MANCHESTER 1824

The University of Manchester

BBSRC Doctoral Training Partnerships

Research Experience Placements 2021/22

Supervisor	Project Title (further details below)
Professor Martin Lowe	Understanding transport vesicle targeting at the Golgi apparatus.
Dr Mato Lagator	Using molecular and synthetic biology to study bacterial evolution.
Dr Rok Krasovec	Density-associated mutation rate plasticity in bacterial community.
Professor Holly Shiels	Micro and Nano plastic toxicity in freshwater fish.
Dr Samuel De Visser	Biodegradation of herbicides by plant and crop enzymes: a mechanistic study.
Professor Simon Turner	CRISPR/CAS9 mediated gene editing of the cellulose synthase complex.
Dr Patrick Gallois	Maximising recombinant protein expression in tobacco plants.
Dr Dongda Zhang	Developing digital tools for sustainable fermentation process modelling and optimisation

Supervisor Details	Professor Martin Lowe Enquiries: martin.p.lowe@manchester.ac.uk 0161 275 5387 https://www.research.manchester.ac.uk/portal/en/researchers/martin-lowe(dee05236-6b35-400b-90df-cd286596f48e).html
Project Title	Understanding transport vesicle targeting at the Golgi apparatus.
Project outline	The secretory pathway plays a fundamental role within our cells, essential for the production of all membrane and secreted proteins, including the extracellular matrix, digestive enzymes, antibodies and numerous hormones and growth factors. The pathway is critical to human health, and many diseases are caused by defects in secretory trafficking, and it is also exploited by pathogenic viruses and bacteria during infection. Cargo proteins are moved within the secretory pathway in transport vesicles that must be correctly targeted to the target destination within the cell. Essential for vesicle targeting are the golgin family of proteins that reside at the central station of the pathway, the Golgi apparatus. Although we know the golgins are important for vesicle targeting, how they mediate this process is poorly understood. This project aims to investigate the mechanism of vesicle targeting by golgins. In vitro biochemistry will be combined with cell-based assays to identify the determinants of targeting, and then to show their functional importance for secretory traffic. Gene editing combined with fluorescence microscopy will be utilised to achieve the latter. Training in these methods will be provided. The results will provide new insight into how the secretory pathway functions at the molecular level, which has broad relevance for human health.

Supervisor Details	Dr Mato Lagator Enquiries: Mato.lagator@manchester.ac.uk 0161 275 5766 https://www.research.manchester.ac.uk/portal/mato.lagator.html
Project Title	Using molecular and synthetic biology to study bacterial evolution.
Project outline	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. Following from this placement the student would 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.

Supervisor Details	Dr Rok Krasovec Enquiries: rok.krasovec@manchester.ac.uk 0161 275 5766 https://www.research.manchester.ac.uk/portal/rok.krasovec.html
Project Title	Density-associated mutation rate plasticity in bacterial community.
Project outline	My group focuses on spontaneous mutations, a fundamental biological process that drives evolutionary innovations and generates the key global challenge of antimicrobial resistance. Group is built on Rok's previous research findings that bacterial pathogens at lower population densities have more than 20-fold higher chance of becoming resistant to multiple antibiotics (Krašovec et al., Nature Commun., http://doi.org/skb; Krašovec et al., Plos Biology, http://doi.org/cb9s). This 'density-associated mutation rate plasticity' (DAMP) critically depends on mutation avoidance proteins and cell-cell interactions. Our approach is inter-disciplinary. We combine microbiology techniques such as fluctuation assay with live fluorescence (super-resolution) microscopy, microfluidics, single-molecule tracking and statistical modelling to measure quantitatively and dynamically the molecular processes involved in DAMP.

