Nottingham BBSRC DTP

Project List 2020

For interview candidates

Contents

Introduction

Dear Candidate,

We are looking forward to welcoming you to your interview for the Nottingham BBSRC DTP. Prior to your interview, we ask that you review this list of projects and identify three projects that you would be interested in undertaking should you be offered a place on the programme. You should do this using the Confirmation of Attendance form by midnight on Sunday 8th March.

Please note that indicating your preference for a project at this point does not guarantee that you will be allocated this project should your application be successful – formal project allocation will take place in summer 2020. This project listing is subject to change.

This booklet contains all of the projects currently on offer for students enrolling on the BBSRC DTP in September 2020. They have been sorted by research area. Some projects are listed in multiple research areas as appropriate. As a DTP student, you would undertake two lab rotations, and you will find that some projects indicate that they are linked to another – this is to indicate where two lab rotations would work especially well together as preparation for a PhD project.

If you would like to find out more about a project, please look up the supervisor on the relevant institution's website, where you will find their staff profile and an email address. Supervisors will be very happy to answer any questions you may have.

Enjoy exploring the projects.

Best wishes,

The Nottingham BBSRC DTP Team

Ageing

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(24) First 3D isogenic model for the study of age-related bone loss

(26) Identifying genetic determinants ensuring correct DNA damage repair pathway choice

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(31) Role of post-translational modifications in DNMT3B activity

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(35) Development of a non-invasive imaging toolbox for investigating muscle physiology and its application to peripheral vascular disease

(42) Inhibition of mechanistic target of rapamycin (mTOR) as a novel therapeutic for maintaining skeletal muscle mass during ageing

(43) The protein secrets of ancient teeth

(47) Epigenetic and epitranscriptomic networks in gene regulation and cancer

(49) The role of E2F7/8 on HIF-1 and cellular response during hypoxia

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(95) Neuroinflammatory responses across the lifespan: Role of Nuclear Receptor SUMOylation

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(105) PARP-1 roles in age-related skeletal muscle metabolic decline

(116) The role of monoamine oxidases (MAOs) in selective vulnerability of brain cells - implications for healthy ageing

(125) mRNA methylation and complex components within healthy, developing and diseased the cardiovascular system

(128) The role of circadian clock genes in cognition

(139) Defining how the spinal cord endothelial cell modulates pain perception

(141) Tuning dietary fibre structure and composition to improve the glucose absorption curve of starchy foods

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(10) Pause for thought: Corticostriatal dopamine and the inhibitory modulation of associative learning

Animal Health

(5) Impact of variation in equine influenza A virus pathogenicity determinants on vaccine effectiveness and interspecies transmission

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(19) Impact of tetracyclines on AMR in race horses

(21) Left-right asymmetry in Hawaiian 'Looking-glass' snails

(41) Induction of autophagy by important animal and human viruses

(53) The genomic and phenotypic basis of a biological invasion

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(142) Drug targets under stress: post-transcriptional regulation of RTKs

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(59) Identifying predicative patterns of activity in spinal pain circuitry characteristic of chronic pain and plasticity

(63) Role of non-coding RNAs in the development of somatosensory systems

(89) Dynamic design and bottom-up assembly of complex 3D micro-environments: Construction and study of the bone fracture callus

(90) The effects of physical activity and social enrichment on healthy brain ageing

(117) Using isogenic induced pluripotent stem cells-derived organoids to model triple negative breast cancer

(133) Elaborating the interactions between mesenchymal stem cells and immune cells in non-union bone fractures using a novel 3D printed in vitro model

(119) Protein tubes for nanodevices

(157) Development of theranostic agents for combined multimodal imaging and targeted therapy

(161) Mapping the 3-Dimensional Architecture of Human Oocyte Chromosomes

(10) Pause for thought: Corticostriatal dopamine and the inhibitory modulation of associative learning

Project Details

(4) Exploring the immune instructive potential of glycosaminoglycans

Primary supervisor: James Hook

Second supervisor: Amir Ghaemmaghami

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

Glycosaminoglycans (GAGs) are a key component of extracellular matrix (ECM) involved within many biological processes including self-renewal, differentiation, growth, protein sequestration, mechanosensing and water retention. In the nine week project, the ability of these molecules to instruct the attachment of monocytes will be explored, with a key aim of optimising a high throughput screening methodology to assess the cellular response through the creation and characterisation of microarrarys of GAGs. A combinatorial library of the six main glycosaminoglycans (chondroitin sulphate, heparan sulphate, keratan sulphate, hyaluronic acid, dermatan sulphate and heparin) will be prepared using non-contact printing using protocols already established at the University of Nottingham within the laboratory of Dr. Hook. This will allow for hundreds of different GAG combinations to be generated rapidly. This array of materials will be assessed for differential attachment responses of monocytes using high content imaging. Monocytes will be isolated (using magnetic cell sorting) from Peripheral blood mononuclear cells (PBMCs), obtained from buffy coats. The cell culture methods are established within the laboratory of Prof Ghaemmaghami. Monocyte attachment and their differentiation to macrophages will be analysed using brightfield and fluorescent microscopy.

Full PhD project description:

Macrophages play a central role in immune regulation and maintaining tissue homeostasis in health and disease (e.g. tissue injury and inflammation). They can adapt a spectrum of different functional phenotypes in response to microenvironmental cues. Such plasticity provides many opportunities for modulating macrophage phenotype thorough changing their environment. Historically there has been a heavy reliance on the use of growth factors and cytokines to influence macrophage behaviour and promote a particular phenotype. However, there is increasing appreciation that other environmental cues could be equally efficient in modulating macrophage phenotype and function. The main aim of this project is to investigate the ability of different Glycosaminoglycans (GAGs), a key component of extracellular matrix (ECM), to modulate human macrophage phenotype and function. GAGs are involved in numerous cell signalling and biological processes and, due to variation in chain length and sulfation patterns, have greater information carrying capacity than the more commonly studied biological polymers, nucleic acids and proteins. However, the use of these biomolecules for biomedical applications is limited due to their complexity

We hypothesise that certain combinations of GAGs could promote generation of pro or anti-inflammatory macrophages. To examine this hypothesis first we will develop a combinatorial library of GAGs (different combinations and ratios) followed by screening their ‘immune-instructive’ ability using a high throughput screening (HTS) strategy. We will seek to explore different combinations of GAGs and include materials with varied molecular weight and sulfation patterns. GAG arrays will be

prepared using non-contact, piezo-driven ink-jet printing to rapidly produce thousands of unique combinations of GAGs on a single glass slide. In particular, this method will enable spots to be mixed in situ, providing a significant reduction to the time required to prepare different solutions. The large number of GAG-cell interactions that HTS will produce will enable the selection of an optimal immuno-instructive GAG composition despite the inherent complexity of these biomolecules.

Monocytes, isolated from peripheral blood mononuclear cells, will be seeded on GAG arrays followed by high content imaging to assess monocyte attachment and their differentiation to macrophages over 6 days. Macrophages polarisation status will be determined using staining for surface markers and intracellular staining for pro and anti-inflammatory cytokines. Any 'hit' (immune-instructive) GAG combinations will be scaled from the array format and used in more detailed functional studies (e.g. phagocytosis, bacterial clearance and wound healing). This will enable assessing the function of pro and anti-inflammatory macrophages beyond surface marker expression and cytokine production.

Identifying novel combinations of different GAGs that are able to induce a desirable immune-responses could pave the way for using them therapeutically in a diverse set of applications including wound healing, inflammatory diseases and medical device filed where controlling macrophage phenotype has been shown to be crucial in determining the clinical outcome. This project will particularly focus on the area of wound healing and, if appropriate, will seek to test the research outcomes within animal wound models.

References to learn more:

1- Engineering Immunomodulatory Biomaterials To Tune the Inflammatory Response. Vishwakarma A, Bhise NS, Evangelista MB, Rouwkema J, Dokmeci MR, Ghaemmaghami AM, Vrana NE, Khademhosseini A. Trends Biotechnol. 2016 Jun;34(6):470-482.

2- Construction and characterisation of a heparan sulphate heptasaccharide microarray. Yang, J., P.-H. Hsieh, X. Liu, W. Zhou, X. Zhang, J. Zhao, Y. Xu, F. Zhang, R. J. Linhardt and J. Liu. Chemical Communications 2017;53(10):1743-1746.

Location of lab rotation: University Park

Location of full PhD project: University Park

(5) Impact of variation in equine influenza A virus pathogenicity determinants on vaccine effectiveness and interspecies transmission

Primary supervisor: Janet Daly

Second supervisor: Toshana Foster

Institution: University of Nottingham

School: School of Veterinary Medicine and Science

Lab rotation description:

Equine and canine influenza A virus gene sequences available at GISAID (an online database of influenza virus sequences) will be downloaded, aligned and analysed to identify mutations that may be associated with changes in pathogenicity. The analysis will focus on genes known to be associated with pathogenicity traits. Canine sequences will be included to study changes that may be associated with adaptation to a new host because there are only a few sequences available from the 1960s when the H3N8 equine influenza virus first emerged in horses. In addition, strains isolated during major epidemics will be compared with inter-epidemic strains.

Full PhD project description:

Influenza A viruses (IAV) are believed to have circulated in horses for centuries and to have crossed species barriers to infect humans in the past. In 2005, the first evidence for transmission of equine influenza A virus (eqIAV) to dogs was reported; the virus subsequently became endemic in the USA. We retrospectively demonstrated that eqIAV had also jumped from horses to foxhounds in the UK in 2002, but did not become established. Experimental infection of ponies with an eqIAV strain isolated in 2003 confirmed the more severe clinical signs seen (including prolonged coughing) than with earlier isolates. This was associated with greater induction of pro-inflammatory cytokines. Genome sequencing of the 2003 strain revealed a truncation in the viral NS1 protein, which modulates the host cell cytokine response. Using reverse genetics to switch the NS gene segment, we confirmed that the truncated NS1 induced a greater cytokine response in infected cells. This suggests that the species jump occurred due to emergence of an eqIAV strain with increased pathogenicity. Interestingly, the NS1 truncation was lost on adaptation to dogs. Vaccines against eqIAV have been available since the late 1960s and vaccination of UK racehorses has been compulsory since 1982. There is a process for updating vaccine strains but there continue to be periodic major epidemics of equine influenza – these have occurred in 1963, 1979, 1989, 2003 and 2019. These major epidemics are usually associated with some deaths among unvaccinated horses. Variation in the haemagglutinin surface glycoprotein as a cause of vaccine breakdown has been extensively studied. The aim of this project is to investigate the role of variation in other viral proteins that determine the pathogenicity of IAV in vaccine breakdown and interspecies transmission.

Reverse genetics is a technique where a recombinant influenza virus can be generated by transfecting eight plasmids each encoding one of the eight viral gene segments into mammalian cells in vitro. This allows specific changes between viruses to be studied individually, for example by swapping only the gene segment encoding NS1 on an ‘isogenic’ background. This technique will be complemented by bioinformatics analysis to compare the genes that determine the pathogenicity of IAV in epidemic eqIAV strains with inter-epidemic strains. The ability of viruses in which genes have been swapped or mutated to replicate and induce or resist pro-inflammatory cytokine responses will be tested in cell culture. The use of established techniques and involvement of collaborators with

complementary skills should ensure any challenges can be addressed and the appropriate risk assessment for this work is already in place.

Identification of patterns associated with the emergence of an epidemic strain could improve the ability of the World Organization for Animal Health (OIE) Expert Surveillance Panel to pre-empt the next equine influenza epidemic.

References to learn more:

1. Woodward A, Rash AS, Medcalf E, Bryant NA, Elton DM. Using epidemics to map H3 equine influenza virus determinants of antigenicity. Virology. 2015;481:187-98.

2. Newton JR, Daly JM, Spencer L, Mumford JA. Description of the outbreak of equine influenza (H3N8) in the United Kingdom in 2003, during which recently vaccinated horses in Newmarket developed respiratory disease. Vet Rec. 2006;158(6):185-92.

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

This is a linked project – we recommend you select project 9 alongside this one.

(8) Project title Synthesis and Evaluation of Harmonine and Analogues for Inhibition of NMDA Receptors

Primary supervisor: Robert Stockman

Second supervisor: Ian Mellor

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

The initial few weeks of the rotation will involve the synthesis of the mixture of harmonine isomers using the synthesis already developed within the Stockman labs.

The student will assess the activity of the mixture of harmonine stereoisomers against NMDA receptors (Mellor lab). This will be important in determining whether both or one of the isomers are active. NMDA receptors containing GluN1-1A and GluN2B receptors will be expressed in Xenopus laevis oocytes through injection of cDNA and subjected to voltage-clamp analysis. Glutamate or NMDA activated ionic currents will be assessed in the absence and presence of harmonine to measure its inhibitory potency; we already have evidence that an alkaloid extract from Harmonia axyridis (harlequin ladybird) containing predominantly (ca. 90%) harmonine is a strong inhibitor of NMDA receptors.

Full PhD project description:

Dementia is a growing human problem and has a massive financial impact due to cost of caring for sufferers. Alzheimer's disease accounts for the majority of dementia cases and at present is lacking suitable treatments. The only options are memantine that is an inhibitor of NMDA receptors and is thought to reduce glutamate mediated excitotoxic cell death, or several acetylcholinesterase inhibitors that improve cognitive function through elevation of acetylcholine levels to combat the deficit of cholinergic neurons. The success of these treatments is quite variable and not always long-lasting.

Our preliminary work using extracts from Harmonia axyridis ladybirds where the major component is harmonine, have indicated strong inhibition of NMDA receptors and this has been confirmed using an almost pure fraction containing harmonine. However, purification of harmonine has proved particularly difficult and produced only very small quantities of the compound, hence the need for synthetic production of the natural compound and the possibility of studying structural analogues. It is known that harmonine is also an acetylcholinesterase inhibitor, thus combining two beneficial therapeutic properties in a single compound. The aim of this project will be to devise an efficient synthesis of harmonine in a way that will also allow the generation of structural analogues and to investigate their mechanism and site of inhibition.

The Stockman group has previously developed a synthesis of a mixture of harmonine stereoisomers. The project will build on this prior work, and investigate cis-selective olefin metathesis (using recent work by Hoyveda), and also investigate other approaches – e.g. ring-closing alkyne metathesis followed by Lindlar reduction. The synthetic approaches will then be used to probe the SAR of harmonine. The synthesis is inherently flexible, thus many alterations can be made, including chain lengths, position and stereochemistry of the alkene, and range of groups around the amines. The total synthesis of harmonine and analogues will provide a thorough training in synthetic chemistry.

(Two previous collaborations on total synthesis / biological evaluation between Stockman and Mellor have proven their track record for producing high quality publications)

We will use an electrophysiological approach to assess the potency of harmonine / analogues against NMDA receptor functioning and to determine their mode and site of action, essential for therapeutic development of these compounds. Human NMDA receptors will be expressed in the Xenopus oocyte subjected to voltage-clamp analysis to measure ionic current in response to glutamate or NMDA in the absence and presence of harmonine and analogues. We will focus our study on GluN1-1a/GluN2B subunit-containing receptors that are considered to be important in mediating excitotoxic cell death. This approach will also enable us to examine several subtypes of NMDA receptors as well as mutants of these subtypes. We will also assess activity at other ionotropic receptors such as AMPARs, nAChRs and GABAARs, and voltage-gated ion channels, all of which are essential components in neurotransmission; this will inform us about selectivity of the compounds. This part of the project will provide the student with extensive training in electrophysiological techniques.

References to learn more:

1. Rosini, M., Simoni, E., Caporaso, R., Basagni, F., Catanzaro, M., Abu, I.F.*, Fagiani, F., Fusco, F., Masuzzo, S., Albani, D., Lanni, C., Mellor, I.R. & Minarini, A. (2019) Merging memantine and ferulic acid to probe connections between NMDA receptors, oxidative stress and amyloid-beta peptide in Alzheimer's disease. Eur J Med Chem, 180, 111-120.

2. Synthesis Of Natural-Product-Like Scaffolds In Unprecedented Efficiency Via A 12-Fold Branching Pathway, D. Robbins, A. F. Newton, C. Gignoux, J.-C. Legeay, A. Sinclair, M. Rejzek, C. A. Laxon, S. K. Yalamanchili, W. Lewis, M. A. O'Connell and R. A. Stockman, Chemical Science, 2011, 2, 2232-2235.

Location of lab rotation: University Park

Location of full PhD project: University Park

(9) Functional characterisation of the molecular interactome of emerging viral haemorrhagic fever arenaviruses

Primary supervisor: Toshana Foster

Second supervisor: Janet Daly

Institution: University of Nottingham

School: SVMS

Lab rotation description:

The Foster lab works on host restriction of arenaviruses and on identifying key interactions involved in virus pathogenesis. This research will map the complex pathways that are responsible for potentiating and antagonising arenavirus infection and functionally characterise key molecular interactions.

During the 9 week rotation, the student will express nucleoprotein (NP) and matrix (Z) proteins of arenavirus strains, including Lassa, with a fluorescent tag in mammalian cells. The cellular localisation of these tagged proteins will be analysed and the biotin-ligase tagged proteins purified from cells in preparation for mass spectrometry. Preliminary mass spectrometry data have identified interactions NP of the Lassa strain; these interactions will be verified by immunofluorescence and immunoprecipitation techniques.

Weeks 1-2: Cell culture techniques and transfection

Week 3: Protein expression in mammalian cells- detection by Immunofluorescence, western blotting

Week 4-6: Purification trials from mammalian cells, coomassie staining, data analysis

Weeks 5-7: Validate key interaction by immunofluorescence and co-immunoprecipitation

Week 8-9: Data compilation, analysis and write-up

Training will be provided in cell culture (sterile technique) and protein expression, protein detection methods (immunofluorescence and western blotting), immunoprecipitation and data analysis. There will also be the opportunity to present the project at lab meetings and to the wider 'One Virology' group.

Full PhD project description:

The recent Ebola and Lassa fever epidemics highlight the immense impact human viral haemorrhagic fevers (VHFs) have on human health and on the socio-economic status of the developing world. Arenaviruses are the largest family of VHF-causing viruses; they have worldwide distribution and are endemic in South America and West Africa, mainly in Sierra Leone, Liberia and Nigeria. The current outbreak by the most common arenavirus, Lassa, in Nigeria, makes it imperative to understand the molecular basis of viral pathogenesis and immune evasion, to identify factors that drive viral emergence and to identify drug targets.

The Arenaviridae family is divided into Old World (OW - endemic in West Africa) and New World (NW - endemic in South America) viruses based on their phylogeny, geographical distribution and serological cross reactivity. Arenaviruses cause persistent infections in their natural rodent hosts and viral transmission to humans occurs through direct contact with infectious materials or exposure to

rodent urine/faeces. Fatality rates are extremely high; there is no vaccine and the few therapeutic options are ineffective. The overarching aim is to map the complex pathways that are responsible for potentiating and antagonising arenavirus infection and, importantly, explore the molecular basis of host adaptation.

Despite possessing a small genome and encoding for only 4 proteins, arenaviruses have developed strategies to maintain high levels of replication and avoid host immune responses, implying multiple viral and host protein interactions. Thus far, few host cell protein interactions have been identified. Current knowledge of the modulation of immune factors is restricted to the viral nucleoprotein (NP) and matrix protein (Z) and their inhibition of type I interferon (IFN) induction. Given, the involvement of these proteins in early to late steps of the viral life cycle, it is unlikely that their immunosuppressive function is exclusively limited to this mechanism. Furthermore, they possess structural characteristics that could be fundamental to their ability to modulate different viral processes central to viral spread.

In order to unravel the arenavirus interactome and the differential mechanisms between OW and NW viruses, the student will aim to:

1) develop proteomic screens for the identification of novel interacting partners of NP and Z. Developing these methods will reveal novel co-factors that define pathogenicity differences. Mutational analysis of NP and Z will inform experiments that can delineate differential mechanisms used by pathogenic and non-pathogenic viral strains. Validation of identified protein interactors will involve CRISPR/Cas9 knockout and CAT4 virus assays conducted via collaboration in Marburg.

Linked to this will be the use of structural methods, i.e. X-ray crystallography and NMR, to

2) elucidate the molecular details of the interactions of NP and Z with host factors; exploiting these interactions is key in the application of this research to the design of therapeutics. Structural information will define the molecular details of novel host interactions and will inform the future design of effective therapeutics. NP and Z are highly amenable for structural studies and interactions revealed in Aim 1 will be exploited to obtain protein complex structures.

References to learn more:

1. Zapata, J.C.; Salvato, M.S. (2013) Arenavirus Variations Due to Host-Specific Adaptation. Viruses 5, 241-278.
2. Yun, N.E.; Walker, D.H. (2012) Pathogenesis of Lassa Fever. Viruses 4, 2031-2048

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

This is a linked project – we recommend you select project 5 alongside this one.

(12) Developing tools for wild relative introgression into wheat where recombination is not possible

Primary supervisor: Surbhi Grewal

Second supervisor: Julie King

Third supervisor: Michael Wilson

Fourth supervisor: Vladimir Nekrasov

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

The student will gain experience on 3 aspects of wheat breeding namely wide crossing techniques, molecular marker development and cytogenetics, all of which are key to the PhD project.

To save on time, we will grow the parental wheat lines which the student will cross to obtain F1 plants. This will give the student an insight into wheat spike morphology and the techniques of emasculation and pollination (3 weeks).

The mini-project will also include development of Kompetitive Allele Specific PCR (KASP) markers between chromosome 4B of wheat and chromosome 4S of its wild relative Aegilops sharonensis. These markers will allow the detection of wild relative introgressions in F1 lines and this will be done by testing existing wheat-wild relative KASP markers and/or developing new ones through sequence analysis (3 weeks).

The student will also be given some wheat-Ae. sharonensis F1 lines (at flowering stage) to study chromosomal integrity/breakage during pollen mitoses using genomic in-situ hybridisation (GISH) and microscopy. This work will provide familiarity with various mitotic stages and wheat chromosome structure (3 weeks).

All these techniques will be used to answer a specific scientific question related to the main PhD project: Does the dosage of wheat chromosome 4B have an important effect on the amount of chromosome damage during gametocidal action?

Full PhD project description:

In the past, the wild relatives of wheat have been successfully exploited as a novel source of genetic variation for traits in wheat breeding programmes. In brief, wheat/alien introgression involves the hybridisation of wheat with a wild relative followed by repeated backcrossing to generate lines of wheat carrying a wild relative chromosome segment on which a target gene is located. If the wheat and wild species chromosomes have similar gene order they can exchange genetic material through recombination, as has been shown successfully at the Nottingham BBSRC Wheat Research Centre (King et al., 2017; Grewal et al., 2018).

However, many wild relatives have rearranged their chromosomes relative to that of wheat making gene transfer difficult, if not impossible, via recombination during meiosis. This reduces significantly the numbers of wild species that can be exploited for wheat/alien introgression. What we want to do is to exploit special genes, known as gametocidal (Gc) genes that are found in some wild species and transmit preferentially to the offspring. Gc genes induce chromosomal breakages which

frequently result in translocations, or exchanges, between the chromosomes of wheat and those of the wild species (King et al, 1991). This strategy provides an alternative route for the transfer of genes from chromosomes of wild species into wheat.

We have identified a simple and rapid assay to isolate mutants at the Gc locus that was introgressed from chromosome 4S of Aegilops sharonensis and translocated to wheat chromosome 4B. One putative EMS (ethyl methansulfonate)-induced Gc mutant was identified.

The specific objectives of the proposed research are to: 1. identify additional mutants at the Gc locus, 2. determine the location of the Gc locus on the 4S segment through sequence analysis of the Gc mutant(s), 3. identify differentially expressed genes in the Gc mutant(s) via RNA-seq, and 4. develop Bobwhite wheat lines carrying the Gc locus for CRISPR/Cas knockout (KO) of candidate genes in collaboration with US Department of Agriculture (USDA). The proposed research is a crucial first step towards the molecular understanding of Gc function, which eventually will lead to the cloning of this gene or gene complex for future use in wheat breeding.

To achieve these objectives, the students will initially spend time making wide crosses between different wheat lines and mutagenizing the resulting seeds with EMS. The progeny will be screened for mutants with a variety of techniques such as phenotypic scoring (visual analysis of spike fertility), cytogenetics (GISH), microscopy (analysis of chromosomal breakage during pollen mitosis) and molecular markers (KASP assays; Grewal et al., 2019). The putative mutants will be sequenced (in collaboration with USDA) and common genes with mutations will be identified through Next–Gen sequencing (NGS) bioinformatics. In another approach to identify candidate genes for the Gc locus, the student will use RNA-seq to analyse differential gene expression between the Gc mutant and non-mutant lines.

There will be an opportunity to visit Rothamsted Research where our project partner has key expertise in applying genome editing technology (CRISPR/Cas) in plants. The student will learn gene cloning techniques while making KO constructs for a few candidate genes for the Gc locus.

To enable downstream functional studies, using the KO constructs, we need the Gc locus carrying segment from Ae. sharonensis to be present in a wheat background that has high-efficiency of wheat transformation. As such, the student will transfer this segment, via crossing, into Bobwhite wheat to produce introgression lines ready for transformation with KO constructs (will be done by project partners in USDA) targeting candidate genes for Gc locus.

Grewal S. et al. 2018. Theor. Appl. Genet. 131 (2) 389-406

Grewal S. et al. 2019. Plant Biotechnol. J. doi: 10.1111/pbi.13241

King, I.P. et al. 1991. Genome 34:944–9

References to learn more:

1. Grewal S, Gardiner L, Ndreca B, Knight W, Moore G, King I.P, and King J (2017) Comparative Mapping and Targeted-Capture Sequencing of the Gametocidal Loci in Aegilops sharonensis. Plant Genome 10(2)
2. Friebe, B., P. Zhang, S. Nasuda, and B.S. Gill (2003) Characterization of a knock-out mutation at the Gc2 locus in wheat. Chromosoma 111:509–517.

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(13) The DEEP-C: Realizing the potential of subsoils for enhanced carbon storage

Primary supervisor: Sacha Mooney

Second supervisor:

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

Following sampling on the UoN experimental farm on two different soil types, the student will determine the amount, quality and spatial distribution of Soil Organic Carbon (SOC) as a function of soil depth and its relationship to the size, composition and activity of the soil microbial community (training by Neal) and chemistry of the mineral fraction. This will take around 2 weeks to complete. The student will then use X-ray Computed Tomography (CT) (training by Mooney) to map the spatial heterogeneity of SOC within the samples (soil aggregate scale) using a new OsO4 staining method. A similar method has already been demonstrated on synchrotron images, but this would be the first time it would be proven from benchtop CT images. We have proof of concept to confirm this is possible (i.e. the OsO4 allows SOM to be segmented in soil images) but a new imaging procedure is needed for analysis (training from French). SOC quality will also be evaluated using a combination of physical fractionation, infra-red spectroscopy, FTIR and NMR at RRes (training by Neal). This will permit understanding of how SOC varies with depth and determine the extent to which SOC persists due to physical (i.e. aggregates) or chemical (i.e. sorption) protection.

Full PhD project description:

Almost all arable soils are suffering a progressive loss of organic matter, leading to a decline in soil quality, release of damaging greenhouse gases, and the sub-optimal delivery of many ecosystem services. This PhD aims to identify the key mechanisms to be exploited to enhance carbon (C) stabilization in soil. Previous attempts at promoting C storage in top soils have had limited success and uptake by end-users. We hypothesize manipulation of the subsoil environment (frequently ignored) offers a better opportunity to deliver and stabilize greater amounts of C, and that this will yield benefits above and beyond those of just C sequestration. Central to achieving our aims will be the study of C from the micro- to macro-scale, the use of the latest analytical platforms, exploitation of national and international datasets and the use of innovative modelling approaches. The focus is on arable systems where there is: (i) greatest potential for C storage due to the lower initial starting point; (ii) greater potential for breeding deep rooted crops with more persistent residues; and (iii) greater opportunities for applying amendments within cultivation schemes without negative impact on yields. The overarching aim of the project is to identify low cost, practical solutions capable of widespread adoption by land managers for enhancing C sequestration in soil. The specific aims are:

O1. Quantify how SOC accumulation in top soils and subsoils are determined by dominant environmental processes.

O2. Quantify the relationships between the quantity, spatial distribution and quality of SOC and (i) microbial functional diversity, (ii) enzyme activity, (iii) management drivers, and (iv) matrix protection in the stabilisation and persistence of SOC in subsoils in comparison to top soils.

References to learn more:

1. Stockmann (2015) Global soil organic carbon assessment Glob Food Sec 6:9-16

2. Minasny et al., 2017 Soil carbon 4 per mille Geoderma 292, 59–86

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

This is a linked project – we recommend you select project 114 alongside this one.

(14) Computational Engineering of the Biosynthesis of the Sand Fly Pheromone Sobralene

Primary supervisor: Nick Besley

Second supervisor: Chris Hayes

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

The lab rotation project will focus on becoming familiar with the computational methods that underpin the project detailed below. The structure, stability, spectroscopy and formation of the target molecules sobralene and taxadiene will be studied using density functional theory exploiting high performance computing resources. These studies will be extended to consider how the chemical environment influences the structure and spectroscopy through the use of quantum mechanics/molecular mechanics approaches to model both solvent and enzyme environments. The student will be situated in the Computational Chemistry group.

Full PhD project description:

Visceral leishmaniasis (VL) is a tropical disease transmitted to humans through the bite of the female sand fly (Lutzomyia longipalpis) which is fatal if untreated and is the second largest parasite killer in the world, after malaria resulting in 20,000-40,000 fatalities per year. Sobralene is a recently discovered sex-aggregation pheromone produced by populations of the sand fly. Sobralene has the potential to be used in pheromone attractant traps as a vector control measure. However, sobralene can only be isolated in very small quantities from the sand flies, and consequently methods need to be developed for its larger scale production. One approach to develop larger scale production is to exploit enzymes to perform key steps in the chemical synthesis. This approach has been successfully applied to the production of closely related molecules such as taxadiene, but its application to the production of sobralene has not been explored.

This project will focus on the use of computational methods to inform the design and engineering of an enzyme that can catalyse the synthesis of sobralene from geranyl geranyl pyrophosphate (GGPP). The work will be guided by the close synthetic relationship between sobralene and taxadiene a biosynthetic precurser to the anti-cancer drug Taxol which was co-isolated with 1 from the sand flies. This suggests that the sobralene synthase will be similar to the taxadiene synthase, providing a starting point for the computational studies.

The project will use modern quantum chemical methods such as density functional theory (DFT) to explore the reaction path and transition states for the conversion of GGPP to sobralene. A key component of the project is to understand how the enzyme environment affects the reaction path. This will be achieved through the use of hybrid quantum mechanics/molecular mechanics (QM/MM) methods and also a new approach called the Atomic Interactions Represented By Empirical Dispersion (AIRBED) developed in the Besley group. Initially the formation of taxadiene will be explored and a detailed understanding of the role of the enzyme established. The active site of the enzyme will be characterised in terms of its shape, electrostatic environment and key non-bonding interactions. Subsequently, the same principles will be applied to sobralene with the aim of developing a synthase for the production of sobralene. The project will work in partnership with

Prof. Chris Hayes who exploit the findings from the computational studies to realise the enzyme in plant based systems.

References to learn more:

QM/MM study of the taxadiene synthase mechanism

J. P. M. van Rijn, A. M. Escorcia and W. Thiel

J. Comp. Chem., 40, 1902-1910, (2019)

Location of lab rotation: University Park

Location of full PhD project: University Park

(15) Molecular modelling of the Androgen Receptor – Elk1 complex, a new target for prostate cancer

Primary supervisor: Jonathan Hirst

Second supervisor: Peter Shaw

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

Biomolecular modelling skills development. Essential computational competencies will be developed in Linux, python programming, molecular docking (using Autodock Vina and OpenEye Fred software), de novo protein structure prediction (using web servers, such as SwissModel), biomolecular visualisation (using VMD software) and molecular dynamics simulations (using NAMD). A series of tutorials and exercises will be used to develop a working knowledge of the various computational tools. These tools will be used to investigate structurally well-defined domains of the Androgen Receptor, i.e., the activation function-2 (AF2) and the DNA-binding domains, and the interaction of known small molecule binders to these domains. Comparison to data available from the literature will allow the application of the modelling approaches to be validated and will build confidence for the full PhD project, where the modelling challenges are substantially more ambitious. Time permitting, various protocols will be evaluated for accuracy and computational efficiency. The biomolecular modelling skills developed in the 9-week lab rotation should be transferable to many different projects across the DTP (and beyond).

Full PhD project description:

The involvement of Androgen Receptor in prostate cancer is well established, both in androgen dependent and castration-recurrent tumours. In hormone-independent tumours, Androgen Receptor upregulates an expression signature enriched for genes promoting the cell cycle and mitosis, which is distinct from genes activated by androgens in normal prostate tissue. In prostate tumour cells Androgen Receptor forms a complex and cooperates with another protein, called Elk1, to drive expression of cell proliferation genes. Removing Elk1 from prostate tumour cells blocks Androgen Receptor function and prevents tumour growth. The dependence of prostate cancer on the Androgen Receptor has been known for some time, whereas the cooperation between the Androgen Receptor and Elk1 has only recently come to light. Thus, Elk1 and its interaction with the Androgen receptor is a relatively unexplored target for prostate tumour therapy. Furthermore, targeting the Androgen Receptor with drugs is potentially problematic due to its involvement in other physiological roles throughout the body. By comparison, cooperation between the Androgen Receptor and Elk1 only occurs in prostate tumours.

Systematic mapping using a mammalian two-hybrid assay and an extensive series of ELK1 deletion and point mutants, and confirmatory co-immunoprecipitation experiments, have identified the two ERK-docking motifs (D-box and DEF motif) in ELK1 as the elements essential for co-activation by the Androgen Receptor. These experiments have shown that the N-terminal domain (NTD) of the Androgen Receptor co-opts extracellular signal-regulated kinase (ERK) docking sites in the ELK1 protein to induce sustained gene activation that supports prostate cancer cell growth.

The Androgen Receptor is made up of both stably folded globular domains, involved in hormone and DNA binding, and regions of intrinsic disorder, including the NTD. Biophysical studies have revealed that NTD has limited stable secondary structure and conforms to a collapsed

disordered conformation. The challenge we face is that of obtaining useful data for rational drug design given the lack of structural information on the NTD or indeed, the C-terminal region of ELK-1.

Given the inherent flexibility of AR, only a structure moulded by its ELK1 target would have value for rational drug design. Thus, any approach would require both interaction partners. We will define regions within AR NTD that bind to the docking sites in ELK1 and we will recapitulate the interactions with pairs of peptides from AR and ELK1. We expect that this will generate enough information to make a de novo folding approach feasible, which will be used as a starting point for fully atomistic molecular dynamics simulations of the protein-protein association. These simulations will provide detailed structural information, which will be used to guide mutational studies to inform the development of new potential drug molecules.

References to learn more:

1. Rosati R; Polin L; Ducker C; Li J; Bao X; Selvakumar D; Kim S; Xhabija B; Larsen M; McFall T; Huang Y; Kidder BL; Fribley A; Saxton J; Kakuta H; Shaw P; Ratnam M. Strategy for Tumor-Selective Disruption of Androgen Receptor Function in the Spectrum of Prostate Cancer. Clin Cancer Res, 24, 6509–6622 (2018).
2. Hussain, A., Shaw, P.E. & Hirst, J.D., Molecular dynamics simulations and in silico peptide ligand screening of the Elk-1 ETS domain. J. Cheminf., 3, 49 (2011)

Location of lab rotation: University Park

Location of full PhD project: University Park

(17) VIROFISH

Primary supervisor: Rachael Tarlinton

Second supervisor: Adam Blanchard

Institution: University of Nottingham

School: School of Veterinary Medicine and Science

Lab rotation description:

Getting to grips with Bio-informatics for Viruses.

The 9 week project will be a crash course in basic bio-informatics (Handling large data sets, data formats and software suites, local blast search and RNAseq mapping to reference genomes) and viral phylogenetics (sequence analysis and construction of phylogenetic trees). Existing data on rodent endogenous retroviruses will be used to get to grips with the basic principles required for the larger PhD project. Prior computing experience an advantage, willingness to have a go and not being afraid of a spreadsheet are essential (we are used to teaching and supervising the non-programmer). This project and the larger PhD project are primarily data analysis rather than lab based but will give you a start in one of the most employable skill sets in biology.

Full PhD project description:

The intensification of aquaculture has accelerated the emergence of new viral diseases in economically important species of fish. Viruses typically spread between individuals but occasionally, a chromosomal integration of viral genes occurs in the germline of the host. These integrations are known as endogenous viral elements. They are frequently activated by events such exogenous viral infection and are often active in stem cell, germ line or neoplastic tissue. They contribute to pathogenicity in complex ways, including triggering inflammatory antiviral responses, activation of nearby genes via viral promoters, swapping gene segments with their exogenous counterparts and insertional oncogenesis from re-mobilised viruses. In a worst case scenario they can form recombinants with exogenous (infectious viruses) causing large scale epizootics. Counter-intuitively they can also sometimes provide protection from their exogenous counterparts by blocking superinfection or blockading parts of the cellular machinery needed for viral replication eg by producing antiviral RNA (e.g. piRNA) and post-transcriptionally silencing exogenous viral mRNA. With the advent of new sequencing technologies there has been an explosion in identification of new viruses associated with disease syndromes as these are typically identified based on sequence information alone. However distinguishing endogenous from exogenous infections and untangling the role played by both has become an increasing problem. Fish genomes differ from mammalian ones with respect to their viral complement in that many of those that have been studied contain a large number of herpesviruses linked to a DNA transposon, a “piggyBac” element. Additionally, there is abundant evidence of retroviruses jumping into new hosts across many classes of amphibious organisms including fish. What is not known is how these elements interact with their infectious counterparts, what effects they have on fish health in general and the risk of further cross species jumps in intensive aquaculture. Of particular concern is what happens with these viruses under conditions of physiological stress common in intensively farmed species and whether this will result in reactivation of endogenous retroviruses and disease in the host or sympatric species. In essence it is not clear in which cases EVEs provide viral element derived immunity or if they themselves can reactivate or recombine with exogenous viruses.

The project will extend earlier work on establishing data mining algorithms for EVE, assessing the most recent data mining approaches for this type of work and building a database of fish viruses and EVE to add to the existing terrestrial vertebrate database. This data mining algorithm will be tested against the Atlantic Salmon, Nile Tilapia and Channel catfish genomes. The species chosen are of major commercial interest, and have both reference genomes available and RNAseq data sets. RNAseq data sets from these species will then be interrogated to establish an expression map (for both tissue and disease states where available) of the EVE to begin to determine under what conditions expression is activated, what risk this poses in terms of resurrection of infectious viruses from the genome and interference (either inhibition or enhancement) with exogenous pathogen infections and innate immune system activation.

References to learn more:

1. Aswad A, Katzourakis A. A novel viral lineage distantly related to herpesviruses discovered within fish genome sequence data. Virus evolution. 2017;3(2):vex016
2. Brown K, Emes RD, Tarlinton RE. Multiple groups of endogenous epsilon-like retroviruses conserved across primates. J Virol. 2014;88(21):12464-71

Location of lab rotation: Sutton Bonington

Location of full PhD project: Sutton Bonington

(18) Structure-based development of a Chagas vaccine using novel protein immunogens

Primary supervisor: Ivan Campetto (NTU)

Second supervisor: Juan Sanchez-Weatherby (Diamond Light Source)

Third supervisor: Rita Tewari (UoN)

Fourth supervisor: Jody Winter (NTU)

Institution: NTU

School: Science and Technology

Lab rotation description:

Chagas disease is caused by the parasite Trypanosoma cruzi and is one of the main neglected tropical diseases, killing 12,000 people per year and affecting 8 million people worldwide. A vaccine is urgently required because current therapies are outdated, produce severe side effects and have limited efficacy.

Campeotto's group aims to develop a Chagas vaccine, using a protein structure-guided approach. The parasite proteins Tc80 has been recently identified as promising vaccine candidate
Tc80 is an enzyme secreted in the blood stream and used to invade host cells by degrading components of the extra-cellular matrix such as fibronectin and collagen1.

Campeotto's group produced Tc80 protein recombinantly and a series of anti-Tc80 monoclonal antibodies (mAbs) have been produced in mice and will be tested for their ability to inhibit enzyme function and to block parasite infection, thus preventing Chagas disease.

During the lab rotation, the student will purify recombinant Tc80 and use enzymatic and functional assays to test the ability of anti-Tc80 mAbs to block enzyme function. The best mAbs will be further characterised biophysically and structurally with the ultimate aim to determine their crystal structures in complex with Tc80 for diagnostic and therapeutic applications (see PhD proposal).

Full PhD project description:

Importance

American trypanosomiasis, also known as Chagas disease, is caused by Trypanosoma cruzi, a protozoan parasite of the Kinetoplastida order that is transmitted by the triatomine insect through deposition of faeces, containing parasites, at the site of the triatomine bite. Chagas disease is considered one of the main neglected tropical diseases; the disease is spreading quickly due to increased human migration and to climate change. Parasite infection manifests as acute and chronic phases with severe cardiac disease being associated with over 30% of chronic infections. No vaccine is available and current therapy is outdated, associated with severe side effects and shows some efficacy only against the acute infection.
Further research is therefore required to develop new Chagas therapies.

Research Background

T.cruzi parasites concentrate in the faeces of the triatomine insects and gain access to the blood stream upon deposition near the insect bite. When the parasites enter the bloodstream, they differentiate into a replicative form, which secretes the 80kDa enzyme prolyl oligopeptidase (Tc80) into the extracellular blood, which allows the invasion of mammalian cells by degrading their extracellular matrix components. Recombinant Tc80 has recently been shown to elicit a strong humoral response in immunised mice, which were also protected from a lethal dose of T. cruzi1

The absolute requirement for Tc80 in the invasion process, together with its conservation across different strains of T. cruzi, make this protein an exciting candidate for the development of a Chagas blocking vaccine.

PhD objectives:

Objective 1: Determine the effects of anti-Tc80 on Tc80 activity and parasite invasion

The student will use biophysical techniques to quantify individual binding affinities and kinetic properties of anti-Tc80 mAbs in vitro, whilst in parallel cell-based assays will be performed using a T. cruzi fluorescent strain, which will allow to select mAbs, which have inhibitory effects on parasite invasion. This part of the project is in collaboration with parasitologist Prof. John Kelly at the London School of Hygiene and Tropical Medicine (LSHTM) in London.

Objective 2: Determine the crystal structures of Tc80 and Tc80 in complex with specific mAbs

The student will pursue the determination of the crystal structures of Tc80 and in complex with the best mAbs screened in objective 1, which will reveal the the molecular details of antibody-epitope recognition. This information will be exploited to guide future immunogen design (Campeotto et al., patent pending). Campeotto's group has routine access to Diamond Light Source synchrotron facilities (DLS, Oxford, UK) and the student will have the possibility of receiving further training at by the PI and by his collaborator at DLS, Dr Juan Sanchez-Weatherby.

Objective 3: Design novel immunogenic molecules for vaccine applications

The student will design in silico immunogens of Tc80 based on the structural information gained from objective 2 and based on bioinformatics and modelling analysis. These immunogens will be cloned, expressed, purified and conjugated to Virus-Like-Particles to boost the production of broadly neutralizing mAbs in mice2. Mice will be subsequently challenged with a lethal dose of the parasite.

References to learn more:

1. Bivona, A. E. et al. (2018). "Trypanosoma cruzi 80 kDa prolyl oligopeptidase (Tc80) as a novel immunogen for Chagas disease vaccine". PLOS Neglected Tropical Diseases, 12(3), e0006384–23. doi: http://doi.org/10.1371/journal.pntd.0006384
2. Brune D. K. and Howarth M. (2018). "New Routes and Opportunities for Modular Construction of Particulate Vaccines: Stick, Click, and Glue". Front.Immunol, https://doi.org/10.3389/fimmu.2018.01432

Location of lab rotation: Clifton Campus

Location of full PhD project: Clifton Campus

(19) Impact of tetracyclines on AMR in race horses.

Primary supervisor: Michael A. Jones

Second supervisor: Stuart Paine

Third supervisor: Sabine Totemeyer

Institution: University of Nottingham

School: School of Veterinary Medicine and Science

Lab rotation description:

The rotation will focus on isolation of E. coli and also use of molecular techniques s to determine presence of specific tetracycline resistance antibiotic resistance genes (ARGs) in race horse samples. The prevalence of specific and types of ARGs and their relationships to strain MICs will be assessed. The methodology will define protocols for subsequent analyses and provide isolates that can be carried in to the subsequent project.

Work will include (times provided estimate dependant on progress):

Assessment of prevalence and levels of Tetr E. coli in race Studies investigating antimicrobial resistance (AMR) in horses have defined a number of risk factors for AMR selection. Veterinary therapy within six months of the assessment is linked to increased gentamicin, tetracycline and other multi drug resistance. The use of tetracycline or gentamicin for respiratory disease was considered an important risk factor for a range of AMRs. International studies have shown resistance to all antimicrobials available for use in equine practices for E. coli and there is evidence for co-selection between antibiotics.

The use of tetracyclines in horses depends on practitioner choice and therapeutic need. Tetracyclines can provide an additional therapeutic property based on immunosuppressive effects. These anti-inflammatory properties have led to the use of tetracyclines not only as antimicrobials but potential prophylactics for immune health in race horse medicine with the inherent risk of selecting for resistance. Analysis of post-race urine samples carried out by European horse racing authorities have shown that 70% of race horses have detectable levels of antibiotics with persistence of oxytetracycline for greater than 50 days due to its elimination profile. So there are long post-therapy periods with sub-inhibitory doses of tetracycline present in the horse.

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Work will include (times provided estimate dependant on progress):

- Assessment of prevalence and levels of Tetr E. coli in race horse samples using differential selective culture technique. (week 1- 2)
- Assessment of isolates of MIC by liquid and agar break point assays to determine (week 2-3)
- Development of direct polymerase chain reaction PCR to assess common Tetr determinants in samples. This will use direct faecal DNA extraction and PCR. (week 4-5)

- Assessment of resistant isolates for presence of plasmids as a risk factor for transferable Tetr (week 5-6)

Full PhD project description:

Studies investigating antimicrobial resistance (AMR) in horses have defined a number of risk factors for AMR selection. Veterinary therapy within six months of the assessment is linked to increased gentamicin, tetracycline and other multi drug resistance. The use of tetracycline or gentamicin for respiratory disease was considered an important risk factor for a range of AMRs. International studies have shown resistance to all antimicrobials available for use in equine practices for E. coli and there is evidence for co-selection between antibiotics.

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It has been suggested that sub-inhibitory concentrations can select for resistances below their normal therapeutic concentrations. These minimal selective concentrations (MSCs) pose a risk for inadvertent AMR selection over an extended number of generations. The implication of the long exposure to MSCs is antibiotics with a prolonged clearance may provide a longer selective window to select for resistance to itself and other antibiotics.

Aim: This project will focus on the potential for selection in the long sub-MIC elimination phases of tetracyclines.

Hypothesis: The increased use of tetracyclines in races horses is leading to selection higher risk antibiotic resistance genes in the commensal bacteria.

This project will investigate AMR of Enterococcus and Enterobacteriaceae (E. coli, Klebsiella sp.) from faeces. It is divided into three main stages. Stages-1 and 2 are descriptive in nature, while stage-3 will develop methodologies to investigate minimal selective concentrations for field isolates using quantitative PCR to calculate relative proportions of strains. The data will then be brought together to give an assessment of risk due to MSCs for selection of specific therapeutic regimens.

The project has the following units of work:

Part-1 Prevalence and proportions of AMR:

(1) To determine the AMR profiles of Enterococci and E. coli horses under different antimicrobial regimens.

Part-2 Correlation and ARG determination:

(2) To determine co-selectivity based on AMR profiles.

(3) To determine correlations of resistance between antimicrobials.

(4) To identify genetic basis of common co-resistances using whole genome sequencing of selected strains.

Part-3: Competition and selection:

(5) To develop mixed culture qPCR method to determine selective coefficients between field isolates.

(6) To determine intrinsic selective/competitive advantages between strains.

(7) To compare minimal selective concentrations (MSC) for tetracyclines on selected resistant and susceptible isolates.

To link MSC values to pharmacokinetic data for selected antimicrobials to provide risk based model for selection.

References to learn more:

1. GULLBERG, E., CAO, S., BERG, O. G., ILBACK, C., SANDEGREN, L., HUGHES, D. & ANDERSSON, D. I. 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog, 7, e1002158.
2. BAHRAMI, F., MORRIS, D. L. & POURGHOLAMI, M. H. 2012. Tetracyclines: drugs with huge therapeutic potential. Mini Rev Med Chem, 12, 44-52.

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(20) Meat and Medicine: Regenerative programming of animal and human stem cells for engineered skeletal muscle

Primary supervisor: James Dixon

Second supervisor: Alvara Mata

Third supervisor: Ramiro Alberio

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

The lab rotation will demonstrate the initial proof-of-concept that delivery of myogenic regulators can convert stem cells as lines (pluripotent or multipotent) or from primary tissue (bone marrow or lipoaspirates) into muscle. This has implications for regenerative medicine and reconstructive efforts for trauma and disease of soft-tissues, and also in future production of synthetic meat for the food industry.

You will culture and program stem cells (iPSCs, MSCs and MNC derived from tissue) with non-viral gene delivery of transcription factors which will trigger the myogenic program and generate myogenic progenitor and terminally differentiated skeletal muscle cells. Cells will be from human or animal iPSC and MSC lines or from cells directly isolated from animal tissues. You will do this in conventional monolayer cultures and also in scaffolds with the aim of generating skeletal muscle tissues in vitro.

Full PhD project description:

Background: Pluripotent (PSC) and multipotent (such as mesenchymal; MSCs) stem cells hold great promise for the treatment of human skeletal muscle trauma and diseases, but also for the generation of new approaches to generate tissue for food consumption. However, it remains challenging to convert PSCs or MSCs to skeletal muscle cells, and the mechanisms by which the master regulatory transcription factors, promotes muscle stem (satellite) cell identity are not yet fully understood. However it is clear that viral or transgenic expression of transcription factors (TFs) such as Pax7 or MyoD can effectively induce the muscle program in these cell types.

Approach: We have developed a non-viral method to deliver genes to tissues, cells or stem cells. This will be the focus of the PhD, to deliver TF genes to stem cells in order to mediate there programming into myogenic precursors and to expand and use those cells to generate tissue of regenerative grafts and as meat-substitutes. You will use PSC and MSC lines as proof of concept, employing iPSCs from pig, sheep and cow to generate species specific muscle tissue for food applications. For clinical use you will employ human iPSCs, MSCs and bone marrow and liposuction aspirates to program these tissue stem cells into muscle for with cell-therapies or for tissue engineering and implant applications. The genes, duration of expression, if serial dosages are required and the culture conditions will require optimisation and assessment. The seeding of scaffolds with generated progenitors or directly programming in 3D will be needed to generate tissue which will also be studied. Interestingly we have electrical stimulation set ups that can be used to physiologically test contraction of engineered muscle and there is potential to move to in vivo testing depending on the project with collaborators at the RCSI (Dublin, Ireland) and JHU (Baltimore, USA).

Location: The project will be carried out in the new state-of-the-art Biodiscovery institute at University Park (James Dixon) and the School of Biosciences at Sutton Bonington Campus (Ramiro Alberio). The use of human and animal cells of this application will be transformative and is likely to have significant commercial value and interaction with stakeholders. Presently the MoD (through the DSTL) are funding bone and fat reconstructive technologies using gene delivery in the Dixon group and this PhD could add impact and synergy to these projects reciprocally.

Techniques and opportunities: The project will use cell/stem cell culture, QPCR, immunolabelling, flow cytometry, single cell RNAseq, vector design and construction, gene delivery, scaffolds, tissue engineering, electrical stimulation, in vivo translation.

References to learn more:

1. Lilja et al. Pax7 remodels the chromatin landscape in skeletal muscle stem cells. PLoS ONE 12(4):e0176190.
2. Darabi R and Perlingueiro R (2016). Derivation of Skeletal Myogenic Precursors from Human Pluripotent Stem Cells Using Conditional Expression of PAX7. Methods in Mol Biol 1357:423-439

Location of lab rotation: University Park/Sutton Bonington

Location of full PhD project: University Park/Sutton Bonington

(21) Left-right asymmetry in Hawaiian 'Looking-glass' snails

Primary supervisor: Angus Davison

Second supervisor: Mark Ravinet

Third supervisor: Ken Hayes (Pacific Biosciences Research Center/University of Hawaii)

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The rotation project will vary depending upon the existing skills and requirements of the student, whether bioinformatics or wet-lab, or both.

One requirement of the project is that the student is able to extract ultra-high molecular weight DNA from snails, for use in nanopore DNA sequencing. It is therefore likely that the first part of the training will involve optimising the methods in DNA extraction for these snails, and then quality testing the resulting material, using a variety of techniques e.g. pulse field gel electrophoresis, spectrophotometry, and possibly a test run on the nanopore (if time allows). Other wet lab techniques that may be used, again according to need, including PCR, cDNA cloning, Sanger DNA sequencing.

In terms of bioinformatics, the student will receive training in the analysis of genomic data, of the sort that might be generated in the main PhD project. Specifically, the student will learn and use phylogenomic methods, making networks and using other methods such as principal components analysis. The student will prepare the groundwork for subsequent whole genome assemblies. As most analyses will require the use of Unix and/or R, then the student will become familiar with both of these environments – useful skills in many different arenas.

Full PhD project description:

While most animal bodies are bilaterally symmetric on the outside, the internal organs usually show a consistent left-right (LR) asymmetry. Defining this LR asymmetry is a critical part of early development, such that left/right positional errors are an important class of human birth defect, and in later life numerous diseases affect apparently symmetric organs in an asymmetric fashion. Yet, in trying to understand how this LR asymmetry is established, it is straightforward to conceive how the LR axis is defined relative to front/back and top/bottom, but more difficult to comprehend how left and right are consistently orientated in the same direction. In the classic view, the solution is that LR asymmetry is signalled by a chiral structure, the fabled "F-molecule", which is directionally orientated relative to the other axes.

To date, a wealth of studies have revealed the genes that promote the propagation of asymmetric signals, but the earliest LR symmetry-breaking events are not clear. In seeking to understand if there is a common pathway, an emerging consensus is that LR asymmetry in diverse organisms originates from the cytoskeletal dynamics that underlie the asymmetric behaviour of individual cells Nonetheless, a central problem remains – how and why are left and right consistently orientated in the same direction? The main approach to understanding this invariance has been to use rare mutants or manipulations in model animals (vertebrates, nematode/fly), to create individuals that are partly or wholly orientated in the opposite direction. This methodology has been fruitful, of

course, but unfortunately, scientists have largely ignored the only animal group – snails – in which ordinary development can produce individuals that are LR orientated in different directions. Studies of LR asymmetry ("chirality") in snails may be key to understanding how and why are left and right consistently orientated in the same direction in nearly all other animals. In this project, we propose to use association mapping and long read genome sequencing to identify the gene that underpins natural variation in the LR asymmetry of Hawaiian snails of the genus Lymnaea or Auricullela. This knowledge will then be used to understand how molecular chirality defines the LR asymmetry of cells, organs and bodies, with implications for understanding human health and development. The project will involve cutting-edge methods in DNA sequencing / bioinformatics, and may also require field or lab work in Hawaii.

References to learn more:

1. Davison, A. (2019). Flipping shells! Unwinding LR asymmetry in mirror-image molluscs. Trends in Genetics, in press (email for a copy).
2. Davison, A., McDowell, G.S., Holden, J.M., Johnson, H.F., Koutsovoulos, G.D., Liu, M.M., Hulpiau, P., Van Roy, F., Wade, C.M., Banerjee, R., et al. (2016). Formin is associated with left-right asymmetry in the pond snail and the frog. Curr. Biol. 26, 654-660.

Location of lab rotation: University Park

Location of full PhD project: University Park

(23) Regulating DNA methylation to enhance neural plasticity

Primary supervisor: Rebecca Trueman

Second supervisor: Federico Dajas-Bailador

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

Active demethylation of DNA following injury may play a key role in repairing the adult brain. In order to develop an understanding of how DNA demethylation induced by hypoxia in neurones is regulated, you will examine the role of HIF1α – the key transcriptional regulator in relation to hypoxia. Within the tumour literature there is discrepancy as to whether the enzymes responsible for DNA demethylation are up or down regulated by HIF1α. This mechanism has not been examined in neurones from the central nervous system. During your 9 week rotation you will perform a study establishing if silencing HIF1α within primary neuronal cultures during hypoxia, prevents the upregulation of Ten Eleven Transferase enzymes (TET) and prevents hydromethylation of DNA. You will learn cell culture, immunocytochemistry and molecular biology techniques.

Full PhD project description:

Neuroepigenetics is a growing field that aims to examine the molecular processes which dynamically modulate gene expression within the nervous system. DNA methylation, a form of epigenetic modification, alters as we age (Keleshian et al, J Neurochem. 2013. 125:63-73; Su et al, Nat Neurosci. 2012. 15(8):1061-2). As such, it is thought that DNA methylation (5mC) may contribute to reduced plasticity and regenerative ability of the aged central nervous system (CNS) when compared to the juvenile, adolescent, or adult brain (Li et al, Nat Neurosci. 2010 Dec;13(12):1496-504). DNA methylation of cytosines (5mC) can be removed by an active process via Ten Eleven Transferase enzymes (TET), into oxidised forms of 5mC which are then replaced. Hydroxymethylation (5hmC) is the first step in the active demethylation process. We have recently shown that enhancing the active demethylation process, by over expression of the TET enzymes increases axonal growth, and preventing demethylation reduces axonal growth (in preparation for publication). We have also demonstrated that focal cerebral ischemia increases hydroxymethylation in cell bodies close to the insult. Interestingly, it also increases in hydroxymethylation at sites which project to and from the infarcted region. These are regions which do not suffer from hypoxia at the time of stroke, but show axonal sprouting following ischemia, in an attempt to repair the brain. We now want to understand the fundamentals of how hypoxia, and the following cascade of signals can be transmitted to the cell body of neurones to alter DNA methylation, and its subsequent influence on axonal growth. This will allow us to understand the observed alterations in in cells which are connected to but are not in the hypoxic region. Results obtained will support the investigation on how to enhance regeneration in the aging brain to improve recovery from insults, such as stroke, brain injury or cognitive decline. Using compartmentalised microfluidic chambers we will build a model of a circuit between cortical regions, and examine different manipulations at different points and locations within the circuitry. This work will examine the effect on DNA methylation, hydroxymethylation, gene expression (including expression of the TET enzymes) and axonal growth when different insults are applied either directly to the cell body, terminals or cell bodies connected to the neurons of interest. We will examine hypoxia at each of the sites, alongside blocking / enhancing the induction of HIF1α – the key transcriptional regulator in relation to hypoxia, to establish if within neurones HIF1α regulates

expression of key enzymes in the DNA methylation / demethylation pathway. We will then look at different components of the ischemic cascade in isolation at these different sites, including inducing and blocking excitotoxicity, examining the role of neuroinflammation by applying key cytokines, and co-culturing with microglia. By building a complete picture of how different components of the ischemic cascade are able to induce changes in epigenetic markers and gene expression of cells connected to (by not directly affect by) ischemia we will gain a greater understanding of the mechanisms of repair following injury to a neuronal circuit.

References to learn more:

1. Weng YL, An R, Cassin J, et al. An Intrinsic Epigenetic Barrier for Functional Axon Regeneration. Neuron. 2017;94(2):337–346.e6. doi:10.1016/j.neuron.2017.03.034
2. Morris-Blanco KC, Kim T, Lopez MS, Bertogliat MJ, Chelluboina B, Vemuganti R. Induction of DNA Hydroxymethylation Protects the Brain After Stroke. Stroke. 2019; 50(9):2513-2521. doi: 10.1161/STROKEAHA.119.025665.

Location of lab rotation: QMC

Location of full PhD project: QMC

(24) First 3D isogenic model for the study of age-related bone loss

Primary supervisor: Livia Santos

Second supervisor: John Hunt

Third Supervisor: Chris Denning

Fourth Supervisor: Morgan Alexander

Institution: NTU

School: Science and Technology

Lab rotation description:

Week 1. Complete mandatory safety training. Incoming students will be required to complete a safety induction and occupation health training. They will learn how to use a biological safety hood, centrifuges, and incubator. They will shadow existing PhD students to exchange ideas and learn good lab practices.

Week 2-3. Develop aseptic techniques. Students will learn to expand and subculture cells without the interference of microbes or fungus.

Week 4-5. Learn to prepare 3D cell cultures. Students will learn how to prepare a hydrogel from collagen solution, encapsulate cells, fix and stain the cell's cytoskeleton and nucleus. They will learn about cell morphology and microscopy.

Week 6. Acquire competences on how to operate a computer-controlled bioreactor. Students will learn how to programme, load samples and operate a computer-controlled bioreactor which mimics the mechanical forces experience by bone or muscle and perform cell culture with cells in 2 or 3D.

Week 7-8. Acquire skills on cell imaging, image acquisition and basic statistics. Students will use the microscope to observe cell morphology and take photos for later image processing (e.g., to quantify dendrimers or count cells). This is an outstanding opportunity to acquire skill in image processing software (e.g., using FIJI) and statistics.

Week 9. Prepare poster/report

Full PhD project description:

A decline in musculoskeletal function due to age-related bone loss is a major threat to the health of older people as it increases the risks of falls and related mortality and morbidity. A better understanding of age-related bone loss will allow the establishment of strategies that support the preservation of bone mass and health across the life course.

Several constraints around the use of human models and the lack of human-like models that accurately represent age-related bone loss have been limiting factors to a better comprehension of this phenomenon. Therefore, the overarching objective of this proposal is to pioneer the development of the first isogenic human cell model for the study of age-related bone loss. Isogenic models are built from selected or engineered cells to accurately represent the genetics of a specific population and are typically composed of cells with one gene mutation and a genetically matched (non-mutated) control. This pioneering model will be developed by combining CRISPR, a gene-editing technology that allows creating cell lines harboring genes implicated in bone loss, with tissue

engineering, to generate in vitro bone-like models.

The work packages (WP) and learning opportunities (LO) are outlined below. Those were specially designed to maximize the scientific discoveries while providing the PhD candidate unique learning opportunities across different disciplines and within an environment of research excellence.
WP1. Produce cell lines with mutations in genes implicated in bone mass regulation (month 6 -18). A loss-of-function mutation/polymorphism of the gene encoding LRP5 will be introduced in iPSCs by CRISPR/Cas9. LRP5 is a key regulator of bone mass with loss-of-function mutations and certain polymorphisms linked to low peak bone mass and bone loss. The student will have the opportunity to spend 6 weeks in Prof. Denning's Lab to deepen knowledge in CRISPR/Cas9. LO1: Acquire and develop skills in CRISPR/Cas9.

WP2. Develop and characterise a human isogenic model in 3D to study age-related bone loss (month 18-30). The cell lines previously developed will be organised in a 3D collagen matrix and allowed to differentiate into bone cells (osteocytes). To stimulate tissue maturation, the construct will be mechanically stimulated using a computer-controlled bioreactor. The expression of bone markers will be confirmed by RT-qPCR and Western blotting. The transcriptome will be investigated by RNA sequencing. LO2. Develop skills in tissue engineering, omics, bioinformatics, and statistics.
WP3. Validate a human isogenic model in 3D to study age-related bone loss (month 30-38). Bone resorption cells (osteoclasts) or bone formation cells (osteoblasts) will be co-cultured at the surface of the 3D model previously developed and bone formation and resorption quantified using established biochemical methods. ToF-SIMS will be used to assess calcium distribution, an important bone quality parameter at Prof. Alexander's Lab. The ageing bone displays higher bone resorption rates and more heterogeneous distribution of calcium. We hypothesise that our in vitro bone model with a mutation/polymorphism in the LRP5 will exhibit greater bone resorption and more heterogeneous calcium distribution. LO3. Develop skills on cell co-culture, biochemistry, and ToF-SIMs.

WP4. Thesis and paper writing (month 38-48).

References to learn more:

1. Ciu et al. Lrp5 functions in bone to regulate bone mass. Nature Medicine 2011;17:684–691
2. Farr et al. Targeting cellular senescence prevents age-related bone loss in mice. Nature Medicine 2017;23:1072–1079

Location of lab rotation: Clifton Campus

Location of full PhD project: Clifton Campus/University Park

(25) Metabolic and functional validation of iPSC-derived neurons and astrocytes

Primary supervisor: Sebastian Serres

Second supervisor: Dan Scott

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The Serres laboratory works on understanding metabolism and cell function in brain cells in health and disease. We work with molecular biology techniques such as quantitative RT PCR, western blotting, and enzymatic assay for genomics and proteomics analysis and state-of the-art analytical techniques such as nuclear magnetic resonance spectroscopy (MRS) for metabolomics analysis. When combined these techniques enable us to measure metabolic and functional changes associated to astrocytes and neurons in response to inflammation and physiological stress in detail. So far, we have applied these techniques to animal models and primary cell culture from animals' brain, but in this project, we want to apply these traditionally techniques to the study of induced astrocyte and neuron cells. In this lab rotation, the student will be involved in culturing and differentiating fibroblast-derived induced pluripotent stem cells into i-astrocytes, protein immunodetection and quantitative metabolic analysis using MRS. At first, these techniques will be used to identify the effects of inflammation and physiological stress on i-astrocytes.

Full PhD project description:

The Serres laboratory works on understanding metabolism and cell function in brain cells in health and disease. We work with molecular biology techniques such as quantitative RT PCR, western blotting, and enzymatic assay for genomics and proteomics analysis and state-of the-art analytical techniques such as nuclear magnetic resonance spectroscopy (MRS) for metabolomics analysis. When combined these techniques enable us to measure metabolic and functional changes associated to astrocytes and neurons in response to inflammation and physiological stress in detail. So far, we have applied these techniques to animal models and primary cell culture from animals' brain, but in this project, we want to apply these traditionally techniques to the study of induced astrocyte and neuron cells. In this lab rotation, the student will be involved in culturing and differentiating fibroblast-derived induced pluripotent stem cells into i-astrocytes, protein immunodetection and quantitative metabolic analysis using MRS. At first, these techniques will be used to identify the effects of inflammation and physiological stress on i-astrocytes.

