# BBSRC Research Experience Placements Summer 2024

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- **Holly Shiels** - Micro-nanoplastics in sea turtles
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# Live imaging of Notch signal responses to gain of function Notch mutants in Drosophila

**Supervisor:** Martin Baron
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**Research profile:** Martin Baron

Notch is a signalling receptor, highly conserved in evolution, that has widespread roles in development and in adult tissue homeostasis, through regulation of cell fate choices, cell proliferation and cell survival and its mis-regulation is linked to numerous diseases including cancer, and developmental disorders.

Notch is deployed in a variety of contexts to regulate cell fate during lateral inhibition, asymmetric cell fate determination and boundary formation. These diverse roles are revealed by the many mutations of Notch that have been uncovered through genetic screens in Drosophila.

Notch has a large extracellular domain (ECD), only a small part of which is involved in binding and activation by ligands. Biological function of other regions of the ECD are revealed by mutations altering single amino acids which produce phenotypes in different tissues of the fly. One particularly interesting region is known as the Abruptex (Ax) domain, and mutations in this part of the Notch protein affect cell fate during boundary formation to determine the width of the wing veins of the fly.

Currently the mechanism of Notch gain of function of the Ax mutations is not known. Our preliminary cell culture work has indicated that Ax mutants do not simply alter the actual level of ligand-induced signalling. Instead we hypothesise that Ax mutations suppress basal ligand-independent activation of Notch that arises following endocytic uptake of the receptor thus increasing the fold increase of Notch activity.

Our research will now test our cell culture findings *in vivo*, in the wing disc tissues that phenotypically affected by the mutation by examining and comparing Notch signalling in WT and Ax mutants using a GFP reporter that responds to Notch activation in real time in live cells in live dissected wing discs.

# Investigating how the transcription factor HAND1 regulates human heart development

**Supervisor:** Dr Matthew Birket
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**Research profile:** Matthew Birket

Cardiovascular diseases are the most common cause of mortality in the Western world. Finding therapeutic solutions is therefore an important area of scientific research. Targeted differentiation of human embryonic stem cells (hESCs) into clinically relevant populations such as cardiomyocytes offers potential sources of transplantable therapeutic cells, as well as a useful model for studying human heart development.

Our lab is interested in the transcription factor HAND1, which is essential for heart development. How HAND1 regulates heart development however is not well characterised. To address this, we have recently performed a large-scale scRNA-seq time course analysis to determine how perturbation of HAND1 influences cell fate using a hESC model of cardiac differentiation.

This project will aim to identify new HAND1 transcriptional targets identified from our transcriptomics analyses to shine light on how HAND1 controls cardiac cell fate. Using our gain-of-function and loss-of-function tools to manipulate HAND1 expression, the student will utilise molecular biology techniques such as RT-qPCR, Western blotting, and immunofluorescence to examine the impact of HAND1 manipulation on putative downstream targets.

By revealing the role of HAND1 in heart development this project will help us understand congenital heart diseases caused by HAND1 mutations and will help support approaches in cardiac regenerative medicine.

# Use of molecular barcoding for identification of plant species

**Supervisor:** Henry Birt
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**Research profile:** Henry Birt

Botanical collections represent an important biological resource supporting a range of subjects: from biotech to evolution and climate change. Firs Botanical Gardens host the largest botanical collection available at The University of Manchester and require proper identification, cataloguing, and maintenance of collections for use in future research.

Part of the collection is a diverse range of Asphodeloideae, some of which are threatened species in the wild (Grace et al., 2013). Despite the potential importance of this collection, clear identification and cataloguing of the accessions are lacking. Using molecular methods, we will identify which species are present in this collection, assess the efficacy of various markers for species identification, and leave a legacy DNA collection that can be used in future experiments.

## Student development

This project will demonstrate the importance of DNA barcoding for plant studies and motivate students to enter a research career in plant sciences. Furthermore, this project is an introduction to the use of molecular methods that are important for a range of biological research fields, including PCR, DNA extraction and storage, and safe chemical handling.

The student will also be shown bioinformatic analysis (sequence alignment and BLAST searches) that are of broad use. This molecular work will be supplemented with an introduction to morphological identification. From this work, we intend to publish a short paper which will develop the student in terms of scientific writing and the publishing process.

