumass method. Free carbohydrate will be measured using a megazyme kit. Training will also be given in other key research techniques such as data presentation and analysis (e.g. using Prism, Genstat and SIMCA) and how to read and critique the scientific literature. They will also have to have rudimentary histology and histochemistry techniques, including TEM to characterise the lipids in situ in organs or foods.

Hypotheses:

1) dry versus wet food has an altered lipid content and profile, that obligate carnivores recognise as 'foreign'

2) altered fats in dry (cf. wet) food are processed for excretion in obligate carnivores via the liver and kidney but accumulate as cytosolic lipid droplets

3) accumulation of cytosolic lipid droplets with a high content of 'foreign' fatty acids in kidney cells of obligate carnivores provokes a localised inflammatory reaction and deposition of fibrotic tissue

Tissue specimens will be obtained through a network of partner practices allied to The School of Veterinary Medicine and Science, University of Nottingham. Tissue and feed lipids will be extracted and characterised using established methods as described above. Novel processing methods to avoid accumulation of 'foreign' lipids will be trialled in Food Sciences, UoN. Cell culture of primary, feline cell lines (cf. canine, MDCK) to demonstrate renal handling of these altered lipids will be established in the School of Veterinary Medicine.

Future food at Nottingham has the mission statement of, 'Better access to healthier, safer, more nutritious food is needed in all countries due the growing prevalence of pollutants, nutrient deficiencies and over-processed foods'. This project directly concerns this statement in that we have uncovered hitherto unseen evidence that certain food production strategies that are of relatively low cost may be subclinically 'poisoning' our companion animals, particularly felines (processing causes accumulation of branched-chain fatty acids). The outcomes of this research will be highly interesting to pet food manufacturers and to any vet treating domestic cats for kidney related problems. It is also possible of course, that increased processing of ANY foods, including those intended for human consumption, may equally become 'contaminated' with such pro-oxidant lipids. This has direct relevance for the increase in western societies of apparent problems with processed food (food sensitization and allergies, low-grade inflammatory conditions or possibly cancer).

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

67 Modified mRNA Caps in gene expression, cancer, and therapeutic RNA

Lead Supervisor: Nathan Archer

Lead School: Veterinary Medicine and Science

DTP Research Area: Bioscience for Health

Lab Rotation Description: The 5' cap is an essential non-coding feature of eukaryotic mRNA. Recent work by we and others has demonstrated new roles for modifications immediately adjacent to the mRNA cap in gene expression, memory and learning, and disease. The rotational project will give the student an opportunity to learn bioinformatics, cell culture and assay, and RNA design and assay techniques. During the project, cell lines deficient in cap-adjacent methyltransferase "writers" will be assayed for changes to cell cycle and survival in response to stressors. This will then be linked to bioinformatic analysis of transcriptome changes (e.g. differential gene expression analysis).

Full Project Description: Messenger RNA undergoes a variety of post-transcriptional processes which regulate its expression. As part of this, eukaryotes modify their messenger RNA during and following its transcription from DNA. Conceptually like epigenetics, these are chemical modifications to the "A", "C", "G", and "U" genetic alphabet and the sugar-phosphate backbone of RNA. These appear to change how the mRNA is treated in the rest of the cell, and thereby affect processes such as RNA transport, decay, and translation. So, broadly, modifications to mRNA affect the timing, extent, or location of protein production from that mRNA. Due to this, the appropriate use of modifications in RNA vaccines has proven essential for efficacy while drugs which target RNA modifications are in development.

Study of this "epitranscriptome" over the last decade has led to the discovery that it has essential roles across biological systems including plants, insects, mammals, and the viruses which infect them. However, understanding of these modifications and their mechanistic role in cells and organisms is still lacking. In effect, the epitranscriptomics revolution is at the stage of the epigenetics revolution of the early 2000's. While there are a handful of DNA modifications, over 150 RNA modifications have been reported alongside approximately 300 RNA modifying enzymes.

In this project, you will examine the role of underexplored modifications found adjacent to the mRNA cap. We and others have recently demonstrated that modifications in this region fine-tune gene expression to enable normal memory and learning, stress responses, and immune responses. Many pathogens co-opt or replicate these structures and associated pathways to manipulate host gene expression machinery. Similarly, by understanding more about these cap-adjacent modifications we will expand the toolkit for RNA therapies and our understanding of how perturbations in this region of mRNA can cause disease.

You will detect these modifications, and the enzymes responsible for them in cancer cells and cancer patient samples, and then use recently established human cell lines deficient in these modifications to learn more about the gene expression mechanisms they are involved in. To do this, you will use learn and use classical tools (e.g. polysome profiling, RNA-sequencing, molecular cloning techniques, bioinformatics, mRNA design, and in vitro transcription), modern technologies (e.g. Nanopore sequencing,), and novel methods established by the supervisory team. This will be alongside widely used lab-based skills in cell line culture, manipulation, and analysis (e.g. flow cytometry, genetic manipulation, RNA interference). You will use these valuable "RNA-world" techniques to define the roles of cap-adjacent modifications in gene expression and quality control.

This project integrates with, and is underpinned by, an over-arching vision to determine how animal cells use cap-adjacent mRNA modifications to fine-tune gene expression; understand their roles in disease, exploitation by pathogens, and to expand the toolkit used to build mRNA therapies and vaccines.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

71 Targeting carbonic anhydrase in ageing

Lead Supervisor: Lisa Chakrabarti

Lead School: Veterinary Medicine and Science

DTP Research Area: Bioscience for Health

Lab Rotation Description: The lab rotation will be an opportunity to get to grips with some of the general methodologies used in the lab while also potentially meeting with collaborators. Currently we have small datasets on carbonic anhydrase levels in human and rodent tissues. These data need to be extended and analysed. Therefore the student will learn and optimise western blotting and immunohistochemistry of tissues and cells from a variety of different organisms that are interesting in terms of ageing. These include micro-bats, Antarctic icefish, long-lived bird specimens and human samples. In addition the student will use different inhibitors of carbonic anhydrases on neuronal and retinal cells which will involve training in continuous cell culture and maybe growing primary cells. Cells and any fresh tissues obtained during the rotation, these may include blood samples will be interrogated for mitochondrial changes due to carbonic anhydrase inhibition. The student will learn to analyse cell and tissue metabolic changes using high resolution respirometry which will reveal a detailed picture of mitochondrial physiology in the samples.

Full Project Description:

Increasing age brings frailty and disease with it. How and why we age are questions to which we don't yet have very good answers. Our approach is to examine the protein profiles of young and older tissues to see if there is a pattern to what changes with increasing years. Other ways to examine this question include extending our studies to other organisms. Bats for example are extremely long-lived considering their size and metabolic rate – how do their organs and tissues maintain good health for so long? We also look at animals that have outlasted the average for their species. In the case of Antarctic icefish, these are unique vertebrates in not having any haemoglobin to transport oxygen. Our cells use oxygen to generate ATP in mitochondria, how do the icefish live for so long without the assistance of oxygen carrying proteins. In addition we model the potential biochemical networks in cell culture systems to see whether we can understand the ageing process and how to modify it at the molecular level. All the work centres on energy production in cell and most specifically the mitochondrial organelle.

Mitochondrial dysfunction is a cellular hallmark of ageing. Carbonic anhydrases (CA) are a group of enzymes that we want to learn more about in the context of ageing. These enzymes are widespread in living organisms performing the crucial and reversible reaction converting carbon dioxide to bicarbonate and protons. Inhibitors of CA are already used in therapy for acute altitude sickness, glaucoma and under investigation for the potential to treat obesity and cancer.

Previously we have shown that Carbonic anhydrase 2 (CA2) levels are increased in mitochondrial fractions isolated from middle-aged mouse brain. We performed proteomic profiling in mitochondrial fractions from young and middle-aged mouse brain and muscle tissues. Several proteins were identified that changed significantly with age in each tissue type. Carbonic anhydrase (CA) isozymes II and III increased in quantity in older brain and skeletal muscle mitochondria respectively. We measured a concomitant increase in CA enzyme activity in older mitochondria. This increase is also apparent in degenerating brain and retina in the pcd5J mouse model of

neurodegeneration(Chakrabarti et al., 2006), thus connecting increased CA with disease. We found increased CA levels cause a decrease in C. elegans lifespan. Others have shown that CA inhibitors (CAI) could have potential in the treatment of Alzheimer's disease (AD)(Fossati et al., 2015). A new connection between CAI, AD and mitochondrial toxicity has also been established(Solesio et al., 2018).

This project will focus on understanding how CA is involved in the process of ageing and how CA inhibitors might be a way to support healthy ageing. The methodologies to be used are rooted in protein biochemistry and mitochondrial physiology which together will give a picture of the ageing process and the results of targeting it to promote the chance of a longer healthspan.

Chakrabarti, L., Neal, J. T. J. T., Miles, M., Martinez, R. A. R. A., Smith, A. C. A. C., Sopher, B. L. B. L., et al. (2006). The Purkinje cell degeneration 5J mutation is a single amino acid insertion that destabilizes Nna1 protein. Mamm. Genome 17, 103–10. doi:10.1007/s00335-005-0096-x.

Fossati, S., Giannoni, P., Solesio, M. E., Cocklin, S. L., Cabrera, E., Ghiso, J., et al. (2015). The carbonic anhydrase inhibitor methazolamide prevents amyloid beta-induced mitochondrial dysfunction and caspase activation protecting neuronal and glial cells in vitro and in the mouse brain. Neurobiol. Dis. 86, 29–40. doi:10.1016/j.nbd.2015.11.006.

Pollard, A., Shephard, F., Freed, J., Liddell, S., and Chakrabarti, L. (2016). Mitochondrial proteomic profiling reveals increased carbonic anhydrase II in aging and neurodegeneration. Aging (Albany. NY). doi:10.18632/aging.101064.

Solesio, M. E., Peixoto, P. M., Debure, L., Madamba, S. M., de Leon, M. J., Wisniewski, T., et al. (2018)

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

79 Repurposing pharmacological inhibitors, 'Epi-drugs' to target epigenetic modulators in prostate cancer

Lead Supervisor: Jennie Jeyapalan

Lead School: Veterinary Medicine and Science

DTP Research Area: Bioscience for Health

Lab Rotation Description: The lab rotation will be based at the multi-disciplinary Biodiscovery Institute, where you, the new researcher will be working within an international, 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 are altered during cancer progression. The question being 'Can we repurpose pharmacological inhibitors identified in other cancers, for prostate 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 prostate cancer cell lines, how to grow them, phenotypic assays we utilise (invasion and proliferation) and using bioinformatic tools (web-tools and RNA-Seq analysis).

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.

Castration-resistance prostate cancer arises from current androgen deprivation (ADT) and androgen receptor signalling inhibitor (ARSI) treatments. Identifying druggable targets for these tumours will help men all over the world. More recently, in prostate cancer the identification that advanced cancers were driven by hypoxia and genomic instability have led to opening up avenues of potential therapeutic targets. This PhD project will look at how alterations in epigenetic factors, specifically histone modifiers targeting H3K4me3,2,1 and H3K36me3,2,1, occur and drive cancer progression. It will also address how hypoxic conditions effect the epigenetic factors levels and function.

The DTP student will undertake to;

1. Assess the expression of histone modulators in publicly available datasets and within our human prostate cancer specimens. This will indicate if the target is tumour promoting or suppressing, the protein location (nuclear or cytoplasmic) and clinical relevance.

2. Identify which epigenetic modulators are altered during hypoxic conditions and does this drive cancer progression, by assaying cell proliferation, invasion and transcriptomic analysis.

3. use of CRISPR technologies to knockout gene of interest and pharmacological inhibitors to target the epigenetic factors in prostate cancer cell lines. They will then determine the effect on androgen regulated gene expression by qRTPCR

4. use RNAseq and splicing analysis to determine the relative role of histone modifiers in alternative splicing and exon utilization of the AR transcriptome.

5. will compare the effects of functional depletion/over expression of histone modifiers on proliferation, invasion and apoptosis of cancer cells.

Collectively these aims will advance understanding of the role of H3K36me and H3K4me modulators in nuclear receptor transcriptomics in disease context.

The DTP student will receive training in the following techniques

1. 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

2. Cell culture, CRISPR techniques, reporter assays, in vitro pharmacology

3. Basic molecular biology, cloning, qRTPCR, western blotting

4. Bioinformatics: our group has optimized pipelines and existing datasets already available for comparison. RNA-seq analysis and splicing analysis.