References to learn more:

1. Vandoorne, T., K. Veys, W. Guo, A. Sicart, K. Vints, A. Swijsen, M. Moisse, G. Eelen, N. V. Gounko, L. Fumagalli, R. Fazal, C. Germeys, A. Quaegebeur, S. M. Fendt, P. Carmeliet, C. Verfaillie, P. Van Damme, B. Ghesquiere, K. De Bock and L. Van Den Bosch (2019).

"Differentiation but not ALS mutations in FUS rewires motor neuron metabolism." Nat Commun 10(1): 4147.2-

2. Joshi, A. U., P. S. Minhas, S. A. Liddelow, B. Haileselassie, K. I. Andreasson, G. W. Dorn, 2nd and D. Mochly-Rosen (2019). "Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration." Nat Neurosci 22(10): 1635-1648.

Location of lab rotation: QMC

Location of full PhD project: QMC

(26) Identifying genetic determinants ensuring correct DNA damage repair pathway choice

Primary supervisor: Stephen Gray

Second supervisor: Thorsten Allers

Institution:

School:

Lab rotation description:

Full PhD project description:

References to learn more:

Location of lab rotation:

Location of full PhD project:

(27) Exploring the neuropathology of alpha-synuclein amyloid variants

Primary supervisor: Kevin Gough

Second supervisor: Thorsten Allers

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

Research within the Gray lab is focused on investigating DNA damage repair dynamics during the process of meiosis. We primarily use the budding yeast Saccharomyces cerevisiae, taking advantage of the plethora of reporter strains, tools and techniques enabling easy genetic manipulation, and the conservation of DNA repair proteins and mechanisms across eukaryotes. Observations made in S. cerevisiae are further developed through in vivo, in silico and in vitro systems.During meiosis, hundreds of DNA double-strand breaks are formed per cell, some of which repair as crossovers between homologous chromosomes, enabling accurate segregation at the first meiotic division. Critical to accurate DNA repair is finding and using the correct sequence, at the correct locus. Using a similar DNA sequence at the incorrect locus leads to the formation of ectopic recombinants, which manifest as chromosomal aberrations, and are observed in many diseases and cancers. During this rotation, the student will generate DNA damage repair mutants in novel and established ectopic recombinant reporter strains to identify genetic determinants driving or inhibiting ectopic recombinant formation. Mutants will be combined with known ectopic regulators to define epistatic relationships, and site directed mutagenesis will be undertaken to investigate the role of phosphorylation on ectopic recombination.

Full PhD project description:

DNA double-strand breaks (DSBs), where both strands of the DNA helix are broken at the same locus, are the most dangerous lesion to occur to the genome. DSBs must be repaired correctly and in a timely manner to ensure genomic stability. One main pathway for DSB repair is homologous recombination, where a similar DNA sequence, usually the sister chromatid at the same locus, is used as a template for repair. However, when homologous recombination repair takes place from a similar DNA sequence at an incorrect locus, ectopic recombination occurs leading to the formation of chromosomal aberrations, manifested as mutations, deletions, additions and translocations. Investigating the mechanisms that inhibit or promote ectopic recombination using the budding yeast Saccharomyces cerevisiae is the aim of this PhD project and is broken down into three elements, described below.Our current knowledge of ectopic recombination inhibitors are DNA repair and DNA damage checkpoint proteins. These proteins appear to regulate DSB repair pathways to prevent ectopic recombination. Intriguingly our results so far indicate that mutation of individual components of the same DNA damage checkpoint lead to different levels of ectopic recombinants. The first element of this PhD project will be to define the types and levels of ectopic recombinants that form in the different checkpoint mutants. This will be undertaken using established Southern and western blot assays, use of a novel fluorescent reporter tool and development of a CRISPR/Cas9 sequencing technique.Our current data suggests that ectopic recombinants can form by different DNA repair mechanisms. To this end, the second element of this PhD project will be to evaluate and characterise the types of ectopic recombinants formed in mutants defective in specific DNA repair pathways.Our understanding of ectopic recombination has so far focused on strategic and directed

investigations, given previously characterised roles of proteins. However, this strategy removes the ability to find novel inhibitors of ectopic recombination. To this end, the final element of this PhD project will to be develop the use of CRISPR/Cas9 targeted mutation in reporter strains. Once established, the student will construct a CRISPR library and undertake a screen allowing for enrichment of cells carrying ectopic recombinants. Following sequencing to identify the gene mutated, further characterisation of the mutant will be undertaken to define the mechanism for ectopic recombination in this background. Observations made throughout this project will be have the potential to be developed in additional model organism systems.

References to learn more:

1. Gray, S., Allison, R.M., Garcia, V., Goldman, A.S.H., Neale, M.J., (2013) Positive regulation of meiotic DNA double-strand break formation by activation of the DNA damage checkpoint kinase Mec1(ATR). Open Biol 3: 130019. doi: 10.1098/rsob.130019 PMID: 23902647, PMCID: PMC3728922
2. Grushcow, J. M., Holzen, T. M., Park, K. J., Weinert, T., Lichten, M., Bishop, D. K. (1999). Saccharomyces cerevisiae checkpoint genes MEC1, RAD17 and RAD24 are required for normal meiotic recombination partner choice. Genetics 153(2): 607-620. PMID: 10511543, PMCID: PMC1460798

Location of lab rotation: QMC

Location of full PhD project: QMC

(28) Applying a synthetic biology platform to sustainable production of chemicals from CO2 using cyanobacteria

Primary supervisor: John Heap

Second supervisor: Klaus Winzer

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

Cyanobacteria have great potential as biocatalysts using light to convert CO2 to products of interest, while avoiding competition with the food chain. Cyanobacteria have been genetically modified to synthesise many non-native compounds, from biofuels to high-value products.C Production using cyanobacteria is not yet commercially viable, partly because it is difficult to rationally design DNA encoding

Full PhD project description:

Cyanobacteria are the simplest and most genetically-tractable organisms capable of oxygenic photosynthesis, using CO2 and sunlight as sole carbon and energy sources. Their photosynthetic yield and growth rate are similar to the fastest-growing microalgae, and greater than te

References to learn more:

1. Sun, T., Li, S., Song, X., Diao, J., Chen, L., Zhang, W. (2018) Toolboxes for cyanobacteria: Recent advances and future direction. Biotechnol Adv. 36(4):1293-1307. doi: 10.1016/j.biotechadv.2018.04.007.2.
2. Jones, P. R. Genetic instability in cyanobacteria - an elephant in the room? (2014) Front Bioeng Biotechnol 2, 12.

Location of lab rotation: University Park

Location of full PhD project: University Park

(30) Epigenomic mechanisms regulating synaptic plasticity and cognitive ageing

Primary supervisor: Helen Miranda Knight

Second supervisor: Nathan Archer

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The rotational project, based at the QMC medical school, is designed to give the student knowledge of brain related molecular processes and RNA biology as well as experience in RNA transcriptomics and bioinformatic analysis. The project will involve the analysis of pre-existing RNA-seq data to characterise patterns of RNA binding events across transcripts and gene ontology functional analysis of identified RNA binding proteins. Additional analysis may include data mining of RNA and protein databases to characterise specific patterns of expression in human brain.

Full PhD project description:

Modification of eukaryote messenger and noncoding RNAs is a process which governs spatio-temporal patterns of gene expression. N6 methylation of adenosines (m6A/m6am) is known to affect RNA stability, translation status, splicing and subcellular mRNA localisation. Failure to regulate the transcriptome at the right place and time, potentially has a profound effect on plasticity and neural processes which, in turn, may influence brain function over the lifespan. Through the development of novel approaches and techniques, the project will seek to determine the methylation machinery and pathways driving synaptic and cognitive function. Using advanced microscopy and biochemical and pharmacological techniques, we will explore the dynamics of RNA modification at synaptic sites. Using a new transcriptomic RNA-seq method and human brain tissue, we will characterise distinct transcript profiles. This project will build upon a cutting-edge programme of research by exploring the role of transcriptomic regulation on synaptic processes which contribute to cognitive decline with age.

References to learn more:

Dominissini, D., S. Moshitch-Moshkovitz, S. Schwartz, M. Salmon-Divon, L. Ungar, S. Osenberg, K. Cesarkas, et al. "Topology of the Human and Mouse M6a Rna Methylomes Revealed by M6a-Seq." Nature 485, no. 7397 (May 10 2012): 201-6.Mauer, J. et al. Reversible methylation of m(6)Am in the 5' cap controls mRNA stability. Nature 541, 371-375, doi:10.1038/nature21022 (2017).

Location of lab rotation: QMC

Location of full PhD project: QMC

(31) Role of post-translational modifications in DNMT3B activity

Primary supervisor: Cristina Montiel-Duarte

Second supervisor: David M. Heery

Institution: Nottingham Trent University

School: School of Science and Technology

Lab rotation description:

The nine weeks lab rotation will be used as a 'mini' project where the student will perform in silico analyses to determine protein domains and putative post-translational modifications as well as binding motives in DNMT3B. The student will also design primers for subcloning and mutagenesis; be trained in bacterial culture, transformation and plasmid extraction; perform site-directed mutagenesis, digestions and DNA extraction from agarose gels as well as interpret sequencing data. With the successful constructs, the student will transfect human cell lines (after being trained in cell culture) and study its expression and effect through western blotting, proliferation and viability assays (using the IncuCyte life cells analysis system and fluorescence microscopy).The overall goal will be the preparation of DNMT3B deletion and point-mutation constructs to later study modifications and map interactions through immunoprecipitations and mass spectrometry, as well as through the preparation of crystals and the analysis of structural data.

Full PhD project description:

Scientific backgroundEpigenetic changes such as post-translational modifications in histones, modifications in the DNA or interactions with non-coding RNA can modulate gene expression. For example, DNA methyl transferases (DNMTs) transfer a methyl group from the donor S-Adenosyl methionine (SAM) to a cytosine, normally in a CpG dinucleotide, and this modification is associated with gene repression.During the aging process there is an alteration in gene expression driven by changes in epigenetic modifications and more specifically, by distinct changes in DNA methylation patterns. In fact, an increase in age is associated with a decrease of DNMT3B mRNA levels (a de novo DNA methyl transferase) in peripheral blood mononuclear cells.However, aging is not the only factor altering DNA methylation. DNMT3B is often overexpressed or deregulated in cancer and certain intestinal microorganisms are associated with specific patterns of DNA methylation in the blood. In addition, changes in nutrition affect the type of microbiota present in the body and, in elderly people, an altered nutrition is quite common. A bit surprisingly, in spite of DNA methylation relevance in gene expression, very little is known about DNMTs regulation through, for example, post-translational modifications (phosphorylation, acetylation, methylation etc.).Preliminary resultsUsing immunoprecipitations and proximity ligation assays, we have confirmed that DNMT3B can be acetylated in vivo. We also have preliminary data suggesting this acetylation leads to a decrease in the amount of DNMT3B and a reduction in its methyl transferase activity. Acetylation is one of the main post-translational modifications, providing a link between protein regulation and the metabolic state of the cell. This modification depends on the availability of acetyl-CoA (which the microbiota can influence) and the action of lysine acetyltransferases and deacetylases.Scientific aimThis project will aim to elucidate the role of post-translational modifications in DNMT3B activity and to identify the relevant enzymes (acetylases, deacetylases, methylases, kinases) involved in these modifications. Additionally, as DNMT3B monomers could be a better target for degradation, we will also aim to answer these questions: (1) does any of the modifications discovered favour the

monomeric state? (2) what are the key residues involved in the interaction between DNMT3B and the identified modifier enzyme/s? Student deliverablesThe project supports a new interdisciplinary collaboration between Dr Montiel-Duarte's group at NTU, Prof Heery's group at the U. of Nottingham and Dr Flaig's group at Diamond Light Source and the Research Complex at Harwell.The student will:• Develop methodologies to express different DNMT3B mutants and identify interacting partners in the context of human cell lines, studying the role of post-translational modifications in the activity and levels of the enzyme as well as in cell proliferation and survival• Prepare crystals for structural analysis via X-ray crystallography and interpret structural data aiming to identify the key residues involved in the interactions observed.The studentship provides a unique opportunity to develop core experimental skills in biochemistry, structural biology and bioinformatics, facilitated by the supervisory team and the excellent research facilities involved.

References to learn more:

1. MICHALAK, E.M., BURR, M.L., BANNISTER, A.J., DAWSON, M.A., 2019. The roles of DNA, RNA and histone methylation in ageing and cancer. Nature Reviews in Molecular Cell Biology, 20(10), pp. 573-589.
2. FIELD, A.E., ROBERTSON, N.A., WANG, T., HAVAS, A., IDEKER, T., ADAMS, P.D., 2018. DNA Methylation Clocks in Aging: Categories, Causes, and Consequences. Molecular Cell, 71(6), pp. 882-895.

Location of lab rotation: Clifton Campus

Location of full PhD project: University Park; Clifton Campus; Research Complex at Harwell; Diamond Light Source;

(32) Understanding how traumatic early life experience shapes brain development

Primary supervisor: Tracy D Farr

Second supervisor: Gareth Hathway

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

During the rotation, the student will have the opportunity to acquire both data acquisition and analysis skills. This will encompass laboratory based in vivo and ex vivo preclinical experiments, and analysis of human imaging neonatal data. The student will participate in experiments that record electrophysiological activity from networks of single neurons in the living adult rat spinal cord. The experiments are designed to understand what cellular mechanisms are responsible for modulating the response to pain in the spinal cord, and how the response changes following traumatic events early on in life. The supervisors use a wide range of animal models of early life traumas. The student will also learn how to handle and process ex vivo rodent brain samples, and perform a variety of histological stains for inflammatory markers. This will be combined with basic microscopy imaging to capture the inflammatory responses to pain, and the student will be trained to analyse the data. The student will also have the opportunity to manipulate in vivo neonatal MRI data from humans and rodents. This will involve some basic image processing and computational techniques to estimate structural and functional connectivity patterns. Depending on the preferences of the student, the project can be adapted to focus more on the laboratory or analysis aspects.

Full PhD project description:

The post-natal period is characterised by rapid change within the central nervous system of the newborn. There is evidence that perception of the intensity of a stimulus (i.e., how strong it is) is different in early life. Traumatic events such as prematurity, painful experiences, and combinations of the two have been shown to alter sensory and emotional responses to noxious stimuli well into adulthood. This project will investigate the neurodevelopmental processes that occur within interconnected brain structures and supraspinal centres which process pain. It will investigate the way in which these dispersed systems work as a functionally interconnected network using a combination of neuroimaging and computational approaches in data from both preclinical animal models of early life trauma and a neonatal clinical data set. The student will also learn to use an integrated suite of approaches to tackle fundamental questions about the neurobiology of the developing brain, including electrophysiology and neuroimaging with the aim to understand the contribution of the inflammatory system to pain processing.

References to learn more:

1. Gursul D*, Goksan S*, Hartley C, Mellado GS, Moultrie F, Hoskin A, Adams E, Hathway G, Walker S, McGlone F, Slater R (2018) Stroking modulates noxious-evoked brain activity in human infants. Curr Biol. 28(24): R1380-81.
2. Bastiani M, Andersson JLR, Cordero-Grande L, Murgasova M, Hutter J, Price AN, Makropoulos A, Fitzgibbon SP, Hughes E, Rueckert D, Suresh V, Rutherford M, Edwards AD, Smith S, Tournier JD, Hajnal JV, Jbabdi S, Sotiropoulos SN (2019) Automated processing pipeline for neonatal diffusion MRI in the developing Human Connectome Project. NeuroImage. 185: 750-63.

Location of lab rotation: QMC

Location of full PhD project: QMC

(35) Development of a non-invasive imaging toolbox for investigating muscle physiology and its application to peripheral vascular disease

Primary supervisor: Nicholas Blockley

Second supervisor: Paul Greenhaff

Institution: University of Nottingham

School: Life Sciences

Lab rotation description: The Blockley laboratory works on the development and application of techniques to quantify physiology using magnetic resonance imaging (MRI) in health and disease. We work with techniques such as arterial spin labelling (ASL) to map tissue perfusion and quantitative BOLD (qBOLD) to map tissue oxygen extraction fraction. When combined these techniques enable the oxygen metabolism to be measured. So far we have applied these techniques in stroke and TBI in the brain, but in this project we want to apply these traditionally neuro techniques to the study of skeletal muscle.In this lab rotation, the student will be involved in MRI data acquisition at the Sir Peter Mansfield Imaging Centre and exercise testing at the David Greenfield Human Physiology Unit. Human participants will undertake exercise within the MRI scanner (repeated plantar flexion) using an MRI compatible ergometer. MRI will be used to measure perfusion and oxygen extraction in the calf at multiple locations. This will be compared with whole calf MRI measures of blood flow and oxygenation acquired in arteries and veins proximal to the muscles of the calf.

Full PhD project description:

Peripheral vascular disease (PVD) of the lower extremities is the third leading cause of atherosclerotic cardiovascular morbidity, after coronary artery disease and stroke. It is estimated that worldwide 202 million people were living with PVD in 2010. Whilst PVD is caused by arterial stenosis or occlusion, the associated pain is centred in the muscle of the affected limb (typically the calf). However, due to loss of muscle mass, PVD patients cannot typically undergo muscle biopsy, which would provide important physiological insights. Therefore, non-invasive techniques are required to aid in the study of PVD and development of new treatments. Magnetic Resonance Imaging (MRI) offers the opportunity to non-invasively assess muscle quality by mapping tissue perfusion and oxygen metabolism. The full PhD project would therefore be targeted at developing and validating such techniques in human volunteers. The project will involve working with human volunteers to test these new developments and to implement improvements. Importantly these measurements must be made during exercise because of the large variation in blood flow demand between rest and physical activity. This is achieved using a range of MRI compatible ergometers to stress the cardiovascular system or to specifically stress the calf musculature. By using such instrumented exercise machines, it is possible to ensure that each participant performs at the same relative level of effort, reducing intersubject variance. However, the performance of exercise during MRI measurements has its down sides, largely due to motion of the participant. Therefore, it will be important to find ways to isolate this motion through consideration of the biomechanics of the exercise and post-processing to remove residual artefacts. Image analysis will also form an important part of the project and you will be working with cutting edge tools which you will be using in novel ways. Finally, the sensitivity of these techniques will be tested by using drugs to vasodilate blood vessels and/or upregulate metabolism. The ultimate aim being to produce a toolbox to investigate PVD. There will also be an opportunity to work across other projects utilising techniques

like dynamic nuclear polarisation (DNP) to assess the cycling of metabolic substrates such as pyruvate.

References to learn more:

1. Wang, C., Zhang, R., Zhang, X., Wang, H., Zhao, K., Jin, L., … Fang, J. (2015). Noninvasive measurement of lower extremity muscle oxygen extraction fraction under cuff compression paradigm. Journal of Magnetic Resonance Imaging, 43(5), 1148–1158. Retrieved from http://doi.wiley.com/10.1002/jmri.25074
2. Englund, E. K., Rodgers, Z. B., Langham, M. C., Mohler, E. R., Floyd, T. F., & Wehrli, F. W. (2018). Simultaneous measurement of macro- and microvascular blood flow and oxygen saturation for quantification of muscle oxygen consumption. Magnetic Resonance in Medicine, 79(2), 846–855. https://doi.org/10.1002/mrm.26744

Location of lab rotation: QMC

Location of full PhD project: QMC

(36) Multidrug pumps in cancer: how do they do it?

Primary supervisor: Ian Kerr

Second supervisor: Steve Briddon

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

ABCG2 is one of three human transporter proteins known as multidrug pumps. In cancer these pumps are overexpressed leading to the resistance of the tumour to chemotherapy. This project aims to better understand the molecular basis of multidrug transport by the human transporter ABCG2.The goals for the rotation are two-fold: Firstly, to get the student experience in purification of ABCG2 from mammalian cells. This will involve training in cell culture, membrane preparation and affinity chromatography. Suitable samples will be analysed by mass spectrometry (peptide mass fingerprinting) in collaboration with Professor Oldham (co-supervisor). This would help shape the remainder of the project by identifying how much of the ABCG2 protein is "visible" in mass spectrometry and what optimisation needs to occur before the carbene footprinting aspect of the project. Secondly, the student would initiate research into using nanoBRET to elucidate how this technique can give information on the binding of diverse pharmaceutical substrates to ABCG2. The student would gain training in confocal microscopy, SMALP solubilisation of membrane proteins and bioluminescence energy resonance transfer (BRET) using a defined fluorescent transport substrate of ABCG2. This would help shape the remainder of the project by identifying the optimisations required for compound library screening.

Full PhD project description:

ABCG2 is one of three human transport proteins known as multidrug pumps. These transporters are incredible in that they use the hydrolysis of ATP to export from cells a vast range of different chemistries. They play important roles in the absorption and excretion of most pharmaceutical compounds used today; in cancer these pumps are frequently overexpressed leading to the resistance of the tumour to chemotherapy. Knowledge of how ABCG2 interacts with many different chemistries is vital to understand the role of this protein in health and disease. Revealing how some chemicals may inhibit the transporter, rather than being a transport substrate will prove beneficial for therapeutic targeting of the transporter (see the two linked reviews for more information). Recent data has provided the groundwork for understanding two incredibly important aspects of ABCG2 function:i) Where on the protein do drug substrates and transporter inhibitors interact? Is this one large site or cavity, or do different chemicals binding to distinct sites before moving through a common transport pathway?ii) How do inhibitors differ in their interaction compared to substrates? Combinations of emerging and powerful techniques will enable you to address this question during your PhD. You will firstly improve the purification of human ABCG2 either into detergent micelles, or into a membrane mimetic environment (known as "SMALPs"); purification of the protein will enable you to study ABCG2 in isolation from possible confounding factors, whilst use of SMALPs will retain ABCG2 in a shell of lipid molecules; this is relevant as most substrates and inhibitors are highly hydrophobic so will interact with ABCG2 through the lipid bilayer. Then you will use bioluminescence energy transfer (BRET) to interrogate the binding of substrates and inhibitors to ABCG2. BRET will enable you to measure in membrane patches or in intact cells the affinities of

substrates and inhibitors for ABCG2 and how changes in the conformation of the ABCG2 transporter (e.g. upon ATP binding/hydrolysis) affects these interactions. Using an existing library of ABCG2 mutants you will be able to start to pinpoint resides on the protein that are essential for substrate and inhibitor interaction.In parallel, you would use purified ABCG2 in a mass spectrometry labelling technique in which the protein is chemically labelled with a probe molecule (carbene) in presence or absence of drug substrates or inhibitors. Where the pre-incubation with a substrate or inhibitor results in masking of a patch of the ABCG2 surface this will be revealed by a change in the carbene footprint. Mapping data from both approaches onto the emerging structural data for ABCG2 will enable you to answer the 2 important questions posed above and make a huge contribution to transporter biology. The project would be jointly supervised by academics with overlapping areas of interest and expertise: Ian Kerr (ABCG2), Steve Briddon and Nicholas Holliday (confocal imaging and BRET) and Neil Oldham (carbene footprinting) and you would get the opportunity to work in all supervisors' labs as well as attend relevant international conferences to present your work.

References to learn more:

1. Kapoor, P., Horsey, A. J., Cox, M. H., and Kerr, I. D. (2018) ABCG2: does resolving its structure elucidate the mechanism?, Biochem Soc Trans 46, 1485-1494.
2. Horsey, A.J., Briggs, D.A., Holliday, N.D., Briddon, S.J. and Kerr, I.D. (2019) Application of fluorescence correlation spectroscopy to study substrate binding in styrene maleic acid lipid copolymer encapsulated ABCG2. Submitted to BBA Biomembranes November 2019

Location of lab rotation: QMC

Location of full PhD project: QMC

(37) Immune profiling the effect of E-Cigarette vapour on human bronchial epithelial cells

Primary supervisor: Lucy Fairclough

Second supervisor: Victoria James

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The nine-week lab rotation will build all the necessary skills to ensure comprehensive analysis of the effects of E-Cigarette exposure to Bronchial Epithelial Cells. The mini project will use commercially available human Bronchial Epithelial cells.Specifically, the student will learn sterile tissue culture techniques to culture bronchial epithelial cells (weeks 1-5), they will practice multicolour flow cytometry (I am Academic Lead for Flow Cytometry at UoN) (weeks 6-7) and they will optimise ELISAs for multiple cytokine measurements (weeks 8-9) (Selvarajah et al, 2014). The aim of this work will be to set up a panel of techniques suitable for doing a broad screen of immune function after exposure to E-Cigarette vapour.

Full PhD project description:

Electronic cigarettes (E-cigarettes) are considered a preferable alternative to conventional cigarettes due to the lack of combustion and the absence of tobacco-specific toxicants. E-cigarettes have rapidly gained in popularity in recent years amongst both existing smokers and previous non-smokers. In fact there are now thought to be 41 million vapers worldwide, with 2.8 million in the UK. But vaping is not without risk and there is little evidence to date about the effect of vaping on the lungs, specifically lung epithelial cells.Exosomes are a subtype of microvesicles which facilitate cell to cell communication. They are released from every cell within the body, and contain molecules such as DNA, miRNA, mRNA, proteins and lipids. In addition to their role in intercellular communication, exosomes are now also considered as agents of immunoregulation that can modulate antigen presentation, immune activation, suppression and surveillance. This PhD will examine the effect on exosome generation and cytokine production of in vitro exposure of bronchial epithelial cells to a variety of E-Cigarette vapes. The techniques used will include cell culture, immunomagnetic separation, ELISA, size exclusion chromatography and novel imaging flow cytometry (using our new ImageStream Flow Cytometer).

References to learn more:

1. Chen, I-L, Todd I and Fairclough LC. Immunological and pathological effects of electronic cigarettes. Basic Clin Pharmacol Toxicol. 2019;1–16.EVs: Tkach, M and Thery, C. Cell. 2016; 164: 1226-1232

Location of lab rotation: University Park

Location of full PhD project: University Park

This is a linked project – we recommend you select project 71 alongside this one.

(38) The perfect storm: how two problematic processes combined can give a plant an advantage. Abiotic stress tolerance in neo-tetraploids.

Primary supervisor: Sina Fischer

Second supervisor: Guillermina Mendiondo

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

The mini-project will focus on training. The student will gain experience in experimental design in laboratory 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: 1) Experimentation with Arabidopsis and crop plants in growth rooms alongside SF. The student will learn to work with A. thaliana. He/She will perform a pilot experiment to assess germination frequency in response to ABA with the goal to determine optimal conditions for the following experiment. ABA responses appear to be regulated differently in neo-tetraploid Arabidopsis plants. Germination in response to ABA will show if genes with altered expression pattern in tetraploids play a role in ABA signalling. 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). The mini project aims at providing preliminary data on the performance of neo-tetraploid Arabidopsis plants which have been shown to have altered responses to abiotic stress. In addition, this mini project will allow the translation of this approach to crop, using barley as a model.

Full PhD project description:

With climate change natural catastrophes such as drought are more and more likely to impact on food production. Neo-tetraploids are resistant to abiotic stresses such as drought and could be utilised to improve yield. The project is connected with the larger projects of the group of SF and GM who's work on improving food production is aligned not only with the University of Nottinghams research strategy for sustainable societies but also the BBSRCs strategic plan to feed a growing population.The student will work on assessing a collection of neo-tetraploid aba mutants and their diploid progenitors. The aim of the project is to unravel the ABA signalling changes which result from whole genome duplication in order to understand the improved response to abiotic stress. The necessary plant material for this project is already present in the lab and the first step is to screen for seed germination in response to ABA and drought tolerance. The student will be able to publish at least one manuscript, detailing their results on ABA signalling and the differences detected between diploids and neo-tetraploids.As a second part of the project the student will translate the knowledge gained in A. thaliana into Barley. To that end the method for whole genome duplication in monocots will have to be optimised. Upon generation of neo-tetraploid Barley lines these will be phenotype under drought to assess abiotic stress tolerance. The student will use the Mendiondo's Lab expertise in barley tissue culture and crop physiology (Year 2 and 3).The student will learn to cultivate A. thaliana under various conditions and assess phenotypes such as germination, wilting, fitness and gene expression. To that end the student will learn the use of good laboratory practices as well as specific techniques to culture and handle plants, extract DNA and RNA, and perform Real

Time PCR analysis. He/she will also learn microscopy techniques. Finally the student will learn to analyse gathered data, including images, extract meaningful information and present them in a scientifically relevant context. Statistical analysis and the use of required programs will be part of the training as well.In addition, training will be provided in the areas of bioinformatics tools in cereals, PCRs, qPCRs and tissue culture. The data generated on the field will be used to train the student in statistical analysis and data presentation (using the dedicated graphics software SIGMA plot). The student will be principally supervised by SF, who has the expertise to work with polyploidy and GM who has the expertise to work in the crop molecular and physiology relating to abiotic stresses in crops (Mendiondo Plant Biotechnology Journal 2016; Vicente/Mendiondo Current Biology 2018;Gibbs et al. Molecular Cell 2014).

References to learn more:

1. Dai-Yin Chao, Brian Dilkes, Hongbing Luo, Alex Douglas, Elena Yakubova, Brett Lahner, David E. Salt. Polyploids Exhibit Higher Potassium Uptake and Salinity Tolerance in Arabidopsis. 2013. Science. 341: 658-659.
2. THIERRY ALLARIO, JAVIER BRUMOS, JOSE M. COLMENERO-FLORES, DOMINGO J. IGLESIAS, JOSE A. PINA, LUIS NAVARRO, MANUEL TALON, PATRICK OLLITRAULT, RAPHAËL MORILLON. Tetraploid Rangpur lime rootstock increases drought tolerance via enhanced constitutive root abscisic acid production. 2013. Plant, Cell and Envir. 36: 856-868.

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(40) Maximizing fitness in the germline

Primary supervisor: Andrew Renault

Second supervisor: Markus Owen

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

Our lab seeks to understand the signals that regulate the migration, survival and competition between germ cells during Drosophila embryonic development. Germ cells are important for fertility because they give rise to the germline stem cells that produce the almost limitless supply of gametes during the lifetime of the adult. Competition between germ cells thus has the potential to improve the fitness of the germline stem cells leading to increased fertility. A former PhD student has recently visualised and published the first complete 3D migration of Drosophila germ cells in vivo using cutting edge light-sheet microscopy. For this rotation we will use this technique to make further movies of wild type embryos and extend it to a number of mutant backgrounds. The student will then track the migration of germ cells using semi-automated techniques. From such a dataset the student will determine a number of useful characteristics about the migration (including average speed and variation, mean distance between germ cells, relative position of dying germ cells). These will be used as part of the full PhD project to generate and refine the mathematical models that we wish to generate to understand the chemotactic signals that underlie migration and survival.

Full PhD project description:

Cell competition is an interaction between cells in which intrinsic cellular properties are compared by neighbouring cells resulting in the selective elimination of a subset of cells ('losers') and expansion of the fitter cells ('winners'). Competitive cell interactions are widespread both during development and in the adult and occur in multiple cell types. Cell competition can be beneficial, acting as a quality control mechanism to promote tissue repair, but it may also be detrimental, for example, by expanding the pool of oncogenic mutant cells during tumour growth.Work in the field is mostly based on analysis of stationary cells in direct contact with each other in an epithelium. This PhD project will uncover the mechanisms of cell competition in migratory cell type, namely the germ cells, which generate the stem cells that produce the gametes of animals. Competition in this cell type would be important for the fertility of the organism and hence the growth potential of the population. This PhD will define the principles and mechanisms of cell competition during migration in germ cells. We already know the key protein, called Wunen, expressed by germ cells that can regulate both their migration and survival, but we don't know how these processes are interlinked and the key parameters underlying competition. During this PhD the student will attempt to answer: How do Wunen levels influence relative germ cell position during migration? How does heterogeneous Wunen expression mediate competitive interactions between germ cells and the emergence of winners and losers? Do winning germ cells become the germline stem cells and therefore make the predominant contribution to fertility?These questions will be addressed using a mixture of genetic, cell biology and mathematical approaches, the relative proportion of which will depend on the strengths and interests of the student.In terms of genetics and cell biology, the student will use genomic tagging to label Wunen with GFP in order to visualise Wunen levels live

during germ cell migration. This will enable the student to test the hypothesis that germ cells with higher Wunen levels migrate faster and are more likely to survive than germ cells with lower levels. We have recently published the first complete 3D migration of Drosophila germ cells in vivo using cutting edge light-sheet microscopy and the student will be able to adapt these protocols to image both the relative position of the germ cells and their Wunen levels. A possible computational approach would involve the student developing 3D mathematical models of germ cell migration based on existing live imaging data and that obtained from the rotation. We would use such a model to make predictions about the shape of the gradient of the germ cell chemoattractant and if and how a single chemoattractant could theoretically regulate both migration and competition. The model would then tested by using it to generate predictions about germ cell behaviour upon alteration of key parameters and comparing these to what is seen when these parameters are manipulated genetically in vivo.

References to learn more:

1. Kenwrick K*, Mukherjee A, Renault AD. Hmgcr promotes a long-range signal to attract germ cells independent of Hedgehog. J Cell Sci. 2019 Nov 12. pii: jcs.232637. doi: 10.1242/jcs.232637. [Epub ahead of print] PubMed PMID: 31719159.
2. Barton LJ, LeBlanc MG, Lehmann R. Finding their way: themes in germ cell migration. Curr Opin Cell Biol. 2016 Oct;42:128-137. doi:10.1016/j.ceb.2016.07.007. Epub 2016 Jul 30. Review. PubMed PMID: 27484857; PubMed Central PMCID: PMC5064876.

Location of lab rotation: QMC

Location of full PhD project: QMC

(41) Induction of autophagy by important animal and human viruses

Primary supervisor: Stephen Dunham

Second supervisor: Christopher Coleman

Institution: University of Nottingham

School: Veterinary Medicine and Science

Lab rotation description:

During the rotation project the student will create a base for the future PhD project. The student will learn the basic techniques involved in the project, including: cloning of viral genes into expression plasmids, tissue culture of immortalised cell lines, Western blotting and confocal microscopy. During the 9-week project the student will be expected to make some progress in the following areas:1) Cloning coronavirus genes into expression plasmids and/or creating mutant versions of coronavirus genes.2) Cell transfections to confirm viral protein expression, determine protein localisation and assess induction of autophagy using appropriate techniques, including Western blot and/or confocal microscopy.

Full PhD project description:

Influenza viruses and coronaviruses (CoVs) are important pathogens of humans and animals, with a number of these viruses posing a serious zoonotic threat. Both contain viruses that infect animals (e.g. avian influenza and infectious bronchitis virus (IBV) respectively) and humans (e.g. influenza A and Middle East respiratory syndrome coronavirus (MERS-CoV)). Human infections range from mild upper respiratory tract infections (e.g. influenza C and hCoV-229E) through to severe, and often fatal, pneumonia (e.g. influenza H5N1 and MERS-CoV). Similarly, animal pathogens cause important diseases in poultry and livestock, with significant morbidity and mortality (e.g. avian influenza and IBV in poultry, porcine coronaviruses). There are currently no approved treatments for coronaviruses and limited treatment options for influenza viruses.The most pathogenic influenza or corona- viruses are CL3 pathogens that require handling in specialist containment facilities. For this project, we will use plasmids expressing viral genes that can be handled in CL2 facilities. Drs Dunham and Coleman have independently discovered an important role for autophagy in the lifecycle of influenza viruses and coronaviruses, respectively. Autophagy, literally 'self-eating', is the process by which cells dispose of damaged cell parts. The main sign of autophagy is the formation of cellular autophagosomes, vesicles that transport damaged cell parts to the degradation pathway.Influenza viruses induce autophagosome formation during virus replication, however, viral matrix protein 2 blocks autophagosome fusion with lysosomes (Gannagé M et al, 2009). Furthermore, the induction of autophagy is thought to enable virus replication and subvert host immune responses. We observed a difference in the cellular response of avian hosts to influenza A infection (Kuchipudi et al, 2012) with duck cells showing increased levels of both apoptosis and autophagy in comparison with chicken cells. One aim of this project is therefore to investigate further the role of autophagy in chicken and duck cells and to determine the importance in each host on virus replication and innate immune response.MERS-CoV non-structural protein (nsp) 6 induces autophagosomes, perhaps as a mechanism to relocate key cellular antiviral proteins away from the site of virus replication (unpublished data). A range of human and animal coronavirus nsp6 proteins are able to induce autophagosomes (Cottam, EM et al, 2011), but the mechanism of action or role in viral pathogenesis is not determined. To investigate this further, nsp6 genes from human and animal coronaviruses will

be cloned into expression plasmids (using gene synthesis where necessary) and tested for induction of autophagy. In both cases, the mechanism of autophagy induction will be determined using a range of molecular and cellular biology techniques, including Western blotting, immunoprecipitation, confocal microscopy (for localisation and co-localisation of viral and host proteins) and electron microscopy (to observe autophagosomes in detail).This project will provide a unique platform to study the role of autophagy in a range of important animal and human viral pathogens. This could lead to novel targets for drugs that target one or both of these virus groups.

References to learn more:

1. Cong Y, Verlhac P, Reggiori F. The Interaction between Nidovirales andAutophagy Components. Viruses 2017, 9(7), 182; https://doi.org/10.3390/v9070182
2. Yeganeh B, Ghavami S, Rahim MN, Klonisch T, Halayko AJ, Coombs KM. Autophagyactivation is required for influenza A virus-induced apoptosis and replication.Biochim Biophys Acta Mol Cell Res. 2018, 1865(2):364-378. https://doi.org/10.1016/j.bbamcr.2017.10.014

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(42) Inhibition of mechanistic target of rapamycin (mTOR) as a novel therapeutic for maintaining skeletal muscle mass during ageing

Primary supervisor: Daniel Wilkinson

Second supervisor: Matthew Brook

Institution: University of Nottingham

School: Medicine

Lab rotation description:

The student will undertake training in novel mass spectrometry, bioinformatics, molecular biology and cell culture during their nine week lab rotation. Starting at QMC in the labs of Dr Brook, the student will undertake training in cell culture and molecular biology assisting with ongoing projects looking at the effects of ribosomal biogenesis on muscle growth. Following this they will transfer to the labs at the Royal Derby Hospital where they will learn mass spectrometry analytical techniques, alongside the application of stable isotope tracers techniques for the measurement of human in vivo metabolism. Finally, remaining at the Royal Derby Hospital the student will undertake training in OMICs and informatics, unique and powerful techniques that can provide a in depth picture of health and disease.

Full PhD project description:

Skeletal muscle is the largest organ in the body, comprising ~50% of total body mass. The function of muscle is best known for its role in locomotion and providing mechanical support to the skeleton. However, muscles are important for maintaining whole-body metabolic health. For example, muscles also act as a sinkhole for glucose disposal acting to maintain appropriate whole-body glycaemic control. In addition, skeletal muscles represent a vast protein store, the amino acids from which can be broken down in time of fasting, infection and disease in order to provide energy to maintain other critical organs. It is on this basis that losses of skeletal muscle mass associated with prevalent diseases (e.g. cancers/organ-failure/diabetes/bed-rest) and ageing, are major socio-economic concerns in terms of morbidity and mortality. The loss of muscle occurring in older age poses a major socio-economic burden and one without current solutions, despite sarcopenia being recognised as a diagnosable condition (https://www.ncbi.nlm.nih.gov/pubmed/30312372). As such, there is a clinical need to find solutions for this, especially given the failure of “novel” pharmaceuticals (most recently, myostatin antagonism). Our potential solution to this is to provide proof-of-concept that targeting hyper-active mTOR in older individuals will preserve muscle. Recent research has shown that in addition to extending lifespan in lower mammalian species, inhibition of mTOR signalling positively influences neuromuscular outcomes - especially when adopted transiently, later in life. These studies indicate that targeting the age-related hyperactivity of mTOR could be of therapeutic value in relation to ageing, and in particular, mitigate muscle ageing. Here we propose to make the first step to address this in humans. Recent phase II trials have demonstrated the safety of Rapamycin (Rapamune, Pfizer) treatment in older-aged humans, presumably with a view to assessing the safety of similar and longer-term trials testing the general efficiency of mTOR inhibition in relation to multiple aspects of healthy human ageing. Here, we propose to undertake the first study in humans charting the physiological, metabolic and molecular effects upon skeletal muscle following medium-term (i.e. 4-months) mTOR inhibition under rested

and contractile-activity (i.e. exercise) conditions. Using state-of-the-art imaging (MRI, at Sir Peter Mansfield Imaging Centre), metabolic (e.g. D2O and muscle metabolism) and molecular (targeted mTOR signalling and RNA-Seq) approaches, this study will determine the underlying mechanisms and efficacy of mTOR inhibition in relation to mitigating skeletal muscle ageing directly in humans.

References to learn more:

1. Joseph, G. A. et al. (2019) 'Partial Inhibition of mTORC1 in Aged Rats Counteracts the Decline in Muscle Mass and Reverses Molecular Signaling Associated with Sarcopenia.', Molecular and cellular biology, 39(19). doi: 10.1128/MCB.00141-19.
2. Kraig, E. et al. (2018) 'A randomized control trial to establish the feasibility and safety of rapamycin treatment in an older human cohort: Immunological, physical performance, and cognitive effects', Experimental Gerontology. Elsevier Inc., 105, pp. 53–69. doi: 10.1016/j.exger.2017.12.026.

Location of lab rotation: QMC; Derby Royal Infirmary;

Location of full PhD project: QMC; Derby Royal Infirmary

(43) The protein secrets of ancient teeth

Primary supervisor: Rob Layfield

Second supervisor: David Boocock

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

In the rotation the student will be trained in aspects of contemporary protein science and paleoproteomics. They will first perform extraction of ancient proteins from archaeological samples of their choice available in the lab e.g. medieval human bones and teeth, dental calculus, calcified tissues from extinct species (mammoth, dinosaur), ancient mortar (for insights into construction techniques), parchments, food residues on ancient ceramics etc.Extracted protein mixtures will be analysed by gel electrophoresis and western blotting for initial QC, then constituents will be identified by hands on proteomic analyses (at NTU) combined with protein sequence analyses and database searching.The overall goal will be to generate a catalogue of ancient proteins present in the given sample(s), with particular attention paid to discovery of protein sequences that have not previously been reported in the field of paleoproteomics.

Full PhD project description:

BackgroundLike its subject matter the fledgling field of paleoproteomics – the analysis of ancient proteins - has laid dormant until recently. However, recent breakthroughs have brought the field to the fore of molecular research. Paleoproteomics recognises that some proteins are exceptionally stable, surviving in mineralised tissues for up to millions of years. Consequently, paleoproteomics offers remarkable opportunities in, for example, molecular reconstruction of ancient lifestyles and in extending direct investigation of health and disease into the human evolutionary past.ContextThe Layfield group recently applied paleoproteomics to diagnose an unusual medieval form of Paget's disease, affecting human skeletons from Norton Priory in Cheshire, based on protein sequencing from 800 year-old bones and teeth [Shaw et al. (2019) PNAS]. Others have detected proteins from disease-causing bacteria in the pulp/dentine of plague victim's teeth [Barbieri et al. (2017) PLoS One]. Further, proteins recovered from dental calculus (plaque) reflect pathogens and host immune responses in the ancient human oral cavity [Warinner et al. (2014) Nat Genet] and also allow reconstruction of paleodiets from identification of food remnants [Hardy et al. (2017) Evolutionary Anthropol]. Finally, recent studies have demonstrated dental enamel preserves proteins for extraordinarily long timeframes – millions of years [Welker et al. (2019) Nature].In unpublished work the Layfield group has also discovered it is possible to purify intact, functional antibodies preserved in human teeth (pulp/dentine) for at least 800 years. We have generated protein sequence information, and importantly demonstrated proxy function from reactivity with contemporary EBV antigens. The potential of ancient antibodies to provide new insights into the long-term association between host immune factors and ancient microbes, and more broadly the natural history of human health and wellbeing, is completely unexplored.The Boocock group has pioneered development of advanced quantitative mass-spectrometry based approaches (SWATH-MS) towards identification of protein biomarkers relevant to contemporary human health and disease. Notably, these cutting-edge MS approaches have never been applied to paleoproteomic analyses. Strategy, Hypothesis, PlanThe studentship provides a new synergy to shape a collaborative paleoproteomic research space

across UoN and NTU. Our overarching hypothesis is that teeth represent incompletely explored storehouses for ancient proteins, with different ‘compartments’ holding different ‘molecular memories’ (see summary table).The student will:1. Develop methodologies allowing identification of ancient proteins from human teeth across time (1000-1700AD), towards simultaneous investigation of pathogen activity, host immunity, local/systemic disease, and ancient diet, integrated at the individual level.2. Interpret the data to inform developments in global food security (e.g. paleobenchmarking of ancient diets) and future medicines (e.g. identification of use of non-nutritive dietary components correlating with putative pathologies).

References to learn more:

Warren M. Move over, DNA: ancient proteins are starting to reveal humanity's history. Nature. 2019 Jun;570(7762):433-436.

Location of lab rotation: QMC; Clifton Campus;

Location of full PhD project: QMC; Clifton Campus

(44) Imaging immune responses to implanted biomaterials

Primary supervisor: Peter Harvey

Second supervisor: Amir Ghaemmaghami

Institution: University of Nottingham

School: Medicine

Lab rotation description:

The student would perform staining of existing tissue samples from foreign body response experiments with antibodies and molecular dyes. The aim is to image biomarkers of inflammation, focusing on immune cell infiltration and macrophage polarisation. The student would generate new data while training in appropriate tissue handling techniques, pipetting, histochemistry, and microscopy.There will also be opportunities to learn synthetic chemistry skills by tagging nanoparticles with antibodies and other targeting vectors. By focusing on pre-synthesised/ commercial magnetic resonance imaging (MRI) contrast-active nanoparticles, the student would be able to obtain antibody-tagged contrast agents in a realistic time frame. These compounds would allow the student to do some hands-on preliminary in vitro MR imaging within the rotation. This rotation would allow the student to develop a number of new skills, while also gaining an understanding and appreciation of the interconnected aspects of the full project. Similar, yet distinct, rotational projects with related skill sets and experiments are currently being carried out as part of another Doctoral Training Program. The successes and limitations resulting from these projects will be used to refine the rotational project offered here.

Full PhD project description:

The development of materials to form medical devices is a fundamental aspect of modern healthcare. In an ideal scenario, the device should not be targeted by the immune system as a foreign material and the interaction between the device and its host environment should accelerate healing and better device integration. Unfortunately, this ideal is frequently undermined by chronic inflammation, foreign body encapsulation, and other detrimental side effects. For patients implanted with medical device, around 20% will develop infection or chronic inflammation as a direct side effect. In order to understand the biological interactions and triggers of inflammation, we require methods to monitor inflammation around medical devices in vivo over time. Magnetic resonance imaging (MRI) is a powerful tool for non-invasive imaging of living subjects, with unparalleled imaging depth and an ideal combination of spatial and temporal resolution. Current MRI approaches are limited by a lack of molecular detail. This PhD project will focus on improving preclinical imaging of inflammation around device implant sites. The primary method will be through the design, synthesis, and application of novel MRI contrast agents that can target and report on acute and chronic inflammation biomarkers. By advancing the imaging tools at our disposal, we will be able to non-invasively monitor inflammation pathology in living subjects in real-time. Initial focus will be on preclinical rodent models in order to guide further development of medical devices by monitoring the effects of different materials/applications. Long-term goals include translation to the clinic with potential applications beyond implant related inflammation. Two approaches will be explored to address this challenge. The first will rely on nanoparticle-based MRI contrast agents coupled to inflammation-specific antibodies. These agents can be topically or systemically administered and selectively accumulate at sites of inflammation via biomarker recognition.

Judicious selection of immune-responsive biomarkers should allow various stages of inflammation to be mapped. The second approach utilises small molecule MRI contrast agents that report on the underlying physiological condition around device implantation. Example targets include pH and temperature, which are both substantially altered during the immune response. More invasive complimentary techniques (e.g. electrical recordings, histology) will be carried out in parallel to validate new techniques and imaging agents.The student will benefit from an excellent supervisory team spanning multiple fields and departments (Medicine/Life Sciences/Pharmacy). The lead supervisor is currently the academic champion for preclinical MRI at UoN and has a strong background in the development and application of MRI contrast agents. The co-supervisors are world-leaders in medical device development and immunology, with substantial supervisory experience. The student will develop a diverse skillset, including synthetic chemistry, nanoparticle development, histology, immunological studies, analytical methods, and animal handling and imaging. It is envisaged the studentship will result in significant outputs and support a number of ongoing and future projects within the field of medical devices and bio-instructive materials. There are a number of international collaborators associated with the project and it is envisaged that the student would be able to conduct a short research placement at MIT as part of the studentship.

References to learn more:

1. D. A. Hammoud, 'Molecular Imaging of Inflammation: Current Status', J. Nucl. Med., 2016, 57, 1161-1165.
2. E. Dondossola, B. M. Holzapfel, S. Alexander, S. Filippini, D. W. Hutmacher, P Friedl, 'Examination of the foreign body response to biomaterials by nonlinear intravital microscopy', Nat. Biomed. Eng., 2016, 1, 0007.

Location of lab rotation: QMC

Location of full PhD project: University Park; QMC

(45) Universal sensors for identifying intracellular allosteric modulators of G protein-coupled receptors (GPCRs)

Primary supervisor: Nicholas Holliday

Second supervisor: Shailesh Mistry

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

In this mini-project (supervised by Dr Holliday), the student will characterise the effects of intracellular allosteric modulators on a representative GPCR, the chemokine receptor CXCR2. This receptor, expressed on a variety of immune cells, in an important therapeutic target for chronic obstructive pulmonary disease and asthma. We will use our in house luciferase complementation methods to explore the effect of candidate compound(s) on CXCR2-G protein and CXCR2-arrestin interactions. We will also use a TR-FRET assay to monitor the effect of IAMs on chemokine binding. Both sets of assays are well established in our lab, and will provide the student with experience in state of the art bioimaging techniques and pharmacological analysis, with opportunities for training in mutagenesis and molecular cloning techniques. The student will establish baseline assay data for commercial allosteric modulators on CXCL1 and CXCL8 chemokine signalling, and learn the associated analysis. In parallel, there will be the opportunity for molecular cloning experience. The student will construct at least one CXCR2 receptor point mutant based on existing receptor models, and then explore the effects of the mutation on orthosteric and allosteric ligand pharmacology.

Full PhD project description:

An exciting recent development in G protein coupled receptor (GPCR) pharmacology – a hugely important family of receptors for drug discovery - has been the emergence of a wide variety of allosteric modulators. These ligands bind receptors at distinct binding sites from the native stimulating hormone or neurotransmitter, and can provide enhanced target selectivity as well as tailored positive or negative effects on function. Intracellular allosteric modulators (IAMs) work by competing with the G protein binding site on the receptor, to switch off signalling, rather than with the messenger molecule directly. IAMs were originally identified for a few important chemokine receptors, but now new examples have emerged for a completely different GPCR, the beta2-adrenoceptor. This means that IAM-type molecules could be promising in future as potential therapeutics for a broad range of different GPCRs, provided we are able to first identify suitable candidate compounds successfully.The aim of this multi-disciplinary project is to develop new fluorescent sensors to study the IAM binding site that are able to work across the GPCR family. These sensors will then be used to understand better the molecular mechanisms by which IAMs exert their effects, and also to establish a binding assay using the latest imaging technology to identify new molecules. You will first synthesise short peptide ligands (GaC), corresponding to the C termini of the different G proteins that can engage with GPCRs. Fluorescent derivatives of each peptide, labelled at the N terminus, will then act as tracers in a novel binding assay for compounds at the G protein interaction site. This method involves TR-FRET (time resolved-Förster resonance energy transfer) technology, in which the tagged receptor is labelled with a donor fluorophore (Terbium). When excited, the fluorescence emission from the donor can be transferred to excite the acceptor fluorophore on the peptide – but only if this peptide is in close proximity (<10 nM) and is

specifically bound to the receptor. In this way you will be able to determine the mechanism by which GaC peptides bind the receptor (for example, if its binding is stimulated by receptor activation), and also identify compound leads that can compete for this binding at the G protein interaction site. Once GaC pharmacology is established, at example GPCRs coupled to each G protein class, you will use the screening assay in earnest for an example receptor to identify new candidate IAM molecules. The screening process will exploit the managed compound collection (MCC) of > 80K compounds (School of Pharmacy, Nottingham), with initial in silico work to define a pharmacophore model using your library of tracer peptides. This is a chemical biology and therapeutics focused project which will particularly appeal to those interested in pharmacology and drug discovery. The multidisciplinary approach will combine high quality training in molecular pharmacology with synthetic chemistry and modelling (prior experience in these areas not required). Your work will provide new insights into how IAMs work, and broaden their relevance to new important members of the GPCR family.

References to learn more:

1. Liu, X., Ahn, S., Kahsai, A. W., Meng, K.-C., Latorraca, N. R., Pani, B., et al. (2017). Mechanism of intracellular allosteric β2AR antagonist revealed by X-ray crystal structure. Nature, 548(7668), 480–484. http://doi.org/10.1038/nature23652
2. Wold EA, Chen J, Cunningham KA, Zhou J (2019) Allosteric Modulation of Class A GPCRs: Targets, Agents, and Emerging Concepts. J Med Chem. 62(1):88-127. http://doi.org/10.1021/acs.jmedchem.8b00875

Location of lab rotation: University Park; QMC;

Location of full PhD project: University Park; QMC;

(46) Yeast diversity and its implications for continuous manufacturing

Primary supervisor: Simon Avery

Second supervisor: Klaus Winzer

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The rotation project offers the experience of key skills associated with the main project. The rotation will be aligned specifically with objective 1 (below), which has the advantage that results can be consolidated into the main project if the student wishes to undertake this for their PhD. The specific objective in this rotation will be to gain experience through study of an exemplar, high-value product in yeast cultures. The product will be human albumin, monitored by ELISA and expression of a fluorescently-tagged version of the protein. The student will test production both by the bulk yeast population as well as by individual cells within it. With microfluidic technology and time-lapse fluorescence microscopy, the project will ask the question whether changes in albumin production over time are uniform across the cell population or whether production phenotypes are individual to cells. The further question to be tackled then is whether individual-cell phenotypes are heritable. With this rotation plan, the student will gain early insight to whether genotypic or non-genotypic diversification may be driving altered production yields by yeasts. The timescale of the rotation will also allow the student to engage fully with the vibrant community of researchers within our laboratories.

Full PhD project description:

The global market for yeast-derived biologics (mostly therapeutic proteins, vaccines, etc) is currently valued at approximately $40Bn per annum. These high-value proteins are produced in yeasts because these organisms that are fast growing, eukaryotic (i.e., suitable hosts for expression of eukaryotic proteins), well characterised, safe and hardy. The current industry trend is increasingly moving to continuous manufacturing processes, where there are no interruptions to production. This approach has obvious economic advantages. However, it also brings to the fore a problem with the yeast system that has to date been side-stepped. That is, that the producing cell population becomes heterogeneous – showing mixed phenotypes – which can lower production yields over time. Theoretically this can occur from within 40-60 generations of growth (potentially only a few days) after a production strain has been established and banked. The metabolic burden of production could account for this decline, as it may create a selection pressure for faster-growing low-yield variants. To date, however, this problem has not been characterised. Given the value of the market and the current market trend, a project addressing this clear problem is timely and overdue. Yeast diversity in this context also presents intriguing academic questions, which this interdisciplinary project will address. The overall aim is to test the hypothesis that high-yield production drives diversification and selection in yeast cultures, while natural genetic diversity could be exploitable to address this problem. The cross-disciplinary supervisory team has expertise in yeast biology, bioinformatics and microbial fermentation, and has excellent contacts with industry including Nottingham-based company Phenotypeca who have agreed to advise here on the relevant industry perspective. There will be outstanding training opportunities for the student, including industry-relevant research and skills that will open different career-path possibilities. The specific

objectives are:1. How rapidly do product-yields decline during continuous passage of yeast? 2. Is altered product-yield due to emergence of novel genotypes or novel phenotypic variants (i.e. non-genotypic heterogeneity, NGH) within cultures? 3. Can alternative yeast genetic-backgrounds or manipulation of NGH be harnessed to ameliorate production over time? The project will focus primarily on the chassis Saccharomyces cerevisiae, with which we have extensive experience and resources, including diverse strain libraries and culture facilities. The student will focus primarily on one product of interest (e.g., albumin), corroborating key findings with another (e.g., hepatitis B as surface antigen). We have access to the relevant constructs and production yeast strains. Analyses will be at the level of bulk population as well as single cells, facilitated by time-lapse microscopy (examining fluor-tagged product in cells) and flow cytometry or FACS. The student will benefit from microfluidics capability (CellASIC) recently introduced to our laboratories for single cell and NGH studies, as well as options for scale-up in the fermentation laboratories. Training will be provided in all of the necessary skills and there will be excellent networking opportunities including with industry contacts.

References to learn more:

Heins AL, Weuster-Botz D (2018). Population heterogeneity in microbial bioprocesses: origin, analysis, mechanisms, and future perspectives. Bioproc Biosyst Engineering 41:889-916

Hewitt SK, Foster DS, Dyer PS, Avery SV (2016). Phenotypic heterogeneity in fungi: Importance and methodology. Fungal Biol Rev. 30:176-84.

Location of lab rotation: University Park

Location of full PhD project: University Park

(47) Epigenetic and epitranscriptomic networks in gene regulation and cancer

Primary supervisor: Nigel Mongan

Second supervisor: Catrin Rutland

Institution: University of Nottingham

School: Veterinary Medicine and Science

Lab rotation description:

The student will learn the fundamentals of bioinformatics, molecular biology and pathology. To this end the student will mine existing RNA sequencing datasets generated in the lab and will use approaches such as qRTPCR, cell culture, western blotting, immunohistochemistry and cell line models of cancer to understand how epigenetics and epitranscriptomics cooperate to regulate steroid signalling in cells, including stem cells.

Full PhD project description:

Epigenetics relates to covalent modifications of chromatin and has long been known to influence gene expression. Epitranscriptomics is the emerging study of covalent modifications of RNA, which is now known to also influence gene expression RNA processing, stability, splicing and translation. Our labs have demonstrated complex roles for both both lysine demethylase epigenetic coregulators and components of the RNA methylation epitranscriptomic complex in gene regulation and alternative splicing. In a recent ground-breaking study in Nature, chromatin modifications of histone H3 lysine methylation were shown to regulate m6A RNA methylation. This is the first evidence of mechanistic links between epigenetics and epitranscriptomics. In previous work, we have shown that epigenetic modifying enzymes such as histone H3 lysine demethylase enzymes and epitranscriptomics modifying enzymes involved in RNA methylation are in involved in gene regulation. However, the exact mechanisms whereby epigenetics and epitranscriptomics cooperate in steroid signalling and cancer are unknown. We propose that epigenetic and epitranscriptomic dysfunction contributes to malignant transformation of normal cells and promoting cancer by altering normal steroid-regulated gene expression. To test this hypothesis, this project will utilize reagents generated in previous PhD projects to determine the mechanistic interactions of epigenetic and epitranscriptomic complexes in androgen and estrogen regulated gene expression in normal and malignant cells. To this end the following specific aims will be completed1. We will interrogate existing RNA sequencing data from cells treated with pharmaco-inhibitors and/or depleted of lysine demethylase transcriptional coregulators and components of the RNA methylation apparatus to assess the functional interdependence of these complexes in androgen signalling.2. The effect of functional inhibition of lysine demethylases and RNA methylation will used to assess reciprocal regulation of expression of these factors in cells.3. We will determine the role of KDMs and RNA modifying enzymes in normal and malignant cell lines.4. We will test the ability of pharmaco-inhibitors of KDM epigenetic enzymes and m6A epitranscritpomic complexes to prevent, reverse or delay resistance to anti-cancer agents in cell lines models. Collectively these aims will address the fundamental mechanisms of cooperation between epigenetics and epitranscriptomics in gene regulation. Furthermore the data will underpin future projects to identify novel diagnostic and therapeutic targets to improve cancer outcomes. The student will learn a variety of techniques and skills in high demand in academic and pharmacuetical research. The student will also join and interact with a large and vibrant multi-dsciplinary international collaborative network of scientists working in the USA, Europe and Japan. Theses and

network will help enable the student to have a productive and successful start to their research career.

References to learn more:

1. https://www.nature.com/articles/nature20577https://onlinelibrary.wiley.com/doi/abs/10.1111/ejh.13367

Location of lab rotation: University Park

Location of full PhD project: University Park

This is a linked project – we recommend you select project 125 alongside this one.

(48) SCRaMbLEing transcriptional regulation in yeast

Primary supervisor: John Heap

Second supervisor: Benjamin Blount

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

With the availability of fresh water highly likely to become an increasing global concern, the ability of strains used in biotechnology to produce molecules of interest in high-salt, or brackish, conditions is highly desirable. Saccharomyces cerevisiae yeast is a biotechnology workhorse and recent advances in synthetic genomics have led to the availability of yeast strains with synthetic chromosomes, into which a system for rapid inducible evolution (SCRaMbLE) has been encoded. Yeast responds to osmotic stress through activation of the HOG MAPK pathway which, in turn, activates several transcription factors associated with increased salt tolerance. In this project, additional copies of the HOG MAPK-activated transcription factors in a SCRaMbLE-compatible format will be engineered into a yeast strain with a synthetic chromosome XI (synXI). The effects of these genes on host salt tolerance, both individually and in combination, will be assessed. A synXI strain with the full set of SCRaMbLE-formatted transcription factor genes will then be rapidly evolved by SCRaMbLE. This will generate a diverse pool of strains with varied gene content and arrangement, both in the formatted transcription factors and in the synthetic chromosomal background. Selection methods will be used to isolate individuals with rearrangements that lead to improved salt tolerance.

Full PhD project description:

BackgroundThe yeast Saccharomyces cerevisiae is an important organism for both fundamental research and biotechnological applications. Advances in our understanding of genetics, genomics and cell biology, combined with improvements in our ability to chemically synthesise DNA, have led to the design and construction of whole synthetic chromosomes in yeast. Designed into these synthetic chromosomes is a system, SCRaMbLE, which allows inducible large-scale chromosomal rearrangements of the synthetic sequences. Recent studies have shown that SCRaMbLE can be used to rapidly generate huge pools of genetically and phenotypically divergent strains, from which individuals with improved characteristics can be isolated. Conventional techniques to engineer strains for desired properties can be powerful but expensive, lengthy and often rely on detailed biological understanding of the organism. Strain improvement using SCRaMbLE could address many of these drawbacks as wide phenotypic diversity can be generated very quickly, notably by altering the chromosomal context of transcription factors. By making comparatively subtle changes to expression of genes involved in regulating fundamental cellular processes, unpredicted and dramatic changes in cellular behaviour can emerge. AimThis project will aim to apply SCRaMbLE genome rearrangement techniques to generate strains with a variety of enhanced target phenotypes. By incorporating selected deregulated transcription factors into SCRaMbLE, the project will seek to discover and characterise alterations in transcriptional regulation that cause notable effects on cell behaviour. ApproachDNA constructs encoding specific transcription factors under the control of unregulated (constitutive) promoters will be incorporated into synthetic yeast strains that will then undergo SCRaMbLE. By sampling the resultant diverse populations and characterising the genetic and genomic rearrangements underpinning phenotypic changes, this project will contribute towards

our understanding of the transcription factor-mediated regulation acting to control these cellular behaviours. Initially, transcription factor-encoding genes involved in the salt tolerance mechanisms of yeast will be coupled with constitutive promoters and chromosomally integrated into multiple synthetic yeast strains via CRISPR-mediated recombination. An approach that combines multiple rounds of SCRaMbLE with selection in increasing salt concentrations will be used to generate and isolate strains with improved salt tolerance. High-performing strains will be phenotypically characterised and sequenced to help determine chromosomal rearrangement events that contribute towards salt tolerance phenotypes.Transcription factors identified as contributing towards the regulation of industrially relevant properties, including heat tolerance, pH tolerance and growth substrate specificity, will then be integrated into synthetic yeast in the same fashion. SCRaMbLE under conditions selecting for the specific phenotypes of interest will again be used to isolate strains with rearrangements resulting in favourable phenotypes.Where changes in transcription factor genes are identified as likely contributors to interesting phenotypes, whether by changes in those genes' regulation, copy number or output, these will be reverse engineered into yeast to further probe genotype-phenotype links. Promising strains from this process will be applied to specific applications. These could vary from the development of yeast and cyanobacteria co-cultures, which would benefit from yeast strains with improved salt-tolerance and sucrose utilisation, through to directly engineering industrial production strains for more efficient production in bioreactor conditions

References to learn more:

1. Annaluru N., Muller H. et al. (2014) Total Synthesis of a Functional Designer Eukaryotic Chromosome. Science. 344(6179):55-8. doi: 10.1126/science.12492522.
2. Steensels J., Gorkovskiy A. and Verstrepen K.J. (2018) SCRaMbLEing to understand and exploit structural variation in genomes. Nat Commun. 9,1937. doi:10.1038/s41467-018-04308-3

Location of lab rotation: University Park

Location of full PhD project: University Park

(49) The role of E2F7/8 on HIF-1 and cellular response during hypoxia

Primary supervisor: Amanda S Coutts

Second supervisor: Alan McIntyre

Institution: Nottingham Trent University

School: School of Science and Technology

Lab rotation description:

The 9 week lab project will involve examining the localisation and the effects of E2F7/8 expression on cell survival and cell cycle regulation during hypoxia. This will involve expression of E2F7 and E2F8 in human cancer cells to allow visualisation by fluorescence microscopy (to observe localisation). The student will also assess the impact of E2F7/8 on cell cycle regulation using siRNA-mediated knockdown or CRISPR knockout cell lines along with flow cytometry to monitor cell cycle stages via DNA staining. Effects on cell survival and proliferation will be monitored during hypoxia using real-time cell impedance measurements (xCELLigence RTCA).