## Broader impact

Our results will be published to the wider scientific community. The resulting DNA collection will start a larger project to collect DNA and catalogue the collection at FIRS. Demonstrating published outcomes and student development at FIRS will help to secure further funding for the facilities at FIRS from the university in the future. FIRS remains an integral facility for a range of research groups at UoM.

# Mutagenesis and DNA repair in microbial communities

**Supervisor:** Rok Krasovec
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**Research profile:** Rok Krasovec

Rok's group focuses on mutations and on a key global challenge of antimicrobial resistance. We showed that microbes at lower population densities have more than 20-fold higher chance of becoming resistant to multiple antibiotics (Krašovec et al.,Nature Commun.2014; Krašovec et al.,Plos Biology,2017). This density-associated mutation rate plasticity (or DAMP) critically depends on how cells deal with the oxidative stress (hydrogen peroxide in particular, Green et al.BioRxiv,doi: 10.1101/2023.09.27.557722).

Our approach in studying mutations and DAMP is inter-disciplinary. We combine microbiology techniques (like high-throughput fluctuation assays) with live fluorescence (super-resolution) microscopy, microfluidics, single-molecule tracking and statistical modelling.

We are imaging spatial and temporal dynamics of mutation avoidance and DNA repair proteins in individual bacterial cells living in (mixed) communities, and test how mutations are affected by cell-cell interactions and microenvironments. We also study single-molecule dynamics of extrachromosomal DNA, such as plasmids and bacteriophages.

Our work is important for the fundamental understanding of evolution and is enabling us to better predict antimicrobial resistance. This shall extend the usefulness of existing antibiotics and inform the development of longer-lasting novel drugs.

Possible project can be a wet lab single-cell study of mutations, plasmids, phages, protein mobility, antibiotic persistence or cell-cell interactions in cells growing in a batch culture or in a small community of up to 1000 cells. The studied microbial community can contain clonal cells or can be a mix of genetically different strains or species.

Student will be based in the state-of the-art microbiology lab facility with access to a range of cutting-edge analytical instruments, bioimaging facility and robotic automation.

Rok's group is part of a wider collective of evolutionary microbiology labs forming the Microbial Evolution Research Manchester (MERMan) grouping. MERMan is one of the largest group of evolutionary microbiologists in the world, comprising 10 group leaders and >60 research staff.


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# Using molecular and synthetic biology to study bacterial evolution

**Supervisor:** Mato Lagator
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**Research profile:** Mato Lagator

The aim of this project is to introduce an enthusiastic and passionate student to the wonders of interdisciplinary work using microbes as a model system. We use a range of molecular and synthetic biology techniques in order to study the basic rules that govern how evolution works. In particular, we are interested in improving the predictability of evolution by understanding how the existing molecular mechanisms in the cell determine the ways in which an organism or a biological system can evolve. We genetically modify and experimentally evolve bacteria to unravel the relationship between mechanisms and evolution.

There is a range of possible projects that would be suitable for a summer student, all aligned with the existing work in the lab. These ongoing projects in the lab include: predicting evolution of multidrug resistance; relationship between the number of tRNA genes and translation efficiency; how multi-drug efflux pumps are regulated; how is bacterial transcription terminated and how do those mechanisms evolve; how does resistance to one antibiotic alter the evolution of resistance to another antibiotic, the relationship between promoter architecture and its evolution, etc. However, I think the best projects are those that closely match the interests of the student, and hence would develop the specific project with student's input rather than pre-define it myself.

No matter what the project, the student can expect to gain experience in at least a few of the techniques commonly used in the lab: molecular cloning, plasmid and chromosome manipulation, flow cytometry, generation of random mutant libraries, experimental evolution, and bacterial fitness assays. We also employ a range of computational and modelling approaches, and would welcome a student who prefers dry over lab work as well.

# Fabrication of lipid membrane models

**Supervisor:** Jian Lu
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**Research profile:** Jian Lu

Cell membrane covers a cell and mediates material and signal exchanges with outside world. Cell membranes are typically a few nanometers thick, but they differ hugely in structure and composition between different cell types. These differences offer a fertile ground for exploring nanotechnology-driven approaches aiming at talking to the cells in different ways, e.g., how to sneak a drug into targeted cancer cells or bacteria without too much distress.