Lab Rotation Location: University Park;Sutton Bonington Campus;

Full Project Location: University Park;Sutton Bonington Campus;

131 Probing the impact of extracellular matrix on cell-cell communication in breast cancer

Lead Supervisor: Jennifer Ashworth

Lead School: Veterinary Medicine and Science

DTP Research Area: Bioscience for Health

Lab Rotation Description: During breast cancer progression, the matrix of proteins and sugars surrounding the tumour undergoes dramatic changes. Collagen I, the key fibrillar protein marking up breast extracellular matrix (ECM), increases in density, alignment, and stiffness. These processes contribute to cancer invasion and its spread beyond the initial tumour. This project will use 3D biomaterial mimics of breast ECM to examine the correlation between collagen I alignment and cancer cell invasion. Based on self-assembling peptide gels, with combined control of collagen organisation, these biomaterials can be designed to match the biochemical and biophysical features of breast tissue.

The student will fabricate a series of biomaterials, ranging from disorganised to highly aligned collagen, representing the changes that occur during tumour progression. Fluorescently-labelled breast cancer cell lines will be cultured within each biomaterial, to monitor the progressive changes in cell invasion over time. This will be correlated to collagen structure, imaged by exploiting the autofluorescence of the collagen molecule. In parallel, immunofluorescent staining and confocal microscopy will allow changes in cytoskeletal structure to be probed within each biomaterial. In this way, cytoskeletal morphology will be mapped to invasion distance, identifying how matrix organisation may determine cell invasion by controlling cell shape and cell-matrix interaction.

Full Project Description: Breast cancer is a highly heterogeneous disease, with substantial cellular variability both between and within patients. Of the cells in this heterogenous mix, only a subset will be intrinsically capable of invading into the surrounding tissue and forming metastases at distant sites. To explore this heterogeneity, our group has developed advanced 3D cell culture methods to replicate breast cancer tissue in vitro. One application of this technology is as cellular “sieves”, separating/sorting a mixed cell population by invasive capacity. This models the role of extracellular matrix (ECM) in breast cancer invasion, allowing downstream analysis of the molecular and phenotypic differences between invasive and non-invasive cells within a tumour.

However, pilot data indicates that highly invasive cells may also use cell-cell and cell-matrix communication to drive the movement/growth of less invasive cell populations. This is dangerous for patient outcome, as heterogeneous tumours are more resistant to therapy and lead to worse prognosis. We therefore need a better understanding of how communication between different tumour cell subpopulations can contribute to cancer progression. This project will test the hypothesis that different subpopulations of breast cancer cells interact with each other and with their surroundings, to drive the invasion of multiple cell populations.

As pilot data indicates that both short-range and long-range cell interactions are crucial in determining breast cancer invasion, this project will examine both, implementing a set of interdisciplinary tools to probe cancer cell interactions with each other and their surroundings. Specifically, the project will address the following aims:

1) Identify key roles of ECM composition and stiffness in determining short-range cell-cell interactions. Fluorescently labelled breast cancer cell lines will be cultured within biomaterial mimics of the tumour microenvironment. Short-range interactions will be probed using advanced microscopy, including light-sheet fluorescence microscopy, to track cell-cell and cell-matrix

interactions in real-time. This will be combined with micromechanical sensing via particle tracking, testing the impact of matrix stiffness on cellular interactions.

2) Explore the role of ECM in determining long-range interactions via extracellular vesicles (EVs). Breast cancer cell lines with differing invasive capacity will be cultured in biomaterials with controlled composition and stiffness, representing normal breast tissue or invasive cancer. EVs will be extracted from cells cultured within each biomaterial, and their RNA/protein cargo analysed and compared. Cells with low invasive capacity will be treated with EVs extracted from highly invasive cells, testing their ability to induce invasion within biomaterials representing each tissue type.

3) Characterise interactions between invasive and non-invasive subpopulations of patient-derived breast cancer cells. By implementing cellular sieving technology on patient-derived tumour cells, the student will isolate subpopulations of breast cancer cells according to their invasive capacity. The phenotypic and molecular characteristics of these subpopulations will be analysed using RNAseq, EV analysis, and immunofluorescence staining. Patient-derived cells will be cultured within biomaterial models of invasive cancer, comparing the invasion response of each subpopulation when cultured together or in isolation.

Through these objectives, this project will identify key mechanisms underpinning invasion via cell-cell and cell-matrix interaction, increasing our understanding of breast cancer progression.

Lab Rotation Location: University Park;Sutton Bonington Campus;

Full Project Location: University Park;Sutton Bonington Campus;

174 Fighting Infection and AMR in broiler farming

Lead Supervisor: Tania Dottorini

Lead School: Veterinary Medicine and Science

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The student will be joining an exciting multidisciplinary team based in Dr Dottorini's lab. Dr. Dottorini is an Associate Professor in Bioinformatics at the University of Nottingham (UK and UNNC) and the Director of the Centre for Smart Food Research in the China Beacons Institute. She is currently the UK academic lead of several national and international research projects (MRC, Innovate UK-China and BBSRC) dedicated to developing new AI-based solutions to study the emergence and spread of antimicrobial resistance in several ESKAPE pathogens.

The supervisory team, together with the strong international links (UNICEF, Bangladesh National Centre for Diarrhoea, Prof R. Colwell University of Maryland) offers a unique combination of expertise in machine learning, bioinformatics, sequencing, cloud computing, microbiology, infection control, post-genomic statistical and computational approaches, microbiology and biofilm formation. During the nine-week rotation, the student will receive training in cutting-edge bioinformatics, high-throughput sequencing and machine-learning techniques applied to study bacterial genomes and their capacity to acquire resistance. In addition, he/she will be trained on how these techniques can be applied to the field of microbiology.

Full Project Description: The fight against enteric infections and antimicrobial resistance represents a major challenge in contemporary broiler farming. Infections caused by Clostridium perfringens, Enterococcus cecorum, Escherichia coli and Salmonella spp. are a significant cause of morbidity, mortality, poor welfare and economical losses in broiler farming. The gut microbiome is composed of harmless symbionts, commensal bacteria, and opportunistic pathogens, all of which play crucial roles in health and disease. In physiological conditions the gut microbiome is stable, but when perturbative events occur (e.g., dietary changes, infections, stress, antibiotic administration) the population of microbiota changes, influencing health and protection against further colonisation. Key to better solutions for surveillance, diagnostics and treatment selection, is exploring the modifications gut microorganisms undergo as a consequence of infection, treatment and development of resistant traits.

With the proliferation of collectable information, research has been gradually moving towards the adoption of the latest technologies in machine learning (ML) and big data mining to implement precision poultry farming. In this project building on methods previously developed by Dr Dottorini, we will explore the broiler gut microbiome, focusing on infection and resistance in relation to pathogens typically found in the gastrointestinal tract of the birds: Clostridium perfringens, Enterococcus cecorum, Escherichia coli and Salmonella spp. The project will utilise a large amount of heterogeneous data from farms, feed and birds, including sequencing, microbiological and sensor data, collected as part of a BBSRC-funded project 6784258.

The aim of this project is to introduce novel ML approaches to precision farming, based on a better understanding of infection and resistance and relationships with the gut microbiome. This will be achieved through three objectives: uncovering the broiler gut microbiome, exposing external correlations, and identifying biomarkers of infection events or resistance development. There will also be the opportunity to experimentally validate any promising biomarker candidates uncovered in the lab of Prof Paul Williams.

WP1: Use infection statuses and resistance profiles as detected from processing of biological samples to tag all the other data collected contextually from environment, birds, water and feed.

WP2: Use the previously illustrated methods and develop new pipelines to uncover correlations with gut microbiome modifications observed via biological sampling. Including the analysis of commensals and opportunistic pathogens, and the modifications of the resident and transient resistomes.

WP3: Extend the correlation analysis to identify relationships between gut microbiome modifications and changes in feed, water, environmental variables,

WP4: Given the knowledge acquired on the correlations between infection, resistance, gut microbiome and other measurable variables (related to birds, feed, water, soil, air, etc.) identify subsets of variables which may act as predictors (i.e., biomarkers) of infection or resistance development. Selection driven by tradeoff between correlation strength and viability of application (technical and economical) within the farm.

For further background see papers:

- Peng et al. 2022. 'Whole-genome sequencing and gene sharing network analysis powered by machine learning identifies antibiotic resistance sharing between animals, humans and environment in livestock farming', PLoS Computational Biology, 18: e1010018.

- Maciel-Guerra et al 2022. 'Dissecting microbial communities and resistomes for interconnected humans, soil, and livestock', The ISME Journal.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

198 Precision-targeting of pathogens using bacteriophage and antimicrobial peptides

Lead Supervisor: Robert Atterbury

Lead School: Veterinary Medicine and Science

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: In this rotation project, students will first learn the media and methods used to culture Salmonella. These methods will be employed to isolate bacteriophage which infect Salmonella Infantis from environmental samples. These phage will be amplified and purified before being characterised using techniques such as host range profiling, in vitro replication kinetics and stability. Restriction digest profiling and protein profiling (using SDS PAGE) may also be performed. The genomes of different phage will be extracted and sequenced. There will be an opportunity to learn bioinformatic techniques used in the assembly, annotation and comparison of phage genomes during this project. Students will also be introduced to the laboratory techniques used in Next Generation Phage Display and the preparation and screening of antimicrobial peptides.

Full Project Description: Salmonellosis is a major food-borne pathogen worldwide, frequently associated with contaminated poultry and eggs. Salmonella Infantis (SI) is the 4th most common serotype in human infections in the EU, and the most frequent serotype in broiler chickens. Worryingly, multidrug-resistant isolates are increasingly common in Europe, particularly those which have acquired a pESI megaplasmid encoding resistance to multiple classes of antibiotics as well as virulence genes and enhanced tolerance of heavy metals and biocides. Alternative approaches to controlling antimicrobial resistance in Salmonella and other pathogens are urgently needed.

Two such approaches are biological control using viruses which specifically target bacteria (bacteriophages) and antimicrobial peptides (AMPs). Both approaches have unique advantages over antibiotics. Bacteriophage are self-replicating and self-limiting – reproducing only when susceptible bacteria are present. Unlike broad-spectrum antibiotics, they target a specific genus, species or strain of bacterium, avoiding potentially harmful dysbiosis in the patient.

AMPs form the basis of effective innate immune responses to bacteria and other micro-organisms in all classes of life. The most common mode of action of these to bacterial cells is thought to be via a destabilisation of the bacterial cell membrane resulting in cell lysis. AMPs can vary in size from less than 10aas to 60-70aas, their small size enabling rapid diffusion, and penetration/destabilisation of bacterial biofilms.

In this project we aim to combine the strengths of both approaches to control SI, with the ultimate aim of using this combination therapy in poultry, and potentially as a model for use in human medicine.

The PhD study is divided into two strands:

1. Isolation and characterisation of antimicrobial peptides (AMPs) specifically targeting SI.

Next Generation Peptide Phage Display (NGPD) will be used to isolate or modify peptides specifically targeting the surface of SI. NGPD couples the vast diversity of phage-peptide libraries with the screening power of next generation sequencing and bioinformatics. SI-targeting peptides will be coupled via peptide linkers to known broad specificity AMPs. These fusions will be expressed and characterised according to their MIC and biofilm penetration/inhibition ability against clinical and laboratory strains of SI, and potential host cell toxicity. The optimal combination of AMPs will be determined by iterative machine learning using parameters we identify as important for the performance of AMPs individually (e.g. MIC, stability, efficacy against a range of pathogenic strains).

2. Isolation and characterisation of SI-specific bacteriophages(phages)

Phages specific for SI will be isolated from environmental samples, then purified, and screened for their ability to lyse a broad range of clinical and laboratory SI strains in our collection. The genomes of promising, strictly-lytic phage will be sequenced and analysed for therapeutic suitability before undergoing high throughput phenotypic and bioinformatic screening to determine the most effective combinations and titres.

The final part of the project will be to combine the AMPs and phages identified from (1) and (2) to determine their synergistic action against SI in biofilm and planktonic cultures, and subsequently ex-vivo models of SI colonisation of chickens. A particular focus will be efficacy against pESI-carrying strains of SI.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

199 Investigating immune responses of bovine mammary gland.

Lead Supervisor: Anna Malecka

Lead School: Veterinary Medicine and Science

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The immune system of the bovine mammary gland is in many ways unique amongst the body systems. In contrast to human or murine research, our knowledge of the function and characteristics of immune cells in bovine milk is very limited leading to delays in the development of novel effective therapeutics to prevent and treat infections of bovine mammary gland.

During the lab rotation the student will learn the methodology of isolation and in vitro culture of bovine milk macrophages and T-cells. During the rotation the student will characterise the phenotype and polarisation of milk immune cells using multi-colour flow cytometry, microscopy, and ELISA.

The rotation will supply initial preliminary data regarding characterisation of milk immune cells and will provide training in culture of primary cells and a range of analytic methods, data analysis and basic statistics. The student will also take part in lab meetings and participate in relevant seminars and presentations.