Full PhD project description:

The cellular response to hypoxia (low oxygen) plays a significant role in solid cancers such as breast and colorectal cancer as well as in human aging which is characterised by a decline in tissue oxygen levels. Hypoxia is highly relevant to cancer progression; as the tumours grow, regions of low oxygen develop, which promotes angiogenesis and is associated with disease progression, metastasis and resistance to therapies. The cellular response during hypoxia is regulated by the transcription factor HIF-1, a heterodimeric complex containing the oxygen-regulated HIF-1 and the constitutively expressed HIF-1 subunits. During hypoxia HIF-1 is stabilised and nuclear HIF-1 binds to hypoxia-responsive elements (HREs) in target genes critical for many important cellular processes including angiogenesis, proliferation, apoptosis and survival. The E2F family of transcription factors are key regulators of cell proliferation with eight family members identified to date. E2F7 and E2F8, the most recently identified family members, form homodimers or heterodimers, and are considered atypical as they contain two DNA binding domains and regulate transcription independent of Rb (retinoblastoma) and DP proteins. E2F7/8 are known transcriptional repressors that influence cell cycle regulation by repressing E2F-1 activity. In mice, E2F7/8 knockout is embryonic lethal due to widespread apoptosis and vascular defects. Research has identified a critical role for E2F7/8 in angiogenesis during foetal development in mice and zebrafish where they form a complex with HIF-1 to regulate vascular development through stimulating VEGFA (vascular endothelial growth factor A) transcription. Interestingly, this unexpected positive transcriptional role occurs via a HIF-1 binding site (HRE) rather than the expected E2F binding site. Moreover, in cancer cell lines E2F7 was shown to regulate VEGFA transcription through an interaction with HIF-1. In contrast, E2F7/8 inhibited angiogenesis and tumour vascularisation in mouse and zebrafish models of cancer, underscoring the context-specific outcomes of E2F7/8 function. Importantly, the role of E2F7/8 in relation to HIF-1 in human cancer has not been fully explored. This project will explore the role of the key cell cycle regulators E2F7/8 and the interplay with HIF-1 during hypoxia in human cancer. This study will provide novel information into mechanisms crucial to human cancer and in understanding basic cell biological mechanisms involved in oxygen sensing and cell cycle regulation.Specific aimsThis study will investigate the interplay between E2F7/8 and HIF-1 during hypoxia in cancer. In particular this project will investigate: E2F7/8 regulation and function during hypoxia and the requirement for E2F-

1 activity. The impact of E2F7/8 on HIF-1 activity and cellular outcome. Identify the E2F7/8 interactome during hypoxia Techniques and Methodology:The student will use a range of techniques to explore E2F7/8 regulation during hypoxia and the interplay with HIF-1 (chromatin-immunoprecipitation, reporter assays, siRNA depletion/CRISPR knockout) in human cancer cell lines. Functional assays will include live cell imaging, real-time impedance and flow cytometry to investigate cell survival, cell cycle regulation, migration and invasion. Mass spectrometry will be used to identify novel interacting proteins and confirm any interaction with HIF-1 during hypoxia in human cells.

References to learn more:

1. Regulatory mechanisms of hypoxia-inducible factor 1 activity: Two decades of knowledge. Koyasu S, Kobayashi M, Goto Y, Hiraoka M, Harada H. Cancer Sci. 109: 560-571, 2018.E2F
2. Transcription Factors Control the Roller Coaster Ride of Cell Cycle Gene Expression. Thurlings I, de Bruin A. Methods Mol Biol. 1342:71-88, 2016.

Location of lab rotation: Clifton Campus

Location of full PhD project: Clifton Campus

(51) The Role of the Neurovascular Unit in Maintaining Normal Blood-Brain Barrier Function and Healthy Ageing

Primary supervisor: Zahraa Al-Ahmady

Second supervisor: Claire Gibson

Institution: NTU

School: Science and Technology

Lab rotation description:

Dr Al-Ahmady laboratory works on understanding blood-brain barrier (BBB) alterations associated with different neurological conditions such as stroke and Alzheimer's disease. Although understanding BBB alterations in those conditions is crucial to develop novel targeted drug delivery systems it is necessary to further determine the function of the BBB under normal physiological conditions and ageing. Prof Gibson's group has also extensive experience in several cerebrovascular disorders including ageing and a wealth experience in different animal models, in vivo imaging and behavioural studies.

In the lab rotation project, the student will carry out cryosectioning, immunohistochemical staining, confocal microscopy, digital analysis and quantification of different cellular and molecular component of the BBB in aged rodents. These include; assessing endothelial cell expression of cell adhesion molecules (ICAM-1, VCAM-1, and E/P Selectins), pericytes coverage and neutrophils infiltration into the brain. The student will also investigate similar parameters in young rodents and mouse model of stroke as a negative and positive control, respectively. This will then be followed by evaluation of BBB integrity by analysing the infiltration of endogenous immunoglobulins into the brain which are normally excluded from the brain parenchyma by an intact BBB.

Full PhD project description:

Healthy ageing is an important goal in modern societies due to the demographic shift in the proportions of older people and the economic burdens associated with that. The brain vasculature is composed of highly specialised cells that form blood brain barrier (BBB) e.g. endothelial cells and pericytes, which play a critical role in maintaining normal brain function. The BBB also restricts the entry of many blood-borne molecules including toxins and inflammatory cells into the brain. However, there is now increased understanding that the structure and function of BBB is altered in many neurodegenerative diseases. Alterations to the BBB can have a direct effect on neuronal and synaptic functions through changes in blood flow, BBB permeability, nutrient supply, faulty clearance of toxic molecules and altered secretion of trophic factors. Interestingly, a recent clinical study demonstrated a strong association between pericyte loss, BBB disruption and cognitive impairment in humans. However, the field is still at the beginning of the journey to fully interrogate BBB alterations in ageing and their implications in neurodegenerative disorders. Based on the above, understanding how to preserve the cellular component and function of BBB through selective targeting approaches may represent a new therapeutic target to promote healthy ageing because of its critical role for normal brain function.

The aim of this project is to investigate the biological changes to key cellular and molecular components of the neurovascular unit (e.g endothelial cells and pericytes) during the ageing process that may compromise BBB function. We also aim to selectively target those regions with nanoparticles to preserve their function. The central hypothesis is that the communications between

different component of the neurovascular unit are critical to maintain the neurovascular functions which are essential for normal brain performance. Our project aims to identify the underlying process during ageing that contribute to the loss of such communication and thus leads to progressive age-dependent vascular damage mediated by BBB disruption and entry of neurotoxic and inflammatory cells into the brain.

To test this, we propose to use normal aged rodents and accelerated ageing models such as SAMP8 mice and combine, at the later stages of the project, with inducible comorbidities such as peripheral inflammation and infection. We will use state-of-the art imaging techniques such as optical live imaging, in vivo multiphoton microscopy, SPECT/CT imaging, quantitative autoradiography and in vitro and in vivo markers of BBB permeability to study the structure and function of the neurovascular unit and the potential of selective targeting using nanoparticles. The proposed project will provide a better understanding of the role of the BBB alteration induced by ageing and the potential of selective targeting approach to provide new diagnostic and therapeutic tools.

References to learn more:

1. Age-Associated Physiological and Pathological Changes at The Blood-Brain Barrier: A Review. Erdő ,Denes,de Lange. J Cereb Blood Flow Metab. 2017 (1):4-24.
2. Selective Liposomal Transport through Blood Brain Barrier Disruption in Ischemic Stroke Reveals Two Distinct Therapeutic Opportunities. Al-Ahmady ZS, Jasim, Ahmad SS, Wong R, Haley M, Coutts G, Schiessl, Allan S, Kostarelos K. ACS Nano. 2019 ;13(11):12470-12486.

Location of lab rotation: University Park; Clifton Campus

Location of full PhD project: Clifton Campus

(52) The glycocalyx on the maternal surface of the human placenta: help or hindrance to materno-fetal nutrient transport?

Primary supervisor: Lopa Leach

Second supervisor: Kenton Arkill

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The student will learn to visualise the placental glycocalyx, using well-established lab techniques and confocal microscopy. Archived and newly acquired human term placenta from normal pregnancies and those complicated by diabetes (over nutrition) and pre-eclampsia (under-nutrition) will be analysed. These analyses will be performed in Life Sciences (Leach Lab). A pilot study on the absence/extent of the glycocalyx and the composition of the glycocalyx (in situ mass spec) will be performed in the School of Medicine (Arkill lab).

Full PhD project description:

The human placenta is an essential organ for the fetus; it protects the fetus from harmful maternal pathogens and immune cells whilst delivering oxygen and vital nutrients from the mother to the fetus. It is haemochorial- placental villi lie bathed in maternal blood and can directly take up nutrients (Fig 1a). These solutes have to cross an outer syncytiotrophoblast layer and an underlying fetal endothelium to enter fetal blood (Fig 1b). Both layers provide resistance in series; pathology here lead to compromised fetal growth and later cardiovascular disease (fetal origin of disease). Pilot studies in Leach lab demonstrated that the syncytiotrophoblast has an extensive glycocalyx layer- a potential third layer of resistance (Fig 1c).

Fig 1a- Placental villi, containing fetal blood vessels, lying bathed in maternal blood; Fig 1b -2 Layers separate maternal and fetal blood; Fig 1c- An extensive glycocalyx (green) cover the outer lining of all placental villi. Note some fetal vessels (red) also have luminal glycocalyx.

In other systems, the endothelial surface glycocalyx (GLX) layer has been proven to provide large resistance to solute transport [1]. GLX carry negative charge, which deter charged solutes. GLX has been shown to initiate vasodilation, via the eNOS and nitric oxide pathway and alter blood flow [2]. Thus, the urgent questions this project will ask are: What is the function of this layer in the placenta? Is it hindering solute transport or filtering what may or may not get across? Does it ensure maximal vasodilatation of maternal flow to the placenta for optimal nutrient delivery?
To address these questions this project will look at the composition of the placental glycocalyx from normal pregnancies and those complicated by diabetes (overnutrition; fetal macrosomia) and preeclampsia (fetal growth restriction; high sheer stress; reduced nutrient transport; reduced eNOS). Using confocal microscopy and in situ mass spectroscopy, the glycocalyx coverage will be correlated with fetal growth and maternal complications. An ex vivo perfused placental model will be used to investigate effects of altered flow on transport of tagged hydrophilic solutes, changes in GLX coverage and composition, altered eNOS and endothelial adhesion molecules. Doppler ultrasound angiography will reveal changes in maternal and fetal blood flows in the placenta. Epigenetic alterations in key genes will be probed.
The data will deliver new knowledge on the role of the placental glycocalyx in regulating nutrient transfer and fetal growth. The impaired mechanisms underlying trans-placental nutrient transfer in

different pregnancy complications will be elucidated and lead to novel approaches to alleviating fetal demise and later cardiovascular complications.

References to learn more:

1. M. GOUVERNEUR B. VAN DEN BERG M. NIEUWDORP E. STROES H. VINK Vasculoprotective properties of the endothelial glycocalyx: effects of fluid shear stress. J of Internal Medicine. https://doi.org/10.1111/j.1365-2796.2006.01625.x
2. N. L. Pillinger (2017). Endothelial Glycocalyx: Basic Science and Clinical Implications. Anaesthesia and Intensive Care. https://doi.org/10.1177/0310057X1704500305

Location of lab rotation: QMC

Location of full PhD project: University Park; QMC

(53) The genomic and phenotypic basis of a biological invasion

Primary supervisor: Mark Ravinet

Second supervisor: Andrew MacColl

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The rotation is flexible depending on the skills that the student has prior to starting; it is designed to ensure skill development in 3D morphometrics and bioinformatics.

Identifying craniofacial evolution during the context of a biological invasion is an important part of the proposed project. Using a dataset of 90 high resolution microCT scans of sparrow skulls, the student will receive training in designing and applying a set of 3D morphometric landmarks. They will also learn photogrammetry and will produce an additional set of 3D skull images. They will then compare the shape variation characterised by the two methods to gain an appreciation of their different uses and applications.

Bioinformatics and population genomics are a core component of the main project. The student will receive training in handling genomic data using the Unix command-line, variant calling, variant filtering and population genomic analyses including population structure, measures of differentiation and selection. The student will map a pilot dataset (single location, with samples from across the time-series) to the house sparrow reference genome and compare it to an already established contemporary dataset from across the native range of the house sparrow.

Full PhD project description:

Human impact has altered the adaptive landscape and has shifted the evolutionary trajectories of a huge number of species. Many species are able to survive, persist and adapt to a human niche, yet our knowledge of how humans indirectly alter traits, genes and the evolutionary processes involved is limited. The house sparrow (P. domesticus) is a widespread, charismatic and well-known example of a human-commensal bird. We have previously shown that the house sparrow spread from its native range in the Middle East into Europe alongside the movement of early agricultural societies during the Neolithic. However, due to deliberate introductions during the 19th Century, house sparrows have established in North America, South America and Australia. Has this more recent spread to new regions been facilitated by the same morphological and metabolic changes that led to the original shift to a human-dependent niche during the Neolithic? Or has it been driven by new adaptations such as changes in cognition or immune response? Fortunately, house sparrow introductions are well-documented and museum collections contain specimens sampled from the time of introduction to contemporary periods. This makes it possible to quantify changes in allele frequencies and phenotypes across the temporal scale that tracks the recent spread of this commensal species, providing evidence of selection and insight into adaptive mechanisms.

We have established a dataset of house sparrows sampled from 5 locations (Melbourne, Rio de Janeiro, New York, Toronto and San Francisco) at 20, 50 and 100-years post-introduction (15-20 samples per time period). Using whole genome resequencing, this project will first seek to test the hypothesis that modern house sparrow introductions have been founded by multiple independent evolutionary lineages. The second part of the project will be to identify genomic signatures of

selection in populations from the introduced range of the house sparrow. In particular, we hope to use our unique time-series data to reconstruct allele frequency changes during the course of the introduction, focusing on a set of candidate genes involving cognition, beak shape, immune response and digestion. Combined with our contemporary dataset of >400 genomes from across the native range of the species, it will be possible to explicitly test whether selection has occurred on the same genes in the more recent spread. In addition to genomic data, we have access to museum collections with 1974 house sparrow individuals collected over the last 141 years. The project will use 3D morphometrics and photogrammetry to identify phenotypic evolution in house sparrows following introduction, focusing on craniofacial morphology.

The overarching aim of this project is to understand the spread of anthrodependent taxa in an evolutionary context and to shed light on the genomic and phenotypic basis of a biological invasion. The successful student can expect training in bioinformatics, population genomics, 3D morphometrics and photogrammetry. There is also potential to work with collaborative partners on investigating immune response and behavioural evolution.

References to learn more:

Ravinet, M., Elgvin, T.O., Trier, C.*, Aliabadian, M., Gavrilov, A. & Sætre, G.-P. 2018. Signatures of human-commensalism in the house sparrow genome. Proceedings of the Royal Society B: Biological Sciences 285: 20181246–10. The Royal Society.

Location of lab rotation: University Park

Location of full PhD project: University Park

(55) Resilience of agriculturally important spiders to insecticides

Primary supervisor: Ian Mellor

Second supervisor: Sara Goodacre

Third supervisor: Emyr Davies (Rothamsted)

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The lab rotation will provide the student with experience in several technical aspects of the full project:

1. Voltage-gated sodium channels and nicotinic acetylcholine receptors from ticks and mites will be expressed in Xenopus oocytes by injection of their cRNA and their functional responses will be assessed using two-electrode voltage-clamp electrophysiology. Associated design of experimental protocols and analysis of data will also be performed to assess important functional parameters that are affected by insecticide exposure.

2. There are annotated genomes currently available on GenBank for spiders from two distinct phylogenetic lineages (Theredіidae and Araneidae) that will be searched using bioinformatic tools for proteins with homology to voltage-gated sodium channels and nicotinic acetylcholine receptors in other arachnids such as Ixodes ticks.

3. Bioassays with selected insecticides (from the pyrethroid and neonicotinoid groups) will be performed on an agriculturally relevant species of spider. Spiders will be exposed to insecticides through contact or by developing an assay where spiders feed on insecticide treated prey. Assays will be designed to determine LD50s but also to investigate behavioural effects.

Full PhD project description:

Spiders are natural pest control agents that have an important role to play in integrated pest management schemes. Populations of certain species can be densely clustered, and some species, such as money spiders, can rapidly disperse and populate large expanses of newly planted areas. Indiscriminate use of insecticides would likely damage these populations and so a more selective approach should be employed.

Pyrethroids and neonicotinoids are two major classes of insecticide consisting of many structural analogues. They target voltage-gated sodium channels (VGSCs) and nicotinic acetylcholine receptors (nAChRs), respectively, with strong selectivity for these targets in arthropods compared to vertebrates. Pyrethroids are particularly diverse and have been developed as effective knockdown agents of insect pests. However, other arthropod pests such as ticks and mites show low sensitivity to many pyrethroids and only a small subset, including tau-fluvalinate and flumethrin, are sufficiently effective against these pests. Our understanding of the pyrethroid binding pocket in VGSCs has led us to a possible explanation for this selectivity. One of the amino acids in the binding pocket, C933, is conserved in all known insect sequences but in ticks and mites it is substituted predominantly by G, but may also be V or A. In vertebrates it is A, and we have previously shown this causes strong resistance to deltamethrin but not to two other tested pyrethroids. Furthermore, G is

found at this site in the bird eating (tarantula) spider and common house spider VGSCs. Neonicotinoids, like many pyrethroids, also have poor effectiveness against ticks and mites and are not used for their control. The likely reason for this is that one site in the ligand binding domain of insect nAChR β-subunits, R81, at which neonicotinoid resistance mutations (R81T) have arisen in many pests, is Q in wild-type ticks and mites. A Q is also found at this position in the Wolf spider and common house spider, suggesting it will be found in most spider species. Additionally, this site provides the basis for vertebrate insensitivity, it being T in nAChR β-subunit sequences.

Hyopothesis. Spiders are arachnids like the ticks and mites described above. We predict that agriculturally important spiders will most likely have VGSC and nAChR sequence characteristics of the ticks and mites and, hence, lower sensitivity to specific pyrethroids and neonicotinoids.

Project aims. The aim of this project will be to clone, sequence and express VGSCs and nAChRs from several agriculturally important spiders of the Linyphiid family. We will go on to characterize the action of a range of pyrethroid and neonicotinoid insecticides through functional electrophysiological studies to confirm the expected target site insensitivity and carry out bioassays (determination of LD50s and behavioural effects) to assess spider insensitivity through other mechanisms. It will also be important to sample spiders from agricultural as well as isolated sites to determine whether exposure to insecticides has given rise to resistance mutations in our target proteins. We will deliver a list of insecticides that may be better choices to allow spiders to act as allies in crop protection.

References to learn more:

1. O'Reilly AO, Williamson MS, Gonzalez-Cabrera J, Turberg A, Field LM, Wallace BA, Davies TGE. 2014. Predictive 3D modelling of the interactions of pyrethroids with the voltage-gated sodium channels of ticks and mites. Pest Management Science 70: 369-377.
2. Erdmanis L, O'Reilly AO, Williamson MS, Field LM, Turberg A, Wallace BA. 2012. Association of Neonicotinoid Insensitivity with a Conserved Residue in the Loop D Binding Region of the Tick Nicotinic Acetylcholine Receptor. Biochemistry 51: 4627-4629.

Location of lab rotation: University Park

Location of full PhD project: University Park, Rothamsted Research

(56) Screening for new antibiotics using native protein mass spectrometry

Primary supervisor: Neil Oldham

Second supervisor: Panos Soultanas

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

Elongation factor-Tu (EF-TU) is responsible for the delivery of aminoacyl-tRNAs (aa-tRNAs) to the ribosomal A site during protein biosynthesis in bacteria. It is an established antibiotic target for the natural product kirromycin, which traps the protein in a compact conformation and prevent its release from the ribosome. This effectively inhibits protein synthesis and kills bacteria. Native electrospray ionisation-mass spectrometry will be used to study the binding of kirromycin and novel natural products to E. coli EF-Tu. This technique is able to reveal drug binding to distinct populations of the protein, for example with and without the presence of GTP co-factor binding, thus enabling the mechanism of binding to be elucidated and new ligands discovered.

The rotation project will include training in protein handling, native mass spectrometry, data processing and presentation.

Full PhD project description:

Increasing antimicrobial resistance is one of the largest threats to our modern urbanised society. Amongst the multifaceted approach to tackling this problem is the need for new antibiotics.

Many new candidate antibiotics come from natural sources, such as microorganisms, where they are present as components of complex mixtures of other compounds. The challenge of identifying active compounds, often of low abundance, from such complex mixtures represents a serious bottleneck. This project aims to exploit a new technique developed by the Oldham group in which the complex mixture from a crude natural product extract is separated into its components using HPLC, and these combined in-line with a selected target protein. Binding is then directly measured by coupled native electrospray ionisation-mass spectrometry (ESI-MS). This approach promises to speed-up the process of drug discovery by flagging-up interesting hit compounds within crude natural product extracts without the need for prior separation.

Native ESI-MS is an existing, specialist, technique able to detect non-covalent complexes between proteins and ligands. Separately, liquid chromatography (LC), especially high performance liquid chromatography (HPLC), is a commonly used for analysing and separating complex mixtures in a sample. LC is routinely coupled to MS, and commercial products are available. The novelty here is the combination of native MS with liquid chromatography. We have shown that the two are compatible, thus allowing complex natural product extracts to be interrogated for binding activity without preceding purification which is both challenging and time consuming.

The full PhD project will develop this platform for screening microbial extracts (provided by our collaborators at the University of Warwick) against antibiotic target proteins to detect ‘hits’ (compounds capable of binding to the target). This will facilitate screening of large numbers of potential antibiotic producing microorganisms for new compounds with activity against validated antibiotic targets.

Hits will be followed up by purification and full chemical/biological characterisation for potential medicinal use.

References to learn more:

1. Song H, et al. (1999) Crystal Structure of Intact Elongation Factor EF-Tu from Escherichia coli in GDP Conformation at 2.05 A Resolution. J. Mol. Biol. 285: 1245-1256.
2. Kosol et al. (2019) Structural basis for chain release from the enacyloxin polyketide synthase. Nat. Chem. 11: 913-923.

Location of lab rotation: University Park

Location of full PhD project: University Park

(57) Investigating the role of APOE-e4 in structural and functional changes to neuromodulatory brain nuclei

Primary supervisor: Christopher Madan

Second supervisor: Andrew Reid

Institution: University of Nottingham

School: Psychology

Lab rotation description:

During the nine-week lab rotation, the Ph.D. candidate will be introduced to important literature linking Alzheimer's disease etiology, noradrenergic and cholinergic systems, the APOE-e4 allele, and associated changes to brain function and morphology. They will be given access to the two large public datasets of interest: the Human Connectome Project and the Berlin Aging Study II, and asked to perform preprocessing steps on neuroimaging data for a subset of these datasets. This will involve using specialised neuroimaging software alongside custom Matlab or Python code, to apply the appropriate processing steps, and instruction will be given on the theoretical and practical aspects of these steps. Finally, multimodal integrative approaches will be discussed and a suitable analytic methodology will be decided upon, as a team including both supervisors and the student. This rotation will allow the student to become acquainted with big data analysis, the use of major neuroimaging software packages, the use of database approaches to organise and filter large data sets, scientific programming using Matlab and Python, and the existing literature and theory underlying a proposed link between AD progression and changes to noradrenergic and cholinergic systems.

Full PhD project description:

Dementia is an age-related, highly debilitating condition. Around 850,000 people in the UK suffer from dementia, at an estimated cost of £26 billion annually. While research has helped us understand some of the biological changes underlying different forms of dementia, there is presently no cure, and its neurodegenerative processes appear to be largely irreversible. As a result, a major focus of current dementia research is on the early changes that occur years or even decades prior to the cognitive deficits that are presently used for diagnosis.

This PhD project will investigate two aspects of early dementia-related phenomena: (1) two neuromodulatory systems – noradrenergic and cholinergic – known to be susceptible to pathology found in Alzheimer's disease (AD; see Ref 1) and other less common forms of dementia; and (2) genetic risk factors for AD and other dementias – in particular, carriers of the APOE-e4 allele, which confers a higher risk of developing AD in later life. We will focus on two major datasets: (1) the Human Connectome Project (HCP), which contains cross-sectional structural and functional neuroimaging, task performance, and whole-genome data from ~1200 healthy young participants; and (2) the Berlin Aging Study II (BAS2), which contains longitudinal data from ~2200 older and ~600 younger participants, including behavioural and whole-genome data, and (for a subset) structural neuroimaging data.

The candidate will analyse these datasets in order to assess differences in the structure, function, and multimodal connectivity between APOE-e4 carriers and non-carriers, in both younger and older cohorts. The use of multimodal approaches will help us focus more closely on the biological mechanisms underlying connectivity changes (see Ref 2). They will learn how to process data

obtained from large, publicly available datasets, containing neuroimaging, neuropsychological, clinical, and genetic information. This will involve the use of Matlab and/or Python programming languages, specialized neuroimaging software including the FSL and SPM packages, and high-performance parallel processing of data using SLURM.

References to learn more:

1. Mather M, Harley CW (2016). The Locus Coeruleus: Essential for Maintaining Cognitive Function and the Aging Brain. Trends Cogn Sci. 20(3): 214-226.
2. Reid AT, Headley DB, Mill Ravi, Sanchez-Romero R, Uddin LQ, Marinazzo D, Lurie DJ, Valdés-Sosa PA, Hanson SJ, Biswal BB, Calhoun V, Poldrack RA, Cole MW (2019). Advancing functional connectivity research from association to causation. Nat Neurosci. 22: 1751–1760.

Location of lab rotation: University Park

Location of full PhD project: University Park

(58) Anticancer therapies from renewable sources

Primary supervisor: Christopher J Hayes

Second supervisor: Rupert Fray

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

This lab rotation is associated with a PhD project that will explore the construction of anti-cancer therapeutics from renewable starting materials. During the lab rotation period, you will become familiar with the chemistry laboratory environment and receive training in a variety of areas. This rotation will suit students who are looking for a project that will involve preparing therapeutically important molecules, so training in the synthesis, purification and characterisation of organic molecules will be provided. At the end of this rotation, you will be able to perform a variety of synthetic experiments, purify your products and collect/analyse spectroscopic data (NMR, IR, Mass Spec etc.).

Full PhD project description:

Well over 50% of anti-cancer drugs take their inspiration from molecules that were isolated from Nature (so called Natural Products). During this PhD project you will explore the preparation of anti-cancer natural products from renewable starting materials. Depending upon your scientific background and previous lab experience, we can offer a selection of specific targets that could be accessed using a blend of synthetic chemistry and synthetic biology. Possible molecular targets are the taxanes (e.g. Taxol), glycosyldiglycerides and/or homoharringtonine (HHT), which represent three distinct structural types (and biosynthetic origins), and display their anti-cancer activity by three different mechanisms (microtubule stabilisation, translation initiation inhibition, translation elongation inhibition). These molecules can all be extracted from plant species, but sustainable/renewable routes for their large scale production are required. During this PhD we will explore preparing these materials synthetically from simpler renewable precursor building blocks, and/or we can exploit protein overexpression techniques to produce them in a host organism (or a combination of the two).

Taxanes: Previous work in our laboratory has shown that taxadiene, the key precursor to Taxol, can be prepared in two different ways using either synthetic chemistry or synthetic biology. In the synthetic chemistry approach, we synthesised taxadiene from the simpler renewable terpene farnesol, and using synthetic biology we were able to overexpresss taxadiene synthase in tomato plants that then produced taxadiene instead of the usual carotenoid pigments. During this PhD project we want to advance this science to explore further steps along the pathway to Taxol, and this will involve exploration of chemical and/or biological oxidation of the hydrocarbon taxadiene scaffold.

Glycosyldiglycerides: Previous work in our laboratory has shown that certain glycosyldiglycerides can be extracted from plant species, and that they show novel anti-cancer activity. Specifically, these molecules are newly discovered selective inhibitors of protein translation, and they have shown efficacy in vivo. These molecules have a modular construction (sugar-glycerol linker-lipid sidechain) and they are readily prepared in the laboratory. During this PhD we plan to synthesise a wide variety of analogues of these molecules to explore their structure/activity relationship. Furthermore, we

will also examine methods to improve their drug-like properties.

Homoharringtonine (HHT): Previous work in our laboratory has resulted in the synthesis of the alkaloid core of HHT. This alkaloid core is called cephalotaxine and it is also produced by the same Japanese Plum Yew tree that produces HHT, and the structures only differ by the presence/absence of an ester side chain. During this project, we will examine the use of biotransformations to attach the ester side chain to cephalotaxine to produce HHT. This will require you to first synthesise the required side chain carboxylic acid, and then explore the use of enzymes for the key biocatalytic esterification reaction.

References to learn more:

1. Howat, S., Park, B., Oh, I. S., Jin, Y-W., Lee, E-K., & Loake, G. J. Paclitaxel: biosynthesis, production and future prospects. New Biotechnology, 2014, 31, 242-245. doi:10.1016/j.nbt.2014.02.010
2. Bhat, M., Robichaud, N., Hulea, L. et al. Targeting the translation machinery in cancer. Nat Rev Drug Discov 2015, 14, 261–278 doi:10.1038/nrd4505

Location of lab rotation: University Park

Location of full PhD project: University Park

(59) Identifying predicative patterns of activity in spinal pain circuitry characteristic of chronic pain and plasticity

Primary supervisor: Gareth Hathway

Second supervisor: Simon Preston

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

During the 9-week lab rotation you will be introduced to the biological and mathematical concepts that underpin this project. You will work with existing data sets to acquire the skills and experience that would enable a student to interrogate new data sets that will be acquired during the PhD project. You will work alongside current members of the research team to learn how biological data is acquired and transfer this knowledge to develop new analytical and statistical tools to assess the existing data sets to address fundamental questions about the physiology and computational processes of spinal sensory circuits.You will work closely with colleagues from both Maths and Life Sciences and will gain a unique training opportunity. You will be embedded within both research environments gaining day-to-day supervision from both sets of academics and their research groups.

Full PhD project description:

The dorsal horn (DH) of the spinal cord, as part of the central nervous system, is a complex network of neurons whose purpose is to respond and process afferent sensory information conveyed to it from the peripheral nervous system. The DH is a layered (laminated) structure, much like the cerebral cortex, with each lamina receiving different sensory inputs. Neurons in each lamina are also highly heterogenous, some are interneurons conveying information to the brain, others are interneurons relaying information from site to site within the DH. The way in which information is processed and flows through the DH is unknown. Previously the physiological and computational properties of the DH have been informed by techniques which rely on the response of one class of neuron which has been considered to be representative of the all others and extrapolating this. We have developed a new technique (Greenspon et al 2018) which permits neuronal activity in all laminae to be sampled simultaneously thus removing the need for inference and extrapolation. This project will form the basis of a unique collaboration between Neuroscientists and Mathematicians to develop new analytical tools to assess the function of the DH activity in health and disease. To date our datasets have been analysed using simple statistical approaches. This project will seek to interrogate these existing and new datasets in new ways. We will compare the basal activity of the DH with that in chronic pain states of different types (inflammatory, neuropathic and osteoarthritic). Currently the way in which afferent sensory information is processed by the different laminae is poorly understood and we have little information about how information passes within and between the laminae, the computational role of interneurons and the impact of pain upon this. The simultaneous recording of multiple laminae produces multi-dimensional time-series data. The correlation within and between these time series will characterise how the neurons respond to stimuli and interact with one another. They will be unpicked initially by data-driven tools of multivariate analysis and time series analysis, and potentially later via mechanistic models of neuronal activity.Pain is a neurological phenomenon that affects the lives of people of all ages and regardless of sex or ethnicity. It blights the lives of people leading to social withdrawal, anxiety and

depression. Central to the experience of pain is the DH and this project will break new ground in understanding how these complex networks function and ultimately lead to behavioural responses and the experienced of pain, with the long-term aim of identifying populations of neurons or signalling pathways that can be manipulated to relieve pain in people and animals.

References to learn more:

GREENSPON CM*, BATTELL EE*, DEVONSHIRE IM, DONALDSON LF, CHAPMAN V, HATHWAY GJ. 2018 Lamina-specific population encoding of cutaneous signals in the spinal dorsal horn using multi-electrode arrays. J. Physiol. 597(2):377-397

Location of lab rotation: QMC

Location of full PhD project: University Park

(61) The role of G protein-coupled receptors in the control of brown adipose tissue

Primary supervisor: Mark Christian

Second supervisor: Michael Symonds

Institution: Nottingham Trent University

School: School of Science and Technology

Lab rotation description:

This research project aims to investigate GPCR signalling in brown adipose tissue (BAT). To elucidate the role of GPCRs in BAT, conditionally immortalised brown adipocytes will be cultured and differentiated. GPR120 is a fatty acid receptor expressed in brown adipocytes. This project will investigate cross-talk between GPR120 and the b3-Adrenergic receptor (b3-AR), which is the main receptor for stimulating expression of UCP1 to facilitate thermogenesis in brown adipocytes. Adipocytes will be treated with ligands for GPR120 and b3-AR separately and in combination. Changes in gene expression of key brown fat genes (Ucp1, Cidea, PGC-1a) will be assessed by q-RT-PCR and Western Blotting. This will reveal whether GPR120 has a role in enhancing or inhibiting the activity of b3-AR-dependent activation of the thermogenic program in brown adipocytes. Preliminary evidence indicates that activation of the b3-AR promotes expression of GPR120. The pathway that mediates this induction will be assessed with the use of inhibitors of known b3-AR downstream signalling components including p38-MAPK and PKA. The lab rotation will provide training in the skills to culture and differentiate brown adipocytes as well as determine changes in gene expression and signalling pathway characterisation.

Full PhD project description:

The increasing prevalence of obesity-related disorders has led to a growing need for therapeutic strategies to reverse diet-induced weight gain. Brown adipose tissue (BAT) contributes to total energy expenditure by burning fat for heat generation by thermogenesis and when maximally activated produces 300 times more heat per unit mass than any other organ in the body. Therefore, interventions that increase BAT activity can be used to reduce obesity. One approach to influence BAT activity is through enhancing or inhibiting signalling through G protein-coupled receptors (GPCRs). Recent studies have shown that the GPCRs GPR43, GPR120, and GPRC5b play an important role in energy metabolism by influencing white adipose tissue activity. However, their full roles in BAT are still to be characterised. This research project aims to investigate GPCR signalling in BAT. To elucidate the role of these GPCRs in BAT, brown adipocytes will be cultured and stimulated with ligands for GPR43 and GPR120. Changes in gene expression (q-RT-PCR), protein content (Western Blotting, immunocytochemistry), lipolysis and mitochondrial metabolism (Seahorse Bioanalysis) will be measured. The signalling pathways activated by the GPCRs will be investigated (cAMP ELISA, Ca2+ Fluo-4-AM imaging) with the use of specific inhibitors of Ga subunits, as well as inhibitors of downstream signalling cascades. GPR43, GPR120, and GPRC5b knockout cell lines will be generated by CRISPR/Cas9 and used to investigate the effect of an absence of GPCR signalling on BAT activity.A key aim of this project is to identify additional receptors that control the function of brown adipocytes by interrogation of available RNAseq and microarray datasets. This analysis will reveal all the GPCRs expressed by brown adipocytes and the set that are enriched in brown compared to white adipocytes. To perform the receptor identification task based on data analytic techniques, we will conduct differential expression analysis followed by pathway/gene set analysis. The focus will be on methods that exploit pathway knowledge in public repositories. In addition, we will also test

state-of-the-art machine learning techniques for this task. Not only do such techniques adopt an integrated and task-driven approach, but they can also combine different data sets and/or different knowledge bases in a principled manner. Candidate GPCRs will be selected from this analysis for investigation of their functional consequences when activated by determining brown adipocyte gene expression and cellular metabolism. Further aims include identifying other receptor subtypes including enzyme-linked receptors that control brown adipocyte activity.This project will provide insight into the role of GPCR signalling in brown adipocytes, which could lead to new therapeutic strategies in activating BAT and reducing obesity.

References to learn more:

Schilperoort M, van Dam AD, Hoeke G, Shabalina IG, Okolo A, Hanyaloglu AC, Dib LH, Mol IM, Caengprasath N, Chan YW, Damak S, Miller AR, Coskun T, Shimpukade B, Ulven T, Kooijman S, Rensen PCN, Christian M. The GPR120 agonist TUG-891 promotes metabolic health by stimulating mitochondrial respiration in brown fat. EMBO Mol Med. 2018. pii: e8047. doi: 10.15252/emmm.201708047. PMID: 29343498.Rosell M, Kaforou M, Frontini A, Okolo A, Chan YW, Nikolopoulou E, Millership S, Fenech ME, MacIntyre D, Turner JO, Moore JD, Blackburn E, Gullick WJ, Cinti S, Montana G, Parker MG, Christian M. Brown and white adipose tissues. Intrinsic differences in gene expression and response to cold exposure in mice. Am J Physiol Endocrinol Metab. 2014. 306(8):E945-964. PMID: 24549398.

Location of lab rotation: Clifton Campus

Location of full PhD project: Clifton Campus

(62) Disease dynamics in freshwater ecosystems: validation of eDNA for informing exposure risk to wild and farmed fish.

Primary supervisor: Hannah Hartikainen

Second supervisor: Stephen Dugdale

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The associated rotation project will conduct a field experiment to test the following hypothesis: Concentration of eDNA in river water correlates with local host biomass and distance to the source population. The project will involve repeatedly visiting focal populations of freshwater bryozoans (benthic, sessile invertebrates, growing on tree roots, rocks and other submerged surfaces). The bryozoans release their own DNA into the water, mainly through faecal pellets and degrading tissue. Bryozoans also release the spores of a highly pathogenic fish parasite (Tetracapsuloides bryosalmonae). Removal of a part of the infected bryozoan population will change the release of both the host and the parasite DNA into water. The student would design such a manipulation experiment, with appropriate controls (e.g. tracers in water), to measure the eDNA release before and after manipulation (gaining training in eDNA extraction protocols and specific quantitative PCR assays for bryozoans and the parasite). To analyse the data, we provide training in the use of statistical models to infer the decay and detection probabilities for both species at various points downstream from the focal population. The results will inform on the power of eDNA sampling to deliver quantitative information on exposure risk to waterborne parasites in natural river systems.

Full PhD project description:

All organisms leave traces of DNA in the environment via sloughed cells, mucous, excretions etc. Such environmental DNA (eDNA) can be extracted from water samples and used to track e.g. rare or invasive species, or to characterise biodiversity more broadly (e.g. fish communities or diversity of invertebrate taxa). Parasites shed from hosts into water can also be detected and quantified simultaneously with the eDNA of their hosts. Such eDNA-based, simultaneous tracking of the host and parasite distributions has the potential to greatly aid in understanding the epidemiology of aquatic diseases. This is important because disease is the major obstacle to the expanding aquaculture industry, and detection, monitoring and surveillance of disease agents via eDNA may provide an important tool for achieving sustainable aquaculture. Aquaculture is closely associated with natural aquatic systems (lochs, rivers, lakes or estuaries) and pathogen spill-over between natural and farmed animals is a constant risk. eDNA may provide particularly important insights to pathogen dispersal, spill-over potential and the temporal variation in exposure risk of farmed animals. This project will focus on eDNA sampling in rivers to track the distributions of parasites and their hosts and assess how parasite transport in rivers affects exposure risk on associated fish farms. The project is interdisciplinary (joint between Life Sciences and Geography) and the student will receive training in: 1a) Field work (eDNA and invertebrate host collections) AND 1b) Molecular lab work (eDNA extraction, quantitative PCR, NGS library preparation and sequencing and bioinformatics). 2) Laboratory experiments (quantifying variation in eDNA and parasite spore decay and release). 3) Spatially explicit analysis/modelling (eDNA and spore transport/decay) and

quantifying the environmental correlates of infection hotspots.Field Collections and Molecular Work: Our previous work on a parasite of salmonid fish has shown that hotspots of high parasite densities in water occur in different parts of a river network. The student will use eDNA sampling to track parasite concentrations across rivers in the UK, and relate the concentrations to the presence of fish and invertebrate hosts (measured from eDNA via “metabarcoding”) to reveal exposure and infection ‘hotspots’. Sampling locations will include sites upstream and downstream from fish farms, in key river systems, to assess the likelihoods of spill-over between natural and farmed settings.Lab experiments: Our preliminary work suggests that parasite detection in water is more variable than detection of host DNA. The student would design and conduct experiments in the lab/field to contrast the detection of eDNA naturally sloughed by hosts with the eDNA originating from parasite spores in water.Spatially explicit models: Understanding the variation in the dispersal, retention and detection likelihoods of parasites is crucial for better reconstructing the distributions of the infected hosts and understanding the effective infective range of spores within networks The student will be trained in geospatial analysis techniques and use state-of-the-art remote sensing to map river networks and habitat features. The aim is to identify the key properties that constrain or facilitate the spread of infectious agents and how river network topology modifies the species distribution information gained from eDNA sampling.

References to learn more:

Bush, A., Sollmann, R., Wilting, A., Bohmann, K., Cole, B., Balzter, H., Martius, C., Zlinszky, A., Calvignac-Spencer, S., Cobbold, C.A. and Dawson, T.P., 2017. Connecting Earth observation to high-throughput biodiversity data. Nature Ecology & Evolution, 1(7), p.0176. https://www.nature.com/articles/s41559-017-0176Carraro, L., Hartikainen, H., Jokela, J., Bertuzzo, E. and Rinaldo, A., 2018. Estimating species distribution and abundance in river networks using environmental DNA. Proceedings of the National Academy of Sciences, 115(46), pp.11724-11729.https://www.pnas.org/content/115/46/11724.short

Location of lab rotation: University Park

Location of full PhD project: University Park

(63) Role of non-coding RNAs in the development of somatosensory systems

Primary supervisor: Federico Dajas-Bailador

Second supervisor: Gareth Hathway

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

This 9-week rotation project will introduce you to the worlds of cell biology and in vivo neuroscience. During the rotation you will learn primary cell culture techniques and you will perform a series of immunohistochemcial and immunocytochemical assays of sensory neuron phenotype from different stages of development.We hypothesise that miRNAs play an important role in controlling the innervation of sensory neurons into the central nervous system, crucially we predict that this process is modifiable, especially by injury or disease and that this results in altered “wiring” of these circuits which in turn leads to sensory abnormalities, developmental disorders and susceptibility to neurological disease later in life.You will be fully embedded in the Dajas-Bailador/Hathway groups, with a strong track-record of collaboration and co-supervision of PhD students. The rotation will enable you to get to know our teams, participate in your own project and observe those which are already taking place.

Full PhD project description:

The dorsal horn (DH) of the spinal cord, as part of the central nervous system, is a complex network of neurons whose purpose is to respond and process afferent sensory information conveyed to it from the peripheral nervous system. The DH is a layered (laminated) structure, much like the cerebral cortex, with each lamina receiving different sensory inputs. Neurons in each lamina are also highly heterogenous, some are interneurons conveying information to the brain, others are interneurons relaying information from site to site within the DH. The way in which the spinal cord matures is central to our experience of pain and our interactions with the environment for the rest of our lives. Pain processing in early life is different to how we experience it as adults, and in large part that is because significant changes in the anatomy and physiology of the DH are still to take place. Crucially sensory neurons undergo significant rearrangement of termination zones in the DH with some neurons extending axons into new DH layers and others retracting or undergoing apoptosis. Painful experiences in early life change the trajectory of these processes and fundamentally alter how we experience pain for the rest of our lives, leaving us with sensory abnormalities and susceptibility to neurological disease.We wish to understand the processes which control the normal maturation of these circuits and how damage can alter them. We have a strong interest and track-record in the role of miRNA in controlling neuronal behaviour and development and this project will apply our skills and expertise to this problem. Our aim is to identify miRNAs which are central to neurodevelopment and whose function in altered by damage, which in turn feeds onto alterations in neuronal function. These pathways can then be exploited to protect the individual from deleterious consequences of pain in early life.

References to learn more:

GREENSPON CM*, BATTELL EE*, DEVONSHIRE IM, DONALDSON LF, CHAPMAN V, HATHWAY GJ. 2018 Lamina-specific population encoding of cutaneous signals in the spinal dorsal horn using multi-electrode arrays. J. Physiol. 597(2):377-397

Location of lab rotation: QMC

Location of full PhD project: QMC

(64) Interferons as pacesetters in cellular ageing

Primary supervisor: Uwe Vinkemeier

Second supervisor: David Scott

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The lab rotation will introduce students into the areas of science that are most relevant forthis work, namely interferon biology, transcriptional regulation and ageing. On a theoreticaland conceptual level, this is achieved through their participation in laboratory seminars, andin their study of topical and landmark scientific articles followed by discussions with thesupervisory team. This will cover molecular underpinnings of interferon signalling and thecontrol of gene expression, the cellular manifestations of senescence and their consequencesfor the health and wellbeing of humans and animals over the course of life. Theexperimental work will familiarise students with mammalian cell culture techniques,methods such as reporter gene assays and qRT-PCR to assess cytokine-mediated geneexpression, as well as fluorescence live cell imaging to assess the mechanisms andregulation of senescence and cell death. At the end of the lab rotation, students will havegained a good understanding of how interferons link transcriptional regulation to cellularsenescence and age-related decline in immunity and cognition. This will prepare them forthe immediate theoretical and practical challenges and inspire a longer-term vision forsuccessful completion of the proposed PhD project entitled “interferons as pacesetters incellular ageing.”

Full PhD project description:

The members of the interferon (IFN) family of proteins alter the behaviour of cells in importantways through changes in gene transcription. Discovered more than 60 years ago for their role in thefirst line of defence against virus infections, these proteins have now more than reached thepotential envisioned by the early discovering virologists. Interferons are commonly used in antihepatitisB virus therapy, and they have found additional therapeutic applications for oncology andMultiple Sclerosis. Interferons enhance innate and acquired immune responses, and modulatenormal and tumour cell survival and death. Studies of interferons have resulted in fundamentalinsights into the mechanisms of cellular signalling, gene transcription and the workings of theinnate and adaptive immune systems. Mutations in STAT proteins and other interferon pathwayconstituents give rise to a spectrum of severe inherited immune system diseases characterised bysusceptibility to infections, autoimmunity and cancers, unambiguously demonstrating the vitalimportance of IFNs to the health of animals and humans.Recent research has demonstrated that IFNs are also crucial in promoting cellular senescence andthat IFN activity in turn underlies detrimental age-related manifestations such as progressivecognitive decline or reduced immune protection. Stable cell cycle arrest, resistance to apoptosis and release of a complex mixture of soluble and insoluble factors are hallmarks of senescence.However, how IFNs control the ageing of cells and organisms and the molecular basis of theseprocesses are incompletely understood. Work of Johnathan Ho, a previous PhD student in the lab,has uncovered an IFN-inhibiting mechanism that slows down the ageing of cells.We propose tocontinue this line of research and explore the molecular underpinnings of IFN-mediated senescenceand their effects on cellular functions.We will focus on the mechanisms by which IFNs regulate theexpression of genes, in particular the striking observation that senescence-

related genes are amongthe most highly interferon-responsive ones. Successful applicants will therefore use nextgenerationsequencing to identify highly IFN-sensitive genes by comparing wild type cells to mutantcells with increased senescence. Bioinformatics tools will be used to distinguish the genes that arelinked to senescence, and characterise the enhancer landscape to delineate shared promoterfeatures. The aim is to recognize patterns that define senescence-related genes, and/or confer highIFN-sensitivity. Students will then go on to assess the functional significance of putativesenescence-associated genes. To this effect, they will use RNAi-mediated gene silencing inconjunction with biochemical assays and fluorescence microscopy to study how the absence of therespective gene products alters manifestations of cellular senescence. As STAT proteins are crucialeffectors of chromatin topology for all IFN-dependent gene regulatory networks including cellularsenescence, students will acquire expertise in the in vitro assembly and DNA binding of STATtranscription complexes using recombinant proteins that are available in the laboratory. Part of thiswork will be done at the Harwell Research Complex, where the co-supervisor is based.We invite imaginative students to explore interferons as pacesetters in cellular ageing. Anintellectually stimulating environment and world-leading facilities are provided.

References to learn more:

1. L1 drives interferon in senescent cells and promotes age-associated inflammation. (2019). Cecco Met al. Nature 566, 73.
2. STAT2 is a pervasive cytokine regulator due to its inhibition of STAT1 in multiple signaling pathways.(2016). Ho J et al. PLoS Biology 14, e2000117.

Location of lab rotation: QMC

Location of full PhD project: QMC;Research Complex at Harwell

(65) Molecular engineering of cell wall targeting chimeric antimicrobials “lighting up the target”

Primary supervisor: Boyan Bonev

Second supervisor: Paul Williams

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The rotation project aims to provide an introduction to the partner labs and the methodology that will be used in the project. These include a combination of structural and biophysical approaches, such as solid state NMR and advanced computing alongside advanced molecular microbiology. It offers an overview of the early stages of molecular design and architectural considerations for target selectivity and specificity, structural considerations for antimicrobial and antibiofilm activity alongside low eukaryotic toxicity. The candidate will investigate the molecular interactions between lead compounds form the designer antimicrobials and their molecular target. To do this, the following step will be undertaken: w1- introduction to the lab and project; w2,3: pro-drugs will be activated following our established protocols; pro-drug and activated drug will be purified and assessed for activity in a comparative manner against standard laboratory strains; w4,5: molecular target, lipid II, will be produced using biosynthetic approach; w6,7: molecular complexes will be studied using high resolution solid state NMR; data analysis w8,9: computational docking, molecular dynamics simulations; writing reports.

Full PhD project description:

The use of antibiotics for bacterial management has proven very successful and has transformed our healthcare and lifestyle. In the second half of the 20th century, the widespread use of antibiotic prophylaxis and use of antibiotics in animals became commonplace and we now appreciate that this has swayed the balance from successful management of bacterial infections in favour of microbial adaptations under sustained antibiotic pressure. One major contribution to this process is the severe lack and desperate need for new lead compounds.Besides human health, antibiotics and antimicrobials are widely used in food preservation, veterinary medicine, packaging and device and carrier protection to prevent and remove biofilms. The molecular architecture of antimicrobials with such diverse application is challenging and is broadly guided by the Lipinski’s rule of five alongside application-specific criteria. Primary objective is to engineer compounds with antimicrobial activity, surface adhesion modulating properties (antibiofilm activity), paralleled by low toxicity and safety. The overall aim of this project is to develop chimeric antimicrobials with multiple mode of action [Hyde et al. PNAS 2006] and broader spectrum of activity, capable of interfering with bacterial division and simultaneously with cell envelope formation, as well as disrupting bacterial plasma membranes [Hasper et al., Science 2006;Bonev et al., FASEB J 2004]. Selectivity in the design relies on recognition of highly specific molecular targets [Breukink et al., Science 2004] unique to bacteria, which are refractive to alteration and resistance [Ling et al., Nature 2015; Bayley et al., Bacterial Resistance to Antibiotics 2019]. Lead compounds will be optimised for favourable pharmacological properties and directed evolution will be used to assess any routes to bacterial adaptation.Your challenge will be to use our new design platform to engineer and characterise the mechanism of

target engagement, antimicrobial and antibiofilm action of a series of binary chimeric antimicrobials. These combine nature-inspired peptidoglycan-targeting epitopes with receptor-independent membrane-disrupting segments. Using NMR you will gather experimental evidence of the molecular details of target engagement by a) the recognition epitope alone, b) of a chimera with the membrane disruptor and c) the membrane disrupting compound alone as control. Using in-house biological assays you will characterise the antimicrobial and antibiofilm activity of these compounds alongside their toxicity to select active and safe leads. Finally, using fluorescently tagged compounds and superresolution microscopy alongside flow cytometry you will investigate target presentation and localisation on bacterial cells to understand the fundamental processes of peptidoglycan biosynthesis, as well as target presentation and availability. One key deliverable is understanding the molecular details of bacterial peptidoglycan synthesis, which remains the most successfully exploited pharmacological target. Our preliminary data suggests that our compounds follow target presentation and engagement strongly coupled to bacterial cell cycle and phenotype, which suggests a novel and uncharacterised mode of action. This offers exciting and unprecedented insights into key stages in the assembly of bacterial envelopes.

References to learn more:

1. Hyde et al., (2006) Nisin-induced changes in Bacillus morphology suggest a paradigm of antibiotic action. PNAS 103, 19896 –901
2. Ling et al. (2015) A new antibiotic kills pathogens without detectable resistance. Nature 517, 455–459

Location of lab rotation: QMC

Location of full PhD project: QMC

(66) Smart adsorbent materials to mitigate anti-microbial resistance in dairy farm wastewater (SAM-FARM)

Primary supervisor: Andrea Laybourn

Second supervisor: Rachel Louise Gomes

Institution: University of Nottingham

School: Engineering

Lab rotation description:

This novel interdisciplinary PhD project involves developing adsorbent materials to treat cattle waste for the reduction of antimicrobial resistance (AMR) and recovery of metal and antibiotic contaminants. The selected student will have an exciting opportunity to work across the Faculty of Engineering, School of Biosciences and visit the University of Nottingham Dairy Farm to introduce them to the materials synthesis and analytical approaches that will be implemented in the linked PhD project. The student will visit materials laboratories (Advanced Materials Research Group) to synthesise novel sorbent materials using proven rapid and efficient microwave heating methods. They will also learn how to characterise the materials produced using a range of analytical techniques.In order to develop effective novel sorbent materials for removal metal and antibiotic contaminants, it is important to identify and quantify the species (biological and chemical) present in cattle waste and to understand their impact on the sorption process. To this end, the student will participate in collecting (at the Dairy Farm) and characterising (Food, Water, Waste Group) samples from cattle waste. The microbial content will be assessed in the microbiology labs where the student will learn techniques used for cell culture, growth and viability.

Full PhD project description:

Project Aim: This project will develop sorbent materials to treat cattle waste for the reduction of AMR and recovery of metal and antibiotic contaminants. Removal of contaminants from cattle waste will enable safe re-use as a fertiliser for crops.The University Dairy Farm currently disposes 4-6 tonnes of metal-containing cattle footbath waste (used to protect against lameness caused by bacteria) into slurry wastewater per annum. This is a potential source of environmental metal pollution (the waste is eventually spread as fertilizer). The waste also contains antibiotic-contaminated milk, urine and faeces. Both metal ions and antibiotics are co-selection drivers for antimicrobial-resistant bacteria.1Metal-organic frameworks (MOFs) are sorbent materials that show great promise for removal of metals and antibiotic drugs from waste.2 Their high porosity and tuneable functionality provides the opportunity for developing highly selective MOFs for sorption of metal and antibiotic contaminants from cattle waste.2 From a cost-benefit perspective, electromagnetic (EM) energy offers two advantages; it has been used for ‘curing’ plasmid DNA3 and can be used to improve sorption through heating4 the waste. The project will involve identifying and quantifying the species present in real-world cattle waste samples. It is important to understand what is present in the waste and in what concentrations as this will strongly impact the sorption process. A range of novel MOFs will be prepared rapidly and efficiently using bespoke microwave technology. The stability of these MOFs will be assessed under temperature and pH conditions relevant to cattle waste. Next, variables which affect the sorption kinetics (ratio of MOF to metal ion and/or antibiotic, concentration, pH, competitive sorption, the impact of microwave heating, MOF

functionalisation and pore size) will be assessed using environmental matrices which mimic the conditions of the cattle waste. Results will enable us to develop MOFs with high sorption capacity and selectivity and to optimise removal of contaminants. The effect of microwave heating on cell lysis and/or plasmid curing will be investigated. The final established process (novel developed MOFs and microwave conditions) will then be tested on real-world dairy farm waste and the impact of this on AMR co-selection will be evaluated using microbiological techniques.This exciting multidisciplinary PhD project offers the unique opportunity to prepare novel materials for AMR mitigation whilst developing a microbiological understanding of AMR co-selection drivers. The project addresses concerns in food security, food safety, and AMR with the potential to deliver economic and societal impact in UK and global agriculture.References:1. BMC Genomics, 2015, 16:964; J. Med. Microbiol., 2015, 64:471-97.2. Chem. Eng. J., 2017, 322:366–374; ACS Appl. Mater. Interfaces, 2016, 8, 30294−30303.3. Arch. Microbiol., 2013, 195:181–188.4. Bioresour. Technol., 2014, 160:3–14.

References to learn more:

1. THOMAS-HILLMAN,* I., LAYBOURN, A., DODDS, C. and KINGMAN, S. W., 2018. Realising the environmental benefits of metal-organic frameworks: Recent advances in microwave synthesis: Journal of Materials Chemistry A, 6(25), 11564-11581.
2. WILLIAMS O, CLARK I,* GOMES RL, PEREHINIC T, HOBMAN J, STEKEL DJ, HYDE R, DODDS C, LESTER E, 2019, Removal of copper from cattle footbath wastewater with layered double hydroxide adsorbents as a route to antimicrobial resistance mitigation on dairy farms, Science of the Total Environment, 655, 1139-1149.

Location of lab rotation: University Park

Location of full PhD project: University Park;Sutton Bonington Campus

This is a linked project – we recommend you select either project 101 or project 107 alongside this one.

(69) Hippocampo-prefrontal-subcortical circuit in cognition and behaviour

Primary supervisor: Tobias Bast

Second supervisor: Paula Moran

Institution: University of Nottingham

School: Psychology

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: Students will be introduced to stereotaxic brain surgery (e.g., to selectively manipulate or record from specific brain regions) and in vivo electrophysiology in rats. They will learn to use dedicated software for the acquisition and analysis of electrophysiological data. 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, and in the design and statistical analysis of behavioural studies. They will learn about software packages used for behavioural data collection (e.g., Ethovision).

•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 relevant neuroscience seminars.

Full PhD 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; Bast et al., 2017, Brit J Pharmacol).Research questionsIn 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 will 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 balanced neural activity - Imbalanced neural activity within the hippocampal-prefrontal-subcortical circuit, caused by changes in inhibitory GABA transmission, have come to the

fore in important brain disorders, including age-related cognitive decline, Alzheimer's disease and schizophrenia (Bast et al., 2017). How do such imbalances affect distinct cognitive and behavioural functions? Can they explain symptoms characterizing these disorders?MethodsTo address these questions, we will combine a wide range of neuroscience methods in rat models. We will pharmacologically modulate specific brain regions by intracerebral drug microinfusions and examine the impact on dedicated tests of specific cognitive and behavioural functions. 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). Additionally, depending on their interests and specific project objectives, students may also work with computational neuroscientists to synthesise experimental findings into neuro-computational models to formalise neurobiological mechanisms; apply 'translational' brain imaging methods to characterise neuronal network changes in a way that enables direct comparison to human brain imaging studies; use modern neural tract tracing methods (involving 'clarity' and light-sheet microscopy) and pharmacogenetic methods.

References to learn more:

3. Bast T, M Pezze, McGarrity S (2017) Cognitive deficits caused by prefrontal and hippocampal neural disinhibition. Br J Pharmacol 174(19): 3211–3225
4. Bast T (2011) The hippocampal learning-behavior translation and the functional significance of hippocampal dysfunction in schizophrenia. Curr. Opin. Neurobiol. 21: 492-501

Location of lab rotation: University Park

Location of full PhD project: University Park

(71) Non-coding RNA transcriptome of extracellular vesicles produced by peripheral blood mononuclear cells following exposure to E-Cigarettes

Primary supervisor: Victoria James

Second supervisor: Lucy Fairclough

Institution: University of Nottingham

School: Veterinary Medicine and Science

Lab rotation description:

The nine-week lab rotation will allow the student to develop the skills necessary to isolate and characterise extracellular vesicles produced by cultured cells. Techniques will include cell culture, size exclusion chromatography, nanoparticle tracking and SDS-Page immunoblots. Specifically, the student will learn sterile tissue culture techniques to collect conditioned media (weeks 1-4), they experience vesicle isolation techniques including size exclusion chromatography (weeks 5-6) and in the final weeks of the project will characterise their isolated vesicles using nanoparticle tracking analysis and western blots (weeks 7-9). The aim of this work will be for the student to develop a series of techniques that are essential to the study of extracellular vesicles.

Full PhD project description:

Electronic cigarettes (E-cigarettes) are considered a preferable alternative to conventional cigarettes due to the lack of combustion and the absence of tobacco-specific toxicants. E-cigarettes have rapidly gained in popularity in recent years amongst both existing smokers and previous non-smokers. In fact there are now thought to be 41 million vapers worldwide, with 2.8 million in the UK. But vaping is not without risk and there is little evidence to date about the effect of vaping on the lungs, specifically lung epithelial cells. Exosomes are a subtype of microvesicles which facilitate cell to cell communication. They are released from every cell within the body, and contain molecules such as DNA, miRNA, mRNA, proteins and lipids. In addition to their role in intercellular communication, exosomes are now also considered as agents of immunoregulation that can modulate antigen presentation, immune activation, suppression and surveillance. This PhD will examine the effect on exosome generation and cytokine production of in vitro exposure of bronchial epithelial cells to a variety of E-Cigarette vapes. The techniques used will include cell culture, immunomagnetic separation, ELISA, size exclusion chromatography and novel imaging flow cytometry (using our new ImageStream Flow Cytometer).

References to learn more:

1. Chen, I-L, Todd I and Fairclough LC. Immunological and pathological effects of electronic cigarettes. Basic Clin Pharmacol Toxicol. 2019;1–16.
2. EVs: Tkach, M and Thery, C. Cell. 2016; 164: 1226-1232

Location of lab rotation: University Park

Location of full PhD project: University Park

This is a linked project – we recommend you select project 37 alongside this one.

(73) Stimulating bacterial natural product synthesis in algal coculture to discover new antibiotics

Primary supervisor: Ellis O'Neill

Second supervisor: Sam Bryan

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

Streptomycete bacteria are the largest source of clinical antibiotics. With the growing problem of antibiotic resistance researchers are developing new tools to find novel compounds. However, it is well known that these bacteria can make many more compounds than have ever been seen in lab conditions. We have recently discovered that coculturing these bacteria with algae from the same environment dramatically changes the profile of metabolites produced. This project will leverage that discovery to explore new bacteria-algal interactions to discover new antibiotics.For the initial rotation, the student will grow five strains of novel Streptomyces, available from the Bryan lab, in the presence of three different algal species, grown in the O'Neill lab. They will then extract the metabolites produced under these different conditions and compare the change in metabolite production. The extracts will be tested for antibiotic activity and analysed by mass spectrometry. These changes will be correlated to provide future leads for the discovery of new antibiotics in the full project.The student will gain training in cell culture, bioactivity testing and advanced mass spectrometry.

Full PhD project description:

In the lab scientists mostly work on isolated strains of bacteria, grown in nutrient rich media. This does not represent the real environment that bacteria find themselves in the wild. In order to fend off competitors, or predators, bacteria produce a wide range of chemical weapons, which we can hijack to use as antibiotics. By growing these bacteria with algae, which are found in the same environment as the bacteria, we set up an interaction that may be at some level competition or symbiosis. In these completely altered growth conditions the biology changes and new metabolites are produced.From the rotation project it will be clear which factors most influence the metabolite profile of the bacteria. This will be expanded upon in the full project, to give a larger range of conditions to maximise the production of the interesting natural products. For compound isolation, the student will increase the cell culturing by large scale fermentation of the cocultures. They will purify the new compounds using HPLC and the structures elucidated using NMR and MS. The student will evaluate the bioactivity of the new compounds and further mode of action studies will be undertaken in collaboration with colleagues.At the same time the biosynthesis of these compounds will be explored in the bacteria through genome sequencing and metabolic labelling. The genomes of these bacteria will be sequenced and the student will study the genes for the biosynthesis of these compounds bioinformatically. This will not only help with structure elucidation, but also allow identification of related compounds in other, distantly related, species and help uncover novel chemistry and biochemistry in natural product biosynthesis.Another aspect of the project will be to understand the nature of the interactions between the species and whether this is an antagonistic or collaborative interaction. The student will explore this interaction through transcriptomics, metabolomics and in competition assays to outline the biological interaction. The

nature of the communication will be investigated to try and identify the active components of the communication system between these cells and the student will investigate whether this can be applied more widely to the discovery of natural products.The student will receive mentoring from the supervisory team, benefiting from their expertise and will gain experience in all aspects of natural product chemistry, including compound isolation and structure elucidation, genomics and biochemical analysis, providing the student with a range of transferable skills, highly prized by employers in the growing bioeconomy.

References to learn more:

1. Crüsemann, M., O'Neill, E. C., Larson, C. B., Melnik, A. V., Floros, D. J., da Silva, R. R., Jensen, P. R, Dorrestein, B. C., and Moore, B. S. Prioritizing natural product diversity in a collection of 146 bacterial strains based on growth and extraction protocols. Journal of Natural Products (2017) 80, 588-597 DOI: 10.1021/acs.jnatprod.6b00722
2. O'Neill, E. C., Saalbach, G., and Field, R. A. Gene discovery for synthetic biology: exploring the novel natural product biosynthetic capacity of eukaryotic microalgae. Methods in Enzymology (2016) 576, 99-120 DOI: 10.1016/bs.mie.2016.03.005

Location of lab rotation: University Park

Location of full PhD project: University Park

(74) Evaluating the effect of training on pupillometry, EEG, and performance on a naturalistic driving task

Primary supervisor: Andrew Reid

Second supervisor: Matias Ison

Institution: University of Nottingham

School: Psychology

Lab rotation description:

The nine-week rotation for this project will consist of a literature review, preliminary experimental design, pilot data collection, and data preprocessing. The literature review will familiarize the student with our present understanding of the noradrenergic system, its role in cognition, and its putative link to early changes underlying Alzheimer's disease (AD). In the experimental design phase, the student will consult with the supervisory team about what variables of interest will be targeted, based on behavioural, pupillometric, and EEG observations, and how we can best test for the effects of interest. This exercise will equip the student to interpret the observed data, as well as anticipate and deal with issues such as confounds and statistical power, early on in the data collection process. Additionally, the student will recruit participants and collect pilot data in order to fine-tune the experimental approach and get hands-on experience with setting up and acquiring data from eye tracking and EEG equipment and running the driving simulation software. Finally, data obtained from these pilot sessions will be preprocessed using Matlab software, allowing the student to become acquainted with the critical steps leading up to statistical analyses.