Cell membrane models facilitate modern measurement techniques (electron microscopy, lasers, X-rays, neutrons based) for observations of membrane structures and the subsequent impact upon exposure to a bioactive, e.g., a biocide or antibiotic.

The Biological Physics Group in P&A, SoNS has leading expertise in undertaking biomembrane research using both biophysical measurements and molecular dynamics (MD) simulations. The activities in the group are highly multi-disciplinary, requiring students to build knowledge and capability in physics, biochemistry and computing.

The student(s) will first be given reading materials on biophysics of lipid membranes, pros and cons of different lipid models, hand-on teaching on how to fabricate them and characterisations.

The student(s) will then be coached on (i) how to produce small unilamellar vesicles (SUVs) and how to form supported lipid bilayers mimicking bacterial and host cells, followed by characterizing their structure and interactions with biocide or antibiotic; (ii) how to load a self-quenching fluorescent dye (CF) into SUVs (in light green) and then how to release the dye by biocide binding (in bright orange), mimicking the process of killing oral bacteria by an active in toothpaste. This work will enable the student(s) to appreciate structural implications underlying their efficacy as well as toxicity to host cells.

The day-to-day supervision will be supported by senior PhD students in the group. Prof Lu is currently the first supervisor to 10 PhDs and 3 PDRAs.

# Investigation of animal behaviour using artificial intelligence

**Supervisor:** Rasmus Petersen
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One of the extraordinary things that our brains do is to infer what is out there in the environment using information from our senses. This is process is fundamental for all our behaviour. Historically, this process was studied in anaesthetised animals which are immobile since it was technically impossible to study sensation in animals that were free to move, behaving naturally. Now that has changed. Progress in Computer Vision and Machine Learning (Artificial Intelligence) makes it possible to quantify how animals move during their natural behaviour. Excitingly, this makes it possible to study sensation in freely moving animals.

The Petersen lab is focussed on using these techniques to investigate tactile behaviour. This placement will give a motivated student the opportunity to learn how to apply these Artificial Intelligence techniques and to use them to get insight into how animals behave. Specifically, the aim is to apply a method known as “DeepLabCut”. No prior experience of computer programming or Artificial Intelligence is required. However, the placement will suit a student who is highly motivated to learn about such approaches to neuroscience.

# Microplastics content in 2 edible bivalves the Pacific oyster and Blue mussel

**Supervisor:** Holly Shiels
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Microplastics (MPs), plastic fragments smaller than 1mm, pose a significant concern in marine environments. This issue is particularly pronounced in in shore areas where rivers carrying MPs enter the sea next to aquaculture facilities for bivalves.

Oysters and mussels, as filter feeders, are especially susceptible to MPs pollution. They filter large volumes of seawater, approximately 6-9 liters per hour, to obtain their food. In the process, they can ingest MP. Research has shown that the ingestion of MPs can have detrimental effects on bivalves, causing physical damage and interfering with their growth and reproduction. MPs can then enter the food chain through a process known as 'trophic transfer' and become part of the human diet when we consume seafood contaminated with MPs.

MPs themselves can be toxic and carry pathogens. As they move higher up the food chain, they can bio-magnify increasing the concern for human consumption. However, the impact of MPs on human health remains unclear. What we do know is that the highest concentrations of MPs in the food chain appear to be in fish, particularly shellfish, like oysters and mussels. This is a pressing issue that requires further research and immediate action.

The aim of this project is for the summer intern to work together with a PhD student learning how to determine the chemical composition of MPs that have accumulated in their body tissues. The student will learn analytical chemistry skills including FTIR to help identify the polymers and associated chemicals on the MPs that have been found in these edible bivalves. This information will inform on their safety for human consumption. As shellfish form a large part of the diet for some communities, this information is key for food security.

## Micro-nanoplastics in sea turtles

**Supervisor:** Holly Shiels
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Microplastics (MPs) are a pervasive marine environmental pollutant, posing serious threats to marine ecosystems and organisms at all trophic levels. Loggerhead sea turtles (Caretta caretta) have been identified as promising plastic indicator species to monitor MP pollution globally; these long lived, wide-ranging species are particularly susceptible to marine MP pollution due to their visual feeding strategies and backward facing oesophageal papillae.

