Biotechnology Project List for 2024 Recruitment

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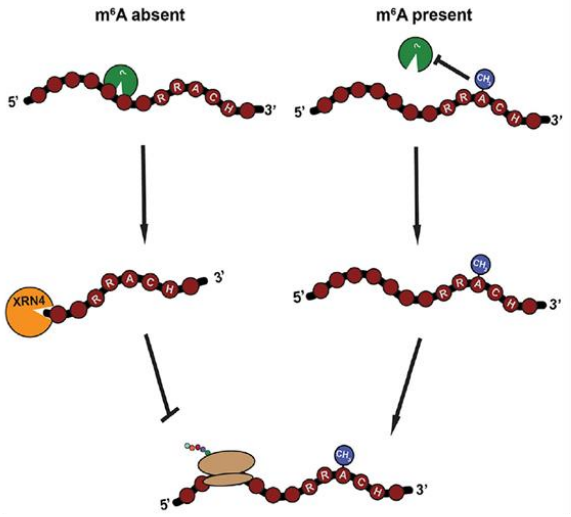
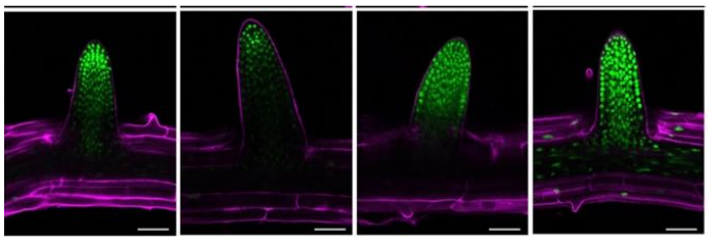
mRNA methylation regulation of gene expression

Project Supervisor: Professor Rupert Fray





School: Biosciences

Description: The methylation of certain adenosines (m6A) in messenger RNA is an ancient process that has been conserved through animal, yeast and plant evolution. In animals, m6A is required for stem cell differentiation and normal development and misregulation of methylation is associated with cancer. In plants m6A is also needed for stem cell differentiation and organ formation as well as responses to environmental stress. The enzymes that put the methylation on mRNA and the "reader" proteins that recognise methylated transcripts are conserved between plants and animals and the extensive genetic resources and ease of gene manipulation make the plant system a great model to address fundamental questions about the process. During the lab rotation, the student will investigate the role of two RNA binding proteins that specifically bind to methylated RNA. They will be trained in vector design, DNA assembly technologies and use of CRISPR gene editing. They will gain experience in confocal microscopy and protein-RNA binding assays.

Location: Sutton Bonington Campus;

Full project description: Methylation of mRNA is a fundamental process that is conserved across eukaryote Kingdoms. It is required for stem cell differentiation and organ formation, and for responses to environmental signals. Misregulation is associated with cancer, but many questions remain as to how this post-transcriptional modification controls gene expression. The Fray, Archer and Mongan groups have collaborated extensively over the past 10 years to address the role of adenosine methylation (m6A) in biological systems including yeast, plants, insects and mammalian cells.

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

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

The student will use CRISPR/Cas9 genome editing, transcriptomic analysis, plant genetic transformation, phenotypic analysis and con-focal microscopy to study the roles of these m6A readers. They will use this information to dissect the role of the different classes of

YTH proteins during normal plant growth and in response to environmental signals such as heat and drought stress and to pathogen attack.

Full project location: Sutton Bonington Campus;

Developing the next generation of immune-modulatory nanomedicine to treat chronic diseases

Project Supervisor: Babatunde Okesola

School: Life Sciences

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

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

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

Week 6 – 9: Student will independently isolate monocytes from human whole blood and will work with Dr Okesola and Prof. Ghaemmaghami's team to learn macrophage differentiation and characterization.

Location: University Park;

Full project description: Project overview:

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

Background and aims: The immune microenvironment is a complex system and plays a critical role in biological processes of some of the most pressing healthcare challenges such as chronic inflammation, wound healing, cancer, and implant integration. Macrophages as a heterogeneous group of immune cells play integral roles in chronic inflammation and metabolic disorders and can adopt various functional states, which are broadly classified into pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes. For example, dysregulation of the M1 macrophage population density and disproportional production of pro-inflammatory cytokines at a wound site can contribute to chronic inflammation and delay wound healing in diabetic patients. The possibility to therapeutically repolarize macrophages, in the form of preventing the M1 phenotype by

controlling tissue environmental factors can be beneficial for the treatment of chronic conditions such as diabetic wounds and cancer.

Like other types of immune cells, macrophages respond to different environmental cues including extracellular matrix (ECM) stiffness, redox state and cytokines and are thereby polarized into specialized functional subsets. The possibility to control macrophage polarization toward either pro-inflammatory or anti-inflammatory phenotypes using matrix stiffness has been demonstrated, however, molecular mechanisms that drive such phenotypical changes have remained elusive. Hypoxia (oxygen shortage) is another important tissue environmental factor. Its impact on macrophage polarization and subsequent modification of the inflammatory microenvironment have not been fully established. Hypoxia can promote macrophages accumulation, polarization and modify the inflammatory microenvironment in chronic conditions such as diabetic wounds, which are generally oxygen-deficient, leading to reduced cell metabolism, retarded angiogenesis, dysregulated inflammation, and anaerobic bacteria colonization. Modification of such microenvironment by molecular oxygen supply can switch macrophage phenotype between the anti-inflammatory and pro-inflammatory phenotypes.

The proposed project aimed to design novel molecular hydrogels that can create synthetic ECM with tunable stiffness, redox sensing and oxygen self-generation ability to modulate macrophage polarization and metabolic reprogramming. This system offers fundamental advantages over state-of-the-art approaches that either rely on the use of cytokines/growth factors or animal-derived matrices such as Matrigel and collagen.

By tuning the mechanical properties of the hydrogels and the concentration of self-generated molecular oxygen, we aim to investigate the crosstalk between the hydrogel mechanics and molecular oxygen level on macrophage polarization and their respective molecular pathways. This information will aid the design of novel immune-instructive nanotechnology with desired immune regulatory properties that could be used for a range of applications including promoting pathogen clearance and treating chronic diseases such as cardiovascular disease, cancer, diabetic wound healing, and arthritis amongst others.

Full project location: University Park;

Identifying novel natural products in the Klebsiella oxytoca complex using genomic and metabolomic techniques

Project Supervisor: Lesley Hoyles

School: School of Science and Technology (NTU)

Description: During the rotation, you will be integrated into the laboratories at Nottingham Trent University (microbiology, genomics) and the University of Nottingham (chemistry). Genomic data available for our in-house collection (n=~100) of clinical, veterinary and intestinal Klebsiella oxytoca complex strains will be searched for biosynthetic gene clusters (BGCs) using antiSMASH, the most widely used tool for detecting and characterising BGCs in microbes. A minimum of six strains showing

diverse BGC profiles, including strains we already know to produce toxic metabolites or potential antimicrobial agents, will be studied using a "one strain many compounds" approach. That is, the strains will be grown in a range of different media and conditions (e.g. changing oxygen availability, temperature and pH) to alter their metabolism.

After completing screening work in the microbiology laboratory at Nottingham Trent University's Clifton campus, analytical work will be undertaken in the Department of Chemistry at the University of Nottingham. The metabolite profiles of spent media will be characterized using mass spectrometry to identify specialized metabolites produced under different conditions. Tools such as GNPS and Pep2Path will be used to identify novel natural products for later structural characterization and bioassays. The overall aim is to connect microbial genes to their chemistry.

Location: University Park;Clifton Campus;

Full project description: Biosynthetic gene clusters (BGCs) are sets of two or more adjacent microbial genes that have the potential to synthesize natural products with diverse functions. Computational methods can be used to identify BGCs in genome sequences, and to systematically explore and prioritize them for laboratory-based characterization, where a "one strain many compounds" approach can be used to identify different molecules produced under different environmental conditions. That is, parameters such as oxygen availability, nutrient content, temperature and pH can be adjusted to alter the metabolism of bacteria. Mass spectrometry and chemical structure elucidation can then be used to identify these natural products, facilitating linkage of microbial genes to their chemistry. Many known natural products (including specialized metabolites) have applications in biotechnology, medicine and agriculture. Others can be toxins. Detailed characterization of these natural products, and their associated BGCs, remains limited to a few model organisms.

The Klebsiella oxytoca complex comprises nine species of bacteria, found in soil, plants, the environment, and clinical, veterinary and intestinal environments. A BGC in plant-growth-promoting strains of K. oxytoca has been exploited to enhance the growth of barley. Some clinical strains of K. oxytoca encode a BGC responsible for producing the cytotoxic metabolite tilimycin, a DNA-damaging agent that is a causative agent of antibiotic-associated haemorrhagic colitis in humans. Recent genomic work done at Nottingham Trent University has shown this BGC to be more widespread within the K. oxytoca complex than first thought. We have also shown, through a combination of computational and laboratory-based work, that members of the K. oxytoca complex encode a wide range of unexplored natural products, including antimicrobial agents.

The PhD will involve bioinformatics-based characterization of the BGCs encoded within genomes of our in-house collection of approximately 100 K. oxytoca complex isolates of clinical, veterinary and intestinal origin. Genomic analyses will be extended to publicly available K. oxytoca complex sequence data to characterize the global diversity of BGCs within these bacteria. In the microbiology laboratory, using high-throughput approaches strains will be grown under different conditions to maximise their production of specialized metabolites. Spent media will then be characterized using mass spectrometry to identify metabolites produced under different conditions. Tools such as Pep2Path and GNPS will be used to identify novel peptidic natural products. These products will then be purified and their structures identified. Bioassays will then be carried out to understand the roles of these natural products as antibacterials or antifungals. Mass spectrometry data will also give us novel insights into the metabolome of K. oxytoca-related species, allowing us to generate improved metabolic models for these bacteria. Depending on the nature of the natural products identified, transcriptomic, mammalian cell-line-based and/or transposon mutagenesis approaches will be used to characterize the functionality

of selected BGCs. The overall aim is to connect microbial genes to their chemistry, to identify novel natural products with wider applications.

Full project location: University Park;Clifton Campus;

Metalloenzymes for the biodegradation of rubbers

Project Supervisor: Anca Pordea

School: Engineering

Description: The project aim is to engineer latex clearing proteins (Lcp's) for the degradation of different synthetic rubbers.

During the rotation, the student will be trained in fundamental techniques required in the project, such as established procedures for the expression of Lcp in E. coli, activity determination using an oxygen consumption assay, and analytical techniques for the characterisation of the mixture of oligomer products (fluorescence assay, NMR and HPLC). Guided by our previous computational analyses, the student will select a suitable position to mutate in the protein sequence, will create a saturation mutagenesis library and will screen this for activity using a colorimetric assay.

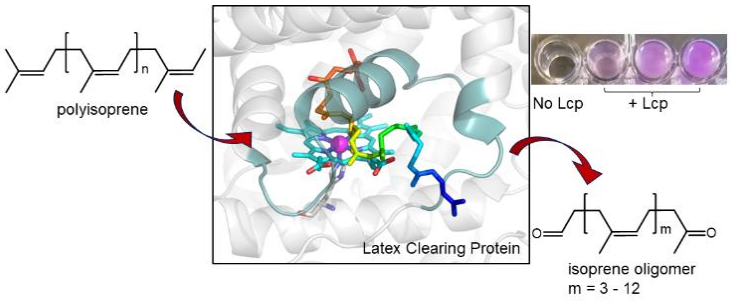
Location: University Park;

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

The vision of this research is to develop metalloenzymes for the cleavage of carbon-carbon double bonds in rubbers. Much exciting research has been published recently, regarding the enzymatic degradation of hydrolysable polymers such as PET. However, the most problematic and abundant polymers have carbon-carbon backbones (polyolefins such as polyethylene, polypropylene and rubbers such as polyisoprene, polybutadiene); these are much more difficult to degrade and recycle than PET.

Based on previous research reported by others and conducted in our own lab, we propose to engineer metalloenzymes to break down synthetic rubbers (polymers with C=C bonds in main chain). Latex clearing proteins (Lcp's) have been shown to degrade natural rubber (latex) into oligomers. Data suggests that synthetic rubbers can also be enzymatically degraded. A recent report demonstrated the increased ability of Lcp K30 to degrade synthetic polyisoprene by using a biphasic system with water, polymer and a hydrophobic solvent. Although a few Lcp's have been characterised so far, the substrate and product spectrum of these enzymes remain largely underexplored.

In our laboratory, we established methods for the expression and purification of two Lcps and we implemented a range of analytical techniques to characterise the rubber degradation, using substrates in biphasic emulsion, powder and film form. In this project, the aim is to engineer Lcps and expand their substrate scope to synthetic polymers such as isoprene rubber (cis-polyisoprene), polybutadiene and trans-polyisoprene.



Full project location:
University Park;

Cultivated meet production using novel hydrogel fibres with encapsulated livestock stem cells

Project Supervisor: Jing Yang

School: Pharmacy

Description: The lab rotation will demonstrate the initial proof-of-concept that livestock stem cells can be encapsulated in hydrogel micro fibres and survive with minimum death and proliferate over time. This is fundamental for this new bioprocess to become a viable approach for producing cultivated meat. Our lab already has some encouraging data on the viability of cell lines in these fibres.

You will first produce hydrogel micro fibres and investigate how processing parameters will affect the formation of these fibres. Then you will encapsulate livestock iPSCs within these fibres during the fibre production process. Finally, you will investigate the viability and proliferation of encapsulated stem cells within these hydrogel fibres. The collaboration between biomaterial expert (Jing Yang), gene therapist (James Dixon) and Animal stem cell expert (Ramiro Alberio) will be key for this project.

Location: University Park;Sutton Bonington Campus;

Full project description: Background: We will have insufficient planetary resources to provide meat to the world population by middle of the century based on the anticipated increase in global demand. Cultivated meat will be an essential part of future protein consumption and will play a critical role in reducing the negative impacts associated with industrial animal agriculture. To realise the potential of cultivated meat, we will need to produce them affordably and efficiently. Growing cultivated meat at scale currently requires the use of bioreactors in volumes up to or beyond several thousands of litres. Continuous stirred tank reactor is the most commonly used bioreactor for culturing meat, which permit growth of cells in suspension via mechanical stirring while maintaining high mass transfer of oxygen. However, this type of bioreactor consumes a large amount of water and energy and has other limitations such as shear stress-induced cell death, contamination risk and associated decontamination cost, and requirement of cell harvesting and subsequent processing to make final meat products. In addition, cell density within stirred tank reactors is in the 2x106 to 1x107 cells/mL range, which is only commercially viable for high-value products such as cell therapies and recombinant proteins. The number of cells required for meat production is much larger than those for cell therapies. To make the production of cultured meat more efficient and commercially viable, new bioprocesses that can handle much higher cell density and use less water

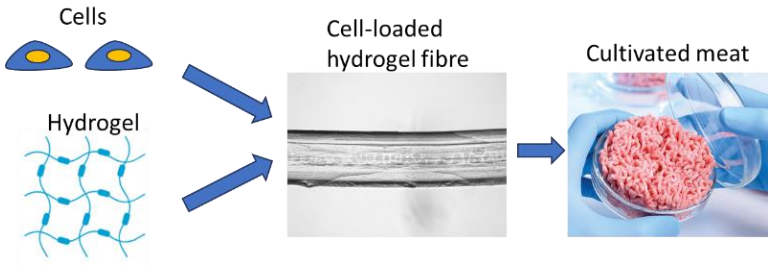
and energy are required. Moreover, these novel bioprocesses will need to produce muscle cells and fat cells quickly and efficiently from stem cells and be able to use sustainable materials within the processes and final products.

Approach: The project will explore a novel way of producing cultivated meat to address the aforementioned limitations associated with current bioprocessing methods. The focus of the PhD will be to produce continuous hydrogel micro fibres with encapsulated livestock stem cells and subsequently differentiate them into muscle and fat cells using a non-viral gene delivery system that has been developed. You will use induced pluripotent stem cells (iPSCs) from pig, sheep and cow to generate species specific muscle tissue for food applications. You will test sustainable and commercially viable materials for making these hydrogel fibres, and optimise the material chemistry, physical properties, and processing parameters in the fibre production process to identify optimal conditions for livestock cell proliferation and differentiation. Means of arranging these cell-containing hydrogel fibres into final meat products will also be investigated.

Location: The project will be carried out in the new state-of-the-art Biodiscovery institute at University Park (Jing Yang & James Dixon) and the School of Biosciences at Sutton Bonington Campus (Ramiro Alberio). The use of livestock stem cells is an advantage of this project and is likely to have significant commercial value and interaction with stakeholders.

Techniques employed: The project will include hydrogel preparation, modification and characterisation, biofabrication, stem cell culture, QPCR, immunostaining, vector construction, gene delivery.

Full project location: University Park;Sutton Bonington Campus;



New Biocatalysts: Identification and directed evolution of a 'clickase' enzyme from a natural protein scaffold

Project Supervisor: Neil R. Thomas

School: Chemistry

Description: 'Click' Chemistry was recognised with the 2022 Nobel Prize for Chemistry. One major use of 'click' reactions is in bio-orthogonal site-specific modification of proteins, nucleic acids and a wide range of organic molecules in the presence of other functional groups. The most common 'click' reaction is the copper(I) catalysed Huisgen azide-alkyne cycloaddition (CuAAC). However, its use is limited for in cellulo reactions due to the toxicity of the copper(I) ions. The uncatalyzed reaction suffers from requiring high temperatures and gives poor regioselectivity, generating a mixture of 1,4 and 1,5-1,2,3-triazole chemically robust products. There are several examples of 'in situ' click reactions where enzymes such as biotin protein ligase have been used to template the Huisgen reaction between an azide and alkyne in order to produce an inhibitor at room

temperature. This observation suggests that if a protein scaffold that allows effective 'click' reaction followed by product release can be identified that it may possess 'clickase' activity which would avoid the need for the toxic copper(I).