Full PhD project description:

The locus coeruleus (LC) system is a small, elongated brainstem structure containing the majority of noradrenergic (NE)-expressing neurons in the brain. This system is critical for the human stress response, but is also involved in long-term potentiation and adaptive aspects of working memory and decision-making processes. Recent studies have identified early-life occurrences of Alzheimer's disease (AD)-related tau pathology, primarily in the LC. Tau is a microtubule-associated protein, and misfolding of this molecule leads to the dysfunction and ultimately structural degradation of affected neurons. This pathological evidence, along with functional and structural changes (see Ref.1, for review), suggest that the LC/NE system is an important factor in the early aetiology of AD. Challenging this system in a way that mitigates such changes is therefore a potential means of interfering with AD aetiology at an early stage, although this remains to be demonstrated.The proposed project will utilise a continuous highway driving task, in conjunction with EEG and pupillometry, to challenge the LC/NE system in a naturalistic way. This paradigm has formerly been developed by Dr. Reid, and will be customised in order to investigate the relationship of LC/NE activity to decision-making processes under varying levels of simultaneous cognitive load. The classical P3 EEG component has been recently proposed as a candidate neurophysiological marker of LC activity (Ref.2). Pupillometry has been strongly linked to LC firing in both animals and humans, and will be used as a proxy measure for LC function in this approach. Preliminary evidence from this approach, obtained from ~40 participants, indicates a strong, transient pupil response during decision points in the task, with stronger responses associated with more difficult decisions. The Ph.D. student will be involved in designing a novel longitudinal implementation of the driving paradigm, in order to investigate the effect of regular training over a 6-month period on decision-

making performance for time-critical simulated driving events. Baseline and follow-up sessions will be obtained from young and older healthy participants, with half of these participants being asked to practice using a brief training version of the task in the intervening period. The student will assess whether training alters performance, pupil dilation locked to decision events, and associated EEG phenomena. The results will constitute a critical first step in determining the feasibility of such training as an intervention to delay cognitive impairment in individuals vulnerable to AD.During the full project, the Ph.D. student will learn about existing theory and knowledge about the LC/NE system and its involvement in AD etiology. They will gain experience of longitudinal experimental design, participant recruitment, and data collection using eye tracking and EEG setups. They will also learn how to use Matlab and/or Python software to preprocess, synchronise, and analyse the resulting datasets. In general, they will conduct a full set of pilot and longitudinal studies to establish whether regular training on a simulated driving task can improve both behavioural performance and pupil/EEG signatures of LC activation.

References to learn more:

1. Mather M, Harley CW (2016). The Locus Coeruleus: Essential for Maintaining Cognitive Function and the Aging Brain. Trends Cogn Sci. 20(3): 214-226.
2. Murphy PR, Robertson IH, Balsters JH, O'connell RG. Pupillometry and P3 index the locus coeruleus-noradrenergic arousal function in humans. Psychophysiology. 48(11): 1532-1543.

Location of lab rotation: University Park

Location of full PhD project: University Park

(75) Effects of peri-conceptional vitamin B12 and folate deficiency on epigenetic programming of metabolic health

Primary supervisor: Adaikala Antonysunil

Second supervisor: Kevin Sinclair

Institution: Nottingham Trent University

School: School of Science and Technology

Lab rotation description: Dietary deficiencies in vitamin B12 (B12) and folate around conception can increase the risk of congenital abnormalities (e.g. neural-tube defects) and long-term cardio-metabolic health in children. This rotation, operating between the labs of Dr Antonysunil (NTU) and Professor Sinclair (UoN), involves a pilot study which will test the hypothesis that the culture of mammalian embryos from fertilisation in different physiological concentrations of B12 and folate up to Day 8, leads to the epigenetic dysregulation of genes that influence later development and health of offspring. Spent culture media at different stages of embryo development (i.e. oocyte maturation, early zygote, morula (Day6) and blastocyst (Day8) stages) will be harvested for LC-MS/MS analyses of 1-carbon(1C) metabolites involved in the linked B12-folate-methionine pathways. This will provide insights into the 1C metabolic requirements of the pre-implantation embryo. Blastocysts will undergo immuno-dissection into epiblast/hypoblast and trophectoderm lineages (the former two give rise to the fetus, the latter to the placenta) from which DNA and RNA will be extracted for pyrosequencing and gene expression. This will identify differences in DNA methylation and mRNA expression for a select group of genes that regulate early development and metabolic health. Training in all methodologies, including data analyses, will be provided.

Full PhD project description:

Background: Dietary deficiencies in B12 and folate around the time of conception can increase the risk of congenital abnormalities (e.g. neural-tube defects) and long-term cardio-metabolic health disorders in children and adult offspring. Procedures used in assisted reproduction, such as ovarian stimulation and embryo culture, can also predispose to these disorders as, for example, most commercially available embryo culture media contain variable, usually undeclared, levels of these and other related metabolites. Hypothesis: Gametes (i.e. eggs and sperm) and pre-implantation embryos are particularly sensitive to B vitamins, which are key co-enzymes involved in 1C metabolic pathways, components of which are not expressed in the ovary, testis or early embryo. We hypothesise that this sensitises gametes and the pre-implantation embryo to dietary/in vitro-culture induced epigenetic dysregulation of genes that determine subsequent adult-offspring cardio-metabolic health. Furthermore, such effects are compounded when these deficiencies extend to the latter stages of gestation and/or during infancy, as often occurs. Aims: The proposed PhD will test this hypothesis using two model systems. These focus on B-vitamin induced modifications to DNA methylation as a key epigenetic regulator of developmental genes in (A) embryonic cells during the peri-conceptional period and (B) target somatic cells representing three target tissue types (i.e. the placenta, liver and adipose tissue). Model System A. In vitro culture of mammalian embryos:Continuing from the pilot studies undertaken during the lab rotation, we will explore varying concentrations of B12 and folate separately and in combination, on epigenetic alterations to DNA methylation in Day 8 embryos, using endpoints described earlier. These studies at UoN will include measurements of genome-wide DNA methylation using Reduced Representation Bisulphite

Sequencing (RRBS). Bioinformatic analyses (at NTU) will identify the location of differentially methylated regions (DMRs) affected by methylation. We have recent data indicating that physiological reductions in a related metabolite (methionine) alters DNA methylation in cell-cycle regulatory genes, key metabolic (including insulin signalling) pathways and at imprinted loci. The bovine model at UoN allows us to transfer treated embryos to normally fed surrogates. These embryos don't implant until around Day 18-21, allowing non-surgically flushing of elongated trophoblasts around Day 16 (onset of gastrulation). Cells isolated from the embryonic disc (representing the three germ layers) and trophoblast will undergo pyrosequencing at NTU to establish DMRs at targeted sites (identified from the RRBS analyses), with expression confirmed by qPCR. Model System B. Human somatic and placental cell lines:Studies similar to those described for embryos above will be undertaken at NTU using BeWo, Chub-S7 and HepG2 cells which will model human placental, adipose and liver tissues, respectively. Our previous studies showed that adipocytes and hepatocytes cultured with low B12 had higher lipid levels, altered DNA methylation and miRNAs regulating lipid metabolism and insulin resistance. Representing the species of primary clinical interest, these lines also provide more cells for biochemical measurements of 1C metabolites by LC-MS/MS, pyrosequencing and qPCR.Collectively, these studies will provide evidence of B12/folate-mediated epigenetic programming of metabolism in embryonic and differentiated cells with implications for maternal nutrition, assisted reproduction and stem-cell biology.

References to learn more:

1. Adaikalakoteswari A, Finer S, Voyias PD, McCarthy C, Moore J, Smart-Halajko M, Bawazeer N, Al-Daghri NM, McTernan PG, Kumar S, Hitman GA, Saravanan P, Tripathi G (2015). Vitamin B12 insufficiency induces cholesterol biosynthesis by limiting s-adenosylmethionine and modulating the methylation of SREBF1 and LDLR genes. Clinical Epigenetics 7(1):14.
2. Clare CE, Brassington AH, Kwong WY, Sinclair KD (2019). One-Carbon Metabolism: Linking Nutritional Biochemistry to Epigenetic Programming of Long-Term Development. Annu Rev Anim Biosci. 15, 7: 263-287.

Location of lab rotation: Clifton Campus

Location of full PhD project: Sutton Bonington Campus; Clifton Campus;

(74) Evaluating the effect of training on pupillometry, EEG, and performance on a naturalistic driving task

Primary supervisor: Andrew Reid

Second supervisor: Steven J Briddon

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

During this rotation the student will become familiar with cell culture, transfection and cell-based and imaging assays. These techniques (widely applicable within biochemistry, pharmacology and cell biology laboratories) will be used to interrogate the action of dopamine receptors in cell lines. Mastering these approaches in cell lines is a key step to progress studies in primary neuronal cultures. The student will i) express dopamine receptors in cells (cell culture and transfection), ii) assess receptor expression using fluorescent ligand binding, Enzyme-linked immunosorbent assay (ELISA) and imaging (confocal) and iii) check their function by measuring G protein activation and cAMP levels using bioluminescence resonance energy transfer (BRET). Together, these will form a solid basis for future work on this receptor to study the action of atypical antipsychotics.

Full PhD project description:

Antagonism of the dopamine D2 receptor (D2R) is required for the efficacy of all clinically used antipsychotic drugs (APDs) for the treatment of schizophrenia. Unfortunately, antagonism of the D2R is associated with extrapyramidal side effects (EPS) such as Parkinsonism, and tardive dyskinesia. Some newer APDs (termed atypical APDs) are thought to have a lower side-effect risk but the mechanism behind these differences is unclear. We have recently shown that the binding association rate of APDs predicts their ability to cause EPS although we lack direct experimental evidence of this phenomenon. Furthermore, in another recent study we have revealed that some but not all APDs can act as pharmacological chaperones to increase cell surface expression of D2Rs. Increases in brain D2R after chronic treatment with APDs have been suggested as the reason for iatrogenic psychoses and for resistance of schizophrenia patients to pharmacotherapy over time. APD-induced up-regulation of D2R has also been implicated in tardive dyskinesia, suggesting that chronic D2R up-regulation can produce permanent adverse alterations in D2R-expressing neurons. However, neither the inverse agonist properties or the chaperone activity have been studied in brain cells relevant to EPS. Hypothesis Understanding the molecular basis of drug rebinding and pharmacological chaperoning activity of APDs at the D2R will reveal differences in antipsychotic drug action that predict their side-effect profile. This project will use a wide range of imaging and biophysical techniques in combination with classical pharmacology and biochemistry approaches to address this hypothesis. We have used Fluorescence Correlation Spectroscopy (FCS) to show that drug interactions with the cell membrane and receptors drives a higher local concentration of drug proximal to the cell membrane and developed a series of fluorescently-labelled D2R antagonists whose binding kinetics properties span the range of those exhibited by APDs. The student will investigate the molecular determinants of drug rebinding. Using FCS in model cell lines expressing different levels of SNAP-D2R in combination with fluorescent D2R ligands with known binding kinetics, we will assess how different levels of cell surface D2R expression can drive local concentrations of drug. Then the student will extend these studies to primary cultures of striatal and

cortical neurons as well as pituitary lactotrophs derived from the SNAP-D2R mouse to understand how these phenomena might impact drug rebinding at endogenously expressed D2Rs in disease-relevant tissues. In parallel, the student will use Tr-FRET binding to measure the binding kinetics of selected unlabelled APDs at natively expressed D2Rs in the above primary neuronal cultures.To investigate the ability of different APDs to drive D2R cell surface expression the student will use BRET to quantify D2R trafficking. These experiments will be complemented with classical confocal, TIRF microscopy and high content imaging. Then we will extend these studies to primary cell cultures expressing the SNAP-D2R to investigate the impact of acute and longer-term application of APDs on D2R expression and trafficking in these disease-relevant cells.

References to learn more:

1. Sykes, D. A. et al. Extrapyramidal side effects of antipsychotics are linked to their association kinetics at dopamine D2 receptors. Nature Communications 8, 763 (2017).
2. Schrader, J. et al. The differential actions of clozapine and other antipsychotic drugs on the translocation of dopamine D2 receptors to the cell surface. The Journal of biological chemistry 294, 5604–5615 (2019).

Location of lab rotation: QMC

Location of full PhD project: QMC

(77) Molecular basis of atypicality in antipsychotic drug action

Primary supervisor: Robert Lane

Second supervisor: Steven J Briddon

Institution: Nottingham Trent University

School: School of Science and Technology

Lab rotation description:

The student will be trained in anaerobic microbiology, and will screen isolates for their ability to ferment a limited range of different amino acids/proteins. They will have the opportunity to undertake metabolomic analyses, and to learn how to generate and analyse whole-genome sequence data. Depending on progress, they will also have the opportunity to undertake some transcriptomic analyses.

Full PhD project description:

The human gut microbiota represents a diverse community of bacteria, archaea, viruses and fungi. The collective genome of the gut microbiota (the 'metagenome') encodes 150 times more genes than the human genome. Enzymes encoded by the metagenome allow gut bacteria to use dietary substrates that escape digestion by human-encoded enzymes in the gastrointestinal tract. Metabolites produced as a result of these microbial processes act on intestinal cells, or are taken up into the blood via the hepatic portal vein and transported around the body. The interaction of microbial metabolites and host cells – the so-called microbial–mammalian metabolic axis – contributes to homeostasis of the human system. Disruption of homeostasis and the gut microbiota is linked with a range of metabolic diseases (obesity, non-alcoholic fatty liver disease, atherosclerosis, type 2 diabetes), inflammatory bowel disease (Crohn's, ulcerative colitis) and neurodegenerative diseases (Alzheimer's disease, Parkinson's disease).We only know the functions of approximately 30 % of the genes that contribute to the metagenome. While much is known about the bacteria responsible for fermenting carbohydrates in the gastrointestinal tract, little is known about the microbes that use proteins, peptides and amino acids in this environment. A diverse range of products (essential amino acids, phenolic compounds, amines, ammonia, short-chain fatty acids, branched-chain fatty acids, gases, sulfides) is produced as a result of microbial-driven synthesis or proteolysis. All these metabolites have the potential to influence host systems in beneficial or detrimental ways. For example, the microbiota of the small intestine contributes 8–17 % and 5–21 %, respectively, of the essential amino acids lysine and threonine found in the bloodstream. Conversely, circulating phenyl acetate produced via bacterial breakdown of phenylalanine contributes to lipid accumulation in the liver of individuals with non-alcoholic fatty liver disease. The aim of this project is to characterize proteolysis in a range of anaerobic gut bacteria. Understanding which gut bacteria are involved in proteolysis will allow us to develop targeted interventions to promote human health.

References to learn more:

1. Yao CK, Muir JG, Gibson PR. Review article: insights into colonic protein fermentation, its modulation and potential health implications. Aliment Pharmacol Ther. 2016 Jan;43(2):181-96. doi: 10.1111/apt.13456. Epub 2015 Nov 2. Review. PubMed PMID: 26527169.

2. Hoyles, L. & Swann, J. (2019). Chapter 18 – Influence of the human gut microbiome on the metabolic phenotype. In The Handbook of Metabolic Phenotyping, pp. 535–560. Edited by John C. Lindon, Elaine Holmes and Jeremy K. Nicholson. Elsevier. https://doi.org/10.1016/B978-0-12-812293-8.00018-9.

Location of lab rotation: University Park; Clifton Campus;

Location of full PhD project: University Park; Clifton Campus;

(75) Effects of peri-conceptional vitamin B12 and folate deficiency on epigenetic programming of metabolic health

Primary supervisor: Adaikala Antonysunil

Second supervisor: Helen Miranda Knight

Institution: University of Nottingham

School: Medicine

Lab rotation description:

The rotational project will lead the student to gain essential knowledge and skills in separating extracellular vesicles (EV) from human plasma, separating plasma EV subpopulations by immunoprecipitation, extracting RNA from EVs, and measuring EV RNA levels using digital droplet polymerase chain reactions (ddPCR). During the rotational project, we will measure IL1B (Interleukin-1-Beta) and CXCL8 (Interleukin-8) mRNA levels in plasma total EVs and in plasma EVs enriched for neuronal origin, in healthy younger (20-40 years) and older (>65 years) people. IL1B and CXCL8 mRNA levels in plasma EVs enriched for neuronal origin will clarify whether immunosenescence or neuroinflammation is associated with ageing.Total EVs will be separated from plasma using size exclusion chromatography. EVs enriched for neuronal origin will be separated using L1CAM immunoprecipitation (https://www.ncbi.nlm.nih.gov/pubmed/28588440). Total RNA from EVs will be purified using the Invitrogen total exosome RNA isolation kit (Thermo Fisher scientific, USA). Quantity and quality of purified RNA will be assessed using the NanoDrop™ spectrophotometer. IL1B and CXCL8 mRNA levels in plasma EVs will be assessed using the QX100 ddPCR system (Bio-Rad, USA) in the UoN. Statistical analyses will be performed with the guidance of the lead supervisor.

Full PhD project description:

Microglia are immune cells within the central nervous system that regulate inflammatory responses and neuronal plasticity. Optimal microglial activation is essential for neuronal survival, synaptic plasticity and healthy ageing. Neuroinflammation due to chronic microglial activation and immunosenescence related microglial dysfunction, are associated with various neurodegenerative disorders. Microglia secrete extracellular vesicles (EV) that carry biologically active RNA and proteins. Small (30-100 nm) EVs can cross blood-brain barrier and they transport microglial origin RNA and proteins to other cells via peripheral circulation. Prior microglial studies have mostly investigated post-mortem brain tissue. Investigating RNA cargoes of microglial origin EVs in human plasma will enhance our molecular level understanding of immunological and other ageing associated changes in living human brain. This may facilitate discovery of diagnostic biomarkers and novel therapeutic avenues for various neurodegenerative disorders.The objectives of the project include, (i) To optimise a protocol for separating EVs enriched for microglial origin from human plasma using IB4 immunoprecipitation, (ii) To profile the transcriptome of plasma EVs enriched for microglial origin, and (iii) To study the transcriptional changes associated with ageing in plasma EVs enriched for microglial origin.EVs will be separated from plasma of younger (20 - 40 years) and older (> 65 years) adults using the Izon qEV size exclusion chromatography system (Izon science Ltd., USA). A protocol for separating neuronally enriched plasma EVs using L1CAM immunoprecipitation (https://www.ncbi.nlm.nih.gov/pubmed/28588440) will be adapted for separating microglial enriched EVs using IB4 immunoprecipitation. The characteristics of separated EVs will be studied

using the Malvern NanoSight nanoparticle analyser and Cryo-Transmission Electron Microscopy (Cryo-TEM). The enrichment of EVs will be verified by Western blotting. In order to profile EV transcriptomes, total EV RNA will be purified from all enriched EV samples using the Invitrogen total exosome RNA isolation kit. Quantity and quality of purified RNA will be assessed using the NanoDrop™ spectrophotometer and the Agilent 2100 Bioanalyzer. A NEBNext Ultra-II (New England Biolabs, USA) directional cDNA library, and another small RNA specific cDNA library will be prepared for each RNA sample (N=20). The cDNA libraries will be mixed (1:1) and then will undergo single-end RNA-Seq (minimum of 15 million reads/ sample) in the UoN Deep-Seq facility. Expression levels of all RNA in the EVs enriched for microglial origin will be documented. The lead supervisor has experimentally validated an edgeR algorithm (https://www.ncbi.nlm.nih.gov/pubmed/26208977) for analysing RNA-Seq data to identify differentially expressed genes (DEG). Statistically significant DEGs and alternative splicing events that are associated with ageing will be identified with appropriate false discovery rate correction. Differential expression levels of identified DEG will be verified by ddPCR using independent biological replicate plasma samples. Functional analyses of identified DEGs will highlight the altered molecular pathways and dysfunctional molecular networks associated with ageing. This project will lead to a cutting-edge research program that will investigate the transcriptional changes in the EVs enriched for microglial origin in plasma of people living with neurodegenerative disorders.

References to learn more:

1. Mustapic M, Eitan E, Werner JK, Jr., et al. Plasma Extracellular Vesicles Enriched for Neuronal Origin: A Potential Window into Brain Pathologic Processes. Front Neurosci. 2017; 11: 278.2.
2. Paolicelli RC, Bergamini G, Rajendran L. Cell-to-cell Communication by Extracellular Vesicles: Focus on Microglia. Neuroscience. 2019; 405: 148-157.

Location of lab rotation: QMC

Location of full PhD project: QMC

(81) Proteins and the human gut microbiota: who does what?

Primary supervisor: Lesley Hoyles

Second supervisor: Klaus Winzer

Institution: NTU

School: School of Science and Technology

Lab rotation description:

The lab rotation will give the candidate experience of growing fungal mycelial mass under 3-D (conventional flask) conditions followed by analysis of the effect of growth conditions (for example sugar source) on cellular characteristics. They will gain experience of using biochemical/ fluorescent stains to map out cellular activity using confocal microscopy to localise the dyes. Further, the mycelial mass generated will be separated into its biochemical components and analysed using standard biochemical assays for protein, carbohydrate and chitin. Correlations will be drawn between the biochemical composition and the cellular characteristics. If time permits samples will be prepared for proteomic/ metabolomic assessment and initial bioinformatics analysis performed. In collaboration with the second supervisor at UoN, training for cultivation of fungi in different growth media will be offered. Furthermore, protoplast transformation methods will be applied to generate fluorescent reporter strains.

Full PhD project description:

Fungi are ubiquitous and are important sources of biologically active molecules including antibiotics (e.g. penicillin and cephalosporins), antifungals (e.g. griseofulvin), anti-cholesterol drugs (e.g. statins) and anti-cancer agents. Alternative applications that include recycling of waste materials include the generation of a range of building materials. The importance of the approach is highlighted by the fact that a Defence Research Agency in the USA (DARPA) in 2017 invested $9.1M for the creation of a 'new class of materials that combines the structural properties of traditional building materials with the attributes of living systems'. The intention was to be able to readily transport materials anywhere in the world to build housing following wars or disasters. There is also more limited research being conducted on the development of building materials in mainland Europe. Although there is a recognition that fungi could provide an effective way to generate new materials there is scant research into the chemical, biological and concomitant structural changes that occur when the conditions in the local environment are modified. Here, we propose to use filamentous fungi to develop engineered living materials (ELM) where our understanding of the effect of growth environment will lead to physical structures that can be controlled, where growth can be directed and thereafter developed into novel materials. This project will use fungal mycelia to (1) understand how living networks form and the role that the local environment has on their biochemistry, structure at both the microscopic and macroscopic scale and physico-chemical/mechanical properties of the resulting biological material and (2) use the materials developed in (1) to fabricate novel bio-inorganic mesoscale composites. The project will combine aspects of microbiology with biological chemistry and analytical science to identify how mycelial biochemistry is modified under different growth conditions. To do this we will design novel growing platforms that can be used for in situ visualization and chemical mapping of the developing hyphal structures. We will apply existing

isolation and coupling technologies to generate additional functionality on the biomolecular components of the hyphal structures to direct the formation of novel mesoscale composites.

References to learn more:

1. Yao CK, Muir JG, Gibson PR. Review article: insights into colonic protein fermentation, its modulation and potential health implications. Aliment Pharmacol Ther. 2016 Jan;43(2):181-96. doi: 10.1111/apt.13456. Epub 2015 Nov 2. Review. PubMed PMID: 26527169.Hoyles, L. & Swann, J. (2019).
2. Chapter 18 – Influence of the human gut microbiome on the metabolic phenotype. In The Handbook of Metabolic Phenotyping, pp. 535–560. Edited by John C. Lindon, Elaine Holmes and Jeremy K. Nicholson. Elsevier. https://doi.org/10.1016/B978-0-12-812293-8.00018-9.

Location of lab rotation: University Park; Clifton Campus

Location of full PhD project: University Park; Clifton Campus

(84) Ageing associated transcriptomic changes in plasma extracellular vesicles enriched for microglial origin

Primary supervisor: Anto Praveen Rajkumar Rajamani

Second supervisor: Helen Miranda Knight

Institution: University of Nottingham

School: Medicine

Lab rotation description:

The rotational project will lead the student to gain essential knowledge and skills in separating extracellular vesicles (EV) from human plasma, separating plasma EV subpopulations by immunoprecipitation, extracting RNA from EVs, and measuring EV RNA levels using digital droplet polymerase chain reactions (ddPCR). During the rotational project, we will measure IL1B (Interleukin-1-Beta) and CXCL8 (Interleukin-8) mRNA levels in plasma total EVs and in plasma EVs enriched for neuronal origin, in healthy younger (20-40 years) and older (>65 years) people. IL1B and CXCL8 mRNA levels in plasma EVs enriched for neuronal origin will clarify whether immunosenescence or neuroinflammation is associated with ageing.Total EVs will be separated from plasma using size exclusion chromatography. EVs enriched for neuronal origin will be separated using L1CAM immunoprecipitation (https://www.ncbi.nlm.nih.gov/pubmed/28588440). Total RNA from EVs will be purified using the Invitrogen total exosome RNA isolation kit (Thermo Fisher scientific, USA). Quantity and quality of purified RNA will be assessed using the NanoDrop™ spectrophotometer. IL1B and CXCL8 mRNA levels in plasma EVs will be assessed using the QX100 ddPCR system (Bio-Rad, USA) in the UoN. Statistical analyses will be performed with the guidance of the lead supervisor.

Full PhD project description:

Microglia are immune cells within the central nervous system that regulate inflammatory responses and neuronal plasticity. Optimal microglial activation is essential for neuronal survival, synaptic plasticity and healthy ageing. Neuroinflammation due to chronic microglial activation and immunosenescence related microglial dysfunction, are associated with various neurodegenerative disorders. Microglia secrete extracellular vesicles (EV) that carry biologically active RNA and proteins. Small (30-100 nm) EVs can cross blood-brain barrier and they transport microglial origin RNA and proteins to other cells via peripheral circulation. Prior microglial studies have mostly investigated post-mortem brain tissue. Investigating RNA cargoes of microglial origin EVs in human plasma will enhance our molecular level understanding of immunological and other ageing associated changes in living human brain. This may facilitate discovery of diagnostic biomarkers and novel therapeutic avenues for various neurodegenerative disorders.The objectives of the project include, (i) To optimise a protocol for separating EVs enriched for microglial origin from human plasma using IB4 immunoprecipitation, (ii) To profile the transcriptome of plasma EVs enriched for microglial origin, and (iii) To study the transcriptional changes associated with ageing in plasma EVs enriched for microglial origin.EVs will be separated from plasma of younger (20 - 40 years) and older (> 65 years) adults using the Izon qEV size exclusion chromatography system (Izon science Ltd., USA). A protocol for separating neuronally enriched plasma EVs using L1CAM immunoprecipitation (https://www.ncbi.nlm.nih.gov/pubmed/28588440) will be adapted for separating microglial enriched EVs using IB4 immunoprecipitation. The characteristics of separated EVs will be studied using the Malvern NanoSight nanoparticle analyser and Cryo-Transmission Electron Microscopy

(Cryo-TEM). The enrichment of EVs will be verified by Western blotting. In order to profile EV transcriptomes, total EV RNA will be purified from all enriched EV samples using the Invitrogen total exosome RNA isolation kit. Quantity and quality of purified RNA will be assessed using the NanoDrop™ spectrophotometer and the Agilent 2100 Bioanalyzer. A NEBNext Ultra-II (New England Biolabs, USA) directional cDNA library, and another small RNA specific cDNA library will be prepared for each RNA sample (N=20). The cDNA libraries will be mixed (1:1) and then will undergo single-end RNA-Seq (minimum of 15 million reads/ sample) in the UoN Deep-Seq facility. Expression levels of all RNA in the EVs enriched for microglial origin will be documented. The lead supervisor has experimentally validated an edgeR algorithm (https://www.ncbi.nlm.nih.gov/pubmed/26208977) for analysing RNA-Seq data to identify differentially expressed genes (DEG). Statistically significant DEGs and alternative splicing events that are associated with ageing will be identified with appropriate false discovery rate correction. Differential expression levels of identified DEG will be verified by ddPCR using independent biological replicate plasma samples. Functional analyses of identified DEGs will highlight the altered molecular pathways and dysfunctional molecular networks associated with ageing. This project will lead to a cutting-edge research program that will investigate the transcriptional changes in the EVs enriched for microglial origin in plasma of people living with neurodegenerative disorders.

References to learn more:

1. Mustapic M, Eitan E, Werner JK, Jr., et al. Plasma Extracellular Vesicles Enriched for Neuronal Origin: A Potential Window into Brain Pathologic Processes. Front Neurosci. 2017; 11: 278.2.

2. Paolicelli RC, Bergamini G, Rajendran L. Cell-to-cell Communication by Extracellular Vesicles: Focus on Microglia. Neuroscience. 2019; 405: 148-157.

Location of lab rotation: QMC

Location of full PhD project: QMC

(85) microRNA function in the cytoplasm and endoplasmic reticulum

Primary supervisor: Catherine Jopling

Second supervisor: Hilary Collins

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

MicroRNAs are short non-coding regulatory RNA molecules. In humans, hundreds of different microRNAs each target a particular set of mRNAs. The mechanism by which microRNAs regulate their targets is still not fully understood. Interestingly, there is evidence that microRNAs located at different sites within the cell may function differently. This project will focus on miR-122, a very highly expressed liver-specific microRNA that has important functions in liver development and disease. In addition to repression of cellular mRNA targets via sites in the 3' untranslated region (UTR), miR-122 has an unusual function in activating translation and replication of hepatitis C virus (HCV) RNA by binding to 5' UTR sites.We have found that miR-122 differentially regulates target mRNAs translated in the cytoplasm and at the endoplasmic reticulum (ER). The rotation project will focus on identifying the protein factors involved in miR-122 repression of cellular targets/activation via the HCV 5'UTR in the ER versus cytoplasm. We will take a candidate protein approach, depleting proteins that are known to be involved in miRNA activity and assessing the effect on ER- and cytoplasm-localised miR-122-targeted reporters. Methods will include cell culture, transfection, cellular fractionation, RNAi, western blotting, qPCR, luciferase assays and immunoprecipitation.

Full PhD project description:

miRNAs generally function by binding, in association with a complex of proteins known as the RNA-Induced Silencing Complex (RISC), to imperfectly complementary sites in the 3' untranslated region (UTR) of their mRNA targets, leading to reduced production of the encoded protein. Despite extensive investigation, the mechanism of miRNA repression remains imperfectly understood. While both mRNA decay and inhibition of translation occur, the extent to which each process contributes to overall regulation and the regulatory proteins involved vary in different studies. One important limitation to our existing knowledge is that most research has been carried out in whole cells, failing to take into account the localised nature of translation within the cell. This project will focus particularly on the endoplasmic reticulum (ER), which is the site of translation of secreted and membrane proteins. Interestingly, some data from model organisms suggests that both the mechanism of miRNA activity and the composition of the RISC may differ between the endoplasmic reticulum (ER) and cytoplasm. We have recently found that the highly expressed liver-specific miR-122 differentially regulates targets located in the cytoplasm versus ER. The overall hypothesis of the project is that the mechanism of miRNA regulation at the ER is different to that in the cytoplasm in human cells. The project will focus specifically on understanding the effect of miR-122 on its target mRNAs in both locations. We will investigate both miR-122 repression of target mRNAs by binding to 3'UTR sites, and miR-122 activation via the HCV 5'UTR. This is an unusual process by which miR-122 positively regulates translation and replication of HCV RNA by binding to two sites close to the 5' end of the viral RNA genome. The mechanism by which miR-122 regulates HCV is not fully understood. Interestingly, HCV replication occurs in association with ER-derived membranes, so is likely to be affected by localised miRNA activity. The aims are:

1. Identify the protein factors involved in miR-122 regulation via 3’ and 5’UTR sites in the cytoplasm and ER.

• This will include using approaches such as CRISPR and auxin-inducible degron technology to knockout and/or rapidly deplete proteins to follow up findings from the rotation project, and characterisation of complexes by fractionation/immunoprecipitation/mass spectrometry.

2. Determine how miR-122 affects RNA stability and translation by binding to 3’ and 5’UTR sites in the cytoplasm and ER.

• This will include an extensive series of approaches to assess the effects of miR-122 inhibition on translation and RNA stability of both ER- and cytoplasm-localised 3’ and 5’UTR-targeted reporters, and endogenous miR-122 targets. Techniques will include confocal microscopy, polysome fractionation and 4-thio-uridine labelling.The project will bring together the expertise of the Catherine Jopling (miRNA regulation) and Hilary Collins (confocal microscopy) to address these aims.

References to learn more:

1. Trabucchi M., Mategot, R. Subcellular heterogeneity of the microRNA machinery. Trends in Genetics (2019) 35:15-28.
2. Gebert L., MacRae I. Regulation of microRNA function in animals. Nature Reviews Molecular Cell Biology (2019) 20:21-37.

Location of lab rotation: University Park

Location of full PhD project: University Park

(86) Investigating the role of cell mechanics in Alzheimer's disease-associated neuroinflammation

Primary supervisor: Graham Sheridan

Second supervisor: Tomas Bellamy

Institution: Life Sciences

School: Life Sciences

Lab rotation description:

Students will first be trained in aseptic cell culture techniques and primary mouse astrocyte culturing protocols. Students will then learn how to perform immunocytochemistry, capture fluorescent images using confocal microscopy and analyse the data obtained using automated image analysis software to quantify changes in protein expression in individual astrocytic cells.The aim of the project will be to measure changes in the expression of intracellular signalling proteins linked to the activation of mechanosensitive ion channels, such as Piezo1. Astrocytes will be 'activated' using inflammatory mediators (e.g. LPS) and exposed to pharmacological compounds that either stimulate or block mechanosensitive ion channels. Students will then measure changes in intracellular protein expression of various mechano-responsive signalling molecules (e.g. Yap transcription factor).Weeks 1 – 3: Grow primary mouse astrocyte cell cultures and treat with pharmacological activators and blockers of Piezo1.Weeks 4 – 6: Investigate if Piezo1 activation modulates nuclear expression of Yap or triggers the SOCS signalling pathway in astrocytes, using immunofluorescence and confocal microscopy. Weeks 7 – 9: Analyse fluorescence images using automated image processing tools (e.g. Cell Profiler) and learn how to present and determine statistically relevant changes in intracellular protein expression in response to various pharmacological compounds.

Full PhD project description:

Project backgroundResearch into neurodegenerative disorders, such as Alzheimer's disease (AD), focuses primarily on genetic, biochemical and electrophysiological disturbances that underlie these complex conditions. Cell mechanics, particularly mechanosensitivity of neurons and glia, is often overlooked as a contributing factor to disease progression. However, changes in mechanical properties of ageing degenerating brain tissue impacts the physiology of glial cells, such as astrocytes and microglia. For example, astrocytes can detect the presence of very stiff peptide aggregates (amyloid plaques) in their surroundings and, in turn, upregulate the mechanosensitive ion channel, Piezo1 [1]. We have shown that activating Piezo1 channels increases calcium oscillations and inhibits the release of cytokines, such as IL-1β and TNFα, from reactive astrocytes in culture [2]; thus dampening neuroinflammation. Although secretion of proinflammatory mediators is attenuated by Piezo1 activation, mechanical stimulation of glia will lead to the release of other gliotransmitters and extracellular vesicles (EVs) that can modulate neurotransmission. As such, chronic dysregulations in glial mechanotransduction may contribute to synaptic plasticity deficits observed in AD. However, very little is known about the composition of gliotransmitters (e.g. sphingosine 1-phosphate) or EV cargo (e.g. neurotrophic factors) secreted following mechanical stimulation of astrocytes and microglia. We aim to identify these cell-to-cell signalling proteins and investigate their effects on neuronal function. Results from this project may help to identify novel drug targets for treating chronic neuroinflammation and reversing AD-associated deficits in synaptic

plasticity. Objectives1. Investigate the mechanisms involved in Piezo1-mediated inhibition of cytokine production. We hypothesise that activation of Piezo1 in amyloid β-reactive astrocytes leads to upregulation of mechano-responsive transcriptional cofactors, YAP/TAZ, and activation of the SOCS (suppressor of cytokine signalling) pathway. To test this hypothesis, primary murine astrocytes will be cultured in vitro, incubated with cytotoxic Aβ42 peptides, and treated with pharmacological activators or inhibitors of Piezo1. Changes in intracellular protein expression following 'mechanical stimulation' of astrocytes will be investigated using mass spectrometry and confirmed by immunofluorescence and image analysis. 2. Investigate gliotransmitters and EV-associated biochemical factors released following mechanical stimulation of glial cells. Murine astrocytes will be grown on 'soft' or 'stiff' mechanical substrates and stimulated with activators or inhibitors of Piezo1 channels. Conditioned media will be collected and the EVs secreted will be isolated and characterised by mass spectrometry and ELISA. Data will be analysed for known modulators of synaptic plasticity.3. Investigate the effects of key gliotransmitters (identified above) on neuronal plasticity. Organotypic hippocampal slice cultures will be used to examine the effects of mechanically-stimulated EVs on spontaneous and glutamate-evoked calcium oscillations in CA1 pyramidal neurons, using live-cell calcium imaging. Moreover, the intrinsic properties and synaptic plasticity of hippocampal neurons will be investigated using electrophysiology techniques. SignificanceThese experiments will increase our understanding of the role of glial cell mechanics in modulating synaptic plasticity in the ageing AD brain. By analyzing pathways activated by Piezo1, as well as the signalling molecules released in EVs as a result of mechanical stimulation of reactive astrocytes and microglia, we hope to identify novel molecular targets for Alzheimer's disease drug development.

References to learn more:

1. Velasco-Estevez M, Mampay M, Boutin H, Chaney A, Warn P, Sharp A, Burgess E, Moeendarbary E, Dev KK, & SHERIDAN GK (2018) Infection augments expression of mechanosensing Piezo1 channels in amyloid plaque-reactive astrocytes. Front Aging Neurosci. 10: 332.
2. Velasco-Estevez M, Rolle SO, Mampay M, Dev KK, & SHERIDAN GK (2020) Piezo1 regulates calcium oscillations and cytokine release from astrocytes. Glia 68: 145-160.

Location of lab rotation: QMC

Location of full PhD project: QMC

(87) Measurement of physicochemical factors and their contribution to reproductive function using optical fibre sensors

Primary supervisor: Raheela N Khan

Second supervisor: Sergiy Korposh

Institution: University of Nottingham

School: Medicine

Lab rotation description:

The project during a 9 week lab rotation will take place in the host labs of both supervisors. At the Royal derby Hospital, the student will initially learn to harvest, isolate and culture human endometrium. Using isolated cells, the student will gain experience using patch-clamp electrophysiology to record bioelectric signals through high -resistance seal formation. This will introduce the student to electrophysiology software and concepts of membrane bioelectrical phenomena as well as some data handling and analysis. For the other 4-5 weeks at University Park, the student will focus on learning the principle of operation of optical fibres, handling optical fibres and development of optical fibre chemical and bio-sensor. This research exploits cutting edge OFS, developed within the Faculty of Engineering, for monitoring physiological parameters in real time for healthcare applications. In particular we will focus on in situ measurements of pH, temperature and Spo2. This project brings cutting-edge technological development to address important biological questions with future clinical application to reproductive health.

Full PhD project description:

Most tissues and cells of the body are exposed to a constantly fluctuating environment of physicochemical factors such as pH, oxygen tension and electrolytes. Inappropriate control/levels of these factors may be associated with disease e.g. cancer, infection/sepsis, diabetes, miscarriage, preterm birth. Not only are accurate measurements of such factors ex vivo and in vitro lacking but also the mechanisms by which physicochemical cues are sensed and transduced into bioelectrical signals by cell membranes to effect function, are poorly understood. A successful pregnancy outcome is dependent an optimal uterine microenvironment. However, some 70% of unsuccessful IVF attempts and 75% of early pregnancy losses by natural conception are attributable to implantation failure due to a 'hostile' microenvironment. Human uterine fluid, with a pH of 6.6-7.6, is characterised by a six-fold higher potassium (K+) concentration than is found in plasma and is postulated to promote conception, implantation, and blastocyst cleavage. Despite this, there is a real lack of reliable, more specific and precise measurements of these parameters due to the limited technology available. Evidence from gene profiling studies and our findings has identified that two-pore potassium channels (K2P), a class of ion channels activated directly by pH, oxygen and temperature are expressed in epithelia of the female reproductive tract and demonstrate pH- and oxygen dependent physiological responses. Thus, in the endometrium (the tissue lining the uterus), K2P channels may be of importance in local homeostasis where slight perturbations in ion transport, pH, or oxygen tension could jeopardize the delicate ambient balance necessary for establishing a pregnancy. The range and significance of these responses in relation to reproductive outcomes remains unknown although, high oxygen levels have been linked to endometrial dysfunction through free radical generation while levels of both oxygen and pH are critical to the success of embryo

cultures as part of routine IVF protocols. Working in collaboration with the world-leading Optics and Photonics Group (Faculty of Engineering, UoN), we aim to utilise optical fibre sensors (OFS) that have wide-ranging but largely unrealized healthcare benefits. These sensors, modified with advanced molecular materials, offer a promising sensing platform, facilitating highly sensitive, selective and fast measurement of real time physiological parameters. With access to well characterised human ex vivo endometrial samples and in vitro models of the endometrium in RNK's lab and for which ethical approval is in place, this PhD project will focus on (i) utilising OFS technologies to measure local levels of oxygen, pH and temperature under a variety of experimental conditions and (ii) to determine the downstream K2P-mediated mechanisms triggered by local, physicochemical changes in endometrial cells using electrophysiology and fluorescence cell-based assays and imaging. It is expected that data generated through this DTP and the opportunity for cross-disciplinary study will fill an important gap in our knowledge of a uterine environment conducive to pregnancy. It will also for the first time provide a direct correlation between physicochemical parameters and their regulation of bioelectrical signals which will help identify new approaches to improve reproductive outcomes. Finally, the project will be an important step towards an application to assess the use of OFS in vivo in women undergoing fertility investigations and also in other common diseases, in order to establish the clinical utility of such measurements.Development of the optical fibre sensors is multidisciplinary in nature and is positioned at the interface between physics/engineering and surface chemistry with realistic translational application to the field of female reproductive health.

References to learn more:

1. Ng BKY, Mingels R, Morgan H, Macklon N, Cheong Y (2018) In vivo, oxygen, temperature and pH dynamics in the female reproductive tract and their importance in human conception: a systematic review. Human Reproduction update 24, 15-34. https://doi.org/10.1093/humupd/dmx028
2. Correia R, James S, Morgan S, Lee S-W, Korposh S. (2018) Biomedical application of optical fibre sensors. Journal of Optics, 20, 073003.

Location of lab rotation: University Park;Derby Royal Infirmary

Location of full PhD project: University Park;Derby Royal Infirmary

(88) Emergence of new genes by gene remodelling in Animals

Primary supervisor: Mary J O'Connell

Second supervisor: James McInerney

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

Transcription-derived gene fusions and CNVsGenomic instability initiates rearrangements giving rise to gene fusions, and different genomic regions can be differentially susceptible to genomic instability. In cancers, recurrent genomic instability leads to gene fusions in key locations, and the resulting fusion transcripts can drive the cell's oncogenic properties. This rotation will explore the relationship between germline genomic instability and transcriptional readthrough, to identify possible examples of incipient transcription-derived gene fusions. The work will begin in humans using high-quality genomic resources; the 1000 Genomes Project sequences will be used to validate reported deletion CNVs, and RNAseq data from the GTEx project will be used to investigate the frequency of transcriptional readthrough across CNV breakpoints. Transcription-derived gene fusions that are polymorphic in the population should create new fusions that are present only in CNV carriers and absent from non-human primate sequences. After description of CNV-associated gene fusions in humans, a successful approach can be extended to other animal species (such as wild mice, domesticated ruminants, fish or insects) for which sufficient genomic resources are available from outbred individuals.

Full PhD project description:

Please provide a description of the full PhD project (500 words)The main aim of the project is to identify, test and apply gene fusions to understanding animal diversity and evolution.In order to investigate this we will ask the following research questions:1. What gene fusion events are in modern day animal genomes?2. Can we use these novel data types and datasets to resolve the animal phylogeny3. What properties can we define for these gene fusions?Animal genomes have evolved (for the most part) in the absence of horizontal gene transfer, i.e. they cannot directly acquire genes from their environment, therefore new functions and phenotypes emerge in the animal group by remodelling existing pieces/sequences into something new. We know that the major drivers of change are the evolutionary processes of mutation, recombination, selection and drift - yet recombination (e.g. remodeling by gene fusion) is probably the least understood process. Gene fusion can affect biological function in many ways altering interactions, pathways, function and expression. For example, the chimeric gene Jingwei that functions in development in Drosophila. Jingwei is remodeled from yellow emperor and alcohol dehydrogenase genes combining activity on both long chain alcohols and diols, including growth hormones and pheromones, and the kua-UEV fusion gene in human has facilitated localization to the cytoplasm of an otherwise solely nuclear localised polyubiquitination co-effector. We want to understand the rules that govern the emergence of new genes by gene fusion in animals (i.e. metazoa). By its very nature the process of gene fusion is non-tree-like because a fusion gene has more than one origin/root. Therefore, the traditional framework of the phylogenetic tree that we are used to applying to studying sequence evolution can't adequately model a gene with multiple origins. We will use network theory and sequence similarity networks to identify genes that connect otherwise unconnected gene families on

graphs. Previously the community has used these networks to split sequence space hierarchically - forcing ‘natural’ homologous families and super families and thereby masking gene fusions as any individual gene is permitted to exist in only one gene family. The reality for gene fusions is that they belong to more than one family.We will determine where and when gene fusions occurred in the history of animals, and whether these new genes have contributed to the major transitions we observe, e.g. non-bilaterian to bilaterian body plans, and the emergence mesoderm, organs/tissues/systems, a body cavity, and the adaptive immune system. Answering the question of whether gene fusions emerge in a gradual or saltational way addresses a long-standing question in biology over the tempo of evolution. Using genomic and transcriptomic datasets, we will analyse and interpret the complex patterns of connectivity that emerge between individual sequences to identify gene fusion events in the animal tree of life. This work will impact on the entire field of evolution by bringing about the necessity to expand our current theories. .

References to learn more:

1. Kaessmann, H. (2010). "Origins, evolution, and phenotypic impact of new genes." Genome Res 20(10): 1313-1326.
2. Haggerty, L. S., et al. (2014). "A pluralistic account of homology: adapting the models to the data." Mol Biol Evol 31(3): 501-516.

Location of lab rotation: QMC

Location of full PhD project: University Park

(89) Dynamic design and bottom-up assembly of complex 3D micro-environments: Construction and study of the bone fracture callus

Primary supervisor: Lee Buttery

Second supervisor: Glen Kirkham

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

Buttery and Kirkham work on cell biology and tools and technology, including optical tweezers, to achieve precision engineering of 3D in vitro cell models. Ghaemmaghami works on immune cells, in particular the monocyte-macrophage lineage and approaches to coordinate shifts between inflammation (M1) and healing (M2) phenotypes.Training will be given on basic culture of human mesenchymal stem cells, osteoblasts and monocyte-macrophages and their phenotypic characterization by molecular markers and morphology. Approaches to 2D and 3D cell culture will include aggregation methods and an introduction to optical tweezers. The main focus will be to investigate differentiation of human osteoblasts in response to nano-vibrational stimulation (NanoKick bioreactor with Prof Matthew Dalby, University of Glasgow). Nano/micromechanical forces are integral to bone formation in health and healing and this project will be one of the first to investigate nanokicking on a 3D cellular structure in vitro. Further work will include exposure to IL-1 and TNF-alpha and effects of the combination of nano-mechanical and pro-inflammatory stimuli on osteogenic differentiation. There will also be an introduction to creating microscale biochemical gradients using microparticles doped with biochemical factors (e.g. cytokines) and incorporation into cellular aggregates or precision positioning around cellular aggregates.

Full PhD project description:

We hypothesize that we can build the first in vitro bone fracture callus microscale model and interactively study how bone tissue repairs itself. We will achieve the systematic, spatial and temporal engineering of an in vitro 3D bone microenvironment model, precision configured to deliver multiple cellular, bio-molecular, micromechanical and vascular interactions and signals. These coordinated inputs are all integral to callus formation and resolution of repair. The key outputs and impact will be highly detailed bone tissue microscale constructs that mimic the fracture callus and a dynamic cell model to recapitulate and study the complex process of bone healing. It will provide new insights on in vivo repair and opportunities to test strategies to support bone repair. It also impacts on the 3Rs and use of bone models in animals and maps onto UKRI priorities on using regenerative medicine to develop new therapeutic approaches to improve tissue repair and healing. We have developed new approaches to coordinate cell-cell and cell-materials interactions and interactively build complex in vitro 3D cellular micro-environments to mimic native tissue histology. Using optical tweezers, controlled aggregation, scaffold microparticles, de-cellularized and de-mineralized tissue fragments, hydrogels and a simple microfluidic device we have established a cellular ‘toolkit’ and 3D assembly platform. Here we will establish a ‘build a bone microenvironment’ toolkit. The project will iteratively progress from a basic microenvironment towards more complex assemblies and mimicking of the fracture callus. The modular nature will enable and encourage flexibility, innovation and enterprise of how components are assembled around the core concept of

dynamic assembly and coordination of multiple cell types and signals, informed by fracture callus histology. Aims1. Building a basic bone microenvironment – the ‘starter kit’.

• Use de-cellularized / de-mineralized bone (e.g. porcine/ovine) fragments to mimic the rudimentary architecture of the fracture and callus and precision assemble human osteoblasts onto this template using optical tweezers.

• Deliver signal gradients (e.g. BMP2) via polymer microparticles precision positioned around the bone fragments

• Stabilize within a hydrogel (e.g. gelatin methacrylate) of varying stiffness (to mimic elements of a haematoma) and culture over days to several weeks.2. Addition of more cell types and challenge with inflammatory mediators – ‘the advanced kit’

• Assemble aggregates of human mesenchymal stem cells (e.g. GFP labelled line) and human monocytes (as precursors to macrophages and also osteoclasts) at definable locations around the microenvironment

• Simulate inflammation by exposure to cytokines (e.g. IL-1, IL-6, TNF-alpha)

• Assemble M1 (inflammation) and then M2 (healing) macrophages into the environment to coordinate inflammation and healing responses3. Nano/micro-mechanical stimulation and induction of vascular responses – ‘the expert kit’

• Deliver nano-vibrational signals (e.g. mini-project) to coordinate cellular responses to healing

• Place 3D microscale callus constructs on the chick chorioallantoic membrane (CAM) assay to introduce vascular signallingQualitative and quantitative analyses of bone formation and cell differentiation will include extensive molecular, biochemical and morphological assays.

References to learn more:

1. Kirkham G, Britchford E, Upton T, Ware J, Gibson G, Devaud Y, Ehrbar M, Padgett M, Allen S, Buttery L, Shakesheff K. Precision Assembly of Complex Cellular Microenvironments using Holographic Optical Tweezers. Scientific Reports 2015;5:8577. https://doi.org/10.1038/srep085772.
2. Alvarez MM, Liu JC, Trujillo-de Santiago G, Cha BH, Vishwakarma A, Ghaemmaghami AM, Khademhosseini A. Delivery strategies to control inflammatory response: Modulating M1-M2 polarization in tissue engineering applications. J Control Release. 2016;240:349-363. https://doi.org/10.1016/j.jconrel.2016.01.026

Location of lab rotation: University Park;Clifton Campus;

Location of full PhD project: University Park;Clifton Campus;

(90) The effects of physical activity and social enrichment on healthy brain ageing

Primary supervisor: Claire Gibson

Second supervisor: Paula Moran

Institution: University of Nottingham

School: Psychology

Lab rotation description:

To explore the core underpinning changes that occur at the cellular and molecular level in response to 'healthy' ageing. Insufficient cardiovascular function, and therefore cerebrovascular supply, is likely to contribute to brain ageing through chronic and systemic inflammation and also through the loss of adequate brain perfusion due to vascular damage. The white matter, in particular, seems vulnerable to these changes. The overall aim of this lab rotation is to contribute to ongoing work identify novel biomarkers of 'healthy' ageing in rodents. Such understanding of biological mechanisms may enhance the identification of drug targets to enhance healthy ageing. Research Techniques: immunocytochemistry, tissue preparation, on going in vivo studies. Relevant reading: [1] Corriveau et al., (2016) Cell Mol. Neurobiol. 36: 281-288; [2] Liu et al., (2017) Ageing Res. Rev. 34: 64-76; [3] Ryan et al., (2019) Front. Aging Neurosci.

Full PhD project description:

A global increase in life expectancy has led to increased incidence of age-related cognitive decline. Cognitive decline is part of the normal ageing process, occurs in the absence of underlying neurodegenerative disease and is a significant contributor to diminished quality of life in an ageing population. It is well accepted that exercise is good for our physical health but it's timely to consider the (beneficial) impact it may have on brain function. Emerging evidence suggests that modifiable lifestyle factors, including physical activity and social engagement, can promote healthy brain ageing and engagement with regular physical activity may even prevent cognitive decline and dementia. Our aim here is to explore, using rodents, how physical activity and social enrichment affect brain function during the normal ageing process by examining cognitive function and changes at the cellular level. Such research may contribute to the identification of relevant cellular and molecular targets to enhance healthy brain ageing. We have recently developed protocols for playpen enrichment whereby rodents are exposed to both physical and social enrichment and have some preliminary data showing the effects of enrichment on brain function. However, our aim here is to compare the types of enrichment i.e. physical exercise and social engagement on brain function during ageing and we will examine if combined enrichment (i.e. physical activity + social) gives any added benefit. We will use ageing rats to examine age-dependent memory impairment and the underlying neurochemical changes following exposure to physical exercise and/or social enrichment or the appropriate control environment. We will also examine the effect of age at which environment exposure is initiated by comparing exposure beginning at young, middle or old(er) age. For the various experimental groups we will make the following measures:i) To determine the effects of environment exposure on general animal behaviour (burrowing, climbing, upright standing, lateral stretching) and animal physiology (food consumption, faecal corticoid levels, body/organ weight, cerebral stress signalling peptide/mRNA levels).ii) Assess the effects of environment exposure on cognitive function – using tasks such as spatial learning, object recognition and operant lever press set-shifting.iii) Investigate the effects on brain function – using

neuroimaging (MRI) and immunocytochemistry techniques to explore neurogenesis, growth factor (e.g. BDNF, VEGF) expression and changes in neurotransmitter (e.g. glutamate, acetylcholine). 3Rs/Animal Welfare Impact: Our aim here is to provide scientific evidence of changes in brain function during ageing which may underlie cognitive improvement following exposure to physical and/or social enrichment. However, this work may also have beneficial, from an animal welfare perspective, for rodents undergoing ageing studies for other studies if we can validate enrichment protocols which provide health benefits.

References to learn more:

1. Dause TJ & Kirby ED (2019) Aging gracefully: social engagement joins exercise and enrichment as a key lifestyle factor in resistance to age-related cognitive decline. Neural Regeneration Research 14(1): 39-42.
2. Stimpson NJ, Davison G, Javadi A-H (2018) Joggin' the noggin: towards a physiological understanding of exercise-induced cognitive benefits. Neurosci Biobehav Rev 88: 177-186.

Location of lab rotation: University Park;QMC;

Location of full PhD project: University Park

(91) Developing iPSC models of the airway epithelium to understand host – virus interactions

Primary supervisor: Ian Sayers

Second supervisor: Nick Hannan

Institution: University of Nottingham

School: Medicine

Lab rotation description:

The nine 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 of iPSC from Human donors and initial characterisation (Lead Hannan)iii) The characterisation of virus species in natural isolates (via Biomedical Research Centre) and the propagation of these and laboratory viruses (Lead Tarr).iv) Initial handling of transcriptomic datasets from pilot data we already have looking at the interaction between Rhinovirus and human bronchial epithelial cells (Lead Sayers).

Full PhD project description:

The airway epithelium acts as the critical interface between the environment and organ physiology. It acts as a barrier to potential pathogens and extraneous particles and helps regulate host defence mechanisms, including the inflammation process. Under normal conditions, the bronchial epithelium is composed of ciliated columnar, mucus-secreting goblet and Clara cells that secrete surfactant. Respiratory viruses including respiratory syncytial virus (RSV), influenza virus and rhinovirus (RV) are common and cause significant illness in children and also exacerbate existing lung diseases. A greater understanding of the molecular basis of the virus-airway epithelial interactions in donors of different genetic backgrounds or in different environmental context will provide mechanistic understanding. Accumulating evidence suggest that genetic variants mediate the extent and nature of the virus interaction, e.g. CDHR3 variants and RV-C (PMID: 24241537, PMID: 30930175).The most common approach to study bronchial epithelial cell – virus interactions in human context is to isolate airway epithelial cells using bronchoscopic brush technique and then culture the cells using air–liquid interface (ALI) differentiation (PMID: 22287976). ALI cells form an epithelial barrier that closely resembles the in vivo architecture and is composed of basal, goblet, and ciliated cells. However, the bronchoscopic procedure is invasive with life threatening risk to the individual, adequate numbers of cells are hardly collected and cells have a limited lifespan. These factors make the development of a non-invasive, sustainable model of the human airway epithelium that incorporates the genetic complexity of human donors for mechanistic studies highly desirable.The differentiation of induced pluripotent stem cells (iPSCs) to mature cell types shows great promise to provide personalized disease modelling including mechanistic studies. Development of iPSC models that encapsulate mature multiciliated cells in a functional airway epithelium with clara, goblet, and basal cells indicative of a polarized epithelial-cell layer have recently been developed (PMID: 24706852). However, more work is needed to advance this area providing a robust representative model the captures the complexity of the airway epithelium and can be used to investigate the host

epithelial – virus interactions representative of that occurs in the human lung.The hypotheses underlying this proposal are i) iPSC models can capture the complexity of airway epithelium, ii) these models will allow the study of RSV, influenza and RV induced responses providing unprecedented insight into the molecular basis of specific and overlapping virus driven effects and iii) by studying viral induced changes in cells derived from donors that carry specific genetic variants and/or introduce changes using CRISPR/Cas9 we will identify potentially new/novel understanding of pathways that could be the target of new anti-viral drug development.Key stages to the project:1. Develop and optimise culture conditions for the generation of airway epithelium in vitro complete with clara, goblet, and basal cells using iPSC and ALI differentiation.2. Compare and contrast at the functional (barrier), morphological, protein and transcriptomic (RNA-seq) level these iPSC derived airway epithelial layers with the current gold standard derived from donor bronchial epithelial cells isolated by bronchoscopy and grown at ALI.3. Investigate virus – epithelial cell interactions in both the iPSC and HBEC models using cells from controls and patients with respiratory conditions e.g. asthma to understand the effects of genetic variation (in genotype donors and/or introduced by CRISPR/Cas9) on these responses. This project represents an exciting PhD opportunity and brings together significant expertise across schools and disciplines including the use of airway models, genetics (Sayers), iPSCs (Hannan) and virology (Tarr).

References to learn more:

Evaluation of differentiated human bronchial epithelial cell culture systems for asthma research.Stewart CE, Torr EE, Mohd Jamili NH, Bosquillon C, Sayers I. J Allergy (Cairo). 2012;2012:943982.

Generation of multiciliated cells in functional airway epithelia from human induced pluripotent stem cells. Firth AL, Dargitz CT, Qualls SJ, Menon T, Wright R, Singer O, Gage FH, Khanna A, Verma IM. Proc Natl Acad Sci U S A. 2014 Apr 29;111(17):E1723-30.

Location of lab rotation: University Park;QMC

Location of full PhD project: University Park

(92) Engineering P450s for monoterpenes oxyfunctionalisation

Primary supervisor: Stephen Hall

Second supervisor: Anca Pordea

Institution: University of Nottingham

School: Engineering

Lab rotation description:

The project aim is to establish a library of cytochrome P450 mutants with the ability to oxyfunctionalise monoterpenes, which subsequently, can be polymerised by chemical catalysis to produce renewable polymers. Following an established procedure, the student will produce mutants of the enzyme cytochrome P450 BM3 with different activities and selectivities using a variety of site-directed mutagenesis techniques. A library of 125 mutants will be constructed by combining five hydrophobic amino acids (alanine, phenylalanine, valine, leucine, methionine) at 3 positions, selected from previous mutational studies in the hydrophobic substrate channel. We will investigate the effect of these changes on the epoxidation vs hydroxylation preference of the enzyme. Analytical methods such as GC-MS will be used to analyse biotransformations of the mutants for functionalised pinene compounds.

Full PhD project description:

The overall project is part of the Green Chemicals Beacon of Excellence, a UoN funded initiative committed to tackling the challenges of sustainable chemicals production and will contribute to establishing an enzyme engineering capability within the beacon.Biocatalytic terpene functionalisation can give access to a wide range of building blocks for chemical and material synthesis. Due to their ability to selectively oxidise inert C-H bonds, cytochrome P450s have been proposed as a viable technology for terpene oxyfunctionalisation. Recent reported data identified a P450 library for the controlled oxidation of monoterpenes (ref 1). Rob Stockman's group has already used oxidised terpen(oid)s for further functionalisation to (meth)acrylate monomers, and their further polymerisation to make renewable polymers. In their published work, the corresponding alcohol derivatives were obtained via a wasteful hydroboration / oxidation protocol. The use of biocatalysis would enable a simpler and more sustainable route to such polymers. The project aim is to optimise, using a rational design approach, a cytochrome P450 for the specific oxidation of two terpenes from wood waste: α-pinene and β-pinene. The enzyme we are using is the well-studied P450 BM3 from Bacillus megaterium. This is the most active P450 known, it is easily expressed in E. coli as a fusion with its reductase partner, and it has a large substrate range. Although there is ample literature on engineering this enzyme in order to modulate its substrate profile and chemo/regioselectivity, the selective oxidation of pinenes to alcohols remains a challenge. The major products reported in the literature are alpha/beta-pinene oxides, but their recovery is non-trivial because they undergo non-enzymatic hydrolysis and rearrangement in water. Thus, engineering P450 BM3 to efficiently synthesise verbenol and pinocarveol, as precursors to functionalised (meth)acrylate monomers will be a priority. We will also investigate the synthesis of pinene oxides, which could potentially be further functionalised to monomers. We have already made a start on protein engineering, first testing the highly active P450 co-solvent resistant variant (W5F5). A small

library of ~10 mutants has been constructed and screening of these for alpha- and beta-pinene oxidation is being performed.The mutations introduced are at 3 positions, substituting WT residues with five hydrophobic amino acids (alanine, phenylalanine, valine, leucine, methionine) based on information obtained from current literature. Eventually, the library will comprise 125 variants and the effect of these mutations on activity will be examined.Mutants are currently being prepared by site-directed mutagenesis and some method optimisation to increase through-put is envisaged. Activity screening with alpha/beta-pinene will be performed using P450-containing cell lysates and a glucose dehydrogenase cofactor regeneration. A colorimetric screen based on Purpald (J. Am. Chem. Soc. 2011, 133, 3242) will be developed using the verbenol and pinocarveol methyl ethers, to quickly assess mutant selectivity towards the corresponding alcohols. To confirm the colorimetric screening results, conversions and product ratios will be also determined on GC-MS. The mutants showing the highest conversions and selectivities will be tested on a preparative scale. To avoid pinene oxide hydrolysis, we will also assess the epoxide-selective mutants in a biphasic system, either using the substrate itself, or a non-miscible solvent.

References to learn more:

1. Hernandez-Ortega A, Vinaixa M, Zebec Z, Takano E, Scrutton N. (2018). A toolbox for diverse oxyfunctionalisation of monoterpenes. Nature Scientific Reports. 8: 143962.
2. Fasan R. (2012) Tuning P450 Enzymes as oxidation catalysts. Catalysis. 2: 647-666

Location of lab rotation: University Park

Location of full PhD project: University Park

(95) Neuroinflammatory responses across the lifespan: Role of Nuclear Receptor SUMOylation

Primary supervisor: Andrew Bennett

Second supervisor: Gareth Hathway

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

Microglia are the resident immune cells of the brain responsible for neuronal pruning in early life and phagocytosis of damaged neurones across the lifespan. SUMOylation of nuclear receptors is reported to reduce inflammatory responses in microglia by inhibiting transcription of inflammatory response genes. We hypothesise that phagocytosis of damaged neurones induces nuclear receptors SUMOylation and that nuclear receptor expression and SUMOylation declines with age leading to increased neuroinflammation. The rotation will involve learning to culture primary rat microglia and cortical neuronal cells. SUMOylation of nuclear receptors (Nurr1 and LXR/) will be detected in response to inflammatory stimuli using SUMO immunoprecipitation assays and western blotting. Phagocytosis assays in which microglia engulf fluorescently labelled apoptotic neurones will be set up and rates of phagocytosis measured by fluorescent microscopy. The change in phenotype of microglia induced by neuronal phagocytosis will be assessed using Taqman Q-RTPCR for pro- and anti-inflammatory genes and ELISA for secreted cytokines

Full PhD project description:

Microglia are the resident immune cells of the central nervous system and can be both neuroprotective and neurotoxic dependent upon their activation state. Dyeing neurones release substances such as ATP, chemokines and cytokines that attract microglia and promote phagocytosis via interaction with cell surface receptors. These mediators, in addition to promoting phagocytosis of apoptotic neurones as part of healthy neuronal homeostasis are also pro-inflammatory, and can promote an activated, neurotoxic microglial phenotype. In the healthy individual, neurones routinely die without the development of chronic neuroinflammation, although the development of low-grade neuroinflammation is associated with ageing. When microglia phagocytose dyeing neurones the neuronal cell contents are taken into lysosomes for degradation. There is a growing body of evidence from study of peripheral blood macrophages that lysosomal processing of cell debris releases molecules, which promote an anti-inflammatory phenotype, preventing uncontrolled inflammatory responses from developing. One of the mechanisms that has been put forward for this anti-inflammatory response is activation of nuclear receptors in response to release of nuclear receptor ligands from lysosomes of phagocytic macrophages. Nuclear receptors such as Nurr1 and LXR are inti-inflammatory; and have been shown to inhibit activation of gene expression by NFkB, the master regulator if inflammatory gene expression. The exact mechanism by which nuclear receptors inhibit inflammatory gene expression is unclear. Nuclear receptors can be SUMOylated post-translationally and this has been proposed to lead to interaction of SUMOylated nuclear receptors with inflammatory gene promoters via protein-protein interaction with nuclear co-repressor proteins. The regulation of SUMOylation in this context is as yet not described. We have shown in preliminary studies that molecules such as oxysterols that are released from lysosomes

post-phagocytosis can induce SUMOylation and are anti-inflammatory. We propose that apoptosis of neurones by microglia leads to nuclear receptor SUMOylation and prevents the development of neuroinflammation. There is evidence that nuclear receptor expression declines with age; we hypothesis that the development of neuroinflammation over the lifespan is due at least in part to reduced expression and SUMOylation of nuclear receptors in microglia. The project will use primary microglia and neuronal cultures, which are established in our laboratories. Microglia taken from rats across the lifespan from neonate to aged animals will be exposed to fluorescently labelled apoptotic neurones and cells that have engulfed neurones sorted by FACS. The phenotype of cells will be assessed by Taqman QRTPCR, western blotting and ELISA. SUMOylation of nuclear receptors will be detected in response to phagocytosis using SUMO immunoprecipitation and western blotting. We will use sequential chromatin immunoprecipitation – in which chromatin is first pulled down with an anti-SUMO antibody and then further purified using anti- nuclear receptor (Nurr1 or LXR) antibodies – followed by RNA-seq to determine which gene promoters are targeted by SUMOylated nuclear receptors. We will then use QRTPCR to conform changes of expression in identified genes. The level of expression of nuclear receptors, response to phagocytosis in terms of SUMOylation and gene expression profiles will be assessed in microglia taken from animals of different ages to determine the involvement of nuclear receptor SUMOylation in development of neuroinflammation with age.