The ability of small MP particles (<10µm) to translocate across the gastro-intestinal membrane and accumulate in distal body tissues have been demonstrated in fish, mice, and humans. A recent call highlighted the need to understand these processes in marine turtles to better protect vulnerable populations globally. Loggerhead turtles in the Mediterranean are an excellent candidate for this as sea water MP concentrations is reported in at 1.25 million fragments per km2. Working together with Oceanogràfic Foundation in Valencia, Spain, we have post-mortem collected samples from several organs of 10 loggerhead turtles that died due to being hit by boats or entangled in nets in the Mediterranean. The internship student would work with a PhD student processing these samples and investigating the amount and type of microplastic present in the tissue.

# Biosynthesis of terpene indole alkaloids by cytochrome P450 enzymes

**Supervisor:** Sam de Visser
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**Research profile:** Sam de Visser

Terpene indole alkaloids are important natural products with medicinal properties. However, the synthesis of these compounds is challenging. In plants many of these compounds are synthesized with enzymes with high stereo- and regioselectivity. In order to utilize these enzymes in biotechnology for the biosynthesis of valuable materials such as drug molecules, we need to understand how these enzymes obtain their high selectivity. As such, the de Visser group uses computational approaches including molecular dynamics and quantum mechanics to gain insight into fast reaction processes in enzymes.[1] These studies have given insight into the rate-determining step of the reaction but also the function of the protein and oxidant in the reaction processes. Using this information enzymes can be engineered for the catalysis of relevant reactions in biotechnology.

In this Summer Placement a computational study will be undertaken into the secologanic acid biosynthesis in plants. This compound has medicinal properties and is synthesized through a chain of enzymatic reactions from geraniol that contain several cytochrome P450 isozymes.[2] The P450s are important enzymes in nature and, for instance, in the human liver are involved in the biodegradation of toxic metabolites but also take part in the biosynthesis of hormones. The student will create a three-dimensional model of the enzyme using AlphaFold and insert heme and substrate.

The student will get access to the Computational Shared Facilities to run supercomputing calculations using available software packages. Molecular dynamics simulations will be done on the model to determine substrate binding, substrate positioning and protein and substrate movements. Thereafter, quantum chemical calculations on the reaction mechanism for formation of secologanic acid products from 7-deoxyloganic acid will be performed. Geometries of possible intermediates will be optimized and a prediction of the mechanism and the role of the protein will be made.

## References

1. a) S. P. de Visser, Chem. Eur. J. 2020, 26, 5308–5327; b) S. P. de Visser, G. Mukherjee, H. S. Ali, C. V. Sastri, Acc. Chem. Res. 2022, 55, 65–74
2. P. Rao, M. A. Yaroslavsky, J. C. Miller, M. A. Schuler, Biochemistry 2023, 62, 2763–2774.

# Developing a novel digital twin for sustainable fermentation process predictive modelling

**Supervisor:** Dongda Zhang
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**Research profile:** Dongda Zhang

Improving the economic competitiveness of industrial bioprocesses for sustainable commodity chemicals production is one of the grand research themes of the 4th Industrial Revolution. Currently, industrial bio-manufacturing systems primarily rely on the use of fermentation technology, with global market demand of their produced compounds estimated to reach £70 billion by 2025. Despite their huge success and impact, however, the conversation efficiency from organic carbon sources to final product is still very low in most of industrial fermentation processes (ranging from <1% to 20% depending on the complexity of metabolic pathways and operating conditions). Moreover, these bioprocesses contravene the concept of circular economy due to substantial CO2 emissions and undesirable waste products generated.

In order to resolve this challenge, developing predictive digital twins to maximise bioprocess efficiency and minimise waste generation has become one of the most critical steps. Given the rapid development of advanced mathematical modelling tools and their potential in future process automation and bioreactor design, this summer placement project aims to construct an accurate kinetic model to analyse industrial bioprocess data and guide optimal design of experiments for the underlying process. Specifically, this project will focus on a fermentation process for high-value biorenewables production. Experimental data has been available at this moment. During the summer internship project, we will build and compare different kinetic model structures to simulate the dynamics of the complex bioprocess and estimate its variability. We will also provide a range of supervisions to support the student, including:

- Assigning a senior PhD student to co-supervise the student;
- Having weekly meetings with the supervisor and the PhD student to update progress;
- Providing valuable resources (e.g. specific code and teaching materials developed within the group) to help improve the student's programming skills;
- Possibility to bring the student to a research workshop/seminar to develop their interest in biotechnology.