MANCHESTER 1824

The University of Manchester

BBSRC Doctoral Training Partnerships

Research Experience Placements 2019

Students successful applying for a BBSRC placement with a Wellcome Trust Supervisor (indicated below) will also take part in the Wellcome Trust Summer School; a programme of weekly seminars, in addition lab work.

Supervisor	Project Title (click link for further details)
Prof Enrique Amaya (Wellcome Trust)	The role of inflammation and immunity during tissue repair and regeneration
Prof Andrew Munro	Biochemical and structural analysis of alkene-producing P450 peroxygenase enzymes
Dr Danna Gifford and Dr Chris Knight	Effects of sub-lethal antibiotics on bacterial spontaneous mutation rate
Prof David Brough	Understanding regulation of the inflammasome
Dr Giles Johnson	Coming in from the cold – how do plants optimise their growth in cold climates?
Dr Gregory Perry	C–H functionalisation of allenes: towards diverse small molecule libraries for chemical genetics
Dr Hamish Gilbert	The role of the nucleus in the cellular response to mechanical stress
Dr Joe Swift (Wellcome Trust)	Investigating the impact of mechanical load on the proteotoxic stress response
Dr Patrick O'malley	Modelling Nature's Production of Oxygen and Hydrogen from Water
Dr Philip Day	The diversity of Rubus species and the evolution of regional endemic complexes within the north of England
Dr Samuel De visser	Fatty acid decarboxylases for biofuel synthesis

Supervisor Details	**Professor Enrique Amaya** **Email: enrique.amaya@manchester.ac.uk** **Tel: 0161 275 1716** **http://www.manchester.ac.uk/research/Enrique.Amaya**
Project Title	The role of inflammation and immunity during tissue repair and regeneration
Project outline	Studies using a variety of model organisms have implicated a critical role for inflammation during wound healing and tissue regeneration through mechanisms that are not entirely understood. The overarching aim of this project will be to address the role of the inflammatory response during tissue repair and regeneration using frog embryos and tadpoles as a model system, given that frog embryos and tadpoles are able to heal wound perfectly and to regenerate complex tissue and appendages (such as tails and limbs) following amputation. We recently used state of the art TALEN mutagenesis techniques to generate null mutations in the *spib* locus in *Xenopus tropicalis*, and we have begun to characterise the phenotypes in the homozygous spib mutant embryos and tadpoles. We have also been investigating the ability of the homozygous *spib* mutant embryos and tadpoles to heal wounds and regenerated appendages, such as their tails, following injury (Love et al., 2011; Chen et al., 2014). Given that our preliminary findings suggest that spib mutant embryos lack primitive myeloid lineages, but has no effect on definitive myeloid lineages, we also plan to generate CRISPR mediated mutations in other critical genes required for the development of inflammatory and adaptive immune cells, such as *pu.1* and *c-myb*. During the summer project that student will take part in this on going work in the lab, will participate in the following specific aims: 1. Further characterization of the phenotypes of embryos, which are homozygous for the *spib* null mutant alleles 2. Further assess the ability of *spib* mutant embryos to heal wounds and regenerate their tails 3. Begin generating CRISPR mediated mutants in *pu.1* and *c-myb* in *Xenopus tropicalis*. The techniques learned during this project, include CRISPR mediated mutagenesis, genotyping embryos, performing whole-mount in situ hybridizations, and performing wound healing assays in embryos and tail regeneration assays in young tadpoles. References: Chen, Y., Love, N.R. and Amaya, E. (2014) Tadpole tail regeneration in Xenopus. *Biochemical Society Transactions*, 42(3):617-623. Costa, R.M.B., Soto, X., Chen, Y., Zorn, A.M. and Amaya, E. (2008) *spib* is required for primitive myeloid development in Xenopus. *Blood* 112(6):2287-96. Love, N.R., Chen, Y., Ishibashi, S., Kritsiligkou, P., Lea, R., Gallop, J.L., Dorey, K. and Amaya, E. (2013) Amputation-induced reactive oxygen species (ROS) are required for successful *Xenopus*

	tadpole tail regeneration. *Nature Cell Biology*, 15:222-228.