References to learn more:

1. Transcriptional control of metabolic and inflammatory pathways by nuclear receptor SUMOylation. EckardtTreuter and NicolasVenteclef. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease Volume 1812, Issue 8, August 2011, Pages 909-918.
2. Synaptic Pruning by Microglia Is Necessary for Normal Brain DevelopmentRosa C. Paolicelli1, Giulia Bolasco1, Francesca Pagani2, Laura Maggi2, Maria Scianni2, Patrizia Panzanelli3, Maurizio Giustetto3,4, Tiago Alves Ferreira1, Eva Guiducci1, Laura Dumas1, Davide Ragozzino2, Cornelius T. Gross1,* Science 2011: Vol. 333, Issue 6048, pp. 1456-1458

Location of lab rotation: QMC

Location of full PhD project: QMC

(96) A comparative analysis of survivin distribution in young, aged and transformed human cells, and its influence on genomic integrity under hypoxia

Primary supervisor: Sally Wheatley

Second supervisor: Stephen Gray

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

Survivin is an inhibitor of apoptosis protein that is upregulated in all cancers. Under hypoxic conditions its expression is upregulated at the mRNA and protein levels and it becomes predominantly localised to the nucleus, rather than the cytoplasm. To date nuclear survivin has been considered unable to inhibit apoptosis, however, all experiments in which this was assessed were carried out under normoxic conditions with survivin forcibly expressed or retained in this compartment1. Preliminary data from the lab suggest that under hypoxic conditions, under which survivin naturally becomes resident in the nucleus, it is in fact cytoprotective. Here we will test the ability of nuclear survivin to protect cancer cells against different forms of genotoxic and metabolic stress, including UV irradiation, TRAIL, doxirubicin and staurosporine treatments under hypoxic conditions. Briefly, cells will be grown in monolayer culture and in 3D hanging drops under hypoxia, survivin expression suppressed using the commercially available survivin specific inhibitor, YM155, and cellular response assessed using FACS-live-dead assay, supported by immunoblotting, immunostaining and live imaging to determine mitotic state and mitochondrial health. 1Connell CM, Colnaghi R, Wheatley SP. (2008) Nuclear survivin has reduced stability and is not cytoprotective. J Biol Chem. 8;283(6):3289-96. PMID: 18057009.

Full PhD project description:

Genome instability increases with age and is a hallmark of cancer, unsurprisingly, therefore, age is the primary risk factor in cancer1. The aim of this project is to compare the genomic integrity (nuclear and mitochondria (mt)) of young versus old fibroblasts and transformed cells that differentially express the inhibitor of apoptosis protein, survivin. Owing to the presence of cell cycle related elements in its promoter region, in normal cells survivin is usually only expressed in G2 and M phase, however, its expression is upregulated under hypoxia, a state that has relevance to both ageing and to cancer. Literature suggests that as cells age, survivin switches from being predominantly nuclear to mainly cytoplasmic2, and that this redistribution enables cells to tolerate stress, giving them a survival advantage. In cancer cells survivin becomes additionally localised to the mitochondria. We have extensive data to show that in cancer cells mitochondrial survivin inhibits mitophagy, the specialist from of autophagy that eliminates damaged mitochondria: consequently the cells bear extensive mtDNA lesions and switch from a metabolic dependency on Oxphos to glycolysis, known as the “Warburg transition”. In this project young, old and transformed cells will be grown under hypoxic conditions, and normoxic conditions, survivin distribution and relative abundance recorded, and suppressed using the commercially available inhibitor, YM155, and the survivin-specific miRNA, miR203. Cell proliferation and viability will be assessed using colony counting and FACS analysis, and a resazurin-resarufin assay used to determine respiratory capacity.

Cell health and mitochondrial integrity will be assessed by live imaging with fluorescently labelled mitotracker, and nuclear DNA repair monitored using anti-H2AX foci in fixed preparations. To determine genomic integrity directly, RNA will isolated from young (passage 2-4) and aged (passage 35) fibroblasts and cancer cells, reverse transcribed and the integrity of the nuclear and mitochondrial DNA monitored using PCR reactions designed to generate short DNA (200bp) and long (7kb) fragments from each genome. Reactions from cells harbouring nuclear and mtDNA lesions will yield fewer long PCR products, but will have many short fragments, while cells with undamaged DNA will deliver plenty of both products. Preliminary data from my lab suggest that survivin overexpression in cancer cells inhibits mitophagy and causes an accumulation of mt lesions (SPW, A. Townley, in preparation), but whether this holds true under hypoxic conditions, or is affected by ageing, has not been addressed. Data from this project will enable us to determine whether survivin expression increases cellular tolerance to nuclear and mtDNA damage, facilitates nuclear DNA repair, and/or accelerates mitochondrial clearance, whether these responses differ with age and how survivin modulates these responses, and whether any intervention to survivin function in these pathways could have therapeutic potential.

References to learn more:

1. Wheatley SP, Altieri DC. (2019), Survivin at a glance. J Cell Sci. 4;132(7). doi: 10.1242/jcs.223826.2
2. Temme et al., (2005) Biochemical and Biophysical Research Communications 327 (2005) 765–773.

Location of lab rotation: QMC;

Location of full PhD project: QMC;

(97) The role of axonal mRNAs in pain perception

Primary supervisor: Cornelia H. de Moor

Second supervisor: Federico Dajas-Bailador

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

Pain is a useful danger signal that can be tuned according to the circumstances. When the pain neurons (nociceptors) are repeatedly activated, they become more sensitive, leading to pain experiences from weaker stimuli, sometimes leading to chronic pain. An important recent insight is that the axons of many neurons contain RNAs that are important for such plasticity. Activation of mRNA translation by polyadenylation, repression by microRNAs and changes in signal transduction are involved in these processes. We have evidence that polyadenylation is important in nociceptor plasticity. Using high-throughput methods we have identified mRNAs that change in a model of nociceptor sensitisation. In this rotation, you will investigate mRNAs that we have found changing in the axons of nociceptors to check if they are regulated by translational control and are affected by signal transduction or inhibition of polyadenylation. You will study primary mouse dorsal root ganglia cultures (which contain the nociceptors) using techniques such as immunohistochemistry, in situ hybridisation, calcium imaging, RNA isolation, quantitative PCR, and poly(A) tests to investigate whether specific mRNAs localised to nociceptor axons are likely to play a role in their sensitisation. The work is likely to contribute to a publication.

Full PhD project description:

Neurons famously can change their electrochemical signalling properties in a process called synaptic plasticity, which is thought to underlie memory formation. The plasticity of neurons is therefore an area of intensive study, with many studies indicating that the local regulation of translation of mRNAs in the dendrites and axons play a key role in this process. The neurons that conduct pain signals, the nociceptors, have been relatively neglected in these studies. Yet these neurons have some of the longest axons, running all the way from the lower spine to the toe, for instance. Nociceptors are also known to display plasticity, which is involved both in normal and neuropathic pain. In an ongoing study funded by Versus Arthritis, we have found that inhibition of polyadenylation reduces sensitisation of nociceptors and identified mRNAs that are localised in the axons of these neurons using RNA-Seq. In this PhD project, you will build on these findings to identify which of the localised mRNAs play an important role in the plasticity of pain neurons. After identifying the most promising candidates in the rotation, you will use neuronal cultures in microfluidic chambers to treat the cell body and axon separately with translation and polyadenylation inhibitors to detect the effects with immunohistochemistry and in situ hybridisation. You will use recombinant viruses to knock down and over express mRNAs of interest, first in culture and then in an animal model, to see if pain signalling and perception is affected. To identify the mechanism by which the mRNA is regulated, you will construct reporter genes with the untranslated regions of the mRNA and knock down or inhibit potential regulators, such as RNA binding proteins, poly(A) polymerases and microRNAs that you will have identified by bioinformatic

analysis. This work will contribute to the fundamental understanding of the plasticity of pain perception and is likely to inform research into improved treatment of chronic pain.

References to learn more:

1. Ashraf, S., Radhi, M.*, Gowler, P.*, Burston, J.J., Gandhi, R.D.*, Thorn, G.J., Piccinini A.M., Walsh, D.A., Chapman, V., De Moor, C.H. (2019) The polyadenylation inhibitor cordycepin reduces pain, inflammation and joint pathology in rodent models of osteoarthritis. Scientific Reports, 9, 4696. https://www.nature.com/articles/s41598-019-41140-1
2. Spatiotemporal regulation of GSK3beta levels by miR-26a controls axon development in cortical neurons. Lucci C*, Mesquita-Ribeiro R*, Rathbone A, Dajas-Bailador F. Development 2020 (in press)

Location of lab rotation: University Park

Location of full PhD project: University Park

(98) Directing fungal growth as the basis for new building materials

Primary supervisor: Carole C. Perry

Second supervisor: Dr Matthias Brock

Institution: University of Nottingham

School: School of Life Science

Lab rotation description:

The student will be trained in anaerobic microbiology, and will screen isolates for their ability to ferment a limited range of different amino acids/proteins. They will have the opportunity to undertake metabolomic analyses, and to learn how to generate and analyse whole-genome sequence data. Depending on progress, they will also have the opportunity to undertake some transcriptomic analyses.

Full PhD project description:

The human gut microbiota represents a diverse community of bacteria, archaea, viruses and fungi. The collective genome of the gut microbiota (the 'metagenome') encodes 150 times more genes than the human genome. Enzymes encoded by the metagenome allow gut bacteria to use dietary substrates that escape digestion by human-encoded enzymes in the gastrointestinal tract. Metabolites produced as a result of these microbial processes act on intestinal cells, or are taken up into the blood via the hepatic portal vein and transported around the body. The interaction of microbial metabolites and host cells – the so-called microbial–mammalian metabolic axis – contributes to homeostasis of the human system. Disruption of homeostasis and the gut microbiota is linked with a range of metabolic diseases (obesity, non-alcoholic fatty liver disease, atherosclerosis, type 2 diabetes), inflammatory bowel disease (Crohn's, ulcerative colitis) and neurodegenerative diseases (Alzheimer's disease, Parkinson's disease).We only know the functions of approximately 30 % of the genes that contribute to the metagenome. While much is known about the bacteria responsible for fermenting carbohydrates in the gastrointestinal tract, little is known about the microbes that use proteins, peptides and amino acids in this environment. A diverse range of products (essential amino acids, phenolic compounds, amines, ammonia, short-chain fatty acids, branched-chain fatty acids, gases, sulfides) is produced as a result of microbial-driven synthesis or proteolysis. All these metabolites have the potential to influence host systems in beneficial or detrimental ways. For example, the microbiota of the small intestine contributes 8–17 % and 5–21 %, respectively, of the essential amino acids lysine and threonine found in the bloodstream. Conversely, circulating phenyl acetate produced via bacterial breakdown of phenylalanine contributes to lipid accumulation in the liver of individuals with non-alcoholic fatty liver disease. The aim of this project is to characterize proteolysis in a range of anaerobic gut bacteria. Understanding which gut bacteria are involved in proteolysis will allow us to develop targeted interventions to promote human health.

References to learn more:

1. Ashraf, S., Radhi, M.*, Gowler, P.*, Burston, J.J., Gandhi, R.D.*, Thorn, G.J., Piccinini A.M., Walsh, D.A., Chapman, V., De Moor, C.H. (2019) The polyadenylation inhibitor cordycepin

reduces pain, inflammation and joint pathology in rodent models of osteoarthritis. Scientific Reports, 9, 4696. https://www.nature.com/articles/s41598-019-41140-1

2. Spatiotemporal regulation of GSK3beta levels by miR-26a controls axon development in cortical neurons. Lucci C*, Mesquita-Ribeiro R*, Rathbone A, Dajas-Bailador F. Development 2020 (in press)

Location of lab rotation: University Park

Location of full PhD project: University Park

(99) The importance of a novel nucleoside rescue pathway for fresh versus processed food

Primary supervisor: Pavel Gershkovich

Second supervisor: Cornelia H. de Moor

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

Nucleosides and nucleotides are essential components of cells, with especially adenosine triphosphate (ATP) playing a large role in energy metabolism and signal transduction as well as being a building block for RNA. ATP is abundant in fresh foods but is degraded to compounds such as hypoxanthine during processing. This has not been thought to affect the nutritional value, as it was assumed that a similar degradation takes place during normal digestion and metabolism. However recent research in our laboratories has shown that an analogue of the intermediate metabolite inosine can be re-converted into the ATP analogue in immune cells. This indicates that some cell types have the enzymes to utilise inosine to make ATP and suggests that these cells could be affected by a diet lacking inosine or its precursors. In this rotation, you will investigate the adenosine metabolism in different tissue culture cells to determine its tissue specificity and investigate if inosine indeed has specific effects on immune responses. You will determine the levels of ATP and inosine in fresh and processed foods and determine inosine levels in blood. You will use cell culture, metabolite analysis with mass spectrometry (metabolomics) and gene expression analysis to investigate the role of inosine in ATP synthesis and the immune response.

Full PhD project description:

Population studies suggest that fresh food consumption improves general health, yet it is not entirely clear what components are critical or which processes are affected. Our recent data indicate that ATP can sometimes be made from inosine precursors, which is a biochemical pathway that had not been previously detected in mammalians. ATP is quite unstable and is rapidly degraded during food processing to the ribose sugar and the hypoxanthine base, which was also thought to be the fate of ATP during the digestion of fresh food. An intermediate that can be formed from ATP during digestion is the nucleoside inosine. We hypothesise that:1. Particular cell types in the body depend this pathway for optimal ATP production and 2. Fresh food gives rise to high circulating inosine, promoting the normal function of cells and organs through his pathway, in contrast to processed food. The project falls into four sections:I. Identifying the cells and organs that can use inosine for ATP production. Using mass labelled and radioactive adenosine or inosine in animals, you will identify the cells and tissues that can make ATP from inosine. You will use scintillation counting, metabolite analysis (chromatography and mass spectrometry) as well as mass-spectrometry imaging to this end.II. Identifying the enzymes required for the conversion of inosine to ATP by siRNA knockdown in tissue culture, followed by metabolite analysis.III. Characterising the responses of relevant human or animal primary cells cultured in the presence or absence of inosine and after knockdown of the enzymes required for the inosine to ATP pathway.IV. Determine ATP metabolites in fresh and processed food and in the blood of animals and/or people fed these foods. You achieve this by doing animal experiments and metabolite analysis.This work will increase our understanding

of the cell type specific metabolism of inosine and ATP. The work may ultimately lead to improved food production and nutritional guidelines.

References to learn more:

1. Ashraf, S., Radhi, M.*, Gowler, P.*, Burston, J.J., Gandhi, R.D.*, Thorn, G.J., Piccinini A.M., Walsh, D.A., Chapman, V., De Moor, C.H. (2019) The polyadenylation inhibitor cordycepin reduces pain, inflammation and joint pathology in rodent models of osteoarthritis. Scientific Reports, 9, 4696. https://www.nature.com/articles/s41598-019-41140-1
2. Lee, J.B.*, Radhi, M.*, Cipolla, E.*, Gandhi, R.D.*, Sarmad, S., Zgair, A., Kim, T.H., Feng, W.*, Qin, C., Adrower, C., Ortori, C.A., Barrett, D.A., Kagan, L., Fisher, P.M., De Moor, C.H., Gershkovich, P. (2019) A novel nucleoside rescue metabolic pathway may be responsible for the therapeutic effect of orally administered cordycepin. Sci. Rep., 9, 15760. doi: 10.1038/s41598-019-52254-x https://www.nature.com/articles/s41598-019-52254-x.pdf

Location of lab rotation: University Park

Location of full PhD project: University Park

(100) Selective editing of cellular protein degradation to target cancer cells

Primary supervisor: Ingrid Dreveny

Second supervisor: Lodewijk Dekker

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

In the lab rotation you will receive training in contemporary protein biochemistry and structural biology approaches and learn how to apply these to the identification of enzyme inhibitors of ubiquitin-mediated processes. The rotation will consist of the following parts:

• Recombinantly express and purify a ubiquitin specific protease (USP)

• Use engineered fluorescent substrates to test the activity and inhibition of a USP

• Conduct binding assays and initial structural characterisation of an inhibitor complex using crystallisations.

Full PhD project description:

Manipulation of a cell's proteome by selectively increasing the longevity of beneficial proteins or removing aberrant or undesirable proteins is highly attractive in basic research, therapy and biotechnology applications. This strategy offers advantages over gene silencing and genome editing techniques in that it impacts directly on the protein levels potentially enhancing precision. In eukaryotic cells, the ubiquitin proteasome system regulates selective protein degradation. Therefore, interference with the ubiquitin system offers valuable avenues for modulating cellular protein clearance and in consequence protein levels. Ubiquitin modification of a substrate protein is reversible, whereby ubiquitin specific proteases (USPs), a class of deubiquitinating enzymes, can salvage proteins from destruction by the proteasome by removing the ubiquitin tag. These cysteine proteases are often dysregulated in cancer and also play a role in neurodegenerative disorders and the host's response to infection. The main aim of this project is the identification and evaluation of novel ubiquitin system modulators that interfere with ubiquitin-mediated protein degradation.To this end we will further develop our (unpublished) approach of using engineered fluorescent ubiquitin variants to test ubiquitin specific protease activity in vitro. We will then use this approach to identify novel USP inhibitors by screening an in-house compound collection in high-throughput assays.As a target we will focus on USP15, a multi-functional protease that has been shown to play a regulatory role in transforming growth factor β (TGF-β) signalling that regulates cell growth, differentiation and apoptosis. In cancer TGF-β signalling promotes tumour development during the advanced stages of tumorigenesis, but induces cell-cycle arrest and consequently tumour suppression in the early stages. For example, high expression of USP15 correlates with certain glioblastoma, breast and ovarian cancers. In addition, USP15 suppresses mitophagy, which in turn is related with Parkinson disease and other dysfunctional mitochondria diseases associated with ageing. USP15 also influences the inflammatory response. We have recently solved the crystal structure of the USP15 N-terminal and catalytic domains. The known crystallisation conditions for

the protein will facilitate the determination of structures of USP15-inhibtor complexes to gain insight into the interactions and in combination with binding assays investigate the specificity of identified inhibitors with regards to related USPs.We will then test the top scoring inhibitors in cellular assays focusing on the impact on viability and protein levels of USP15 substrates such as the E3 ubiquitin ligase SMURF2 that targets the TGF-β receptor (TβR) complex for ubiquitin-mediated degradation and the tumour suppressor p53.Together, the project will offer skill development in an interdisciplinary setting including protein engineering, fluorescent assays, structural biology and cellular assays and will deliver novel insights into USP activity, structure and inhibition and create tools for the manipulation of protein degradation to ultimately target cancer cells.

References to learn more:

1. Ward, S. J., Gratton, H. E., Indrayudha, P., Michavila, C., Mukhopadhyay, R., Maurer, S. K., Caulton, S. G., Emsley, J. and Dreveny, I. (2018) The structure of the deubiquitinase USP15 reveals a misaligned catalytic triad and an open ubiquitin-binding channel. J Biol Chem. 293, 17362-17374
2. Moon, S. and Lee, B. H. (2018) Chemically Induced Cellular Proteolysis. Mol Cells. 41, 933-942

Location of lab rotation: University Park

Location of full PhD project: University Park

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

Primary supervisor: Samantha Bryan

Second supervisor: Begum Tokay

Institution: University of Nottingham

School: Engineering

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.

Full PhD 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 CO2 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 CO2 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 CO2 challenge. This project aims to develop a carbon sequestration and conversion platform utilizing metal organic frameworks (MOFs) to capture, concentrate and release CO2 directly to cyanobacteria, which will then convert the CO2 to high value products. Metal organic frameworks (MOFs) are adsorbent materials that have already been utilised for the selective capture of CO2 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 CO2 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 CO2, fixing 1.83 kg of CO2 per 1 kg of biomass. Therefore, they are excellent chassis for carbon capture, sequestering CO2 into high value by products.The primary objectives of the PhD project are;

1. Design and evaluate several different MOFS to assess CO2 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 CO2 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.

References to learn more:

1. R. Ricco, C. Pfeiffer, K. Sumida, C.J. Sumby, P. Falcaro, S. Furukawa, N.R. Champness and C.J. Doonan, CrystEngComm, 2016, 18, 6532–6542.2.
2. A. Kumar, C. Hua, D. G. Madden, D. O'Nolan, K.-J. Chen, L.-A. J. Keane, J. J. Perry and M. J. Zaworotko, Chem. Commun., 2017, 53, 5946–5949.

Location of lab rotation: University Park;Sutton Bonington Campus

Location of full PhD project: University Park

This is a linked project – we recommend you select either project 66 or project 107 alongside this one.

(103) Evolutionary and population genomics of adaptation of a small plant with a big future!

Primary supervisor: Levi Yant

Second supervisor: Matt Loose

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

Here you can gain experience in genome bioinformatics, physiology, ecology, and evolutionary biology working with a broadly adaptable candidate for synthetic biology and future agriculture, spirodela polyrhiza (duckweed).Primary rotation aims include Next Generation Sequencing (NGS) library preparation and analysis that will be incorporated into a PhD, including both RNAseq and whole genome resequencing of large numbers of individuals to perform demographic analysis and genome scans for selection (GWAS). Firstly, you'll have the opportunity to phenotype, isolate DNA and generate Illumina NGS libraries of a panel of duckweeds. After local sequencing (no time lag) you'll learn to analyse whole genome data, processing it through established pipelines in my lab (learning basic Python to run our pipeline along the way: there is no need to know coding ahead of time; just have a desire to learn and you are set!). Finally, you can run demographic analyses and selection scans on the data. This is feasible in the rotation period, as we have well established pipelines in place as well as ample support to help you in the Yant Lab and the Future Food Beacon.

Full PhD project description:

There's an urgent and growing worldwide need for simplified plant systems for food production and synthetic biology applications. Accordingly, there's been increasing interest in duckweeds, eminently tractable little plants that get big quickly, with doubling times of as little as 30 hours, and untapped worldwide diversity. We will harness duckweed's synthetic biology potential, focussing on their small, fully sequenced 150 megabase genomes (>21x smaller than ours!), and amenability to genetic manipulation. Applications include remediation of polluted water and use as a food resource, especially in developing nations where most needed. Duckweed aquaculture fits into systems managed by small farmers or commercial scales, not requiring arable land and has the ability to remediate wastewater, minimizing fertilization requirements. Amazingly, managed duckweed cultures yield up to 30 tons dry weight/ha/year, yielding up to 43% protein (with a better amino acids profile than most other sources). Additionally, duckweed has a high concentration of trace minerals, K, P and pigments, particularly carotene and vitamins A and B. However, much can be done to optimize the use of duckweed, as well as to leverage this system for fundamental studies in the evolutionary genomics of adaptation, as different duckweeds show striking variation in the relative accumulation of many essential nutrients. This project merges the population genomics of adaptation and elemental accumulation ('ionomics') to explore the genomic basis of adaptation in duckweeds and to harness their genomic potential. Working with us, you will do fundamental and applied science on duckweed diversity to understand the genomic basis of adaptations to various environments, from rainforests to tundra. Aim 1. Characterise duckweed adaptation to extreme environmental conditions. This includes phenotyping hundreds of accessions in a novel high-

throughput robotic phenotyping platform and collecting duckweed from across Europe and Africa, if you enjoy travel (we do a lot of field work; it's great fun, but not required). Phenotyping approaches multi-elemental 'ionomics' and growth parameters in diverse growth conditions using automated high-tech phenotyping platforms using machine learning in collaboration with several groups in Biosciences (We are embedded in the Future Food Beacon, but also in the COMgen division of Life Sciences.). These data will feed into Aim 2.Aim 2. Population genomics of adaptation in duckweeds. What is the genomic basis for the ionomic, morphological, and growth variation in duckweeds we observe? What genomic alleles mediate these adaptations and what are the precise genomic changes that functionally underlie adaptations? What is the effect of whole genome duplication on all these phenotypes? Can we use genome duplication as an engineering tool for making new duckweeds? We have a quality reference genome and established pipelines for state-of-the-art bioinformatic analyses, as well as easy, high-throughput NGS library protocols in robotics systems for resequencing of thousands of genomes. We begin by sequencing the most phenotypically extreme ~500 genomes for demographic analysis and selection scans and assays for gene flow and hybridisation. This will produce candidate alleles for functional follow-up studies in the last phase of the thesis.

References to learn more:

An, D., Li, C., Zhou, Y., Wu, Y. & Wang, W. Genomes and transcriptomes of duckweeds. Front. Chem. 6, (2018). https://doi.org/10.3389/fchem.2018.00230

Baduel, P., Bray, S., Vallejo-Marin, M., Kolář, F. & Yant, L. The 'Polyploid Hop': Shifting challenges and opportunities over the evolutionary lifespan of genome duplications. Front. Ecol. Evol. 6, (2018). https://doi.org/10.3389/fevo.2018.00117

Location of lab rotation: University Park;Sutton Bonington Campus

Location of full PhD project: University Park;Sutton Bonington Campus

(104) Investigating the use of Bdellovibrio bacteriovorus as a 'living antibiotic' to control Salmonella in pigs

Primary supervisor: Laura Hobley

Second supervisor: Robert Atterbury

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

Foodborne Salmonella spp. infections are a major burden to global human health, a major source of these infections is from contaminated pork products. Salmonella is also a pathogen of pigs, and thus a non-antibiotic treatment to reduce the burden of Salmonella infection in pigs will be beneficial to animal health and safe food production. The predatory bacterium Bdellovibrio bacteriovorus is proposed as such a treatment. The rotation project will begin to investigate the potential of Bdellovibrio for use against porcine-derived Salmonella. The main aims of the rotation project are:1) Test the in vitro efficiency of Bdellovibrio predation on a range of Salmonella spp. (all isolated from infected pigs), to determine the range of Salmonella spp. that are susceptible to Bdellovibrio predation. 2) To correlate the results with genomic data about the Salmonella strains, along with experimentally-derived information regarding prey motility, size and burst size of progeny Bdellovibrio. This will illuminate prey-factors that may play a factor in the outcome of any Bdellovibrio therapeutic uses.The rotation will provide training in maintenance and analysis of predatory Bdellovibrio, including predation assays, microscopy as well as bioinformatics analyses of Salmonella genome sequences. These are essential skills for the proposed subsequent PhD project.

Full PhD project description:

Globally, non-typhoidal Salmonella spp. is responsible for 93 million human infections perannum, ~27% due to consuming contaminated pork. Salmonella is also a pathogen of pigs, and costs the EU pig industry ~€600 million per annum. Multidrug resistant (MDR) strains of Salmonella are increasingly responsible for infections in pigs, and are associated with higher morbidity and mortality in humans1. Alternative approaches to antibiotics are desperately needed. One such approach is the use of the predatory bacterium Bdellovibrio bacteriovorus.Bdellovibrio preys upon a range of Gram-negative bacterial pathogensincluding Salmonella spp.. We previously demonstrated that Bdellovibrio can significantly reduce Salmonella Enteritidis in the caeca of chickens; accompanied by improvements in clinical symptoms2. Subsequently, Bdellovibrio has been applied to treat infections caused by Yersinia3, Shigella4, Klebsiella5 and Vibrio6. Bdellovibrio has advantages over antibiotics: they are self replicating and self-limiting; replicating only within susceptible bacteria; and resistance is a transient event linked to phenotypic plasticity7. Bdellovibrio does not harm animals during therapeutic trials8, at worst eliciting a mild and temporary inflammation9. Bdellovibrio is present, at least transiently, in the intestinal tracts of animals and humans10,11; we have evidence that Bdellovibrio can be isolated from the intestinal tract of pigs.The aim of this project is to move towards applying Bdellovibrio therapeutically in pigs: by isolating Bdellovibrio from the intestine of pigs, characterising these bacteria with respect to prey preference and determining their survival and predatory efficiency against Salmonella in ex-vivo models of different pig intestinal

compartments. In addition, the delivery of Bdellovibrio into the intestinal tract will be optimised by testing a range of micro- and nano-encapsulation techniques.Main objectives:1. Isolation of Bdellovibrio from different gut compartments of pig intestine samples (obtained from commercial and on-site abattoirs). 2. Genetic and phenotypic analysis of Bdellovibrio isolates, including prey range and predation efficiency on a range of porcine-derived Salmonella spp.. 3. Testing Bdellovibrio predation on Salmonella in ex-vivo gut models. Also analysis of Bdellovibrio preparation methods including micro and nano-encapsulation to optimise Bdellovibrio delivery.4. Determining the effect of Bdellovibrio on the microbiota of the pig intestine by metagenomics analyses before and after Bdellovibrio treatment.This project will broaden our knowledge about Bdellovibrio's ability to colonise themammalian intestine, and affect target pathogenic species in a complex environment. It will allow us to further explore the effect of Bdellovibrio on the intestinal microbiota of pigs and help us to optimise Bdellovibrio preparations for use in an in vivo therapeutic trial (outside the scope of this project). Given the increasing presence of multi-drug resistant Salmonella in both pigs and humans, this project may lead to a new, alternative treatment for such infections which may be recalcitrant to conventional antibiotic chemotherapy.References1.Parisi, Foodborne Pathog. Dis. (2018).doi:10.1089/fpd.2017.2403; 2.Atterbury, Appl Env Microbiol (2011)77,5794–5803; 3.Russo, Microorganisms (2018).doi:10.3390/microorganisms7010002; 4.Willis, Curr. Biol. (2016).doi:10.1016/j.cub.2016.09.067; 5.Shatzkes, MBio (2016).doi:10.1128/mBio.01847-16; 6.Li, Int. J. Food Microbiol. (2011).doi:10.1016/j.ijfoodmicro.2011.07.036; 7.Shemesh, Environ. Microbiol. (2004). doi:10.1046/j.1462-2920.2003.00530.x; 8.Westergaard, Appl Env Microbiol (1977) 34,506–511; 9.Shatzkes, Sci. Rep. (2015). doi:10.1038/srep12899; 10.Schwudke, Syst. Appl. Microbiol. (2001).doi:10.1078/0723-2020-00042; 11.Iebba, PLoS One (2013).doi:10.1371/journal.pone.0061608.

References to learn more:

1. R.J. Atterbury, L. Hobley, R. Till, C. Lambert, M.J. Capeness, T.R. Lerner, A.K. Fenton, P. Barrow, R.E. Sockett. (2011) "Studying the effects of orally administered Bdellovibrio bacteriovorus upon the well-being and caecal microbiota of poultry". Applied and Environmental Microbiology 77(16):5794-803D.
2. Negus, C. Moore, M. Baker, D. Raghunathan, J. Tyson, R.E. Sockett. (2017) "Predator Versus Pathogen: How Does Predatory Bdellovibrio bacteriovorus Interface with the Challenges of Killing Gram-Negative Pathogens in a Host Setting?" Annual reviews of Microbiology 71:441-457

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(105) PARP-1 roles in age-related skeletal muscle metabolic decline

Primary supervisor: Craig L. Doig

Second supervisor: Paul Greenhaff

Institution: Nottingham Trent University

School: School of Science and Technology

Lab rotation description:

The Doig laboratory work on two distinct molecules that likely have overlapping physiological actions. These are; the glucocorticoid known as cortisol and the essential coenzyme Nicotinamide Adenine Dinucleotide (NAD+). Existing evidence indicates tissue NAD+ levels decrease during chronic ill health and as part of the ageing process. In parallel, chronic excess exposure to glucocorticoids (endogenous or exogenously driven) is a key driver of tissue atrophy and reduced metabolic flux. This laboratory rotation project will conduct LC-MS based measurement of the post-translational modification ADP-ribosylation made by the cells major NAD+ consuming enzyme PARP-1 (poly(ADP-ribose) polymerase-1). The 9 week project will use an existing archive of skeletal muscle tissue from aged and juvenile mice exposed to chronic glucocorticoid excess or vehicle controls. Currently, the protein targets of PARP-1 mediated ADP-ribosylation are poorly defined, as are the impact of ageing and sustained glucocorticoid treatment upon PARP-1 actions. Identification the skeletal ADP-ribosyl-ome in ageing along with delineation of the impacts of glucocorticoid excess will provide important knowledge for the future treatment of age-related diseases. The data from this project will be used to underpin future translational work on human health and skeletal muscle mass maintenance.

Full PhD project description:

Background: A progressive loss of skeletal muscle mass and functional capacity occurs throughout adult life and is exacerbated by age-related chronic non-communicable diseases. Despite widespread evidence, and decades of work revealing the molecular mechanisms determining skeletal muscle maintenance, identification of a pharmacologic treatment to preserve muscle function and prevent skeletal muscle loss during ageing remains an urgent unmet need. ADP-Ribosylation is a post-translational modification applied by the NAD+ (Nicotinamide Adenine Dinucleotide)-dependent PARP enzymes to proteins modifying their biological activity. With a key role in the DNA Damage Repair Response the ability of PARP-1 to interact with the genome has been extensively studied. However, recently, definitive biological roles for site specific PARP-1 mediated ADP-Ribosylation upon proteins with transcriptional and translational capacity have been revealed. Identification of these mechanisms within skeletal muscle is yet to be conducted. Additionally, characterisation and study of PARP-1 mediated ADP-Ribosylation within the contexts of aging and chronic disease are unknown. Significance: Despite a rising lifespan, human 'healthspan' fails to achieve the same trajectory. Observed during aging and chronic illness is a progressive loss of skeletal muscle mass and decline in metabolic function. Despite widespread evidence exhibiting this, pharmacologic treatments to preserve muscle function with age remain elusive. Research Hypothesis: PARP-1 mediated ADP-ribosylation of proteins is fundamental to skeletal muscle function and whole body metabolic health. The project will use in vitro molecular biology (qPCR, proteomics & CRISPR) and metabolic measurement (High-Resolution Respirometry of mitochondria

and radiolabelled glucose metabolic tracing) techniques to study the placement of ADP-ribosylation on specific proteins and better understand the biological implications of these marks. Existing work from our lab shows that PARP-1 may be important to skeletal muscle architecture and a potential marker of muscle mass maintenance. Abrogation of PARP-1 through chemical inhibition or CRISPR-CAS9 deletion has demonstrated profound changes in satellite cell mediated myogenesis (muscle regeneration) and genes critical in metabolic adaptation to physiological stress. The PhD project will explore these impacts in further detail, elucidating the implicated signalling pathways. Additionally, this project will utilise human skeletal muscle taken from humans of varying age from the Translational Human Research Lab of Professor Paul Greenhaff (University of Nottingham).

References to learn more:

Alemasova & Lavrik. Poly(ADP-ribosyl)ation by PARP1:reaction mechanism and regulatory Proteins. Nucleic Acids Research 2019.

Ryu et al. Metabolic regulation of transcription through compartmentalized NAD+ biosynthesis. Science 2018.

Location of lab rotation: Clifton Campus

Location of full PhD project: Clifton Campus

(106) Why does the human retina have a cone-enriched rim?

Primary supervisor: Timothy Ledgeway

Second supervisor: Paul McGraw

Institution: University of Nottingham

School: Psychology

Lab rotation description:

Many visual tasks, such as the resolution of a letter, show a decrease in performance with increasing eccentricity from the fovea. This performance loss results from changes in the density of retinal ganglion cells and is characterized by calculating the parameter E2 – eccentricity where stimulus size must double to match performance at the fovea. Psychophysical estimates of E2 show good agreement with anatomical and physiological measures of cortical magnification. A problem is that these estimates are derived from observations that are limited in the extent of the visual field tested (typically 0-50 deg). Here we will measure the performance change to see if there is any significant deviation from the predicted pattern as we approach the extreme edge of the visual field. The student will become familiar with the use and calibration of large-field visual displays, gain experience in experimental design, programming, visual psychophysical methods, data analysis and visualisation. The student will develop an understanding of how the human retina samples visual space and how this representation is altered in visual cortex.Weeks 1-2: Literature reviewWeeks 2-5: Display calibration and experimental designWeeks 5-7: Data collectionWeeks 7-9: Data analysis and write-up

Full PhD project description:

In primate retina, photoreceptors have a highly distinct geographical distribution that supports the duplex nature of vision. The central retina (fovea) is dominated by retinal cones that are tightly packed forming a foveal mosaic, endowing the central visual field with a high-resolution 'spotlight'. As retinal eccentricity increases, cone density declines dramatically and the number of rod receptors, which are completely absent at the fovea, increases. In the mid-peripheral retina rods outnumber cones by a factor of 25:1, which increases the retina's ability to capture light and endows the retinal periphery with vastly superior light sensitivity. The dominance of rods over cones was thought to extend to the very edge of the sensory retina. However, histological evidence from human retinal tissue has revealed that the ratio of cones to rods rises sharply towards the edge of the retinal surface (ora serrata), and is particularly pronounced in nasal retina. Indeed, this cone-enriched rim at the edge of the retina contains more than three times as many cones as the fovea. At present, the functional role of this rim is unknown, although several theories have been advanced. These include;

1) alerting and orienting:

2) encoding optic-flow from locomotion:

3) estimation of colour illuminants;

4) circadian regulation.

However, there is no convincing empirical data to support any of these hypotheses. Psychophysical experiments at the edge of the visual field are technically challenging and consequently little data currently exist. This behavioural project will use a unique display system to identify the functional correlates of the cone-enriched rim in human vision. Nottingham hosts the NITES (Nottingham Integrated Transport Environment Simulators) facility which has a large, horizontally circular (180 deg.) display (radius 2.5 m). The display is spanned by 3 separate high-resolution digital light processing projectors. The projectors are controlled by rendering software (Sol7) that is used to geometrically warp images for projection to the circular surface and remove luminance artifacts from areas where the projection areas overlap. This system allows us to generate a high resolution continuous large-field display, which can be used to deliver visual stimuli for human psychophysical experiments. This system is unique in the UK. By using this display we will be able to present visual stimuli at the very edge of the visual field, whilst monitoring eye position centrally (via video-based eye tracking). This will allow us to test each of the functional accounts proposed for retinal cone-enriched rim. This is an area of growing interest. It has recently been proposed that the extreme retinal periphery projects to a single morphological class of ganglion cells, unlike the rest of the retina, and the output of these ganglion cells project to specific region in the limbic cortex – termed the prostriata (Yu et al., Current Biology, 2012). This unique physiological arrangement suggests that the extreme retinal periphery serves a particular function not fulfilled by more central retinal structures and signals from this area can rapidly access multiple brain systems.

References to learn more:

1. Yu, H. H., Chaplin, T. A., Davies, A. J., Verma, R., & Rosa, M. G. (2012). A specialized area in limbic cortex for fast analysis of peripheral vision. Current Biology, 22(14), 1351-1357.
2. Mollon, J. D., Regan, B. C., & Bowmaker, J. K. (1998). What is the function of the cone-rich rim of the retina?. Eye, 12(3b), 548.

Location of lab rotation: University Park

Location of full PhD project: University Park

(107) Antimicrobial Films Based on Metal-Organic Framework (MOF)/Biopolymer Composites

Primary supervisor: Begum Tokay

Second supervisor: Andrea Laybourn

Institution: University of Nottingham

School: Engineering

Lab rotation description:

The student will spend time in the BS2 labs becoming familiar with the techniques for rapid analysis of antimicrobial activity of polymers. This will include training in pathogen handling (up to BS level 2) and the culture of different bioluminescent derivatives of representative of Gram-positive and Gram-negative bacteria. Once familiar with these techniques the student will be trained in the use of imaging techniques to quantify bioluminescent output as an indication metabolic activity and the high throughput culture and screening methods used to rapidly evaluate the potential antimicrobial activity of novel antimicrobials. Once compounds of interest have been identified the student will use a range of bacteriological methods, including fluorescent microscopy, to investigate the mode of antimicrobial action and to identify the susceptible sites in the affected bacterial cells. This project will provide the student with experience of the methods to be used as part of the full PhD project.

Full PhD project description:

Background: Mycobacterium tuberculosis and M. bovis are the main causes of tuberculosis (TB) in humans and animals respectively. The World Health Organisation (WHO) reported that 1.5M people (including 251K with HIV) died from TB and 10M fell ill in 2018, the highest of any infectious disease. Increasing occurrences of drug resistance to the drugs used to treat TB include strains showing extreme drug resistance (XDR-TB) have been observed. DEFRA reported that 2,805 herds of cattle in Great Britain tested positive for bovine TB in the 12 months to Sept 2018, with 305K cattle having to be slaughtered in GB over the last decade at the cost of £500M. Methods of treating or eradicating TB are therefore crucial to both human and animal health.Mycobacteria have an unusual and more complex cell wall structure than other Gram-positive bacteria. The exterior surface of the bacterium is formed from a hydrophobic, waxy coat of mycolic acids (~C60 long chain fatty acids) (Alderwick et al. Cold Spring Harb Perspect Med. 2015, 5, a021113) that acts as a pseudo-outer membrane. This sits on a polysaccharide structure, arabinogalactan that contains several rare sugars (galactofuranose/arabinofuranose/rhamnose) which is then attached to the peptidoglycan layer. This ‘stealth’ coat masks the bacteria from its host’s defences and allows them to colonize mammalian tissues and form biofilms without triggering a host response. We can selectively target several enzymes unique to Mycobacteria with inhibitors/drugs with a reduced chance of off-target toxicity. Research Project: This will involve the design, synthesis and evaluation of new compounds as putative inhibitors of selected target enzymes: InhA (an enoyl reductase) involved in mycolic acid biosynthesis; UGM and GlfT2 involved in galactan biosynthesis. There are high resolution X-ray structures available for these enzymes allowing new inhibitor designs to be tested in silico using protein-ligand docking programs including GOLD or OEDocking prior to their synthesis. For InhA, the clinically important mutations that cause resistance are known and new compounds can be docked

against these mutants to test if their binding will be impacted. The majority of TB cases are in the third world, therefore new drugs need to be easy to produce and be orally active to minimise their cost.The research will focus on one/two of the enzymes mentioned above. The student will express the enzyme(s) from vectors available in the group and synthesize their assay substrates as necessary. Initial test compounds will be designed by docking existing small molecule/fragment libraries (size < 500 amu) and selecting the best binders. These will then be synthesized and their activity against the purified enzyme determined. Those compounds with promising inhibitory activity will be tested against whole M. bovis in Dr Rees's laboratory (training will be provided in handling pathogenic organisms) and M. tuberculosis including drug resistant strains in Birmingham. Based on these results, lead generation studies will be undertaken through the synthesis of focused small libraries for QSAR studies. Co-crystallization of the best inhibitors with their target enzymes will be undertaken with Dr Martin Walsh at the Diamond Light Source, Oxfordshire.

References to learn more:

Elangovan et al, Ind. Eng. Chem. Res. 2011, 50, 11136–111422- Li et al, Chemical Engineering Journal 160 (2010) 378–382

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: University Park;Sutton Bonington Campus

This is a linked project – we recommend you select either project 66 or project 101 alongside this one.

(108) Rational, structure-based inhibitor design, synthesis and evaluation as a first step towards the discovery of new anti-tuberculosis drugs

Primary supervisor: Neil R. Thomas

Second supervisor: Cath Rees

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

We are interested in designing, synthesizing and evaluating new inhibitors of Mycobacterial enzymes that have been validated as suitable drug targets due to them being essential to the life cycle of the M. tuberculosis and M. bovis. These include the enzyme, InhA an enoyl reductase involved in the biosynthesis of the mycolic acids that give the bacteria a hydrophobic exterior, masking them from the host's immune system and the enzymes UDP-galactopyranose mutase (UGM) and UDP-galactofuranose transferases (GlfT1/2) that biosynthesize the arabinogalactan layer supporting the mycolic acids which is also essential. The rotation project can have one of several starting points depending on the background and interests of the student:1. In silico docking studies against one of the target enzymes using a library of compounds or fragments preselected for their drug-like properties.2. Synthesis a 'hit' compound identified in an in silico screen/designed based on our understanding of the enzyme mechanism.3. Purification of the target enzyme, synthesis of its substrate(s) and evaluation of the inhibitory properties of putative inhibitors generated previously.4. Evaluation of compounds with significant inhibitory activity against whole Mycobacteria.5. Lead generation: Synthesis of a focused library of compounds to undertake QSAR studies and structural optimisation.

Full PhD project description:

Background: Mycobacterium tuberculosis and M. bovis are the main causes of tuberculosis (TB) in humans and animals respectively. The World Health Organisation (WHO) reported that 1.5M people (including 251K with HIV) died from TB and 10M fell ill in 2018, the highest of any infectious disease. Increasing occurrences of drug resistance to the drugs used to treat TB include strains showing extreme drug resistance (XDR-TB) have been observed. DEFRA reported that 2,805 herds of cattle in Great Britain tested positive for bovine TB in the 12 months to Sept 2018, with 305K cattle having to be slaughtered in GB over the last decade at the cost of £500M. Methods of treating or eradicating TB are therefore crucial to both human and animal health.Mycobacteria have an unusual and more complex cell wall structure than other Gram-positive bacteria. The exterior surface of the bacterium is formed from a hydrophobic, waxy coat of mycolic acids (~C60 long chain fatty acids) (Alderwick et al. Cold Spring Harb Perspect Med. 2015, 5, a021113) that acts as a pseudo-outer membrane. This sits on a polysaccharide structure, arabinogalactan that contains several rare sugars (galactofuranose/arabinofuranose/rhamnose) which is then attached to the peptidoglycan layer. This 'stealth' coat masks the bacteria from its host's defences and allows them to colonize mammalian tissues and form biofilms without triggering a host response. We can selectively target several enzymes unique to Mycobacteria with inhibitors/drugs with a reduced chance of off-target toxicity. Research Project: This will involve the design, synthesis and evaluation of new compounds

as putative inhibitors of selected target enzymes: InhA (an enoyl reductase) involved in mycolic acid biosynthesis; UGM and GlfT2 involved in galactan biosynthesis. There are high resolution X-ray structures available for these enzymes allowing new inhibitor designs to be tested in silico using protein-ligand docking programs including GOLD or OEDocking prior to their synthesis. For InhA, the clinically important mutations that cause resistance are known and new compounds can be docked against these mutants to test if their binding will be impacted. The majority of TB cases are in the third world, therefore new drugs need to be easy to produce and be orally active to minimise their cost.The research will focus on one/two of the enzymes mentioned above. The student will express the enzyme(s) from vectors available in the group and synthesize their assay substrates as necessary. Initial test compounds will be designed by docking existing small molecule/fragment libraries (size < 500 amu) and selecting the best binders. These will then be synthesized and their activity against the purified enzyme determined. Those compounds with promising inhibitory activity will be tested against whole M. bovis in Dr Rees's laboratory (training will be provided in handling pathogenic organisms) and M. tuberculosis including drug resistant strains in Birmingham. Based on these results, lead generation studies will be undertaken through the synthesis of focused small libraries for QSAR studies. Co-crystallization of the best inhibitors with their target enzymes will be undertaken with Dr Martin Walsh at the Diamond Light Source, Oxfordshire.

References to learn more:

1. PARTHA SK, SADEGHI-KHOMAMI, A, CREN, S, ROBINSON RI, WOODWARD, S, SLOWSKI, K, BERAST, L, ZHENG, B, THOMAS, NR and SANDERS, DAR, 2012. Identification of Novel Inhibitors of UDP-Galactopyranose Mutase by Structure-Based Virtual Screening, Mol. Informatics. 30(10), 873-883 (https://doi.org/10.1002/minf.201100085)
2. CAMPANICO, A; MOREIRA, R; LOPES, F. 1018, Drug discovery in tuberculosis. New drug targets and antimycobacterial agents Campanico, Andre; Moreira, Rui; Lopes, Francisca Eur. J. Med. Chem. 150, 525-545 (https://doi.org/10.1016/j.ejmech.2018.03.020)

Location of lab rotation: University Park

Location of full PhD project: University Park;Sutton Bonington Campus;Diamond Light Source

(109) Characterisation of complex bacterial communities and the host response in ovine footrot

Primary supervisor: Sabine Tötemeyer

Second supervisor: Fiona Lovatt

Institution: University of Nottingham

School: Veterinary Medicine and Science

Lab rotation description:

Ovine underrunning footrot results in lameness and is the greatest welfare and economic concern for sheep farmers and veterinarians worldwide. Footrot is caused by the bacterium Dichelobacter nodosus. Current available vaccines have limited efficacy and management is costly and stressful for sheep. We found recently, that in addition to D nodosus, in bacterial communities from footrot samples a number of bacterial genera and species were found at high levels, compared to healthy feet (Maboni et al 2017) and may hence play a role in establishing or exacerbating footrot.In this rotation we aim to use ovine dermal fibroblast tissue culture to develop a stimulation model to study the host response to bacteria other than D nodosus, such as Porphyromonas ssp. Expression of inflammatory mediators such as dermal cytokines, chemokines and metalloproteases will be investigated Methodology will include general cell culture, cell stimulation, cell lysis, RNA isolation, cDNA synthesis, PCR and quantitative PCR as well as data analysis and presentation skills. If time permits, bacterial isolation and/or preparation of tissue sections for imaging can be included.

Full PhD project description:

Ovine underrunning footrot results in lameness and is the greatest welfare and economic concern for sheep farmers and veterinarians worldwide. In England, 95% of sheep flocks have footrot, with a mean daily prevalence of ~10%2 and an estimated cost to the UK sheep industry of £24-£84 million/annum. Footrot is a two-stage disease characterised by interdigital dermatitis (ID) that in the presence of virulent D nodosus progresses to the separation of skin and hoof horn.The commercial multivalent vaccine licensed in the UK contains 9 serogroups of D. nodosus and has limited efficacy (60%) with a maximum duration of protection of 5 months. Its use is currently recommended as a component of integrated management strategies that include individual sheep treatment for footrot (EBLEX, FAI). These management strategies include quarantine of infected animals, foot trimming and use of antibiotics; and are labour intensive and stressful to the sheep. Farmers have identified that an effective vaccine would be an ideal management of footrot. The limited and partial protection of the current vaccine are not fully understood. In contrast, monovalent vaccines induce good immunity and have been used to assist in eradication of footrot when there is only one or two serogroups of D. nodosus present. However, most sheep flocks have several serogroups of D. nodosus thereby limiting vaccine efficacy. We found recently, that in addition to D nodosus, in bacterial communities from footrot samples, four bacterial genera were found at high levels compared to healthy feet: Mycoplasma (20%, $p=0.0009$), Corynebacterium (19%), Psychrobacter (18%) and Treponema (14%, $p<0.0001$) compared to healthy samples (Maboni et al 2017). Similar bacterial populations have also been found in Merino sheep footrot lesions in Australia (McPherson

et al 2019). Overall hypothesis: Bacteria highly prevalent in footrot lesions in addition to D. nodosus contribute to host response and hence may make good vaccine targets.

Aims

(1) To identify bacteria of interest (in addition to Mycoplasma fermentans, Treponema ssp.) by analyzing metagenomic data and the literature

(2) Confirm species of bacteria using species specific PCR

(3) Investigate the host response to the bacteria listed & identified in (1) and (2) by using primary ovine dermal keratinocytes and fibroblastsRNA isolation/cDNA synthesis/qPCR for pro-inflammatory markers as well as ovine cytokine ELISA

(4) Test ovine serum samples from healthy and footrot affected sheep for the presence of antibodies against bacteria listed & identified in (1) and (2) by ELISA

(5) Investigate the host response to the bacteria identified in aims (1-3) in the interdigital skin ex-vivo organ culture (EVOC) model (Maboni et al 2017) in the presence of Il1b to mimic inflammation. Viability of the EVOC model will be determined by histology score and/or TUNEL AssayHost response by ovine cytokine ELISA Quantification of bacterial invasion by qPCR

(6) Development of a Skin equivalent model (3D structured model re-seeded with primary epidermal keratinocytes and/or dermal fibroblasts) and investigate the host response the bacteria listed & identified in (1) and (2)

References to learn more:

1. Maboni, G, Davenport, R, Sessford, K, Baiker, K, Jensen, TK, Blanchard, AM, Wattegedera, S, Entrican, G, Tötemeyer, S, 2017 A novel 3D skin explant model to study anaerobic bacterial infection, Frontiers in Cellular and Infection Microbiology, 7, 404 DOI: 10.3389/fcimb.2017.004042.
2. Maboni, G, Blanchard, A, Frosth, S, Stewart, C, Emes, R and Tötemeyer, S, 2017 A distinct bacterial dysbiosis associated skin inflammation in ovine footrot, Nature Scientific Report 7:45220 DOI: 10.1038/srep45220

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(110) Recruiting functional brain networks to improve sight after stroke

Primary supervisor: Ben Webb

Second supervisor: Denis Schluppeck

Institution: University of Nottingham

School: Psychology

Lab rotation description:

In the UK, about 110,000 people have a stroke every year. One third of stroke survivors are left with sight loss on one side of the visible world. The aim of the rotation will be to identify brain networks that have survived the stroke that have the potential to engender conscious visual perception in the "cortically blind" visual field. To estimate the capacity of residual brain pathways to generate vision, the student will use magnetic resonance (MR) brain imaging to measure the structure and responsiveness of spared cortex in the occipital lobe. Under our supervision and guidance, the student will be trained to:Week 1: Review the neuroscience and vision literature on the visual field deficits after stroke.Week 2: Generate hypotheses on visual field coverage of spared visual cortex after stroke.Weeks 3-4: Design and code visual stimuli in Matlab or python.Week 5: Acquire MR anatomical, diffusion tensor and functional brain scans.Weeks 6-8: Using established analysis pipelines: (i) reconstruct the representation of visual space in different cortical areas within the visual brain; (ii) map regions of the visual field covered by intact visual brain networks. Weeks 8-9: Present results to Stroke Vision Group.

Full PhD project description:

In the UK, approximately 150,000 people a year have a stroke. Twenty to thirty percent of stroke survivors are left with sight loss on one side of the visual field, and have difficulty reading, driving and navigating unfamiliar environments. Currently, there are no effective rehabilitations for treating these visual field scotomas. Perceptual retraining can be effective, but visual improvements tend to be variable across individuals and do not generalise beyond trained stimuli and visual field locations. Sight loss after stroke is caused by injury to visual brain pathways. We therefore need to identify brain networks that survived the stroke and which have the capacity to generate visual perception in the visual field scotoma. The PhD student will develop a multidisciplinary approach that ensures perceptual retraining is targeted at functional visual brain networks that survived the stroke. To stratify the capacity of stroke survivors for visual rehabilitation, they will use anatomical and functional magnetic resonance imaging to estimate cortical tissue in visual brain pathways that responds to visual input. To map the visual field regions covered by functional visual brain networks, they will estimate the location and size of population receptive fields in visual cortex. These estimates of visual field coverage are used to target retraining of visual detection and discrimination at locations inside the scotoma with stimuli that we know elicit responses from visual cortex. To develop this targeted approach for clinical benefit, they will apply this approach to a heterogeneous group of stroke survivors with different patterns of visual and cortical loss.

References to learn more:

1. Melnick, M. D. et al. (2016) Relearning to See in Cortical Blindness. Neuroscientist 22, 199-212.
2. Papanikolaou, A. et al (2014). Population receptive field analysis of the primary visual cortex complements perimetry in patients with homonymous visual field defects. Proceedings of the National Academy of Sciences USA 111, E1656.

Location of lab rotation: University Park

Location of full PhD project: University Park

(111) Harnessing the enzymatic potential of Streptomyces strains for biomass degradation

Primary supervisor: Luisa Ciano

Second supervisor: Samantha Bryan

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

During the 9-week lab rotation, the student will acquire the basic skills necessary to carry out the PhD project. The lab rotation project will focus on deepening our understanding of the interaction between cellulose-active enzymes and their natural substrate to improve current enzymatic cocktails used in biomass degradation. The project will involve the production of known cellulose-active glycoside hydrolases which carry the aminoacid sequence for the formation of a formylglycine handle (to gain skills in protein expression and purification). The enzymes will be tagged with a spin label and/or a fluorescent probe (basic synthetic chemistry skills) and the activity tested (biochemistry assays, mass-spectrometry). The interaction between the enzyme and cellulose will be monitored using spectroscopy (introduction to Electron Paramagnetic Resonance and Fluorescence spectroscopies).

Full PhD project description:

Finding alternatives to fossil fuels is an imperative of modern society. In this context, biomass waste offers a sustainable and renewable source of energy for the production of second-generation biofuels, but polysaccharides are very resistant to degradation. Enzymatic breakdown of biomass is the current method of choice to obtain biofuels and commodity chemicals, as many organisms have evolved a variety of proteins, such as glycoside hydrolases (GHs), carbohydrate binding modules (CBMs) and lytic polysaccharide monooxygenases (LPMOs), which synergistically act on the crystalline substrate to break it down to smaller components. Despite a number of breakthroughs led by both industrial and academic research, which have progressively improved the composition of the enzymatic cocktails used in bioreactors, progress has been slow and much more can still be done to harness the full energetic power stored in polysaccharides. Two main strategies can be envisaged to tackle such a challenge: the discovery of new enzymes able to degrade cellulose and other polysaccharides, and/or the optimisation of enzymatic cocktails via an improved understanding of the enzymatic molecular action on the substrate. The aim of this PhD project is to address such a challenge and develop a method to identify novel carbohydrate-active enzymes in Streptomyces strains, with particular focus to metalloenzymes. Building up from previous studies, the successful candidate will design and synthesise suitable spin/fluorescent probes, which will be inserted on the protein targets by exploiting the formation of a formylglycine handle. This chemical modification is a facile reaction which occurs in vivo by the action of the Formylglycine-Generating Enzyme (FGE), which recognises a specific aminoacid sequence and converts a cysteine residue in this sequence into a formylglycine. The formylglycine, in turn, provides an anchor for the addition of the tag by a facile reaction. As carbohydrate-active enzymes are usually secreted in the medium by the organism which produces them, the enzymes can be followed during the interaction with the substrate by monitoring the fluorescence/paramagnetism given by the tag. The newly identified proteins will be

purified and their interaction with the polysaccharide analysed through a variety of methods, e.g. Electron Paramagnetic Resonance (EPR) spectroscopy. Enzymatic activity and protein-protein interactions will also be subjected to analyses (e.g. biochemical assays, mass-spectrometry, EPR) with the goal of gaining insights into enzymatic polysaccharide degradation. In parallel to these studies, the synthetic method developed for the tags will be adapted to introduce different modifications to the proteins (e.g. artificial catalysts).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, developing a wide variety of Chemistry and Biochemistry skills.

References to learn more:

1. A. Levasseur et al, Biotechnol. Biofuels, 2013, 6, 41, doi: 10.1186/1754-6834-6-412.
2. P. Wu et al, PNAS, 2009, 106, 3000, doi: 10.1073/pnas.0807820106

Location of lab rotation: University Park

Location of full PhD project: University Park

(112) Development of aptamers for the detection of microbial spoilage in the beverage industry

Primary supervisor: Stephen Lawrence

Second supervisor: Chris Powell

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

The student will be trained in general laboratory techniques, including working with yeast and bacteria cultures. They will be expected to complete 2 tasks which will provide skills required for the remainder of the project:1. Selection and characterisation of aptamers for beverage spoilage microorganisms using a cell-SELEX procedure. Following negative selection on a culture yeast, the aptamer library will be incubated with spoilage bacteria (Lactobacillus) / yeast (Brettnanomyces) to select aptamers that specifically recognise the target cell. Multiple rounds of cell-SELEX (a minimum of 5) will be completed.2. Cloning of isolated aptamers into Escherichia coli. Positive clones will be sequenced to identify individual aptamer sequences. Aptamer sequences will be analysed by a number of open access software programmes to determine sequence similarity and to predict secondary structures, which are important for aptamer binding.

Full PhD project description:

Food safety is about ensuring that products provided to the consumer are fit to ingest. This covers the safety of the raw materials used, maintaining a hygienic manufacturing environment and ensuring that the final product is clear of chemicals or microorganisms that could cause harm or impact on quality. The global beer market was valued at £297 billion in 2017, and is projected to reach £688 billion by 2025. A significant proportion of this growth will be driven by developing markets such as Africa and South America. This growth puts increasing pressure on available raw materials and greater demands on the hygiene of brewery plants. The industry is going to face increasing pressure to ensure the food safety of their products. This is further necessitated by the growth of craft breweries, who are less skilled in hygiene and food safety, and the rise in popularity of low and no-alcohol beers which have a greater risk of spoilage. Spoilage of ingredients or in-process products usually only leads to a direct financial loss, the consequences are more severe if it concerns a final product which has reached the market. On top of the costs of lost product and its recall, customer confidence and brand image may be seriously affected.To avoid this, new techniques are needed to optimise quality assurance (QA) in manufacturing plants to ensure the food safety and quality of products. These new techniques should support rapid identification of microorganisms in the raw materials and final product.This project will investigate the potential of a new platform based on aptamers to provide fast, reliable and sensitive identification and quantification of spoilage organisms in brewery samples (wort, beer, CIP). Currently there are no methods available to the industry that allow rapid direct detection and identification of spoilage organisms. This project will provide the technology for the next generation of QA tools.Aptamers are short oligonucleotides, either RNA or DNA, which have been successfully used to specifically target a range of different cell types or molecules. The nucleotide sequence of the aptamers causes it to fold

into a specific three dimensional shape, which allows it to bind to specific targets. As such, aptamers are similar to antibodies, but possess key advantages including size, increased stability, reduce immunogenicity and simple conjugation chemistry. Aptamers can be used in conjunction with a sensor to produce aptasensors which enable detection of a designated target through the measurement of changes in optical, electrochemical or mass properties. Aptamers are selected from a random library of oligonucleotides through a process of systematic evolution of ligands by exponential enrichment (SELEX). The key outcomes of the project: 1. Develop cell-SELEX aptamer selection buffer and method optimised for brewhouse, fermentation and CIP rinse water samples2. Selection and characterisation of aptamers for key brewing spoilage microorganisms3. Conjugation of aptamer with appropriate tag (e.g. fluorescence)4. Assessment of isolated aptamers for their effectiveness for detection of spoilage organisms in industrial samples5. Development of aptasensorThis research will lead to the development of a new technique for rapid identification and quantification of potential spoilage contamination. It will also provide the groundwork to expand this technology from detection to prevention of contamination, reducing food waste.We are using brewing as a model system due to my experience in the industry but this project has practical application to industries which are subject to spoilage during production or in final product including carbonated soft drinks, energy drinks, fruit juice and wine.

References to learn more:

Song et al (2019) Aptamer based detection methodology studies in food safety. Food Analytical Methods. 12: 966-990

Kaur (2018) Recent developments in cell-SELEX technology for aptamer selection. BBA - General Subjects. 1862: 2323-2329

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(113) Multiscale mechanochemical drivers of airway hyper-responsiveness in asthma

Primary supervisor: Bindi Brook

Second supervisor: Simon Johnson

Institution: University of Nottingham

School: Mathematics

Lab rotation description:

The student will construct a computational model of an individual airway smooth muscle cell and its interactions with its environment. This will require extension, and coupling, of models of: (i) dynamic cell-ECM and cell-cell adhesion (Irons et al, 2018); (ii) contractile force generation, incorporating acto- myosin cross-bridge cycling (Brook 2014); (iii) subcellular adaptor protein signalling that links adhesion, contractile force generation and cytoskeletal organization. This will require some understanding of cell mechanics and biophysical models of protein interactions. The computational models and simulations will be developed and carried out using Matlab. The resulting model will be validated against existing AFM data, and have the potential to inform the design of future novel experiments that will be carried out as part of the full PhD project

Full PhD project description:

Background: Despite significant research effort, the genesis of airway hyper- responsiveness (AHR), a cardinal feature of asthma, remains poorly understood (Lutchen, 2016). In particular, it is not clear if AHR is the cause of the disease or a consequence of the asthmatic inflammatory and airway-remodelling response. Key to AHR is airway smooth muscle cell (ASMC) contractile force (An et al, and its transmission through the extracellular matrix (ECM), which itself plays an important regulatory role. Hypothesis:The dynamic microscale mechanochemical interactions between ASMC and ECM are responsible for maintaining normal airway function, and AHR emerges out of perturbations to this finely-tuned homeostasis. Aims: Emergence of AHR has never been investigated as a multiscale phenomenon, in part due to the inherent complexity of the biomechanical and biochemical ASMC- ECM interactions. We will develop innovative in silico models, using sophisticated mathematical approaches that can accommodate features on multiple lengthscales (sub-cellular to whole tissue). This will be guided by data from specifically-tailored, imaging experiments in isolated cells and cell culture (Ballestrem lab), as well as existing extensive in vivo datasets (from our previous MRC grant MR/M004643/1 BSB), to determine the drivers underpinning AHR. The specific aims are: Aim 1. Quantify the contributions of mechanochemical processes mediated by cell- cell and cell-ECM adhesion complexes and associated signalling pathways that regulate transmission of force from internal contractile machinery to the extra-cellular environment. Aim 2. Quantify the effect of biochemical and mechanical stimuli on signalling and ASMC force generation in a multicellular environment in silico, in cell culture and in intact murine precision-cut lung slices (PCLS) to determine whether hyper- responsiveness emerges from these collective interactions. Experimental methods and research plan.The required combination of modelling and experiments will exploit the supervisory team's multidisciplinary expertise, encompassing multiscale modelling in biomechanics and mechanobiology, ASMC imaging, cell biology and intact lung slices, supporting the student to pursue the following research plan:

1. Construct a computational model of the individual ASMC and its interactions with its environment by extending models that describe: (i) dynamic cell-ECM and (ii) acto-myosin-mediated force generation, coupled via adaptor protein signalling (in particular vinculin, talin and integrins that form focal adhesions) between cell and ECM. These coupled models will enable prediction of the combined effect of internal force generation and external mechanical stimuli on individual cell-ECM interactions.

2. Couple virtual ASMCs and ECM within a vertex-based multicellular computational model to investigate emergence of hyperresponsiveness in a multicellular context. Macroscopic constitutive laws that describe ASMC and ECM mechanics will be obtained via computational or asymptotic 'coarse-graining' approaches. These models will be validated by in vitro measurements on ASMC monolayers using AFM combined with photo activation methods and TFM.

3. Through an MRC-funded study, BSB, RDO and SRJ have generated a large dataset with unprecedented quantitative detail, characterizing the structural and functional changes occurring during murine airway remodelling in vivo. This existing data set will be integrated into models developed in 2, providing realistic computational models of the intact airway, and thereby identify factors contributing to the transition to AHR.

