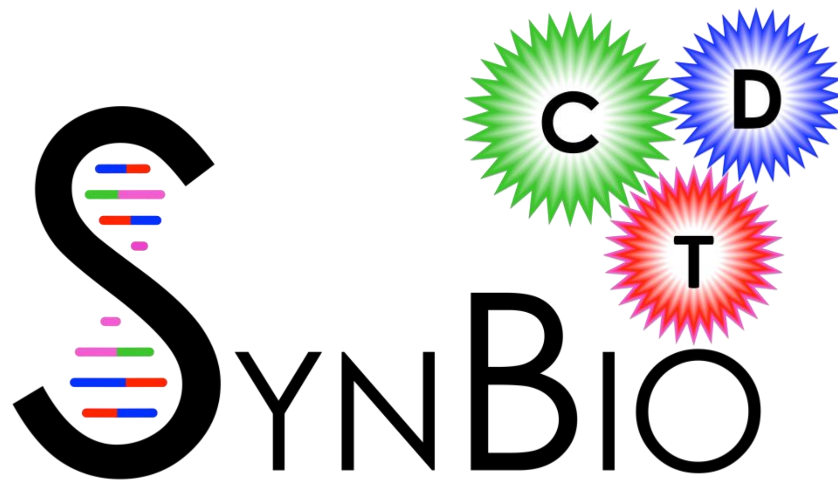
EPSRC & BBSRC Centre for Doctoral
Training in Synthetic Biology



EPSRC & BBSRC
Synthetic Biology
Centre for Doctoral Training

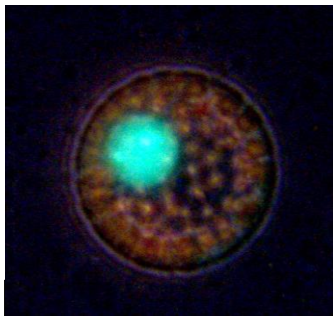
EPSRC Annual Reporting Booklet 2015

Controlling Recombination in Plants using CRISPR

Luke Cartlidge

My project aims to investigate the potential to artificially manipulate patterns of genetic recombination in plants, to provide an advance in crop breeding technology. CRISPR/Cas9 constructs will be used to make targeted alterations in recombination cold-spots in plant genomes, in order to artificially stimulate recombination.

In sexually reproducing species, homologous recombination during meiosis creates genetic variation in offspring. Segments of DNA are exchanged between homologous chromosomes via crossover events. Recombination shuffles genes to create novel allele combinations, and plant breeding relies on this diversity to improve crops. Recombination of alleles is often limited because genes are located in close proximity or in recombination cold-spots. This limits the ability of crop breeders to improve traits or to introgress useful genes from wild relatives (1). The ability to manipulate the rate and locations of recombination events within plant genomes would therefore provide a great breakthrough for crop improvement. Recombination is initiated by the Spo11 protein catalysing double strand breaks in DNA, which is followed by strand invasion, DNA synthesis and cutting to release the homologous chromosomes (2). The sites where Spo11 creates breaks are non-random and are concentrated in genomic hotspots. It has been demonstrated that recombination rate can be increased by randomly creating double strand breaks (3). CRISPR/Cas9 technology allows targeting of a specified DNA sequence, a short RNA guides the Cas9 nuclease to a specific DNA sequence by simple base pairing (4). CRISPR/Cas9 offers an accessible method to make targeted double strand breaks that may trigger recombination. By placing CRISPR/Cas9 downstream of meiosis specific promoters, experiments will be conducted to determine whether recombination rates can be increase and if Spo11 deficient mutants can be rescued. Additionally inactive Cas9 will be expressed as a fusion protein with Spo11, as well as other proteins implicated in recombination, to attempt to encourage recombination. Initial work will be conducted in *Arabidopsis thaliana* . Promising methods will then be selected to trial in wheat. The overarching goal is the ability to manipulate recombination in bread wheat (*Triticum aestivum)* .



tagged Cas9 concentrated in the nucleus

Supervisor(s): Prof Keith Edward
Prof Claire Grierson

References:

(1) Wijnker, E., de Jong, H. Managing meiotic recombination in plant breeding. *Trends Plant Science*. 2008, 13: 640–646.
(2) Grelon M., Vezon D., Gendrot G., Pelletier G. AtSPO11-1 is necessary for efficient meiotic recombination in plants. *The EMBO Journal* . 2001, 20: 589–600.
(3) Sanchez-Moran, E., Santos, J-L., Jones, GH., Franklin, FCH. ASY1 mediates AtDMC1-dependent interhomolog recombination during meiosis in *Arabidopsis* . *Genes & Development* . 2007, 21, 17: 2220-2233.
(4) Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, JA., Charpentier, E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012, 337: 816–21.