We have identified two small protein scaffolds that have the potential to be evolved into effective 'clickases' for the ligation of peptides, because of their conformational flexibility. The 9-week project will involve expressing one of the protein scaffolds and several mutants which will then be characterised by CD and ITC (no previous experience of expressing proteins is required) and the synthesis of azide and alkyne substrates to evaluate the 'clickase' catalytic activity. The design of the substrates will be guided by some computational 'docking' studies.

Location: University Park;

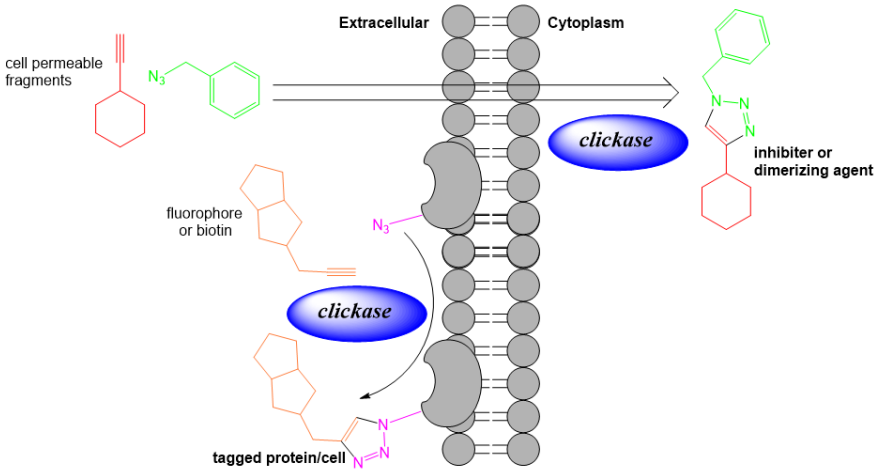
Full project description: The copper(I) catalysed azide-alkyne Huisgen 1,3-dipolar cycloaddition 'click' reaction (CuAAC) has been used extensively for the last two decades in the preparation of new drug conjugates, imaging probes and the conjugation of ligands to biopolymers and nanostructures since its discovery by Meldal and Sharpless. The toxicity of the copper(I) has limited its use with living cells and resulted in the development of a number of metal(catalyst)-free 'click' reactions including the strain-promoted cyclic alkyne-azide cycloaddition (SPAAC) by Bertozzi and the inverse demand Diels-Alder cycloaddition (IEDDA) between 1,2,4,5-tetrazines and cyclic-strained alkene dienophiles. Whilst the SPAAC and IEDDA reactions are compatible with proteins and cells, they suffer from much slower reaction kinetics than the CuAAC reaction, require multistep syntheses to prepare the reaction partners and generate bulky products. The creation of a catalyst that will accelerate the azide-alkyne cycloaddition reaction to give a single 1,4- or 1,5-triazole at room temperature in aqueous neutral conditions that is both biocompatible and can be used in cellulo using simple commercially available azide and alkyne substrates is therefore highly desirable. To generate such a 'clickase' or 'Huisgenase' catalyst we propose to repurpose an existing enzyme scaffold using site directed mutagenesis and directed evolution approaches. The protein scaffolds we have chosen are readily expressed in E. coli and of human origin hence they would not elicit an immune response if used in human cells and can be produced sustainably.

It has previously been shown that copper free in situ azide-alkyne 'click' chemistry can be used to generate competitive inhibitors of the bacterial enzyme biotin protein ligase under ambient conditions. In this case, because of the high affinity of the triazole product for the enzyme, the reaction is limited to a single turnover and hence not catalytic. The reaction does demonstrate good regioselectivity forming the 1,4-substituted triazole in preference to the 1,5-substituted triazole because of the control exerted by the biocatalyst's active site. Building on this and other in situ click reactions, we have identified two candidate enzymes that undergo significant conformational changes during their normal catalytic cycles which should facilitate product release and hence allow multiple turnover events with the azide-alkyne reaction. Both enzymes have been extensively studied as drug targets and hence have large numbers of enzyme-inhibitor and enzyme substrate crystal structures available, which will facilitate our site-directed amino acid mutagenesis experiments and also help rationalise any rate enhancements observed from directed evolution experiments.

Effective directed evolution requires the ability to screen or select proteins with the desired catalytic properties from the largest library of mutants possible. In our case the ligated products can be detected using a Fluorescence Resonance Energy Transfer (FRET) high throughput assay which will allow us to survey very large libraries, increasing the probability of identifying effective biocatalysts.

The project will involve synthetic chemistry to produce the azide and alkyne substrates together with standard synthetic biology (molecular biology; protein expression and engineering) and enzymology methods. Both molecular modelling and structural biology will also be used to elucidate the mechanism of any catalyst developed.

Full project location:
University Park;



Potential applications of a 'clickase' enzyme in chemical biology

In-situ cryo-electron tomography of arenavirus entry pathways

Project Supervisor: Dr Toshana Foster

School: Veterinary Medicine and Science

Description: The student will generate CL2 Mopeia (MOPV) arenavirus by infection of Vero cells and titration by plaque assay. Arenavirus infection assays in the presence of arenavirus restriction factors will be used to assess susceptibility to these host innate factors that can limit arenavirus entry and replication. The host restriction factors of interest, ZMPSTE24 and interferon induced transmembrane proteins (IFITMs), modulate cell membrane fluidity and the student will assess this using membrane probes that are sensitive to the biophysical environment of the membrane and measure these changes by the super-resolution microscopy technique- Fluorescence Lifetime Imaging microscopy (FLIM). The student will have the opportunity to spend 1 week at the MRC CVR in Glasgow to gain training in cryo-electron tomography (cryo-ET) methodologies.

Key methodologies/timeline:

- Weeks 1-2: Cell culture techniques, and MOPV virus production.
- Weeks 3-4: MOPV infection assays in cells ectopically expressing innate restriction factors- measure differences in infection by qPCR.
- Weeks 5-6: Given the impact of these host innate factors on membrane dynamics, perform fluorescence lifetime imaging microscopy to measure changes in membrane tension in cells that ectopically express the innate factors that limit arenavirus infection.
- Week 7: 1 week stay at the MRC CVR to train in cryo-ET methodologies.
- Weeks 8-9: Final data analysis and write-up

All relevant lab training will be provided and there will be the opportunity to present the project at lab meetings and to the wider One virology group. References related to the project:

- Stott-Marshall RJ, Foster TL. Inhibition of Arenavirus Entry and Replication by the Cell-Intrinsic Restriction Factor ZMPSTE24 Is Enhanced by IFITM Antiviral Activity. Front Microbiol. 2022 Feb 18;13:840885. doi: 10.3389/fmicb.2022.840885. PMID: 35283811; PMCID: PMC8915953.

- Vijayakrishnan S*, McElwee M, Loney C, Rixon F, Bhella D. In situ structure of virus capsids within cell nuclei by correlative light and electron microscopy. Nat Sci Rep. 10:1-10 (2020, * corresponding author). doi: 10.1038/s41598-020-74104-x

Location: School of Veterinary Medicine and Science- Sutton Bonington Campus with a training week spent at the MRC CVR, Glasgow;Sutton Bonington Campus;

Full project description: This joint project bridges across the virology and structural biology research programs in the Foster and Vijayakrishnan groups, focussing on elucidating the entry pathways of arenaviruses by cryo-correlative light and electron microscopy (cryo-CLEM) and cryo-electron tomography (cryo-ET) of arenavirus infected cells. Through this approach, we will visualise how the host restriction factors, ZMPSTE24 and the interferon induced transmembrane proteins (IFITMs) mediate their restriction activity through modulation of the biophysical properties of cell membranes.

The Foster group focusses on the largest family of haemorrhagic fever causing viruses known as arenaviruses. These zoonotic viruses are rapidly expanding in their genetic diversity leading to increased annual outbreaks in endemic regions and to sporadic imported outbreaks in globally, including recent cases in the UK. Understanding how host antiviral proteins block arenavirus replication is imperative to addressing current knowledge gaps on the viral lifecycle processes that are key for the development of effective vaccines and treatments.

Using the super-resolution microscopy technique known as Fluorescence Lifetime Imaging microscopy (FLIM), the Foster lab has shown that ZMPSTE24 and IFITM proteins act to modulate the membrane integrity of the host cell and in this way limit arenavirus entry processes. Cryo-ET will allow 3D visualisation of the early events of arenavirus infection in host cells- elucidating key structural and mechanistic details of this process at nanometer resolution in a near native state. This technique will fill critical gaps in our knowledge of arenavirus membrane fusion and will allow us to visualise how fusion events are affected by expression of ZMPSTE24 and IFITM proteins.

We therefore propose to observe the fusion events of containment level 2 arenavirus Mopeia (MOPV) at different stages of infection, prior to and after membrane fusion and in the absence or presence of ZMPSTE24/IFITMs. To localise and identify the restriction factors in the crowded cellular landscape by cryo-ET, we will use fluorescence to guide image acquisition and interpretation, i.e. ZMPSTE24 and IFITM proteins will be expressed in cells with a molecular tag (Halo-Tag) that can be fluorescently labelled for direct macromolecular localisation in cryo-electron tomograms. In addition, Mopeia and cellular membranes will also be fluorescently labelled to enable spatial localisation and aid in easy correlative searches within the dense environment of the cell.

Cryo samples will be prepared by plunge freezing fluorescently labelled MOPV-infected cells in the presence and absence of the fluorescently tagged restriction factors to enable subsequent imaging by cryo-ET. Samples will then be screened by cryo-CLEM to check for sample integrity and obtain spatially localised fluorescence signals. Guided by the fluorescent cryo-CLEM, cryo-ET will be performed, exploiting the power of correlative imaging. This will provide detailed ultrastructure of MOPV entry into cells, and structural insights into modulation of membrane integrity and fusion under the influence of ZMPSTE24/IFITM proteins at macromolecular resolution. Combining cryo-ET with computational methods like subtomogram averaging (STA) will allow elucidation of high-resolution structural details of arenavirus fusion and host restriction processes.

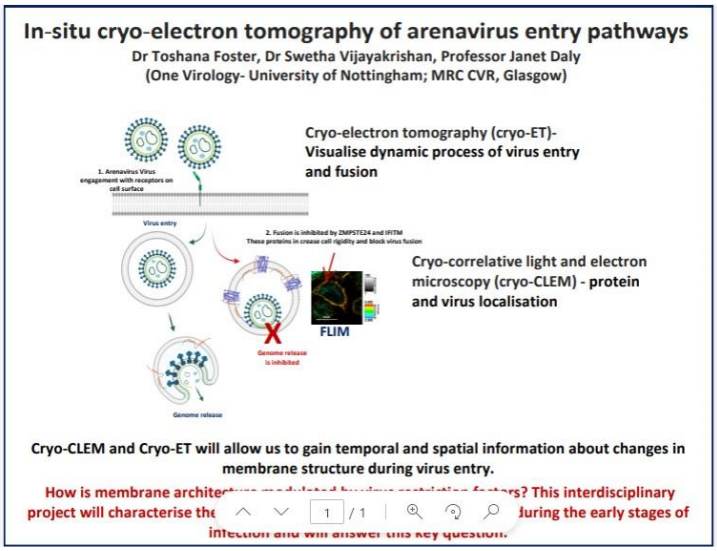
Thus, this interdisciplinary project aims to:

Characterise the dynamic behaviour of arenavirus particles during the early stages of infection
Assess the impact of host innate restriction factor expression on the dynamics of arenavirus entry
Gain temporal and spatial information about the multiple steps of arenavirus entry, information on how host membrane fluidity impacts on fusion of the viral and host-membranes and on the virus interactions with the host cell components during the early steps of the life cycle.

Full project location:
Sutton Bonington Campus;

School of Veterinary Medicine and Science- Sutton Bonington Campus

MRC Centre for Virus Research, University of Glasgow;



Manufacturing Precious Metals from Waste Streams via Microbial Biotechnology

Project Supervisor: Helena Gomes

School: Engineering

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

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

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

Location: University Park;

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

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

Full project location: University Park;

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

Project Supervisor: Andrea Laybourn

School: Engineering

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

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

Location: University Park;

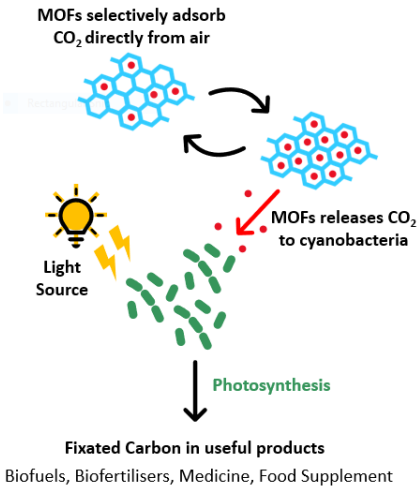
Full project description: The need to develop sustainable, renewable Industrial processes is crucial and one of the foremost global challenges facing humanity. Traditional manufacturing processes are unsustainable, utilising non-renewable feedstocks, and releasing large quantities of greenhouse gases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



Full project location: University Park;

Type 5e two-partner secretion systems of Gram-negative bacteria: a novel secretion pathway with biotechnological potential

Project Supervisor: Jack C. Leo

School: School of Science and Technology (NTU)

Description: ZirTSU is an antivirulence factor of the gastrointestinal pathogen Salmonella enterica. ZirT is an inverse autotransporter, and outer membrane-embedded adhesin with a large extracellular region (the passenger). ZirT and ZirU are smaller soluble proteins that are secreted into the extracellular space in a ZirT-dependent manner. Enteropathogenic E. coli also encodes a homologous system, called SinHI, where SinH is the inverse autotransporter and SinI a putative secretion substrate. SinH is known to be involved in adhesion to host cells, host cell invation, and biofilm formation, but the role of SinI is not known.

In this project, the student will determine whether SinI is indeed secreted and whether the secretion depends on SinH. This will involve cloning the sinH and sinI genes, either together or separately (weeks 1-4), followed by protein expression and subcellular localisation studies (weeks 5-9). The latter will include separating whole-cell, periplasmic, outer membrane and extracellular fractions from bacterial cultures of E. coli. Establishing SinI as a secreted protein present an opportunity to exploit the secretion mechanism for biotechnological applications.

In this project, the student will learn the following techniques:
- molecular cloning
- protein production
- subcellular fractionation
- western blotting

Location: Clifton Campus;

Full project description: Type 5 secretion systems, or autotransporters, are the most widespread protein secretion systems in Gram-negative bacteria. Most characterised autotransporters are virulence factors contributing to the ability of pathogenic bacteria to colonise tissues and cause disease. Autotransporters consist of an outer membrane embedded beta-barrel domain and an extracellular effector region called the passenger. The beta-barrel domain is responsible for the secretion of the passenger, which is the original reason these proteins were termed autotransporters.

There are several subtypes of autotransporter. The type 5e secretion systems (also termed inverse autotransporters) are adhesins or cell invasion molecules exemplified by intimin of enterohaemorrhagic E. coli and invasin of enteropathogenic Yersinia species. Salmonella enterica also encodes a type 5e autotranspoter called ZirT. Interestingly, ZirT appears to secrete not only its own passenger but two other proteins, ZirS and ZirU. This is reminiscent of type 5b (two partner) secretion systems, where the beta-barrel and passenger are separate polypeptides. Enteropathogenic E. coli also encodes a ZirT homologue, SinH, which presumably secretes a protein called SinI and possibly a second protein termed RatA.

In this project, the student will characterise the secretion mechanism of type 5e two-partner secretion using ZirTSU and SinHI-RatA as model systems. Questions to answer include:

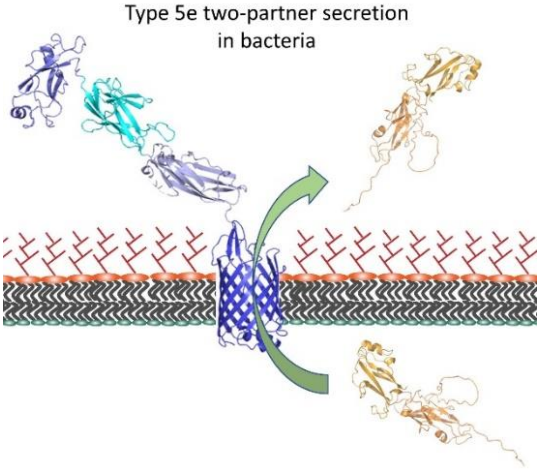
- Are SinI and RatA secretion substrates for SinH?
- Which domains in the proteins are required for secretion? How do they interact?
- What is the sequence of events? Does secretion of accessory proteins take place before or after passenger secretion?
- There is evidence to suggest that ZirSU interact with the ZirT passenger after secretion. Do SinI and RatA also bind to SinH once secreted?
- How do the different proteins affect cell adhesion and biofilm formation?

In addition, the project will include exploring the ability of these systems to secreted foreign proteins for use in biotechnological applications. Questions to be answered in this part are:

- Can foreign proteins/domains be efficiently secreted if fused to substrate proteins?
- If so, what requirements do such proteins/domains have in terms of size, stability, and structure?