References to learn more:

1. Lutchen. Airway smooth muscle stretch and airway hyperresponsiveness in asthma: have we chased the wrong horse? J Appl Physiol 116: 1113–1115 (2014)
2. An et al. Airway smooth muscle dynamics: a common pathway of airway obstruction in asthma. Eur Respir J. 29(5):834-60 (2007)

Location of lab rotation: University Park

Location of full PhD project: University Park

(114) Rooting Deep: New strategies for improved rooting in subsoils

Primary supervisor: Jonathan Atkinson

Second supervisor: William Whalley

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

The student will conduct a pilot study following on from research conducted in Atkinson et al., (2019). Soil will be sampled from the UoN experimental farm, dried and repacked it into small soil columns at 4 bulk densities representative of field topsoil and subsoils. Artificial macropores will then be created in these columns of varying diameters using a 3D printed jig. The student will then quantify wheat root responses to the artificial macropores using X-ray Computed Tomography (CT) (training by Atkinson). This experiment has the potential to generate a stand-alone research paper.

Full PhD project description:

Wheat is a crop of global importance accounting for around 20% of global calorie consumption. Roots are a key for water and nutrient uptake, and thus have a direct impact on wheat yields. Despite this, our understanding of crop roots still lags behind aerial tissues, due to inherent difficulties in studying them and the complex relationship between roots and soil. Deep roots are beneficial for accessing water deeper in the subsoil, conferring higher yields and resistance to drought in many scenarios. Ideotypes such as steep root growth angle or growth rate are often cited, but these fail to account for the relationship between roots and soil, and the impact of compaction in the subsoil.This project will study the interactions between wheat roots and macropores in subsoils. We hypothesise that wheat roots cannot pass through subsoil levels of compaction, and thus utilise macropores as a means to bypass compact layers to explore deeper into the soil profile. There is evidence in some crop species that roots not only utilise macropores, but actively seek them out through a process termed trematotropism. The environmental signal behind this phenomenon is still unknown but is likely under genetic control, and thus has potential utility as a breeding target to generate higher yielding or drought resistant wheat varieties.This project will aim to:1) Quantify the specific soil conditions in which wheat roots colonise macropores (ie compaction level, pore size, etc). 2) Establish if wheat roots actively seek out macropores in subsoils (trematotropism), and if so, what is the signal driving this tropism. 3) Quantify the potential yield gains in wheat varieties able to better colonise subsoil macropores. 4) Establish the possibility of utilising root-macropore interactions as a target for breeding more resilient wheat varieties; is trematotropism a heritable trait?

References to learn more:

Atkinson JA, Hawkesford MJ, Whalley WR, Zhou H, Mooney SJ (2019) Soil strength influences wheat root interactions with soil macropores. Plant, Cell and Environment. DOI: 10.1111/pce.136592. C

olombi T., Braun S., Keller T., & Walter A. (2017) Artificial macropores attract crop roots and enhance plant productivity on compacted soils. Science of the Total Environment. 574, 1283–1293. http://dx.doi.org/ 10.1016/j.scitotenv.2016.07.194

Location of lab rotation: Clifton Campus

Location of full PhD project: Clifton Campus

This is a linked project – we recommend you select project 13 alongside this one.

(116) The role of monoamine oxidases (MAOs) in selective vulnerability of brain cells - implications for healthy ageing

Primary supervisor: Aslihan Ugun-Klusek

Second supervisor: Rob Layfield

Institution: Nottingham Trent University

School: School of Science and Technology

Lab rotation description:

Brain function relies on neurons communicating with and modulating each other. Extracellular vesicles (EVs) and their cargo are known to facilitate intercellular communication between cells. In vitro studies suggest neuron derived EVs can be taken up by other neurons and glial cells and could modify signal transduction and protein expression in receiving cells. This rotation project will use MAO-A overexpressing human SH-SY5Y neuroblastoma cells and will explore the role of MAO-A levels/activity on the proteomic cargo of EVs. It will introduce you to a range of techniques that will be essential for the project.Week 1: Laboratory inductions and introduction to mammalian cell cultureWeek 2: Cell culture and microscopyWeeks 3&4: Preparation and characterisation of EVs (marker proteins with Western blotting, size analysis with ZetaView particle analyser) Weeks 5-7: Sample preparation for proteomics analysis. MAO-A overexpressing and mock transfected control cells will be incubated with an amine substrate to stimulate MAO activity. MAO inhibitor treated cells (prior to amine substrate stimulation) will be used as additional controls. EVs will be isolated and tryptic digests will be prepared for analysis of their proteomic cargo Weeks 8 &9: Data analysis (MetaCore pathway analysis) and report write-up

Full PhD project description:

Ageing is a complex process involving a multitude of contributing factors; however, alterations in mitochondrial function are considered as one of the main drivers. Mitochondria have key roles in metabolism, calcium buffering and redox/protein homeostasis, controlling overall cellular health. Brain, especially neurons are highly susceptible to mitochondrial dysfunction and disrupted redox homeostasis. Mitochondrial function differs in different tissues and recent research suggests cell-type specific regulation of the mitochondrial proteome even within the same tissue. This diversity allows mitochondria to contribute to unique functions of each cell but may also contribute to selective vulnerability during ageing and age-related diseases.Accumulating data support that protein oxidation, build-up of oxidised proteins accompanied by the impairment of the protein degradation pathways (ubiquitin-proteasome system (UPS), autophagy (including mitochondrial-autophagy (mitophagy)) plays a major role in ageing. The electron transport chain (ETC) is considered the main source of reactive oxygen species (ROS) in the mitochondria but ROS production also occurs outside the ETC by monoamine oxidases (MAOs). MAOs are mitochondrial (outer membrane) enzymes that catalyse the oxidative deamination of amines (including dopamine, serotonin), producing hydrogen peroxide and aldehydes as by-products. There are two isoforms, MAO-A and MAO-B. In the human brain, MAO-A is located largely to neurons whereas MAO-B is localised in glial cells. Our recent work was first to demonstrate that sustained ROS production by MAO-A in neuronal cells initiates mitochondrial fragmentation, activates autophagy and promotes

mitochondrial clearance via mitophagy to maintain viability. The effects of increased MAO-A levels however depend on availability of amine substrates and when substrates are in excess, cell viability is reduced. A variety of cellular stressors including ETC complex inhibition are known to induce MAO levels/activity but cell-type specific effects of a sustained increase in MAO levels and MAO generated ROS/aldehydes have not been investigated. Using neuronal and glial cell models, this project will test the hypothesis that MAO-A is an important mitochondrial sensor/regulator, which through its catalytic by-products modulates mitochondrial function, protein and mitochondrial clearance and contributes to selective vulnerability of different brain cells. This will be achieved through the following:

• Generate MAO-A overexpressing cells from different lineages, this will include LUHMES (to examine effects on differentiated neurons), glial cells (A-172) and non-neuronal cells as controls. Overexpression of catalytically inactive MAO will be an additional control.

• Develop and optimise reporter assays to monitor MAO generated ROS/aldehydes. Real-time monitoring of aldehydes in cells will require development of novel techniques using fluorescent probes.

• Investigate mechanisms/effects of MAO generated ROS/aldehydes (in the presence/absence of exogenous amine substrate) on mitochondrial function, calcium signalling and protein degradation pathways (UPS, autophagy).

• Investigate whether MAO levels (via changes in redox homeostasis and proteostasis) influence the cargo of extracellular vesicles (protein, miRNA) in different cell types (signalling between cells).This interdisciplinary project will use a variety of techniques including mammalian cell culture, biochemical, molecular biology and proteomics/bioinformatics techniques. It will provide insight into how MAO levels influence signalling molecules in different brain cells and whether MAO contributes to cell specific vulnerability under stress, which has relevance to ageing.

References to learn more:

Ugun-Klusek A, Theodosi TS, Fitzgerald JC, Burté F, Ufer C, Boocock DJ, Yu-Wai-Man P, Bedford L, Billett EE. Monoamine oxidase-A promotes protective autophagy in human SH-SY5Y neuroblastoma cells through Bcl-2 phosphorylation (2019) Redox Biology, 20:167-181. doi:10.1016/j.redox.2018.10.003

Fecher C, Trovò L, Müller SA, Snaidero N, Wettmarshausen J, Heink S, Ortiz O, Wagner I, Kühn R, Hartmann J, Karl RM, Konnerth A, Korn T, Wurst W, Merkler D, Lichtenthaler SF, Perocchi F, Misgeld T. Cell-type-specific profiling of brain mitochondria reveals functional and molecular diversity (2019) Nat Neurosci. 22(10):1731-1742. doi: 10.1038/s41593-019-0479-z.

Location of lab rotation: Clifton Campus

Location of full PhD project: Clifton Campus

(117) Using isogenic induced pluripotent stem cells-derived organoids to model triple negative breast cancer

Primary supervisor: Cinzia Allegrucci

Second supervisor: Anna Grabowska

Institution: University of Nottingham

School: Veterinary Medicine and Science

Lab rotation description:

The rotation will introduce the student to the topic of the project and to a range of laboratory techniques which are essential for the project. The student will acquire cell culture and molecular biology skills, including designing and cloning CRISPR constructs. Week 1: Lab inductions/Introduction to cell culture Week 2-3: Cell culture and cell viability assaysWeek 4-5: CRISPR design and cloningWeek 5-6: CRISPR cloning and transfectionWeek 7-8: Selection and screening of gene-edited clones Week 9: Writing report

Full PhD project description:

Please provide a description of the full PhD project (500 words)Models systems that recapitulate the malignant state both at the cellular and organism level are at the basis of cancer research. To this end, animal models have significantly contributed to the progress of our understanding of the mechanisms responsible for cancer development. However, recently patient-derived cancer models have increased in relevance, due to both an increased understanding of the complexity of the disease and a drive to reduce the use animals for research. The aim of this research proposal is create an effective human in vitro system to model cancer initiation and progression for the replacement of animals in cancer research. The project will focus on the reprogramming of normal cells harbouring genetic mutations found in primary and metastastic breast tumours into induced pluripotent stem cells (iPSCs) followed by directed differentiation into 3D organoids. This approach will be used to establish a platform for drug screening in order to replace the use of animal models, including genetically engineered mice, mouse xenografts and mouse/rat models of chemical carcinogenesis. We will use a novel approach based on the establishment of normal immortalised-non tumorigenic breast cells gene edited with CRISPR/Cas9 to create isogenic mutant cell lines that, after reprogramming to iPSC and differentiation to mammary organoids, will model triple negative breast cancer. Targeted therapy for this aggressive type of breast cancer is currently not available, so the project aims to satisfy an unmet clinical need, whilst reducing and replacing the use of animals for research.The objectives of the proposal are to:

1) Reprogram mammary cell lines carrying genetic mutations to create an isogenic iPSC cancer progression model to represent triple negative breast cancer.

2) Establish an effective mammary organoid differentiation protocol from iPSC

3) Fully characterise the iPSC-differentiated mammary organoids to ensure phenotypic (histopathological analysis, growth and cancer stem cell profile, invasion, drug resistance) and genotypic cancer subtype identity (lineage marker expression)

4) Validate the in vitro replacement model by assessing mutant iPSC and organoid- differentiated mammary cells tumorigenicity in vitro and in vivo.

References to learn more:

Rowe RG and Daley GQ. Induced pluripotent stem cells in disease modelling and drug discovery. Nat Rev Genet 2019. 20 (7), 377-3882)

Ryan C Smith , Viviane Tabar. Constructing and Deconstructing Cancers Using Human Pluripotent Stem Cells and Organoids. Cell Stem Cell 2019. 24 (1), 12-24

Location of lab rotation: University Park

Location of full PhD project: University Park

(118) Engineering sustainable pathways to plastic recycling in Cyanobacteria

Primary supervisor: Dong Hyun Kim

Second supervisor: Samantha Bryan

1) Rowe RG and Daley GQ. Induced pluripotent stem cells in disease modelling and drug discovery. Nat Rev Genet 2019. 20 (7), 377-3882) Ryan C Smith , Viviane Tabar. Constructing and Deconstructing Cancers Using Human Pluripotent Stem Cells and Organoids. Cell Stem Cell 2019. 24 (1), 12-24

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

During the lab rotation the student will learn to grow and manipulate cyanobacteria, initially expressing two small depolymerase candidates recently identified in Dr Bryan's laboratory, a lipase and a hydrolase in both the model cyanobacterium Synechocystis sp. PCC 6803 and Nostoc sp. The student will assess the growth of both WT and the lipase/hydrolase expressing strains of Synechocystis sp PCC 6803 and Nostoc sp. on PET and PE (Polyethylene). The student will assess physiological fitness of the bacteria on the plastics and evaluate metabolite production/utilisation and the properties of the plastic using advanced metabolomics methods. This will involve the optimisation of the existing liquid chromatography (LC)-mass spectrometry (MS)-based metabolite profiling developed in Dr Kim's group. The student will utilise several different techniques during the rotation including CRISPR, SDS-PAGE, PAM fluorimetry, SEM and LC-MS-based metabolite profiling. They will also participate in the weekly group meetings.

Full PhD project description:

Plastic usage will double by 2036, yet >70% is unrecyclable and >94% is still produced from virgin hydrocarbons. Despite growing social momentum for a plastics circular economy, re-processing of mixed plastic waste (MPW) is extremely challenging. Waste-to-energy (W2E) technology offers a steppingstone technology, extracting value from MPW but at high environmental cost (CO_2 production) and is a fundamentally open-loop process (low on the waste hierarchy). The remaining plastic waste sits in the environment or ends up in landfill 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 311m tons. Around 41m tons of PET was produced in 2013 and this is projected to increase to 73m tons by 2020. PE production currently exceeds 100 million tons accounting for 34% of the plastics market, with worldwide usage of plastic bags alone exceeding 1 trillion a year. It is imperative that we find an efficient and green solution to tackle the global plastic problem.

Cyanobacteria are exemplars of the first microorganisms capable of oxygenic photosynthesis and capable of fixing 1.83 kg of CO_2 per 1 kg of biomass.

This project aims to utilise waste plastics such as PET and PE and produce biodegradable plastic and high value chemicals in a cyanobacterial chassis.

Objectives
The PhD project will have three main objectives; the first objective being to generate a strain capable of enhanced plastic degradation through the expression of novel lipases and hydrolases in cyanobacteria. These strains will be fully characterised utilising a wide variety of techniques to monitor the physiological state of the cell, plastic degradation and biofilm formation on the plastic. The properties of the plastic will also be assessed using NMR and FT-IR. Engineered strains will then be subject to metabolomic characterisation (metabolic phenotyping) to estimate intra and extracellular metabolic fluxes. Conventional LC-MS-based metabolomics and stable isotope-assisted metabolic pathway profiling, coupled with 13C flux analysis will be utilised to predict in vivo enzyme reaction rates, unravelling key metabolism and providing exemplar kinetic data, allowing for the development of designer strains with improved plastic degradation.

The second objective will focus on utilising these strains as chassis to produce 2,5-PDCA from PET, which is a bioplastic building block. Metabolic engineering utilising CRISPR and CRISPRi coupled with metabolomic analysis will allow flux to be diverted to 2,5-PDCA production. This will then be utilised to produce bioplastics in collaboration with Biome Bioplastics (industrial collaborator).

The third objective will focus on isolating novel cyanobacterial strains from the environment particularly environments contaminated by plastic pollution. Strains will be isolated on both PET and PE and characterised via genomics, transcriptomics, proteomics and metabolomics to identify novel enzymes which can be utilised in Obj. 1 and 2.

The student will receive dedicated mentoring from the supervisory team and will benefit from their substantial expertise and the multidisciplinary nature of the project.

References to learn more:

1. Danso, D., Chow, J., Streit, W. R. (2019) Plastics: Environmental and Biotechnological Perspectives on Microbial Degradation. Applied and Environmental Microbiology, 85(19), e01095-19.

2. Sarmah, P., Rout, J. (2019) Cyanobacterial degradation of low-density polyethylene (LDPE) by Nostoc carneum isolated from submerged polyethylene surface in domestic sewage water. Energy, Ecology and Environment, 4(5), 240-252.

Location of lab rotation: University Park

Location of full PhD project: University Park

(119) Protein tubes for nanodevices

Primary supervisor: Frankie Rawson

Second supervisor: TBC

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

The student will undergo a training project to develop skills on knowledge in the analytical tool of choice electrochemistry and click chemistry. This chemistry will be used to adhere modified bacteria with protein tubules to facilitate adherence to gold electrodes. The electrodes will then be appraised using cyclic voltammetry to assess electron transfer behaviour between the modified bacteria and the underlying electrode. Thus this will establish the beneficial behaviour of functionalising biology/electrodes to facilitate the two way electrochemical communication of biology with the electrode and vice versa.

Full PhD project description:

Bioelectrochemical systems (BESs) are capable of converting electrical energy into chemical energy and vice-versa by employing microbes as catalysts. In this project we will address one of the limitations of traditional BESs; the low efficiency of electron transfer to and from living cells to the surface of the electrodes. We propose to use protein nanotubes for templating of nanowires for direct coupling of living cells to electrodes:

Many biological processes are mediated by macromolecular structures, evolution has provided a plethora of protein nanotubes such as pili, flagella, gas vesicles and viral capsids. These could be utilised as templates for nanomaterials due to their ease of manipulation via protein engineering and assembly under physiological conditions. We intend to engineer the tips of such nanotubes so they can directly bind to modified electrode surfaces and/or living cells to act as a robust interface between cells and electronic devices. Collaboration with NBIC will facilitate the analysis of the cell/device interfaces once they are established.

Location of lab rotation: University Park

Location of full PhD project: University Park

(120) Capturing ubiquitin system complexes by single-particle cryo-electron microscopy

Primary supervisor: Jonas Emsley

Second supervisor: Ingrid Dreveny

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

In the lab rotation you will receive training in contemporary protein chemistry and structural biology approaches and learn how to apply these to study the molecular basis of ubiquitin-mediated processes. The rotation will consist of the following parts:
• Mutagenesis to create a "substrate-trapping" ubiquitin specific protease mutant
• Recombinantly express and purify the created mutant ubiquitin specific protease (USP)
• Prepare a substrate trapped complex using the recombinant USP protein
• Initial structural characterisation of the complex using crystallisations and electron microscopy negative stain imaging.

Full PhD project description:

Ubiquitination is a key posttranslational modification for protein degradation and numerous signalling pathways and influences virtually all cellular functions including gene expression and cell proliferation in health, ageing and disease. Ubiquitin specific proteases (USPs) are multi-domain enzymes that regulate cellular protein levels as well as signalling pathways by specifically removing the ubiquitin tag from target proteins that otherwise would mark them out for destruction or other fates. These enzymes need to be highly specific in order to ensure ubiquitin removal from the correct target protein. However, for the majority of USPs we do not currently understand how they select substrate proteins or other binding partners, which USP regions are involved in the interactions and how similar USPs differentiate between binding partners.

Here, we will use the latest structural biology techniques including single-particle cryo-electron microscopy to decipher the molecular basis of the function of ubiquitin specific protease complexes that modulate the life time and activity of substrate proteins specifically focusing on a complex important in health and tumourogenesis.

In a collaborative project we will take "snapshots" of USP complexes in the absence and presence of substrates to visualise the structure of a full-length USP and the impact of substrate binding on the conformation of this deubiquitination machinery. Through mutagenesis and domain-swapping we will identify which regions are essential for USP function. The structures of USP complexes will be visualised using the latest cryo-electron microscopy techniques. The field of structural biology has undergone a revolution as the structural details of macromolecules and complexes can be determined to unprecedented resolutions with the new generation of cryo-EM instruments. For these break-through developments the 2017 Nobel Prize in Chemistry was awarded. We have access to the very latest FEI Titan Krios G3 300 kV transmission electron microscope with state-of-the-art detectors by being part of the Midlands Regional cryo-EM facility.

We will combine this structural knowledge with complementary structural techniques, biochemical

and cell-based functional assays to gain a detailed understanding of how these proteases exert their physiological function, recognize their substrates, function in disease processes and how they can be manipulated for therapeutic interventions in particular cancer.

References to learn more:

1. Raunser, S. (2017) Cryo-EM Revolutionizes the Structure Determination of Biomolecules. Angewandte Chemie 56, 16450-16452

2. Mevissen, T. E. T., and Komander, D. (2017) Mechanisms of Deubiquitinase Specificity and Regulation. Annu Rev Biochem 86, 159-192

Location of lab rotation: University Park

Location of full PhD project: University Park

(121) Outside-in signaling: extracellular matrix proteins regulate miRNA biogenesis during inflammation

Primary supervisor: Anna M. Piccinini

Second supervisor: Keith Spriggs

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

Immune cells quickly respond to infection and injury relying on regulatory checkpoints, including miRNAs which regulate gene expression. However, it is unclear how the innate immune response becomes dysregulated in disease and how miRNAs themselves are regulated. We found that the extracellular matrix glycoprotein tenascin-C regulates the production of the pro-inflammatory cytokine TNF-a in macrophages activated by the bacterial component lipopolysaccharide. Remarkably, tenascin-C does this by regulating the expression of miR-155. This is in line with work reported by others using mice lacking or overexpressing miR-155 that display uncontrolled TNF-a production.

We recently identified the receptor which mediates miRNA regulation by tenascin-C. Characterization of the receptor/ligand interaction and downstream signalling that are activated by tenascin-C and culminate in the induction of the inflammation-associated and oncogenic miR-155 during infection and cancer will provide a new area of drug research which might impact on infection, cancer and/or neuroinflammation.

During the lab rotation you will receive the training necessary to 1) design and validate CRISPR single guide RNA molecules for mutagenesis studies to map the ligand/receptor interaction; 2) co-immunoprecipitate the ligand and the receptor and potential co-receptor(s); and 3) analyse the activation of candidate kinases following knock-down of tenascin-C and receptor by siRNA.

Full PhD project description:

Macrophages are white blood cells that are a key part of the innate immune system. In addition to sensing and clearing invading pathogens, they play a major role in regulating the body's inflammatory response. Like most cells, macrophages are surrounded by a complex extracellular matrix (ECM) which contains molecules that can convey signals to the cells. Infection, cancer and tissue damage induce changes in the ECM that enable effective host responses, mediated by cytokine signalling. An important advance in understanding this pathway is the discovery that ECM components such as the protein tenascin-C signal changes in the levels of microRNAs, which are key regulators of gene expression in the cell (Piccinini AM, Cell Reports, 2012). The microRNAs in turn control the inflammatory response by regulating cytokine synthesis. This novel level of inflammatory gene regulation has the potential to explain why inflammation is not resolved in disease. Research needs to be progressed so that the findings can be exploited to modulate inflammatory responses in different diseases, including sepsis, autoimmune disorders and cancer.

By combining molecular biology, biochemical and cell biology techniques, this project will characterise the interaction of tenascin-C with the receptor which has recently been identified in our lab that transduces the ECM signal into regulation of miRNA biogenesis inside the cell. You will use

siRNA technology and co-immunoprecipitation experiments combined with proteomics analysis to define the ligand-receptor interaction, and identify any co-receptor(s) in macrophages. CRISPR genome editing will help you to carry out mutagenesis studies to map binding sites. Additionally, you will use biophysical tools such as surface plasmon resonance to characterise the binding in vitro. Furthermore, you will unravel the signalling pathways induced upon this ligand-receptor interaction using a combination of phosphospecific antibodies for western blotting and immunofluoresce. You will be encouraged to exploit the information obtained in the early part of the project in order to design therapeutic strategies that will manipulate inflammatory and oncogenic miRNA and cytokine levels by inhibiting or enhancing the interaction of tenascin-C with its receptor.

The project will be carried out within the Gene Regulation & RNA Biology laboratory, which currently consists of 6 principal investigators and around 25 full time researchers with well-equipped laboratory facilities. The School of Pharmacy is the highest ranked School for quality and impact of research in the UK (REF2014). The School provides excellent cross-disciplinary support and training facilities for PG students. You will have access to a wide range of different sources of training, both in advanced scientific techniques and transferable skills. You will attend division and PG meetings and will be expected to attend seminars within the School and those relevant in the wider University. Subject-specific training will be received through our group's weekly supervision meetings.

References to learn more:

1. Piccinini A.M. and Midwood K.S. (2012). Endogenous control of immunity against infection: tenascin-C regulates TLR4-mediated inflammation via microRNA-155. Cell Reports 2 (4), 914-26.

2. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. J Cell Sci. 2010;123(Pt 24):4195–4200. doi:10.1242/jcs.023820

3. Piccinini A.M. and Midwood K.S. (2014). Illustrating the interplay between the extracellular matrix and microRNAs. Int J Exp Pathol 95 (3), 158-80.

Location of lab rotation: University Park

Location of full PhD project: University Park

(122) Computational enzyme engineering for sustainable applications: Electric field catalysis

Primary supervisor: Christof Jaeger

Second supervisor: Anca Pordea

Institution: University of Nottingham

School: Engineering

Lab rotation description:

During the lab rotation, the student will learn how to use basic computational chemistry approaches to gain insights into enzymatic functions and dynamics.

Depending on the background of the student the lab rotation will either target investigations into enzyme flexibility and substrate binding through methods of molecular docking and molecular dynamics simulations, or the student will start applying quantum chemical DFT calculations to understand the reaction mechanism of chosen transition metal complex catalysts and will use molecular docking and equilibration techniques to investigate how artificial metal complexes can bind into native enzyme structures and how redox centres are effected by their local environment. Both approaches will subsequently be linked to the PhD aim of engineering enzyme functionality and turnover by investigating single amino acid mutations in silico and their effects on flexibility and functionality. This ultimately then leads into detailed and systematic investigations of electric field effects in the enzymes and the application of combined quantum mechanical and molecular mechanics (QM/MM) calculations to understand and interpret experimental observations.
The student will gain insights into a wide variety of computational modelling tools in a biochemistry environment with strong links to ongoing experimental efforts in the group.

Full PhD project description:

Enzymatic reaction catalysis is controlled by many factors enabling the enzymes to perform chemical transformations hard or even impossible to perform by standard synthetic techniques. Within protein engineering we aim to further improve the catalytic performance of enzymes for specific transformations or re-engineer them to facilitate reactions not known to be catalysed by enzymes at all. This eventually leads to industrially viable and sustainable bio-catalysts with use in industrial biotechnology.

In order to rationally improve enzyme catalysis we need to understand the reaction and control mechanisms in the enzymes. Here, computational methods are on the forefront of getting insights into this reaction control which informs and guides experimental advances in enzyme engineering. One very interesting key influencing parameter for many examples in enzyme catalysis is the internal electrostatic field in the enzyme active site, often referred to as electrostatic preorganization. This effect has been discussed a lot and very recently it has been demonstrated how externally orientated electric fields can influence biocatalytic reaction rates by orders of magnitude.
Within this project we aim to investigate in close collaboration with experimental partners how oriented electric fields influence enzyme catalysis, with the target to be able to influence this factor and improve enzymatic catalysis.

The project will first focus on reactions within radical SAM enzymes. These enzymes incorporate

hydrogen abstraction reaction steps facilitated by highly redox reactive metal clusters within these enzymes which are known to be influenced by changes in the surrounding electrostatic field.
We will investigate how the internal electric field influences different reaction steps during enzyme catalysis and will computationally manipulate the electric field by point mutations within the enzyme scaffold. By this we aim to influence enzyme catalysis by a novel rational approach which will subsequently be tested experimentally via collaborative approaches. In a next step it will also be evaluated how an externally applied electric field could influence and alter enzyme catalysis with the aim to gain control over enzymatic catalysis by fine tuning this external variable.

Subsequently the project will be expanded to engineering redox reaction active metallo enzymes such as carbonic anhydrases in a similar fashion by applying methodologies developed in the first part of the project.

References to learn more:

1. Electric Fields and Enzyme Catalysis, Stephen D. Fried and Steven G. Boxer, Annual Review of Biochemistry, 2017, 86, 387.

2. Oriented electric fields as future smart reagents in chemistry, S Shaik, D Mandal and R Ramanan, Nat. Chem., 2016, 8, 1091 (DOI: 10.1038/nchem.2651).

Location of lab rotation: University Park

Location of full PhD project: University Park

(123) Disease intervention targets for Group B Streptococcus infections

Primary supervisor: Adam Blanchard

Second supervisor: Sharon Egan

Institution: University of Nottingham

School: School of Veterinary Medicine and Science

Lab rotation description:

The nine week rotation project will focus on developing skills (wet lab and bioinformatics) which will enable the student to make quick progress if this project is chosen.

Initially this will consist of growth of bacterial isolates, understanding aseptic techniques and good laboratory practice. This will also include DNA isolation, PCR and preparing samples for sequencing. This rotation will also allow the student to develop bioinformatics techniques using PIMMS. PIMMS allows the students to use software for identifying essential genes from bacterial genomes but also understand best practices in managing large data sets and quality control measures. Prior computing experience will be an advantage but this will give any student a good introduction to one of the most employable skill sets in biology.

Full PhD project description:

Group B Streptococcus (GBS) is the leading cause of neonatal meningitis in the western world and typically leads to meningitis or bacterial sepsis, with further infection-related complications including cerebral palsy, deafness, blindness and serious learning difficulties. Approximately 30% of women carry GBS as natural flora and prevention of infection requires administration of large doses of antibiotics during labour. GBS is also increasingly associated with invasive disease among the elderly and immuno-compromised worldwide. Prevalence in South East Asia is significantly under reported, however some data suggests that infection conditions mirror those reported in the UK.

Identification of the key components of the bacterium that are involved in the infection process will allow the development of alternative therapies that prevent transmission from mother to baby during birth. Alternately, these components may be incorporated into diagnostic tests to identify those strains naturally carried by the mother that are more likely cause neonatal infection, allowing a more targeted approach for antibiotic treatment in those situations where transmission leading to disease is more likely. Either approach would greatly reduce the incidence of GBS infection in the new-born and thus reduce mortality associated with this disease in infants. GBS causes significant economic and stock losses in the aquaculture industry and is a threat to the emerging dairy industry; both identified as key targets for continued intensification and food security. Antibiotic control of disease is becoming less feasible due to growing resistance and the threat of gene transfer within this zoonotic pathogen and alternative control strategies are urgently required.

The growing human:cattle:fish interface, driven by intensification of these livestock sectors in developing countries, is a driver for increased prevalence of zoonotic disease. Limited strain, prevalence and sequence information exists for zoonotic Group B Streptococcus (GBS) and characterisation of these strains will provide key virulence related information for potential future control based strategies.

This project will characterise genes/proteins essential for GBS pathogenesis in humans, fish and cattle using a combination of random mutagenesis and high throughput sequencing. Individual bacterial mutant pools will be generated for representative strains using the pGh9:ISS1 insertional mutagenesis system, and a bespoke laboratory and bioinformatic analysis programme we have developed called PIMMS. PIMMS will be used to map insertions within the bacterial genomes and identify conditionally important / essential sequences that will be translated to metabolic pathways and biochemical processes for further comparative analysis.

The selected bovine, fish and human isolates will be phenotypically tested for growth in the presence of antimicrobial peptides, essential oils and other alternatives. This will allow us to identify modes of action against the streptococcal isolates and investigate potential antimicrobial alternatives.

References to learn more:

1. Blanchard AM, Leigh JA, Egan SA, Emes RD. Transposon insertion mapping with PIMMS - Pragmatic Insertional Mutation Mapping System. Front Genet 2015;6:139. doi:10.3389/fgene.2015.00139.

2. Blanchard AM, Egan SA, Emes RD, Warry A, Leigh JA. PIMMS (Pragmatic Insertional Mutation Mapping System) laboratory methodology a readily accessible tool for identification of essential genes in Streptococcus. Front Microbiol 2016;7:1–12. doi:10.3389/fmicb.2016.01645.

Location of lab rotation: Sutton Bonington

Location of full PhD project: Sutton Bonington

(125) mRNA methylation and complex components within healthy, developing and diseased the cardiovascular system

Primary supervisor: Catrin Rutland

Second supervisor: Nigel Mongan

Institution: University of Nottingham

School: School of Veterinary Medicine and Science

Lab rotation description:

However, our unpublished work shows that domesticated chicken genome only contains the METTL14 homologue, and it appears that it is this enzyme that is required for the mRNA methylation in this case. In addition we have carried out many immunohistochemistry experiments on murine and chicken heart and vasculature from a variety of tissues exploring whether the key components of the RNA methylation complex are expressed. Based on these positive findings (which were also part of a successful Masters project) we propose that the complex plays vital roles in cardiogenesis, angiogenesis, and normal heart and blood vessel function. Therefore it could play a vital role in healthy aging and disease states.
In order to fully understand m6A methylation, you will investigate where the key components of the METTL14 and METTL3 complex are situated. Including tissue expression of METTL14, METTL3, WTAP, m6A and viruliser, which species show complex components expression and whether they are differential expressed during key developmental stages. This will mainly be undertaken using immunohistochemistry and microscopy methods.

Full PhD project description:

Please provide a description of the full PhD project (500 words)
The presence of m6A in mRNA is a common modification across the eukaryotes, and its presence is necessary for developmental decisions, and responses to nutritional cues. Our previous work has demonstrated that the pathway for mRNA methylation cross-talks to the TOR pathway, a major, conserved signalling pathway between environmental nutritional changes and translation, in the cell. Most metazoans, plants and yeast have two related methyltransferases, METTL3 and METTL14, of which the METTL3 homologues have been shown to be crucial for mRNA methylation.

The key objectives are to investigate whether mRNA methylation and the key pathway components are differentially expressed in relation to both abundance and localisation in 1) tissues within the cardiovascular system, 2) in the developing heart, 3) in diseased versus healthy hearts in 4) differing species.

Preliminary evidence: Our collaborative team have already produced preliminary evidence in order to develop the concepts involved in this PhD. This work includes testing antibodies for immunohistochemistry and western blots on avian and mammalian cardiovascular system tissues, TLCs have been carried out to show basic expression mRNA methylation, bioinformatics and expression studies have already shown differing expression patterns within differing species, therefore this project builds upon that work and answers vital questions relating to mRNA methylation in the cardiovascular system.

Techniques used to deliver objectives: A number of techniques will be used to investigate the mRNA

expression and key components of the mechanism. Immunohistochemistry (single-triple staining) will be utilised to showed localisation. Western blotting, RNAseq, qPCR and TLCs will be utilised for quantification of these mechanistic RNAs and proteins. Bioinformatics will be used in conjunction with both RNAseq and used to compare sequences from the different species. All of the techniques are used routinely within the group and provide excellent training opportunities for both the rotation and entire project. Our students have excellent publication records and we expect more high quality, novel publications to arise from this project.

Tissues from the chicken, turkey and mouse will be utilised in addition to numerous cell lines in vitro. We have also collected canine pathology tissue from healthy and diseased hearts. The study will mostly use tissues already collected which enables us to reduce the use of animals in research and address the BBSRC 3Rs policy. This research directly addresses BBSRC areas as it seeks to understand physiological processes that will enable increased lifelong health, welfare and longevity in birds and mammals and can positively affect global food security.

References to learn more:

1. Simpson S*, Dunning M, Brownlie S, Patel J*, Godden M*, Cobb M, Mongan NP and Rutland CS. (2017) Multiple Genetic Associations with Irish Wolfhound Dilated Cardiomyopathy. BioMed research international. 6374082

2. Haussmann I, Archer N*, Bodi Z, Nallasivan M, Sanches-Moran E, Mongan N, Fray R, Soller M.2018. Deciphering the role of mRNA methylation in Drosophila sex determination and the nervous system FEBS OPEN BIO. 8, 23-23

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

This is a linked project – we recommend you select project 47 alongside this one.

(127) Exploiting genetic diversity of essential fatty acids in duckweeds for human nutrition

Primary supervisor: Rahul Bhosale

Second supervisor: Anthony Bishopp

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

Title: Molecular profiling of duckweed species collected across the UK

Duckweeds represent a small family of aquatic floating plants consisting of 37 species, which are well adapted to different geographic and climatic zones worldwide. They are very fast growing and can cover ponds or lake within a few days under favourable conditions. They have been long consumed as an inexpensive source of proteins and omega-3 fatty acids in a few Asian countries. Thus, duckweeds are now being investigated prominently to address if they have the potential to be adopted by other Asian and western countries as a dietary supplement.

For the 9-week DTP rotation, we will use 10 different duckweed species collected across the UK and leverage recent investement in molecular phenomics platform at Univesrity of Nottingham (UNot) to
• grow and produce enough biomass using established tissue culture techniques (Weeks 1&2).
• profile proteins, lipids and carbohydrate content using hyperspectral imaging (Week 3).
• quantify individual fatty acids using Gas Chromatography–Mass Spectrometry (Week 4&5).
• measure 23 elements using high-throughput Inductively Coupled Plasma-Mass Spectrometry (Week 6&7).
• assess molecular profiling data, review of literature and write report (Week 8&9)

Output: Understanding of variation of macro and micro-nutrients in duckweed species in the UK.

Full PhD project description:

Background:
Duckweeds are fast growing aquatic plants that have excellent nutritional qualities, such as high protein content, essential omega-3 fatty acids (EFA) and micronutrients, suggesting their potential for human and animal nutrition. Recent research suggest that there is a significant genetic variation between and within individual species of duckweeds for these nutritional qualities. However, the genetic basis of such variation remains unclear.

Aim:
We aim to pinpoint the genetic basis underlying the variation of nutritional qualities in duckweeds towards developing toolkit for selecting or breeding of suitable duckweed genotypes for future applications in human and animal nutrition.

Work-plan:
In this DTP-studentship, we will primarily focus on the nutritional quality of EFA (alpha linolenic, ecosapentaenoic and docosahexaenoic acids), which are important in a variety of physiological processes in human and animal health. Towards this, we will exploit (i) recently sequenced diversity

collection of 200 Spirodela polyrhiza accessions collected from Europe, North America and South East Asia (available through our collaborators) supplemented by additional accessions from Kenya (collaboration with S Villiers, Pwani University) and (ii) core expertise of the supervisory team, collaborators and resources available at UNot and its partner institutions.

Year1
Objective-1: FA profiling of diversity collection of Spirodela polyrhiza accessions.
Fresh biomass of each accession will be used for total FA extraction and FA methyl esters preparation, which will be profiled using UNot GC-MS facility.

Objective-2: GWAS to pinpoint genes controlling content of EFA.
FarmCPU will be used to perform GWAS on individual FA. Hit SNPs will be used to pinpoint genes, which will be prioritised using cross-species functional annotation.

Objective-3: RNAsequencing of two genotypes with contrasting EFA.
To complement Obj2, transcriptomes of two accessions with contrasting EFA content (from Obj1) will be profiled using RNAsequencing (DeepSeq facility, UNot) to obtain a list of differentially expressed genes involved in regulating EFA content.

Output: List of prioritised candidate genes (intersection of obj2 & obj3 outputs) controlling EFA content in duckweeds. FA profiling and RNAseq datasets.

Year2
Objective-1: Developing RNAi and over-expressor lines for three prioritised candidate genes.
RNAi and over-expressor lines in Spirodella polyrhiza background will be generated to study candidate genes' functions. Alternatively, CRISPR-Cas9 gene-editing approach (which is not yet widely employed for duckweeds) will be attempted to generate mutants. Confocal/LighSheet microscopy will be used to screen generated lines.

Output: RNAi/CRISPR and over-expressor lines and molecular resources.

Year3
Objective-1: FA profiling of RNAi and over-expressor lines.
Profiling will be performed as in Year1/objective1 to validate functions of candidate genes.

Objective-2: Assessing assimilation of EFA in duckweeds to shrimp food-chain
Student will visit UNot Malaysia campus to conduct trials of feeding duckweeds (contrasting genotypes, developed RNAi/CRISPR and over-expressor lines) to larvae and then feed larvae to shrimps and performing FA profiling of shrimps.

Output: Funtionally validated candidate genes that control EFA content in duckweeds. Potential of duckweed as a food for freshwater shrimp to improve their nutrient value for human consumption.

Overall, this DTP project will help develop gene-toolkit for breeding suitable duckweeds for future applications in human and animal nutrition.

References to learn more:

1. Nutritional Value of the Duckweed Species of the Genus Wolffia (Lemnaceae) as Human Food. Front. Chem., 29 October 2018. Doi: 10.3389/fchem.2018.00483

2. Mass Production of Lemna minor and Its Amino Acid and Fatty Acid Profiles. Front Chem. 2018; 6: 479. doi: 10.3389/fchem.2018.00479

Location of lab rotation: QMC; Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(128) The role of circadian clock genes in cognition

Primary supervisor: Paula Moran

Second supervisor: David Heery

Institution: University of Nottingham

School: Psychology

Lab rotation description:

The Heery lab can provide training in a wide range of molecular and cellular biology techniques, such as protein expression, protein-protein interaction assays, yeast two hybrid, mammalian cell transfection, reporter assays, Split YFP system, qPCR, western blots, PCR genotyping of CRISPR edited clones. We assign rotation students to work with a task supervisor on a current project within the team. This is decided just before the rotation begins, ensuring the short project goals are relevant and in line with the current research priorities of the team. In past rotations, students have performed site-directed mutagenesis to study Nuclear receptor /cofactor interactions in yeast two-hybrid; used PCR and subcloning techniques to generate CRISPR constructs to disrupt a gene, followed by genotyping of edited cells to confirm the targeting events; purified wild type and mutant proteins for histone acetylation assays or performed immunofluorescence staining analysed by confocal microscopy. The student would work within the Gene Regulation & RNA Biology laboratories (25-30 full time researchers) receive local induction and instruction in health & safety procedures, SOPs, risk assessments etc. Depending on the experiments that are ongoing, the Moran laboratory will additionally familiarise the student with the in-vivo testing environment for genetically altered mice and whichever circadian rhythm experiments are current. If Home office licence training is taken in advance of the rotation, hands-on experience in behavioural studies in mice can be provided as part of this rotation.

Full PhD project description:

Memory performance is known to fluctuate with time of day. This is most markedly observed in Alzheimer's disease where patients may show severe exacerbation of symptoms in the evening (known as 'sundowning'). Ageing is also associated with memory decline and has a concomitant disruption of circadian patterns of activity and sleep, while many conditions such as depression and autism display both circadian sleep disturbances and cognitive abnormalities. There is behavioural evidence that circadian cycles interact with cognitive performance but it is unknown what the biological mechanism of this might be. At the molecular level circadian rhythms are established by "clock genes". A network of transcriptional/translational feedback loops involving these genes generate and sustain these rhythms.

The aim of this project is to investigate the function of NPAS2 in circadian gene expression and memory. NPAS2 is a "clock" gene that is expressed in the forebrain and hippocampus brain regions that are important for cognitive function. CLOCK/BMAL1 heterodimers are central to maintenance of circadian rhythms, but recent evidence shows NPAS2/BMAL1 can partially compensate for loss of CLOCK, although NPAS2 may have other independent roles. In this project the molecular mechanisms of how NPAS2 regulates circadian gene expression, interacts with other clock genes and influences circadian rhythms in memory and activity will be explored. We have a colony of mice with conditional deletion of NPAS2 and are currently investigating how this deletion impacts circadian variation in cognition and other behaviours.

References to learn more:

1. Molecular mechanisms and physiological importance of circadian rhythms
   Nature Reviews Molecular Cell Biology (2019)
   doi:10.1038/s41580-019-0179-2

2. Cell-Type-Specific Regulation of Nucleus Accumbens Synaptic Plasticity and Cocaine Reward Sensitivity by the Circadian Protein, NPAS2. J Neurosci. 2019 Jun 12;39(24):4657-4667.
   doi: 10.1523/JNEUROSCI.2233-18.2019.

Location of lab rotation: University Park

Location of full PhD project: University Park

(129) Carrion specialising: adaptations and welfare outcomes among vulture species to carcass scavenging

Primary supervisor: Katherine Whitehouse-Tedd

Second supervisor: Geert Janssens

Institution: NTU

School: School of Animal, Rural and Environmental Sciences

Lab rotation description:

MHC diversity
The Major Histocompatibility Complex (MHC) comprises a series of structural proteins associated with pathogen recognition and initiation of the vertebrate immune system. Loss of genetic diversity due to inbreeding has long been associated with loss of MHC function and reduced evolutionary potential in species. Here we offer exciting opportunities for students to study MHC diversity in a range of exotic mammalian species to characterize MHC diversity and identify new alleles. Students will gain experience in DNA extraction, PCR, gel electrophoresis, sequence alignment and data analysis.

Full PhD project description:

By disposing of decomposing carcasses, vultures (Aegypiinae and Gypaetinae) provide essential ecosystem services of importance to ecology, human health, and agriculture. The 23 extant vulture species all have a high dietary pathogen challenge in common, but differ largely in feeding strategy, ranging from general carcass parts to bone marrow specialisation. Evolutionary drivers for these differences are uncovered, but insights are crucial to understand drivers of host-diet interactions and their role in animal health and welfare. Recently, our research group has identified important species- and husbandry-specific factors shaping the gut microbial community of Old World vultures. Prey type fed to captive vultures influenced the gut microbial profile, and we documented microbial alignment with species-specific feeding strategies. Using a comparative physiology angle, this project now wants to identify how the differences in feeding ecology and scavenging behaviour among vulture species are associated with particular adaptations in digestive processing and intestinal microbiome.

To that aim, four species have been identified with distinct feeding ecologies. The Endangered lappet-faced vulture (Torgos tracheliotos) is a cartilaginous tissue specialist, often feeding on fibrous tissues including tendons, ligaments, and skin of mammalian carcasses. The Endangered Egyptian vulture (Neophron percnopterus sp.) is a food generalist, shown to eat a variety of feed items depending on availability and geographical location. The bearded vulture or lammergeyer (Gypaetus barbatus) is a Near Threatened species with a unique feeding strategy; as bone specialists they feed on the marrow of the long bones of prey carcasses. Lastly, the griffon vulture (Gyps fulvus; a Least Concern species) is predominantly a muscle and offal eater. Bearded and Egyptian vultures are a monophyletic group, whilst the lappet-faced and griffon vultures are related by paraphyly. Falcons (Falconiformes) or owls (Strigiformes) will be used as the outgroup due to their low evolutionary relatedness to vultures. New World vultures (Cathartiformes) may also be used for comparison due to their occupation of similar ecological niches in the western hemisphere. Based on our studies in carnivorous mammals, we anticipate that the natural diets of these four vultures vary largely in fibrous components, exerting differences in digesta passage rate and intestinal fermentation

profiles. Our team was the first to identify the impact of animal fibre on health in carnivorous mammals, but this is undocumented in avian carnivores.

Using indirect sampling techniques (records of diet composition, collecting excreta, and moulted feathers) we will quantify nutritional and inflammatory markers (signalling proteins) and systemic stress (stress hormones), and characterise the gut microbiome among these four species. Because of the particular nature of the vulture's natural diet (animal matter in varying stages of decomposition), we hope to document a unique intestinal microbial ecosystem, and show how feeding ecology drives digestive processing with the concomitant modulation of this microbiome, with implications for animal welfare when dietary provision is not aligned with digestive biology.

References to learn more:

1. Gaengler, H., and Clum, N. (2015). Investigating the impact of large carcass feeding on the behavior of captive Andean condors (Vultur gryphus) and its perception by Zoo Visitors. Zoo Biol. 34, 118–129. doi:10.1002/zoo.21202.

2. Kraimi, N., Dawkins, M., Gebhardt-Henrich, S. G., Velge, P., Rychlik, I., Volf, J., et al. (2019). Influence of the microbiota-gut-brain axis on behavior and welfare in farm animals: A review. Physiol. Behav. 210, 112658. doi:10.1016/j.physbeh.2019.112658.

Location of lab rotation: Sutton Bonington

Location of full PhD project: Brackenhurst Campus

(130) The KAT in the HAT: Exploring a Novel Critical DNA Binding Function in Chromatin Regulator KAT6A/MOZ

Primary supervisor: David M Heery

Second supervisor: Hilary Collins

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

The Heery lab can provide training in a wide range of molecular and cellular biology techniques, such as protein expression, protein-protein interaction assays, yeast two hybrid, mammalian cell transfection, reporter assays, RTqPCR, western blots, RNA seq analysis and CRISPR CAS9 genome editing. Ongoing projects are relevant to cancer research and neurodevelopmental disorders. Rotation students are assigned to work with task supervisor(s) on a current project within the team. This is decided just before the rotation begins, ensuring the short project goals are relevant and in line with the current research priorities of the team. In past rotations, students have performed site-directed mutagenesis to study Nuclear Receptor /cofactor interactions in yeast two-hybrid; used PCR and subcloning techniques to generate CRISPR constructs to disrupt NR genes; performed genotyping allele sequencing of edited cells to confirm the targeting events; performed western blots and co-immunoprecipitations, purified wild type and mutant proteins for histone acetylation assays or performed immunofluorescence staining of proteins analysed by confocal microscopy. The student will join the Gene Regulation & RNA Biology laboratories (25-30 full time researchers) receive necessary instruction in health & safety procedures, SOPs, risk assessments etc, as solid preparation for a PhD project in molecular cell biology.

Full PhD project description:

This project is based in the Gene Regulation RNA Biology group and would suit students interested in developing research skills in molecular and cellular biology related to human health.

Our interests are in gene/protein structure and function, in particular those proteins involved in regulation of gene expression, i.e transcription factors and chromatin regulators. Our goal is to understanding how mutations in the genes encoding these proteins alter their function leading to catastrophic outcomes such as neurodevelopmental syndromes or cancer.

For several years PhD students and other researchers in my group have studied a protein called MOZ or KAT6A. This is a histone acetyltransferase that regulates other genes by adding acetyl groups to histones in gene promoter regions, increasing gene expression. MOZ/KAT6A is a key regulator in developing blood cells, and rare chromosomal breaks that fuse MOZ to other genes produce oncogenic hybrid proteins associated with acute myeloid leukemias. We have used protein-protein interaction studies and mutagenesis to identify functional domains in MOZ oncogenic proteins necessary for leukemia. For example, we were the first group to define the structure of the Double PhD Finger domain of MOZ/KAT6A and show how it ‘grabs’ the histone tail to read or add modifications i.e a key feature of the ‘histone code’.

Recently, it was discovered that some children with neurodevelopmental syndromes have mutations in the MOZ/KAT6A gene, for which there is currently no treatment. PhD students in my lab have

used CRISPR CAS9 editing of cell lines to mimic these clinical mutations and assess the impact on MOZ/KAT6A function; we also generated cell lines in which lacking MOZ/KAT6A expression, so we can add back altered MOZ proteins to assess which functions are essential for gene expression or disease (papers in preparation).

The specific project will focus on a previously unstudied domain of MOZ/KAT6A that we discovered functions in DNA recognition. It was previously assumed that other proteins recruit MOZ/KAT6A to promoters. This project can take a number of directions including:

Structural studies of the novel domain with our collaborators Dr Dreveny (Pharmacy) and Dr Flaig at Diamond Light Source. A previous student has optimised purification of the domain for crystallisation trials which can start early in the project. Once crystals are obtained, structure determination with the target DNA sequence will be performed.

We have partially characterised the DNA sequence recognised by the novel MOZ domain. Further validation will use EMSA assays, ITC assays, biochemical assays and confocal microscopy using mutants that are disrupted for DNA binding.

CRISPR CAS9 will be used generate an epitope-tagged version of MOZ so we can follow it more easily in cells. This could be used with chromatin IP or RIME to validate genes that are regulated by MOZ/KAT6A- we already have RNA Seq data from CRISPR edited WT and KO cell lines which can integrate with this approach.

A long term aim is to develop this research towards in vitro assays to discover small molecule inhibitors of MOZ/KAT6A domains with colleagues in medicinal chemistry.

References to learn more:

Related paper from the lab:
Dreveney I, Deeves SD, Fulton J, Yue B, Messmer M, Bhattacharya A, Collins HM & Heery DM (2014). The DOUBLE PHD Finger Domain of MOZ/MYST3 induces a-helical fold of the histone H3 tail to facilitate acetylation and methylation sampling and modification. Nucleic Acids Research 42(2):822-835

Detailed review of KAT6A:
Huang F, Abmayr SM, Workman JL. (2016) Regulation of KAT6 Acetyltransferases and Their Roles in Cell Cycle Progression, Stem Cell Maintenance, and Human Disease. Mol Cell Biol. 36(14):1900-7.

Location of lab rotation: University Park

Location of full PhD project: University Park

(132) TRAP: A platform for characterising Targeted RNA Interactome at high resolution

Primary supervisor: Aditi Borkar

Second supervisor: Rahul Bhosale

Institution: University of Nottingham

School: School of Veterinary Medicine and Science

Lab rotation description:

Title: Purification and characterisation of 7SK, a master regulator of gene transcription in the cell

7SK is the most abundant RNA-protein complex in the cell. It sequesters a major transcription factor in an inactive form and thus acts a master regulator of all gene transcription by RNA Polymerase II. Thus, 7SK has been extensively studied for understanding its function in a healthy cell and its dysregulation in diseases such as cancer and HIV pathogenesis. Yet, fundamental challenges associated with the biochemical heterogeneity and conformational flexibility of native 7SK have prevented high-resolution structure determination of the complex using conventional techniques.

For the DTP lab rotation, we will use established techniques in our lab to
1) Enrich native 7SK from mammalian cell lysates using Co-Immunoprecipitation (Weeks 1-4)
2) Characterise it using biochemical and biophysical techniques such as SDS PAGE and Western Blotting (Weeks 4-5), Mass Spectrometry and Negative Stain Transmission Electron Microscopy (Weeks 6-8)
3) Validate its function in vivo and in vitro using over-expression of mutant 7SK RNA defective in binding to its protein partners (Weeks 8-9).

This rotation will give the DTP student a taster for the full methodology of the proposed project.

Full PhD project description:

Importance
Regulatory RNA-protein complexes contribute substantially to cellular health and diseases. However, such complexes are often large, dynamic and short-lived, which makes it challenging to characterise their structure and function using conventional techniques. Thus, despite their importance, we have limited understanding of the role of individual non-coding, regulatory RNAs in maintaining cellular homeostasis or disease progression.

Aim
Through this BBSRC DTP programme, we aim to develop an integrated platform for enriching targeted native RNA-protein complexes (RNP) from cross-kingdom sources (including human, viral and plant systems) and characterising their structure and function using state-of-art biochemical and biophysical techniques. Towards this, we will leverage the core expertise of the supervisory team, the collaborators and the resources available at University of Nottingham and its partner institutions as described above. This project will be the first step towards our long-term goal of constructing a comprehensive and high-resolution structure-function interactome of native regulatory RNAs.

Work plan

Year 1:

Objective 1.1: Identify representative target RNPs from viral, human and plant systems
Methods: Cross-disciplinary literature review, bioinformatics analysis of existing databases

Objective 1.2: Enrich the selected RNPs in solution and on chip
Methods: Co-immunoprecipitation, RHyTEM.

Novelty: RHyTEM is a proprietary technique for solid phase enrichment that completely bypasses the heterologous expression, purification and in-vitro reconstitution methods for purifying molecules for structure determination. It is thus highly suitable for short-lived and heterogeneous systems, such as native RNPs, that cannot survive the conventional pipeline.

Risk Mitigation: Conventional purification techniques (Analytical Ultracentrifugation, affinity-based purification, UV-crosslinking and RNA-interactome capture) will be used where RHyTEM leads to inconclusive results.

Year 2:

Objective 2: Characterise the enriched RNPs
Methods: biochemical and biophysical techniques including RNA sequencing, Mass Spectrometry, 3D-OrbiSIMS and high-resolution Cryo-Electron Microscopy.

Risk Mitigation: Conventional Liquid Chromatography-Mass Spectrometry for proteomic analysis, negative stain Transmission Electron Microscopy (TEM) and computational modelling techniques will be used where high-resolution structural characterisation is not possible.

Year 3:
Objective 3.1: Validate RNP function
Methods: in vitro and in vivo functional assays such as enzyme kinetics, phenotyping, over-expressor cell lines or light microscopy.

Objective 3.2: Develop proof of concept for Targetted RNA-Protein Interactome (TRAP) Platform
Methods: Integrate RHyTEM enrichment technique with on-chip characterisation methods such as 3D-OrbiSIMS and TEM

Outcomes
1. Structure-function insights into key regulatory RNPs.
2. Development of novel, high-throughput platform for enrichment and characterisation of native RNPs.
3. High-impact publications and translation into Intellectual Property Rights protection for future commercialisation.

Host environment and project suitability
At UNot, we are equipped to perform protein-RNA biochemistry including all in-vitro assays and computational studies while at nmRC and RCaH, the student will be specifically trained in biophysics and EM techniques. This project is also part of a broader international programme for understanding the regulatory role of RNAs in health and diseases. Thus, the DTP student will be working within a highly dynamic, intellectually stimulating and resourceful environment and will benefit from cross-institution supervision in multidisciplinary techniques (interface of experimental biochemistry, biophysics, proteomics, systems biology and structural biology) from world experts.

References to learn more:

1. R. A. Knoener, J. T. Becker, M. Scalf, N. M. Sherer, and L. M. Smith, “Elucidating the in vivo interactome of HIV-1 RNA by hybridization capture and mass spectrometry,” Sci. Rep., vol. 7, no. 1, p. 16965, Dec. 2017.

2. D. Giss, S. Kemmerling, V. Dandey, H. Stahlberg, and T. Braun, “Exploring the interactome: microfluidic isolation of proteins and interacting partners for quantitative analysis by electron microscopy.,” Anal. Chem., vol. 86, no. 10, pp. 4680–4687, May 2014.

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(133) Elaborating the interactions between mesenchymal stem cells and immune cells in non-union bone fractures using a novel 3D printed in vitro model

Primary supervisor: Yasser El-Sherbiny

Second supervisor: Jing Yang

Institution: NTU

School: School of Science and Technology

Lab rotation description:

The first 9-week project will focus on hydrogels and 3D bioprinting. This project will involve encapsulating mesenchymal stem cells in various hydrogels including gelatin methacrylate, collagen, fibrin and the 3D bioprinting of cell-laden hydrogels. Rheological and mechanical properties of cell-laden hydrogels will be characterised. Cell viability, proliferation and differentiation will be characterised at different time points. Various assays that measure cell viability, cell number and differentiation towards osteogenic and chondrogenic lineages will be utilised. Confocal microscopy will be used to image cell morphology. The architecture of the 3D bioprinted cellular constructs will be purposely altered to study how the structure may affect cellular responses.

The second 9-week project will focus on immunology and immuno-cell biology interaction of MSCs and immune cells, in order to be validated in the 3D context. In this lab programme, the student will have a rota of multiple introductory techniques to learn and apply including mesenchymal stem cells extraction and co-culture with immune cells, MSCs multi-lineage differentiation mainly into Bone (osteogenic) and immunoregulation function determination using phenotyping by FACS laser analysis (flow cytometry) and advanced molecular techniques, selective immune cells extraction and purification, multiparameter FACS immunophenotyping and sorting, Immunological functional assays, cell cytotoxicity & tissue proliferation assay, microscopy, imaging and molecular procedures for gene expression using RT-PCR. Various assays will be applied to determine the secretome, transcriptome and proteomic profile of MSCs and immune cells associated with inflammatory milieu and reflecting the impact of 3D encapsulation and 2D culture impact on differentiation biomarkers and lineage commitments as well as immune cell function and fate.

Full PhD project description:

Bone is one of the tissues that normally heal without a scar. However, in some conditions, due to trauma or underlying systemic or local diseases, bone healing fails and becomes hard to treat. The time, effort and cost (£7,000- £79,000 per patient) needed to treat non-union bone fractures is considerably high. Therefore, a deep understanding of the biological events of bone healing is essential in order to introduce better therapies or to reduce or completely avoid complications of bone healing.

Bone healing is a natural spontaneous process happens after injury or tissue damage with three stages; inflammation, repair and remodelling. The inflammation phase is usually short (few days) and involves the formation of clotted blood hematoma at the site of bone fracture/loss, as a result of an associated vascular and tissue damage. The repair phase involves the differentiation of mesenchymal stem cells (MSCs) into chondroblasts forming a cartilaginous callus then into osteoblasts forming a hard callus. The remodelling phase involves a balanced osteogenic

differentiation of MSCs with the generation of bone-forming cells, osteocytes and the bone resorption by osteoclasts.

Focusing on the inflammatory phase, different immune cells are present within a haematoma and function to clear the damaged area from microbes and debris. Interestingly, recruitment and proliferation of MSCs at formed haematoma are facilitated by immune responses. MSCs can, in turn, limit the inflammatory response mediated by immune cells helping the progression to repair phase. Indeed, prolonged or chronic inflammation is always associated with complicated healing of bones. However, the exact sequences of events and molecular details on how MSC-immune cells interaction at the hematoma, as well as when populate scaffolds used for therapy, are largely unclear. Interestingly, isolated MSCs from the fibrous tissue of non-union fractures can differentiate into osteogenic and chondrogenic lineages in vitro. Therefore, it is intriguing to find out the molecular mechanisms that are responsible for the inability of these cells to form bone. Despite being important, the interactions between MSCs and immune cells particularly within hematoma or early repairing bone tissues is poorly understood.

Our hypothesis that the type and quality of cross-talk between immune cells and MSCs in non-union bone fractures contribute to the failure of bone healing.

A 3-dimensional in vitro model recapitulating the microenvironment where MSCs interact with innate and adaptive immune cells will be a powerful tool to understand the molecular details that control processes such as repair. Biomaterials such as fibrin, collagen and gelatine are able to affect cellular responses via their chemical and physical properties. 3D bioprinting is a versatile tool to rapidly fabricate in vitro models that recapitulate the chemical, physical and architecture of non-union fractures. This project will combine immunology and 3D bioprinting in a non-union context to study interactions between MSCs and immune cells, particularly macrophages and T helper type 2 (Th2) cells.

References to learn more:

1. Bajada S1, Marshall MJ, Wright KT, Richardson JB, Johnson WE. Decreased osteogenesis, increased cell senescence and elevated Dickkopf-1 secretion in human fracture non-union stromal cells. Bone. 2009 Oct;45(4):726-35. doi: 10.1016/j.bone.2009.06.015. Epub 2009 Jun 18.

2. El-Jawhari JJ, Kleftouris G, El-Sherbiny Y, Saleeb H, West RM, Jones E, Giannoudis PV. Defective Proliferation and Osteogenic Potential with Altered Immunoregulatory phenotype of Native Bone marrow-Multipotential Stromal Cells in Atrophic Fracture Non-Union. Sci Rep. 2019 Nov 22;9(1):17340. doi: 10.1038/s41598-019-53927-3.

Location of lab rotation: Clifton Campus; University Park

Location of full PhD project: Clifton Campus; University Park

(135) Investigation of the biological mechanisms governing meat chicken growth and development in early life

Primary supervisor: Cormac O'Shea

Second supervisor: Emily Burton

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

Title: Quantifying the impact of egg laying sequence and incubation conditions on early post-hatch development in meat poultry.

While the linked PhD focuses on understanding and quantifying impacts of the pre- and post-incubation conditions in a highly controlled commercial hatchery on body weight and chick uniformity, this project explores the impact of egg sequence and altering incubation temperature and humidity on hatch weight, early post-hatch behaviour and digestive enzyme activity in broiler chicks. Recent pilot data obtained from working with the largest chicken meat producer in the UK (P.D. Hook) show that the sequence of egg in a clutch may be a strong, and as yet undescribed, source of variation in the subsequent growth of broiler chicks.

Project outline:
Incubators will be used to incubate batches of chicken eggs to day 16, when a small adjustment to either temperature or humidity will be made for 5 days and compared to a control.

Project will involve:
conducting incubator adjustments during the final week of incubation
behavioural assessment of activity budgets during first week post hatch
post mortem assessment of digestive enzyme activity and related gene expression via qPCR.

Based at the NTU and UoN.

Full PhD project description:

Chicken meat provides an economical, safe and nutritious source of protein for the human food chain: currently 1 billion birds are reared for food each year in the UK alone. However, the poultry meat sector faces substantial challenges associated with both bird welfare and infectious diseases associated with food-borne pathogens. Control of uniformity in growth is critical as low bodyweight animals are more vulnerable to infection from zoonoses, aggression from heavier birds, and represent a loss in the efficient conversion of feed to meat for the production cycle.

The growth and development of broiler chickens is strongly influenced in early life with pre- and post- incubation events governing final bodyweight and uniformity. The development of the chick in the 1st week of life is a critical window which dictates the subsequent growth trajectory. Despite this understanding, there is a lack of multi-disciplinary research conducted to identify the key events and drivers which influence chick outcomes in the post-hatch period. Producers, nutritionists, veterinarians and technical advisors are restricted to an understanding of broilers as groups of animals in order to implement strategies to improve broiler health and growth outcomes.

This PhD project proposes to adopt an experimental model whereby individual birds, ranked on the basis of early life growth and development, will be isolated and studied as individuals and in groups. The overall aim of this project will be to characterise the immune status, microbiology and digestive physiology of birds which exhibit extremes of performance in early life and implement targeted strategies based on these findings to improve overall flock growth, health and uniformity. This approach will integrate methodologies from several disciplines encompassing immunology, microbiology and nutrition to provide a more holistic overview of the factors impacting broiler flocks.

The initial stages of this PhD will be to characterise the growth and morphometry of broiler chicks in early life. Distinct sub-groups of broilers who exceed or fail to achieve predicted growth and development trajectories in early life will be formed and investigated in more detail. Assessment of intestinal transcriptomics, gastrointestinal physiology and caecal microbiome will be undertaken. In addition, behavioural aspects related to appetite, energetics and social hierarchy will be monitored. The latter portion of the PhD will use dietary and management strategies derived from the initial studies to influence and improve flock development from early life onwards.

The overall objectives of this PhD are to:

i) Understand the environmental and biological factors governing meat chicken development and growth in early life

ii)Develop dietary and management strategies to improve broiler flock uniformity, health and welfare.

References to learn more:

1. Metzler-Zebeli, B. U., A. Molnár, M. Hollmann, E. Magowan, R. J. Hawken, P. G. Lawlor and Q. Zebeli (2016). "Comparison of growth performance and excreta composition in broiler chickens when ranked according to various feed efficiency metrics1." Journal of Animal Science 94(7): 2890-2899.

2. Lindholm, C., J. Jönsson, A. Calais, A. Middelkoop, N. Yngwe, E. Berndtson, J. J. Lees, E. Hult and J. Altimiras (2017). "Growth heterogeneity in broiler breeder pullets is settled before the onset of feed restriction but is not predicted by size at hatch." Journal of Animal Science 95(1): 182-193.