Full Project Description: Bacterial infection of the mammary gland leading to mastitis, one of the most common diseases of dairy cattle, impacts animal welfare and global milk production. Reduced

milk production results in the requirement for a greater number of animals to maintain the milk supply. This leads to higher emissions of greenhouse gases. Preventative methods to control infection/mastitis are mostly ineffective resulting in a wide use of antibiotics which increases the risk of developing antibiotic resistance. Therefore, there is an urgent need for novel effective therapeutic approaches utilising immune system such as vaccination and/or immunotherapy. However, progress in this field is severely delayed by the limited understanding of immune responses in the mammary gland and lack of validated in vitro models to test the interventions.

The milk of healthy cows contains a wide range of immune cells the numbers of which increase rapidly during infection. These cells are crucial for recognising and fighting pathogens in the mammary gland. Interestingly, the milk immune cells face multiple challenges which are unique compared to those in other tissues. They are removed at every milking and therefore are present in the lumen of mammary gland only for few hours. As the main role of the mammary gland is to produce milk (which must be maintained even in the face of pathogen invasion) the immune response must be carefully balanced to avoid interruptions in milk production but also efficiently remove the pathogen.

We have shown that milk macrophages are indispensable to initiate the response to Streptococcus uberis - one of the most common pathogens infecting mammary gland in dairy cattle. This project will build on our established methods to improve our understanding of the role of immune cells in preventing and fighting infections and will investigate the usefulness of in vitro models in testing therapeutic interventions.

Aim1: The student will characterise the pro- and anti-inflammatory phenotypes of milk immune cells and compare them to their blood counterparts via variety of techniques including flow cytometry, gene expression analysis, western blotting and ELISA.

Aim2: Immune cells do not act alone but are in constant crosstalk with their environment which strongly affect their behaviour. The student will investigate the importance of milk immune microenvironment and cell-to-cell crosstalk by comparison of the response of immune cells to S.uberis infection in single and multi-cellular models utilising our extensive library of S.uberis strains of known and differing virulence.

Aim3: Based on the results from aims 1&2 the student will develop an in-vitro organoid model of mammary gland. This will allow investigation in the context of the specific architectural organisation of mammary gland and more closely reflect complex interactions within living organism.

Results from this project will provide a basic understanding of the immunology of the mammary gland in response to infectious challenge and how this may be reflected in vitro. This will inform strategies to develop novel therapeutics to prevent and treat bovine mastitis. The student will benefit from working in a friendly team, recently initiated international collaborations, and attending seminars and conferences.

Lab Rotation Location: Sutton Bonington Campus;

Full Project Location: Sutton Bonington Campus;

Nottingham Trent University – School of Science & Technology

5 Shining new light on NMR spectroscopy for a quantum leap in nuclear hyperpolarisation

Lead Supervisor: Philippe B Wilson

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation Description: The student will have the opportunity to engage in linked rotations in the labs of Prof Wilson and Prof Mather. These will focus on:

- Wilson: biofluid analysis at high and low field NMR and tools and techniques required to elucidate the metabolites therein, including fundamentals in multivariate statistics. The student will be attached to the Lead NMR Postdoctoral researcher for this period and will work with them on the range of projects across human and animal medicine available. Furthermore, the student will have the opportunity to experience current hyperpolarisation techniques available through our MRI-linked project.

- Mather: herein, the student will be able to learn the fundamentals of ultra-low field NMR and optically detected NMR using the techniques Mather and Wilson developed throughout their CRUK and EPSRC-funded projects in the early detection of cancer.

Following these rotations, the student will be able to shape the project more on fundamental technology development or application based on their learning and interests.

Full Project Description: Recent scientific breakthroughs have demonstrated nuclear spin HP via polarisation transfer from unpaired electrons in negatively charged paramagnetic NV defects in diamond (Figure 1). This approach employs optical pumping to rapidly generate significant electron polarisation (over 92%5) and presents several advantages over conventional DNP, namely replinishable hyperpolarisation, operation at ambient temperatures and using modest resources (low optical and microwave powers). The entire polarisation process is achievable within microseconds and can be repeated to enable polarisation build up within the typical millisecond scale longitudinal relaxation time for NV defects in bulk diamond. Recently both experimental and theoretical studies have demonstrated the principle of polarisation transfer from NV defects to adjacent nuclear targets exploiting the magnetic dipole-dipole coupling between these spins. Experimental studies report remarkable progress towards effective transfer of NV polarisation to stationary nuclear spin baths inside diamond (13C inside diamond with enhancement of the order of 105 times over thermal equilibrium polarisation6) and small scale polarisation to nuclear spin baths external to diamond (1H nuclei in oil molecules surrounding a shallow NV defect in diamond7). Amongst the growing number of theoretical investigations there are studies presenting strategies to enhance polarisation transfer through consideration of multiple spin transfer mechanisms, diamond lattice quality and diffusivity of target nuclear spins5. Significantly, theoretical studies predict average nuclear spin polarisations exceeding 10% can in principle be generated over macroscopic sample volumes (>□l) using NVs with adequate quantum coherence properties8. Collectively these studies provide compelling evidence that NV based optically pumped DNP methods will enable spin transfer to nuclei in external liquids in a replensiable way, boosting NMR signals by orders of magnitude

Establishment of robust strategies and experimental protocols for efficient nuclear spin HP via optically pumped NV defects in diamond will be transformative

To establish the optical pumping of NV defects in diamond as an effective method for nuclear spin HP the efficiency of existing approaches must be significantly enhanced and the challenge of transferring polarisation to bulk liquid states addressed. Allied to this is the need to develop material specifications and processing protocols to produce diamond samples with high concentrations of NVs, to enable spin transfer at scale, whilst maintaining sufficiently good quantum coherence properties, especially in the case of near surface NVs. Further, to increase the volumes over which spin is transferred, engineering strategies must be implemented to maximise diamond-sample contact area which is achievable through the use of micro- and nano- sized diamond particles or high aspect ratio micro- and nano- scale surface structuring on bulk diamond samples8. Another promising strategy to improve HP performance is to exploit multiple spin transfer pathways. To date, most studies have investigated the direct transfer of spin between the NV and nuclear spin, however, recently reported alternative routes demonstrate significant performance improvements using intermediary spin sources to relay spin between the NV and target nuclear spin6,9. Notably this approach may remove the need for stringent magnetic field alignment when spin ½ nuclei are used in the relay process as demonstrated in studies harnessing 13C spins naturally present in diamond.

The overarching ambition of this research is to advance the current state-of-the-art in optically pumped spin transfer methods for nuclear spin HP using NV defects in diamond.

Lab Rotation Location: University Park;Clifton Campus;

Full Project Location: University Park;Clifton Campus;

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

Lead Supervisor: Sophie Benjamin

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation 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 current COVID-19 pandemic highlights 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.

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 current pandemic highlights 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.

Lab Rotation Location: Clifton Campus;Sutton Bonington Campus;

Full Project Location: Clifton Campus;Sutton Bonington Campus;

27 Developing molecular epidemiology frameworks using whole genome sequence data for pathogenic mycobacteria in animals.

Lead Supervisor: Conor Meehan

Lead School: School of Science and Technology (NTU)

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The purpose of the rotation mini project will be to train the student in the current state of the art molecular epidemiology pipelines for mycobacteria. There are standard whole genome sequencing assembly pipelines for M. tuberculosis human lineages and the student will be trained in the theory of these processes and given test datasets to learn the practical computer skills for applying them.

Once the student is familiar with the processes, we will investigate their applicability for the animal variants of M. tuberculosis. Public datasets will be used to assess the pipelines for their accuracy in reconstructing recent transmission of M. tuberculosis variants bovis and orygis, similarly to what was done previously for lineage 4 (see Meehan et al, EBioMedicine, 2018). This extended training will include introductions to phylogenetics, including Bayesian approaches, as well as molecular epidemiology and visualisation of outputs.

Skills learned by the student in rotation: UNIX, R, genome assembly, comparative genomics, introduction to Bayesian phylogenetics, transmission clusters and reconstruction, genetics of mycobacteria. All of these are transferable skills and highly sought after in future employment opportunities.

Full Project Description: Infection of both agricultural and wildlife animals with different species of mycobacteria is a large burden, both in terms of animal health and associated economic costs. Two of the primary mycobacteria infecting such animals are Mycobacterium tuberculosis variants bovis and orygis infecting cattle, sheep, deer, badgers and rhino and Mycobacterium avium subspecies paratuberculosis infecting cattle, sheep, goats, rabbits, deer and bison.

The frameworks used for tracking mycobacterial infections are almost exclusively built around tracking Mycobacterium tuberculosis in humans. They rely on whole genome sequencing data from the pathogens which are then compared to see how many mutations each pair differ by. If this difference is less than five single nucleotide polymorphisms, the two isolates are said to be in a transmission cluster together. However, these approaches have not been tailored for any other mycobacteria, who have different genome sizes and mutation rates.

This project aims to create gold standards for tracking transmission of animal-associated pathogenic mycobacteria similar to those in place for M. tuberculosis in humans. This will be achieved through various work packages including:

- Analysis of mutation rates and genome comparison approaches for M tuberculosis animal variants and M. avium subsp paratuberculosis
- Create adapted computational frameworks for undertaking track and trace of these pathogens using clinically derived whole genome samples
- Extension of these pipelines to look for drug resistance and virulence factors

- Use these pipelines to better understand the transmission of pathogenic mycobacteria between animals, with a particular focus of transmission corridors between agricultural and wildlife animals.

The project will be almost wholly computational and develop the student in skills such as:

- Bioinformatics pipeline construction
- Comparative genomics
- Molecular epidemiology, in particular Bayesian phylogenetics
- Clinical bacteriology, in particular mycobacteriology
- Scripting languages such as python, UNIX and R

Prior bioinformatics training is desired but not essential as all relevant training will be provided during the project.

Lab Rotation Location: Clifton Campus;

Full Project Location: Sutton Bonington Campus;Clifton Campus;

35 Understanding the role of actin nucleation in the cellular stress response

Lead Supervisor: Amanda Coutts

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation Description: The lab rotation will provide the student with key skills related to this project including, cell culture, CRISPR technologies, immunofluorescence, PCR based cloning, and flow cytometry. We will use CRISPR technologies to design specific constructs in order to endogenously tag target genes using fluorescence.

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.

We have uncovered roles for nuclear actin in the DNA damage response where it influences transcriptional regulation and splicing. As well have shown that actin nucleation influenced cell survival during autophagy (enables cell survival during metabolic stress). We want to further understand how nuclear is regulated during the DNA damage response and how this impacts on cell survival. This project will study the role of actin regulators and how they influence cell responses

such as cell survival (autophagy and apoptosis) and nuclear activities (such as DNA repair, transcriptional regulation and splicing) during the DNA damage response. This research is expected to uncover novel pathways related to cell survival and reveal new components involved in nuclear activities. 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.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;

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

Lead Supervisor: Jessica Piasecki

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;

43 Functional Metasurfaces for Early-Stage Non-Invasive Disease Diagnosis

Lead supervisor: Lei Xu

Lead School: School of Science & Technology (NTU)

DTP Research Area: Biotechnology

Lab Rotation Description: First, the student will learn relevant techniques for fabricating/characterising optical nanosensors for four weeks. He/she will be involved in hands-on

experiments with the cutting-edge state-of-the-art facility at both NTU and UoN, including but not limited to Electron Beam Lithography, Scanning Electron Microscopy, and Dark-field Spectroscopy.

Subsequently, at NTU, the student will run a relevant pilot project for five weeks. He/she will learn the basics of the simulation techniques for modelling the optical properties of nanoscale antennas and arrays of nanoparticles, so-called metasurfaces. He/she will then use a pre-designed dielectric metasurface sample to monitor and compare the level of glucose in his/her sweat droplets before and after several different physical conditions, e.g. exercise, having a meal, etc, as well as the influence of the environmental parameters including lighting, temperature, noise level. The benefits of this project are the following:

1. it will familiarise the student with the basics of nanoscience, as well as non-invasive diagnosis techniques based on optics and photonics.

2. it will enable the student to gain knowledge in optical bio-sensing principles, approaches for experimental data analysis, storage, and retrieval, and working effectively as part of a team and independently.

Full Project Description: Optical bio-sensing is a versatile approach to diagnosing diseases. Particularly, recent research is heavily concentrated on developing technologies where patients can take the lab with them via portable and non-invasive bio-marker monitoring. This is a promising solution to continuously monitor biochemical markers to reduce patients' need for physical attendance in medical centres. Such an innovative approach will enable early diagnosis of many diseases.

With recent developments in nanotechnology in the past decades, nanoscale sensors have emerged as novel high-throughput biomedical sensors with enhanced sensitivity and low cost. In this PhD project, we will develop customised optical biosensors for monitoring bodily excretions and secretions, such as sweat and tears, as they contain biomarkers associated with several diseases such as lung cancer, diabetes and breast cancer (Sci. adv.5,eaax0649, 2019) at very early stages of disease development.