Supervisor Details	**Professor Andrew Munro** **Email:** Andrew.Munro@manchester.ac.uk **Tel: 0161 306 5151** https://www.research.manchester.ac.uk/portal/andrew.munro.html https://www.research.manchester.ac.uk/portal/en/researchers/andrew-munro(8eb33a03-e35b-451d-b248-789d0f6c35b2)/publications.html
Project Title	Biochemical and structural analysis of alkene-producing P450 peroxygenase enzymes
Project outline	The project will focus on the expression, purification and characterization of novel cytochrome P450 enzymes of the peroxygenase class, which we have shown to catalyse oxidative decarboxylation of fatty acids of different chain lengths, leading to production of terminal alkenes with potential applications as biofuels. The student will purify and analyse a group of these peroxygenases to identify the most efficient enzymes and to establish which have the highest activities in alkene production. Genes encoding novel peroxygenase enzymes will be selected from diverse microbes, including thermophilic bacteria, with the aim of producing robust biocatalysts that can produce large quantities of alkenes. The student will be trained in P450 peroxygenase protein expression in *E. coli*, leading to cell breakage and peroxygenase purification by column chromatography using a combination of affinity and gel filtration chromatography using a high resolution AKTA purifier. Following purification of targeted peroxygenases, these enzymes will be used in analytical studies of lipid decarboxylation by HPLC-MS. The affinities of the various purified peroxygenases for different types of lipids (e.g. saturated and unsaturated fatty acids of different chain lengths) will be determined by UV-visible spectroscopic titrations in which the peroxygenase heme undergoes a substantial spectral shift on binding lipids, enabling data fitting to yield binding constants (K_d values) in each case. Purified peroxygenases will be crystallized using the MIB crystallographic facility in work to obtain structural data for peroxygenases in substrate-bound and substrate-free forms. The student will receive guidance and training from senior staff in the group and will learn techniques including protein expression and quantification, determination of substrate binding affinity for the peroxygenases, analytical studies to identify formation of alkenes and other side-products, and X-ray crystallography. The student will thus gain several new skills relevant to key BBSRC areas, including enzymology/biocatalysis, structural biology, biological chemistry and synthetic biology.

Supervisor Details	**Dr Danna Gifford and Dr Chris Knight** **Email: danna.gifford@manchester.ac.uk chris.knight@manchester.ac.uk** **Tel: 0161 275 5376** **https://www.research.manchester.ac.uk/portal/Danna.Gifford.html** **https://www.research.manchester.ac.uk/portal/Chris.Knight.html**
Project Title	Effects of sub-lethal antibiotics on bacterial spontaneous mutation rate
Project outline	Spontaneous mutations in DNA are the fuel of evolution. Our work focuses on how the environments organisms experience influence their mutation rate. We recently discovered relationships between mutation rate and population density via the types and amounts nutrients given (Krašovec et al. 2017, 2018). These environmental effects can change mutation rates by more than an order of magnitude. We aim to determine how other environmental factors influence and interact with this phenomenon. Importantly, sub-lethal concentrations of a particular antibiotic can increase mutation rates (Long et al. 2016). However, it is unclear whether this is driven by direct effects of the antibiotic on its target, or indirect effects on growth, interacting with the mechanisms we have discovered. To distinguish between direct and indirect effects, we will measure spontaneous mutation rate at varying levels of nutrient availability and antibiotic concentrations, using the model bacterium Escherichia coli. For the 'direct' hypothesis, we predict that the effect of antibiotic concentration on mutation rate will depend on the specific antibiotic, and not interact with nutrient supply. For the 'indirect' hypothesis, we predict that the effect of antibiotic concentration on mutation rate will not depend on the specific antibiotic considered, but act via another factor affected by the antibiotic, such as population density. Discovering how sub-lethal antibiotics affect mutation rates is instrumental to understanding the impacts of antibiotics in the wider environment, such as in waste water treatment and agricultural run-off. This project will help to determine whether these environments could be hotbeds for the evolution of key bacterial traits like antibiotic resistance. Krašovec, R., Richards, H., Gifford, D.R., Hatcher, C., Faulkner, K.J., Belavkin, R.V., Channon, A., Aston, E., McBain, A.J. and Knight, C.G. (2017) Spontaneous mutation rate Is a plastic trait associated with population density across domains of life. PLoS Biology, 15, e2002731. http://doi.org/cb9s Krašovec, R., Richards, H., Gifford, D.R., Belavkin, R.V., Channon, A., Aston, E., McBain, A.J. and Knight, C.G. (2018) Opposing effects of final population density and stress on Escherichia coli mutation rate. The ISME Journal. doi:10.1038/s41396-018-0237-3 http://doi.org/cst8 Long et al. (2016) Antibiotic treatment enhances the genome-wide mutation rate of target cells. PNAS 113(18): E2498-E2505. http://doi.org/f8n6fq