Finally, the project will involve structural characterisation of ZirTSU and/or SinHI-RatA complexes to determine how the proteins interact and whether these systems could be used as molecular docking scaffolds for biotechnological applications.

The student will learn a wide variety of techniques in this project, ranging from molecular cloning and mutagenesis, protein expression and purification, subcellular fractionation, adhesion assays, crystallising proteins and protein complexes, and protein detection using western blots. In addition, the project will include cutting edge techniques such as (super-resolution) fluorescence microscopy, structure determination by X-ray crystallography, and biophysical methods for characterising protein-protein interactions. The bulk of the project will be carried out at Nottingham Trent University, but structural biology and microscopy will be performed at the University of Nottingham.



Full project location: Clifton Campus;

Differentiation of human amniotic epithelial stem cells into beta islet cells using conditional cellular reprogramming.

Project Supervisor: Abdolrahman Shams Nateri

School: Medicine

Description: During the project rotation, students will develop practical research skills in 2D and 3D tissue culture methods, as well as transferable skills such as the ability to critically evaluate others' work.

Practical skills in culturing epithelial cell lines in both 2D and 3D cultures will be honed through daily laboratory work, dedicating 7-8 hours per day for 3 weeks. Once proficiency in these skills is attained, the focus will shift to the isolation of human amniotic epithelial cells for 4 weeks. During this training period, supervision will primarily be provided by Dr. Nateri and Dr. Jones (Medicine), with support from the Cancer Genetics and Stem Cells Group within the BioDiscovery Institute.

Given the limited number of researchers working in this specialized field internationally, a comprehensive understanding of the existing literature is essential before embarking on the proposed research project. Consequently, students will spend two weeks exclusively conducting a literature review under the guidance of Dr. Nateri and Dr. Nia Jones (Medicine).

The laboratory rotation is designed to equip students with the knowledge and confidence necessary to incorporate the skills required for their connected PhD studies effectively.

Location: University Park;

Full project description: Background: Pancreatic islet isolation and engraftment are currently offered to patients with difficult-to-control type 1 diabetes (T1D) as a substitute for inactive or destroyed beta cells, aiming to prevent glycemic instability. The remarkable progress in successful islet transplants over the past three decades is a result of continuous research that has led to improvements in islet cell processing, transplantation technology, immunosuppression regimens, and patient selection and management [1]. Presently, the available options for sources of islets for islet transplant surgery encompass the patient's own pancreas (autotransplant), islets from brain-dead cadavers, or the utilization of biopsied pancreatic islets from living related donors (allogeneic transplant).

Challenges such as inflammatory and noninflammatory damage to post-transplanted islet cells and the vascular deployment of islet cells in the liver further complicate the process. Consequently, the broader implementation of islet transplantation hinges on overcoming technical limitations, including an insufficient supply of islet cells and the necessity for chronic systemic immunosuppression, which entails risks for both the islet graft and the patient [2].

Human amniotic epithelial cells (hAECs), derived from pluripotent placental epiblasts, possess immune-privileged properties and the ability to maintain multilineage differentiation potential [3]. They also demonstrate the capability to modulate the local immune response, suggesting exciting prospects for therapeutic applications in tissue regeneration [4]. Unlike induced pluripotent stem cells (iPSCs), hAECs do not give rise to tumours or teratomas and exhibit immunomodulatory and anti-inflammatory characteristics. This presents notable ethical, technical, and scientific advantages over other stem cell resources. Studies involving mouse models have exhibited the potential

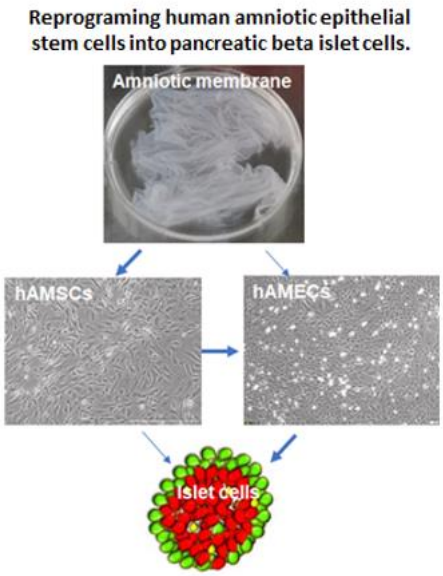
of hAECs to differentiate into surfactant-producing alveolar epithelial cells and hepatocytes [5].

The limited availability and survival of islet cells, along with the prolonged immunosuppression regimens required for allogeneic transplantation, pose significant constraints on utilizing islet transplantation to treat patients with T1D. Moreover, the viability of transplanted islet cells is impeded by immune-mediated and inflammatory reactions. hAECs derived from the placenta present a potential solution, as they exhibit tolerogenic, non-immunogenic, and non-tumorigenic and stem cell characteristics. Preliminary evidence suggests that these cells can differentiate into islet beta-like cells capable of insulin production, offering a promising source for islet replacement.

Nevertheless, a key hurdle in employing stem cells for islet transplantation has been the challenge of scalability. The therapeutic application demands a substantial quantity of cells, necessitating the use of seven-term placentas to treat a single patient. The controlled replication (CR) method emerges as a promising strategy to expand hAEC cultures substantially, all the while preserving their immune-privileged attributes and their potential to differentiate into insulin-producing beta cells.

Hypothesis:

hAECs isolated from the placenta can be propagated using the humanized CR methodology, all the while retaining their primary phenotype, which includes pluripotency. They also exhibit promising characteristics for development into endoderm lineages, including pancreatic beta cells.



Specific Aims:
1. Isolation of human amniotic epithelial cells (hAECs) from the placenta using the Gramignoli protocol.
2. Cultivation, Expansion, and Characterization of hAECs.
3. Differentiation of hAEC-CRs into Insulin-Producing Beta Cells.
4. Validation of Functional Activity of hAEC-CRs through Co-transplantation with Human Islet Cells in a Diabetes Mouse Model.
Full project location: University Park;

Development of high-throughput engineered human muscle for regenerative medicine and drug discovery

Project Supervisor: Livia Santos

School: School of Science and Technology (NTU)

Description: Week 1. Lab induction. Candidates will be required to complete a lab and safety induction. They will learn how to use a hood, a centrifuge and an incubator. They will shadow existing PhD students to exchange ideas and consolidate good lab practices.

Week 2-6. Develop basic skills in cell culture. Candidates will learn how to grow and subculture cells while avoiding contaminations from microbes or fungi. They will become familiar with assays to estimate cell proliferation/viability such as the MTT. They will learn how to sterilise lab ware and solutions.

Week 6-7. Hydrogel preparation. Candidates will learn how to prepare hydrogels previously developed in the Dixon lab to support the culture of cells in 3D.

Week 7-9. Learn to prepare 3D cell cultures. Candidates will become familiar with the protocols to produce tissue in 3D using a cell culture insert recently developed by the Santos lab. Specifically, they will learn to produce tissue-engineered muscle using a muscle cell line (C2C12). They will also understand how to prepare cells for advanced imaging (confocal microscopy) and analyse obtained data (Excel and SPSS).

By the end of this rotation, the candidates will have the essential lab skills for their PhD and some preliminary data.

Location: Clifton Campus;

Full project description: Current therapies to treat injured or diseased muscles are scarce because of our limited understanding of muscle regeneration. This is further inhibited by the suboptimal models traditionally utilised across discovery - 2D cell muscle culture and animal models.

Human-engineered muscle can be implanted into injured muscle or utilised as a physiologically relevant muscle model for drug discovery holding great promise for regenerative medicine and pharmaceuticals, respectively. Despite this, current approaches to generate engineered muscle lack scalability, which has been holding back the discovery of regenerative medicine approaches, the development of novel effective pharmaceuticals and the significant impact on the 3Rs. Specifically, current methods only allow to produce tens when hundreds are needed in high-throughput strategies.

Our lab recently invented a transformative technology which allows us to upscale the generation of tissue engineering muscle by 10 times, into hundreds of tissues This BBSRC DTP project is pivotal to advance this research and technology further to be impactful for our knowledge of muscle physiology and regeneration.

To advance the research further, the following work packages (WP) will be implemented.

WP1. Develop and characterise tissue-engineered muscle (months 1-12). This is important to demonstrate that the proposed technology can generate human and contractile muscle, and is thus appropriate for regenerative medicine. Briefly, the muscle will be generated in our patented inserts and contractility measured after electric stimulation. Two types of electric stimuli will be delivered - a short, lasting 10ms to induce a twitch contraction and a more prolonged lasting 1s in train mode to trigger a tetanic contraction. Muscle protein synthesis (MPS) will be measured using the SUnSET assay.

WP2. Assess muscle function and growth in response to pharmacological interventions (months 12.24). This WP important to demonstrate that engineered muscle responds to drugs similar to native muscle and is thus appropriate for drug discovery. To achieve this, the muscle model developed in WP1 will be treated with glucocorticoids and statins for up to 4 weeks. Glucocorticoids are beneficial for the treatment of muscle wasting diseases as they improve muscle function and strength, while statins, a drug class widely prescribed to manage coronary artery disease can induce significant muscle weakness as early as 2 weeks of use. In brief, the engineered muscle will be exposed to culture medium supplemented with these drugs and contractility and MPS measured as described in WP1.

WP3. Characterise muscle function and growth in response to non-pharmacological interventions (months 24-36). Adequate protein intake and exercise are recommended by the NHS for patients with muscle trauma or disease. This WP is important to

demonstrate that the muscle model previously generated responds to protein-based (amino acid L-leucine) interventions like native muscle does. In brief, the engineered muscle will be exposed to culture medium (which by default contains ~800 µM of L-leucine) supplemented with an additional 5 mM or 20 mM L-leucine and contractility and MPS estimated as described in WP1.

In the long term, we expect to accelerate discoveries to provide patients earlier access to new and better regenerative medicine approaches and pharmaceuticals.

Full project location: Clifton Campus;

Discovering new components and functions of plant oxygen sensing

Project Supervisor: Michael Holdsworth

School: Biosciences

Description: A mechanism of plant oxygen-sensing has been discovered by the host laboratory (Gibbs et al Nature 2011, Nature Communications 2018, 2023), but key components of this mechanism still remain to be discovered, and evidence points to the existence of other oxygen sensing systems in plants (Holdsworth, Nature Plants 2017). Using already available resources the student will combine physiology, molecular biology and Mass Spectrometry (MS) approaches to investigate the nature of oxidation changes to key transcription factors and other regulatory proteins that control the plant oxygen sensing system. The rotation will include

- physiological analysis of the hypoxia (low oxygen) tolerance of plants containing mutations in potentially novel components of the oxygen-sensing system, that will provide new components for biochemical analysis (Holdsworth Biosciences).
- Biochemical investigation of oxygen sensing by key transcription factors using an in-vitro plant extract system, investigating the influence of inhibitors of oxygen-utilising enzymes on bacterially expressed transcription factors through MS analyses (Oldham Chemistry)

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

Location: Sutton Bonington Campus;University Park;

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

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

Four objectives will address this hypothesis, using Arabidopsis thaliana novel genetic components of the known pathway of oxygen-sensing (through oxygen-regulated Ubiquitin-mediated degradation of ERFVII transcription factors), to identify novel substrates of oxygen-sensing, and to transfer this information to crop species.

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

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

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

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

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

Full project location: University Park;Sutton Bonington Campus;

Green hydrogen from microbial fermentation

Project Supervisor: Simone Morra

School: Engineering

Description: Using hydrogen as a sustainable fuel and energy carrier requires tackling several technical challenges, including the development and implementation of clean production technologies from renewable resources. H2 is produced in nature by several microorganisms, as a biproduct of the energy metabolism. As such, numerous biotechnological routes have been explored to generate green hydrogen from several feedstocks. This offers obvious benefits over conventional production of hydrogen from fossil fuels, particularly when waste biomass is used.

Dark fermentative hydrogen production (DFHP) is generally preferred to photosynthetic and photo-fermentative hydrogen production due to simpler bioprocess operation, low

energy requirements, and the possibility to be carried out using pure or mixed cultures. Despite this, current yields are not sufficient to meet industrial application demand, and improvements to the process are required.

This project aims at understanding and improving microbial hydrogen production, by focusing on hydrogenases, the enzymes that catalyse the final reaction step, and their involvement in energy metabolism.

During the rotation project the student will assess changes in the metabolism of Clostridium beijerinckii and mutants overexpressing specific hydrogenase genes.

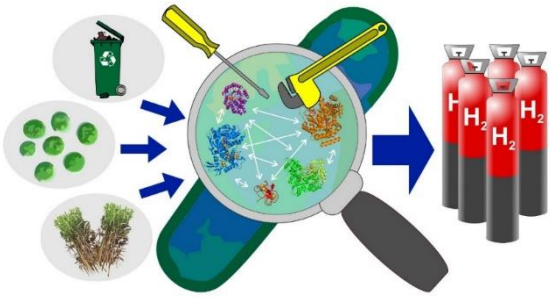
Location: University Park;

Full project description: This project aims at understanding the role of multiple hydrogenase genes in Clostridium and improving hydrogen production by using this information to design targeted synthetic biology strategies. This research will also explore the production of other valuable chemicals (solvents and organic acids), whose metabolism is directly linked to H2, and assessing the economic aspects of the process towards industrial applications.

Microbial hydrogen metabolism relies on hydrogenases, specialised metallo-enzymes that catalyse H2 production as a means of dissipating excess of reducing equivalents during anaerobic fermentation (i.e. energy metabolism in the absence of oxygen as the final electron acceptor). The diversity of these enzymes is massive, and numerous isoforms are found in each genome of those species that produce hydrogen. This makes hydrogen metabolism a complex process that is not easily manipulated. This project will target this knowledge gap by shedding light on the specific functional role of each hydrogenase, leading to improved production of H2 and other valuable metabolites.

The student will use genetic engineering tools that have been developed in the Heap group to generate systematic knockout strains and assess changes in physiology. In the Morra group, the student will analyse changes in the production of H2 and other metabolites (including acetone, butanol, ethanol, acetic acid, butyric acid) when growing on standard nutrients (glucose) and when growing on waste biomass. Finally, Prof McKechnie will support the student to perform a techno-economical assessment of the fermentation to assess its potential as a large scale waste valorisation process.

The student will work in state-of-the-art facilities and receive extensive support and training by the supervisors. This project is part of an established collaboration between Dr Morra, Dr Heap and Prof McKechnie with existing postdoctoral researcher already doing work that the student will be able to build upon.



Full project location: University Park;

One-Pot, Cascade Reaction for production of pharmaceutical intermediates

Project Supervisor: Parimala Shivaprasad

School: Engineering

Description: Chemo-Enzymatic catalyst system for one-pot, cascade reaction

This project involves developing a robust protocol to immobilise a metal organic catalyst and an enzyme onto a wool support. A key challenge to achieving multi-catalytic

reactions in one-pot is the catalyst incompatibility when used in homogeneous environment. Catalyst immobilisation allows for catalyst compartmentalisation allowing for the chemo and enzyme catalyst to retain their individual activities. This also allows from easy separation and re-usability of the catalyst at the end of the reaction. The catalysts will be immobilised on wool, which is a natural fibre with rich surface functional groups and allows for easy immobilisation of catalysts.

Catalyst immobilisation will be achieved through a series of pre-treatment techniques on wool and the protocol will be optimised to achieve a stable catalyst loading. The catalyst cloths will be characterised using EDX, SEM and FTIR. The immobilised catalyst system will first be tested individually for nitroaldol reaction (chemo catalysed) and kinetic resolution (enzyme catalysed) reactions. The reaction conversion will be measured by collecting samples and analysing them using gas chromatography. The catalyst cloths will then be tested in one-pot for the kinetic resolution of nitroaldol condensation reaction products. The enantiomeric excess and catalyst cloth re-usability will be measured for individual and cascade reaction systems.

Location: University Park;

Full project description: One-pot, cascade reaction has the potential to be the greener and a more sustainable alternative for production of chemicals. The increasing research interest in cascade reactions is due to the potential reduction in the number of reaction steps and elimination of intermediate product purification, catalyst recovery, resulting in better control over chemical equilibrium and reduced energy consumption. For a one pot reaction in tandem, it is important to design a suitable catalyst system and identify a reactor design which can support reactions in tandem. Catalyst compartmentalisation and site isolated catalyst has been gained increasing attention over the last decade to mitigate the challenge around multi-catalyst compatibility.

The aim of this project is to develop a supported chemo-enzymatic catalyst system for one-pot cascade reaction and test the catalyst for reaction scale-up in a continuous reactor configuration. Dynamic Kinetic Resolution (DKR) of nitroaldol condensation reaction will be used as the model reaction to demonstrate the cascade process. DKR is a reaction wherein a pure racemic mixture can be converted to enantiopure compounds. The reaction involves differentiating two enantiomers by the rate at which each reacts under the influence of a chiral catalyst or a reagent. The reaction products from DKR are high purity enantiomers and have important application as active pharmaceutical intermediates in the pharmaceutical sector.

The first part of this project will focus on optimising the reaction parameters for the DKR reaction on a bench scale. The reaction requires a chemo-enzymatic catalyst combination and catalyst separation will be achieved by immobilising the two catalysts on separate supports. The immobilised catalysts will be used to optimise the reaction parameters such as substrate – catalyst loading, temperature and solvent selection. Reaction kinetic studies will be performed using the optimised conditions to determine the overall reaction rate.

