Cambridge BBSRC DTP – Bioscience for Health sample projects

(Students are provided with a list of ~150 projects to select from, these are just a sample)

Rotation projects

Minimizing genome-wide CRISPR/Cas9 screens

Wellcome Trust Sanger Institute

The CRISPR/Cas9 system has revolutionized research in cell biology. DNA can now be edited easily and accurately, enabling experiments ranging from understanding gene function and establishing mutations that cause disease, to correcting inherited genetic defects. However, as all locations cannot be targeted equally well, many gRNAs are used to edit one gene, which limits the scale of the experiments that interrogate large numbers of genes at once. This motivates the need for more efficient screening reagents.

The current industry standard libraries contain nearly 100,000 gRNAs, each gene targeted by 4-5 of them. We have established models of CRISPR gRNA efficacy that allow us to limit the number of sites targeted for each gene, and an experimental approach to use more than one perturbation in a single cell. Together, these promise to reduce the size of a genome-wide library to 20,000, thus saving 5-fold on experimental costs.

This rotation project would test and implement this strategy on large scale by designing the gRNAs used for a set of positive and negative controls, and confirming that the minimized screen results conform to the conventional ones.

Importance of colour saliency in Drosophila melanogaster

Department of Physiology, Development and Neuroscience

Currently the neural basis for the colour opponent process in animal visual systems is poorly understood. It is known in honey bees that signals leaving the second optical neuropile, called the medulla, already have signals indicating colour receptor information is compared upstream.

This rotation project aims to test the hypothesis that inner and outer receptor types (R1-6 and R7-R8 respectively) enable colour discrimination by comparing broadband receptors and colour specific receptors. Flies with specific pairs of opsins will be genetically engineered to allow these comparisons. Thus to determine precisely which neurons perform these computations, 2-photon imaging of neural activity indicators (GCaMP6 or ASAP1) will be used in combination a panoramic visual system stimulator.

The experimenter will have control of which colour and pattern can be delivered, so that spectral and spatial receptivity maps can be measured. By using a combination of transgenically manipulated flies (already produced), the outcome of this rotation is to determine which colour opponent comparisons are made and the neurons responsible for these tasks.

Full training will be given for a dedicated 2-photon microscope, the visual stimulation system and the image processing needed to produce data for this project.

The candidate student will be required to perform precise dissections to mount the fly in a holder prior to imaging, setup and run experiments using custom imaging and visual simulation software and undertake data analysis and figure production using Matlab.

Translational control in response to stress

Department of Biochemistry

Eukaryotic cells respond to stress by enhancing the translation of specific mRNAs.

We have identified a transcription factor gene that is absolutely required for the response to amino acid starvation. We have found that this gene is translationally upregulated in response to stress, and that this regulation is mediated through sequences in its 5' UTR.

The rotation project will involve the use of reporter systems to study how this gene is regulated by stress conditions, and how this regulation is important for its in vivo function. The model system will be the fission yeast Schizosaccharomyces pombe.

Effect on working memory performance of optogenetic stimulation and inhibition of cholinergic neurons

Department of Physiology, Development and Neuroscience (PDN)

Persistent neuronal activity in the prefrontal cortex is required during the delayed phase of working memory tasks. In slice preparations from the prefrontal cortex, application of a cholinergic agonist triggers persistent activation of pyramidal cells. We hypothesise that modulating cholinergic tone in the prefrontal cortex affects the performance in a delayed working memory task. To test this hypothesis, the student will use an optogenetic strategy to either activate or silence cholinergic cells during a T-maze alternation task.

First, mice expressing either light-activating or light-silencing protein in cholinergic cells will be trained to perform the task. Next, under supervision of a post-doc, the student will implant optic fibres above the cholinergic nuclei. This will allow control of the activity of cholinergic cells using light. Finally, they will characterise the effect of stimulating or inhibiting the cholinergic neurons during the delay phase of the alternation task. Specifically, the student will test different delay durations and characterise how excitation or inhibition promotes or impairs task performance. The project will help understand the role of cholinergic activity in working memory.

Breast implant associated ALCL (BIA-ALCL) is a transformation of Th17 cells

Department of Pathology

In this project, the student will investigate the potential origin of this rare breast-implant associated T cell lymphoma. Employing a collection of 60 primary cases, 3 cell lines and 1 patient-derived xenograft, the student will assess isolated RNA for the presence of transcripts associated with a Th17 cell phenotype. These data will be confirmed using a tissue microarray to validate expression by immunohistochemistry. In addition, the array of cytokines produced by the cell lines and secreted into tissue culture media will be analysed using a flow cytometry technique.

PhD projects

Developing small peptide inhibitors of RAMP-GPCR interactions

Babraham Institute

Receptor activity-modifying proteins (RAMPs) are single pass transmembrane (TM) proteins initially identified by their ability to determine the pharmacology of the calcitonin receptor-like receptor (CLR), a family B G protein-coupled receptor (GPCR). It is now known that RAMPs can interact with a much wider range of GPCRs including family A and family C GPCRs. RAMPs influence receptor expression at the cell surface, trafficking, ligand binding and G protein coupling. Thus the GPCR-RAMP interface offers opportunities for drug targeting, illustrated by examples of drugs developed for migraine.

In this PhD project we propose to design novel single span peptides that block RAMP-GPCR interactions. Mammalian cells express three different RAMPs, but they all share the same structure: the N-terminal domain modulates ligand binding whereas the short C-terminal intracellular domain has no known function; the TM domain is thought to facilitate the interaction with GPCRs, and it is this domain that we will target for interference. Using short peptides that we will design based on the TM helix we propose to block RAMP-GPCR interactions.

