Research Experience Placements 2016

*Please note: Students must discuss their application with their chosen supervisor prior to making an application.*

*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.*

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**Supervisor Details**

Professor Enrique Amaya, Faculty of Biology, Medicine and Health

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**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 previously showed that knocking down the transcription factor, *spib*, using antisense morpholino
approaches, results in the elimination of the inflammatory cells in early embryos. However, we were unable to use this strategy to eliminate the inflammatory response during wound healing and tissue regeneration, because the knockdown strategy was too variable and the effect was only transient. In order to overcome these disadvantages we recently used state of the art TALEN mutagenesis techniques to generate null mutations in the spib locus in Xenopus tropicalis. The aim of this project will be to generate homozygous mutants of spib, characterise the phenotype of these mutant embryos, and assess the ability of these mutant embryos and tadpoles to heal wounds and regenerated appendages, such as their tails, following injury (Love et al., 2011; Chen et al., 2014). The techniques that will be learned during this project, include genotyping embryos, performing whole-mount in situ hybridizations, using a variety of markers for innate and adaptive immune cells, performing wound healing assays in embryos and tail regeneration assays in young tadpoles. The specific aims of the project will be:

1. Characterization of the phenotype of embryos, which are homozygous for the spib null mutant alleles
2. Assess the ability of spib mutant embryos to heal wounds
3. Assess the ability of spib mutant tadpoles to regenerate their tails

References:

**Supervisor Details**
Dr Alex Casson – Faculty of Science and Engineering
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**Project Title**
3D printed EEG electrodes

**Project outline**
This placement will contribute to the ‘technology development for the biosciences’ BBSRC priority, within ‘Basic Bioscience Underpinning Health’. EEG (electroencephalography) is the process of placing small metal electrodes on the scalp to non-invasively monitor the brain. It is a widely used neuroimaging tool, and Dr Casson is exploring its use in a number of ‘healthy ageing across the life course’ applications, including to better understand the role of sleep, and manipulations of sleep in maintaining a healthy brain and slowing the cognitive aging process. This placement will help us to create personalised EEG electrodes that are quicker and easier to set up, to get better quality data in basic bioscience experiments.
In particular, Dr Casson has recently shown it is possible to 3D print EEG electrodes for the first time ([http://www.mdpi.com/1424-8220/16/10/1635](http://www.mdpi.com/1424-8220/16/10/1635)). This means that electrodes can be personalised: the size, shape, coating, and similar can be changed for each person to allow a better connection through hair, giving better data quality.

To date we have demonstrated 3D printing of rigid plastic electrodes coated with Silver. The student in this placement will advance our wider agenda in this area. Specific tasks will include:

- Comparing the performance of electrodes coated with: Silver; Silver/Silver Chloride; and directly conductive 3D printer filaments.
- Investigating the bio-compatibility of directly conductive 3D printer filaments.
- Advancing the design of our ‘head phantom with hair’ to allow the long term testing of bio-compatibility.
- Developing new electrodes using flexible printed elements, and assessing their performance compared to the above.
- Creating customised electrode designs for different types of hair and demonstrating these can be quicker/easier to set up and/or offer better recording quality.

It is likely that two areas can be tackled in the time available, giving potential to tailor the work to the interests of the selected student.

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**Supervisor Details**

Dr Patrick Gallois – Faculty of Biology, Medicine and Health

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**Project Title**

Deciphering the programmed cell death pathway during plant pathogen interaction

**Project outline**

Plants can activate a cell-suicide-programme called Programmed Cell Death (PCD) in specific stress or developmental conditions. In particular, upon attack by pathogens, plants activate a local cell death to help stop the infection. The *Arabidopsis* KOD locus, discovered in Manchester (Blanvillain et al. 2011), encodes a novel peptide that positively regulates plant PCD. Over expression of KOD is sufficient to induce a rapid PCD in plant seedlings and leaves. Characterizing the mode of action of KOD in inducing PCD is a unique opportunity to make discoveries that can translate into agro-industrial approaches. Pathogen induced PCD is under the control of salicylic acid and salicylic acid induces the expression of KOD. The aim of the project is to understand how the salicylic acid receptor NPR1 regulates the activation of the KOD gene using mutants, western analysis, confocal microscopy and mining transcriptomic data.
Supervisor Details

Professor Simon Hubbard and Dr Joe Swift – Faculty of Biology, Medicine and Health Sciences