The optimum reaction conditions will be used to test the reaction scale-up in a spinning mesh disc reactor (SMDR). The SMDR uses centrifugal forces to allow an even spread of a thin film across a spinning horizontal disc covered by a replaceable cloth. The cloth can have a range of catalysts immobilised onto it, protecting the catalyst from hydrodynamic forces and also help in better mixing leading to faster reactions. Reactor parameters such as spinning speed and flow rate will be optimised for the DKR reaction. The overall reaction rate in the SMDR will be bench marked against the batch system to determine the reactor productivity.

This is largely an experimental project with ample training opportunities in analytical and characterisation techniques, reactor development and optimisation. The student will be supported by current PhD students from two research groups and will have opportunities for additional skills development through participation in seminars and conferences, industry and public engagement events.

Full project location: University Park;

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

Project Supervisor: Klaus Winzer

School: Life Sciences

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

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

The rotation student will:

- Receive training in general microbiological methods, modern genetic approaches including HiFi gene assembly, state-of-the-art microbioreactor systems, and the use of high-throughput cultivation facilities
- Generate and test a range of reporter plasmids carrying e.g. green and turquoise fluorescing GFP protein derivatives.
- Transfer the generated reporters into two different Acetobacter strains and validate their performance by monitoring for constitutive and induced expression under various conditions including nitrogen and oxygen limitation.

Location: University Park;

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

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

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

Experimental approach:

1. Regulation of nitrogen fixation

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

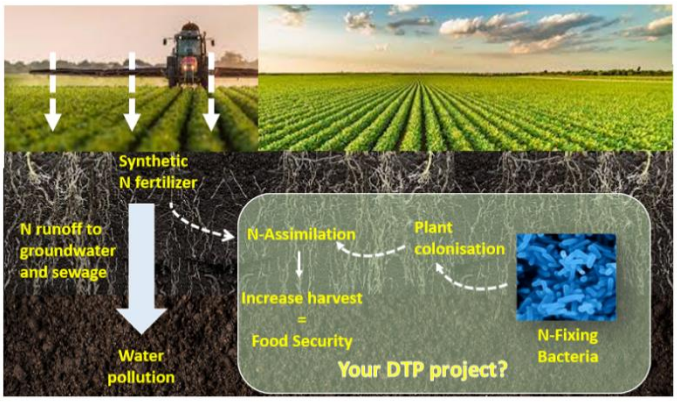
2. Promoting nitrogen fixation

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

3. Plant hormones

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

Full project location: University Park;Sutton Bonington Campus;Genetics, transcriptomics and in vitro studies: Within the labs of the BBSRC/EPSRC Synthetic Biology Research Centre at the Biodiscovery Institute Evaluation of strains and constructs in planta: School of Bioscience



Investigation of the receptor Paqr9 in metabolic health and identification of its novel ligands

Project Supervisor: Mark Christian

School: School of Science and Technology (NTU)

Description: Analysis of the expression and subcellular localisation of Paqr9 in brown adipocytes

Brown adipocytes serve to burn fat and therefore have an important role in maintaining metabolic health. Identification of the receptors that control the process of fat burning and heat generation in brown fat has great potential for the treatment of metabolic disease. Paqr9 mRNA is highly induced in brown adipocytes following their differentiation. In this rotation you will culture brown adipocyte cell lines and analyse PAQR9 protein expression by Western Blotting as well as immunocytochemistry and confocal microscopy. This will allow assessment of the subcellular localisation of this receptor. You will also clone the Paqr9 gene into a plasmid expression vector to facilitate its labelling and expression in cell lines for the purpose of preparing a system for ligand screening. These approaches will provide key skills training in molecular biology techniques and generate research tools that will be applied in the PhD project.

Location: Clifton Campus;

Full project description: Receptors are the most important drug targets in medicine. Therefore, identifying receptors and their ligands is central to advancing biomedical science.

Interventions that increase brown fat activity are promising pathways to reduce obesity and associated diseases that include type 2 diabetes and a range of cancers. Brown adipose tissue is a healthy fat deposit that contributes to metabolic health by generating heat through the process of thermogenesis. It is detected in adult humans and increases energy expenditure and fat burning following exposure to cold or after food intake. Therefore, identifying new pharmacological approaches to activate brown fat is of great scientific interest.

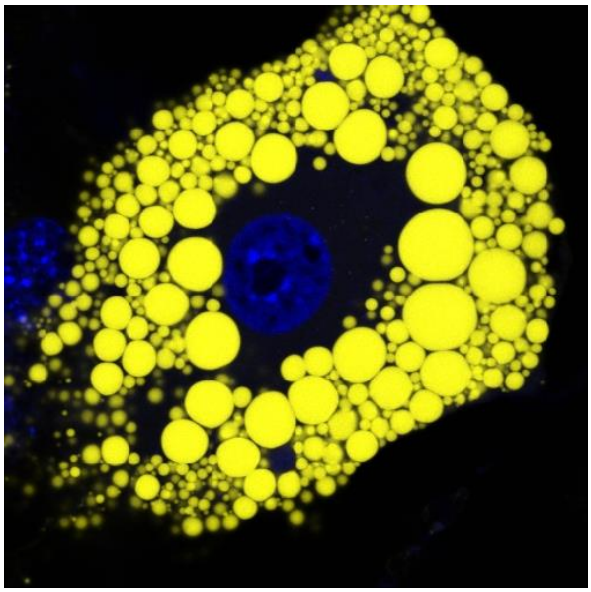
This project will focus on investigating Paqr9 as a modulator of brown adipocyte action and determine the ligands that activate it with potential for clinical application. Paqr9 (Progestin And AdipoQ Receptor Family Member 9) is a 7-transmembrane receptor that is highly expressed in activated brown adipose tissue. White adipose tissue, which lacks the capacity for thermogenesis and when in excess is associated with poor metabolic health, expresses lower levels of Paqr9. Pilot data reveal that Paqr9 is highly induced following the differentiation of brown pre-adipocytes to mature adipocytes. Furthermore, it is highly increased in mice following cold exposure when brown fat is activated. The ligands that activate Paqr9 and its signal transduction mechanism have not yet been determined.

The overall aim of this PhD project is to understand the role of Paqr9 in brown adipocytes and identify ligands able to bind/activate it. This will be assessed in cell lines and tissues by applying a range of molecular techniques. The collaborative nature of project with the Kellam/Mistry labs at the University of Nottingham (UoN) brings complementary expertise in developing ligand-based chemical biology approaches for membrane receptors. Furthermore, the student will have access to the UoN Medicinal Chemistry Compound Collection (MCCC), comprising >80K drug-like compounds that can be dispensed for screening in well-based format. The existence of x-ray crystal structure (XRCS) for the related AdipoR1/AdipoR2 proteins also offers the opportunity to generate a homology model for Paqr9, against which the MCCC library could be virtually screened before validation through pharmacological assay.

The main aims are:
1) Determine the subcellular localisation of PAQR9 in brown adipocytes

2) Determine the role of PAQR9 in brown adipocyte differentiation and metabolic function
3) Generate a reporter cell line to screen for ligands that activate PAQR9
4) Generate a Paqr9 homology model based on the reported AdipoR1/AdipoR2 XRCS and use this to virtually screen the UoN MCCC
5) Use the reporter cell platform to validate UoN MCCC virtual hits

This project is multidisciplinary providing training in cell culture and molecular biology techniques such as q-RT-PCR, confocal microscopy, immunohistochemistry, siRNA knockdown, Western Blotting, gene cloning and mutagenesis. Virtual screening and hit identification. Cellular metabolic analysis will be determined using Seahorse bioanalysis.



The project will reveal the role of Paqr9 and through the identification of ligands for this receptor will have potential for therapeutic approaches targeted to adipocytes and other Paqr9-expressing cells and tissues.

Full project location: Clifton Campus;QMC;

Psychedelic drugs and serotonergic modulation of functional connectivity in neuronal microcircuits

Project Supervisor: Michael Okun

School: Life Sciences

Description: Over the course of the rotation, you will be introduced to in vivo experiments taking place in our laboratory, in which we record the neuronal activity of hundreds of neurons using high-density silicon Neuropixels probes and/or wide-field imaging. You will learn about the hardware and software tools that we use, surgical methods and methods for processing and analysing electrophysiological and imaging data. Examples of topics that can be covered include spike-sorting (e.g., Buccino et al., Prog. Biomed. Eng. 2022), calculation of power-spectrum of EEG data, analysing spike trains of neurons and neuronal populations using scripts in high-level programming languages such as Matlab or Python. This training will be tailored to your background and previous knowledge and will provide a foundation for the main project or any other research in the systems neuroscience area.

Location: QMC;University Park;

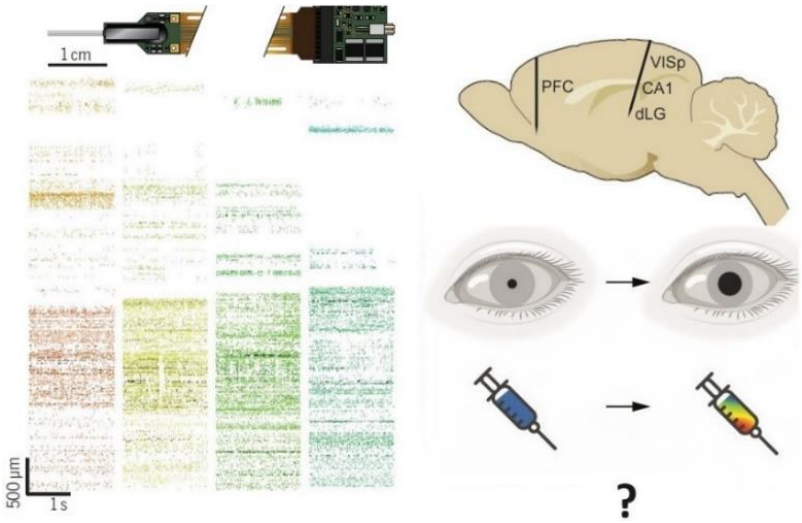
Full project description: Psychoactive drugs result in profound alterations of our state of consciousness. Well known examples of such drugs are general anaesthetics, in use since mid-19th century, and psychedelics, which are consumed by different societies from times immemorial. The way these drugs operate is far from understood, although last decades witnessed a significant progress on this question. Most of the research has focused on the cellular mechanisms of action of such drugs in the central nervous system, elucidating the receptors and intracellular molecular pathways that are involved.

However, knowing how a neuron is affected when considered in isolation (typically in an in vitro preparation), does not explain how the drug affects the intact brain, where neurons are highly interconnected.

The aim of the present project is to further the systems level understanding of the changes in cortical activity under the effect of classical psychedelic compounds, exerted primarily by activation of serotonergic 5HT-2A receptors, and related drugs.

Our aim is to determine the changes in spontaneous and sensory evoked activity produced by 5-HT2A agonists at the level of single neurons and neuronal populations in sensory and frontal cortex (Dearnley et al., Cell Reports, in press). Towards this end, we will use high-density multi-electrode arrays (Steinmetz et al., Science 2021,) imaging and advanced computational methods to record and analyse spontaneous and sensory evoked activity of large neuronal populations in mice.

There is a scope to tailor the specific research questions to your interests and strengths. In particular, the research can have a stronger emphasis either on the experimental or the computational research components. The former is typically more appropriate if your background is in biology, neuroscience, pharmacology, medicine or psychology, while the latter is particularly suitable if you have a background in programming or quantitative subjects, e.g., mathematics, statistics, physics or computer science and engineering. The decision on your second/third supervisor(s) will also be based on the specific research questions that we decide to explore.



You will gain expertise in cutting-edge methods in systems and computational neuroscience and will help advancing our understanding of a fundamental question at the intersection of neuropharmacology and systems neuroscience.

Full project location: QMC;

Computational modelling of the axolotl spinal cord during regeneration

Project Supervisor: Osvaldo Chara

School: Biosciences

Description: During this lab rotation, the student will be introduced to computational modelling and programming languages and, specifically, Python. The student will learn different types of mathematical modelling approaches and will focus on computational models that are suitable to simulate biological tissues: agent-based models, in particular, cell-based models. The student will learn how to simulate state-of-the-art cell-based computational models, in the context of development and regeneration of tissues. The student will participate in the Journal Club and Group meetings where she or

he will learn how to present and discuss exciting new preprints as well as seminal articles of development and regeneration of tissues from a quantitative perspective.

Location: Sutton Bonington Campus;University Park;

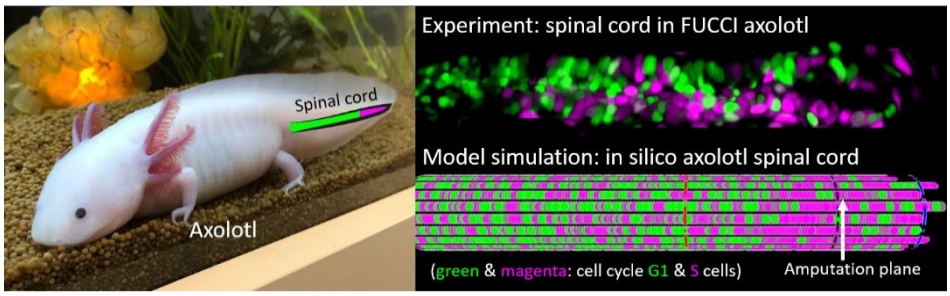
Full project description: Context: In contrast to humans, salamanders like the axolotl can resolve severe and extreme injuries of spinal cord throughout complete and faithful regeneration. Although more than 250 years have passed since the original discovery of salamander tail regeneration after amputation by Spallanzani, the governing mechanisms underlying these unparalleled regeneration capabilities are not yet understood. In this interdisciplinary PhD project, we will investigate how the axolotl regenerates the spinal cord after amputation. This project is part of an international collaboration between the lab of Elly Tanaka, a world leader in regeneration in the axolotl at the Institute of Molecular Pathology in Vienna and the Chara and Band modelling labs at UoN. Recently, Tanaka and Chara labs demonstrated that tail amputation leads to a particular spatiotemporal distribution of cycling cells in the axolotl (Rost et al., 2016. eLife.5:e20357; Rodrigo Albors et al., 2015 eLife.4:e10230). By combining a new transgenic axolotl using FUCCI technology (AxFUCCI) with the first cell-based computational model of the regenerative spinal cord, both labs found that regeneration is orchestrated by a particular spatiotemporal pattern of neural stem cell recruitment along the anterior-posterior (AP) axis (Cura Costa et al., 2021. eLife.10:e55665). This pattern is consistent with a Reaction-Diffusion signal spreading along the spinal cord tissue during regeneration (Caliaro et al., 2023. bioRxiv).

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

Significance and outcomes:

- While the immediate goal of this project is to mechanistically address how spinal cord regenerates in salamanders, the ultimate goal is to understand why this trait is absent in humans. Thus, the knowledge gained would pave the way for future therapeutic strategies within the emergent field of regenerative medicine.
- Furthermore, the AI-based image-analysis and modelling methodology developed in this project will be of wide interest for multi-scale modellers. The techniques developed are relevant to many contexts where tissue-scale growth depends on cell proliferation, such as embryo development, cancer and plant growth.
- The project is highly interdisciplinary and international (with two supervisors from two different Schools and collaboration with a leading experimental group in Vienna) broadening the research within the University and in this field.

Full project location: Sutton Bonington Campus;University Park;



Life on Plastic: Is it really Fantastic?

Project Supervisor: Samantha Bryan

School: Engineering

Description: During the lab rotation the student will learn some of the core techniques required for the project focusing on identifying bacterial communities from the environment and evaluating the physical and chemical properties of plastic and begin to explore how microbial communities are influenced and impacted by plastic pollution. The student will be introduced and learn to utilise a wide range of different techniques including DNA isolation, metagenomic analysis, GC-MS, LC-MS, FTIR, NMR and SEM. They will also participate in the weekly group meetings and wider research seminars, offering opportunities to engage with many PhD and postdoctoral researchers.

Location: University Park;

Full project description: Anthropogenic plastic pollution is a serious problem impacting almost every habitat on earth. 400Mt of plastic waste is generated every year, with 175Mt entering landfill and the environment, sufficient for rebuilding the Great Wall of China every year. Microorganisms have been identified living on plastic, a term coined "the plastisphere". Yet the role these individual microorganisms play on plastic waste still remains unclear, with focus to date having centred on microbial colonisation versus utilisation as a substrate and thus degradation of plastic. Microbial members of niche environments such as waste plastic often give rise to discrete abundant populations. Yet a detailed understanding of the functional diversity and metabolic capacity of these communities remains elusive and it is also unclear how plastic influences microbial adaptation and evolution.

This becomes more complex when considering that the term 'plastic' encompasses a vast compositional range (the polymer type e.g., polyethylene (PE), polyethylene terephthalate (PET)), whether plastic is virgin (new) or recycled, or indeed modified due to environmental processes that cause chemical and morphological changes in the condition of the plastic. This may also lead to cascade pollution from chemical additives in the plastic that are subsequently leached or secondary metabolites produced by the microorganism that are known to drive antimicrobial resistance. Therefore, despite the scale of plastic waste and the identification of some microorganisms, which can colonise plastic, we still have no fundamental understanding of how individual microorganisms and the wider community transition to "life" on plastic.

This project aims to evaluate what affects microbial community composition and function on plastic, and how the condition of the plastic drives this process. Linking the individual directly to the community and providing a comprehensive understanding of the role played by microorganisms in plastic degradation.