First, we will employ artificial intelligence and machine learning for modelling and designing customised bio-sensors via metasurfaces. The building blocks of the metasurfaces will be atomically thin two-dimensional (2D) materials and/or quantum dots, which can support strong photoluminescence or nonlinear optical effects. This strategy will enable a background-free sensing platform with light generation over a large spectral range. Meanwhile, metasurfaces will be designed to selectively confine the generated light of different colours into nanoscale gaps, so-called hot spots, to push the detection sensitivity.

Subsequently, the bio-sensors will be fabricated and examined at well-equipped labs at NTU and UoN. By introducing the biomarkers to the customised metasurfaces, the variations in the optical properties of the emitted light, from the spectral domain in the optical spectrum and from the spatial domain in the far-field radiation patterns, will be characterised and analysed. To interpret the collected data, we will employ artificial intelligence and machine learning one more time to study the light properties in different spectral and spatial domains to retrieve the biomarker information. This project will target lung and breast cancers, known for realising various biomarkers in body excretions at early stages (Sci. adv.5,eaax0649, 2019).

The combination of multidisciplinary research areas, including biology, optical physics, and materials science, together with the frontiers in nanotechnology and nanofabrication, will significantly enhance the sensitivity and improve the specificity of detection. The project's objectives are:
(1) Design and integrate light generation and detection based on nonlinear nanoantennae and metasurfaces;
(2) Developing an innovative platform for employing artificial intelligence and machine learning for modelling the metasurfaces, as well as interpreting the collected characterisation data;
(3) Realising an innovative, non-invasive biosensing and bioimaging platform for identifying biomarker conformation evolution.

IMPACT: Developing and innovating low-cost, highly sensitive diagnostic technologies for bio-sensing and bio-imaging will enable early diagnosis of deadly diseases and could improve the health of at-risk populations worldwide.

SKILLS: The student will gain simulation and experimental skills in optics and photonics, nanotechnology, biophysics, machine learning and data analytics.

References:

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2. H. Ceren Ates, Peter Q. Nguyen, L. Gonzalez-Macia, E. Morales-Narváez, F. Güder, J. Collins & C. Dincer. "End-to-end design of wearable sensors." Nature Reviews Materials, 7, 887–907 (2022)

Lab Rotation Location: University Park, Clifton Campus

Full Project Location: Clifton Campus

76 Understanding the Effect of Ischemic Stroke on Peripheral Immune Cells Response to Infections

Lead Supervisor: Zahraa Al-Ahmady

Lead School: School of Science and Technology (NTU)

DTP Research Area: Biotechnology

Lab Rotation Description: Stroke-associated infections (SAI) represent a serious complication affecting a third of stroke survivors and have a strong association with poor outcome and mortality as such are considered in the top 10 priorities for stroke research. Therefore, understanding the effect of ischemic stroke on peripheral immune cells' response to infections, which is the focus of this work, will allow the development of potential therapeutic tools to tackle those changes at a preclinical level and hence prevent SAI.

In the lab rotation project, the student will carry out flow cytometry, cryosectioning, immunohistochemical staining, confocal microscopy, digital analysis and quantification of different cellular and the molecular component of peripheral immune cells (e.g spleen, and blood) from

rodents after inducing ischemic stroke. Samples from other sham and healthy mice will be included as controls.

Primary assessment parameters include; assessing immune cells apoptosis, lung infection, spleen size and circulating cytokines. This will then be complemented by assessing secondary outcome measures of brain damage (infarct volume with Nissl stain) and behaviour (neuroscore and foot-fault-test).

Full Project Description:

Stroke is a leading cause of death and disability worldwide and carries a substantial socioeconomic burden. In the UK, this is currently estimated at £26 billion a year with a projection to triple over the next 20 years. Stroke patients' recovery is often compromised by secondary infections which have rapidly become an area of unmet clinical need.

The timing of SAI in the first few days after stroke, suggests that deficits in innate rather than adaptive immunity are most likely responsible for the initial susceptibility to infections. The mechanisms behind the increased susceptibility to infection in stroke patients are not fully defined. Dysphagia and aspiration are key risk factors for stroke-associated pneumonia, however, their absence in half of the patients indicates that other immunological mechanisms are involved. This is also supported by a recent study showing that stroke-induced immunosuppression and dysphagia are independent predictors of SAI.

No effective treatment is currently available to prevent SAI. The results of two recent randomized phase III clinical trials demonstrated that using prophylactic antibiotics showed no clear benefit. Therefore, there is an urgent need to have a better understanding of the impact of ischemic stroke on peripheral immune responses that could inform the development of new therapeutic strategies to reduce the incidence and the impact of SAI.

Splenic atrophy and a general reduction in lymphocyte numbers in the blood and other lymphoid organs have been repeatedly reported in experimental stroke models and patients. Functional deficiencies in T cell, NK cell and monocyte populations have also been described. Of special interest is a subset of innate-like lymphocytes that reside at the interface between the circulation and the immune system termed marginal zone B cells (MZ B cells). MZ B cells have a crucial role in antibacterial defence within 1-3 days after bacterial exposure. This effect is mediated by the rapid production of polyreactive immunoglobulin M (IgM), which recognises bacterial capsular polysaccharide antigens and initiates potent neutralising and complement-activating functions. This effect happens independently from T-cell and follicular B cell. Recent evidence proved that MZ B cell function is compromised after stroke and has a strong link to stroke-induced immunosuppression and SAI.

Evidence from previous studies indicated that splenic shrinkage and loss of immune cells such as NK cell loss and susceptibility to SAI are mediated by a synergistic effect of sympathetic and hypothalamic pituitary adrenal (HPA) axis activation. However, the impact of those pathways on MZ B cell loss and function after stroke is not fully understood. Therefore, this project aims to elucidate the impact of sympathetic and HPA activation on innate-like immune cells' response after stroke and susceptibility to infection.

To achieve this aim, our program of work includes:

1. Establish an induced infection model after stroke by intranasal administration of bacteria after inducing stroke with a middle cerebral artery occlusion model.

2. Establish the impact of sympathetic and HPA-axis activation on MZ B cell loss by using pharmacological inhibitors and RNA interference approach.

Lab Rotation Location: Jubilee Campus;

Full Project Location: Clifton Campus;University Park;

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

Lead Supervisor: Maria Rosa Domingo Sananes

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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 sequence evolution and divergence, thanks to a combination of increasingly sophisticated statistical models and 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). This project aims 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 biology's most important outstanding questions: 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.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;

89 Investigating tRNA fragments as biomarkers of amyloidosis and alpha-synucleinopathies

Lead Supervisor: Marion Hogg

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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 project will begin with 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. Small non-coding RNA sequencing will be carried out on an exploratory cohort of samples, and differentially expressed tRNA fragments will be validated in an independent cohort using high throughput quantitative PCR and northern blotting techniques.

The student will then investigate the molecular processes which lead to biogenesis of tRNA fragments using induced pluripotent stem cell (iPSC)-derived neuronal models. Here dopaminergic and cortical neurons will be differentiated and 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. We will define the ribonuclease(s) responsible for generating tRNA fragments and analyse the effect of overexpression and depletion on neuronal activity. Finally, we will investigate the function of identified tRNA fragments using synthetic tRNA fragments and standard biochemical techniques, and will determine whether tRNA fragments interact directly with proteins and RNAs implicated in alpha synucleinopathy or amyloidosis pathology.

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. 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.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;

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

Lead Supervisor: Rebecca Dumbell

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation 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 forward in the PhD.

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.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;

97 Controlling and modelling blood-flow-induced shear stress on endothelial cells to unlock unique signalling and metabolic pathways important in vascular homeostasis and pathophysiology

Lead Supervisor: Mark Paul-Clark

Lead School: School of Science and Technology (NTU)

DTP Research Area: Biotechnology

Lab Rotation Description: Students will learn practical, theoretical and computational aspects of biological fluids and their flow behaviour. They will be trained in the use of a state-of-the art rheometer to measure the flow properties of various test samples. Once they are familiar with the use of the equipment, students will investigate the properties of defibrinated horse blood, adjusting the experimental parameters to match the flow rates and physical properties of micro- and macro-vessels. In the second stage students have significant experimental freedom to investigate fluids of their own choosing, including food, beauty or hair-care products, allowing each student to gain a deep understanding of fluid properties and characterisation. Previously students have studied the temperature-dependent flow properties of honey; a comparison of shampoos and soaps and how viscosity changes with dilution; the melting behaviour of chocolate; and the flow of water and corn-starch mixtures. Having fully characterised the fluid, students will use syringe drivers and/or peristaltic pumps to drive the fluids through microfluidic devices, and measure the resulting fluid dynamics. They will use computational modelling, based on their rheological characterisations, to calculate the expected flow and compare with their observations.

Full Project Description: Endothelial cells, which line all blood vessels, are continually subjected to flow of blood across their surface. This flow applies a hemodynamic stress that varies from one region of vasculature to another. The cells acclimatise to the flow in both their morphology and cytoprotective response: in laminar flow in large vessels such as the aorta, endothelial cells are elongated in the direction of flow with evidence of improved protection from inflammation; conversely oscillatory flow leads to randomly orientated cells with a cobblestone appearance, that are more susceptible to inflammation and atherosclerosis. Currently, most in vitro studies assessing endothelial cell physiological responses are performed under static conditions, even though production of key endocrine hormones is shear-stress regulated. It has been proposed that endothelial cells have mechanical memory through cytoskeletal reorganization and conformational change, implicating memory of environment in their responses to both physiological and inflammatory stimuli. This indicates that the environment where endothelial cells grow plays a strong role in determining their response to shear stress and inflammatory insult.

Studying endothelial cells under shear would provide relevant functional data and lead to an improvement of our understanding of healthy and pathological responses. However current methodologies are less than ideal: many flow chambers do not permit cells to be grown under shear conditions prior to experimentation; other methods using orbital shakers allow endothelial cells to be grown under only poorly controlled shear conditions [A] making detailed omics studies difficult to perform.

The aim of this project is to develop an experimental apparatus in which endothelial cells can be grown under controlled shear to model the conditions found in macro- and micro-vessels.

This will be achieved by using a rheometer allowing application of a controlled and uniform shear stress. The initial challenge is to design, build and test the setup in which cells grow under shear for several days. Cellular physiology will then be assessed under macro- and micro-vessel conditions by measuring mechano-sensing ion channel Piezo 1 and through acute Ca2+ responses and vasoactive hormone production. Once the experimental system has been established it will be used to assess how changes in shear effect physiological and pathophysiological stimuli. We aim to monitor these changes in signalling pathways through transcriptomic and proteomic analysis, metabolic analysis using Seahorse and Oroboros, and functional exploration using pharmacological interventions.

In parallel to the experimental work, we will use computational simulations of the fluid flow to predict the shear stress at a cell-by-cell level. We will calculate the fluid flow using the Navier-Stokes equations and capture the interactions with the endothelial cells using an embedded template technique. Such models have been used successfully to simulate fluid-particle interactions under a variety of flow conditions [B]. Initially the cells will be assumed to be immobile, providing a complex boundary condition for the shear flow. Further model extensions will incorporate deformation and movement of the cells in response to the fluid. The numerical results will be validated against the controlled shear experiments and used to provide detailed insight into the role of fluid flow in cell development.

Lab Rotation Location: Clifton Campus;University Park;

Full Project Location: University Park;Clifton Campus;

98 Effect of antimicrobial selection on collateral susceptibility and mutation selection windows

Lead Supervisor: Alasdair Hubbard

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation Description: With the increasing incidence of antimicrobial resistance (AMR) globally, there is a growing need to limit the emergence of AMR and keep the antibiotics we currently have available working. To achieve this, we must understand how bacteria evolve AMR during treatment of an infection.

Mutations within the bacterial genome occur naturally and frequently in large populations. In the presence an antibiotic, those mutations which cause resistance will be selected for. One strategy to help overcome AMR is to take advantage of a phenomenon where mutations leading to resistance to one antibiotic may result in susceptibility to a second antibiotic, termed collateral susceptibility.

However, it is important to also determine if mutations causing AMR to one antibiotic also influences the probability for selection of AMR-causing mutations to a second antibiotic. This can be estimated by measuring mutant selection windows - the range of antibiotic concentrations for which AMR-causing mutants can be selected.

In this project, we will select for resistance to trimethoprim in clinical isolates of Escherichia coli and assess the impact these mutations on collateral susceptibility and mutant selection windows to other classes of antibiotics. All training will be provided.