Host-circuit interaction modelling in synthetic circuits

Alexander Darlington, University of Warwick

Training programme. At the beginning of the year I attended a range of training courses in maths, computer science, engineering and lab methods used modern synthetic biology. As a cohort we considered the ethical and societal implications of synthetic biology, culminating in the production of a review considering the effect of speculation on synthetic biology. Additionally, I presented both a talk and poster at the inaugural Synthetic Biology CDT miniconference.

Resource allocation in synthetic gene circuits. Cells have a finite cellular resources. Expression of foreign genes require the host cell's machinery. We used a recently published host-circuit model [1] to investigate the behaviour of gene circuits in the context of the host. We showed that the behaviour of even simple circuits can change based on the resources which can be allocated by the cell.

Evolution of gene circuits. The expression of foreign genes *in vivo* generates a burden on their host cell. This results in a selective pressure against the circuit. Using the host-circuit interaction model we modelled the evolution of simple gene circuits. The model qualitatively replicates the findings of Sleight *et al*. [2]. Simple modifications were able to more than doubles evolutionary stability.

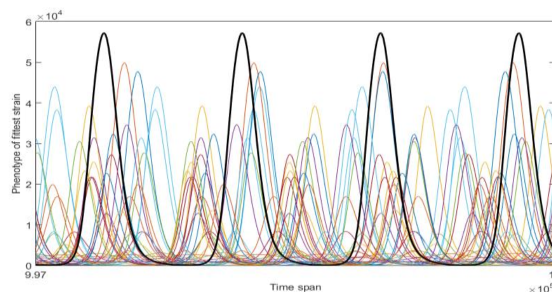
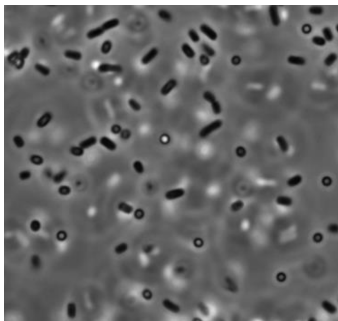


Figure: The oscillations breakdown (from black initial value) due selection against foreign gene expression.



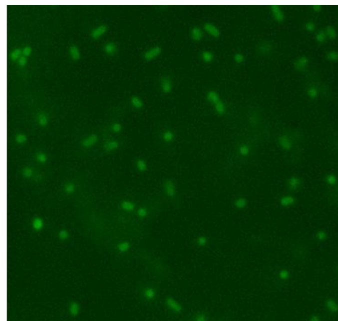


Figure: Phase contrast image of *E. coli* expressing a GFP containing circuit *(left)* Fluorescence microscopy of the same cells showing GFP production *(right)*.

Implementing protein-based circuits *in vivo*. Protein-based systems provide numerous advantages over gene-based systems, such as increased rate of response. We aimed to implement the *S. cerevisiae* osmosensing system into *E. coli*. This system has previously been shown to exhibit ultrasensitivity [3]. We modelled the ability of the certain implementations of the system to show logical behaviours.

Supervisors

Prof. Declan Bates, School of Engineering, University of Warwick

Prof. Orkun Soyer, Warwick Integrative Synthetic Biology Centre, University of Warwick

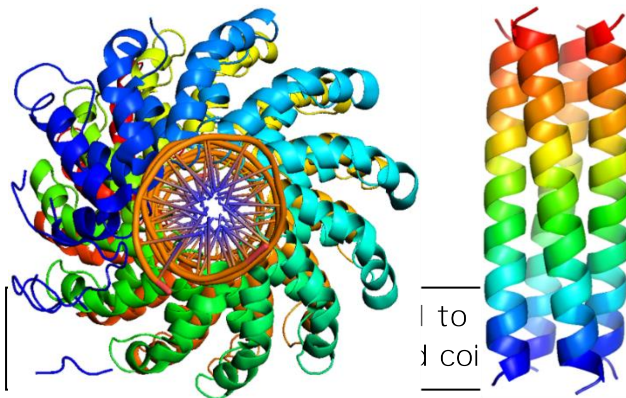
References [1] Weiße *et al.* (2015) Mechanistic links between cellular trade-offs, gene expression, and growth. *Proc. Natl. Acad. Sci., 112(9)*, E1038-E1047. [2] Sleight *et al.* (2010) Designing and engineering evolutionary robust genetic circuits. *J. Biol. Eng. 4(1)* 12 [3] Amin *et al* (2014) Phosphate sink containing two-component signalling systems as tunable threshold devices. *PLoS Comput. Biol. 10* e1003890.