Objectives:

The primary objective being to evaluate how communities compete and cooperate on plastic and what impact the state of the plastic e.g., virgin, weathered, degraded has on this community structure and function. The student will identify community members directly from the environment and use this information to seed synthetic communities on plastic. The student will look at the impact the plastic has on community dynamic and on the leaching of chemical additives and the impact this has through potential cascade pollution in the environment. The student will utilise a range of techniques including metabolomics, proteomics, transcriptomics, and imaging (multiplex FISH, 3DOrbiSIMs), FTIR, NMR and GC-MS.

Full project location: University Park;

Controlling the function of individual neurons within a neural circuit

Project Supervisor: Joseph Sollini

School: Medicine

Description: In this lab rotation students learn skills involving cutting-edge neuroscience tools and help to protype the development of an exciting new tool that will be able to control the function of genetically targeted individual neurons while recording the activity of 100s of potentially connected neurons. In addition, students will gain the following experience:

- Neurosurgical techniques – Learn about high density electrode recordings (Neuropixels), viral injections (to allow opto/chemogenetics) and mouse brainstem measurements (ABRs).
- Data analysis - Learn about the data processing tools (such as spike sorting) and analysis programs (such as Matlab) used to understand neural data.
- Practice neural implantation – Using ex-vivo brains gain experience implanting optical fibres and electrodes. Students will test the ability of different optical fibres (varying the dimensions, coating and taper) to withstand the implantation process.
- Histology and microscopy training - Students will receive training to fix, slice and mount brain slices on slides as well as image slides to measure the damage caused by different implantation techniques.
- Image neurons using optical fibres – We will test the imaging and stimulation properties of the selected fibres by imaging neurons that express fluorescent proteins.
- Attend regular lab meetings.

Location: University Park;

Full project description: A fundamental aim in neuroscience is to understand how neurons in our brain interact to give rise to our thoughts, emotions and perception. To understand these processes, we need to build a "circuit diagram" detailing the types of neurons in each circuit, how they are connected and how these connections change activity in other neurons. In theory this sounds like a simple task requiring just three steps: 1) go through the circuit identifying the different types of neuron 2) then activate each neuron, one at a time (i.e. single cell control) and 3) measure how this affects all the other neurons it is connected to (record from large populations of neurons). In practice this is far from simple. Optogenetics is a revolutionary technique (Häusser, 2014, Nature Methods) that allows scientists to genetically target specific neuron types (Step 1) and control their function by shining a light on them (Sollini et al. 2016, J

Neuroscience). However, neurons in the brain are tiny (often between ~0.005 and ~0.03mm) and the light used scatters to many neurons, meaning optogenetic approaches typically alter the function of large numbers of neurons rather than single neurons one at a time. However, recent advances in fibre-optic technology now make it possible to target light onto single neurons (Accanto et al. 2023, Neuron), meaning we can use this approach to control the function of single neurons (Step 2). Sadly, using an optical fibre to control neural activity solves the problem of single cell control but means we can also only measure activity, using that fibre, from a small number of neurons (i.e. neurons within 0.1-0.2mm of that neuron). By contrast cutting-edge high-density electrodes can allow 100s of neurons to be recorded from simultaneously over large areas of the brain (Steinmetz et al, 2021, Science, Step 3). By combining optogenetics, optics and cutting-edge electrodes we intend to control individual neurons (using advanced fibre optic techniques) while measuring the impact (via high-density electrodes) in large populations of neurons and, hence, create a tool to vastly improve our ability to understand the way in which neurons interact with one another.

Our work focusses on understanding:

- How brain regions involved in cognition (such as memory and attention) alter our perception of sound
- How hearing-loss changes the function of neural circuits leading to problems in auditory and general cognition (such as dementia and Alzheimer's)

Based on the your preferences this technique will be applied to a circuit involved in one of these areas. During this project you will develop skills to use cutting-edge neuroscience technologies (see Sollini et al., 2016, J Neuroscience and Sollini, Chapuis, et al. 2018, Nature Communications) such as: fiber photometry and stimulation, optogenetics, high-density extracellular electrodes as well as the surgical approaches for their use in mice. In addition, you will learn how to image neural tissues via microscopy (e.g. confocal microscopy), apply histological techniques (anti-body labelling, brain fixing and clearing), analyse large neural data sets, program in Python/Matlab and apply statistics.

Full project location: University Park;

Haploid genetic screens for better delivery systems of therapeutic nucleic acids

Project Supervisor: Snow Stolnik

School: Pharmacy

Description: The main hurdle for RNA therapies beyond vaccines is the fact that human cells are very inefficient in taking up RNA. Even if the RNA has been packaged in the latest nanocarriers less than 1% of RNA manages to make it into the cytoplasm an produce protein. It is not well understood why this is. In this project, you will test if haploid cell screens can be used to investigate this problem. Haploid genetic screens are a novel method to do genetics in tissue culture, allowing the mapping of mutations in selected cells. You will clone the open reading frames for Neomycin/G418, Blasticidin, Zeocin or Hygromycin C resistance genes into vectors with T7 promoters and synthesise RNA in vitro. You will then transfect these RNAs into eHAP cells and optimise a protocol to select cells that express the resistance gene. After one or more rounds of selection, you will test if the selected cells have increased their receptivity for transfection by transfecting mRNA encoding green fluorescent protein and counting the number of green cells in selected and unselected populations. This work will contribute to better tools for the development of RNA medicines.

Location: University Park;

Full project description: Nucleic acid therapy was used in the Covid vaccines, but also has great potential to cure untreatable rare genetic diseases as well as more widespread afflictions such as diabetes and cancer.

The main barrier to these new therapies is the reluctance of human cells to take up nucleic acids. To overcome this, nucleic acids have incorporated into a variety of designed nanoscale structures (nanocarriers), as for the Moderna and Pfizer vaccines. Nanocarriers are effective in delivering nucleic acids to a few cells, which is sufficient for vaccines. However, the nucleic acid delivery is very uneven, with some cells having very high levels and others none. In many cases, less than 10% of cells has effective uptake of the nucleic acids. Moreover, for unknown reasons, each nanocarrier has its preferred cell types. If we knew what causes this variation, we could design nucleic acid medicines which enter a larger percentage of the correct cells and are incorporated in the right protein complexes. This would lead to larger therapeutic effects as well as fewer side effects.

In this project, you will develop methods to find out what makes a cell receptive for a certain nanocarrier, to help improve its efficiency and cell specificity. You will use two of the latest techniques in gene expression: metabolic labelling of RNA and haploid genetic screens.

For the metabolic labelling, you will use a commercial nanocarrier to transfect mRNA for an enzyme called UPRT, which allows the conversion of thiouracil to thiouridine. In this way, only cells that express this enzyme will be able to incorporate thiouridine into their RNA. By using a method called SLAM-Seq, you can then identified the labelled RNAs through changes in their sequence. This will allow you to compare the genes that are on in cells that are successfully expressing the transfected mRNA with those that do not, hopefully giving clues to what makes cells receptive for RNA transfection.

Haploid cells have been recently engineered, allowing the efficient knockout of genes, as only one copy of each gene is present. They are surprisingly normal cells, with haploid embryonic stem cells being capable of generating all main tissue types, allowing a variety of near normal cells to be tested. By randomly inserting transposons into such cells, we have generated a so-called cell library, where typically one transposon is present in each cell and inactivates the gene it is inserted in. You will transfect an mRNA encoding resistance to an antibiotic into this library and select cells that have efficiently taken up by treating with the antibiotic over multiple rounds. Sequencing of the insertion sites in the surviving cells allows identification of the mutated genes and generate suggestions on how to persuade cells to take up and express mRNA efficiently.

Once one or more of these method are established you will collaborate with students developing new RNA nanocarriers to test them in your system. This work is likely to ultimately lead to better RNA medicines for a wide variety of applications.

Full project location: University Park;

Non-Invasive Early-Stage Disease Diagnosis via Optical Nano-sensors

Project Supervisor: Mohsen Rahmani

School: School of Science and Technology (NTU)

Description: Initially, the student will learn common experimental techniques for four weeks. He/she will be familiarised with the facility at NTU and UoN for fabricating

nanostructures, 100s of times thinner than a human hair and microscopy of them. Subsequently, at NTU, the student will be involved in one of the ongoing projects on fabricating the nanoscale sensor via electron beam lithography and characterisation of the sensor via a homemade Koehler illumination system. Koehler Illumination is a process that enables maximum contrast and resolution by focusing and spreading the light path evenly over the field of view. The student's involvement in this project will familiarise the student with diagnosis techniques via nanoscale sensors. It will expose the student to appropriate techniques for experimental data analysis, storage and retrieval.

Location: University Park;Clifton Campus;

Full project description: STATE-OF-THE-ART AND SIGNIFICANCE:

Chronic diseases are responsible for as many as 60% of annual deaths worldwide [Heal. Expect.18, 312–324 (2015)]. Today, most chronic diseases can only be diagnosed via blood analysis, which requires specialised training and expensive laboratory facilities. However, recent studies have shown that breath contains biomarkers associated with several diseases such as diabetes [J. Breath. Res.8,014001(2014) & Sci. adv.5,eaax0649(2019)] at very early stages of disease development. However, due to the ultra-low concentration of such biomarkers in breath, current technologies cannot measure them with sufficient accuracy to replace blood analysis.

In this PhD project, we will push the frontier of biosensor technology via developing ultrasensitive optical materials. We will draw inspiration from an existing widely available technology that uses metallic nano-particles to achieve ultra-high sensitivity: home pregnancy tests. Metallic nano-particles exhibit strong local near-field enhancements that are very sensitive to the change of environment. Thus, when exposed to a high concentration of biomarkers trapped in a liquid medium, such as urine for pregnancy testing, they induce a change in the colour of scattered light that can be detected. However, these near-fields' short propagation distances, combined with ohmic losses of metallic nano-particles have created a bottleneck for further applications.

In this project, hybridised metallo-semiconductor nano-particles will be developed to detect ultra-low concentrations of biomarkers. Metallic nano-particles enhance the optical near-fields, and semiconductor nano-particles extend and transfer the near-fields while reducing losses. This combination both greatly enlarges the sensing area and significantly enhances sensitivity. We have already published proof-of-principle works on this technique in 2D [Adv. Mater.30,1800931(2018)] and 3D [Adv. Mater.32,2002471(2020)] scaffolds. Modelling and simulation suggest that engineering hybridised scaffolds can significantly enhance sensitivity. This innovation will be extended, developed, and demonstrated experimentally during this PhD project. The targeted disease will be type II diabetes, which produces known biomarkers in bodily fluids at early stages [Diagnostics8,12(2018)].

AIMS AND OBJECTIVES:

The proposed research's over-arching aim is to develop pocket-sized sensors that allow users to quickly and reliably diagnose early-stage disease from home. The optical sensing can be done using conventional room lighting and visual examination, much like existing pregnancy tests, and thus does not require bulky or expensive electronic equipment. The main objectives are:

(1) Design and integrate a hybrid network of nano-particles for optical detection of analytes at concentrations of several parts per billion (ppb). We have previously

achieved 100 parts per million (ppm) [Adv. Mater.32,2002471(2020)], so this represents a significant improvement.

(2) Customise optical sensors to make portable, non-invasive biomarker sampling devices targeting diabetes biomarkers.

Accurate, low-cost, and pocket-size testing kits for early diabetes detection will help people detect and treat such chronic illnesses much more effectively. Therefore, this project's outcomes will save many lives and significantly reduce healthcare systems' associated costs.

Full project location: Clifton Campus;

Polyadenylation and Signal Transduction: Is RNA in charge?

Project Supervisor: Cornelia de Moor

School: Pharmacy

Description: Our recent research indicates that the fundamental process of mRNA polyadenylation is much more extensively regulated than previously suspected, especially during the changing conditions related to health and disease. We have shown that a natural compound derived from the famous parasitic fungus Cordyceps, cordycepin, is a polyadenylation inhibitor which represses inflammation and growth signalling and has therapeutic properties in models of osteoarthritis and cancer. Indeed, the cordycepin analogue NUC-7738 is currently in phase II clinical trials for solid tumours.

This project uses the insights from a dozen high throughput experiments to test hypotheses on the link between polyadenylation and signal transduction. You will compare the effect of cordycepin and NUC-7738 and investigate genes proposed to link polyadenylation and signal transduction using CRISPR-Cas9 and RNA silencing and/or investigate protein-RNA associations by immunoprecipitation. In addition you will measure poly(A) tail sizes using PAT-PCR. You probably will be involved in the preparation for or the evaluation of a haploid genetic screen and/or participate in Nanopore sequencing to determine poly(A) tail sizes genome wide and could do some bioinformatic analysis. Your work is likely to contribute both to the fundamental understanding of gene expression and to the development of a new class of medicines.

Location: University Park;

Full project description: Our laboratory has demonstrated in recent years that proteins that mediate mRNA polyadenylation deadenylation have unexpected roles in growth factor and inflammatory gene regulation. In addition, we have shown that inhibition of polyadenylation by the natural compound cordycepin represses signal transduction, especially in the PI3K signalling pathway. Excitingly, we have recently become capable of measuring poly(A) tail sizes in the whole transcriptome using Nanopore sequencing, which is likely to lead to novel insights.

In this PhD project you will use 3 approaches to determine the role polyadenylation plays in the regulation of signal transduction:

1. Determine the RNAs associated with proteins that could link signal transduction and poly(A) metabolising enzymes. A previous PhD students has performed Orthogonal Organic Phase Separation, a method that allows the determination of changes in RNA binding proteins. This has led to a list of potential candidate proteins which change their

association with RNA upon treatment with cordycepin. In addition, haploid genetic screens for cordycepin and NUC-7738 has generated a list of genes which are required for sensitivity of cells to cordycepin. By examining the RNA association of these proteins with immunoprecipitation followed by RT-qPCR and RNA-seq, you will determine which mRNAs are likely to play a role in signal transduction. In addition, you will test these candidates using gene knockout or knockdown and test for altered responses to cordycepin and growth factor stimuli.
2. Nanopore sequencing for gene expression and poly(A) tail size to determine the short term effect of cordycepin, NUC-7738 and signal transduction inhibitors of the MEK and PI3K pathways. Bioinformatic analysis of these data will determine which RNAs have poly(A) tails that are immediately affected by these treatments. These are candidates for being RNA regulators of signal transduction, which can be tested by RNA silencing or CRISPR-Cas9 knockout.
3. Use the Cellular Thermal Shift Assay and immunoprecipitation to examine changes in the protein complexes involved in growth factor signal transduction and poly(A) tail regulation. If the above approaches are still inconclusive, you will conduct a CETSA proteomic screen to determine changes in protein complexes after short-term cordycepin treatment.

This project is the culmination of a 20 year long research program and will lead to fundamental changes in our understanding of the regulation of gene expression. In addition, it will contribute to the development of polyadenylation inhibitors as medicines. This is likely to lead to a very good publication.

Full project location: University Park;

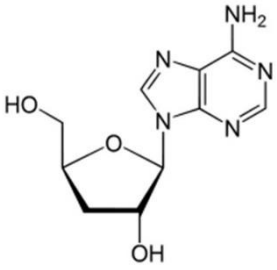
Streamlining synthetic genomes for designer organisms

Project Supervisor: Dr Benjamin Blount

School: Life Sciences

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

Cordyceps militaris (© Daniel Winkler)



Cordycepin

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

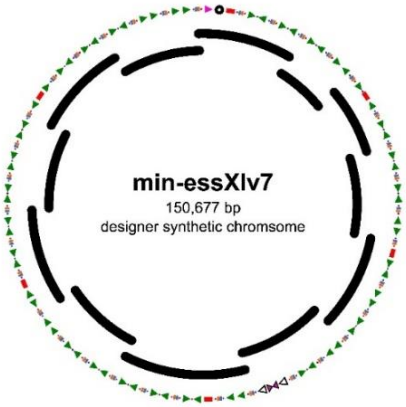
In the rotation project, the student will use CRISPR to show that essential genes can be deleted when our new synthetic chromosome is present. They will then show that the SCRaMbLE system can now be used to delete previously protected genes. The rotation

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

Location: University Park;

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

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



Supervision Team:

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

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

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

Full project location: University Park;

Controlling miR-122: a master regulator of liver health and disease

Project Supervisor: Catherine Jopling

School: Pharmacy

Description: MicroRNAs are tiny RNA molecules that bind to target mRNAs and regulate protein production, with important consequences for development and the maintenance of a healthy organism. MicroRNA-122 (miR-122) is crucially important in the liver, where it is required for hepatitis C virus infection, but also has an essential role in preventing liver cancer. Moreover, miR-122 functions to coordinate cellular senescence and is thus important in healthy ageing.

miR-122 is produced in liver cells at an exceptionally high level. The factors controlling this very high, tissue-specific expression are poorly understood. Interestingly, miR-122 is produced as part of a long noncoding (lnc)RNA. LncRNAs are a recently discovered class of RNA molecules which are an emerging area of research for biotechnology due to their gene regulation potential.

In the rotation project, you will investigate the role of promoter elements in driving the very high expression of miR-122 using CRISPR/Cas9. Using reagents that have been prepared for you, you will generate and characterise a CRISPR-modified cell line to test the effects of targeted promoter modification on miR-122 production. In parallel, you will use a range of molecular biology techniques to investigate the effect of specific genetic elements on miR-122 transcription and processing.