Supervisor Details	**Professor David Brough** **Email: David.brough@manchester.ac.uk** **Tel: 0161 275 5039** **https://www.research.manchester.ac.uk/portal/en/researchers/david-brough(3ca9a2e5-2794-4f9d-b6bd-1000e6295745).html**
Project Title	Understanding regulation of the inflammasome
Project outline	Inflammasomes are cytosolic multimeric protein complexes formed in inflammatory cells in response to pathogenic infection and tissue injury. Activated inflammasomes drive the processing of pro-inflammatory cytokine precursors such as pro-interleukin-1β to its mature secreted form (IL-1β) and initiate inflammation. The most commonly studied inflammasome is composed of the cytosolic pattern recognition receptor (PRR) NACHT, LRR and PYD domains-containing protein 3 (NLRP3). NLRP3-dependent inflammation is associated with the worsening of non-communicable diseases including atherosclerosis, Alzheimer's disease, type-II diabetes, arthritis and others, so a research priority has been to understand the mechanism of NLRP3 inflammasome activation. Despite this our understanding of the regulation of the NLRP3 inflammasome remains incomplete. Here we propose a project where we will study the dynamic nature of NLRP3 inflammasomes and how we will exploit this information to open new avenues of investigation. This proposal addresses the following aims: 1. To understand the mechanisms of dynamic ASC oligomerisation and deoligomerisation. The successful student will learn a range of cell and molecular techniques and will form part of a dynamic research environment.

Supervisor Details	**Dr Giles Johnson** **Email: Giles.johnson@manchester.ac.uk** **Tel: 0161 275 5750** **http://personalpages.manchester.ac.uk/staff/giles.johnson/**
Project Title	Coming in from the cold – how do plants optimise their growth in cold climates?
Project outline	As the UK climate changes through the next century to be more Mediterranean, there will be a need to shift plant growth to times of the year when water is secure but temperatures suboptimal. If we want to breed plants to a shifted growth seasons, we will need to understand the limits on photosynthesis across different conditions. This project will explore how plants allocate photosynthetically fixed carbon in response to different temperatures. The student will learn how to measure photosynthesis using infrared gas analysis and how to carry out metabolite assays to measure carbon storage compounds in leaves. The aim of the study will be to carry out these analyses using different Arabidopsis mutants, to understand how carbon resources are allocated under changing temperature conditions.