	This project will be a wet lab study of mutations and antimicrobial resistance in a large microbial community (containing millions of cells) by using a fluctuation assay (Krašovec et al., JoVE, https://doi.org/dj9n) or within a small community of up to 1000 cells, where live cell microscopy and microfluidics are needed. There are open questions that demand answers, for instance, what is the role of density in mutagenesis & DNA repair? Is there a density-associated accumulation of antimutagenic compound(s) or density-associated removal of mutagenic compound(s) The 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. My group (a postdoctoral researcher, technician and a PhD student) is a part of a wider collective of evolutionary microbiology labs forming the Microbial Evolution Research Manchester (MERMan) grouping. MERMan is one of the largest groups of evolutionary microbiologists in the UK, comprising 9 group leaders and >30 research staff all working on microbial ecology and evolution projects. We share laboratories and write-up spaces and form an exciting and cohesive community of likeminded scientists.

Supervisor Details	Professor Holly Shiels Enquiries: Holly.shiels@manchester.ac.uk 0161-275-5092 https://www.research.manchester.ac.uk/portal/holly.shiels.html
Project Title	Micro and Nano plastic toxicity in freshwater fish.
Project outline	The river Tame that runs through greater Manchester is the most microplastic polluted riverbed in the world but the impact on the benthic fauna are not well understood. This project would involve investigating microplastic accumulation in fishes collected at different stretches of the river where microplastic burden has already been characterised. The student would learn field work, chemical and enzymatic tissue digestion techniques, and microscopy including bright field, florescence, and IR imaging **References:** 1. Woodward J, Li J, Rothwell J, Hurley R. Acute riverine microplastic contamination due to avoidable releases of untreated wastewater. Nature Sustainability. 2021 May 13:1-0. 2. Hurley R, Woodward J, Rothwell JJ. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. Nature Geoscience. 2018 Apr;11(4):251-7.

Supervisor Details	Dr Samuel De Visser Enquiries: Sam.devisser@manchester.ac.uk 0161 306 4882 https://www.research.manchester.ac.uk/portal/sam.devisser.html
Project Title	Biodegradation of herbicides by plant and crop enzymes: a mechanistic study.
Project outline	Herbicides are common chemicals used in agriculture to improve crop yield. Often, however, these chemicals cause toxicological and ecological problems to the environment. As such, research has been devoted into biodegradable herbicides or environmental friendly alternatives. Thus, herbicide biodegradation is important in agriculture and particularly from a human health perspective, whereby plants metabolize excess herbicide and prevent these chemicals from entering the human body or the environment. Interestingly, in recent years several nonheme iron dioxygenases have been identified that can activate common herbicide molecules in plants such as maize and rice. In particular, the aryloxyalkanoate dioxygenase is able to hydroxylate the herbicide 2,4-dichlorophenoxyacetic acid, which then triggers its further metabolism in the plant cell.[1] Recently, research reported on the aryloxyphenoxypropionate tolerance (FT) enzyme highlighted the fact that these enzymes can activate auxin herbicides like 2,4-dichlorophenoxyacetic acid and its analogues. In this Summer placement the structure and reactivity of the FT enzyme will be investigated using computational tools.[3] Based on the available crystal structure coordinates of the enzyme, a substrate-bound reactant model will be created and mechanisms will be calculated (using quantum chemical techniques) leading to various products by calculating local minima and transition states and finding reaction pathways. This should give insight into the possible biodegradation pathways of the substrate in the enzyme. These studies will give insight into fast reaction processes and will help to gain understanding of the potential of these enzymes and how they should be engineered further to make them more efficient. **References:** 1. J. R. Chekan, C. Ongpipattanakul, T. R. Wright, B. Zhang, J. M. Bollinger Jr, L. J. Rajakovich, C. Krebs, R. M. Cicchillo, S. K. Nair, *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 13299–13304. 2. C. T. Larue, M. Goley, L. Shi, A. G. Evdokimov, O. C. Sparks, C. Ellis, A. M. Wollacott, T. J. Rydel, C. E. Halls, B. Van Scoyoc, X. Fu, J. R. Nageotte, A. M. Adio, M. Zheng, E. J. Sturman, G. S. Garvey, M. J. Varagona, *Pest Manag. Sci*. **2019**, *75*, 2086–2094. 3. S. P. de Visser, Y.-T. Lin, H. S. Ali, U. K. Bagha, G. Mukherjee, C. V. Sastri, *Coord. Chem. Rev*. **2021**, *439*, 213914.