Location of lab rotation: Sutton Bonington; Brackenhurst Campus

Location of full PhD project: Sutton Bonington; Brackenhurst Campus

(136) Synthesis and Application of Hybrid Peptide-Polyoxometalate Clusters as Novel Therapeutic Agents

Primary supervisor: Graham Newton

Second supervisor: Nicholas Mitchell

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

This rotation will investigate the grafting of a range of therapeutically-relevant peptides onto polyoxometalate (POM) clusters, and the exploration of the properties of these novel hybrid nanomaterials. The candidate will develop skills in solid-phase peptide synthesis (SPPS), metal oxide chemistry, electrochemistry, analytical chemistry (HPLC, mass spec.) and reaction development. This rotation would suit a candidate interested in the application of materials science to therapeutics and biotechnology.

During the rotation, several peptides will be prepared that will enhance the cytotoxicity of POM clusters and enable cellular targeting and uptake; e.g. cell-penetrating peptides, antimicrobial peptides and sequences targeting the cell-surface protein receptor, HER2. Initially, these peptides will be used as model fragments to enable the development of a general ligation method that can be applied to graft peptides, and even proteins, to POM clusters.

Peptide ligation techniques will be applied to chemoselectively attach the peptides to the cluster. Such techniques are routinely used to provide access to large peptide/small protein sequences, however the application of this chemistry to materials science is underexplored. Once a ligation method has been successfully developed and optimised, the spectro-electrochemical properties of the material will be fully explored using electrochemistry and UV/Vis spectroscopy.

Full PhD project description:

Polyoxometalates (POMs) are nanoscale molecular metal oxides, typically constructed from tungsten or molybdenum. They exhibit rich electrochemistry and can act as reservoirs for multiple electrons and protons simultaneously. When reduced, POMs exhibit specific absorption spectra that act as spectrochemical ‘fingerprints’. They can be controllably functionalised to yield organic-inorganic materials whose solution-phase behaviour, electrochemistry, and supramolecular chemistry can be conveniently controlled. The ability to ‘tune’ the properties of these highly redox-active materials has led to the diverse application of these clusters within materials science.

Recently, attention in this field has turned towards the biological applications of POMs; various clusters have demonstrated cytotoxicity towards cancer cell lines, viruses, and bacteria. As well as inherent toxicity, POMs can be reduced using UV light to access highly reactive excited species that may be able to interrupt vital cellular processes. Furthermore, it has been demonstrated that certain POMs are able to cross the blood-brain barrier, presenting the possibility to target pathology in the brain using the POM as the therapeutic agent directly, or as the delivery vehicle. Whilst the biomedical applications of POMs are now being realised, this area is still in its infancy, with little understanding regarding the biological mode of action of these clusters. The literature precedence mainly involves unmodified POM clusters, however, the ability to graft organic molecules onto the

POM framework presents an opportunity to prepare hybrid materials that may find impactful application as therapeutic agents. The biological specificity and activity of peptides make these biopolymers the ideal partner to enhance the biologically-relevant properties of POMs.

This project seeks to develop a novel general ligation method to enable the chemoselective grafting of any peptide, regardless of amino acid sequence, onto the POM structure. Due to the diverse array of chemical functionality displayed by the 20 canonical amino acids, many standard bioconjugation techniques can be employed to attach peptides to POMs. However, an effective and reproducible method, that retains the native activity of the sequence, must be employed to enable libraries of peptide-POM conjugates to be prepared. Peptide ligation is a powerful synthetic method, routinely used to assemble multiple peptides to provide access to large peptide sequences and small proteins. The application of this chemistry to materials science is underexplored; the repurposing of this reaction for our requirements would enable the attachment of any peptide sequence by its C-terminus, regardless of sequence. Therefore, this single method can be used for any peptide without compromising the secondary/tertiary fold of the sequence or limiting access to the peptide for binding partners. This technique can even be applied to the attachments of large proteins, including antibodies.

To explore the utilisation of POMs in therapeutics, a number of biologically-relevant peptides will be synthesised and grafted to a small library of POMs using this strategy. Cell-penetrating peptides, cytotoxic sequences, antimicrobial peptides and peptides that bind to cell-surface proteins overexpressed in numerous cancer types, such as HER2, will be prepared. Once optimised, a general method of ligation will be used to attach these sequences to a range of POMs. The cytotoxicity of these conjugates will be accessed in bacteria and a range of cancer cell lines via established collaborations within the University of Nottingham.

References to learn more:

1. D. Ni et al., Nano Lett. 2017, 17, 3282−3289

2. C. Yvon et al., Angew. Chem. Int. Ed. 2014, 53, 3336 –3341

Location of lab rotation: University Park; Jubilee Campus

Location of full PhD project: University Park; Jubilee Campus

(138) Application of a Novel Site-Selective Protein Bioconjugation Method in Cancer Therapeutics and Imaging

Primary supervisor: Nicholas Mitchell

Second supervisor: Robert Layfield

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

This rotation will involve the expression of a selenocysteine-containing antibody (selenomab) and the utilisation and evaluation of a novel bioconjugation method, previously developed by the group, to prepare protein-drug and imaging agent conjugates for evaluation. The project will suit a biosciences/molecular biology graduate or a chemist with an interest in protein chemistry.

An anti-HER2 selenomab containing two selenocysteine (Sec) residues will be expressed in mammalian cell lines and our novel conjugation technique optimised on this protein with a model probe. The conjugation is rapid, operationally simple and chemoselective for Sec over all other native proteinogenic functionality. Once quantitative modification has been confirmed, a number of bespoke probes will be utilised to functionalise the protein with a range of desired moieties including a peptide that allows penetration of the blood-brain barrier, a cytotoxic drug, stabilising agents such as PEG polymers and a group that can be readily radiolabelled with iodine-123/125.

The rotation can be tailored to the skills of the candidate; a chemistry graduate will have the opportunity to synthesise the bespoke probes while these probes can be provided for a biosciences graduate if preferred. Full training in all the relevant techniques will be provided.

Full PhD project description:

The value of the biologic drugs market is estimated to be in excess of $220 bn; currently, seven of the top ten best-selling drugs on the market are proteins. As the healthcare industry increasingly moves towards a reliance on biologics the development of synthetic tools to enable the functionalisation of proteins becomes increasingly relevant. The chemoselective functionalisation of the native amino acids has been a long-standing goal of the synthetic chemistry community. The introduction of contrast agents, cytotoxic groups, affinity probes, and stabilising agents enable researchers to further explore and exploit the remarkable selectivity and affinity inherent to proteins.

A broad range of powerful methods that target many of the canonical amino acids has been reported in the literature. The most well-utilised methods target cysteine (Cys); the enhanced nucleophilicity of the thiol group of this residue allows for chemoselective functionalisation and the low level of expression of Cys across the proteome ensures production of minimal modified isoforms. Cys mutagenesis can be employed to incorporate non-native Cys residues into a protein sequence for modification, however, due to the importance of native Cys residues in protein activity and folding, off-target modification of these residues can be detrimental to the efficacy of the protein.

An underexplored avenue towards site-selective peptide and protein modification involves

application of the natively expressed Cys surrogate, selenocysteine (Sec). Sec, known as the 21st proteinogenic amino acid, offers the chemoselectively of Cys with enhanced nucleophilicity. Present in peroxidase and reductase enzymes, the Sec residue plays a key role in protecting the cell from oxidative damage, enabled via a low redox potential (-381 mV). Native expression is rare, to-date only 25 selenoproteins have been identified in the human proteome. Ribosomal incorporation of this amino acid is controlled by the opal stop codon UGA when present with the selenocysteine insertion sequence (SECIS) RNA motif and a Sec-specific synthase. Exploitation of appropriately engineered tRNA enables the installation of Sec via recombinant expression in mammalian cell lines.

We have developed a Sec-selective bioconjugation reaction that is rapid, operationally simple and versatile. This PhD project seeks to further develop and apply our novel bioconjugation chemistry to the functionalisation of proteins with a focus on radio-labelling for application in medical imaging (cancer) and drug-conjugation for utilisation in cancer therapeutics. Antibodies bearing Sec residues (selenomabs) will be expressed using mammalian cell lines and modified with a range of bespoke probes to enable targeted delivery of cytotoxic agents, polymers to enhance stability, groups to facilitate rapid radiolabelling and peptide sequences to enable penetration of the blood-brain barrier. The protein conjugates will be fully characterised and the stability of the linkers evaluated in biological serum. The efficacy of the various conjugates in targeting and killing a range of cancer cells will be assessed, in vitro. Radiolabelling of the conjugates will allow targeted imaging using tumour models (spheroid aggregates). Ultimately, this project will assess the suitability of our technology as a versatile method to prepare therapeutic agents and diagnostic tools for the clinic.

References to learn more:

X. Li et al., Cell Chem. Biol. 2017, 24(4): 433–442

Location of lab rotation: University Park; QMC

Location of full PhD project: University Park; QMC

(139) Defining how the spinal cord endothelial cell modulates pain perception

Primary supervisor: Richard Hulse

Second supervisor: Jeanette Woolard

Institution: NTU

School: School of Science and Technology

Lab rotation description:

Pain is an integral protective mechanism however in certain instances pain is detrimental to the individual. Pain is highly prevalent in the United Kingdom population, and unfortunately is increasingly prevalent due to an ageing population, which is increasingly susceptible to metabolic disorders such as diabetes and obesity, factors that exacerbates the onset of pain. The microvasculature is essential for maintaining a healthy nervous system permitting normal neuronal function. An adequate tissue blood supply is maintained by microvessel perfusion, where a failure in coupling neuronal metabolic demand with capillary perfusion underpins the development of neurodegenerative disease. Our rodent models of vascular degeneration have demonstrated that loss of endothelial cell Vascular Endothelial Growth Factor receptor 2 signalling leads to reduced capillary perfusion of the dorsal horn in the spinal cord and consequently development of pain. During this lab rotation research project primary cell culture of rodent spinal cord endothelial cells will be utilised to evaluate how these cell types respond (QPCR expression profiling of metabolic and angiogenic markers, cytoarchitectual changes through confocal microscopy) to changes in blood perfusion termed shear stress and these responses will be compared to an adipokine rich environment (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 pain. These experiments will directly feed into outlined PhD program of work through acquisition of laboratory training and working in the outlined research areas.

Full PhD project description:

28 million people in the United Kingdom suffer from pain, with the prevalence increasing with age. The level of susceptibility to pain development is further exacerbated by concomitant metabolic pathological conditions such as diabetes mellitus and obesity. Unfortunately, current painkillers provide poor pain relief, whilst these drugs also causing significant adverse effects.

It is increasingly 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 smooth muscle cells and pericytes, which act in harmony with the endothelium to regulate tissue perfusionand quiescence (state of non-proliferation). However, dysfunction in this coordinated communication is implicated in age and metabolic disturbances in neurological disease. Pericytes are widely regarded as integral in maintaining capillary architecture, with pericyte dysfunction characteristic of vascular disease. Recent evidence also highlights that pericyte mediated contractility underlies reduced neural tissue perfusion and neurodegeneration.

The vasculature is highly adaptable in response to a number of stimuli, undergoing vascular expansion and remodelling during development and pathological conditions. However, vascular regression, which curtails such expansion, arises in areas of reduced blood flow due to occlusion or

narrowing (ie constriction or blockage) of microvessels induces degeneration of neighbouring endothelial cells; initiating vascular pruning. This is attributable to impaired angiogenic response through for example reductions in VEGF-A/VEGF receptor 2 signalling. Our work has identified that spinal cord sensory neurodegeneration and chronic pain arises due to a loss of endothelial derived VEGFR2 signalling in the spinal cord. We have identified that capillary occlusion is mediated by pericyte derived vasoconstriction to instigate the development of vascular pruning. This work highlights that the capillary network in the dorsal horn is essential in the regulation of pain perception.

Our hypothesis is that disturbances in pericyte activation induces vasoconstriction and cessation of blood flow in the dorsal horn at the level of the spinal cord, inducing pain. This project will investigate key concepts associated with pericyte mediated cessated blood flow and the adaptive stress response of the spinal cord endothelial cells. Training will be provided in primary cell culture, biochemical assays and invitro assays to model blood flow to evaluate the interplay between pericytes and endothelial cells in relation to capillary integrity. Rodents models of vascular degeneration (inclusive of high fat, type 2 diabetes rodent models) and intravital imaging of the nervous system will be utilised to evaluate how the endothelium responds to pericyte mediated occlusion to underlie sensory neurodegeneration. The supervisory team have a diverse array of experience in vascular biology and neuroscience to evaluate those mechanisms outlined in this proposal.

References to learn more:

1. *N. Ved, M.E. Da Vitoria Lobo, *S.M. Bestall, C.L. Vidueira, N. Beazley-Long, K. Ballmer-Hofer, D.O. Bates, L.F Donaldson and R.P. Hulse* (2018) Diabetes-induced microvascular complications at the level of the spinal cord; a contributing factor in diabetic neuropathic pain. J Physiol. PMID: 29774557 DOI: 10.1113/JP275067
2. Nicholas Beazley-Long, William Robert Ashby, Catherine Elizabeth Moss, Samuel Marcus Bestall, Fatimah Almahasneh, Alexandra Margaret Durrant, Andrew Vaughan Benest, Zoe Blackley, Kurt Ballmer-Hofer, Masanori Hirashima, Richard Philip Hulse, David Owen Bates, Lucy Frances Donaldson VEGFR2 promotes central endothelial activation and the spread of pain in inflammatory arthritis (2018) Brain, Behavior, and Immunity. PMID: 29548992. DOI: 10.1016/j.bbi.2018.03.012

Location of lab rotation: Clifton Campus

Location of full PhD project: QMC; Clifton Campus

(140) Metabolomic profiling of neurodevelopmental programs in healthy brain which promote dysregulated neural progenitor growth

Primary supervisor: Ruman Rahman

Second supervisor: Dong-Hyun Kim

Institution: University of Nottingham

School: Medicine

Lab rotation description:

Title: How does the brain extracellular matrix (ECM) affect the metabolism of neural cells?

To determine how biochemical signals from the brain microenvironment influences the metabolic viability of healthy or dysregulated, transformed neural stem cells, decellularised extracellular matrices will be prepared from cadaver human brain. Both healthy and cancerous human neural stem cells and lineage-restricted astrocytic progenitors will be grown on decellularized brain ECM and metabolic rate measured quantitatively.

Electron-microscopy will be used to visualise ligand-receptor-mediated interactions between neural (healthy or transformed) cells and decellularized brain ECM, which underpin anticipated differences in metabolic rate.

Next, to investigate whether the brain microenvironment promotes neurotransmission, fluorescently-tagged neural stem cells and non-tagged brain cancer stem cells will be co-cultured on decellularized brain ECM and separated by fluorescence-activated cell sorting after 1-week. Quantitative RT-PCR will be used to measure the expression of key glutamatergic (Gria1, Gria 2) and dopaminergic (Drd3, Drd4) genes and compared statistically to gene expression from monocultures.

Skills and knowledge acquired during rotation: neural stem cell culture; metabolic viability assays; electron microscopy, quantitative RT-PCR.

Full PhD project description:

Background:

Recent 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 neural progenitor cells. This is most strikingly exemplified by synaptic neurotransmission between neurons and immature cancerous cells in the brain which resemble neural stem/progenitor cells [1].

Whilst neurotransmitters such as glutamate released from healthy neuronal structures in the brain, can enhance the survival and infiltration of cancerous cells into the surrounding brain parenchyma [2], the underlying functional relationship remains unclear. Metabolomics is the study of chemical processes involving metabolites and is an integral technology for understanding the function of biological systems. To date however, no metabolomic studies to elucidate fully the biochemical communication between healthy and dysregulated brain cells have been conducted.

Importance of study:
For malignant brain cancers such as glioblastoma, patients are asymptomatic during early stages of disease, likely explained by cancer cells associating functionally with healthy neural cells. This means that for improved therapy, we need to study how neuroscience and aberrant neural stem cell/progenitor biology promotes phenotypes (migration and invasion) characteristic of brain cancer.

Hypotheses:
Neural communication between healthy astrocytes and dysregulated, transformed neural progenitors is characterised by aberrant metabolomic signatures.

Workplan:
(Months 1-24): Aim 1: Develop 3D co-cultures of human astrocytes and infiltrative glioblastoma cells which can be interrogated to reveal the underlying metabolomes.
We have established a complex 3D cellular model which recapitulates the interaction between normal brain and post-surgical glioblastoma cells. CRISPR-Cas9 was used to stably introduce a transgene containing the glial fibrillary acid protein promoter and enhanced yellow fluorescent protein (YFP) gene, into human neural stem cells, which express the GFAP transgene upon astrocytic maturation. To ensure physiologically-accurate signalling from the tumour microenvironment, cells are placed onto decellularized human brain extracellular matrix within.
To investigate whether astrocytic-cancerous communication varies during distinct neurodevelopmental states, patient-derived glioblastoma cells will be maintained in conditions which enrich either neural stem cells or lineage-committed progenitor cells. Based on YFP transgene expression, cancer stem/progenitor cells and astrocytes will be isolated by fluorescence-activated cell sorting (FACS) upon periods of co-culture to promote biochemical cross-talk and neurotransmission. Liquid-chromatography mass spectrometry (LC-MS) will be conducted on sorted astrocytes and cancerous cells to determine the metabolome of each cell type
(Months 25-42): Aim 2: Validate results by analysing the metabolome of astrocytes and cancer stem cells isolated from surgically resected tissue of human glioblastoma patients.
5-aminolevulinic acid (5-ALA), a metabolic substrate given to glioblastoma patients prior to surgery, is converted to a fluorescence-emitting product in cancer cells only and permits the isolation of infiltrative diseased tissue which blends into healthy brain parenchyma. Tissue isolated in this manner, will be subjected to FACS to separate brain cancer and healthy astrocytic cells. LC-MS will determine metabolomic profiles which reflect a molecular snapshot when residual cancer cells interact directly with normal neurodevelopmental signals in the surrounding brain.

Outputs:
• Graduate immersed with inter-disciplinary cellular biology and analytical bioscience expertise.
• Two high-impact publications.
• Presentation at global neurobiology conference.

References to learn more:

1. Venkatesh HS et al. Electrical and synaptic integration of glioma into neural circuits. Nature 573(7775): 539-545 (2019).

2. Venkataramani V et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. Nature 573(7775):532-538 (2019).

Location of lab rotation: University Park

Location of full PhD project: University Park

(141) Tuning dietary fibre structure and composition to improve the glucose absorption curve of starchy foods

Primary supervisor: Gleb Yakubov

Second supervisor: Huw Williams

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

The project will examine in vitro starch hydrolysis in the presence of different fraction of Plantago (psyllium) dietary fibre. Within 9 week, the student will perform extraction, fractionation and enzymatic modification of Plantago dietary fibre. Using an in vitro hydrolysis reactor, the student will perform quantification of starch hydrolysis kinetics as a function of dietary fibre concentration and composition. The student will also be familiarised with rheological techniques which are necessary to develop solution with 'matching' viscosity in order to control the effect of viscosity on the rat of hydrolysis. In addition, the student will perform microscopic analysis of starch granules to establish differences in the morphology of starch granules during hydrolysis in the presence of dietary fibre. Microscopy tools will be utilised to examine non-specific binding of amylase to the dietary fibre molecules and microparticles. The outcomes of this project will form basis of future research and will equip student with key physicochemical techniques used in food and nutrition research.
TRAINING POTENTIAL
Student will receive in depth training in the area of food, nutrition, carbohydrate characterisation and rheology. Further they will be introduced to the key concepts of advanced microscopy, NMR, MR imaging.

Full PhD project description:

1. RESEARCH EXCELLENCE
Two of the most common health problem in developed countries are diabetes mellitus and irritable bowel syndrome (IBS). Dietary control of diabetes aims to reduce variability in blood glucose. One of the methods for controlling glucose release is dietary fibre, which may slow down starch hydrolysis and decrease the rate of small sugar absorption from the small intestine into the blood stream. However, the mechanisms by which dietary fibre influence diabetes and IBS lead to a conflicting scenario, whereby reduction in the starch hydrolysis that reduces blood glucose worsens symptoms of IBS. When DF and undigested starch pass to the colon, they are exposed to the colonic microbiota, causing excessive gas production due to rapid fermentation leading to bloating and discomfort. Thus, an ideal dietary fibre would slow down starch hydrolysis and reduce trans-mucosal transport of small carbohydrates, while limiting rapid colonic fermentation.
Dietary fibre (DF) is the complex mixture of polysaccharides that are resistant to digestion and absorption in the small intestine. DF vary in their ability to bind (non-specifically) digestive enzymes and to modify the viscosity and hydration of the chyme. However, viewing ''dietary fibre'' as a single dietary component hinders progress in targeted utilisation. The recent review by Gidley & Yakubov (2019) highlighted that a simple, chemistry-based classification of dietary fibre into soluble and insoluble has numerous deficiencies, hampering the mechanism-based understanding of the mechanisms of dietary fibre function.
This project aims to identify a physicochemical mechanism by which dietary fibre interfere with the starch hydrolysis and influence the trans-mucosal transport of small carbohydrates. In addition, the

project is set to explore the mechanisms of starch encapsulation by dietary fibre polymers in order to provide new food structuring avenues for controlling the rate of colonic fermentation.
1.1. Hypothesis. DF control blood glucose via (i) non-specific binding of DF to starch surfaces, which shields it from amylase; (ii) non-specific binding of DF to amylase; and (iii) interaction of DF with mucus, which leads to a more selective barrier that renders slower trans-mucosal transport of simple sugars.
1.2. Importance & Timeliness. Xylan-based DF can be designed with targeted binding properties by altering the motif of side-chain substitution.
1.3. Transformative potential. Fundamental insights will enable identifying novel DF structures that specifically target diabetes and IBS. Opportunity to translate to the human population, particularly in older individuals, and thereby inform policy and the potential benefits of ingredients that are currently under-utilised.
1.4. Innovation. 1) Utilisation of Plantago xylan platform developed by PI Yakubov. 2) Characterisation methods including high resolution NMR (Huw Williams). 3) Application of MR imaging to monitor gas production (Giles Major).
2. OBJECTIVES
Objective 1. Establish the effect of DF structure and composition on the adsorption of DF onto the starch surface. Evaluate how DF adsorption influences amylase-assisted hydrolysis in vitro.
Objective 2. Establish the effect of DF structure and composition on the binding with amylase under simulated intestinal environment.
Objective 3. Explore the effect DF structure and composition on the barrier properties of mucus, and ascertain the parameters of trans-mucosal transport of model simple sugars (glucose, sucrose).
Objective 4. Explore the effect of DF adsorption onto the starch surface on the hydrolytic activity of bacterial glycosidases.
Objective 5. In healthy volunteers, use MR imaging of GI content to show colonic gas production after ingestion of starch/DF formulations, while ascertaining glucose absorption profile.
4. SUPPORTING FRAMEWORK
The project will share several aspects with the funded BBSRC grant BB-T006404-1 ‘Biophysical defence in the mammalian gut'

References to learn more:

1. Gidley MJ & Yakubov GE ‘Functional categorisation of dietary fibre in foods: Beyond ‘soluble’ vs ‘insoluble’’, Trends in Food Science & Technology, 86, 563-568 (2019)

2. Major G 'Fermentable Carbohydrates in IBD - Trouble Brewing?', Journal of Crohns and Colitis, 11, 1405-1406 (2017)

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: University Park, QMC, Sutton Bonington Campus

(142) Drug targets under stress: post-transcriptional regulation of RTKs

Primary supervisor: Keith Spriggs

Second supervisor: Anna Piccinini

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

Protein production is typically switched off when cells are stressed. However, some protein synthesis is required for a cell to mount an appropriate response to stress. Many stress response proteins, when expressed incorrectly, are associated with disease. We recently identified a mechanism by which EGFR (epidermal growth factor receptor) evades the normal stress induced global translation shutdown, and how it can continue to drive proliferation. EGFR is one of a group of related receptor tyrosine kinases (RTKs), but less is known about the post-transcriptional regulation of the rest of the family despite many of them being implicated in diseases, notably cancers. The lab rotation will be structured as follows Week 1-3: Amplification and cloning of RTK family members into luciferase reporter constructs Week 3-6: Cell culture, transfection of reporter constructs and assay. The effects of several cell stresses will be compared. Week 7-9 Mutagenesis and further hypothesis testing, directed by the student based on data obtained. In parallel, the student will explore some bioinformatic analysis of receptor tyrosine kinase sequences, looking for associations with disease and for conservation of potential regulatory elements.

Full PhD project description:

Regulatory elements in the untranslated regions (UTRs) of messenger RNAs allow rapid responses to cellular stresses and other stimuli. This type of regulation allows cells to respond to stresses, but can also be linked with inappropriate gene expression and disease. However, a better understanding of the regulation of genes associated with disease also allows us to identify novel drug targets and to design improved therapies for proliferative disease. The principle hypothesis is that genes required in the response to cell stresses will be resistant to the downregulation of gene expression that typically accompanies cell stresses. This project will use a combination of bioinformatic and laboratory tools to investigate how receptor tyrosine kinases respond to cell stresses. You will clone the human genes for these growth factor receptors and use a combination of reporter assays and mutational analysis to identify regulatory elements in the 5’ and 3’ UTRs. You will get training in a broad range of molecular biology techniques, in addition to specialised methods such as polysome profiling. Training in bioinformatic methodologies will add an extra dimension to your research and give you much sought after skills in this rapidly growing area of pharmacy research. You will be encouraged to design strategies to target the regulatory elements identified in the early part of the project, for example using modified complementary oligonucleotides. In the unlikely event that no post-transcriptional control is observed in response to stress (which itself would be a notable finding), we will expand the search to include other cancer drug targes. The project would take place in a well-resourced laboratory shared with several other research groups interested in gene regulation and RNA biology. We foster a supportive and collegial research environment where honest communication and constructive discussions are encouraged, and where expert advice is readily available.

References to learn more:

1. Spriggs KA, Bushell M, Willis AE. Translational regulation of gene expression during conditions of cell stress. Mol Cell. 2010 Oct 22;40(2):228-37. doi: 10.1016/j.molcel.2010.09.028. Review. PubMed PMID: 20965418.

2. Le Quesne JP, Spriggs KA, Bushell M, Willis AE. Dysregulation of protein synthesis and disease. J Pathol. 2010 Jan;220(2):140-51. doi: 10.1002/path.2627. Review. PubMed PMID: 19827082.

Location of lab rotation: University Park

Location of full PhD project: University Park

(143) Precision programming of cell fate by 'optical air-brushing'

Primary supervisor: Amanda Wright

Second supervisor: James Dixon

Institution: University of Nottingham

School: Engineering

Lab rotation description:

The lab rotation will continue to develop the initial proof-of-concept that genes can be delivered to cells at a sub-cellular resolution using magnetic nanoparticle technology and optical pressure by automated microscopy. This has implications for generating tools to interrogate developmental biology and stem cell niches on a single cell level. The collaboration between optics expert (Amanda Wright) and gene therapist (James Dixon) will be key for of the development of this project.

You will culture and program stem cells (iPSCs, MSCs) with non-viral gene delivery initially of reporter genes then transcription factors which will trigger the terminal differentiation spatially and in a patterned manner. You will do this in conventional monolayer cultures that have been adapted for this optical patterning of the gene therapy with the aim to use the technology to mimic gene expression during embryogenesis and in stem cell niches of adult tissues. A powerful technology to drive the expression of genes in specific patterns and cell locations in a culture, scaffold or embryo will be vital in understanding and manipulating potency and stem cell biology.

Full PhD project description:

PROJECT AIM: Problem & Solution: The stem cell and regenerative medicine research communities are currently lacking technologies that control cell-fate at the single-cell level. Such approaches are crucial to successfully mimicking of the cellular micro-environment and processes such as embryogenesis (embryo formation and development) in-vitro. This collaboration and PhD project aims to make real-time single-cell patterning of stem cell-fate a reality.

Approach: Magnetic nanoparticles (MNP) show great promise as therapeutic delivery vehicles, delivering drugs to stem cells that influence their structure and function. Current demonstrations of MNPs operate on the cell culture as a whole, and do not possess the positional dexterity required to influence individual cells. We are establishing a revolutionary new tool for 'optically air-brushing' MNPs onto the surface of single-cells, resulting in controlled patterning of stem cell behaviour with micron-scale position. We aim to will generate a step-change in capability by demonstrating user-defined positional control of stem cell-fate.

PROJECT OUTLINE: Technologies for controlling cell-fate have revolutionised in-vitro models, providing vital improvements in our ability to generate patient-matched stem cells and mimic the complex cell-cell microenvironments encountered in-vivo. However, these powerful genetic re/programming methods are at the resolution of entire cultures. This is in stark contrast to the in-vivo case where embryo and organ formation are intricately controlled with single-cell precision. Mimicking such processes are important in developmental biology and regenerative medicine, which strive to, for example, engineer authentic tissues or to repair tissue in-vitro.

Nanoparticle (NP) technology can increase drug delivery to cells but not at resolutions needed to

recreate complex biology. We have an efficient drug-delivery system using magnetic (M)NPs termed Glycosaminoglycan-binding enhanced transduction (GET) (Dixon et al.). In an exciting recent discovery, the optical scattering force from a laser was used to generate a jet-stream of MNPs, propelling the particles forward, in the direction of laser propagation, and air-brushing them onto the surface of a single-cell. This discovery is timely, and has the ability to make an important contribution to stem cell and regenerative medicine research effort at Nottingham. You will work on further development of the technology. The optics team (lead Dr Wright: AJW) has developed a new module, compatible with an existing microscope, maximising the optical scattering force & enabling high-resolution efficient positioning of MNPs. The biological team (lead Dr Dixon: JED) is conducting cell programming experiments. The teams will supervise the student to maximise MNPs delivery, cell viability & positional dexterity; culminating in the demonstration that single pluripotent cells (induced pluripotent stem cells; iPSCs) can be pushed to resemble cells seen in early embryos.

Location: The project will be carried out in the new state-of-the-art Biodiscovery institute at University Park (James Dixon) and the Faculty of Engineering, Optics and Photonics Research Group (Amanda Wright).

Techniques and opportunities: This work will include some or all of the following techniques, depending on the ability of the student and their progress: Optical MNP manipulation, Gene transfection, Peptide-mediated cell transduction, Reporter gene analyses, Directed differentiation, Flow cytometry, Stem cell culture/differentiation

References to learn more:

1. Dixon JE et al. PNAS. E291-9, 2016,

2. Kirkham G et al. Sci Rep. 5. 8577. 2015

Location of lab rotation: University Park

Location of full PhD project: University Park

(144) How are cellular auxin levels regulated at transcriptional level?

Primary supervisor: Ute Voss

Second supervisor: Anthony Bishopp

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

Auxin is a developmental signalling compound in land plants. Maintaining correct cellular auxin concentrations is critical for almost all aspects of normal plant development. This is achieved through an interplay of auxin synthesis, degradation, transport, and perception. However, we still have little insight into how degradation contributes to controlling cellular auxin levels.

The project will start with some theoretical introduction to plant molecular physiology and auxin biology and introduce the student to a broad range of molecular biology and phenotyping techniques:

Week 1-4: Initially the student will use Arabidopsis thaliana auxin degradation mutants and compare their performance (growth, survival, root and shoot architecture) to wildtype. This will introduce the student to:
- Growing and monitoring plants in controlled conditions (plant physiology).
- Semi-automated phenotyping.
- Basic image analysis techniques, including statistical data analytics and numeracy skills.
- Plant genotyping (DNA extraction and PCR).

Week 4-9: Based on results from week1-4, we will select mutants for initial analysis of the molecular processes underlying this auxin phenotypes. This will include
- Compare how expression levels of candidate genes change in wildtype and mutants (RNA extraction, qRT-PCR).
- Microscopy to follow how and where gene expression and auxin levels change on cellular level.

Full PhD project description:

Background
Auxin is an important developmental signalling compound in land plants. Maintaining correct cellular auxin concentrations is critical for almost all aspects of normal plant development. This is achieved through an interplay of auxin synthesis, degradation, transport, and perception. Even though breakthroughs have been made dissecting the molecular basis of auxin synthesis, transport, and perception, we still have little insight into how degradation contributes to controlling cellular auxin levels.

Auxin degradation takes 3 main forms. The first two are conjugation, to either sugars or amino acids. However, metabolic data suggest that auxin oxidation is the major auxin degradation pathway. We recently identified the two major auxin oxidising enzymes in the model plant Arabidopsis thaliana: DAO1&2. Despite auxin oxidation being reduced by 70% in dao1 mutant seedlings, overall auxin levels remain similar to wildtype, as the loss of DAO1 is compensated by increased auxin conjugation to amino acids. Mathematical modelling and metabolic data showed that also auxin synthesis is

upregulated in dao1 mutants indicating a tight interaction of synthesis, oxidation and conjugation pathways. This finding is unintuitive; one would not expect an increase in synthesis to compensate for a deficit in degradation. These data highlight not only the fundamental importance of localized auxin degradation in root tips, but also indicate the complexity of the underlying regulatory network fine-tuning cellular auxin concentrations.

Aims
This project is based on a Royal Society funded RNAseq dataset comparing transcriptional responses in wildtype and dao1 mutants root tips with and without auxin treatment. Using an interdisciplinary approach, including Molecular Biology, Microscopy, Semi-throughput Phenotyping, Metabolic Profiling (Analytical Chemistry), Bioinformatics and Mathematical Modelling this project will achieve:

• Answering how cellular auxin concentrations are regulated at transcriptional level.
• Use the generated data to predict the auxin homeostatic network involved in compensating gain or loss of auxin oxidation.
• Testing these predictions by generating and analysing mutants of key genes in this network.

In summary this project will identify which auxin homeostatic pathways compensate for disturbances to auxin homeostasis. In addition, it will identify novel candidates for regulating cellular auxin homeostasis in root tips, such as transcription factors.

References to learn more:

1. Casanova-Sáez R, Voß U. Auxin Metabolism Controls Developmental Decisions in Land Plants. Trends Plant Sci. 2019 Aug;24(8):741-754.

2. Porco S*, Pěnčík A*, Rashed A*, Voß U*, Casanova-Sáez, R*, Bishopp A, Golebiowska A, Bhosale R, Swarup R, Swarup K, Peňáková P, Novák O, Staswick P, Hedden P, Phillips AL, Vissenberg K, Bennett MJ, Ljung K. The dioxygenase-encoding AtDAO1 gene controls IAA degradation and homeostasis in Arabidopsis. PNAS. Sep 27;113(39):11016-21.

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(145) Understanding myosin motors from the malaria parasite

Primary supervisor: Alistair Hume

Second supervisor: Rita Tewari

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

This rotation project will lay the ground work for biochemical analysis of the motor properties of myosin motors from the malaria parasite Plasmodium. The student will use PCR to amplify the six myosin cDNAs from plasmodium and sub-clone these into plasmid vectors allowing generation of virus vectors that will allow their transient expression as fusion proteins to GFP and organelle targeting proteins (Sytl2a – synaptotgamin-like protein 2-a) in mammalian skin pigment cells. The fusion proteins will then be expressed in mammalian cells and their function will be measured by live cell microscopy and tracking analysis of pigment distribution and movement. This provides a convenient read-out of motor protein function in a living cell.

Together with mutagenesis studies this will allow determination of motor directionality, speed and run length plus it will allow mapping of motor domain and lever arm that are essential for motility. Myosin motors are important for the pathogenicity of the malaria parasite. Thus they are potentially interesting therapeutic targets. Although their high divergence from mammalian myosins supports this possibility, it also means that their structure, function and mechansims are poorly understood. Work in this rotation will start the process of understanding the motor functions and mechanisms of these promising targets for therapy.

Full PhD project description:

Aim: The aim of this project is elucidate the cellular and molecular mechanism of molecular motors (myosin) in malaria parasite cell proliferation. This help us to identify the potential of these myosins as drug targets for malaria.

Background: Plasmodium, the causative agent of malaria, is the most fatal of vector borne protozoan parasites transmitted by infected female mosquitoes. It kills 584,000 people annually (WHO 2014). Measures to control exposure to the mosquito vector have met with limited success, and drug resistance is emerging rapidly. A greater understanding of the fundamental biology of the parasite proliferation and especially of the process of parasite cell division is needed to identify potential intervention candidates.

Hypothesis: The hypothesis of the project is that the myosin motor protein family identified by data mining in malaria parasite may give us the lead toward drug targets that could be used to inhibit cell proliferation. The myosin family has been little studied in the malaria parasite and hence the studies on the cellular localisation and function of kinesins in unicellular malaria parasite will unravel their unique motor properties and regulatory mechanism in cell proliferation in malaria parasite.

Myosin are a superfamily of ATP-dependent, actin filament (AF)-dependent nano-motors; they are defined by their conserved motor domain that contains both ATP and AF-binding sites. Many myosins are involved in cell proliferation, motility, invasion and many other cellular function,

emphasising both the importance and diversity of functions they perform.

Experimental Methods and Research Plan- Using complimentary expertise between the group of Dr Alistair Hume (Molecular Motors 1,2) and Prof Rita Tewari (Parasite cell division, signalling and development-3,4) the role of six parasite specific myosins will be studied in assays that allow characterisation of motor properties during stages where parasite proliferate endo-mitotically e.g. scizogony, within red blood cells, male gametogenesis giving rise to flagella and oocyst stage within mosquito vector to generate sporozoites.

The three main objectives will be:
1. To characterise the motor activity of myosin motors from Plasmodium. This will use cell and in vitro assays that allow biochemical characterisation of myosin motors from plasmodium.

2. To define the localisation, dynamics and interacting partners of myosin motors during endo mitotic stages of parasite cell proliferation. Fixed and time lapse high resolution imaging technologies like confocal, super resolution and TIRF will used with transgenic parasite expressing Pb Kinesins as either GFP or RFP.

3. To determine the function(s) of Plasmodium myosins during parasite proliferation? The 6 myosin genes in plasmodium were previously disrupted and in this project we will characterise their effects on the parasite life cycle and thus better characterise their function.

Impact and outcome
The outcome of this project will be a good fundamental understanding of cell proliferation of malaria parasite stage and role of molecular motors in these processes. The impact will be to give us clue towards novel intervention strategies.

References to learn more:

1. Systematic analysis of Plasmodium myosins reveals differential expression, localisation, and function in invasive and proliferative parasite stages. Wall RJ, Zeeshan M, Katris NJ, Limenitakis R, Rea E, Stock J, Brady D, Waller RF, Holder AA, Tewari R. Cell Microbiol. 2019 Oct;21(10):e13082. doi: 10.1111/cmi.13082. Epub 2019 Jul 23. PMID: 31283102

2. Compositional and expression analyses of the glideosome during the Plasmodium life cycle reveal an additional myosin light chain required for maximum motility. Green JL, Wall RJ, Vahokoski J, Yusuf NA, Ridzuan MAM, Stanway RR, Stock J, Knuepfer E, Brady D, Martin SR, Howell SA, Pires IP, Moon RW, Molloy JE, Kursula I, Tewari R, Holder AA. J Biol Chem. 2017 Oct 27;292(43):17857-17875. doi: 10.1074/jbc.M117.802769. Epub 2017 Sep 11. PMID: 28893907

Location of lab rotation: QMC

Location of full PhD project: QMC

(146) Reconstitution of regulated mRNA deadenylation by components of the microRNA repression machinery

Primary supervisor: Sebastiaan Winkler

Second supervisor: David Scott

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

In this rotation, you will focus on protein assemblies involved in eukaryotic mRNA degradation, which play a pivotal role in microRNA-mediated repression. Bacterial co-expression systems will be employed as an enabling technology to obtain protein complexes. The purified protein complexes will subsequently be used for the reconstitution of RNA degradation processes and the structural characterisation using various biophysical methods available at the Research Complex at Harwell (RCaH).

Specifically, active sub-modules of the Ccr4-Not deadenylase as well as the complete protein complex will be reconstituted following methodology established in the lab. Following initial evaluation of purity and homogeneity, the protein complexes will be further characterised using analytical ultracentrifugation and gel filtration coupled to multi-angle light scattering available at the RCaH. Subsequently, single protein particles will be analysed by electron microscopy (EM) with negative stain, and where suitable moving to cryo-EM.

The training includes transferable skills focussing on DNA cloning and protein techniques, such as (restriction enzyme-free) DNA cloning, protein expression and purification, gel electrophoresis and western blotting, as well as the characterisation of biochemical samples using biophysical techniques. The project offers the opportunity to work at world-class facilities at the Research Complex at Harwell in Oxfordshire.

Full PhD project description:

Background - Cytoplasmic mRNA in eukaryotes is characterised by a long poly(A) tail, which has a typical length of 50-100 nucleotides in human cells. The tail is important for efficient translation and prevents unregulated degradation of mRNA. Particularly since the discovery of microRNAs, it has become increasingly evident that control of mRNA degradation is a critical step in eukaryotic gene regulation.

The project will focus on the multi-subunit Ccr4-Not deadenylase complex, which is involved in the shortening and removal of the mRNA poly(A) tail (deadenylation), the initial and often rate-limiting step in regulated mRNA deadenylation. The Ccr4-Not complex is recruited to target mRNAs by binding directly to RNA-binding proteins. These include proteins that are known to regulate mRNA stability such as tristetraprolin (TTP/ZFP36), PUM2 and Nanos, which recognise specific sequences in the 3’ end of the mRNA; proteins containing the YTH domain, which recognises RNA containing the covalently modified N6-methyladenosine base, and GW182 (TRNC6), a component of the microRNA repression complex.

Aim - The aim of this project is to reconstitute regulated deadenylation of mRNA in a test tube using

defined components, purified proteins and synthetic RNA molecules. This approach will refine current models of mRNA degradation and is designed to reveal unexpected behaviour and characteristics of the proteins involved.

Experimental plan - The project will involve the expression and purification of recombinant proteins, including sequence-specific RNA binding proteins (such as the protein TTP and the silencing domain of GW182) and a minimal Ccr4-Not deadenylase module using bacterial and/or baculovirus co-expression systems. In addition, a synthetic RNA substrate containing a long poly(A) tail (50-75 bases) will be prepared. Using structure-guided site-directed mutagenesis, the mechanism of deadenylase recruitment will be investigated using biochemical methods based on fluorescence-based detection of the RNA substrate.

Part of the experimental work may be completed at the Research Complex at Harwell (RCaH), which has excellent facilities for large-scale protein expression and purification. In addition, a full suite of biophysical instruments is available for sample characterisation. All facilities are staffed by expert technical staff with post-doctoral qualifications who are available for training and experimental design during the course of this studentship. The RCaH is adjacent to both the Diamond Light Source (protein crystallography, small angle X-ray scattering) and eBIC, the national EM facility, which opens up opportunities to apply these techniques for the structural characterisation of human Ccr4-Not complexes.

The experimental data will provide insight into the mechanism of regulated RNA degradation and the structural dynamics of the Ccr4-Not complex. In addition to a detailed theoretical understanding of regulated mRNA degradation and gene regulation, this project will provide in-depth training in recombinant DNA techniques, protein expression and affinity purification using various expression systems, and quantitative analysis of enzymes. The results obtained will be a significant step towards the understanding of regulated mRNA degradation at the molecular level.

References to learn more:

1. Wahle E, and Winkler GS (2013) Deadenylation by the Ccr4-Not and Pan2-Pan3 complexes. BBA Gene Regulatory Mechanisms, 1829, 561-570

2. Doidge R, Mittal S, Aslam A, and Winkler GS (2012) Deadenylation of cytoplasmic mRNA by the mammalian Ccr4-Not complex. Biochemical Society Transctions, 40, 896-901

Location of lab rotation: University Park; Research Complex at Harwell

Location of full PhD project: University Park; Research Complex at Harwell

(148) Hybrid Multifunctional Scaffolds for Guided Tissue Regeneration: combating Periodontitis and Associated Infections

Primary supervisor: Dmitry Volodkin

Second supervisor: Veeren Chauhan

Institution: NTU

School: School of Science and Technology

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 (Jack Campbell, Anna Ferreira) 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 PhD 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.

References to learn more:

1. Feoktistova, N.A.; Vikulina, A.S.; Balabushevich, N.G.; Skirtach, A. and Volodkin, D., Bioactivity of catalase loaded into vaterite CaCO3 crystals via adsorption and co-synthesis. Materials & Design, 185, 108223 (2020).

2. Elsharkawy, S. and Mata, A., Hierarchical Biomineralization: from Nature's Designs to Synthetic Materials for Regenerative Medicine and Dentistry. Advanced Healthcare Materials, 7, e1800178 (2018).

Location of lab rotation: Clifton Campus

Location of full PhD project: University Park; Clifton Campus

(150) The importance of biofilm formation to drug-resistant Cholera in Bangladesh

Primary supervisor: Tania Dottorini

Second supervisor: Michelle Baker

Institution: University of Nottingham

School: School of Veterinary Medicine and Science

Lab rotation description:

The student will be joining an exciting multidisciplinary team comprising Dr. Tania Dottorini, Prof. Miguel Camara and Dr. Michelle Baker. Dr. Dottorini, with wide expertise in bioinformatics and machine learning is the PI of a recently awarded ~£1.5M InnovateUK-China ABR- and cloud computing-related grant: FARMWATCH. Two research fellows including Dr Michelle Baker, with expertise in bioinformatics and machine learning, are currently working in this project. Dottorini's group share office space with the Advanced Data Analysis Centre (ADAC consisting of 20 staff of which Dottorini is the academic lead in Bioinformatics. The group also hosts the computational unit (3 fellows) of the Green Chemicals Beacon of Excellence headed by Dottorini. Miguel Camara is a Professor of Molecular Microbiology and Co-Director of the National Biofilm Innovations Centre (one of the BBSRC-DTP partners). Two research fellows from NBIC (Dr. Manuel Romero and Dr Shaun Robertson) will support the candidate PhD to carry out the biofilm analysis. Michelle Baker is an experienced research fellow with expertise in machine learning and modelling of bacterial systems. The supervisory team, together with the strong international links (UNICEF, Bangladesh National Centre for Diarrhoea, Prof R. Colwell University of Maryland) supported by the recently awarded grants (GCRF and InnovateUK) offers a unique combination of cutting-edge expertise in machine learning, bioinformatics, sequencing, cloud computing, microbiology, infection control, post-genomic statistical and computational approaches, microbiology and biofilm formation. Finally, the candidate will benefit from the links to both the UoN Future foods and UoN Green Chemicals beacons (Dr. Dottorini is a member of the Leadership teams).

Full PhD project description:

Cholera is caused by ingestion of food or water contaminated with the bacterium Vibrio cholerae. Worldwide, 1.3 billion people are estimated to be at risk and approximately 1.3 to 4 million cases occur annually with 21,000 to 143,000 resulting in death. In Bangladesh alone, where cholera is endemic, an estimated 66 million people are at risk of cholera with at least 100,000 cases and 4,500 deaths per year. Moreover, theThe indiscriminate use of wide-spectrum antibiotics creates the additional threat represented by the appearance and diffusion of antibiotic resistance (ABR) profiles in the pathogen population. Much of the work on AMR profiles and behaviour of V. cholerae to date has considered only planktonic (free moving) bacteria but there it is a recognised need to understand how these bacteria behave in biofilm. Many of the mechanisms for biofilm formation in V. cholerae have been limited to a relatively small number of strains of serogroup O1 and O139 where high variability in biofilm regulation has been found. Hence there is an urge to study the genetic variation of biofilm determinants in a broader range of clinical isolates and how this impacts on biofilm formation and AMR which will be at the centre of this PhD project, which falls under the National Biofilms Innovation Centre theme Biofilm Manage.

The PhD project will rely and build on the data collected from a recently awarded GCRF grant: CARE Bangladesh - Cholera Antibiotic REsistance in Bangladesh: big data mining and machine learning to

improve diagnostics and treatment selection, of which Dr. Dottorini is the PI. Specifically, a wide repertoire of V. cholerae strains circulating across three areas including hyper-endemic and endemic settings will be collected between February 2020 and August 2020, from a cohort of 600 individuals. Conventional culture-based screening, antibiotic susceptibility tests and whole genome DNA sequencing (WGS) will be carried out on each isolate. Data mining and statistical modelling powered by machine learning will be used to scan the genomes of V. cholerae strains and find correlations with the ABR profiles to identify the genomic markers that distinguish different populations of the V. cholerae. (stratification of strain and ABR profiles). The PhD project will comprise of an initial and significant computational component where novel machine learning methodologies are developed and applied to identify important mutations for antibiotic resistanceABR and biofilm formation in the strains collected in Bangladesh. Clusters of isolates showing interesting ABR and biofilm genomic features will be either sent to the National Biofilms Innovation CentreNBIC or developed directly in the Centre using CRISPR/Cas9 techniques and tested under different antibiotic conditions to determine the impact of these mutations on biofilm formation and their evolution under antibiotic stress. The development of such approach will be a unique, innovative andinnovative and objective tool for investigating resistance dynamics of biofilms obtained from V. cholerae strains circulating in endemic and hyperendemic settings.

Working with academics in both SVMS and NBIC and by collaborating with an outstanding International consortium comprising UNICEF, the national Centre for Diarrhoea (Icddr,b) and Prof. Colwell (University of Maryland) a world-renowned global infectious disease scientist who served as the eleventh director of the National Science Foundation (NSF), the studentship offers a unique opportunity to gain expertise in machine learning, bioinformatics, sequencing, cloud computing, post-genomic statistical, computational approaches, microbiology including the use of biofilm models and their imaging analysis, infection control, surveillance, epidemiology and healthcare.

References to learn more:

1. Penesyan, A., Nagy, S.S., Kjelleberg, S. et al. Rapid microevolution of biofilm cells in response to antibiotics. npj Biofilms Microbiomes 5, 34 (2019) doi:10.1038/s41522-019-0108-3

2. Nguyen M, Long SW, McDermott PF, Olsen RJ, Olson R, Stevens RL, Tyson GH, Zhao S, Davis JJ. Using machine learning to predict antimicrobial MICs and associated genomic features for nontyphoidal Salmonella. Journal of clinical microbiology. 2019 Feb 1;57(2):e01260-18.

Location of lab rotation: Sutton Bonington

Location of full PhD project: University Park; Sutton Bonington

(151) Incorporation of catalytic functionalities into apoferritin for controlled catalysis in the living cells

Primary supervisor: Anca Pordea

Second supervisor: Neil Thomas

Institution: University of Nottingham

School: Engineering

Lab rotation description:

The use of metal complexes for in vivo catalysis is attractive for the development of catalytic drugs. The challenge is the inactivation of metal catalysts by the high concentration of functional groups within cells. Furthermore, the delivery and the spatial localisation of the catalyst remain difficult to control. This project aims to incorporate metal complexes into the hollow core of apoferritin, a protein nanocapsule that can 1) encapsulate metal complexes and organic molecules and 2) specifically deliver synthetic molecules to tumor cells, due to its ability to bind the human TtR1 receptor, which is overexpressed in these cells. The apoferritin cage will act as a shield for the metal catalyst in a biological environment. The resulting bioconjugates will be used as catalysts, for example of prodrug activation in vivo. Other potential applications include delivery of therapeutic metal complexes, or integration into chemo-enzymatic cascade reactions.

During the rotation project, the student will synthesise a metal catalyst (based on gold or ruthenium) and characterise it using common chemical analytical techniques. The metal complex will be incorporated into apoferritin by encapsulation. The student will optimise the encapsulation conditions and analyse the resulting bioconjugates by spectroscopy, ICP-MS and electron microscopy. Cytotoxicity studies with the characterised compounds will be performed in vitro.

Full PhD project description:

Artificial metalloenzymes (ArMs) are built by incorporating non-biological metal catalysts within biomolecular hosts and have been used to expand the catalytic functionality of biomolecules towards non-biological transformations. They offer great opportunities for the integration of chemical catalysis with the biological environment. Challenges with the development of biocompatible (biooorthogonal) catalysts include their inactivation in the presence of biological material, as well as the difficulty to control the delivery and the spatial localisation of the catalyst. The development of a technology to target ArMs to specific cells or cell compartments would address these limitations. To achieve this, the biomolecular host must be able to incorporate and shield the metal catalyst, as well as to target its delivery to the desired site of action. Such a technology would ensure spatial control over the in vivo synthesis / activation of molecules such as prodrugs, with potential medical applications.

Apoferritin is a protein cage used for targeted drug delivery due to its modifiable surface, its biocompatibility as well as its disassembly-reassembly into its 24-subunits according to different pH conditions. In this project, we aim to use the hollow core of apoferritin, in order to incorporate metal complexes that can catalyse chemical transformations in the presence of biological material. These artificial metalloenzymes will be used as systems for the targeted delivery and catalytic activation of prodrugs. Apoferritin can specifically deliver synthetic molecules to tumor cells, due to its ability to bind the human TtR1 receptor, which is overexpressed in these cells. Therefore, this

approach offers the exciting potential to precisely localise non-biological reactions within cells and tissues of interest. Furthermore, the use of apoferritin as nanoreactor will provide a platform for the development of biocompatible transition metal catalysed reactions, to be combined with cellular metabolism for the synthesis of organic chemicals.

Building on previous work in our team on the preparation and characterisation of metal complexes encapsulated into apoferritin, metal catalysts with suitable functionalisation handles and catalytic properties will be synthesised and characterised. Metal complexes will be conjugated to apoferritin, using two different approaches: encapsulation within the apoferritin cage, and covalent binding to a single cysteine residue. A range of non-biological model reactions (Au-catalysed alkyne hydroarylation; Pd- and Ru-catalysed O-allyl carbamate hydrolysis; Ru- and Ir-catalysed transfer hydrogenation; and Pd-catalysed cross coupling) will be tested with the apoferritin-based ArMs and used to synthesise fluorogenic compounds, to enable a simple activity screen. The ability of the metal-protein constructs to act as therapeutic metal complexes will also be tested.

The model ArM-catalysed transformations developed above will be tested in living cells. Apoferritin binds the human TtR1 receptors, which are overexpressed in certain human cancer cell lines. We will test the ArM activity in normal and in cancerous human cell lines, by comparison with passive cell diffusion. The fluorescent catalysis products will also enable us to assess the intracellular catalyst distribution during in vivo catalysis. We will apply the novel ArMs to (pro)drug delivery to cancer cell lines and subsequent activation in the cytoplasm.

This is a highly interdisciplinary project combining supervisors from 3 different schools, with a wide range of different expertise. The project is relevant to the biotechnology area of the DTP, in that it develops novel protein engineering and chemical biology tools with applications in pharmaceuticals, biocatalysis and synthetic biology.

References to learn more:

1. Vidal, C.; Tomas-Gamasa, M.; Destito P.; Lopez F.; Mascarenas J. L., Concurrent and orthogonal gold(I) and ruthenium(II) catalysis inside living cells. Nature Communications 2018, 9 (1), 1913.

2. Ghattas, W.; Dubosclard, V.; Wick, A.; Bendelac, A.; Guillot, R.; Ricoux, R.; Mahy, J.-P., Receptor-Based Artificial Metalloenzymes on Living Human Cells. Journal of the American Chemical Society 2018, 140 (28), 8756-8762

Location of lab rotation: University Park

Location of full PhD project: University Park

This is a linked project – we recommend you select project 157 alongside this one.

(153) CRISPR CAS9 targeting of MORF: Exploring Molecular functions of a protein associated with genetic disorders

Primary supervisor: Hilary Collins

Second supervisor: David Heery

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

The rotation project will involve training in molecular cloning techniques and also confocal microscopy. We will use PCR cloning to generate MORF cDNA expression vectors for mammalian/ and /or yeast expression. If time allows we will begin to generate mutations in these constructs by site-directed mutagenesis. We will use transfection of mammalian cells to assess the ability of MORF and other proteins (BRPF1; KDM4C) to associate in the nucleus using immunofluorescence staining and confocal microscopy. We will also generate CRISPR targeting strategies for the main project.

Full PhD project description:

CRISPR CAS9 targeting of MORF: Exploring Molecular functions of a protein associated with genetic disorders.

MORF is a histone acetyltransferase encoded by the KAT6B gene that is known to be required for neurogenesis and bone development. Patients presenting with the overlapping neurodevelopmental disorders such as Ohdo syndrome, genitopatellar syndrome (GPS) and Say-Barber-Biesecker-Young-Simpson syndrome were found to have pathogenic mutations in KAT6B gene.

MORF is a multidomain protein containing several functional domains including histone binding motifs and also a histone acetylation activity. MORF also interacts with a number of other nuclear proteins such as BRPF1, and thus is likely to act within a multiprotein gene regulatory complex. While MORF is known to be required for normal mammalian development and functions as a regulator of gene expression, little is known about the molecular mechanisms of MORF function, or how mutations associated with genetic syndromes impact on these functions.

The aim of this project will be to advance our understanding of the molecular functions of MORF/KAT6B using a variety of techniques including expression construct and mutagenesis, yeast two hybrid interactions, colocalisation studies using confocal microscopy. We will also use CRISPR editing of cell lines to try to generate clones lacking expression of MORF/KAT6B or which carry mutations mimicking those found in patients. Western blots, qPCR and analysis of nonsense-mediated decay of the KAT6B transcripts.

This is a new project in the lab and will be performed in collaboration with the Prof. Heery's team with the Gene regulation & RNA Biology Group

References to learn more:

1. De novo KAT6B mutation, Say-Barber-Biesecker-Young-Simpson syndrome, and specific language impairment.

Fernández-Mayoralas DM, Calleja-Pérez B, Álvarez S, Fernández-Jaén A.
Neurologia. 2019 Jul 17.

2. MOZ and MORF acetyltransferases: Molecular interaction, animal development and human disease.
Yang XJ.Biochim Biophys Acta. 2015

Location of lab rotation: University Park

Location of full PhD project: University Park

(155) Hetero-synaptic plasticity in health and disease

Primary supervisor: Mark von Rossum

Second supervisor: Steve Coombes

Institution: University of Nottingham

School: Psychology

Lab rotation description:

It is well established that the brain adapts and stores memories mainly through changing the connections between neurons (a process known as synaptic plasticity). There have been many studies characterizing how synaptic connections change depending on the input that a neuron receives.
Very recent calcium imaging data from our experimental collaborator (Wiegert et al 2018), shows that neighbouring synapses are also affected by synaptic plasticity, that is, if one synapse becomes stronger the neighbouring synapse could become stronger as well. The goal of this project is to use computational and mathematical models to examine how this influences memory storage, which could either be improved or impaired, due to this effect. In particular we are also interested in how these processes change in fragileX (a genetic form of autism), which leads to memory and developmental impairments.

In the shorter 9 week project we use traditional correlation measures combined with highly simplified models of neural memory formation to study cross-talk in synaptic plasticity.

Full PhD project description:

It is well established that the brain adapts and stores memories mainly through changing the connections between neurons (a process known as synaptic plasticity). There have been many studies characterizing how synaptic connections change depending on the input that a neuron receives.
Very recent calcium imaging data from our experimental collaborator (Wiegert et al 2018), shows that neighbouring synapses are also affected by synaptic plasticity, that is, if one synapse becomes stronger the neighbouring synapse could become stronger as well. The goal of this project is to use computational and mathematical models to examine how this influences memory storage, which could either be improved or impaired, due to this effect. In particular we are also interested in how these processes change in fragileX (a genetic form of autism), which leads to memory and developmental impairments.

In the PhD project we will develop both phenomenological statistical models of synaptic plasticity that will for the first time include cross-talk terms in the plasticity. The main supervisor has extensive experience in developing statistical synapse models.
Second we will develop more mechanistic underpinnings of these models, splitting the contribution of pre and post-synaptic effects in the plasticity (Wiegert et al 2018, Costa et al 2017).
We will then use these plasticity models in simulated networks of neurons that store memories, and will examine how the cross-talk affects memory capacity and memory persistence of artificial memory patterns.
Finally we will then modify the parameters of these models to mimic synaptic defects as observed in

Fragile X syndrome, which is known to leads to memory impairments on the functional level and less stable synapses on the neuronal level.

References to learn more:

3. Wiegert, J. S., Pulin, M., Gee, C. E., & Oertner, T. G. (2018). The fate of hippocampal synapses depends on the sequence of plasticity-inducing events. Elife, 7, e39151.

4. Costa, R. P., Mizusaki, B. E., Sjöström, P. J., & van Rossum, M. C. (2017). Functional consequences of pre-and postsynaptic expression of synaptic plasticity. Philosophical Transactions of the Royal Society B: Biological Sciences, 372(1715), 20160153.

Location of lab rotation: University Park

Location of full PhD project: University Park

(156) Substrates of Recognition Memory in Mice

Primary supervisor: Charlotte Bonardi

Second supervisor: Jasper Robinson

Institution: University of Nottingham

School: Psychology

Lab rotation description:

This rotation uses a variant of the novel object recognition (NOR) procedure, in which mice explore an object for a short period of time and are then allowed to explore it and a second, novel object. Preferential exploration of the novel item is evidence of recognition. The rotation tests a key assumption underlying a new account of recognition, based on a well-established theory of associative learning (SOP: Wagner, 1981). SOP asserts that every stimulus is mentally represented as a set of elements. When the stimulus is presented these elements are activated, allowing them to provoke exploratory responding - but this tendency can be attenuated by two types of priming:
(i) if the stimulus has recently been presented, its subsequent presentations elicit less responding
(ii) if the stimulus is associatively retrieved, this limits its ability to elicit responding

This rotation provides expert training on behavioural and in vivo training techniques. As such it offers training on what have been identified by MRC and BBSRC as key vulnerable skills. More specifically, the student will be trained to use generic experimental software (AnyMaze) to analyse the observed behaviour, and Excel to extract and manipulate key data into a form suitable for statistical analysis.

Full PhD project description:

RESEARCH AREA 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, which underpins development of therapies designed to alleviate 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. The NOR task is an 'off-the-peg' procedure routinely used to evaluate the effects of brain manipulations on recognition memory in mice. However, the theoretical interpretation of performance on this task is controversial. Translational theories of recognition are typically based on the distinction between recollection and familiarity drawn from human work. This approach is not only relatively loose in the predictions it can make, but is also being increasingly questioned on theoretical and methodological grounds, making translation between animal and human domains problematic. Thus we have argued (e.g. Robinson & Bonardi, 2015) for a different approach. Rather than taking theories of human recognition as a starting point, 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.

AIMS 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. SOP asserts that every stimulus is mentally represented as a set of elements. When the stimulus is presented these elements are activated,

allowing them to provoke behaviour, but this tendency can be attenuated by two types of priming:

(i) if the stimulus has recently been presented, its subsequent presentations will elicit less responding - self-priming (a transient effect)
(ii) if the stimulus is associatively retrieved, this limits its ability to elicit responding - retrieval-priming (a longer-lasting effect)

SOP explains performance on the NOR task in terms of both self-priming and retrieval-priming. A familiar item may have been experienced recently and suffer self-priming; also its elements may be associatively retrieved by surrounding arena cues, or other elements of the item itself, producing retrieval-priming. Both reduce the exploration response, the measure of perceived novelty on this task. We aim to evaluate the key proposal that associations underlie recognition, and confirm that these three mechanisms underlie NOR performance.

HYPOTHESIS The overall hypothesis is that self-priming and retrieval-priming are the building blocks of recognition, and that performance on variants of NOR tasks will conform to the predictions of this associative model - for example, showing evidence of paradigmatic associative phenomena such as overshadowing and blocking. These predictions are neither anticipated nor addressed by the recollection/familiarity based interpretations of NOR performance.

References to learn more:

1. Bonardi, C., Pardon, M-C., & Armstrong, P. (2016). Deficits in object-in-place but not relative recency performance in the APPswe/PS1dE9 mouse model of Alzheimer's disease: Implications for object recognition. Behavioural Brain Research, 313, 71-81.

2. Robinson, J., & Bonardi, C. (2015). An Associative Analysis of Object Memory. Behavioural Brain Research, 285, 1-9. https://doi.org/10.1016/j.bbr.2014.10.046

Location of lab rotation: QMC

Location of full PhD project: QMC

(157) Development of theranostic agents for combined multimodal imaging and targeted therapy

Primary supervisor: Lyudmila Turyanska

Second supervisor: Tracey Bradshaw

Institution: University of Nottingham

School: Engineering

Lab rotation description:

The development of multifunctional theranostic agents could advance emergence of novel therapeutic agents into the clinic by enabling simultaneous imaging of biodistribution and non-invasive efficacy monitoring. In this project, imaging labels and therapeutic agents will be combined in one structure by encapsulation in biocompatible nano-capsules based on proteins. The surface of the capsules will be used to attach targeting molecules (e.g. folic acid; HerX/EGFR specific affibodies) and peptides, while the interior surface of the capsules will used to load therapeutic and imaging agents.

During the rotation project, the student will design, synthesise and characterise nanoparticles with optical and magnetic properties suitable for in vivo fluorescence and MRI imaging.
These nanomaterials will be encapsulated into apoferritin. The student will optimise the encapsulation and purification methods to achieve a high yield of putative theranostic agent. Optimised formulations will be taken forward for in vitro cytotoxicity studies. By the end of the project, the student will have gained a strong understanding of the multidisciplinary approaches and techniques used for the development and characterisation of nanoscale theranostic agents

Full PhD project description:

Development of theranostic agents has attracted considerable attention in recent years and has potential to impact and accelerate the use of both existing and novel therapeutic agents into the clinic. The overall aim of this ambitious project is to develop a strategy for on-demand development of theranostic agents for the targeted delivery of therapeutic agent with multimodal imaging opportunities. The specific objectives are:
1) To develop theranostic agents that incorporate within a protein nanocapsule both colloidal nanocrystal for near-infrared fluorescence and MRI, and a range of therapeutic molecules; and to achieve controlled drug retention/release time.
2) Establish methods for attachment of targeting peptides onto the exterior of the capsule specific to certain cancer types.
3) In vitro assessment of generated theranostic agents: examination of therapeutic activity and application in non-invasive deep tissue imaging. External funding will be sought for pilot in vivo imaging and potential therapy assessments of selected theranostic agents.
The first phase of the project (Year 1) builds on existing expertise in the group on encapsulation of nanoparticles and therapeutic agents into protein capsules, surface functionalization of proteins with targeting proteins and development of nanomaterials for bioimaging. The encapsulation strategies will be developed to combine colloidal nanocrystals and anticancer drugs inside the protein capsule. Methods for complete characterization will be established, including studies of optical and magnetic properties, effect of encapsulation on therapeutic activity, etc. Various therapeutic agents will be examined in this study in order to explore the effect of charge, polarity,

etc of encapsulation efficiency and agent retention/release. By the end of the Year 1, protein encapsulated theranostic agents will be developed and characterised, and will be made available to collaborators for in vivo assessments. In the Year 2, the project will focus on comprehensive in vitro assessment of therapeutic activity and on determination of imaging (near-infrared fluorescence and MRI) capability. Targeting labels will be attached to the exterior of the protein capsule and targeting efficiency will be examined. In Year 3, the library of therapeutic molecules encapsulated will be expanded aiming to develop a model linking encapsulation efficiency, drug retention and release with the properties of the molecules, hence enabling development of a method for prediction and preselection of capsule/molecule pairs. Support from theoretical modelling will be sought through existing collaborations. The theranostic agents with promising imaging and therapeutic activity in vitro will be selected for pilot in vivo studies in collaboration with long term collaborators of supervisory team.