Full Project Description: Antimicrobial resistance (AMR) is a global health crisis, with 1.27 million deaths directly attributed to AMR in 2019 alone. As the discovery and development of new antibiotics to treat antimicrobial-resistant infections are at an all-time low, it is becoming increasingly important to better understand AMR development to maintain efficacy in antibiotics currently available. Random mutations occur continuously and are common in large bacterial populations. Some of these mutations can cause increased resistance to antibiotics, and these mutations can be selected for when the antibiotic is present. One strategy to limit the evolution of AMR is to take advantage of a phenomenon where mutations leading to resistance to one antibiotic may result in susceptibility to a second antibiotic, termed collateral susceptibility. However, before this strategy can be employed, we need to further understand the effect that AMR-causing mutations have on the activity of other antibiotics to ensure multidrug resistance is not selected for. An important evolutionary concept can be applied to improve this knowledge, termed the mutation-selection windows (MSW). MSW is an antibiotic concentration range in which AMR-causing mutations can be selected for by the antibiotic. Below this range, the concentration of the antibiotic is not high enough to select for these mutations and above the range, the concentration is too high for the bacteria to survive. The MSW can be specific for each bacterial strain-antibiotic combination as it is dependent on the strain's genetic background. Importantly, like collateral susceptibility, mutations that cause resistance to one antibiotic may change the MSW to further antibiotics. That is, mutations can shift the MSW up or down the concentration gradient or make the MSW wider or narrower. This can have significant effects on the likelihood of selection of secondary AMR-causing mutations which confer resistance to a second antibiotic.

In this project, we will use an experimental evolution approach to further understand collateral susceptibility and the effect that AMR-causing mutations may have on the probability of further mutations arising. To produce data which will be clinically relevant we will use clinical isolates of Escherichia coli and Klebsiella pneumoniae isolated from urinary tract infections (UTI) and antibiotics recommended for use in UTIs. We will first use an evolutionary ramp approach to select for AMR-causing mutations in increasing concentrations of first-line antibiotics used for UTIs. We will then assess the effect that these initial mutations have on both susceptibility and the MSW to other first and second-line antibiotics. Secondly, we will determine whether the initial antimicrobial-resistance mutations increase or decrease the probability of their descendants establishing in the presence of other antibiotics.

It is anticipated the data from this project will be used to determine the likelihood of the collateral susceptibility strategy succeeding in the clinical environment. Additionally, the data will be used to predict antibiotic choice and treatment order that will lead to collateral susceptibility and a low probability of establishment of the resistant population. This will guide prescription policy to limit the emergence of AMR.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;

104 Modelling human gut inflammation and therapeutics in organs-on-chip

Lead Supervisor: Christos Polytarchou

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation Description: The project will test the hypothesis that intestinal epithelial cells contribute to gut inflammation by intestinal epithelial barrier dysfuntion upon exposure to inflammatory stimuli.

Department of Biosciences and John Van Geest Cancer Research Centre, Nottingham Trent University (6 weeks)

The student will gain proficiency in intestinal epithelial cell culture, using 2D monolayers and 3D enteroids. The student will address the effects of cytokines and bacterial toxins on paracellular permeability (FITC-dextran) and transepithelial electrical resistance (Volt/Ohm meter). Training will include immunofluorescent staining for the analysis of cells tight junctions and RT-qPCR analysis for proinflammatory proteins and non-coding RNAs. Using existing transcriptomic datasets, the student will gain bioinformatic skills using MetaCore user-friendly software. Network analyses will focus on cell survival, inflammatory signaling and cell-cell interactions. This expertise will be applicable to transcriptomic datasets generated in the PhD project.

Biodiscovery Institute and Nottingham Digestive Diseases Centre, University of Nottingham (3 weeks)

The student will be trained on flow cytometry to quantitatively assess changes in cell cycle/survival and stem-cell like properties using the in vitro cell models. Will be processing human patient samples, performing correlations using existing data from inflammatory diseases pre and post therapy, and statistical analyses of data collected throughout the project.

Full Project Description: Context:

Normal homeostasis of the gut largely relies on the intestinal barrier function. Disruption or of this barrier results in local consequences associated with direct contact of bacteria/bacterial products with the epithelial cells, translocation in the systemic circulation and activation of the inflammatory response. Local intestinal inflammation underlies the development of several gastrointestinal diseases, whereas the systemic consequences link with liver, heart and neurologic diseases. The currently used in vitro assays and animal models revealed mechanisms potentially involved in the deregulation of intestinal barrier, however, they have failed to produce targeted therapeutic approaches.

The study of intestinal barrier function based on animal studies is hampered by their differences in microbiome and the innate immune responses of intestinal epithelial cells in humans, while in vitro assays overlook the importance of blood flow, mechanical forces, cell-cell interactions and in vivo cytoarchitecture. Therefore, it is paramount that research focuses on organ-on-chip approaches, to unravel key mechanisms pertaining intestinal barrier function, its role in local and systemic inflammation, and evaluate clinically relevant drugs.

Hypothesis:

Organ-on-chip approaches will facilitate the holistic characterisation of mechanisms involved in intestinal epithelial barrier function in health and disease.

Aims:

Utilise organ-on-chip to elucidate cellular mechanisms which regulate intestinal epithelial barrier function upon inflammatory insults and use as platform to evaluate novel therapeutics.

Work plan:

Months 1-12- Development and validation of reproducible gut-on-chip: Using primary and immortalised non-tumorigenic intestinal epithelial cell lines in combination with primary intestinal endothelial cells will be employed to develop a reproducible organ-on-chip. The Emulate Organ-On-A-Chip platform allows the study of the effects of laminar flow on complex cell systems in an in vivo-relevant tissue architecture using ready-to-populate chips. The immortalised intestinal epithelial cell lines will be compared to epithelial cells derived from primary organoids in their ability to reproduce barrier function under physiological mechanical forces. Comparisons will include permeability, epithelial tight junction immunostaining, cell confluency and survival.

Months 13-24 – Evaluation of inflammatory stimuli effects on barrier function: The gut-on-chips will be used to characterise the alterations in molecular mechanisms involved in endothelial/epithelial barrier integrity and morphology upon exposure to inflammatory stimuli, by measurements of permeability, tight junction immunostaining, confluency and cell death, secretion of chemokines/cytokines, RNA and microRNA. Additional experiments will utilise sera, PBMCs and faecal filtrates collected from patients with intestinal inflammatory diseases before and after therapy.

Months 25-42 - Pathway analysis and identification of druggable targets: RNA and microRNA profiles will be subjected to MetaCore pathway analysis to identify common gene networks deregulated by inflammatory insults in intestinal epithelial cells. Druggable targets will be evaluated using commercially available agonists or inhibitors. New experimental therapeutics, small molecules, microRNA-loaded nanoparticles, metabolites will be tested on this platform against the inflammatory insults. Selected barrier insult experiments and therapeutics will be further evaluated using Emulate's ready-to-use (gut) biokits.

Anticipated outputs:

- Graduate pioneering the gut-on-chip approaches combined with expertise in cellular, molecular biology and bioinformatics.
- Two high impact publications, presentations at international conferences.
- A new platform for evaluation of therapeutics for inflammatory diseases.
- NC3Rs: animal replacement in research

Lab Rotation Location: University Park;QMC;Clifton Campus;

Full Project Location: QMC;Clifton Campus;University Park;

107 Novel quantitative assays for measuring Alpha-synuclein oligomerisation and screening RNA therapeutic targets in Synucleinopathies

Lead Supervisor: Shreyasi Chatterjee

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation Description: Alpha-synuclein regulates synaptic vesicle trafficking and neurotransmitter release under physiological conditions. Under pathological conditions, Alpha-synucleinopathies include the second most prevalent form of neurodegenerative dementia such as Dementia with Lewy Bodies (DLB), and Parkinson's disease (PD). Neuronal Lewy bodies are the pathological hallmarks of these diseases, and Alpha-synuclein oligomerisation is the key initial step in Lewy body formation. However, the molecular mechanisms underlying Alpha-synuclein oligomerisation remain uncertain. We have generated neuroblastoma cell lines and Drosophila models overexpressing both wild type and mutant Alpha-synuclein. During this rotation project, the student will develop assays to monitor Alpha-synuclein oligomerisation in vitro and in vivo using immunohistochemistry and Western Blotting techniques.

Week 1 and 2: Transfection of Alpha-synuclein wild-type and mutant plasmids (AsynA53T) in neuroblastoma cell lines.

Week 3 and 4: Detection of Alpha-synuclein oligomers by immunohistochemistry and Western blotting.

Week 5 and 6: Setting up crosses of Drosophila expressing wild-type and mutant Alpha-synuclein (AsynA53T) with the pan-neuronal Elav-GAL4 Driver to overexpress this protein in all neurons.

Week 7 and 8: Immunohistochemistry and Western blotting analysis on Alpha-synuclein overexpressing flies

Week 9: Project report

1. Chowdhury A, Rajkumar AP. Acta Neuropsychiatr. 2020;32(6):281-292.

2. Chatterjee S et al. Frontiers in Neuroscience, 2019 August (13):1-16.

Full Project Description: Background:

Alpha-synuclein is essential for neuronal survival and regulates synaptic vesicle trafficking and neurotransmitter release. Alpha-synucleinopathies include the second most common type of neurodegenerative dementia such as Dementia with Lewy Bodies (DLB), and Parkinson's disease (PD). Oligomerisation of "neuro-toxic" Alpha-synuclein is the initial step in the formation of Lewy bodies that are found in the brains of these patients. Alpha-synuclein oligomers from the brain tissue or cerebrospinal fluid of people with DLB can induce Lewy pathology in H4 neuro-glioma cells and in mice models. Although, we do not have any disease-modifying treatment for these diseases, genetic association and gene expression studies have identified Alpha synuclein as one of the therapeutic targets. Hence, developing a sensitive, rapid and reliable quantitative assay for measuring Alpha-synuclein oligomerisation is an essential pre-requisite for developing drug-targets against these toxic species.

Drosophila melanogaster has been widely used for modelling neurodegenerative diseases including Tauopathies and Synucleinopathies. Notably, Drosophila models overexpressing Alpha-synuclein

recapitulate salient features of Alpha-Synucleinopathies. The over-arching aim of the project is to develop quantitative assays for measuring Alpha-synuclein oligomerisation using a combination of neuroblastoma cells and Drosophila models.

Aim-1: Systematically reviewing prior evidence

We will conduct a systematic review of studies that have investigated quantitative assays for measuring Alpha-synuclein oligomerisation and post-translational modifications. At least five online databases will be searched and relevant data will be extracted.

Aim-2: Developing and validating a quantitative assay for measuring Alpha-synuclein oligomerisation in neuroblastoma cells.

Methodology: Neuroblastoma cells will be transfected with Alpha-synuclein-wildtype (wt) and Alpha-synuclein-mutant (A53T) plasmids. Alpha-synuclein oligomerisation will be measured by Western blot (Alpha-synuclein oligomer specific antibody Syn33) and immunofluorescence microscopy.

Aim-3: Developing and validating quantitative assays for measuring Alpha-synuclein oligomerisation in Drosophila eyes and brain tissue.

Methodology: Alpha-synuclein-wt and mutant (A53T) Drosophila lines will be crossed to the eye-specific GMR-Gal4 driver. These flies will be aged from 0 weeks to 6 weeks and the fly extracts will be collected at each time point. Alpha-synuclein oligomerisation will be measured by Western blotting, Immunofluorescence microscopy, Atomic Force Microscopy and Cryo-Transmission Electron Microscopy.

Aim-4: Behavioural assays for investigating changes in motor functioning of Drosophila.

Methodology: Alpha-synuclein-wt and mutant (A53T) Drosophila lines will be crossed to the pan-neuronal Elav-Gal4 driver. These flies will be aged from 0 weeks to 6 weeks and the progressive behavioural dysfunctions (longevity, climbing assays and learning/memory assays) will be performed according to standard protocols.

Aim-5: Investigating translational significance of potential RNA targets.

Methodology: The quantitative assays developed in neuroblastoma cell lines and Drosophila models would be used to evaluate the ability of antisense oligonucleotides (ASO) or miRNAs or small interfering RNAs (siRNA) for reducing Alpha-synuclein oligomerisation. The models will be transfected with the ASO, siRNA or miRNA mimics, and the changes in Alpha-synuclein oligomerisation will be measured.

The student will be supervised by Dr. Shreyasi Chatterjee at NTU and Dr. Anto Praveen Rajkumar Rajamani at the University of Nottingham. Dr. Chatterjee is an expert in developing Drosophila models of Neurodegenerative Diseases while Dr. Rajamani's proficiency lies in dementia research and the development of RNA-based biomarkers.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;

124 Black hydrochar for green agriculture – A comparative study on productivity improvement

Lead Supervisor: Wenbin Zhang

Lead School: School of Science and Technology (NTU)

DTP Research Area: Biotechnology

Lab Rotation Description: Students are expected to spend around 3 weeks at each of the three partners involved in this project:

Nottingham Trent University (NTU):

Students will develop their problem-solving skills and gain hand-on experiences through small project-based work by designing products using specialized software and computational skills, manufacturing the product in the workshop or using 3D printers and conduct pilot studies on the testing machines.