The *de novo* design of coiled coil-based transcriptional regulators

Caitlin Edgell

My first rotation project, which was supervised by Professor Ben Davis, investigated possible mechanisms to control the binding of nanobodies, a type of single domain antibody, to their target antigen. This can be achieved by introducing reversible chemical modifications to their complementarity-determining regions (CDRs). These modifications can act by blocking or promoting binding of the nanobody to its antigen, such that the nanobody may be switched between binding and non-binding states. Previous work in this area has included introducing pH-sensitive [1] and phosphorylated [2] amino acids to the CDRs, while my project investigated introducing redox-sensitive amino acids.

My second rotation project, which was co-supervised by Nigel Savery and Dek Woolfson, has led to my substantial PhD project. I will be attempting to build artificial transcription factors (ATFs) by combining *de novo* designed coiled proteins with transcriptional activator-like effectors (TALEs). The TALEs, which are DNA-binding proteins that can be designed to bind to any desired DNA sequence, can be brought together in various ways by using coiled coils as protein-protein interaction domains. Coiled coils are α-helical proteins that have been widely investigated as protein design targets, and it is (to an extent) possible to control their oligomeric state, helix orientation and partner selection state. Various constructs consisting of these parts may be envisaged. For example, fusing each helix of a heterodimeric coiled coil to a TALE designed to bind a different DNA site may lead to looping in a DNA molecule when the construct is fully assembled. This may be useful, for example, in creating a repressing ATF.



Ultimately, these constructs may find use in synthetic circuit design, providing components that may be designed to have specific characteristics (e.g. DNA-binding specificity) and that are orthogonal to natural host systems due to their artificial nature.

[1] Tawfic *et al* (1994) Protein Eng 7(3) 431-434
[2] Gunnoo *et al* (2014) Protein Nat Commun 30 (5) 4388

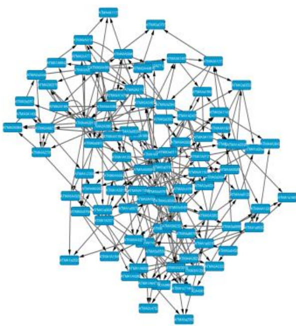
Supervisor(s): Professors Dek Woolfson and Nigel Savery, University of Bristol

Re-wiring signalling pathways to enhance stress tolerance in Arabidopsis

Iulia Gherman

For my first exploratory project under the supervision of Wei Huang and Gail Preston, I presented a functional salicylic acid biosensor in minicells as proof of concept for the use of minicells as chassis for synthetic biology. As the work was very preliminary, I focused on improving minicell purification protocols and testing for GFP activity with fluorescence microscopes and other instruments as a way of quantifying the induction of GFP upon addition of various concentrations of salicylic acid.

For my second project, under the supervision of Katherine Denby and David Wild, I became familiar with the theoretical background behind Causal Structure Inference (CSI), a nonparametric Bayesian learning algorithm that uses Gaussian process regression to identify the relationship between nodes. The aim of this project, and my PhD, is to re-wire transcription factors of defence networks to enhance the Arabidopsis response to stresses such as *Botrytis cinerea* infection. CSI infers networks from dynamic datasets, resulting, in this case, in a network of transcription factors. In doing so, I also simulated gene expression levels of the network containing 96 transcription factors under various perturbations. Additionally, the effects of re-wiring can be simulated through Approximate Bayesian Computation (ABC), following manipulation of the covariance function of CSI.



The directed network composed of transcription factors pertinent to abiotic and biotic stress response i Arabidopsis.

This will lead to the construction of a newer model based on microarray and NanoString data for infected and uninfected wild type and knockout Arabidopsis phenotypes. Subsequently the predictions will be tested out in the laboratory on protoplast systems. The results of the high throughput quantitative analysis will be used to improve and refine the model.

In addition to working on both wet and dry laboratory projects, I also had the chance to explore industry. I gained valuable experience participating in the SynbiCITE 3-day MBA for synthetic biology start-ups and learned about industry opportunities at the SynBioBeta conference in London.

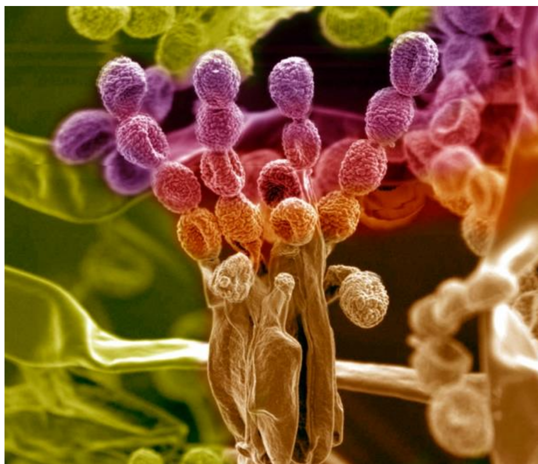
Supervisor(s): Katherine Denby, Declan Bates

Engineering a Synthetic Symbioses between Nitrogen-Fixing Bacteria and Major Crop Species

Kyle C Grant

In my first rotation project I spent ten weeks working in the lab of Professor Philip Poole. My aim was to build and characterise a rhizosphere-specific genetic switch that would switch between two target metabolic pathways when induced.

