Honours Projects available in the Shearwin Laboratory in 2016

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Our research integrates biochemistry, genetics and mathematical modelling to characterise fundamental mechanisms of gene control and how these elements are combined to create gene regulatory circuits with complex functions. Having a toolbox of well characterised genetic components allows us to ‘rewire’ them in a rational way in order to construct new genetic circuits with predictable behaviour for use in Synthetic Biology applications. The lessons learnt in the construction of artificial genetic circuits in turn give us a deeper understanding of how natural biological systems work.

Our primary experimental systems are two E. coli bacteriophages, lambda and 186. These temperate phages can replicate their genomes using alternative developmental pathways, lysis and lysogeny, and are some of the simplest organisms to make developmental decisions. Despite their relative simplicity, the phage systems combine a wide range of gene control mechanisms in complex ways and have many lessons to teach us. Bacteriophage lambda continues to be a key model system for many molecular biological processes; phage 186 is less well characterised but provides a powerful comparison with lambda, as it achieves similar outcomes using different regulatory circuits. The fundamental biochemistry shared by all living things means that the study of any organism, from phage to humans, continues to illuminate universal principles that apply to all organisms.

Here are some examples of projects available in 2016:

1. Development and application of improved Synthetic Biology tools for rapid cloning into bacterial chromosomes

Compared with using plasmids, insertion of DNA into bacterial chromosomes for synthetic biology purposes has several key advantages such as are needed for construction of large gene assemblages e.g. metabolic pathways. Cell-to-cell copy number variation is reduced and there is a lower metabolic burden on the host cell, with proteins able to be expressed at normal physiological levels. Critically, for industrial and medical applications, insertions are generally very stable, obviating the need for continuous antibiotic selection. The one-step-integration-plasmid (pOSIP) system that we have developed (1) fulfils a number of these criteria.

This Honours project will involve expanding and improving the OSIP system. For example, a large collection of new phage integrases and their corresponding attB and attP sites have recently been described (Voigt laboratory, doi:10.1038/nmeth.3147). You will identify appropriate genomic locations in E. coli and
perhaps other bacterial species, and introduce by recombineering a new set \textit{attB} sites to create a new standard OSIP ready chassis strain.

2. Building a low noise protein expression system

To study gene regulation in bacteria, proteins are often expressed from plasmids and the response of a reporter gene is assayed, usually as an average across the whole bacterial population. However, protein expression from plasmids is often noisy, or variable, from cell-to-cell leading to a smoothing out of the true cellular response. One source of this noise is plasmid copy number variation from cell to cell. A second source of noise is the lack of feedback in the expression system, so that stochastic variations in protein expression in an individual cell do not self-correct.

In this project we will design and construct a single copy, feedback-controlled protein expression system. It will be based on the naturally occurring negative feedback control inherent in the lytic/lysogenic switch region of bacteriophage 186, which needs to maintain a tightly controlled concentration of CI in order to maintain lysogeny, yet be able to efficiently and rapidly switch to lytic development in the event that its host cell is damaged. We will construct a series of bacterial strains (1, 2, 3) each of which will contain a chromosomally-integrated, single copy of a circuit specifically designed to produce a tightly controlled concentration of any protein of interest, where expression levels are under 186CI feedback regulation.

3. Design and construction of a modular bacteriophage to facilitate scar-less, site-specific alterations in the phage genome

One of the limiting factors in studying the function of specific components of a genetic circuit in the context of the whole organism is the ability to make small, precise and scarless changes to the DNA sequence. This is particularly true for small, highly compact genomes such as phage where even a single basepair of DNA may have a number of roles in gene circuit regulation. With the advent of Gibson assembly and relatively inexpensive gene synthesis, it should now be possible to use a synthetic biology approach to assemble an entire bacteriophage genome, where one or more of the fragments is chemically synthesised to incorporate the desired change(s). Segments of DNA with overlapping functions can be separated out in the reconfigured phage. This Honours project will involve designing and constructing a complete set of DNA modules which, when assembled together, will generate a functional bacteriophage 186. We will then use this “flat-pack” phage to easily mutate specific regulatory elements in the phage, and study the outcomes at the whole organism level.

4. Structural biology of bacteriophage 186 proteins

Knowing the three dimensional structure of a protein allows us to make very specific predictions about the function of that protein, and the role of specific amino acids. We have a crystal structure of the CI repressor from bacteriophage 186, which forms a wheel-like complex of 14 subunits (4). We have computationally predicted the
structure 186 CII and modelled how it might interact with DNA (5); we are currently trying to confirm the CII structure experimentally. We would like to survey a number of other proteins from bacteriophage 186 to assess their suitability for structure determination. This project would involve cloning, expression, purification and crystallisation trials of some candidate proteins. We would simultaneously develop genetic screens and activity assays for these proteins, in order to isolate mutants, and so validate any structural information obtained.

**Techniques used in these three projects may include:**
- synthetic biology techniques, such as DNA based circuit design, PCR, sequencing, Gibson assembly
- recombineering to make precise DNA sequence changes
- bacterial protein expression and purification
- SDS polyacrylamide gel electrophoresis and western blotting

**Key References**


2. Lun, C., Murchland, I., **Shearwin, K.E.** and Dodd, I.B. (2013) Enhancer-like long-range transcriptional activation by lambda CI-mediated DNA looping. *Proceeding of the National Academy of Sciences USA (PNAS) 110 (8), 2922-2927.*