University of Nottingham (UoN):

Students will have the chance to enter the laboratory in Department of Chemical and Environmental Engineering which is ranked 3rd in the UK. They are going to be trained on the selected state-of-the-art scientific apparatus and analysis equipment which are essential for characterization of physicochemical properties of different samples.

Rothamsted Research Centre (RRes):

The skills to be developed in RRes will include preparation and analysis of soil and water samples for properties including soil bulk density, pH, available and total nutrient contents, organic carbon content and isotopes of N and P. They will have the opportunity to witness and participate in controlled environment plant growth pot trials, and become familiar with the wide range of monitoring equipment that is deployed on the most monitored farm in the world.

Full Project Description: The widely distributed non-point sources from organic biomass-based wastes are emitting significant amount of agricultural CO2 emissions, making agriculture section one of the most difficult sectors to be decarbonized. Converting biomass substrates into char, the CO2 bound in the parent biomass will be no longer liberated via atmospheric decomposition, but bound to the final solid carbonaceous structure which is highly stable against decay. This represents an efficient way of taking the CO2 out of the carbon cycle and directly from the air, known as a negative carbon emission pathway.

The char produced from biomass (known as biochar) has unique physicochemical properties such as porous structure, large surface area, strong carbon stability and active surface functional groups. These favourable properties provide biochar with multiple functionalities including improving soil fertility, enhancing microbial activities and immobilizing heavy metals and organic pollutants.

Traditional biochar is produced by a pyrolysis process in an oxygen-limited environment. However, self-sustainable operation of this process is difficult to achieve due to the energy intensive drying needed for high moisture containing biomass. Biochar production through an emerging hydrothermal carbonization technology (referred as hydrochar) is attracting increasing attention owing to less energy consumption, no de-water processing requirement, more environmentally friendly and higher yield. The resulting hydrochar has as favourable behaviour as the coal in thermal conversion processes but much more environmentally friendly than the coal. The high-pressure

compact design makes the production more economically feasible and more attractive to agriculture practitioners.

The overarching objective of this project is to produce a high value added and multi-functional sustainable material for various applications including soil amendments, plant growth improvement and particularly adsorption of greenhouse gas emissions from soil. The following challenges will be addressed in this project:

Challenge 1: Development of a novel highly efficient hydrothermal process with less energy requirement and higher carbon yield;

Both types of carbohydrates and lignocellulosic biomass wastes will be selected as the precursors. Hydrochar will be produced in a purposely-built reactor in Nottingham Trent University (NTU). Char samples will be characterized and analysed using the state-of-art facilities located in University of Nottingham (UoN).

Challenge 2: Further exploration of the underlying reaction mechanisms between hydrochar and soil-plant systems;

Comparative studies between the traditional biochar and hydrochar on plant growth with and without addition of char samples will be conducted. Relationship between various input parameters and the performance in soil improvement will be revealed.

Challenge 3: Longer-term performance and risk assessment of the hydrochar

Near-term pot experiments, as well as Medium-long (1-4 years) term field monitoring studies will be conducted on the sustainable agriculture platform (NTU) and the farm/yield research facilities (Rothamsted Research) to evaluate the stability of physicochemical properties, sorption affinity, pollutants control functionality of the hydrochar.

The expected outcome of this project is to develop a highly cost-effective and energy-efficient production of hydrochar which acts as a carbon storage sink to mitigate the global warming issues, as well as a promising additive to increase soil fertility and raise agricultural productivity.

Lab Rotation Location: Jubilee Campus;Clifton Campus;Rothamsted Research;

Full Project Location: Jubilee Campus;University Park;Clifton Campus;Rothamsted Research;

126 Towards improving the health of aged joints; the effect of chronic damage mediators on mesenchyme stromal cells

Lead Supervisor: Jehan El-Jawhari

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation Description: The student will spend five weeks at NTU (Dr El-Jawhari lab) and four weeks at UoN (Dr Carter lab) according to the below plan:

NTU lab rotation:

- Training on basic human cell culture techniques and specific protocols related to human mesenchymal stem/stromal cell culture.

- Training on fundamental cell proliferation and survival assays.
- Training and characterising surface phenotype characterisation of human mesenchymal stem/stromal cells using flow cytometry and its analysis software.
- Training on cell imaging technique: Incucyte Live-Cell Analysis.

UoN lab rotation:

- Training on one-dimensional and two-dimensional separation techniques and protein staining.
- Training on immunolocalisation (Western blotting) and quantification of protein levels using ImageLab (BioRad) and Image J (NIH) software.
- Training on spectrophotometric quantitation of reactive oxygen species and protein carbonyl content for measuring oxidative stress and oxidised proteins.

Full Project Description: Background

The most prevalent ageing change in large joints is cartilage damage associated with chronic low-grade inflammatory status, described clinically as arthritis. Osteoarthritis (OA) is a leading cause of arthritis in a third of all 40+ years old UK populations with significant health and socioeconomic burdens. In most OA cases, chronic pain and limited mobility affect the life quality of these patients and can lead to joint replacements with further complications.

Mesenchymal stromal/stem cells (MSCs) are conventionally known as stromal cells delivering repair functions. This knowledge has been updated with a long trail of research studies showing that MSCs have immunomodulatory capacity. Interestingly, MSCs extracted from OA joints were found to have pro-inflammatory potential and skewed function towards bone formation rather than cartilage repair. How the OA damage milieu could alter MSC biological capacities remains to be answered.

In OA patients, several damage mediators show high expression levels in their joint tissues and blood samples. Interestingly, these high levels of damage mediators are positively associated with cartilage loss, inflammation, and extra bone formation shown in patients and animal models. However, no clear link has been established between these damage mediator roles and MSC biology within the OA milieu.

The project aims

- To unravel the undefined effects of OA-linked damage mediators on human MSCs via a detailed functional and molecular analysis.
- To test the effectiveness of targeting damage mediators as a novel approach for enhancing MSC chondrogenic and anti-inflammatory functions toward improving OA therapy.

The project objectives and techniques

- Assessment of damage mediator effects on MSC functions; This part of the study will be conducted at Nottingham Trent University (NTU), supervised by Dr Jehan El-Jawhari. An in vitro tissue culture technique will be utilised to maintain human MSCs derived from bone marrow and umbilical cord tissues. The work includes a specific assessment of the proliferation, tri-lineage differentiation, and pro/anti-inflammatory functions of MSCs when exposed to S100 proteins damage mediators, with/without oxidation.

- Investigating molecular mechanisms of damage mediators on MSCs: This work will be conducted at the University of Nottingham (UoN), supervised by Dr Wayne Carter. The mRNA sequencing, proteomics, and related signalling pathway analysis will be performed to investigate the mechanisms by which damage mediators affect MSC functions.

- Targeting damage mediator effects on MSCs: This part will be conducted at NTU and UoN to assess if and how using damage mediator-neutralising antibodies/inhibitors can reverse the aberrant effects of damage mediators on MSC functions and underlying molecular signatures.

The impact of the project

The project involves a plethora of cell and molecular biology skills and enriched knowledge joining the medicine and biosciences research. This research is an essential step toward improving the health of aged osteoarthritic joints. Revealing how damage mediators can cause a biased differentiation/function of MSCs will present a novel side of stromal cell biology and clarify hidden perspectives of OA pathogenesis. The data will potentially introduce a novel approach augmenting OA regenerative therapies and can be widely applied to treating other joint and musculoskeletal degenerative diseases.

Lab Rotation Location: Clifton Campus;Derby Royal Infirmary;

Full Project Location: Clifton Campus;Derby Royal Infirmary;

140 Use of Short Interfering RNA to Reduce Antimicrobial Resistance in MRSA

Lead Supervisor: Jonathan Thomas

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation Description: In June 2022, the handlebars of e-scooters from five sites around Nottingham were swabbed and bacterial contaminants grown on a non-selective medium. All morphologically distinct colonies were subsequently grown on mannitol salt agar to select for any staphylococci present. 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, biofilm assays and finally Illumina whole-genome sequencing of a subsample of these strains.

You will gain experience of classical microbiological techniques, sequencing library preparation and of the bioinformatics pipelines required to assemble and analyse a bacterial genome sequence.

Full Project Description: Staphylococcus aureus is a leading cause of hospital-acquired infections including bacteraemia, endocarditis and osteomyelitis and has developed resistance to a wide range of antimicrobial agents including methicillin, resulting in methicillin-resistant S. aureus (MRSA). In 2019, 1.27 million deaths globally were attributed directly to antimicrobial resistant infections, with 178,000 deaths caused by Staphylococcus aureus alone.

Short interfering RNA (siRNA) are synthetic non-coding RNA molecules, 20 to 25 nucleotides in length that can be used to downregulate the expression of genes of interest. Designed to bind selectively to target mRNA, siRNA interfere with translation of the targeted gene and reduce production of the encoded protein. When applied to genes encoding antimicrobial resistance (AMR)

determinants, use of siRNA may provide the potential to make strains susceptible to, or “rescue”, antibiotics for which there is already widespread resistance in a species, thereby reducing the need for novel antibiotics.

Recent work in our lab has used siRNAs to downregulate genes encoding AMR determinants in S. epidermidis, a close relative of S. aureus. This PhD project will seek to extend that work in both S. epidermidis and MRSA, designing siRNAs to target genes encoding both virulence and AMR genes while also examining the effect of using multiple siRNA in a single strain, targeted to both a single and multiple genes. As part of the project you will also perform Nanopore and Illumina sequencing of strains to determine their genomic composition, before selecting which AMR and virulence genes to target.

Having identified siRNAs that result in increased susceptibility of a strain to particular antibiotics, you will genetically modify a lysogenic phage to introduce these siRNAs to a strain’s chromosome and express them continually. This will require an investigation of the genetic diversity of staphylococcal bacteriophage, which will be the culmination of your project.

Overall, this project should provide the opportunity to gain experience with cutting-edge molecular biology techniques, while developing tools that could be used to combat antimicrobial resistance in species causing nosocomial infections.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;

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

Lead Supervisor: Warren Cross

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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

Area 2. peptide-drug conjugates

Area 3. covalent binding peptide probes.

Area 1. 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. 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. 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.**Lab Rotation Location:** Clifton Campus;

Full Project Location: Clifton Campus;University Park;

159 Surface modification of biomedical patient-specific cranial-and maxillofacial implants to enhance biocompatibility and host tissue integration

Lead Supervisor: Yvonne Reinwald

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation Description: The Candidate will enjoy a rota of multi-disciplinary techniques for testing the biocompatibility of surface modified polyether ether ketone (PEEK). Initially the lab rotation will commence with trial runs targeted towards testing the biological response of stem cells. Therefore, the student will be trained on stem cell culture, brightfield and fluorescence microscopy, and relevant biochemical assays in YR lab at NTU to learn how to grow human stem cells and to assess PEEK's biocompatibility. Then the candidate will have a meander on imaging techniques employed for monitoring the viability and biological response of stem cells in tissue culture formats, in the lab of Prof MM at UoN. The candidate will gain skills in multiple imaging modalities including multimodal diamond-based quantum microscopy. Finally, the candidate will be trained on operating high power laser apparatuses for treating the surface of PEEK with a view to modify the surface morphology and/or decorate it with nanoparticles or functional coatings in the lab of DK at NTU. The candidate will gain an insight to micro- and nano-technology fabrication, processing, and characterisation techniques, commencing familiarisation with laser micromachining (e.g., micro-drilling, micro ablation, etching), surface topography (e.g., AFM), surface roughness (e.g., contact angle and optical 3D profilometry).

Full Project Description: Cranial defects caused by trauma, diseases, infection, or malignancy, are either repaired naturally or through autologous, allogenic, or xenogeneic implants. These approaches bear numerous shortcomings, which custom cranial implants, 3D printing and novel biomaterials such as polyether ether ketone (PEEK) aim to overcome. PEEK implants are predicted to become a large industry in the future for orthopaedic, facial, and cranial surgeries and prostheses. Rapid prototyping patient specific cranial implants would increase the surgeon's satisfaction and patient's comfort.

This project is part of a multidisciplinary collaboration with researchers and clinicians from the UK and India. In a previous study, the biocompatibility of PEEK was assessed in comparison with standard tissue culture formats. Data have shown that PEEK is a very promising biomaterial when used for the culture of osteoblast-like cells.

Following on from this work, we propose to surface modify PEEK 3D printing filaments to enhance the material's biocompatibility. Therefore, a variety of surface modification techniques, including high-power lasers will be investigated to identify suitable processing conditions to create desired surface morphologies for enhanced biocompatibility. The biocompatibility of surface-modified PEEK will be tested by investigating the biological response of human mesenchymal stem cells. Cell responses will be evaluated via biochemical assays, histological stains, brightfield and fluorescent imaging as well as diamond-based quantum microscopy using Nitrogen Vacancy defects in diamond. This multimodal approach will provide a unique data set delivering insight into morphological,

structural, chemical, and magnetic properties of samples under study. With the use of reference grid spatially correlated imaging will be performed for intercomparison of imaging modalities.