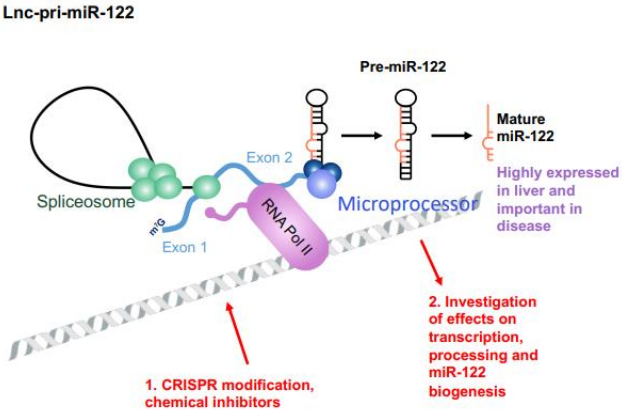
Location: University Park;

Full project description: The full PhD project brings together the expertise of the Jopling lab in microRNA biology and the Heery lab in transcriptional control and chromatin. You will carry out an in depth investigation of the factors that drive high miR-122 expression in liver, informed by bioinformatic analysis of publicly available datasets and genome-wide data on transcription and RNA processing in liver cells generated in the Jopling lab. You will use CRISPR modification to manipulate defined elements in the miR-122 promoter, enhancer and gene and use RNA and molecular biology approaches, including 4-thio-uridine labelling of newly synthesised RNA and quantitative real time PCR, to investigate the consequences for miR-122 transcription and processing. The role of chromatin modifications will also be investigated by chromatin immunoprecipitation (ChIP). You will also apply some of the same techniques to investigate the production of other biologically and medically important microRNAs that are generated from lncRNAs. This is also likely to include genome-wide analysis by RNAseq.

In the longer term, the results of this project may be important in providing new avenues for therapeutic manipulation of miRNA expression, with potential relevance to cancer, viral infection and healthy ageing.

Both the Jopling and Heery groups form part of the Gene Regulation and RNA Biology labs within the School of Pharmacy, a supportive and collaborative environment with a number of research groups working in related fields. Your project will be supported by Angela Downie as Assistant Supervisor. She is a BBSRC-funded postdoc who has been carrying out closely related work and will provide training and support in the lab to ensure your project is successful and to enhance your skills development. You will have the opportunity to attend and present your data at national and/or international conferences.

Full project location: University Park;



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

Project Supervisor: John Heap

School: Life Sciences

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

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

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

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

Location: University Park;

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

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

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

POTENTIAL AND CHALLENGE: We can build designer E. coli strains, repurposing native cellular machinery for safe production of glycans as vaccines - which are greatly needed worldwide. However, this is more complex than the simple way DNA sequences specify the sequence of amino acids in proteins: The variety of glycans is much greater, polymerisation can be linear or branched, and chirality of the sugars can be crucial to an effective immune response. Consequently, many more enzymes (and large gene clusters) are required for the formation of specific glycan structures, and we have to learn to 'speak glycan' like a new language. Conventionally, glycan gene clusters have been cloned whole ('en bloc') from the native organism into E. coli, with original regulatory sequences intact. However, this approach has often failed, because expression of these clusters has been optimised by natural selection for synthesis in the native host and is unlikely to be well-suited to production in an unrelated organism, where it may cause toxicity, metabolic burden, mutations and failure to express.

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

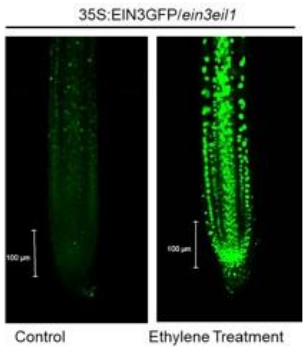
AIM: The student will use the new platform technology to build novel synthetic glycans in E.coli, both advancing the underlying platform capability and making useful vaccine candidates.

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

Libraries will be analysed via our deep sequencing pipeline, and results used to optimise vaccine candidates.

Full project location: University Park;

Does soil compaction trigger an ethylene response in root tissues?



Pattern formation in cyanobacteria: the earliest organised life-forms

Project Supervisor: Lucas Goehring

School: School of Science and Technology (NTU)

Description: Cyanobacteria are one of most successful forms of life on Earth, with origins dating back over two billion years, and can form into complex aggregates called biofilms that lie at the border of unicellular to multicellular life. Despite their importance to the history of our planet, along with their commercial and environmental potentials for biofuel production, little is known about how cyanobacteria biofilms form, how their mechanical properties arise, or how these properties could be engineered.

In this rotation you will make use of advanced microscopy methods to map the development of cyanobacterial colonies as they grow from a few individual cells into a collective structure of a biofilm, as might be present on the surface of a stromatolite, or in the bed of a biodiesel bioreactor.

The cutting-edge imaging methods that you will learn allow for real-time, high-resolution and three-dimensional imaging of macroscopic biological materials, and will allow for unprecedented insight into the dynamical structure of biofilms. The results of the rotation are intended to form an integral part of the full PhD project, which will then explore how to modify or engineer these structures.

Location: Clifton Campus;Sutton Bonington Campus;

Full project description: Cyanobacteria are one of the earliest and most successful forms of life on Earth, dating back over two billion years. They are ubiquitous in nature, finding habitats in most water bodies, and in extreme environments such as the polar regions, deserts, brine lakes and hot springs. They evolved the original mechanisms of photosynthesis, which transformed the early Earth and gave rise to our oxygen-rich atmosphere.

They have also evolved surprisingly complex collective behaviours that lie at the boundary between single-celled and multi-cellular life. For example, many species live in long chains of cells that bundle together into larger biofilms. They can form collective structures like stromatolites that provide a rigid, stable environment for their communities of bacteria. In addition, cyanobacterial biofilms can act as bioreactors, using photosynthesis to produce a wide range of green chemicals, including biofuels like biodiesel and ethanol.

Despite their importance to the history of life on Earth, and their commercial and environmental potentials for biofuel production, little is known about how cyanobacteria biofilms form, how their mechanical properties arise, or how these properties could be engineered.

In this project, you will investigate the biomechanics of filamentous algae, such as Oscillatoria (stromatolite-building species) and Anabaena (used for biofuels). These species consist of filaments one cell wide, and hundreds of cells long. They move by a slow gliding motion. Using state-of-the-art methods allowing for real-time non-invasive imaging you will characterise how the filaments move and change shape in response to their environment, including their interactions with other filaments. As the filaments grow, you will also determine under what conditions they organise into rigid, elastic structures. By relating microscopic properties, like the stiffness or 'stickiness' of individual filaments, to their collective response, you will provide predictive models for the macroscopic properties of biomats. Various proteins have been implicated in biofilm formation, including filament formation; in order to understand their influence, you will create mutant lines to knock out these genes in cyanobacterial species to determine which are essential for biofilm formation, as visualized by microscopy. This research will then enable a more rational engineering of these properties in applications like bioreactor design.

This project is highly interdisciplinary and involves using dynamical systems methods – tools that have already had great success in describing phenomena like the flocking of birds, or swarming of insects – to the problems of the collective behaviour of microorganisms. In it, you will be supported by a broad, interdisciplinary team involving regular supervision by Lucas Goehring (Nottingham Trent, physics) who has an experimental background in the dynamics of complex systems, including modern and fossil biofilms, and Ellen Nisbet (Nottingham, microbiology) who examines the genetics of photosynthetic microbes. Additional guidance will be provided by Marco Mazza (Loughborough) who has extensive expertise in theory and modelling of soft and active matter (such as microbial motility), and from Sourav Ghosh (Loughborough/National Biofilm Innovation Centre) who is a multidisciplinary engineering scientist working on biofilms.

Full project location: Clifton Campus;Sutton Bonington Campus;

The strong arm of CRISPR-Cas systems

Project Supervisor: Ronald Chalmers

School: Life Sciences

Description: The student will join a postdoc and two PhD students working on related projects. They will receive training in the techniques of molecular biology (cloning, sequencing, PCR etc.), which is an excellent foundation for work in almost any branch of the biomedical sciences. The student will also become familiar with genetic screening and genomic recombineering, which are the foundation of Synthetic Biology and Vaccinology, and will be crucial for the main project.

Location: QMC;

Full project description: All organisms have innate immune defences. Examples include the simple barrier-function of a cell wall and the production of antibiotics or anti-microbial peptides. However, the adaptive immune system of vertebrates is much more sophisticated. Not only does it produce antibodies to fight a new infection but it also

stores a memory of past battles to provide long-lasting immunity against repeat encounters. Only recently, it was discovered that bacteria also have an adaptive immune system called CRISPR-Cas, which defends against viruses and other forms of mobile DNA.

Although bacteria are physically simple, they are sophisticated at the molecular level. They provide many health and eco-environmental benefits, and are important in the move towards sustainable development. This project fits within the BBSRC vision for tackling strategic challenges in bioscience for renewable resources, which feeds into the area of sustainable agriculture and food. Large scale bioprocessing is used in agriculture, green-fuel feedstock production and in the pharmaceutical sector. The batch sizes are very large and contamination by viruses and other mobile DNA elements incurs significant costs. We are therefore investigating the molecular mechanism of adaptation, the process by which immunological memories are created and stored in the CRISPR locus. The final step in adaptation, which integrates viral DNA into the bacterial chromosome is catalysed by the Cas1-Cas2 complex. We are also developing hyper-active variants of this enzyme complex to better protect the host cells against invasion.

To pursue these aims, we have developed a new high-throughput assay for CRISPR immunization, which we are using to identify DNA and protein factors that alter its rate. This will allow us to address fundamental questions about the genes required for CRISPR immunization and the evolutionary battle raging between bacteria and their viruses. Although this work is at an early stage, we have already identified genes involved in other aspects of DNA metabolism, and discovered interactions between CRISPR and unexpected areas of cellular physiology. After discussions with the supervisors, the successful applicant will be able to pursue the genes and proteins to discover their precise role in CRISPR immunization and the underlying molecular mechanisms.

Many viruses are already known to protect themselves using anti-CRISPR systems, such as Cas9 inhibitors. Another avenue of work in the host laboratory is to use the high-throughput assay to search meta-genomic DNA-libraries for Cas1-Cas2 inhibitors that would prevent the host from becoming immunized against the viral attacker in the first place. To date no such system has been discovered.

https://www.nature.com/articles/s41579-018-0071-7

https://pubmed.ncbi.nlm.nih.gov/26949040/

https://pubmed.ncbi.nlm.nih.gov/25981466/

https://pubmed.ncbi.nlm.nih.gov/28869593/

https://pubmed.ncbi.nlm.nih.gov/28871175/

Full project location: QMC;

Novel ubiquitin fusions for cancer drug discovery

Project Supervisor: Ingrid Dreveny

School: Pharmacy

Description: Protein engineering can generate protein variants with tailored properties and new functionalities for diagnostic applications, the use as novel research tools and drug discovery. Ubiquitin specific proteases (USPs) can rescue proteins from destruction by the proteasome by reversing ubiquitination and are promising drug targets in cancer. In the lab rotation you will receive training in contemporary protein engineering,

recombinant protein production and biochemistry approaches and learn how to apply these to the characterisation of a ubiquitin specific protease. The rotation will consist of the following parts:

- Reading of scientific literature on the topic and general lab induction
- Generate a novel ubiquitinated substrate mimetic according to protein expression and purification protocols available in the lab.
- Conduct enzymatic and inhibition assays with recombinant USP4 and the generated substrate.
- Rationalise interactions, set up a USP4-substrate complex for crystallisation and/or predict ubiquitin-fusion interactions with AlphaFold.
- Data analysis

Location: University Park;

Full project description: Protein engineering is important for the manufacturing of protein variants with tailored properties and new functionalities, biopharmaceutical production, diagnostic applications, and the use as novel research tools. Ubiquitination is a major posttranslational modification involved in virtually all cellular processes. Ubiquitin specific proteases (USPs) remove ubiquitin from substrate proteins or remodel ubiquitin chains. As such, these proteases can prevent protein clearance by the proteasome or alter signalling pathways to regulate cell proliferation, apoptosis, and DNA damage repair. Dysregulated de-ubiquitination is associated with cancer, neurodegenerative disorders, and the host's response to infection. The tagging of specific substrate proteins with the small modifier protein ubiquitin in vivo is accomplished by a cascade of three enzymes and is not straight forward to replicate in vitro. Engineered substrates offer a unique way to interrogate the function of these enzymes and develop novel assays.

We have recently discovered a new way of generating USP substrate mimetics using recombinant techniques. As proof-of-principle we designed and generated two ubiquitin fusions and were able to show that they are suitable for inhibitor screening (Dreveny & Dekker labs, unpublished results). Modulation of deubiquitinase function offers valuable avenues for therapeutic intervention and biotechnological applications.

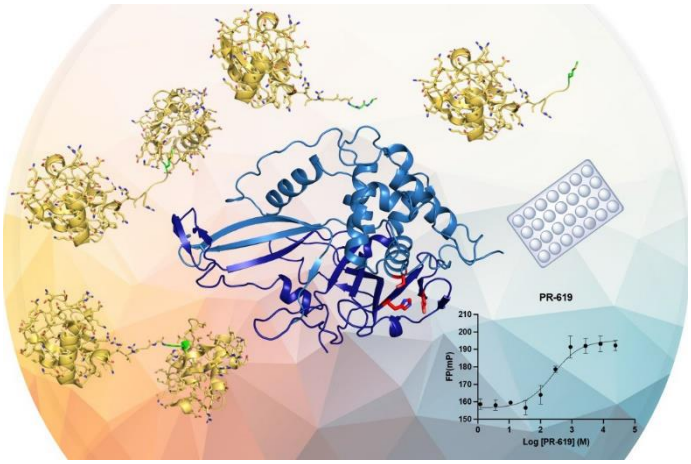
In this project we will explore the exciting opportunities that this discovery provides. The main aim of the project is to exploit and design novel engineered ubiquitin fusions for USP functional analysis and inhibitor discovery.

The project will consist of the following parts: (a) Generation of available and novel ubiquitin fusion substrates with in-house protocols (b) Use of the generated ubiquitin fusions in binding and enzymatic assays to test the substrate scope and specificity of USP4. USP4 fulfils an important regulatory role in TGF-β signalling, a pathway that controls numerous cellular functions including proliferation, apoptosis, and differentiation and plays an important role in carcinogenesis (c) Screening for inhibitors of USP4 (d) Exploitation of the information gained to modulate USP4 function in cancer cells.

Together, the project will offer skill development in an interdisciplinary setting including protein engineering, biochemistry, drug discovery and in vitro and in cellulo assay techniques and will deliver novel insights into ubiquitin specific protease function, selectivity and inhibition to target cancer cells. In addition, the creation of novel ubiquitin variants and fusions with new functionalities will provide a toolkit for the study of other enzymes in the ubiquitin system.

Lange, S.M., Armstrong, L.A., Kulathu, Y. (2022) Deubiquitinases: From mechanisms to their inhibition by small molecules, Mol Cell, 6;82(1):15-29

Full project location: University Park;



Investigating biocide mechanism of action in antimicrobial surfaces on bacterial pathogens.

Project Supervisor: Felicity de Cogan

School: Pharmacy

Description: Surfaces represent a key route through which bacterial infections can spread while also acting as an environment for the spread of antimicrobial resistance. To address this issue many antimicrobial surface technologies have been developed, however they require particularly long contact times for microbial killing (>18 hours). We have recently developed an antimicrobial surface technology that permanently bonds the broad spectrum biocide chlorhexidine to steel, plastics or glass. These antimicrobial surfaces are able to kill both chlorhexidine sensitive and resistant bacteria within minutes of contact, therefore being appropriate for high-frequency touch surface application (e.g. door handles). In addition, the permanent bonding of the biocide enables stability and longevity of the coating.

This project will optimise the concentration of chlorhexidine required to maintain maximal antimicrobial efficacy while also testing other biocides for the capacity to bond the surface.

Skills to be learnt: antimicrobial surface coating, surface characterisation, ToF SIMS, microscopy, Microbiological techniques will include: Minimum inhibitory concentration testing, antimicrobial surface testing, bacterial morphology analysis by fluorescence microscopy, confocal microscopy.

Location: University Park;

Full project description: The increase in antimicrobial resistance is one of the biggest threats to global public health. Forecasts predict that by 2050, it will lead to 10 million preventable deaths, and cost the global economy >£66 trillion. We have developed a novel antimicrobial surface that incorporates biocides onto surfaces, is very durable and can last for years. We have demonstrated that the surfaces are effective in killing bacteria, fungi and viruses (including SARS-CoV-2) in seconds. Our initial studies have also demonstrated that when surfaces incorporate biocides such as chlorhexidine digluconate they are as effective at killing chlorhexidine resistant bacteria as they are at

killing sensitive bacteria. The technology has been shown to be efficacious in both lab-based and "real world" studies.

Despite the progress made so far, the widespread use of biocides raises concerns for the potential of antimicrobial resistance and further work is required. The aim of this project is to investigate the mechanism of action of chlorhexidine and the mechanism by which surfaces inhibit bacterial survival. We will also investigate whether this differs from the effect of the biocide in liquid culture.

Depending on progress, the Ph.D. candidate can choose to target the following research objectives:

Objective 1: Compare the mechanisms by which pathogens develop resistance to chlorhexidine in either liquid medium or on surfaces

The student will use lab-based evolution and genome sequencing to compare the mechanisms by which key pathogens (Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus) develop resistance to chlorhexidine in liquid medium. Resistant strains will then be tested against our novel chlorhexidine surface technology. Training opportunities: Surface coupling, microscopy, bacterial culture, lab-based evolution, genome sequencing.