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<th>Molecular chaperone regulation in the cellular mechano-response.</th>
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<td>Project outline</td>
<td>Molecular chaperones are proteins capable of refolding client proteins in order to maintain their functionality, or isolating unfolded proteins to inhibit harmful aggregation. These roles are important in managing the correct folding of newly translated protein, but also crucial to the cellular stress response. Stress response mechanisms are fundamental to the maintenance of cellular functionality in a diverse range of cell types and organisms. Consequently, increased expression of chaperone proteins can protect cells from many distinct stresses, such as heat shock, chemical or oxidative stress, or mechanical loading. Earlier work has shown that expression of the key chaperone HSP70 (heat shock protein 70) is increased following exercise, and that it can protect against muscle injury and muscle dysfunction in ageing. Furthermore, cellular stress response becomes increasingly misregulated during ageing, and may compound an age-associated decline in the mechanical integrity of tissues. Although the mode of action of HSP70 is broadly understood – it binds to the exposed hydrophobic residues of unfolded proteins – less is known about the client proteins it interacts with, or its role within the individual organelles of mammalian cells.</td>
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It is the aim of this project to optimize delivery and expression of a fluorescently labelled HSP70 construct (GFP-HSP70) in mechanically sensitive cells, and to observe how the location of the protein is affected by thermal or mechanical stress. The student will receive training in a broad range of wet lab techniques: culture of primary human mesenchymal stem cells (a widely used model for studying mechano-signalling, with potential applications in tissue engineering); application of strain cycle to cells in culture using FlexCell apparatus; fluorescence microscopy; and molecular biology methods to prepare and deploy GFP-HSP70 constructs.

This project will be offered as part of the Wellcome Trust Quantitative and Biophysical Biology (QBB) Summer School scheme.
Supervisor Details

Dr Chris Knight – Faculty of Science and Engineering

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Project Title
Evolution of multi-drug-resistance: prediction and reality

Project Description

The current crisis of antimicrobial resistance is not so much down to a lack of effective antimicrobials, as a lack of understanding. Specifically, understanding and control of the evolutionary processes that underlie the origin and spread of resistant phenotypes. We are looking at the fundamental evolutionary processes around antimicrobial resistance and their molecular bases, for instance the process of spontaneous mutation to resistance [1].

One of the most worrying aspects of the antimicrobial resistance crisis is the spread of multi-drug-resistant strains. We have developed a simple mathematical model for the origin and spread of multiple drug resistances in bacterial populations. This shows some surprising results about the effects of a changing antibiotic environment (as experienced by microbes through a course of antibiotics) and mutation rate on the speed at which multi-drug resistance arises in a population. The aim of this project will be to use wet-lab assays with model bacteria and two antibiotics (rifampicin and nalidixic acid) to test whether the predictions of the model work out in reality. This will be achieved via two objectives:

1) Growth assays of resistant and sensitive strains at different concentrations of antibiotic to ensure that what goes in to the computer model is biologically realistic.

2) Use experimental evolution of antibiotic resistant strains to test whether the modelled predictions for the appearance of multi-drug-resistant strains work out in practice.

Whether or not the bacteria evolve as predicted, this work will give a clearer (and highly publishable!) understanding of processes underlying the evolution of multi-drug-resistance. Downstream, this may be applied to the design of better antibiotic usage regimes.

### Supervisor Details
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<th>Modelling Photosynthetic Water Oxidation</th>
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**Project outline**  
Green plants algae and cyanobacteria oxidise water to oxygen and hydrogen ions in an enzyme called Photosystem II. The oxygen produced by this thermodynamically demanding reaction, which evolved 3 billion years ago, fuels our planet permitting development of complex life-forms. Development of artificial analogs is the holy grail for solar energy hydrogen production from water. This project will attempt to find out the mechanism for this reaction using electronic structure molecular modelling calculations on models developed from high resolution crystal structures. The electronic structure calculations will be used to interpret complex magnetic resonance data obtained experimentally for the natural enzyme. Combining experimental measurements and modelling will lead to a detailed atomic-level understanding of the reaction. Finding the detailed mechanism of the reaction as performed by the manganese, calcium oxo complex in Photosystem II is the key to developing efficient solar energy artificial catalysts.

### Supervisor Details
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<th>Project Title</th>
<th>Accurate energy evaluation of receptor-ligand interaction</th>
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**Project outline**  
Drug design routinely uses existing computational methods to evaluate the interaction energy between receptor (protein) and ligand (drug) in molecular docking. The problem with these methods is that they are not reliable and accurate enough. One Perspective1 squarely asks “why docking remains so primitive that it is unable to even rank-order a hit list”.

A more realistic and accurate force field will make the so-called scoring functions that docking uses more reliable. Our lab has a deep knowledge of a next-generation in-house force field called FFLUX2,3. This force field is much more realistic than a point charge based force field such as AMBER. Moreover, FFLUX “sees the electrons” and is hence closer to the underlying quantum 2 mechanics that ultimately governs the behaviour of all matter. FFLUX also introduces multipole moments, which is essential for accurate electrostatics4.

There is a modern and accurate energy partitioning method called *Interacting Quantum Atoms (IQA)5*, which offers a step change in the rigour of atomistic energy analysis. IQA is a parameter-free method that is intuitive but, at the same time, very close to the quantum mechanical character of atoms themselves. Originally rooted in small molecules, our lab has demonstrated that IQA can be now feasibly used for...
systems up to 370 atoms. However, we have shown in work to be published that smaller systems (~150 atoms) suffice to obtain rigorous insight.

Our in-house program ANANKE operates on a sequence of structures with varying distances and orientations of the receptor and ligand. ANANKE is able to highlight which fragments act like the total system, in terms of the various energetic contributions (both in type and locale). This is how, for the first time, a pharmacophore will be actually computed.