Lab Rotation Location: Clifton Campus;University Park;

Full Project Location: Clifton Campus;University Park;

161 Pattern formation in cyanobacteria: the earliest organised life-forms

Lead Supervisor: Lucas Goehring

Lead School: School of Science and Technology (NTU)

DTP Research Area: Biotechnology

Lab Rotation Description: Cyanobacteria are one of most successful forms of life on Earth, with origins dating back over two billion years, and can form into complex aggregates called biofilms that lie at the border of unicellular to multicellular life. Despite their importance to the history of our planet, along with their commercial and environmental potentials for biofuel production, little is known about how cyanobacteria biofilms form, how their mechanical properties arise, or how these properties could be engineered.

In this rotation you will make use of advanced microscopy methods to map the development of cyanobacterial colonies as they grow from a few individual cells into a collective structure of a biofilm, as might be present on the surface of a stromatolite, or in the bed of a biodiesel bioreactor.

The cutting-edge imaging methods that you will learn allow for real-time, high-resolution and three-dimensional imaging of macroscopic biological materials, and will allow for unprecedented insight into the dynamical structure of biofilms. The results of the rotation are intended to form an integral part of the full PhD project, which will then explore how to modify or engineer these structures.**Full Project Description:** Cyanobacteria are one of the earliest and most successful forms of life on Earth, dating back over two billion years. They are ubiquitous in nature, finding habitats in most water bodies, and in extreme environments such as the polar regions, deserts, brine lakes and hot springs. They evolved the original mechanisms of photosynthesis, which transformed the early Earth and gave rise to our oxygen-rich atmosphere.

They have also evolved surprisingly complex collective behaviours that lie at the boundary between single-celled and multi-cellular life. For example, many species live in long chains of cells that bundle together into larger biofilms. They can form collective structures like stromatolites that provide a rigid, stable environment for their communities of bacteria. In addition, cyanobacterial biofilms can act as bioreactors, using photosynthesis to produce a wide range of green chemicals, including biofuels like biodiesel and ethanol.

Despite their importance to the history of life on Earth, and their commercial and environmental potentials for biofuel production, little is known about how cyanobacteria biofilms form, how their mechanical properties arise, or how these properties could be engineered.

In this project, you will investigate the biomechanics of filamentous algae, such as Oscillatoria (stromatolite-building species) and Anabaena (used for biofuels). These species consist of filaments one cell wide, and hundreds of cells long. They move by a slow gliding motion. Using state-of-the-art methods, allowing for real-time non-invasive imaging, you will characterise how the filaments move and change shape in response to their environment, including their interactions with other

filaments. As the filaments grow, you will also determine under what conditions they organise into rigid, elastic structures. By relating microscopic properties, like the stiffness or 'stickiness' of individual filaments, to their collective response, you will provide predictive models for the macroscopic properties of biomats. Various proteins have been implicated in biofilm formation, including filament formation; in order to understand their influence, you will create mutant lines to knock out these genes in cyanobacterial species to determine which are essential for biofilm formation, as visualized by microscopy. This research will then enable a more rational engineering of these properties in applications like bioreactor design.

This project is highly interdisciplinary and involves using dynamical systems methods – tools that have already had great success in describing phenomena like the flocking of birds, or swarming of insects – to the problems of the collective behaviour of microorganisms. In it, you will be supported by a broad, interdisciplinary team involving regular supervision by Lucas Goehring (Nottingham Trent, physics) who has an experimental background in the dynamics of complex systems, including modern and fossil biofilms, and Ellen Nisbet (Nottingham, microbiology) who examines the genetics of photosynthetic microbes. Additional guidance will be provided by Marco Mazza (Loughborough) who has extensive expertise in theory and modelling of soft and active matter (such as microbial motility), and from Sourav Ghosh (Loughborough/National Biofilm Innovation Centre) who is a multidisciplinary engineering scientist working on biofilms.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;Sutton Bonington Campus;

169 Post-translational regulation of RNA through ADP-Ribosylation

Lead Supervisor: Craig Doig

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation Description: The rotation work will involve key molecular and chemical techniques used in the study of RNA and ADP-ribosylation. These will include RNAseq, mass spectrometry and realtime recording of ADP-ribosylation using a novel split-luciferase system. The project will study cellular application of ADP-ribosylation to RNA species under conditions of stress. Consequences upon RNA stability, processing and protein abundance will be measured. Project will provide key training in these techniques and bioinformatic support.

Full Project Description: ADP-ribosylation is a post-translational modification of DNA, RNA and proteins. Identified over sixty years ago it is found to be central in the function of the PARP enzyme family. As a consequence, ADP-ribosylation is a determinant of our physiological state and shifts of it are attributed to various cancers, neurological diseases and metabolic dysfunction.

The RNA targets of ADP-ribosylation and their impact over biological behaviour remain unknown. This project will generate new data fundamental that can be used to generate treatments for diseases impacted by ADP-ribosylation. It will study how different RNA specifies carry ADP-ribosylation and define specific RNAs targeted by the PARP enzymes. Using a combination of murine and cellular models the contribution of this post-translational modification will be determined as well as its role in disease progression. This project will use state-of-the-art techniques and comprise

of a collaborative visit to University of Texas Southwestern Medical Center to work with global leaders in ADP-ribosylation biology.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;

178 Hybrid Multifunctional Scaffolds for Guided Tissue Regeneration: combating Periodontitis and Associated Infections

Lead Supervisor: Dmitry Volodkin

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation 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.

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 (CaCO3 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.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;University Park;

184 Chemogenetic silencing of astrocyte mediated disruption of the blood spinal cord barrier and sensory neurodegeneration

Lead Supervisor: Richard Hulse

Lead School: School of Science and Technology (NTU)

DTP Research Area: Bioscience for Health

Lab Rotation Description: An adequate blood supply is essential for maintaining a healthy and functional nervous system. A failure in coupling neuronal energy demands with capillary perfusion underpins neurodegenerative disease and long-lasting pain development. Adverse health related complications that occur more frequently in an aged and obese population. Our work highlights that high fat diet induces neurodegenerative mechanisms and pain by damaging spinal cord blood vessels. Vasomodulatory factors such as Vascular Endothelial Growth Factor-A and Angiotensin II control blood vessel health in the nervous system, and are released by surrounding mural cell types that closely interact with blood vessels. This includes astrocytes, which when activated are associated with damage to the sensory nervous system. During this lab rotation project, training in astrocyte isolation and cell culture will be provided to investigate how astrocytes are activated and how they influence blood vessel function. Calcium imaging of astrocytes will be measured alongside evaluating the astrocyte proteome in differing experimental environments including hyperglycaemia and adipokine rich media (adipocyte conditioned media). These experiments will provide insight into how the capillary network in the somatosensory nervous system responds to those underlying stress factors that initiate the onset of neurodegenerative disease and pain.**Full Project Description:** Neurodegeneration and pain are highly prevalent adverse health related complications in the United Kingdom 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 supporting capillary network i.e. tissue ischaemia (reduced blood flow). The microvasculature 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. 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 and microvessel health. Furthermore, astrocyte dysfunction (termed astrogliosis-astrocyte activation) is characteristic of neurodegenerative disease and is a pathological hallmark of damage to the sensory nervous system. Astrocytes are a potent source of inflammatory response, impacting upon vessel integrity and function. In rodent models fed high fat diet, the level of astrogliosis is elevated in the dorsal horn versus age matched sham controls, alongside presentation of chronic pain, increased expression of vasomodulatory factors and damage to dorsal horn blood vessels.

However, it remains unclear whether modulation of capillary perfusion through mediation of astrocyte activity is fundamental in the causation of sensory neurodegeneration and long-lasting pain. In this research project we propose to manipulate astrocyte activity specifically, through utilising a spatiotemporal mouse model to clearly delineate the role of astrocytes in the onset of sensory neurodegeneration at the level of the spinal cord and pain induction. By using virally delivered chemogenetic DREADD driven control of astrocyte activity in the spinal cord, will allow us to deduce specifically the processes by which astrocytes mediate disturbances in spinal cord vascular health to induce

Project Objectives:

Using chemogenetic control of astrocytes alongside rodents fed high fat diet, key factors associated with astrocyte mediated cessation of blood flow will be explored. Key objectives include (1) structural and functional evaluation of the spinal cord capillary network and (2) determine astrocytic mediated mechanisms that induce vascular degeneration and neuronal damage to cause pain.

Electron microscopy, confocal microscopy and intravital imaging of the spinal cord will be utilised to evaluate how the endothelium responds and interacts to astrocyte activation to underlie sensory neurodegeneration. Offline 4D computational image analysis will be performed to determined alterations in capillary structure, perfusion and permeability, inconjunction to determining proximity and abundance of astrocyte interaction. Virally delivered astrocyte fluorescent reporters will allow isolation of astrocytes to allow transcriptome evaluation in rodent models (DREADD activation, high fat diet) of astrocyte activation, to identify novel mechanisms that underpin sensory neurodegeneration and pain. The supervisory team have a diverse array of experience in vascular biology and neuroscience to evaluate those mechanisms outlined in this proposal. Training will be provided in primary cell culture, biochemical assays and invitro/in vivo assays to model blood flow to evaluate the interplay between astrocytes and endothelial cells in relation to capillary integrity.

Lab Rotation Location: Clifton Campus;QMC;

Full Project Location: QMC;Clifton Campus;

187 Investigation of the receptor Paqr9 in brown adipocytes and identification of its novel ligands

Lead Supervisor: Mark Christian

Lead School: School of Science and Technology (NTU)

DTP Research Area: Biotechnology

Lab Rotation Description: Analysis of the expression and subcellular localisation of Paqr9 in brown adipocytes

Brown adipocytes serve important roles in maintaining energy balance. Identification of the receptors that control the process of thermogenesis in brown fat has great potential for the treatment of metabolic disease. Paqr9 mRNA is highly induced in brown adipocytes following their differentiation. In this rotation you will culture brown adipocyte cell lines and analyse PAQR9 protein expression by Western Blotting as well as immunocytochemistry and confocal microscopy. This will allow assessment of the subcellular localisation of this receptor. You will also clone the Paqr9 gene into a plasmid expression vector to facilitate its labelling and expression in cell lines for the purpose of preparing a system for ligand screening. These approaches will provide key skills training in molecular biology techniques and generate research tools that will be applied in the PhD project.

Full Project Description: Brown adipose tissue is a healthy fat deposit that contributes to metabolic health by the capacity to generate heat through the process of thermogenesis. It is detected in adult humans and increases energy expenditure and fat burning following exposure to cold or after food intake. Interventions that increase brown fat activity can be used to reduce obesity. Therefore, identifying new pharmacological approaches to activate brown fat is of great scientific interest to enhance metabolic health.

This project will investigate the 7-transmembrane receptor Paqr9 (Progestin And AdipoQ Receptor Family Member 9) and its role in brown adipocytes along with identification of its ligands. Paqr9 is highly expressed in activated brown adipose tissue and present at lower levels in white adipose tissue which lacks the capacity for thermogenesis and when in excess is associated with poor metabolic health. Pilot data from the Christian lab reveal that Paqr9 is highly induced following the differentiation of brown pre-adipocytes to mature adipocytes. Furthermore, it is highly increased in

mice following cold exposure when brown fat is activated. In reproductive cells the steroid hormone progesterone can activate this receptor and there is evidence that it is G-protein-coupled. However, the ligands that activate Paqr9 in brown adipocytes and signal transduction mechanism have not yet been determined.

The overall aim of this PhD project is to understand the role of Paqr9 in brown adipocytes and identify ligands able to bind/activate it. This will be assessed in cell lines and tissues by applying a range of molecular techniques. The collaborative nature of project with the Kellam/Mistry labs at the University of Nottingham (UoN) brings complementary expertise in developing ligand-based chemical biology approaches for membrane receptors. Furthermore, the student will have access to the UoN Medicinal Chemistry Compound Collection (MCCC), comprising >80K drug-like compounds that can be dispensed for screening in well-based format. The existence of x-ray crystal structure (XRCS) for the related AdipoR1/AdipoR2 proteins also offers the opportunity to generate a homology model for Paqr9, against which the MCCC library could be virtually screened before validation through pharmacological assay.