<table>
<tr><td>Supervisor Details</td><td>**Dr Gregory Perry**
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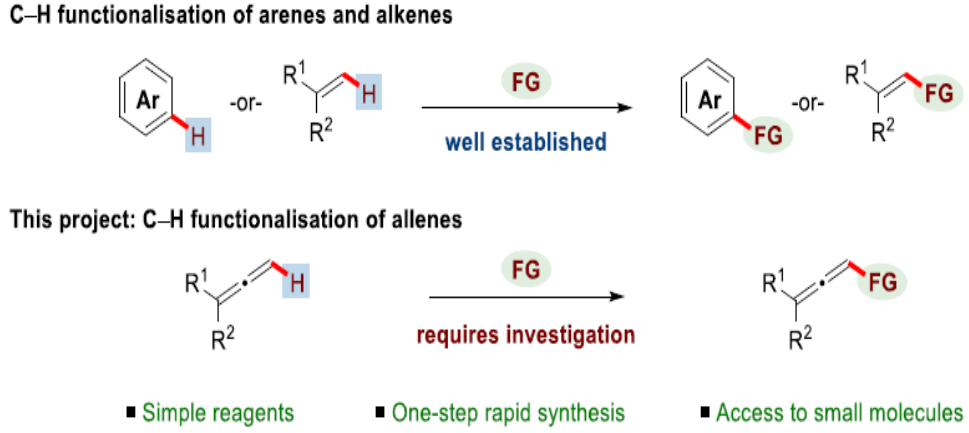
Tel: 0161 275 4676
https://www.research.manchester.ac.uk/portal/en/researchers/gregory-perry(34e1b82f-8446-4424-9174-c34cef61b919).html</td></tr>
<tr><td>Project Title</td><td>C–H functionalisation of allenes: towards diverse small molecule libraries for chemical genetics</td></tr>
<tr><td>Project outline</td><td>
The chemical genetic approach involves the screening of many small molecules from a chemical library in order to discover potential bioactive targets. Accessing a diverse range of these small molecules hinges on the development of new chemical methodologies. C–H (carbon-hydrogen bond) functionalisation holds potential in this area as it represents a means for rapidly accessing a variety of small molecules from simple precursors.[1]
C–H functionalisation has proved successful when transforming C–H bonds of arenes and alkenes, however, a related set of compounds, allenes, have received less attention (Scheme 1).[2] In this project we look to establish *new procedures for the direct C–H functionalisation of allenes*. In doing so, we will rapidly gain access to a variety of functionalised allenes, *ideal for the construction of diverse small molecule libraries*. Our approach to allene C–H functionalisation will rely on the reaction of nucleophilic allenes with electrophilic trifluoromethyl (CF_3) radicals.[3] The installation of the CF_3 group is particularly interesting due to the well-known ability of fluorine to improve drug efficacy.
Scheme 1. C–H functionalisation as a tool for accessing small bioactive molecules. (FG= functional group, e.g. CF_3)


As this project represents a new research venture, 10 weeks funding has been requested. This project will focus on the development of new chemical methods, however, direct collaboration across the fields of chemistry and biology is hoped for in the future.

Timeline and skills gained by student:
<table>
<tr><td>Week 1</td><td>Week 2</td><td>Week 3</td><td>Week 4</td><td>Week 5</td><td>Week 6</td><td>Week 7</td><td>Week 8</td><td>Week 9</td><td>Week 10</td></tr>
<tr><td colspan="4">Preparation of starting materials
Skills gained - literature reviewing, handling/preparation of chemicals, large-scale reactions.</td><td colspan="4">C–H trifluoromethylation of allenes
Skills gained: small scale-reactions, methodology development.</td><td colspan="2">Towards small molecule libraries (progress dependent)
Derivatization of allene products,
Skills gained: application of chemistry to other fields</td></tr>
</table>
</td></tr>
</table>

	References: [1] Murakami, K.; Perry, G. J. P.; Itami, K. Aromatic C–H amination: a radical approach for adding new functions into biology- and materials-oriented aromatics, *Org. Biomol. Chem.* **2017**, *15*, 6071. [2] Allene C–H functionalisation, only 3 reports: (a) Zeng, R.; Wu, S.; Fu, C.; Ma, S. Room-Temperature Synthesis of Trisubstituted Allenylsilanes via Regioselective C–H Functionalization, *J. Am. Chem. Soc.* **2013**, *135*, 18284; (b) Nakanowatari, S.; Ackermann, L.; Ruthenium(II)-Catalyzed C–H Functionalizations with Allenes: Versatile Allenylations and Allylations *Chem. Eur. J.* **2015**, *21*, 16246; (c) Zhang, G.; Xiong, T.; Wang, Z.; Xu, G.; Wang, X.; Zhang, Q. Highly Regioselective Radical Amination of Allenes: Direct Synthesis of Allenamides and Tetrasubstituted Alkenes, *Angew. Chem. Int. Ed.* **2015**, *54*, 12649. [3] Nagib, D. A.; MacMillan, D. W. C.; Trifluoromethylation of arenes and heteroarenes by means of photoredox catalysis, *Nature*, **2011**, *480*, 224