The project builds on current advances in nanomedicine and imaging and aims to combine imaging, therapy and targeted delivery within one nanocapsule. Successful development of non-invasive imaging of biodistribution and efficacy monitoring of therapeutic agents is of paramount importance and has potential to accelerate emergence of novel drugs into the clinic. An additional advantage of multifunctional formulations is controlled drug release at the site of interest triggered by environmental or by external stimuli (e.g. illumination), offers therapeutic benefits including selective site-specific treatment and reduced side effects

References to learn more:

1. A. F. Breen, D. Scurr, M. L. Cassioli, G. Wells, N. R. Thomas, J. Zhang, L. Turyanska, T D. Bradshaw
   Protein encapsulation of experimental anticancer agents 5F 203 and Phortress: towards precision drug delivery. International Journal of Nanomedicine 14, 9525-9534 (2019).

2. L. Turyanska, F. Moro, A. Patanè, J. Barr, W. Köckenberger, A. Taylor, H. M. Faas, M. Fowler, P. Wigmore, R. C. Trueman, H. E. L. Williams, and N. R Thomas, Developing Mn-doped lead sulfide quantum dots for MRI labels. J. Mater. Chem. B 4, 6797-6802 (2016) DOI:10.1039/C6TB02574A

Location of lab rotation: University Park; Jubilee Campus

Location of full PhD project: University Park; Jubilee Campus

This is a linked project – we recommend you select project 151 alongside this one.

(158) Speech Processing in the Ageing Brain

Primary supervisor: Riikka Mottonen

Second supervisor: Martin Schuermann

Institution: University of Nottingham

School: Psychology

Lab rotation description:

Over the course of the lab rotation, students will be introduced to brain stimulation, brain imaging and behavioural experiments in humans on the basis of ongoing experiments. Riikka Mottonen's main research area is speech communication and language learning. The ongoing experiments are likely to be related to this topic.

Brain stimulation: Students will be introduced to Transcranial Magnetic Stimulation (TMS) and how it can be used to investigate neural mechanisms of perception and cognition. The students will learn to stimulate the different areas of the motor cortex (e.g., lip and hand representations) and record motor-evoked potential from the target muscles using electromyography (EMG).

Brain imaging: Students will be introduced to electroencephalography (EEG) and how it can be used to investigate neural mechanisms of perception and cognition. The student will learn to record and analyse EEG data.

Behavioural tests: Students will be introduced to various tests that can be used to investigate perception and cognition. Student will learn to run experiments and analyse the data.

Full PhD project description:

Difficulty in understanding speech in noisy environment is a common problem in older adults. This difficulty results from age-related peripheral hearing loss and decline of neural mechanisms that support speech perception. The proposed project will focus on the effects of aging and hearing loss on auditory and motor systems and their interaction during speech processing. Evidence shows that auditory-motor interactions contribute to phonemic categorization of speech sounds in young adults (for a review, see Liebenthal and Mottonen, 2018). The motor system is also likely to contribute to perception of prosody of speech, which is crucial for understanding the emotional state of the speaker. It has been proposed that upregulation of the motor processes can compensate for the decline of auditory processes in older adults. Our recent study shows, however, that older adults with hearing loss within the speech frequency range (up to 4 kHz) show a reduction in recruitment of the motor system during listening to continuous speech signals, which may contribute to their speech perception difficulties (Panouilleres and Mottonen, 2018).

The main goal of the proposed project is to determine how ageing and peripheral hearing loss affect auditory-motor speech processing. We will investigate speech processing in the young adults with normal hearing and in older adults with normal and impaired hearing in the speech-frequencies (up to 4 kHz). Importantly, we will simulate hearing loss in participants with normal hearing in order to match the auditory input with participants with hearing loss. We will collect a large data set (including 60 young adults and 120 older adults) using a variety of complementary methods: electroencephalography (EEG), transcranial magnetic stimulation (TMS) and behavioural tests. These

techniques will allow us to measure speech processing in auditory and motor systems and address the contribution of the motor system to processing of prosodic and phonemic properties of speech.

Significance: The population of the U.K. is aging rapidly and it has been estimated that 60% of over 65 year olds have a hearing impairment (www.actiononhearingloss.co.uk). Investigation of age-related decline of speech perception and development of new rehabilitation programs are important, because communication difficulties can lead to social isolation, depression and cognitive decline. The ability of communicate effectively and effortlessly is essential for heathy and happy late adulthood.

References to learn more:

Panouillères MTN, Möttönen R. (2018) Decline of auditory-motor speech processing in older adults with hearing loss. Neurobiology of Aging. doi.org/10.1016/j.neurobiolaging.2018.07.013

Location of lab rotation: University Park

Location of full PhD project: University Park

(159) Assessing sustainability of reindeer husbandry in a warming Arctic

Primary supervisor: Sofie Sjogersten

Second supervisor: Nigel Kendall

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

The rotation will involve a detailed study of the Red Deer population and habitat in Wollaton Park, Nottingham as it offers a realistic model system for training in methods to be used in the PhD project. The student will investigate deer behaviour, forage choices, and carry out analysis of forage quality across known gradients in grazing intensity and vegetation cover. This work will involve monitoring deer behaviour over two week period using observational and camera trapping techniques. This will be followed by determining vegetation composition and biomass in areas of different grazing pressure. Samples of the different plant species found in preferred grazing areas and areas of lower grazing intensities will be collected and subsequently analysed for their nutritional value e.g. mineral nutrient, protein and fiber content. Training in remote sensing data processing will be provided in the reindeer research uniting at Umea University, Sweden by co-supervisor Johan Olofsson. This work will be done using existing airborne data from drones flown across areas of different grazing intensities in the study area in northern Sweden. This gives the student an opportunities to develop an understanding of distribution of vegetation across the land scape and how this relates to preferred reindeer grazing areas.

Full PhD project description:

Semi-domesticated reindeer husbandry is an important source of livelihoods for indigenous communities in northern Europe. Reindeer husbandry is strongly impacted by the dramatic warming of the arctic as it impacts reindeer forage, i.e. quantity, quality and species composition as the vegetation responds to climate change. Furthermore, reindeer-stocking density strongly impacts the productivity and nutrient cycling of pastures. To ensure long-term sustainable production it is important to understand i) the interactions between vegetation responses to heating and stocking densities, and ii) any resultant changes in reindeer foraging behaviour as they together determine the carrying capacity.

This project will use long-term field experiments simulating (i) climate change (warming and snow additions experiments) and (ii) grazing experiment (i.e. controls and large plots from which herbivores are excluded) across the reindeer grazing areas of northern Scandinavia to address the following research questions:
1. How does vegetation responses to climate change impact on the abundance and quality of forage for reindeer?
2. How does reindeer impact on the productivity of their preferred forage via browsing and altered soil nutrient cycling?

Does vegetation response to climate change lead to altered reindeer foraging behaviour?
The project will use air and satellite borne vegetation mapping systems to track temporal changes in vegetation addressing the questions:
3. What are the landscape scale climate change driven shifts in vegetation over the last decade?

4. What are the implications of this for landscape-level reindeer carrying capacity?

Work programme
Year 1. Quantification of vegetation and foraging behaviour changes resulting from long term warming and snow addition experiments. This work will include field measurements of plant species composition and growth rates in a series on long-term climate change experiments. Samples of a range of plant species will be collected for proximate, C, N and ICPMS analysis (to determine e.g. protein, fiber, carbohydrates, phenolics, mineral nutrients content). Soil nutrient availability will be assessed to test if the experiments impact available plant nutrients (a known driver of forage quality and productivity). Reindeer foraging behaviour will be monitored via direct observation and GPS tagging. This work will address research questions 1 and 3.

Year 2. The second part of the field programme will involve the same plant measurements and foraging behaviour observations as those carried out in the climate change experiments (i.e. proximate analysis and soil nutrient analysis). The experiments cover a range of habitat types used by reindeer from productive meadows to high alpine heath and in each set of experiments browsing levels will be assessed in control plots. This work will address research questions 2 and 3.

Year 3. To determine the landscape level changes in vegetation in reindeer-grazed areas in response to climate change over the last two decades we will use a combination of satellite data including long-term LandSat data and more recent high-resolution Sentinel-2 data. To ground truth the space borne data we will use a series of pre-existing airborne optical data collected using UAVs flown over the study areas. The LandSat data will be used to assess long term changes in land cover of different vegetation types, the Sentinel-2 data will be used to investigate impacts of climate change on productivity as well as small scale changes in the vegetation composition.

References to learn more:

1. Olofsson J & Post E 2018 Effects of large herbivores on tundra vegetation in a changing climate and implications for rewilding. Phil Trans R Soc B 20170437

2. Väisänen M.*, Ylänne H., Kaarlejärvi E.*, Sjögersten S., Olofsson J., Crout N., Stark S. 2014. Consequences of warming on tundra carbon balance determined by reindeer grazing history. Nature Climate Change, Published Online 16 MARCH 2014 DOI: 10.1038/NCLIMATE2147

Location of lab rotation: University of Umea, Sweden; Sutton Bonington Campus; Clifton Campus;

Location of full PhD project: CIRC, University of Umea; Sutton Bonington Campus;

(160) Identifying key players in intestinal cell differentiation

Primary supervisor: Paloma Ordóñez Morán

Second supervisor: Abdolrahman Shams-Nateri

Institution: University of Nottingham

School: Medicine

Lab rotation description:

Our previous work revealed a novel mechanism showing that induction of intestinal stem cell differentiation impacts directly on tumour progression. We found HOXA5 transcription factor being repressed by the Wnt pathway as a strategy to enhance the self-renewal potential of stem cells. In vivo, HOXA5 reduces cancer stem cells (CSCs) and abrogates metastasis formation. We showed that retinoids can reverse the Wnt-mediated HOXA5 inhibition and block cancer growth in vivo (Ordóñez-Morán et al., 2015). It is important to target CSCs because it has been proven to be resistant to conventional therapy and responsible for tumour relapse. Thus, our goal is to deplete CSCs through controlled, drug-induced differentiation that may prolong patient survival.

Interestingly, we found that HOXA5 expression is also an independent prognostic factor in breast cancer. HOXA5 is downregulated in patient samples vs normal tissue and high expression levels of HOXA5 can identify patients with an improved probability of relapse-free survival. These and some more results from our lab point out a relevant role of HOXA5 in breast cancer. So, during this rotation project, we will study HOX transcription factors role in stemness, differentiation processes and cell plasticity in breast CSCs. We will also test drug-induced differentiation by retinoids. The student will study mouse and human biology by using common lab protocols and specifically will learn how to culture cells in 3D. We have broad experience of these methods in our lab, Naterí's lab (2nd Supervisor) and Allegrucci's lab (3rd Supervisor).

Full PhD project description:

Background
We have found that the HOX differentiation role is context-dependent defined by its interaction with the different binding partners MEIS, and PBX sub-families of TALE factors. In cancer, PBX and MEIS function is poorly described. In our lab, by single cell-RNAseq we have detected that the transcription factor PBX1 is highly expressed in intestinal stem cells and transit-amplifying progenitors but less in more specialized cells (Schwallie et al., 2017, Haber et al., 2015). We think that MEIS1, PBX1 and binding partners are key drivers of stemness in intestinal cancer cells which are the tumour-initiating cells responsible for tumour relapse of patients. So, the objective of this studentship will be to study what is the relevance of these transcription factors in cancer stemness and induced-differentiation.

Outline of research plan and experimental methods
We will study the complex or machinery relevant for cell fate, and we will identify the role of PBX1 and MEIS1 proteins in cell differentiation. We will use a mouse model of colorectal and intestinal cancer (ApcMIN) that will be bred with the Lgr5-GFP knock-in mouse model (cancer stem cells are identified by GFP). By FACS, we will isolate Lgr5-GFPhigh cells from tumours to identify the proteins that bind to HOXA5 in cancer stem cells. To that end, we will use a modified yeast-two hybrid Ras-recruitment System using a mouse-embryonic cDNA library (RRS, optimized in Nateri's lab (2nd

Supervisor): Nateri et al., 2004, Muhammad et al., 2018). These results will be validated in 3D organoids generated from human biopsies (methodology already established in our labs: Muhammad et al., 2018; Ordóñez-Morán et al., 2015, Gjorevski et al., 2016; Sato et al., 2009) and by the mouse intestinal gene transfer method (iGT) that allows expression or depletion of the gene of interest in vivo in intestinal stem cells (Imajo et al., 2014; Ordóñez-Moran et al., 2015). By all these methods, we will study the role of these candidates in stemness, differentiation processes and cell plasticity in cancer cells.

In collaboration with Mata's group (4th supervisor) we will optimize 3D organoid methodology. To this end we will create a 3D synthetic niche exhibiting both well-defined physical features including stiffness, porosity, and topography, as well as specific signalling epitopes such as the cell adhesive peptide Arg-Gly-Asp-Ser (RGDS) to identify and optimize physical environmental features able to maintain stem cells in vitro. This scaffold utilizes self-assembling peptides to recruit and organize key extracellular matrix molecules as both signalling and structural elements of hydrogels, similarly to their function in vivo (Hedegaard et al., 2019).

Outcomes and Impact
A precise balance between self-renewal and differentiation of stem cells is essential to maintain homeostasis. Loss of this balance tends to lead to uncontrolled cell growth or pre-maturation and thus results in tumours, cancers, or tissue defects. Understanding how PBX1 and MEIS1 regulate gene expression in cancer stem cells will enhance our understanding of how specific lineages are established as well as how cell fates can be manipulated for inducing cancer cell differentiation. The experimental demonstration providing a rational for differentiation therapy of cancer patients may improve current chemotherapies.

References to learn more:

1. Gjorevski N, Sachs N, Manfrin A, Giger S, Bragina ME, Ordóñez-Morán P, Clevers H, Lutolf MP. Designer matrices for intestinal stem cell and organoid culture. Nature. 2016 Nov 24;539(7630):560-564.

2. Ordóñez-Morán P, Dafflon C, Imajo M, Nishida E, Huelsken J. HOXA5 Counteracts Stem Cell Traits by Inhibiting Wnt Signaling in Colorectal Cancer. Cancer Cell. 2015 Dec 14;28(6):815-829.

Location of lab rotation: University Park

Location of full PhD project: University Park

(161) Mapping the 3-Dimensional Architecture of Human Oocyte Chromosomes

Primary supervisor: Daniel Booth

Second supervisor: Ramiro Alberio

Institution: University of Nottingham

School: Medicine

Lab rotation description:

This rotation will provide the student with an appropriate knowledge-base and skills-set in preparation for the full PhD project. This will include training in chromosome biology and other aspects of cell division in cancer cells. More specifically, they will learn how to introduce structural defects in mitotic chromosomes and investigate how this can influence cell fate, for example the induction of aneuploidy (a feature of many cancers and birth defects). This will be performed using both super-resolution light microscopy and correlative light electron microscopy (CLEM). These are highly desirable techniques, but CLEM in particular has only a few practitioners world-wide. Finally, you will become familiar with handling and imaging oocytes (female eggs) derived from both cattle and human. The opportunity to work with human oocytes, via collaborators in Warwick, is particularly exciting as we are one of only a handful of groups in the UK to have access to this precious material.

A majority of 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.

Full PhD project description:

PROJECT SNAPSHOT: Develop advanced correlative light electron microscopy (CLEM) pipelines and use them to define, for the first time, the 3D architecture of all human oocyte (female egg) chromosomes, in oocytes undergoing both normal and aberrant division.

WHY THIS IS IMPORTANT: For largely unknown reasons ~half of human oocytes display chromosome segregation errors, a leading cause of aneuploidy. This directly contributes to the 13% of women with fertility problems, 25% of women that will suffer a miscarriage and 0.3% of live births that carry a constitut¬¬¬ional aneuploidy. In most other systems defective chromosome structure is a known cause of aneuploidy, but in human oocytes this remains almost completely unexplored. This is because of three main obstacles: A) Restricted access to human oocytes; B) limited availability of established techniques/tools, particularly those for ultra-structural analyses; C) lack of reference for “normal” human oocyte division – i.e. it is difficult to fully understand features of aberrant division without fully characterising normal oocyte division first.
Our lab is one of the few in the UK that does have access to human oocytes. Therefore, this project provides a unique and exciting opportunity to perform ground-breaking research by addressing points B and C above.

AIMS:
AIM1 - Establish CLEM imaging pipelines. Mammalian oocyte chromosomes occupy <2% of the total oocyte volume. A classic challenge in analysing these small and randomly positioned organelles by

electron microscopy (EM) has been finding them. Conventional EM approaches would require the capture and imaging of nearly 1000 consecutive sections - an impossible economic and equipment burden. We will address this long-standing problem by developing bespoke correlative imaging pipelines, allowing the Euclidian position of chromosomes to be recorded within the oocyte using a light microscope, prior to EM processing. Essentially this provides a more realistic target for 3D electron microscopy imaging and the pathway for successful CLEM. Additional EM methods to be explored will include cryo-CLEM, an equally powerful, but potentially more future-proof approach. In the first instance these techniques will be established using pig oocytes, an excellent substitute for those of human.

AIM2 - Define the 'meiotic chromosome atlas'. The methods established in aim1 will next be optimised for analysing human oocytes. Here we will generate nanometre accurate digital reconstructions of each and every human oocyte chromosome. This will be performed during all stages of meiotic division allowing the retrieval of the basic structural parameters surrounding chromosome compaction, condensation and global morphology. This dataset will provide the first ever reference for chromosome ultra-structure during normal human oocyte division.

AIM3 – Investigate the geometry of aneuploid chromosomes. Finally, we will now be in a position to exploit the 'atlas' and compare differences in chromosome ultra-structure, between oocytes undergoing normal and aberrant division. A full geometric survey will be performed using computational analyses and modelling.

OUTCOMES: Studying the triggers of aneuploidy in human oocytes is widely regarded as one of the most important and rapidly emerging fields in research. This high-impact work will contribute to our understanding of the molecular mechanisms underpinning infertility, spontaneous abortion and birth defects. Furthermore, the advanced imaging tools and data-sets embedded within will become a pioneering resource, not only for those in closely-related fields, but also for the wider cell and developmental biology community.

References to learn more:

1. Booth, D. G. et al. 3D-CLEM Reveals that a Major Portion of Mitotic Chromosomes Is Not Chromatin. Mol Cell 64, 790-802, doi:10.1016/j.molcel.2016.10.009 (2016).

2. Patel, J., Tan, S. L., Hartshorne, G. M. & McAinsh, A. D. Unique geometry of sister kinetochores in human oocytes during meiosis I may explain maternal age-associated increases in chromosomal abnormalities. Biol Open 5, 178-184, doi:10.1242/bio.016394 (2015).

Location of lab rotation: University Park

Location of full PhD project: University Park (90%), Sutton Bonington (5%), Warwick Medical School (5%)

(162) Iron homeostasis in the maintenance of intestinal stem cells

Primary supervisor: Abdolrahman Shams Nateri

Second supervisor: Paloma Ordóñez Morán

Institution: University of Nottingham

School: Medicine

Lab rotation description:

Iron functions as a critical cofactor for many proteins involved in oxygen transport, cellular respiration, or DNA synthesis. Conversely, iron catalyses the Fenton reaction that results in the generation of the hydroxyl radical, one of the most detrimental ROS (reactive oxygen species) in vivo that damages many biological macromolecules. It is, therefore, important that cellular iron levels undergo strict regulation.
Interestingly, through an NC3Rs award, we have recently found that lack of FBXL5 gene, an F-box E3-ligase protein that senses iron and oxygen levels, resulting to the failure of a healthy intestinal 3D-organoid formation and accumulation of iron regulatory proteins (IRP) and the iron-responsive element (IRE) signalling pathway in cell culture.
So, during this rotation project, we will further study the role of Fbxl5 in already generated knocked out Fbxl5-organoid line (please see below background) and cell lines. We will also test their response to iron and oxygen deficiency (hypoxia). The student will be using common lab protocols and specifically will learn how to culture cells and 3D organoids (broad experience of these methods in our lab, Nateri's lab (lead supervisor), Ordóñez-Morán (2nd Supervisor) and McIntyre's lab (3rd Supervisor).

Full PhD project description:

Background
An F-box-protein binds to the specific recognition of target protein(s) for SCF (Skp1-Cul1-F-box-protein)-E3 ligases function, by which control the levels of proteins by promoting ubiquitin-dependent-proteolysis. F-box-proteins (74-genes) regulate substrates in diverse biological pathways; cell growth, development and differentiation, signalling responses, cell survival/death and also stem cell-niche maintenance during homeostasis and regeneration. Thus the dysregulation of F-box-protein-mediated ubiquitylation has been implicated in many pathologies [1-2]. For example, we have extensively studied FBXW7, an F-box gene, commonly mutated in human tumours [3-5]. However, the role of many other F-box-proteins not fully demonstrated. Recently, through an NC3Rs award, we have successfully used CRISPR/Cas system and screened gRNAs-mediated knockouts of F-box genes in the healthy intestinal-organoids (Jinks N. et al., & Kashfi S. et al.; in-revision). Among others, we showed that lack of FBXL5 gene leading to the failure of a healthy organoid formation.

Objectives
Iron functions as a critical cofactor for many proteins involved in oxygen transport, cellular respiration, or DNA synthesis. Conversely, iron catalyses the Fenton reaction that results in the generation of the hydroxyl radical, one of the most detrimental ROS (reactive-oxygen-species) in-vivo that damages many biological macromolecules. It is, therefore, important that cellular iron levels undergo strict regulation [6]. Notably, FBXL5-protein senses iron and oxygen levels through

the Fe-O-Fe centre of its haemerythrin-like domain. It emerges as a novel regulator of cellular iron homoeostasis by targeting iron-regulatory-protein-2 (IRP2) for degradation [7-8]. Despite these advances, the importance of cellular iron homeostasis in the intestine has been unrecognized.

Aim
In this project, we aimed to demonstrate that iron regulation mediated by FBXL5 is required for the maintenance of intestinal-stem cells.

Hypothesis
Conditional ablation of FBXL5 gene in mouse intestinal-stem cells results in cellular iron overload, leading to stem and progenitor cell alterations in the intestine.

Outline of the research plan and experimental methods
We will use a conditional deletion of FBXL5 (floxed-Fbxl5) mouse that will be crossed with the Lgr5-GFP knock-in transgenic model [9]. We will:
• Compare GI-tract for apoptosis levels, crypt size and mitotic-index from H&E-stained sections
• Use IHC for the Ki67-proliferation marker & ISH of Olfm4 and Lgr5 (ISC-markers), supplemented by single and continuous BrdU-incorporation and BrdU-chasing experiments.
• Assess differentiation by staining for cell-types expressing Chromogranin-A (Enteroendocrine cells), Alkaline-phosphatase (Enterocytes), Lysozyme (Paneth) markers by IHC and Alcian-blue (Goblet) by histochemistry.
• Isolate FACS sorted Lgr5-GFP cells for transcriptomic analysis (RNA-seq) and 3D-organoids studies to test their response to iron and oxygen deficiency (hypoxia) and deregulated genes.

Outcomes
If we determine that FBXL5 exerts iron and oxygen-dependent regulation of intestinal-stem cell self-renewal, differentiation and proliferation, findings could lead to new research developments in stem cells niche editing and tissue engineering area. We would also be able to suggest new approaches to treating the pathogenesis of inflammatory diseases and cancer of the gastrointestinal tract.

References
1) Nat Rev Cancer. 2014;14(4):233-47.
2) Nat Rev Drug Discov. 2014;13(12):889-903.
3) Science. 2004;303(5662):1374-8.
4) J Exp Med. 2011;208(2):295-312.
5) Oncogenesis. 2019;8(3):13.
6) Cell. 2010;142(1):24-38.
7) Cell Metab. 2011;14(3):339-51.
8) Science. 2009;326(5953):722-6.
9) Nature. 2007;449(7165):1003-7.

References to learn more:

1. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of Mammalian iron metabolism. Cell. 2010 Jul 9;142(1):24-38.
2. Moroishi T, Nishiyama M, Takeda Y, Iwai K, Nakayama KI. The FBXL5-IRP2 axis is integral to control of iron metabolism in vivo. Cell Metab. 2011 Sep 7;14(3):339-51.

Location of lab rotation: University Park

Location of full PhD project: University Park

(163) Identifying cryptic ligand binding sites in GPCRs by combining machine learning with chemical probes

Primary supervisor: Charlie Laughton

Second supervisor: Shailesh Mistry

Institution: University of Nottingham

School: Pharmacy

Lab rotation description:

In the lab rotation project the student will be introduced to state-of-the-art methods in computational structural biology and machine learning/AI. The student will begin by learning techniques for the high-throughput automated analysis of protein structural data (from the Cryptosite database) to characterise protein conformational changes that reveal cryptic binding pockets (ones that are only visible in the structures of protein-ligand complexes, because the apo-protein must undergo an unexpected conformational change to generate them). The project will then move on to the development of machine learning methods, trained on this data, to predict the location and/or probability of cryptic pockets from a knowledge of apo-protein structure alone. The project will provide training in basic molecular modelling skills and in machine learning methods.

Full PhD project description:

On occasion, structure determination of a protein-ligand complex reveals the ligand bound to a site that is “invisible” in the apo- structure of the same protein. Cryptic binding pockets represent a major challenge for structure-based drug design (if the pocket is not visible in the apo form of the protein, how might its existence have been predicted?), but also a major opportunity (if a pocket can be predicted, it may render a protein regarded as “undruggable” druggable, and the prediction of such a pocket could provide the discoverer with information not in the public domain, and so with a competitive advantage).

In response to this situation, methods for the prediction of cryptic binding pockets have become a hot topic. A variety of computational approaches have been proposed. Some are based on molecular simulations, while others make use of pure informatics (machine learning) methods. The simulation-based approaches tend to be slow but give structural models of cryptic sites that can be used directly for drug discovery activities, while the machine learning methods are much faster, but give much less structure-based information, and so are harder to leverage. In some preliminary work we have shown that a hybrid method, that refines machine-learned 'hits' with high quality simulations, has a good performance when applied to test data sets and it is the development and testing of this approach that will be the focus of the PhD.

G protein-coupled receptors (GPCRs) pharmacology are a hugely important family of receptors for drug discovery. Historically, drug discovery campaigns have targeted the orthosteric site of these membrane proteins (i.e. where the endogenous ligand binds), however, more recently allosteric ligands (acting through a topographically distinct site) have been discovered for a range of GPCRs. Whilst allosteric ligands have the potential to offer a number of benefits over orthosteric ligands (e.g. spatiotemporal signalling, a ceiling effect to activity, improved subtype selectivity), in many cases, their receptor binding sites remain unknown or poorly characterised, often due to the dynamic nature of GPCRs, particularly in the loop regions. This flexible dynamic nature means that

ligand binding sites may not be observable in x-ray crystal structures, i.e., they are cryptic. Indeed, the importance of cryptic pocket formation in allosteric modulator pharmacology at the muscarinic M1 receptor has recently been reported (Hollingsworth, Nat. Commun., 2019, 10(1) 3289).

The conformational behaviour of GPCRs can be described through the Monod-Wyman-Changeux (MWC) mechanism (Canals, J. Biol. Chem., 2012, 287 (1), 650-659, whereby receptors exist in equilibrium between at least two conformational states (active and inactive), each of which can be stabilised by ligands (i.e. agonists and antagonists respectively). In addition, it has been demonstrated that positive allosteric modulators (PAMs) bind cooperatively with agonists, and can further stabilise an active GPCR conformation.

By applying the computational methods described above to GPCR structures with an orthosteric agonist bound, we will improve the chance of exposing cryptic allosteric pockets which promote the binding of PAMs. Thus, the aim of this project will be to extend some recent work we have done (Emtage, J. Biomol. Struct. Dyn., 2017, 35, 2604-2619) and develop new ways for identifying putative allosteric binding sites in GPCRs. The novel aspect of this work will be the combination of two computational techniques – molecular dynamics simulations and machine learning - with experimental work – chemical synthesis and pharmacological evaluation of small molecule probes.

References to learn more:

1. Withers, J. Chem. Inf. Model., 2008, 48, 1448-1454

2. Emtage, J. Biomol. Struct. Dyn., 2017, 35, 2604-2619

Location of lab rotation: University Park

Location of full PhD project: University Park

(164) Imaging coral bleaching: Mitochondrial stress response of dinoflagellate algae, essential symbionts in coral reefs

Primary supervisor: Ellen Nisbet

Second supervisor: Catarina Gadelha

Institution: University of Nottingham

School: Biosciences

Lab rotation description:

Coral reefs are extremely important ecosystems, hosting a huge diversity of marine life, and underpin large scale subsistence fishing and tourism. Coral (an animal) lives in symbiosis with photosynthetic dinoflagellate algae. These algae provide up to 90% of the nutritional requirements of the coral. Unfortunately, dinoflagellates are extremely sensitive to temperature, and the coral-dinoflagellate symbiosis breaks down with rising sea temperatures. A harmful effect of global warming is the death of coral reefs due to coral bleaching, with devastating consequences.

We have recently developed the first genetic modification technology for dinoflagellate algae, and are able to insert foreign genes into their chloroplast genome. This project will make use of this technology to introduce DNA sequences encoding red, green and cyan fluorescent proteins into the chloroplast genome. Once the genes are inserted, the cells will be imaged. This will allow us to determine which fluorescent proteins work in dinoflagellate algae, which have a significant levels of auto-fluorescence. This research will be the first step in developing an in vitro sensor for dinoflagellate stress response, which is key to understanding coral bleaching. The results of this project will directly influence the direction of the full project, as the same results will be applicable to the mitochondrion. The rotation project will include DNA cloning, genetic manipulation, cell culture and fluorescence microscopy.

Full PhD project description:

Dinoflagellate algae are essential symbionts in corals. Coral reefs are extremely important marine ecosystems, hosting a huge diversity of aquatic life, and support subsistence fishing and tourism. Coral (an animal) lives in symbiosis with photosynthetic dinoflagellate algae. These algae provide up to 90% of the nutritional requirements of the coral. Unfortunately, dinoflagellates are extremely temperature sensitive, and the coral-dinoflagellate symbiosis breaks down with rising water temperatures and pollution. This is known as coral bleaching. As sea temperatures rise with global warming, coral reefs will be lost due to bleaching.

Dinoflagellates are photosynthetic eukaryotes, and thus contain a nucleus, a mitochondrion and a chloroplast. Recently, redox imbalance in the dinoflagellate mitochondrion has been strongly implicated in the breakdown of the symbiosis, resulting in the expulsion of the algae from the coral. This leads to coral bleaching. Understanding how and why when redox imbalances occur may provide us with a method to save the coral reefs.

This project will develop tools to allow dinoflagellate mitochondrial genome modification. Once genetic modification has been achieved, the student will image algae under stress conditions. Together, these techniques will allow us to study and manipulate redox imbalances in dinoflagellates under stress conditions. The research will complement work we are already undertaking to

understand the biochemistry of the dinoflagellate chloroplast.

The project will first test dinoflagellate sensitivity to commonly used mitochondrial selective agents. These will allow the choice of a selectable markers for transformation. Vectors will then be designed to introduce the selectable marker, allowing for double or single recombination events. A similar strategy has been successfully used to transform the mitochondrial genome in the green algae Chlamydomonas. DNA will be introduced to the dinoflagellate cells using biolistics (Nimmo et al 2019, eLife 8:e45292).

Once the student has successfully introduced foreign DNA, we will modify the constructs to introduce genes for fluorescent proteins, including redox-sensitive versions (e.g. roGFP). These genes will be put under the control of upstream regions of mitochondrial genes, and as fusion proteins.

We will work together with imaging specialists, in order to measure the redox changes in the mitochondrion, using the redox-sensitive fluorescent proteins. If time permits, in later years of the project we will allow the genetically modified dinoflagellate algae to form symbiosis with either coral (Galaxea) or sea anemone, which is commonly used as a model organism for coral. We will then stress the symbiosis, and image in hospite (within host). The ability to transform the mitochondrial genome will allow us to study how the dinoflagellate alga responds to stress.

The project is a collaboration between Ellen Nisbet (primary supervisor, Nottingham) who has worked on dinoflagellate genetics for many years, Catarina Gadelha (second supervisor, Nottingham), who has expertise in transformation of single-celled eukaryotes and imaging, with assistance from Chris Howe (University of Cambridge), a world-leader in dinoflagellate genetics and organelle biochemistry, and Pietro Cicuta (University of Cambridge), a physicist who is developing real-time automated imaging for algal cultures and host-parasite systems.

References to learn more:

1. Nimmo IC, Barbrook AC, Lassadi I, Chen JE, Geisler K, Smith AG, Aranda M, Purton S, Waller RF, Nisbet RER, Howe CJ. (2019) Genetic transformation of the dinoflagellate chloroplast eLife 8:e45292. doi.org/10.7554/eLife.45292

2. Hughes T et al (2017) Global warming and recurrent mass bleaching of corals. Nature 543, 373–377

Location of lab rotation: Sutton Bonington

Location of full PhD project: Sutton Bonington

(167) Future protein from low cost and sustainable methane gas

Primary supervisor: Ying Zhang

Second supervisor: Ben Dickins

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

During the nine-week rotation, the student will become familiar with the growth and manipulation of methanotrophic organisms and the use of the various gene tools available.
Specifically, they will:
(i) learn how to cultivate methanotrophic organisms using methane as the sole carbon source;
(ii) learn how to electro-transform and conjugate from E. coli into methanotrophic organisms, how to screen putative mutants by colony PCR and agarose gel electrophoresis; how to assemble complex operons via Gibson/USER assembly; become familiar with DNA analysis software, CLC Bio WorkBench, DNASTAR and Vector NTI.
(iii) undertake CRISPR/Cas9-mediated gene knock-out.

This translational project will be carried out within the BBSRC/EPSRC Synthetic Biology Research Centre (SBRC) at Nottingham which comprises 90+ graduate and postdoctoral researchers (http://sbrc-nottingham.ac.uk/). The study will allow for training in a unique multidisciplinary environment, incorporating gas fermentation, Synthetic Biology, microbial physiology, metabolic engineering and computer modelling.

Full PhD project description:

Healthy poultry require high quality sources of protein and carbohydrates. Modern feeds for poultry consists largely of grain and protein supplements such as soybean, which can be in direct competition with human food consumption. Countries like Canada and US have recently been active in producing high-protein insect feed ingredient (Enterra Feed Corporation), and have granted national regulatory approval for its use in broiler feed and pet food, the first ingredient of its kind to do so. In the EU, processed insect protein is currently not eligible to be included in the feed of food-producing animals.

To meet poultry feed market demand in a way that tackles both environmental and economic concerns, we propose a novel route to poultry feed using methane (CH_4) fixing bacteria, methanotrophs. These bacteria naturally produce lipids and have a proven commercial track record for single cell protein production (for fish feed). Methanotrophs also utilize methane as the sole source of carbon and energy, providing significant advantages in both process economics and sustainability. Methane is an abundant and cheap carbon resource, but is currently under-utilised as a feedstock for industrial biotechnology and is more associated as a greenhouse gas (GHG) pollutant. Surplus methane is often released during oil extraction and simply flared. Methane is also a major component of biogas produced on a large scale by anaerobic digestion, technology well established in the EU. Today, most biogenic methane is burnt for energy and has relatively little value. Since it is a low-cost and sustainable feedstock, and given the many sources available and current wastage and associated GHG ramifications, methane provides an exciting feedstock opportunity for fermentation and conversion into high value biochemical metabolites (lipids, proteins and feeds).

Methylococcus capsulatus is the model organism in this process, which has already been approved in the EU for feeding to farmed fish and livestock such as pigs. Methane fermentation on a large scale would reduce the demand for land to grow food for livestock. However, more research is needed on methane-based poultry feed.

Our group recently isolated a novel M. capsulatus (designated as Iso-14) which shows major differences in its DNA sequence from published type strain. Furthermore, it possesses distinctive granules (assumed to be glycogen), a feature absent in other strains. We speculate that feed made from it will have higher carbohydrate composition compared to the commercial feed from the type strain, which will make it potentially more suitable to be used for poultry feed.

In this project, we will explore the possibility of using methane as a feedstock to produce alternative protein source for poultry feed; to improve the rates and energy efficiencies of methane uptake, as well as approaches to engineer high-productivity methane conversion organisms:
(i) identify the biomass composition and nutritional value of the newly isolated Iso-14, using type strain as control, to determine its potential to replace soybean;
(ii) implementing the requisite gene technologies for modifying the organism;
(iii) using synthetic biology to engineer the strain to produce an exemplar nutritional compound such as vitamin or long chain fatty acid;
(iv) subject Iso-14 to adaptive laboratory evolution (ALE) to improve its fitness for biotechnology purposes.

References to learn more:

Strong PJ, Kalyuzhnaya M, Silverman J, Clarke WP. A methanotroph-based biorefinery: Potential scenarios for generating multiple products from a single fermentation. Bioresour Technol. 2016 Sep;215:314-323

Location of lab rotation: University Park

Location of full PhD project: University Park

(168) Investigating mechanisms which control blood vessel formation and function: how does GPCR signalling by Calcrl/Ramp2 control permeability of the blood-brain barrier?

Primary supervisor: Robert Wilkinson

Second supervisor: Martin Gering

Institution: University of Nottingham

School: Life Sciences

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 PhD 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 thus protects the brain from injury and disease. Loss of barrier properties during diseases including stroke, diabetes and vascular dementia contribute to underlying pathology and disease progression. 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 high molecular weight fluorescent dyes within the developing brain vasculature at stages where these vessels are normally intact and the BBB is normally functional. Interestingly, leakage of fluorescent dye was contained within large numbers of microvesicles, suggesting vascular hypermerpeability is an active process in these mutants. We hypothesise that calcrl/ramp2 mutants display an activation of transcellular permeability pathways leading to increased permeability of the BBB. ramp2 mutants display a strikingly similar phenotype to calcrl mutants and this can be rescued using low dose Vascular Endothelial Growth Factor (VEGF) receptor inhibition. Since VEGF is known to make blood vessels leaky and activate transcellular permeability pathways, we hypothesise that under normal circumstances Calcrl/Ramp2 signalling functions to limit vascular permeability by suppressing VEGF receptor signalling.

To test these hypotheses, this project will utilise RNA sequencing to identify the molecular mechanisms underlying calcrl/ramp2-mediated vascular permeability. To test candidate genes, we will employ cutting edge CRISPR/Cas9 and CRISPR interference technologies developed within our group. To determine how Calcrl/Ramp2 control BBB permeability, we 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.

References to learn more:

3. Savage, A.M., Kurusamy, S., Chen., Y, Jiang, Z., Kim, H.R., Wilson H.L., van Eeden F.J.M., Armesilla A.L., Chico T.J.A., Wilkinson R.N. tmem33 is essential for VEGF-mediated endothelial calcium oscillations and angiogenesis. Nature Communications 2019 10:732 doi: 10.1038/s41467-019-08590-7
4. O'Brown, N.M., Megason, S.G., Gu, C. Suppression of transcytosis regulates zebrafish blood-brain barrier function. Elife. 2019 Aug 20;8. pii: e47326. doi: 10.7554/eLife.47326

Location of lab rotation: QMC

Location of full PhD project: QMC

(169) Impact of a crucial regulator of mitosis on the motility and proliferation of cells

Primary supervisor: Claire Friel

Second supervisor: Amanda Wright

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

In this mini project you will use molecular biology techniques to generate the DNA constructs required to express variants of the Mitotic Centromere Associated Kinesin (MCAK) in cells. The Friel lab have created and characterised a suite of variants with a range of microtubule depolymerisation activities. This work will allow us to utilise the power of these molecular tools to understand the role of microtubule stability in cell division and cell motility.

This project will provide training in the fundamental molecular biology and cell biology techniques that underpin biological sciences research, and which are vital skills that are widely applicable in the biological sciences.

Full PhD project description:

Cancer cells are more deformable than healthy cells and deformability is likely linked to their metastatic potential. For instance, it is well-known that stiffness and elasticity of tissues change upon the presence of a tumour; what is less clear is the origin of these changes at the subcellular level and how these relate to the biology of a tumour cell.

The cytoskeleton is a complex composite of microtubules, actin and intermediate filaments which helps to maintain the rigidity of cells and is critical to processes requiring movement and deformation of cells. The stability of the cytoskeleton is controlled by a variety of regulatory proteins. The microtubule depolymerizing kinesin, MCAK (KIF2C) is a major regulator of the microtubule cytoskeleton. In healthy cells, MCAK plays an important role in regulating microtubule length, particularly during mitosis and meiosis, where it also has a role in correction of chromosome-microtubule attachment errors. Knockdown of MCAK has a greater impact on cancer cells than healthy cells and alteration in expression mediates sensitivity to anti-microtubule cancer therapies. MCAK is overexpressed in breast, colorectal and gastric cancers and elevated expression correlates with increased metastasis and poor patient prognosis. We hypothesise that elevated levels of MCAK in cancer cells leading to destabilisation of the microtubule cytoskeleton increases cell deformability and in turn, metastatic potential. Hence, the overall aim of this project is to study the effect of perturbing the microtubule cytoskeleton, through manipulation of MCAK activity, on the mechanical properties of cells. Two different but complementary physical techniques will be used to quantify the mechanical properties of individual cells and these measurements compared with results from established cell biological assays which report on cell motility, deformability and proliferation. Although a body of evidence exists to connect overexpression of MCAK to progression of cancer, the link between MCAK activity and the mechanical properties of cells has not been investigated. Therefore, it is necessary and timely to use the biological tools now available to titrate MCAK activity and combine these with cutting edge physical techniques that report on cell mechanics to uncover the relationship between MCAK expression in cancer cells and increased metastatic potential and/or poor patient prognosis.

MCAK is a target for new chemotherapeutic development, particularly to combat resistance to current anti-microtubule cancer drugs. The proposed work will add to our knowledge of the impact of MCAK in cancer cells and assist the development of drugs targeting MCAK activity. Further, the project will aid development of cutting edge biophysical techniques which will advance the use of cell mechanical properties as a novel label-free biomarker and diagnostic tool in the treatment of cancer.

References to learn more:

1. Friel, C.T. and Welburn, J.P. (2018) Parts List for a Microtubule Depolymerising Kinesin. Biochemical Society Transactions 46: 1665-1672

2. Pachenari, M.et al. (2014) Mechanical properties of cancer cytoskeleton depend on actin filaments to microtubules content, Journal of Biomechanics 47, 373-379.

Location of lab rotation: QMC

Location of full PhD project: University Park; QMC

(170) Robust platforms for the development of bovine, equine and canine monoclonal antibodies to inform future emerging virus therapies and vaccines

Primary supervisor: Jonathan Ball

Second supervisor: Patrick McClure

Third supervisor: R Urbanowicz

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The rotation project will explore different methodologies available for identifying antigen-specific B cells in peripheral blood obtained from different animal species (e.g. sheep, cattle, dogs), including direct antigen staining, plasma blast/memory cell sorting. They will also gain experience of the molecular methods used to clone single B-cell antibody repertoires (i.e. monoclonal antibodies) using single-cell RT-PCR and cloning. If time allows the student will also gain experience of protein expression systems to produce active antibodies as well as virus killing assays, such as pseudovirus neutralisation assays. If the student prefers, they can explore the use of phage display to select for antigen-specific monoclonal antibody Fab fragments. This will involve phage library construction, biopanning and then subsequent characterisation of selected phage displayed antibodies using DNA sequencing and a variety of immunoassays (including ELISA and virus killing assays).

Full PhD project description:

We have established highly successful research programmes focussing on human monoclonal antibody technology and its use in therapeutic and vaccine design and have more recently extended these technologies to establish a bovine mAb discovery platform. The immediate aim of this proposal is to provide proof of concept using vaccinated animals to isolate antigen-specific mAbs.

A variety of techniques are available for the generation of mouse and human monoclonal antibodies, some of which have been applied to other species. Traditional myeloma fusion methods are limited by low transformation and cloning efficiency and are relatively cumbersome and inefficient. More recently, mice harbouring human Ig repertoires have been created and these are starting to deliver lead mAbs. Production of these transgenic mice is not a trivial undertaking and has not extended beyond human antibodies. Antibody-display libraries have yielded important antibodies for a range of important pathogens – this has proven a valuable approach in identifying pathogen-specific antibodies. An alternative and often more robust method is to generate and screen antibodies directly cloned from single B cells (either antigen-stained or following short-term culture) or antibody secreting cells. This is a powerful means to interrogate an antibody repertoire and is amenable to high throughput screening using a variety of methods, including functional assays such as virus-killing.

Through previous MRC, we have developed a high throughput platform for rapid generation of virus-specific human monoclonal antibodies. Our methods were initially developed to study the antibody response induced following natural infection with Hepatitis C virus (HCV) in humans. With a BBSRC-funded pump-priming grant and current EU funding we are now applying these techniques to cattle

and sheep. Knowledge of virus-neutralising antibody responses will inform rational vaccine design for global threats such as bluetongue virus or CCHF, as well as variety of emerging viruses of both animal and human importance (e.g. Filoviruses, Nipah viruses and Coronaviruses).

In addition to B-cell cloning, we are also developing a bovine antibody Fab-fragment display technique focussing on antibodies with ultra-long CDR3 regions. These are proving very potent in their ability to kill a broad range of genetically distinct viruses (for example, we have evidence that cows being immunised with Lassa fever (LFV) virus-like particles are generating antibody repertoires capable of neutralising highly divergent LFV isolates as well as other members of the old-world arenavirus genus (e.g. LCMV). This neutralisation breadth is remarkable and probably a consequence of these ultra-long CDR3 antibodies.

References to learn more:

1. Cao Y, Li K, Wang S, Fu Y, Sun P, Li P, Bai X, Zhang J, Ma X, Xing X, Zhou S, Bao H, Li D, Chen Y, Li Z, Lu Z, Liu Z. Implication of Broadly Neutralizing Bovine Monoclonal Antibodies in the Development of an Enzyme-Linked Immunosorbent Assay for Detecting Neutralizing Antibodies against Foot-and-Mouth Disease Virus Serotype O. J Clin Microbiol. 2019 Nov 22;57(12). pii: e01030-19. doi: 10.1128/JCM.01030-19. Print 2019 Dec. PubMed PMID: 31578261.

2. Sok D, Le KM, Vadnais M, Saye-Francisco KL, Jardine JG, Torres JL, Berndsen ZT, Kong L, Stanfield R, Ruiz J, Ramos A, Liang CH, Chen PL, Criscitiello MF, Mwangi W, Wilson IA, Ward AB, Smider VV, Burton DR. Rapid elicitation of broadly neutralizing antibodies to HIV by immunization in cows. Nature. 2017 Aug 3;548(7665):108-111. doi: 10.1038/nature23301. Epub 2017 Jul 20. PubMed PMID: 28726771; PubMed Central PMCID: PMC5812458.

Location of lab rotation: Sutton Bonington Campus

Location of full PhD project: Sutton Bonington Campus

(171) Proteins in Alien Environments

Primary supervisor: Anna Croft

Second supervisor: Ricky Wildman

Third supervisor: Christof Jaeger

Institution: University of Nottingham

School: Engineering

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 PhD 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 will then be selected based on the combined properties for printing and tes

Location of lab rotation: University Park; Jubilee Campus

Location of full PhD project: University Park; Jubilee Campus

(172)

Primary supervisor: Rachel Gomes

Second supervisor: Anca Pordea

Third supervisor: Jon Hobman

Institution: University of Nottingham

School: Engineering

Lab rotation description:

Full PhD project description:

References to learn more:

Location of lab rotation:

Location of full PhD project:

(173) Decoding tumour-infiltrating T cell heterogeneity in acute myeloid leukaemia (AML) by single-cell genomics

Primary supervisor: Sergio Rutella

Second supervisor: Anbarasu Lourdusamy

Institution: NTU

School: School of Science and Technology

Lab rotation description:

In the lab rotation project, the student will get experience on nucleic acid isolation, quantitation using various assays (Nanodrop, Qubit, Bioanalyzer), flow cytometry and flow sorting of CD4 and CD8 T cells, and functional assessment of purified populations of T cells. The student will also get an opportunity to participate in optimising NanoString-based gene expression assays and in big data analysis.

Full PhD project description:

Acute myeloid leukemia (AML) is characterized by the abnormal clonal proliferation of myeloid cells accumulating in the bone marrow and circulating in the blood. AML is cured in 35% to 40% of adult patients aged <60 years using chemotherapy. The main cause of treatment failure is disease relapse. The immune system plays a key role in restraining tumour progression, as extensively shown in solid tumours. Immune escape mechanisms that are operational in haematological malignancies have been less extensively characterised. Thus far, studies have demonstrated that the presence of T cells in AML tumour microenvironment (TME) may correlate with worse overall survival, implying the occurrence of AML-driven immune evasion/T-cell dysfunction.

Until now, attempts to fully characterise the T-cell compartment of AML with single cell resolution have been very limited. This project will therefore aim to 1) assess the transcriptional and functional heterogeneity of tumour infiltrating T cells (TILs) by utilising cutting-edge technological platforms (Year 1); 2) dissect the composition of the AML immune TME (Year 2); and 3) identify targets for immunotherapy (Years 2-3). TILs will be isolated from primary bone marrow samples obtained at diagnosis and relapse. T cells will be purified using fluorescence activated cell sorting, and single cell barcoded mRNA libraries will be created using the 10X Genomics Chromium platform (single cell libraries up to 8,000 individual CD4 and CD8 T cells). A portion of single cell libraries will be used to assess T-cell repertoire diversity using targeted PCR amplification. The libraries will be sequenced using Illumina next generation sequencing (NGS) platforms and data will be analysed using standard bioinformatics workflows. Cell barcode filtering, alignment of reads and unique molecular identifier (UMI) will be performed using the CellRanger software (10X Genomics). Expression levels will be quantified and used to filter out low-quality cells. Pearson correlation coefficients between expression profiles of cells that passed quality filtering will be calculated using centred gene expression levels and cells will be ordered by hierarchical clustering. In addition, transcriptional programs and cellular hierarchies will be determined using machine-learning approaches.

Location of lab rotation: Clifton Campus

Location of full PhD project: Clifton Campus

(33) Optimising CRISPR-based genetic editing in human cells by tweaking DNA repair

Primary supervisor: Edward Bolt

Second supervisor: Thorsten Allers

Institution: University of Nottingham

School: Life Sciences

Lab rotation description:

The aims of the 9-week rotation will be to introduce the student to the methods that edit genomes in living cells, and to trial a new editing enzyme that we have designed.

(a) Training − the student will gain the skills to edit a genetic locus on the chromosome of cells using the established Cas9 and CasX CRISPR editing enzymes. To do this, the student will learn transfection and nucleofection methods for delivering nucleic acids or proteins directly into cell nuclei.

(b) Preliminary testing of a proof-of-principle – the student will use these methods to deliver a new, rationally designed, CRISPR editing enzyme into cell nuclei, for preliminary assessment of its activity at replacing a genetic locus. The purpose of this test is to give the student hands-on experience of original research in CRISPR editing that extends standard methods using Cas9 and CasX.

Full PhD project description:

The research project fits within the BBSRC areas “Bioscience for Health” and “Biotechnology”. Editing of genomes using enzymes adapted from naturally occurring CRISPR-Cas systems (e.g. Cas9) is widely used for synthetic biology and genetic engineering, but has drawbacks that has so far curtailed its use in medicine. One drawback is that although Cas9 triggers editing very easily, the downstream events needed to complete successful editing are inefficient and unpredictable, because they require complex DNA repair processes that are poorly understood.

Recent studies have indicated that CRISPR-based DNA editing in human cells might be improved by manipulating human DNA repair enzymes that promote the types of DNA repair desirable for completing the editing processes. One line of enquiry involves manipulation of the replication-coupled DNA repair helicase HelQ and its interacting ‘Rad51 paralogue’ proteins: The focus of this PhD project is determining the roles of these proteins in promoting higher efficacy of genome editing, and developing ways to utilize them for improved CRISPR editing methods.

The supervisor labs (Bolt-Allers) are expert in CRISPR-Cas, CRISPR editing and DNA repair by HelQ-like enzymes. This ensures that the DTP student will gain a cutting-edge understanding of the biotechnology and molecular biology related to these processes. The student will combine cell biology, genetic engineering and protein biochemistry to characterise and measure the ability of cells to undergo genome editing when manipulated for HelQ and the Rad51 paralogues.

References to learn more:

1. Richardson et al Nature Genetics (2018). PubMed ID number (PMID) 30054595.
2. Lau et al Essays in Biochemistry (2019). PMID 31186288.

Location of lab rotation: QMC

Location of full PhD project: QMC

(39) Determination of the structure and catalytic mechanisms of Pseudomonas aeruginosa secreted proteins

Primary supervisor: Kim Hardie

Second supervisor: Jonas Emsley

Institution: University of Nottingham

School: Life Sciences

Lab rotation description: The aim is to purify some AaaA and/or pyocin S3 DNAse without the Immunity protein, and become trained in the biochemical protocols of protein purification and peptidase activity assay.

Week 1: Use the existing genetic construct to overproduce the proteins from E. coli
Week 2: Use the existing successful pilot protocol to purify small scale
Week 3: Assay the activity of the proteins.
Week 4-8: Scale up and optimize purification: investigate the efficiency of affinity purification via the His-tag, ionic exchange, selective precipitation using ammonium sulphate and refolding after denaturation in Guanidine or Urea.

Week 1-8 in parallel: Clone the truncated aaaA into the plasmid pME6032 for inducible overproduction in a aaaA deficient P. aeruginosa mutant as an alternative source of purified protein which we hypothesize may be more soluble as this is the homogenous host bacterium.

Week 9: Assay AaaA produced from pME6032 construct, organize samples being stored from the project and write up report.

Full PhD project description:

Secreted virulence factors are ideal targets for novel antimicrobials: they are readily accessible to externally applied chemicals.

Bacteria secrete proteins that specifically target and kill other bacteria (Bacteriocins): potentially useful novel antimicrobials.

Both types of protein could serve as a chassis to export proteins for biotechnological uses thereby reducing downstream purification.

This project seeks to characterize the structure and function two Pseudomonas aeruginosa virulence factors. P. aeruginosa is a major opportunistic pathogen for CF, bronchiectasis, COPD and wound patients, is intrinsically resistant to antibiotics, and almost impossible to eradicate from hospitals in part due to its ability to grow as biofilm communities.

We recently characterized the surface tethered arginine-specific aminopeptidase AaaA. AaaA is required for chronic skin wound infections, and in its absence biofilm formation is impeded. AaaA releases arginine from the amino-terminus of peptides and proteins. AaaA enables P. aeruginosa to grow when the sole source of nitrogen is a peptide with an amino terminal arginine. Thereby, AaaA provides a mechanism for P. aeruginosa to survive in environments limited for oxygen such as anoxic

pockets in the lungs of CF sufferers or biofilms. Before it is of pharmaceutical use, the identity of the substrate of AaaA and its catalytic mechanism need to be characterized. If inhibition of AaaA successfully prevents biofilm formation or leads to biofilm destruction, targeting AaaA will augment current antimicrobial therapy which is often thwarted by their inability to penetrate biofilms. An AaaA-inhibitor may be effective alone or in combination with another drug. AaaA would also be exploitable as a protease.

Pyocin S3 is a bacteriocin produced by P. aeruginosa that kills other species of P. aeruginosa. A cognate immunity protein binds to the pyocin in the producing cell to prevent suicide. We have shown that pyocin S3 is a DNase, and have solved the crystal structure of the DNase domain in complex with its cognate immunity protein, revealing its uncharacteristic primary sequence still maintains an active site bba secondary structure motif without the typical DNase HNH arrangement. In addition, a novel octopus grasp of the DNAse by the cognate immunity protein was discovered. We also have unusual cubic crystals of the S3 DNase in its native form with the receptor binding and translocation domains attached.

This project aims to purify and crystallize:

1. AaaA. The new structure, or the currently available predicted structure, will be docked in silico to the 80,000 compounds available in the CBS library to search for potential inhibitors. The inhibition of activity and biofilm formation will be assessed.

2. Pyocin S3 DNase domain without its cognate Immunity protein to reveal the presence of bound metal ion at the active site motif and in its native full length form. The purified DNase will then be used to investigate the mode of action of DNA catalysis.

3. The Pyocin S3 cognate Immunity protein.

For both AaaA and pyocin S3, site directed mutagenesis will be used to inactivate residues predicted to be implicated in the function to increase our understanding of their functional domains.

References to learn more:

1. Luckett, J.C.A, Darch, O., Watters, C., AbuOun, M., Wright, V., Paredes-Osses, E., Ward, J., Goto, H., Heeb, S., Pommier, S., Rumbaugh, K., Camara, M., and Hardie, K.R. (2012) A novel virulence strategy for Pseudomonas aeruginosa mediated by an autotransporter with arginine-specific aminopeptidase activity. Plos Pathogens. 8(8):e1002854.
2. Michel-Briand, Y. and Baysse C. (2002). The pyocins of Pseudomonas aeruginosa. Biochimie 84(5-6):499-510.

Location of lab rotation: University Park

Location of full PhD project: University Park

(10) Pause for thought: Corticostriatal dopamine and the inhibitory modulation of associative learning

Primary supervisor: Helen Cassaday

Second supervisor: TBC

Institution: University of Nottingham

School: Psychology

Lab rotation description:

The rotation will interface with an ongoing project funded by the BBSRC, so there will be help and support at all times in the lab. Academic supervisions will be scheduled weekly. The rotation will introduce techniques relevant to the PhD project, e.g. behavioural testing and intra-cerebral drug administration.

1) Behavioural experiments. Students will receive 'hands-on' training in animal handling and welfare, experimental design and scheduling.

2) Surgical procedures. Students will observe regulated procedures. There will be opportunities to assist with health monitoring post-operatively and with intra-cerebral drug administration via indwelling cannulae.

3) Data analysis. Students will be trained in the statistical analysis of behavioural data. There may also be opportunities to assist with the histology procedures used to verify the placement of the cannulae or electrodes (used for intra-cerebral drug administration or to record neural activity).

4) Seminars and meetings. The students will be expected to attend the group lab meetings and relevant seminars.

The ethical and legal framework and the principles of the 3Rs will be embedded in the training provided. The student will either conduct, assist with or observe the in vivo aspects of the research, depending on whether s/he has a Home Office personal licence.

Full PhD project description:

Background: Conditioned inhibition is a form of learning seen when an otherwise expected event does not occur in the presence of the inhibitor. Such inhibitory modulation is fundamental to many aspects of normal psychological function, such as the control of food intake, while impairments in this process underpin a wide variety of mental health conditions, e.g. we found impaired inhibitory learning in humans with schizophrenia. We have therefore adapted the experimental design for use in rats, with a refined appetitive procedure, to investigate the role of the dopamine (DA) system and interconnected cortical structures, specifically medial prefrontal cortex (mPFC). We use this translational task to analyse the role of corticostriatal DA in inhibitory modulation, with a view to developing novel therapeutic strategies. The mPFC and DA systems have been independently identified as being involved in aspects of inhibition. The proposed plan of work will advance on these findings by testing for dissociable effects in mPFC sub-regions. Moreover, the mPFC projects topographically to nucleus accumbens (NAc) in the striatum, which is also a functionally heterogenous structure. Results obtained using (1) regional inactivation, (2) electrophysiological and (3) optogenetic methods (e.g. transduction of ChR2 combined with mCherry tracing) in NAc and corresponding mPFC sub-regions, will identify functional interactions between these structures and

their microcircuitry. We will investigate the role of particular DA receptor sub-types in a series of micro-infusion studies at the coordinates identified in the regional inactivation studies. We will define the wider molecular networks via the use of proteomic and RNA analysis using mass spectrometry and network approaches. This will allow us to examine the modulatory role of DA in the key brain regions of interest. The use of a translational task in a rat model allows for localised intervention studies to establish cause and effect, combined with correlational studies of interconnectivity of small sub-regions.

Aims: This project involves in vivo neuroscience and behavioural techniques to determine the substrates of inhibitory learning. Specifically, to (1) define the components of corticostriatal circuitry necessary for the inhibitory modulation of appetitive associative learning and (2) investigate the role of different dopamine receptor sub-types and other molecular networks in the corticostriatal circuitry.

Hypotheses: This project addresses a number of hypotheses and specific predictions. For example, based on the hypothesised functional interplay between PFC and striatum, inactivation of prelimbic PFC or NAc core is predicted to impair acquisition of conditioned inhibition. Based on his/her literature review of the cognitive processes underlying inhibition and their neuropharmacological substrates, the student will be encouraged to formulate his/her own hypotheses and experimental predictions.

Fit with BBSRC remit: The project sits within the Brain Science and Mental Health and Lifelong Health and Wellbeing cross-research council transdisciplinary programmes. Moreover, we address two BBSRC responsive mode priorities: Food, Nutrition and Health, because food intake is often triggered by external cues and identified inhibitory cues provide us with cognitive-behavioural strategies to keep body mass index in a healthy range; and Healthy Ageing as the loss of cognitive inhibition in ageing populations is well documented.

References to learn more:

1. He, Z.*, Cassaday, H.J., Park, S.B.G. & Bonardi, C. (2012). When to hold that thought: An experimental study showing reduced inhibition of pre-trained associations in schizophrenia. PLoS ONE, 7, e42175.

2. He, Z.*, Cassaday, H.J., Bonardi, C. & Bibby, P.A. (2013). Do personality traits predict individual differences in excitatory and inhibitory learning? Frontiers in Psychology: Personality Science and Individual Differences, 4, Article 245.

Location of lab rotation: University Park, QMC

Location of full PhD project: University Park, QMC, Sutton Bonington Campus

(11) The human DNA helicase HelQ is recruited in N6-methyladenosine (m6A)-containing mRNA:DNA transcriptional R-loops and helps in their resolution and repair

Primary supervisor: Panos Soultanas

Second supervisor: TBC

Institution: University of Nottingham

School: Chemistry

Lab rotation description:

During the 9-week rotation the incoming student will be carrying out a two-pronged mini-project.

1. The student will be trained in cell culture techniques so that he/she will be able to carry out immunostaining experiments to visualise in vivo m6A-marked mRNA-DNA R-loops. Once this is done the student will then proceed to carry out experiments in appropriate cell lines: (i) a HelQ knock-out, (ii) GFP-labelled HelQ, (iii) a Walker mutant inactivated in the ATPase/helicase activity and (iv) a control cell line with fully active HelQ, in order to compare and contrast the localisation of HelQ in m6A-containing R-loops.

2. The student will also get trained in the expression and purification of the human HelQ protein in order to purify good amounts of functional HelQ and then carry out standard in vitro ATPase/helicase assays with a range of synthetic oligonucleotide substrates in order to assess the preferred substrates of HelQ.

Full PhD project description:

Introduction
mRNA-DNA R-loops created during transcription by stalling RNA polymerases pose a considerable obstacle to DNA replication and need to be cleared off for a smooth progression into the S phase of the cell cycle when the genome is replicated. Otherwise, the accumulation of R-loops can lead to genome instability. In a recent paper, Ruzov and collaborators discovered that N6-methyladenosine (m6A)-containing R-loops tend to accumulate during the G2/M phase and are depleted during the G0/G1 phase of the cell cycle. Furthermore, they showed that the m6A-binding protein YTHDF2 also associates with R-loops and a ydhdf2 knockout leads to increased R-loops levels, cell growth retardation and accumulation of double strand DNA breaks in mammalian cells.
The Soultanas and Bolt groups have been working collaboratively with a human DNA repair helicase known as HelQ. HelQ is an important DNA repair protein, highlighted by multiple effects of helq gene loss, including elevated mutation rates, increased cancer incidence and sterility. Genetic analyses have led to models implicating HelQ in controlling and limiting homologous recombination when DNA replication is being repaired, so that replication can resume without genetic rearrangements occurring. But biochemical mechanisms for how HelQ helicase contributes to these events are unknown. HelQ is an ATP-dependent DNA helicase that translocates single stranded DNA (ssDNA) with 3’ to 5’ polarity.

Recent collaborative work in the Soultanas, Ruzov and Bolt labs has resulted in the exciting discovery that HelQ appears to localize in m6A-containing R-loops, suggesting that HelQ may also be involved in processing and removing R-loops, clearing the way for replication to occur unimpeded.

Hypothesis
We hypothesize that problematic R-loops created by stalled transcription during the G2/M phase of the cell cycle are marked by m6A for removal during the G0/G1 clearing the genome from obstacles that might interfere with DNA replication during the S phase. HelQ is recruited at these m6A-containing R-loops to unwind them and clear them off.

Project
The main aim of this project is to provide in vivo and in vitro evidence in support of our hypothesis.
1. We will carry out immunostaining experiments with appropriate cell lines, wildtype and helq-knockout, to confirm and provide additional in vivo data that HelQ co-localizes with m6A-containing R-loops.
2. We will carry out immunostaining of m6A on WT and HELQ KO cells in the presence of No RNases, in the presence of RNase A only, and in the presence of both RNase A +H. This may allow us to discern whether the distribution and levels of m6A modified R-loops is altered following HELQ depletion.
3. We will use the gammaH2AX double strand marker to carry out immunostaining experiments to assess whether depletion of HelQ leads to increased DNA damage.
4. We will carry out complementation experiments to assess whether expression of HelQ rescues HelQ-depleted cells.
5. We will investigate whether HelQ co-immunoprecipitates with RNA polymerase.
6. We will express/purify the human HelQ protein and use different synthetic oligonucleotide substrates to study its activities.

References to learn more:

1. Abakir A*, Giles TC, Cristini A, Foster JM, Dai N, Starczak M, Rubio-Roldan A, Li M, Eleftheriou M, Crutchley J, Flatt L, Young L, Gaffney DJ, Denning C, Dalhus B, Emes RD, Gackowski D, Corrêa IR, Garcia-Perez JL, Klungland A, Gromak N, Ruzov A. (2019) N6-methyladenosine regulates the stability of RNA:DNA hybrids in human cells. Nat Genet. In Press.

2. Northall, S., Ivančić-Baće, I, Soultanas, P. & Bolt, E.L. (2016) Remodeling and control of homologous recombination by DNA helicases and translocases that target recombinases and synapsis. Genes, 7, pii:E52.

Location of lab rotation: University Park, QMC

Location of full PhD project: University Park, QMC