Supervisor Details	Professor Simon Turner Enquiries: Simon.turner@manchester.ac.uk 0161 275 5751 http://www.manchester.ac.uk/research/Simon.turner/
Project Title	CRISPR/CAS9 mediated gene editing of the cellulose synthase complex
Project outline	Cellulose is the most abundant component of plant cell walls that constitute the majority of lignocellulosic material, an important feedstock for new generation of biofuels. Despite its importance, an understanding of how plants make cellulose is far from complete. Arabidopsis provides an excellent model system to study cellulose biosynthesis and a vast amount of progress has been made in the identification of genetic components of Arabidopsis cellulose biosynthesis machinery. CESA proteins are the core component of the cellulose synthase complex and three different CESA proteins are required to make cellulose and progress has been helped by the availability of mutants that disrupt the genes encoding these CESA proteins,. These mutants are caused by the insertion of a large T-DNA sequence and while useful, have these T-DNA mutants has limitations. Widespread success of CRISPR/CAS9 based gene editing has opened up possibility of using this technology to generate new alleles. We have taken advantage of new CRISPR vectors that allow simultaneous targeting of multiple genes at the same time. We have made two constructs which both target independent regions within the CESA genes. These constructs were transformed into wild type Arabidopsis. The proposed research experience placement will screen the next generation of these plants and identify the mutation(s) in the plants showing irregular xylem (irx) phenotype, an easily identifiable phenotype caused by loss of CESA protein function. The student will have an experience of growing Arabidopsis plants, (q)PCR based genotyping and DNA sequencing. The objectives of the project are: 1. Identify transformed plants that exhibit the irx phenotype. 2. Use qPCR melt curve analysis to identify insertion/deletion within the target genes. 3. Use DNA sequencing to determine nature of any insertion/deletion.

Supervisor Details	Dr Patrick Gallois Enquiries: patrick.gallois@manchester.ac.uk 0161 275 3922
Project Title	Maximising recombinant protein expression in tobacco plants.
Project outline	Plants are attractive platforms to express recombinant proteins of high value for applications in industrial biotechnology. High expression level of high-value proteins in tobacco leaves can lead to local Programmed Cell Death (PCD) and therefore limit the yield of the recombinant protein of interest. Reducing PCD induction in plants is expected to allow the design of improved protein-expression strategies. The project will test a new expression vector with the aim of increasing the yield of recombinant proteins in plants. The placement student will become familiar with the technique of agro-infiltration of tobacco leaves as a method to express recombinant proteins in non-

	GM plants. The student will then test a new vector design and various expression parameters to maximise expression of a chosen target protein. Techniques to be used will include microbiology techniques, PCR analysis and western analysis.

Supervisor Details	Dr Dongda Zhang Enquiries: dongda.zhang@manchester.ac.uk 0161 306 5153 https://www.research.manchester.ac.uk/portal/dongda.zhang.html
Project Title	Developing digital tools for sustainable fermentation process modelling and optimisation
Project outline	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 CO_2 emissions and undesirable waste products generated. In order to resolve this challenge, developing predictive modelling tools to maximise bioprocess efficiency and minimise waste generation has become one of the most critical steps. Given the rapid development of machine learning techniques and their potential in future process automation, this summer placement project will investigate a range of advanced modelling tools to analyse industrial bioprocess data and guide optimal design of experiments for the industrial partner. Specifically, this project will focus on a sugarcane biowaste derived Acetone–butanol–ethanol (ABE) fermentation process for biomass valorisation and renewable biofuel production. Industrial data has been available at this moment. During the summer internship project, we will test different modelling approaches (kinetic modelling, machine learning based data-driven modelling, hybrid modelling) to identify the best way to simulate and optimise the dynamics of the underlying bioprocess. We will also provide a range of supervision 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; • Possibility to visit the industrial plant and discuss with the process engineers