Supervisor Details	**Dr Hamish T J Gilbert** **Email: Hamish.Gilbert@manchester.ac.uk** **Tel: 0161 275 1524** **https://www.wellcome-matrix.org/people/joe-swift/**
Project Title	The role of the nucleus in the cellular response to mechanical stress
Project outline	Cells are responsive to the mechanical properties of their environments and translate physical forces into biochemical signals, in a process termed mechanotransduction. Cells residing in mechanically loaded tissues (muscle, bone, cartilage etc.) require mechanisms in order to maintain homeostasis under high-levels of mechanical stress. Musculoskeletal pathologies often begin at sights of aberrant loading, therefor understanding these mechano-protective mechanisms, and whether they're altered in disease/ageing, is important for development of future therapies against musculoskeletal diseases. Furthermore, investigating how mesenchymal stem cells (MSCs), cells proposed for medical applications in musculoskeletal tissue repair/regeneration, cope in high mechanical stress environments will be important in deciphering whether these cells need to be pre-conditioned prior to implantation into these mechanically complex tissues. Work within our group has identified an increase in the expression of chaperone proteins (proteins with a role in the cellular stress-response), including heat-shock protein (HSP) 70, which was shown to translocate out from the nucleus to the

	cytoplasm (opposite to that reported with heat-shock), in MSCs treated with strain. However the mechanism behind this HSP70 translocation, and its functional consequence, remains unknown. The aim of this project will be to investigate the regulation of strain-induced HSP70 translocation and expression, in human MSCs exposed to mechanical stress. This will be achieved by administering strain (Flexcell system) to MSCs in the presence and absence of nuclear import/export inhibitors, and siRNAs against Sun2 protein (shown by our group to be important in mechanotransduction to the nucleus) and Hikeshi protein (HSP70-specific import protein active during heat-shock). High-content imaging will then be used to observe the expression and localisation of HSP70, as well as changes in cell and nuclear morphology. This work will enable us to elucidate the mechanisms of mechanical stress-induced chaperone regulation.

Supervisor Details	**Dr Joe Swift** **Email: joe.swift@manchester.ac.uk** **Tel: 0161 275 1162** **https://www.wellcome-matrix.org/people/joe-swift/**
Project Title	Investigating the impact of mechanical load on the proteotoxic stress response
Project outline	Molecular chaperone proteins are responsible for maintaining the functionality of the proteome. They have an important role in assisting the folding of new proteins into their active forms, but are also essential in rectifying cellular damage caused by stresses such as heat shock, chemical or oxidative stress, or mechanical loading. Stress response mechanisms are fundamental to the maintenance of function in a diverse range of cell types, tissues and organisms. For example, expression of chaperones increases following exercise and is thought to protect against muscle injury and muscle dysfunction. Furthermore, the cellular stress response goes awry during ageing, and may compound an age-associated decline in the mechanical integrity of tissues. Work in the Swift lab has quantified the dynamics of the stress response in vitro. Using a combination of mass spectrometry proteomics and imaging methods, we have found that cells subjected to short periods of heat stress rapidly upregulate a network of chaperone proteins. However, this response was found to be attenuated in senescent cells, which we use as a model of ageing. We have also shown that senescent cells behave as if they are ‘stiffer’, and are less responsive to mechanical stimulation. The aim of this project will be to characterize the impact of mechanical challenge on the ability of cells to sense and respond to proteotoxic stress. Primary human mesenchymal stem cells will be subjected to repeated cycles of stretching that mimic tissue activity. We will then characterise the dynamics of the cellular response to proteotoxic stress using immunofluorescence microscopy. Temporal imaging will be complemented by proteomic analysis, using mass spectrometry data and a

	combination of mathematical graph theory and network bioinformatics to elucidate the role of mechanical structure in maintaining tissue health.