The main aims are:

1) Determine the subcellular localisation of PAQR9 in brown adipocytes

2) Determine the role of PAQR9 in brown adipocyte differentiation and metabolic function

3) Generate a reporter cell line to screen for ligands that activate PAQR9

4) Generate a Paqr9 homology model based on the reported AdipoR1/AdipoR2 XRCS and use this to virtually screen the UoN MCCC

5) Use the reporter cell platform to validate UoN MCCC virtual hits

This project is multidisciplinary providing training in cell culture and molecular biology techniques such as q-RT-PCR, confocal microscopy, immunohistochemistry, siRNA knockdown, Western Blotting, gene cloning and mutagenesis. Virtual screening and hit identification. Cellular metabolic analysis will be determined using Seahorse bioanalysis.

The project will reveal the role of Paqr9 in brown adipocytes and through the identification of ligands for this receptor will have potential for therapeutic approaches targeted to adipocytes and other Paqr9-expressing cells and tissues.

Further reading:

McNeill BT, Suchacki KJ, Stimson RH. MECHANISMS IN ENDOCRINOLOGY: Human brown adipose tissue as a therapeutic target: warming up or cooling down? Eur J Endocrinol. 2021 May 4;184(6):R243-R259. doi: 10.1530/EJE-20-1439. PMID: 33729178; PMCID: PMC8111330.

Tang YT, Hu T, Arterburn M, Boyle B, Bright JM, Emtage PC, Funk WD. PAQR proteins: a novel membrane receptor family defined by an ancient 7-transmembrane pass motif. J Mol Evol. 2005 Sep;61(3):372-80. doi: 10.1007/s00239-004-0375-2. Epub 2005 Jul 21. PMID: 16044242.

Lab Rotation Location: Clifton Campus;

Full Project Location: Clifton Campus;University Park;

208. Fabrication and validation of ultrasensitive next-generation immune-biosensors; toward a potent tool for personalised medicine

Lead supervisor: Yasser M. El-Sherbiny

Lead School: School of Science & Technology

DTP Research Area: Bioscience for Health

Lab Rotation Description:

In this lab programme, the student will enjoy having a rota of multi-disciplinary techniques for building ultrasensitive next-generation immune-biosensor fabrication and validation; towards powerful tool for personalised medicine. First, lab rotation will focus on optics, optical biosensors strategies related lab skills. The student will be trained on the first-rate equipment at the Optics and Photonics group, UoN and develop optical fibre biosensors using long-period gratings. Including gaining skills in handling optical fibres, modifying the surface of the optical fibre, with the sensitive coating and calibration by exposure to the known concentrations of the target analyte as described in (https://doi.org/10.1016/j.rio.2021.100172).

Then student will focus on learning skills related to thin-film fabrication, laser fabrication of metal nanoparticles, plasmonics, optical characterisation of nanoparticle templates, structural characterisation of nanoparticles (SEM, AFM).

Then student will have to have a lab focus on immunological techniques and assays & Molecular biology skills, including related to biosensor validation and comparing gold standard immune detection techniques e.g. ELISA. this conveys cell culture, nucleic acid extraction, Molecular gene expression, e.g. RT-PCR, multiparameter immuno-phenotyping (FACS) laser analysis, Immunological biomarker functional assays, microscopy, and Electron microscopy imaging

Full Project Description:

All of the health disorders need the quick and reliable detection of minute amounts of analytes or chemicals. Traditional approaches have considerable limitations, such as time-consuming sample preparation and expensive instrumentation. As a result, faster and more sensitive procedures are necessary to overcome these drawbacks.

The need for innovative methods to detect and monitor the evolution of rheumatological illnesses as immuno-sensors for disease state, progression, and response to therapy remains a challenge.

In Interferon-mediated illnesses, this idea applies to the pre-Rheumatoid stage. No “Gold standard method” exists for detecting and monitoring IFN-mediated illnesses. In reality, all recent research has relied on molecular analyses of patient blood or tissue samples, which seldom represent the patient's true illness immunological state. These approaches have poor correlations with disease activity, which affects the therapy plan. Recent breakthroughs in predicting IFN status biomarkers permitted better patient classification and revealed predictive molecules for disease recurrence and progression (El-Sherbiny et al., 2019, El-Sherbiny et al., 2018).

The project is an excellent example of cross-disciplinary collaboration between biomedical sciences, engineering, and the healthcare sector to develop a solution to the problem of quickly and accurately detecting immunity signatures in patients without the need for sample collection and processing in the lab.

Photonics technologies are essential in the development of better biosensors and next-generation diagnostic tools for medicine. They have the potential to transform healthcare through enhanced sensing and as novel analytical tools in biological sciences. In the era of digital health, there is a high need for novel methodologies and medical equipment for illness monitoring, particularly in chronic disease states produced by difficult-to-trace mediators. As a result, it is expected that the validated biosensor/s prototypes created will have a major influence on clinical disease practice and therapeutic options.

Candidates who join this programme will be able to develop expertise and skills in a training programme that encompasses different disciplinary approaches in optics, physics, nanotechnology, and immunology in order to build prototypes of sensitive immunobiosensors and validate and develop their application for lab to industry and health sector applications. Presenting discoveries and outcomes at international conferences and in high-impact publications in world-renowned journals

References:

El-Sherbiny YM, Md Yusof MY*, Psarras A*, … Emery P, Vital EM. B cell tetherin: a flow-cytometric cell-specific assay for response to Type-I interferon predicts clinical features and flares in SLE. Arthritis Rheumatol. 2019 Dec 5. https://doi.org/10.1002/art.41187

Correia, R., James, S., Lee, S.W., Morgan, S.P., and Korposh, S. (2018). Biomedical application of optical fibre sensors. Journal of Optics 20(7), 073003-073003. DOI: 10.1088/2040-8986/aac68d.

Lab Rotation Location: University Park;Clifton Campus;

Full Project Location: University Park;Clifton Campus;

Nottingham Trent University – School of Animal, Rural & Environmental Sciences

204 Metaphenomics as a tool for assessing soil health

Lead Supervisor: Marcello Di Bonito

Lead School: School of Animal, Rural and Environmental Sciences (NTU)

DTP Research Area: Agriculture and Food Security

Lab Rotation Description: The activities will be carried out across the two institutions and provide an understanding of the challenges with the soil metaphenomic approach.

1 - NTU - ARES

Soils with different histories and landuse (e.g., pasture, no tillage, tillage, and forest soil as control) will be assessed and defined as a habitat in the field. Soils will be sampled, prepared, archived and quality controlled to measure key physical and chemical indicators, including: structure, texture, temperature and water content; C, N, and S pools and fraction (by Organic Elementar analyser vario EL cube).

2 - UoN - SB

Classical soil microbiological techniques will be utilised to determine soil biological processes (e.g. soil enzyme assays), microbial biomass (using chloroform-extraction methods followed by chemical analyses for microbial C, N and P), ergosterol measurements to evaluate fungal presence and arbuscular mycorrhizal fungal quantification through root staining procedures. Metabolic potential will be measured using Ecoplates as an introduction to soil metabolic processes.

3 - NTU - SST

Sequencing libraries will be prepared from DNA extracted from soils samples by PCR of the V3V4 region of 16S rDNA, and quality control checked using an Agilent tape station. Microbial taxa will be classified using specialist R metataxonomic packages.

Full Project Description: This project will investigate the use of metaphenomics approach to provide a better understanding of complex soil systems in different environmental conditions. Metaphenome is defined as the product of expressed functions encoded in microbial genomes (metagenome) and the environment (resources available; spatial, biotic and abiotic constraints). The soil metaphenome is dependent on the combined genetic potential encoded by the soil member genomes, the physiological status of the member populations, their access to resources, contact with other organisms and signalling molecules, combined with their genetic capacity to respond to environmental cues. We will use an integrated multiomic approach to evaluate the physiological responses produced by the soil's microbiome to external conditions as well as their pathway of interactions with plants and/or microorganisms. We will examine a variety of soil types, according to their classification, landuse, and health status by using state of the art untargeted Mass Spectrometry, Nuclear Magnetic Resonance (NMR) spectroscopy and Stable Isotope techniques and mapping these characteristics across the soil spectrum, both spatially, temporally and functionally.

Soil metaphenomics has recently demonstrated the potential to reveal the complex molecular network and metabolic pathways operating in the soil microbial communities and a means of evaluating soil function. It is an important area of research in ecology and environmental sciences that can be applied to agricultural sciences because it could offer tools and answers for soil conservation, food security (e.g., crop yields and nutrient value), climate change (e.g., soil respiration), greater biogeochemical cycles (C, N, micronutrients), and ultimately ecosystems functions (soil health). However, soil metaphenomics presents unique challenges, because soil spatial complexity and highly dynamic distribution of nutrients (C, N, S, and micronutrients) and other resources can result in hot spots for growth of microbial consortia, for example within micro-aggregates and/or the rhizosphere. Understanding the fine scale distribution of microbes and resources is required to predict species physiology and metabolic interactions among community members, that comprise the collective soil metaphenome.

This project addresses several existing knowledge gaps and research priorities all the above themes.

In order to investigate the use of metaphenomics on soils, we need to deliver three aims:

1. Understand how the use of metabolic models can help to predict phenotypic responses of micro-organisms to different environmental conditions.

2. Build relevant databases to fill gaps on metabolite composition of soils.

3. Begin to map metabolite composition of soils across different soil types and conditions.

The proposed project/proof of concept will use sensitive mass spectrometry platforms to predict the identities of metabolites, which will be combined with advanced computational approaches, and help increasing the number of soil metabolites assignments in databases.

We will achieve our aims by carrying out three objectives:

1. Test the validity of flux-based analysis (FBA) on different types of soils to decipher specific metabolic pathways (in specific microbial communities).

2. Assess stable isotope probing (SIP) and multi-omics approaches to assess functions carried out by members of soil microbiome that result in the soil metaphenome.

3. Assess soil-specific genera/species presence by sequencing regions of 16S rRNA.

Lab Rotation Location: Sutton Bonington Campus;Brackenhurst Campus;Clifton Campus;

Full Project Location: Brackenhurst Campus;Clifton Campus;Sutton Bonington Campus;

Nottingham Trent University – Department of Engineering

61 Hybrid Optical Materials for Non-Invasive Early-Stage Disease Diagnosis

Lead Supervisor: Mohsen Rahmani

Lead School: Engineering

DTP Research Area: Biotechnology

Lab Rotation Description: Initially, the student will learn common experimental techniques for four weeks. He/she will be familiarised with the facility at NTU and UoN for fabricating nanostructures, 100s of times thinner than a human hair and microscopy of them. Subsequently, at NTU, the student will be involved in one of the ongoing projects on fabricating the nanoscale sensor via electron beam lithography and characterisation of the sensor via a homemade Koehler illumination system. Koehler Illumination is a process that enables maximum contrast and resolution by focusing and spreading the light path evenly over the field of view. The student's involvement in this project will familiarise the student with diagnosis techniques via nanoscale sensors. It will expose the student to

appropriate techniques for experimental data analysis, storage and retrieval.

Full Project Description: STATE-OF-THE-ART AND SIGNIFICANCE:

Chronic diseases are responsible for as many as 60% of annual deaths worldwide [Heal. Expect.18, 312–324 (2015)]. Today, most chronic diseases can only be diagnosed via blood analysis, which requires specialised training and expensive laboratory facilities. However, recent studies have shown that breath contains biomarkers associated with several diseases such as diabetes [J. Breath. Res.8,014001(2014) & Sci. adv.5,eaax0649(2019)] at very early stages of disease development. However, due to the ultra-low concentration of such biomarkers in breath, current technologies cannot measure them with sufficient accuracy to replace blood analysis.

In this PhD project, we will push the frontier of biosensor technology via developing ultrasensitive optical materials. We will draw inspiration from an existing widely available technology that uses metallic nano-particles to achieve ultra-high sensitivity: home pregnancy tests. Metallic nano-

particles exhibit strong local near-field enhancements that are very sensitive to the change of environment. Thus, when exposed to a high concentration of biomarkers trapped in a liquid medium, such as urine for pregnancy testing, they induce a change in the colour of scattered light that can be detected. However, these near-fields' short propagation distances, combined with ohmic losses of metallic nano-particles have created a bottleneck for further applications.

In this project, hybridised metallo-semiconductor nano-particles will be developed to detect ultra-low concentrations of biomarkers. Metallic nano-particles enhance the optical near-fields, and semiconductor nano-particles extend and transfer the near-fields while reducing losses. This combination both greatly enlarges the sensing area and significantly enhances sensitivity. We have already published proof-of-principle works on this technique in 2D [Adv. Mater.30,1800931(2018)] and 3D [Adv. Mater.32,2002471(2020)] scaffolds. Modelling and simulation suggest that engineering hybridised scaffolds can significantly enhance sensitivity. This innovation will be extended, developed, and demonstrated experimentally during this PhD project. The targeted disease will be type II diabetes, which produces known biomarkers in bodily fluids at early stages [Diagnostics8,12(2018)].

Lab Rotation Location: Clifton Campus;University Park;

Full Project Location: Clifton Campus;University Park;