Our goal is develop peptides that target all three RAMPs. Initially we will extend our RAMP2 screens (in the rotation project) to other GPCRs and then develop new peptides that interfere with RAMP1- and RAMP3-GPCR interactions. Further, we envisage extending the use of these peptides to block recruitment of other GPCR accessory proteins such as β-arrestin proteins. The ability to block individual signalling pathways will provide critical data to aid our on-going efforts to computational model RAMP-mediated agonist bias. Thus the project is multi-disciplinary, combining peptide chemistry, cell biology and in silico modelling. The focus can be adapted to suit the student's background and interests.

Establishing a functional network for the emergence of neural and mesodermal cell fates in vivo

Department of Genetics

By obtaining quantitative imaging-based data for a few key markers of neural and mesodermal cell fates, the candidate will have developed a rudimentary model of how these cell states arise in a timely manner during development.

From this point, there are many interesting follow-up questions: How does expression levels of these genes correlate with other genes across the genome? Are the observed heterogeneities a consequence of differences in the dynamics of gene expression or are they stable through time? What are the key signal and gene regulatory interactions driving this process?

Complementary approaches in my group include the following:

- Transcriptome wide analysis of single neuromesodermal progenitors (NMps) isolated from the embryos at successive phases of axis elongation at to relate this to the spatial information derived from imaging based approaches.
- Live imaging of gene expression dynamics at both mRNA and protein levels to associate specific gene expression signatures with the eventual fate of the cells.
- Spatio-temporally controlled loss and gain of function approaches to modulate signal and gene regulatory inputs into the NMp regulatory network.

Therefore, as a PhD candidate, the student will be in an exciting position to incorporate both genome-wide and dynamical information into the model.

In addition, the candidate will lead a project using functional experiments to test predictions of this model by modulating specific nodes of the hypothesed regulatory network and assessing the impact of this on neural and mesodermal cell specification in vivo.

Using biophysical methods and chemical biology to explore function and design in the tandem-repeat protein class

Department of Pharmacology

The major focus of our research is a class of proteins, known as tandem-repeat proteins, with very distinctive architecture (e.g. ankyrin, tetratricopeptide and armadillo repeats). These proteins are frequently deregulated in human diseases such as cancers and respiratory and cardiovascular diseases. The individual modules of repeat proteins stack in a linear fashion to produce highly elongated, superhelical structures, thereby presenting an extended scaffold for molecular recognition. The term 'scaffold' implies a rigid architecture; however, as suggested by their Slinky spring-like shapes, it is thought that repeat arrays utilise much more dynamic and elastic modes of action. For example: stretching and contraction motions to regulate the activity of a bound enzyme; reversible nanosprings to operate ion channels; transporters that wrap around their cargoes to carry them in and out of the nucleus.

The modular architecture of repeat proteins makes them uniquely amenable to the dissection of their biophysical properties as well as the rational redesign of these properties. We are interested in understanding how the process of folding and unfolding of this distinctive protein class directs their functions in the cell. We are also looking at developing small molecule and peptide-based agents to modulate the functions of these proteins in the cell.

Lastly, we are exploiting the extraordinary design-ability of repeat proteins to create artificial proteins and protein-based materials with applications in medicine, nanotechnology and synthetic biology.

Research in our group is at the interface between biology and chemistry, we also have close collaborations with several other groups in Cambridge, and therefore students will be able to acquire expertise in multiple disciplines. We have a number of projects that would be suitable for a rotation and also for extension into a PhD. The student will learn some or all of the following approaches:

1. Molecular biology to make mutant variants of a protein.
2. Protein expression and purification techniques.
3. Biophysical methods (e.g. fluorescence and circular dichroism spectroscopies, thermal and chemical denaturation, stopped-flow and isothermal titration calorimetry) will used to investigate stability, folding and binding properties of the proteins.
4. Synthetic chemistry methods to incorporate additional functionalities onto repeat proteins.
5. For certain projects, complementary approaches will be used, for example single-molecule fluorescence, force microscopy, cell biology.

The causes of genetic variation in susceptibility to infection in Drosophila

Department of Genetics

The aim of this project is to understand why individuals within populations vary in their susceptibility to infection. This variation determines the burden of disease, and allows populations to evolve resistance. For researchers, it can provide insights into host-parasite coevolution and the functioning of immune systems. This project will use Drosophila as a model system to understand this variation, which allows us to have an unparalleled range of genetic tools to understand variation in natural populations.

In Drosophila melanogaster, populations of Drosophila can rapidly evolve resistance to parasitoid wasps by increasing the numbers of blood cells in circulation. These cells are the main immune cells that kill the parasites, so genetically resistant flies are likely maintaining their immune systems in a permanently activated state. We have recently found that several related species of Drosophila also evolve resistance by increasing

the numbers of circulating blood cells. The aim of this project would be to understand the genetic basis of this remarkable case of evolutionary convergence. Furthermore, this will provide a unique way to understand how cellular immune systems function.

To identify the genes underlying the evolution of resistance, you will artificially select populations of flies for resistance, and then use high-throughput genome sequencing to identify the variants that have changed in frequency in the selected populations. The function of these genes can then be tested by creating genetically identical flies that differ only in the variants in question. Combining microscopy and genetic manipulations, these flies can then be used to investigate how these genetic changes alter immune responses.