Supervisor Details	**Dr Patrick O'malley** **Email: Patrick.omalley@manchester.ac.uk** **Tel: 0161 306 4536**
Project Title	Modelling Nature's Production of Oxygen and Hydrogen from Water
Project outline	Enough solar energy strikes the earth in one hour to power the planet for one year at current consumption rates. Nature via photosynthesis uses this energy to split water into oxygen and hydrogen and manufactures high energy fuel molecules by reducing carbon dioxide with the hydrogen released from water. The key reaction is the splitting of water molecules into oxygen and hydrogen. Unfortunately, using sunlight to split water isn't so easy - unless you're a leaf. The goal is to synthesise a special molecule - a photocatalyst - that would initiate that first step when mixed with water and zapped by sunlight. But designing such a molecule requires a fundamental understanding of the chemistry inside a leaf, and nobody fully understands photosynthesis. This project therefore first aims to understand nature's catalyst which is a Mn4O5Ca complex located in the Photosystem II protein complex of green plants and algae. This will be investigated using a combination of computational chemistry modelling and high level Electron Paramagnetic Resonance (EPR) spectroscopy. A Cobalt-based catalyst which has already been shown to be able to split water into hydrogen when incorporated into an "artificial leaf" will also be investigated by synthesis, structural characterisation and molecular modelling. The mechanism of water splitting by this synthetic catalyst is believed to be similar to the natural system and we aim to improve the catalyst performance by incorporating design principles based on our investigation of the natural system.

Supervisor Details	**Dr Philip Day** **Email: Philip.j.day@manchester.ac.uk** **Tel: 0161 275 1621** **https://www.research.manchester.ac.uk/portal/en/researchers/philip-day(20a78b1b-664c-4cd5-9ec1-84e0183d2462)/publications.html**
Project Title	The diversity of Rubus species and the evolution of regional endemic complexes within the north of England
Project outline	This exciting study aims to extract DNA from an extensive national archive of the bramble Rubus (black berry) housed at the Manchester Museum. In association with a colleague at the Czech Crop Research Institute we wish to associate the sequence diversity of brambles with phenotype and the evolution of regional endemic complexes within the north of England. The student will be trained in DNA extraction, plastid haplotype and internal transcribed spacer (ITS) analyses, qPCR and related data analysis software. The student will also be expose to a parallel protein study addressing how coding sequence changes relate to fitness for bramble growth, fruit

	and leaf changes and ability to grow in harsh environments.

Supervisor Details	**Dr Samuel de Visser** **Email: Sam.devisser@manchester.ac.uk** **Tel: 0161 306 4882** **http://www.manchester.ac.uk/research/sam.devisser/research**
Project Title	Fatty acid decarboxylases for biofuel synthesis
Project outline	With dwindling amounts of fossil fuels in the Earth crust available, there is urgent need for alternative fuels. One option would be to utilize natural processes, including enzymes for biotechnological synthesis of hydrocarbons. In recent years several enzymes have been identified that selectively react with substrates to form hydrocarbons. One such example is the cytochrome P450 peroxygenase OleTJE that utilizes hydrogen peroxide on a heme-iron centre and converts linear fatty acids into terminal olefins.[1] Unfortunately, its reactivity with substrates gives considerable amount of by-products, which makes its application in biotechnology limited. Interestingly, very few non-heme iron enzymes are known to react as decarboxylase, but some were identified in recent years. One of those is the 1-undecene biosynthesis enzyme UndA that takes lauric acid as a substrate and converts it into 1-undecene selectively with limited amount of by-products. As such this system could have potential in industry for the enzymatic production of hydrocarbons from fatty acids. However, since little is known on its catalytic mechanism and substrate scope due to the fast reaction processes involved. Therefore, an urgent computational study is needed that elucidates the mechanism and gives understanding on the reaction rates, but also will clarify if the enzyme has potential for biotechnological applications. In this Summer Placement, the student will set-up models of the UndA enzyme and calculate the mechanisms for the conversion of substrates into products and determine the key details of the reaction mechanism and how by-products are avoided. Initially small model complexes will be investigated, but in the later stages full protein models will be included. References: [1] A. S. Faponle, M. G. Quesne, S. P. de Visser, Origin of the regioselective fatty acid hydroxylation versus decarboxylation by a cytochrome P450 peroxygenase: What drives the reaction to biofuel production? Chem. Eur. J. 2016, 22, 5478–5483. [2] Z. Rui, X. Li, X. Zhu, J. Liu, B. Domigan, I. Barr, J. H. D. Cate, W. Zhang, Microbial biosynthesis of medium-chain 1-alkenes by a nonheme iron oxidase. Proc. Natl. Acad. Sci. USA 2014, 111, 18237–18242.