Objective 2: Characterise the gene networks required for bacterial survival in the presence of chlorhexidine

We will use high-throughput genetics approaches, such as Transposon Directed Insertion-site Sequencing-Xpress (TraDIS-Xpress), to identify the genes that become essential for bacterial survival in the presence of chlorhexidine in liquid or on surfaces. We will also use gene over-expression libraries to identify targets for chlorhexidine killing of bacteria. These gene "hits" will be investigated further using a range of biochemistry and molecular microbiology approaches. Together, the results of Objective 1, Objective 2 will define the mechanism of action for chlorhexidine against Gram-negative pathogens, which is an existing knowledge gap.

Training opportunities: TraDIS-Xpress, data analysis, biochemistry.

Objective 3: Screen environmental/clinical isolates for resistance to chlorhexidine

The first two objectives will address lab-based evolution of resistance within closed genetic systems, the final objective is to identify mechanisms of resistance within the environment. We will use chemical genomics and robotic handling systems to screen an existing bank of environmental/clinical isolates for resistance to chlorhexidine and characterise these mechanisms of resistance by genome sequencing. We will then identify whether any of these strains are capable of survival on antimicrobial surfaces and recapitulate the resistance mechanisms within a sensitive background.

Training opportunities: Experience of work with a range of microorganisms, robotic handling systems, high-throughput screening, genome manipulation.

Full project location: University Park;

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

Project Supervisor: Begum Tokay

School: Engineering

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.

Location: University Park;

Full project description: This project will deliver antimicrobial films consisting of metal-organic framework/biopolymer composites to prevent bacterial growth and reduce the threat of antimicrobial resistance. This will be the first to exploit properties and preparation of such smart antibacterial biocomposite films for a wide range of applications including wound dressing and food packaging.

The project will address the development, characterisation and applications of metal-organic framework (MOF) materials formed into nanocomposite films, which will comprise:

- a polymer support, such as chitosan, that will enhance and support film production;
- a MOF material that is itself antibacterial (e.g., the MOF is constructed from bioactive metals such as zinc (Zn2+) and silver (Ag+) ions and linkers such as cyclo-dextrin and adenine);
- a pre-formed MOF loaded with antibacterial microorganisms

The student will manufacture composite films of antibacterial MOFs in biopolymers using film casting technique. The chemical, physical and mechanical properties of MOFs and composite films will be determined. Inclusion of a biocidal microorganisms and into the MOF pores will be investigated and the subsequent materials will fully characterised. Film fabrication conditions (e.g., mixing time) will be tuned to maximise the performance of bioactive films. The efficiency of bacterial growth inhibition of films will be evaluated. This will be achieved via analysis of release kinetics, growth curve studies and zone inhibition tests. Tests will be focused on genetically engineered gram positive and negative bacterial strains for antibacterial screening as they are the most common cause of diseases and AMR. Student will compare bioactivity of MOF/chitosan films i.e., with commercial wound healing products such as, Chitoderm® Plus and TegaDerm 3M.

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.

Full project location: University Park;

Bacterial nanowires for Bioelectronic devices

Project Supervisor: Katalin Kovacs

School: Pharmacy

Description: The PhD will focus on electrochemical coupling of Cupriavidus metallidurans to electrodes for efficient electron transfer and high value chemical synthesis from waste gases. During the rotation, the student will become familiar with the growth and manipulation of the microbial host, C. metallidurans. and the use of the various genetic tools (ie. CRISPR) available. Specifically, they will: (1) learn to design and build synthetic genetic parts, modules and assemble them into vectors. (2) learn to genetically manipulate the host, creating and characterising insertion and deletion mutants via a range of molecular biology techniques, microscopy and flow cytometry.

Location: University Park;

Full 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 the bacterial respiratory chain to the surface of the electrodes using the industrially relevant bacterium Cupriavidus metallidurans. Cupriavidus metallidurans CH34 has shown to exhibit extraordinary metabolic versatility, including chemolithoautotrophic growth; degradation of BTEX (benzene, toluene, ethylbenzene, xylene); high resistance to numerous metals; biomineralisation of gold, platinum, silver, and uranium; and accumulation of polyhydroxybutyrate (PHB). These qualities make this organism a highly valuable host for biotechnological applications such as bioremediation, bioprocessing, and for potential generation of bioelectricity in microbial fuel cells (MFCs). We propose an electrochemical coupling mediated by surface expressed proteins that will improve the extracellular electron transfer (EET). The formation of conducting nanowires will be used to bridge the redox machinery of the bacteria to a conducting substrate within a BES system; a second generation microbial fuel cell. We have previously shown that expression of modifiedType 4 pili in Cupriavidus metallidurans can improve EET but current densities still need to be improved for efficient BES. Recent studies have established alternative EET systems, such as extracellular cytochrome nanowires, such as those based on OmcS that we intend to incorporate into our current C. metallidurans strains to improve current densities in BES.

We will use a synthetic biology approach to modify the organism and initiate biofilm formation on electrodes which will establish a direct electron-conducting link between Cupriavidus and the electrode surface in our fuel-cell-based bioreactors. The engineered strains with synthetic biochemical pathways will be used to convert captured CO2 into platform chemicals, such as novel bioplastic monomers and polymers.

This is a collaborative project between the Synthetic Biology Research Centre (SBRC-Nottingham), School of Pharmacy and School of Biosciences and is therefore a truly cross disciplinary project giving the student training in a broad background of techniques and fields ranging from microbiology, systems and synthetic biology, gas fermentation, biochemistry, materials science, electrochemistry and nanotechnology. This should provide the student with a diverse skill set in a unique multidisciplinary environment, ideally placing them for a future career in Industry or academia.

Full project location: University Park;Sutton Bonington Campus;

Exploring the role of VGF-derived peptides and their potential therapeutic use in Alzheimer's disease

Project Supervisor: Preeti Jethwa

School: Biosciences

Description: VGF nerve growth factor inducible (VGF) and its derived peptides have been associated with a number of neuroendocrine roles. They have also been highlighted as potential therapeutic targets for Alzheimer's disease (AD) as several lines of evidence suggest that reduced VGF-derived peptide levels correlate with the development of AD.

AD can be effectively modelled in the fruit fly, Drosophila melanogaster and these models have previously been used for the testing of novel therapeutics. However, this approach is yet to be applied for the study of VGF and its derived peptides in AD. To enable this, first a method for the effective delivery of VGF-derived peptides into the fly brain needs to be established. Therefore, this rotation project aims to do that via the steps and timeline outlined below.

Week 1: Learn how to maintain fly stocks and anesthetise them for analyses using standard Drosophila husbandry techniques.

Weeks 2-3: Optimise the methods for extraction and western blot analysis of protein from fly heads.

Weeks 4-9: Assess VGF-derived peptide uptake via feeding and microinjection strategies, using the optimised western blot analysis methods to determine whether the peptides reach the fly brain

Location: Sutton Bonington Campus;

Full project description: The main aims of this project are to investigate whether the observed decline in VGF and its derived peptides are mechanistically involved in the pathogenesis of AD and explore their potential as therapeutics, using the fruit fly, Drosophila melanogaster, as an in vivo model system.

The vgf gene is highly conserved across species including from humans to mice and its expression, plus of the derived peptides, are found throughout the brain, in peripheral tissues including the pituitary gland, adrenal glands and pancreas, and in endocrine cells of the gastrointestinal tract. VGF mRNA in the adult mouse hippocampus decreases with age and is associated with reduced spatial awareness and short-term memory, concomitant with a decrease in age-related neuronal degeneration and the synaptic plasticity markers. Further, vgf mRNA is up-regulated by activities such as learning, neuronal activity, seizures and lesions, while VGF-null mice have impaired: induction of long-term depression, hippocampal-dependent spatial learning, and contextual fear conditioning. Moreover, a number of biomarker studies revealed reduced VGF-derived peptides (GGGE-7, PGH, TLQP-62 and NERP-1) in the cerebrospinal fluid (CSF) and brain cortex of AD patients but not control individuals. TLQP-62 also enhances synaptic activity, induces neurogenesis, and blocking its release via antibodies prevents memory formation following learning tasks. Together, these data indicate that decreased expression of VGF and/or its derived peptides in the brain and CSF are associated with the pathogenesis of neurodegenerative disease, particularly AD.

Further research is now needed to fully understand their roles and investigate whether they could act as treatments; the purpose of this project. The three main objectives are outlined below.

• Objective 1: Develop an effective method for increasing the amount of VGF-derived peptides in the Drosophila brain. This will involve administering small peptides into adult flies via different methods, including microinjection and micro-feeding. Expression of VGF in the brain via genetic techniques could also be explored as an alternative approach. Assessment of peptide presence in the brains will be conducted using western blot analysis with head protein extracts from treated/manipulated flies.

• Objective 2: Examine the effects of increasing various VGF-derived peptides in wildtype flies. This will include phenotypic analyses via a number of techniques, such as: lifespan assays, climbing assays, rough eye analysis, and changes in food intake and glucose metabolism (as obesity and dysfunctional glucose metabolism are associated with the development of AD).

• Objective 3: Examine the effects of increasing various VGF-derived peptides in fly AD models. This enables exploration of the potential of different peptides as novel treatments for AD. There are numerous models that could be utilised here, such as Amyloid beta or Tau models. In addition, resulting changes in gene and protein expression could be analysed by using quantitative PCR, immunohistochemistry and/or western blot methods to interrogate the molecular mechanisms at play.

By the end, you will have gained the following:

• Knowledge: Detailed understanding of Drosophila AD models and related methods.
• Skills: numerous laboratory skills (Drosophila husbandry and genetic techniques, behavioural assessments, protein assay, qPCR, western blot, immunohistochemistry) and literature searching/reading.

Full project location: Sutton Bonington Campus;

Synthesis and Exploration of Hybrid Photo-Responsive Biomaterials

Project Supervisor: Nicholas Mitchell

School: Chemistry

Description: This rotation will investigate the design and synthesis of hybrid organic-inorganic electro/photo-responsive biomaterials. The organic scaffold will be peptide-based, with a range of architectures open for exploration. The inorganic component will be a polyoxometalate (POM) cluster with tunable electronic properties. Such functional hybrid nanostructures have potential applications in biotechnology, imaging, and therapeutics.

The candidate will develop skills in solid-phase peptide synthesis (SPPS), synthetic chemistry (including conjugation chemistry), metal oxide chemistry, electrochemistry, and analytical chemistry (HPLC, mass spec., UV/Vis, and electron microscopy including atomic-force microscopy). This rotation would suit a candidate interested in the application of materials science to biotechnology.

During the rotation, several peptides will be prepared, based on literature precedence, and their formulation into higher-order supramolecular assemblies investigated using a range of analytical techniques including electron microscopy. In parallel, hybrid POMs carrying appropriate groups for peptide conjugation will be prepared. The peptide constructs will then be 'decorated' with the POMs and the unique spectro-electrochemical properties of the material will be fully explored.

Location: University Park;

Full project description: The self-assembly of biomolecules such as peptides and proteins enables the formation of vital biological structures on which life depends. Due to their ease of synthesis, biocompatibility, and controllable self-assembly, peptides are ideal synthetic building blocks with which to construct artificial biomaterials.

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 a diverse application of these clusters within materials science. Furthermore, POMs can be reduced using UV light to access highly reactive excited species; thus the ability to graft POMs onto a supramolecular peptide framework presents an opportunity to prepare photo-responsive hybrid biomaterials that will find impactful applications in biotechnology, imaging, and medicine.

The formation of nanofibres, nanogels and nanotubes using a variety of peptide sequences has been thoroughly explored in the literature, however, the further functionalisation of these structures to enable the preparation of responsive biomaterials is an underexplored theme. Due to the diverse array of chemical functionality displayed by the 20 canonical amino acids, many standard bioconjugation techniques can be employed to attach POMs to individual peptides or peptide self-assemblies. Potentially more effective bioorthogonal approaches such as 'Click Chemistry,' tetrazine-alkene ligation and native chemical ligation (NCL) can also be explored.

This PhD project will focus on the investigation of novel peptide architectures, and the preparation of spectro- and electrochemically responsive biomaterials through the grafting of tunable POMs to the peptide scaffolds. The unique properties of these materials will be characterised and applications in biotechnology as photo-responsive biomaterials fully explored.

Full project location: University Park;

Sustainable fertilizer production with negative CO2 emissions

Project Supervisor: Wenbin Zhang

School: School of Science and Technology (NTU)

Description: Students are expected to spend around 3 weeks at each of the three partners involved in this project:

Nottingham Trent University (NTU): Students will develop their problem-solving skills and gain hand-on experiences through small project-based work by designing products using specialized software and computational skills, manufacturing the product in the workshop or using 3D printers and conduct pilot studies on the testing machines.

University of Nottingham (UoN): Students will have the chance to enter the laboratory in Department of Chemical and Environmental Engineering which is ranked 3rd in the UK. They are going to be trained on the selected state-of-the-art scientific apparatus and analysis equipment which are essential for characterization of physicochemical properties of different samples.

Rothamsted Research Centre (RRes): The skills to be developed in RRes will include preparation and analysis of soil and water samples for properties including soil bulk density, pH, available and total nutrient contents, organic carbon content and isotopes of N and P. They will have the opportunity to witness and participate in controlled environment plant growth pot trials, and become familiar with the wide range of monitoring equipment that is deployed on the most monitored farm in the world.

Location: Rothamsted Research;Jubilee Campus;Clifton Campus;University Park;

Full project description: Carbon capture, utilization and storage (CCUS) is the separation, capture and conversion of carbon dioxide (CO2) from emissions of various sectors prior to release into the atmosphere. It has been recognized as one of the most promising net zero decarbonization technologies. Bioenergy with CCS, known as BECCS, is the most scalable negative emissions technology available today to remove CO2 from the atmosphere. It is also the only carbon removal technology that can provide sustainable energy from biomass such as agricultural waste. To avoid the leakage risks and high transportation infrastructure costs associated with storage of CO2, conversion of CO2 to useful chemicals have received noticeable research in recent years.

The widely distributed non-point sources from organic biomass-based wastes are emitting significant amount of agricultural CO2 emissions, making agriculture section one of the most difficult sectors to be decarbonized. Fertilizers that are used by agriculture and in gardens are leading to numerous environmental issues. There is a huge demand for sustainable fertilizers to further address emissions from the negative impact of excessive phosphate-based fertilizer use.

The proposed project aims to use captured carbon dioxide from biomass conversion processes to stabilize a wide variety of materials from agricultural waste streams and produce green fertilizer products with significantly lower carbon and resource footprints comparing with the conventional fertilizer production. It will demonstrate the intensified production route of urea and ammonium nitrate fertilizers from sustainable feedstocks, namely biomass wastes, water, nitrogen, and carbon dioxide emissions from bioenergy conversion processes. The urea and ammonium nitrate will be synthesized in a purpose-built reactor under considerably milder conditions. Fibrous materials such as organic agricultural residues are used as substrates to formulate the products, further improving nutrient delivery to crops and locking additional carbon into the soil. Particularly, the addition of biomass ashes and their phosphorus fertilizing effects on different crops will be evaluated. In growth studies, the effects of synthesized urea fertilizer produced from various biomass precursors will be assessed under controlled conditions and compared with the commercial fertilizer.

This project is expected to contribute to the UN Sustainable Development Goals (SDG's) on Damaging Carbon impact of convention fertilisers (SDG15.5, 13.1) and can bring multiple environmental and technological benefits:

- Transformation of low-value waste resources (biomass waste and CO2) into predictable and high-value products, promoting a circular economy;
- Produce sustainable urea fertilizer with much lower carbon emissions comparing to the conventional fossil-derived fertilizer;
- Ensuring high yields, improved soil fertility and low costs to facilitate a just net zero transition in agriculture sector;
- Reutilization of biomass ashes to create nutrient cycles and to save fertilizer

The expected outcome of this project is to use waste resources to develop an innovative, cost-effective and environmentally friendly fertilizer which increases soil fertility and raises agricultural productivity, while limiting the negative impact of unsustainable

fertilizers. With the combination of bioenergy resources and CO2 utilization technologies, this project provides a possibly negative emission pathway for decarbonizing agriculture and farming, contributing to the UK 2050 net zero goals.

Full project location: University Park;Jubilee Campus;Clifton Campus;Rothamsted Research;

Fabrication and validation of ultrasensitive next-generation immune-biosensors; toward a potent tool for personalised medicine

Project Supervisor: Yasser El-Sherbiny

School: School of Science and Technology (NTU)

Description: In this lab programme, the student will enjoy having a rota of multi-disciplinary techniques for building ultrasensitive next-generation immune-biosensor fabrication and validation; towards powerful tool for personalised medicine. First, lab rotation will focus on optics, optical biosensors strategies related lab skills. The student will be trained on the first-rate equipment at the Optics and Photonics group, UoN and develop optical fibre biosensors using long-period gratings. Including gaining skills in handling optical fibres, modifying the surface of the optical fibre, with the sensitive coating and calibration by exposure to the known concentrations of the target analyte as described here.

Then student will focus on learning skills related to thin-film fabrication, laser fabrication of metal nanoparticles, plasmonics, optical characterisation of nanoparticle templates, structural characterisation of nanoparticles (SEM, AFM).

Then student will have to have a lab focus on immunological techniques and assays & Molecular biology skills, including related to biosensor validation and comparing gold standard immune detection techniques e.g. ELISA. this conveys cell culture, nucleic acid extraction, Molecular gene expression, e.g. RT-PCR, multiparameter immuno-phenotyping (FACS) laser analysis, Immunological biomarker functional assays, microscopy, and Electron microscopy imaging

Location: University Park;Clifton Campus;

Full project description: All of the health disorders need the quick and reliable detection of minute amounts of analytes or chemicals. Traditional approaches have considerable limitations, such as time-consuming sample preparation and expensive instrumentation. As a result, faster and more sensitive procedures are necessary to overcome these drawbacks.