**Aim and Objectives:**

- Learn and use the program GAUSSIAN and obtain *ab initio* wave functions for all necessary structures of the system.
- Learn and use the atomistic energy partitioning program AIMALL and obtain intra- and interatomic energies of the type (kinetic, exchange, Coulomb and correlation).
- Learn and use the program ANANKE.
- Gather, organise, process and interpret the large amount of energy contributions into a coherent interpretation of the chemistry (e.g. steric, hydrogen bonding, electrostatics) of receptor-ligand interaction.

The receptor to be studied will be the Hepatitis C Virus Non-Structural Protein RNA Polymerase (NS5B) binding at least two inhibitors (filibuvir, GS-9669).

value' and are treated as equivalent by both molecular and palaeontological studies. Furthermore, we have no idea about the distribution or influence of these phenomena. As such, our understanding of a range of evolutionary events is undermined, and our ability to reconstruct evolutionary history is limited. To directly address the limitations of morphology, this project aims to: 1) Build a database of existing morphological datasets of extant clades from across the Arthropoda (i.e. crustaceans, insects, arachnids and myriapods); 2) Use meta-analysis to quantify the extent and distribution of the non-independent and incongruent characters across arthropod clades and across morphological modules. The outcomes will address specific evolutionary hypotheses, and provide powerful tools, workflows and guides for future analyses by providing new ways of working. This project will work alongside the BBSRC funded project “Overcoming the morphology problem of phylogenetics” (Sept 2016-Sept 2019).

### Supervisor Details

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### Project Title
Mathematical modelling of collagen fibril viscoelasticity

Collagen is the most abundant protein in the human body and the most important structural component of many biological soft tissues. Collagen based soft tissues exhibit an extremely wide array of mechanical properties to fulfil a variety of functions throughout the body. Some soft tissues, such as ligaments, are required to be stiff to keep joints stable, whereas others, like skin, are necessarily highly flexible. Viscoelasticity is fundamental to their mechanical behaviour and their stress-strain responses are highly dependent on strain-rate, with fast deformations resulting in much stiffer responses than slow ones. It is not fully understood how these tissues display such a wide range of mechanical properties despite being largely composed of the same materials; however, it has been widely hypothesised that the three-dimensional arrangement of collagen plays a crucial role.

Many mathematical models of collagenous soft tissues assume that the collagen fibril is the basic building block whose mechanical properties effectively determine the behaviour of the tissue on the macroscale. In order to validate such models, it is vital to know the constitutive behaviour of single fibrils; therefore, the aims of this project are to:

1. Obtain stress-relaxation data on rat tail tendon (RTT) fibrils and fibrils reconstituted from collagen I monomers via atomic force microscopy,
2. Evaluate how well this data can be modelled within a linear viscoelastic framework using 1- and 2-term Prony series, and to determine quantitatively the constitutive parameters that fit the data,
3. Compare the RTT and reconstituted fibril data qualitatively and quantitatively,
4. Use this information in an existing mathematical model of the macroscale viscoelastic behaviour of RTT fascicles to determine whether the non-linear effects observed experimentally can be accounted for as being entirely due to the spatial distribution of their linear viscoelastic fibrils.
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Project Title
Chemoenzymatic synthesis of a ‘Click analogue’ of the deubiquitinating enzyme inhibitor WP1130 and investigation of its ability to induce accumulation of polyubiquitinated proteins.

Project outline
Post-translational modification of proteins with ubiquitin regulates virtually every process within eukaryotic cells, and thus both the addition (ubiquitination) and removal (deubiquitination) of ubiquitin must be very precisely regulated in space and time. Deubiquitinating enzymes (DUBs), which remove and remodel ubiquitin chains attached to target proteins, play a central role in cellular homeostasis and altered DUB activity is associated with a range of pathologies. Hence there is much interest in the development of small molecule DUB inhibitors, both as research tools and for therapeutic purposes. The overarching goal of this work is to identify the intracellular targets and mode of action of the candidate DUB inhibitor WP1130.

The proposed vacation project bridges research investigations being carried out during two current DTP PhD studentships and which involve the three members of the supervisory team. There are three strategic goals to be achieved:

i) The successful exploitation of recently developed amine oxidase enzymes for the synthesis of a novel alkyne-containing chiral amine (R-1).

ii) Synthetic conversion of R-1 into the corresponding alkyne-containing analogue of WP1130 (2).

iii) Biological analysis of the new WP1130 variant involving treatment of cells with 2 and measurement of the resulting level of ubiquitinated target proteins using SDS-PAGE and immunoblotting to provide a readout of DUB inhibition.

On-going research being carried out in the Turner/Whitehead groups has demonstrated the feasibility of the chemoenzymatic synthetic approach outlined above with the successful synthesis of a related alkyne-containing chiral amine (R-3). This amine is currently being converted to a ‘Click variant’ of WP1066 (4) which will be biologically assessed in the Swanton lab. Expertise will therefore be in place by the summer of 2017 to enable a successful outcome to the project.