The need for innovative methods to detect and monitor the evolution of rheumatological illnesses as immuno-sensors for disease state, progression, and response to therapy remains a challenge.

In Interferon-mediated illnesses, this idea applies to the pre-Rheumatoid stage. No "Gold standard method" exists for detecting and monitoring IFN-mediated illnesses. In reality, all recent research has relied on molecular analyses of patient blood or tissue samples, which seldom represent the patient's true illness immunological state. These approaches have poor correlations with disease activity, which affects the therapy plan. Recent breakthroughs in predicting IFN status biomarkers permitted better patient classification and revealed predictive molecules for disease recurrence and progression (El-Sherbiny et al., 2019, El-Sherbiny et al., 2018).

The project is an excellent example of cross-disciplinary collaboration between biomedical sciences, engineering, and the healthcare sector to develop a solution to the problem of quickly and accurately detecting immunity signatures in patients without the need for sample collection and processing in the lab.

Photonics technologies are essential in the development of better biosensors and next-generation diagnostic tools for medicine. They have the potential to transform healthcare through enhanced sensing and as novel analytical tools in biological sciences. In the era of digital health, there is a high need for novel methodologies and medical equipment for illness monitoring, particularly in chronic disease states produced by difficult-to-trace mediators. As a result, it is expected that the validated biosensor/s prototypes created will have a major influence on clinical disease practice and therapeutic options.

Candidates who join this programme will be able to develop expertise and skills in a training programme that encompasses different disciplinary approaches in optics, physics, nanotechnology, and immunology in order to build prototypes of sensitive immunobiosensors and validate and develop their application for lab to industry and health sector applications. Presenting discoveries and outcomes at international conferences and in high-impact publications in world-renowned journals

Full project location: University Park;Clifton Campus;

Bacterial cellulose production from spent coffee grounds (SCG) and its potential applications

Project Supervisor: Konstantina Kourmentza

School: Engineering

Description: The 9 weeks rotation will serve as an introduction to the production of bacterial cellulose from microorganisms available in our lab.

Week 1-3: Training in microbiological methods and techniques such as the use of high purity carbon sources and defined media for growth and selection of acetic acid bacteria (AAB), and generation of bacterial cellulose.

Week 4-7: You will be working with spent coffee grounds (SCG), serving as carbon source replacement of high purity sugars, to test and compare bacterial cellulose yield and productivity. You will be trained on methods for the pre-treatment and hydrolysis of SCG. You will monitor the feedstock consumption by liquid chromatography (HPLC) and determine the concentration of bacterial cellulose.

Week 7-9: You will be trained to recover and purify produced bacterial cellulose pellicles (downstream process). Bacterial cellulose will be characterised in terms of thermal and physicochemical properties using SEM, TGA, FTIR etc. According to properties, potential applications in bioremediation will be identified.

Through the above procedures, the student will learn the basic techniques necessary to undertake the project. This will include principles for developing a biorefinery design for the sustainable production of bacterial cellulose from SCG.

Location: University Park;

Full project description: Cellulose is the most abundant natural biopolymer on earth. It can be primarily recovered from wood, grasses, agroforestry and agricultural residues

as well as from tunicates and algae. It has been estimated that from 1 ha of eucalyptus with a basic density of 500 kg/m3, it would take 7 years (from planting to cultivation) to produce around 80 tons of cellulose. One of the most promising alternatives is the production of bacterial cellulose (BC) using acetic acid bacteria (AAB).

BC is a remarkably versatile biopolymer. It possesses enhanced water holding capacity (WHC) and permeability to gases due to the 3D conformation of its ultrafine fibril network. Other properties such as high thermomechanical response, purity, increased surface area, high polymerization degree and crystallinity render it valuable in active food packaging (as reinforcer, and nanocarrier), water bioremediation (adsorption of heavy metals and flocculation capacity), biomedicine (tissue engineering and controlled drug delivery), additive manufacturing (3D Printing), electronics and biosensors (enhanced conductivity). So far, the major factors that limit BC up-scale production are the high cost of the fermentation media and the low productivity values achieved so far.

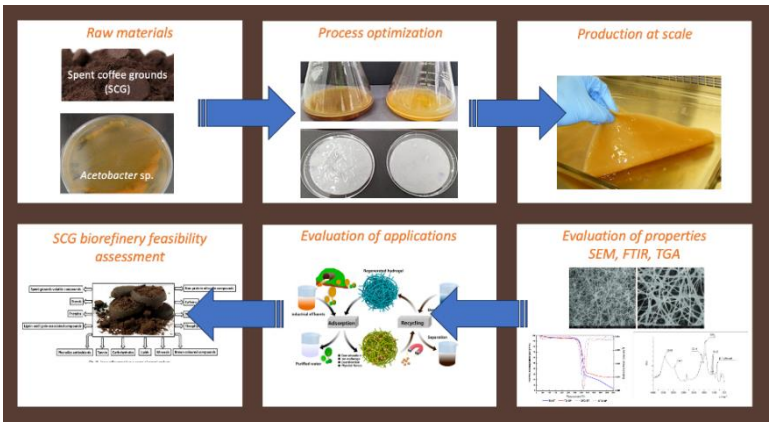
The valorization of renewable resources combined with newly isolated strains that can assimilate a variety of carbon sources and are tolerant to inhibitors, could improve the economics of the BC production bioprocess. A suitable and renewable substrate for sustainable BC production is spent coffee grounds (SCG), which is considered very promising for bottom-up and top-down biorefinery development, involving sustainable fractionation and fermentation. The consumption of coffee is an everyday habit, and even a cultural custom in many countries. Thus, coffee trade and use has been continuously increasing worldwide, generating huge amounts of solid residues, in return. For the year 2020/2021 global coffee consumption was estimated at 166.6 million of 60 kg bags characterized by an annual growth rate of 1.3% compared to 2019/2020. An estimated percentage of around 90% of the brewed coffee ends up though in the form of so-called Spent Coffee Grounds (SCG). SCG is characterized by high organic content, therefore, it has been used as a substrate for the production of biodiesel, bioethanol, biogas and biopolymers, among others.

Bacterial cellulose production through AAB is possible. However, there are still challenges to overcome for producing BC from cheap and sustainable feedstocks at high yield and productivities.

SCG can be used as low-cost carbon source, available in the abundance required to develop a multi-product biorefinery design focused on the production of bio-based commodity polymers at a scale that could replace existing chemical refineries. Recent advances in the pre-treatment and hydrolysis of SCG as well as in fermentation process and control will enable the bioprocess design to improve yield and productivities of BC production in AAB.

AIM: In this project, we will develop a bacterial cellulose production bioprocess from SCG, aiming to tackle the following challenges: 1) The basic research on AAB bacteria not previously reported for BC production, 2) Develop and design of the optimal upstream process (SCG pre-treatment and hydrolysis) both in economic terms and efficiency, 3) Define the optimal fermentation conditions to enhance BC yield and productivity, 4) Identify potential breakthrough applications for BC.

Full project location:
University Park;



Monitoring Effects of Radiation Therapy within a 3D Cancer In Vitro Model using Fluorescent Quantum Sensors

Project Supervisor: Ahmad Serjouei

School: School of Science and Technology (NTU)

Description: The Candidate will enjoy a rota of multi-disciplinary techniques for creating a complex 3D cancer in vitro model composed of healthy and cancer cells. Initially, the lab rotation will involve the preparation of cancer organoids. Therefore, the candidate will be trained on cell culture, brightfield and fluorescence microscopy, and relevant biochemical assays in Dr Yvonne Reinwald's lab at Nottingham Trent University (NTU). Then, the candidate will monitor cell response pre-radiation to establish the concentration of free radicals in untreated samples. This will take place at Prof Melissa Mather's lab at University of Nottingham (UoN), where the candidate will gain skills in multiple imaging modalities including multimodal diamond-based quantum microscopy. Finally, the candidate will mechanically characterise this model using mechanical tester in Dr Ahmad Serjouei's lab at NTU. Here, the student will learn how mechanical properties of in vitro models will change with prolonged culture periods to further establish a mature 3D model and to validate the effectiveness of radiation therapy as cancer treatment.

Location: Clifton Campus;University Park;

Full project description: Ionising radiation (e.g., X-rays, protons, gamma rays) can damage biological tissues and DNA when it interacts with living organisms. Increased exposure to ionising radiation elevates the risk of disease such as cancer and genetic mutations. At the same time, ionising radiation has therapeutic benefits with almost half of cancer patients receiving radiation therapy during the course of their treatment. There is a pressing need to understand the way in which ionising radiation leads to cell damage from a biological aspect and how different modalities elicit these changes.

Currently, most in vitro investigations into the mechanism of X-ray or proton radiations have been with 2D cell structures rather than the more physiologically relevant 3D tissue structures. There is growing evidence that 2D models are ineffective in capturing the mechanisms behind the biological damage, particularly as studies show significant differences in tissue response in tumour spheroids (in 3D) as compared to the 2D cell culture after investigating the effect oxygen depletion has on radiation response.

This project will combine cutting edge 3D cell culture systems, models of radiation and quantum sensing to establish a framework for investigating and uncovering biological mechanisms core to the manifestation of radiation damage.

At Nottingham Trent University (NTU), a novel 3D in vitro model will be created and characterized. This model will then be radiated using radiotherapy modalities to investigate the biological response of healthy and cancer cells. Within the University of Nottingham (UoN) team, quantum sensing to directly detect free radicals generated as a result of radiation will be employed. The team have an existing protocol combining spin probes to capture and stabilise short lived radicals and using fluorescent quantum diamond sensors to record free radicals. The spin probe enables selective detection of target radical species. Combined with the NTU team, nanosized quantum diamond sensors will be chemically conjugated to the polymer backbone of 3D matrices enabling spatial mapping of free radicals captured through augmentation of existing commercial fluorescent microscopes available both at NTU and UoN.

Full project location: Clifton Campus;

Competition in co-cultures of Streptomyces – Identification of novel natural products from the fight for survival

Project Supervisor: Huw Williams

School: Chemistry

Description: Streptomyces 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 co-culturing 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.

During the rotation, you will be integrated into the laboratories at the Biodiscovery institute.

For the initial rotation, the student will grow strains of Streptomyces, in the presence of 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.

Analysis will be done using NMR derived metabolomic techniques. The Highfield NMR facilities provide state of the art capabilities and can link changes in metabolite composition and concentration to the conditions. This powerful technique allows metabolites to be identified from their effect rather than requiring prior knowledge of the metabolite class.

Students will gain training in cell culture, bioactivity testing and advanced NMR.

Location: University Park;

Full 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 natural products that we can exploit to investigate novel 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 you will have gained knowledge of the factors involved that influence metabolite profiles. This will be expanded in the full project, to give a larger range of conditions to maximise the production of interesting natural products.

Using NMR derived metabolomic techniques and access to state-of-the-art high field NMR systems, these complex metabolite mixtures can be analysed and lead metabolites identified. Building on experience gained in both metabolomic and lipidomic studies we have extensive experience of using high-resolution 2D and 3D NMR to gain detailed insights.

NMR metabolomics produces a wealth of rich data, traditional methods involve using statistical techniques such as principle component analysis to identify changes between groups. In this project we will also investigate the use of modern machine learning techniques to augment this approach. Work by O'Neill previously has used databases of MS/MS data to dereplicate natural products. We will look at developing similar approaches to focus the analysis and to avoid know classes of compound.

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 techniques.

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.

Further aspects 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 gain experience in all aspects of natural product chemistry, including compound isolation and structure elucidation, genomics and biochemical advanced analytical skills, bioinformatics and machine learning.

Full project location: University Park;

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

Project Supervisor: Stephen Lawrence

School: Biosciences

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

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

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

Location: Sutton Bonington Campus;

Full project description: The inclusion of dietary fibre in the diet has been shown to have numerous health benefits, helped in part by the wide range of structures and compositions of known dietary fibres. There are multiple mechanisms for fibre functionality through the digestive system that are related to their induced health benefits. These relate to the properties of the fibres including bulking, viscosity inducing and, of key interest in this project, fermentability. These fermentable fibres act as a source of energy for microorganisms in the large intestine, contributing to gut microbiota growth and diversity and the release of physiologically important metabolites such as sc-fatty acids (contribute to intestinal/systemic health). Despite the health benefits, the average daily intake of dietary fibre remains below recommended amounts. Education can help but there is also an opportunity to develop products to fill this "fibre-gap" in modern diets. Of interest to this project is the incorporation of ingredients that contain significant amounts of dietary fibre into fermented beverages (beer). Due to its ABV, beer with an ABV>0.5% cannot support nutritional claims. However, the popularity of non-alcoholic beer is growing, and there is great interest in their potential to contribute to the intake of dietary fibre. Work in my lab has looked at the introduction of dietary fibre using non-traditional ingredients to expand the range of fibre types in non-alcoholic beer. The occurrence of these non-starch polysaccharides in brewed beverages has sparked discussion as to whether they could have physiological impacts. Many of these non-starch polysaccharides are defined as prebiotics which have the potential to act as substrates for gut microbiota. This community of microbes ferments indigestible fibres, producing by-products of metabolism that are known to benefit intestinal (butyrate) and systemic health (propionate/acetate). However, information on the fermentation of non-starch polysaccharides in the gut is limited. The aim of this project is to investigate the potential of dietary fibres and polyphenols present in non-alcoholic beer to modulate the gut microbiota. This knowledge will benefit the growing health/wellness category present in most leading brewing companies and help with the promotion of non-alcoholic beer as part of a healthier lifestyle.

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

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

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

WP4–Analysis of metabolic products generated during in-vitro fermentation. The breakdown of

fibre/polyphenolic compounds will be characterised using untargeted metabolomics. Metabolomic data will be integrated with omics data to allow network-based analyses of microbiome responses to different dietary substrates. Outputs from this work will give mechanistic insights as to how different beer-derived substrates are broken down by gut bacteria.

Full project location: Sutton Bonington Campus;

Autonomous Bioactivity Searching

Project Supervisor: Connor Taylor

School: Engineering

Description: The project aim is to autonomously search for bioactive small molecules. This will involve the high-throughput synthesis of molecules, followed by bioactivity measurements of those molecules, with the goal of unifying these methods in an automated way. During the rotation the student will be trained in the fundamental techniques required in the project, such as running high-throughput chemical experiments, chemical analysis techniques (such as HPLC, MS) and bioactivity measurements (fluorescence assays, affinity-based chromatography). The student will also gain familiarisation in the relevant laboratory equipment including flow chemistry, liquid-handling and high-throughput apparatus.

Location: L4 (Faculty of Engineering) and/or Energy Technologies Building (Jubilee Campus)

Full project description: The aim of this research is to develop an autonomous platform for bioactive molecule discovery. The project combines aspects of chemistry, chemical engineering and biotechnology to automatically synthesise drug-like molecules and evaluate their affinity for specific proteins. Development will begin on a high-throughput chemical experimentation platform, then on the optimal bioactivity screening methods, followed by final process automation steps to couple the processes. This project will thereby help to accelerate bioactive molecule identification and significantly reduce costs, enabling autonomous drug-candidate discovery for many diseases - including rare diseases and diseases of the developing world that are currently economically unfeasible to research. Initially, a poorly-binding molecular fragment is used as a starting point; reactions are then scheduled to exploit multiple growth vectors, yielding a highly-diverse product set of functionalised molecules that can be iterated further. The reactions are then conducted utilising microscale plates to efficiently run hundreds of reactions simultaneously, using advanced robotics and liquid handling systems, whilst only consuming minimal amounts of reaction material. The resultant products will then be screened against the protein of interest for binding affinity, with techniques including (but not limited to): fluorescence assays, inline flow-based assays and affinity-based chromatography. Specific protein targets will be assigned internally at various points in the project to differentiate between proof-of-concept and 'new target' work. Finally, the automation of these making/testing stages will be paired yielding an autonomous bioactivity searching platform that can explore chemical space without the time- and labour-intensive unit operations typically associated with them and thereby greatly reducing costs within the design-make-test-analyse sequence of molecular discovery. This cost reduction will thereby increase the feasibility of drug discovery against rare/orphan diseases, which are currently underexplored. This project is well

suited for students with an interest in how interdisciplinary research can drive drug-like molecular discovery, with areas of overlap including laboratory automation, high-throughput chemistry and analytical chemistry/biochemistry. Many skills and techniques will be learnt during the project, but a willingness to tinker with lab equipment and familiarisation with analytical techniques is preferential.

- Year 1: HTE reactor platform development. Control software implemented and paired with liquid handling robots. Proof-of-concept synthetic and reproducibility studies. Delivering: a bespoke and optimised HTE reactor platform for automated reactions. -Year 2/3: Bioactivity measurement optimisation. Initial strategies are conducted using well-understood proteins, comparing each bioactivity measuring technique for suitability for automation. Purification strategies identified if necessary. The targeted strategy is optimised, and reproducibility studies conducted. Delivering: a bespoke and optimised bioactivity measurement platform.
- Year 3: Integration. Both aspects of the project are combined into an automated platform for bioactivity searching. Initial case studies are conducted to test the system and determine suitability. Delivering: a fully automated reaction and bioanalysis platform.

Full project location: L4 (Faculty of Engineering) and/or Energy Technologies Building (Jubilee Campus)