The target organism was the *Rhizobium sp.* IRBG74, a known rice endophyte. The genetic circuit was constructed to switch nitrogen assimilation from the high affinity enzyme glutamine oxoglutarate aminotransferase (GOGAT) to the low affinity glutamate dehydrogenase (GDH). In doing so it was predicted that the pool of available ammonia within the cell would diffuse out of the community and into the rhizosphere. Thus causing plant promotion effects due to the higher available nitrogen concentration within the rhizosphere. By the end of the rotation both a Taurine and Hesperetin inducible genetic circuit were successfully created and characterised yielding the desired switch characteristic.



From there I spend ten weeks working on enzyme metabolons and kinetics with Professor Sweetlove. I was exposed to several new methods and techniques and a lot of data analysis that was unfamiliar to me. I successfully showed that by immobilising enzymes on carbon nanoparticles, it is potentially possible to induce a level of metabolite channelling between enzymes in a cascade.

For my DPhil project I am continuing my first ten week rotation and also adding an element of essential modelling with Professor Sweetlove to try and characterise and elucidate future targets for engineering in the bacterium *Azorhizobium caulinodans* . This free-living, nitrogen-fixing plant endophyte is the main target in my research as it would be applicable to a wide variety of plant species and so on and so forth.

Supervisor(s): Professors Philip Poole and Lee Sweetlove

Bacteriophage Engineering

Aurelija Grigonyte

Conferences attended during the training period

During the training period in the year one I had an opportunity to attend SynbiCITE 3-Day MBA course in synthetic biology where I learnt the basics about biotech startups, including synbio related patenting process and how to write a business plan for a startup when pitching to investors. In addition, I attended Synbiobeta London 2015 (http://synbiobeta.com/conferences/synbiobeta-london-2015/). Here I had even a greater exposure to synbio related biotech as well as the newest technologies being developed in the field of synthetic biology.

Short project 1

A m Z] f g h f c h U h] c b d f c ^ Y W h k U g University of Warwick. D f c Z > U f U a] ` `

The main goal of bacteriophage therapy is to be able to specifically target any given pathogen. In my first rotation project I have applied rational engineering to enable bacteriophage T7, which normally infects *E. coli* via LPS, to infect *E. coli* via Ail and OmpF outer membrane proteins, which are found on *Yersinia pestis* outer membrane. I have designed a chimeric version of bacteriophage T7 tail fiber protein fused with receptor binding domain of Yep-phi bacteriophage (which targets *Yersinia pestis*). In addition a construct for Ail and OmpF heterologous expression in *E.coli* was designed. CRISPR-Cas9 system was applied to select for the chimeric bacteriophage, this was achieved by creating three guide RNAs that target and cleave only a region in wild type T7. Successful design of Ail-OmpF outer membrane proteins as well as design and insertion of chimeric T7 construct into a vector construct were achieved. Three guide RNAs for chimeric T7 selection were constructed and tested using plaque assays.
During this rotation I have learn the basic techniques for working with bacteriophages including T7 purification and plaque assays. I have also written a review as a second author on 'Approaches for targeting pathogens with engineered phages', which has been recently submitted to the journal of Integrative biology. I have also started a paper on use of CRISPR-Cas9 methodologies for bacteriophage engineering. My PhD project will be based on the research carried out in Prof Jaramillo's lab.

Supervisor(s): Prof Alfonso Jarami

Short Project 2

My second research project was carried out at Prof Collins lab at Massachusetts Institute of Technology.

Cholera is water born disease caused by *V. cholerae* . According to the 2012 – 2013 data collected by World Health Organisation cholera outbreaks were found in the central and south Africa as well as most parts of India. New approaches for sensing and targeting the pathogen are urgently needed. My second rotation project focused on engineering *Lactococcus lactis* to target *V. cholerae* and to induce its death. Here two *V. cholerae* 'killing' strategies have been explored. The first strategy focuses on combining antimicrobial peptides (AMPs) delivery with fragments of bacteriophage tail fiber proteins to increase target-binding specificity. The second explores a possibility of assembling and placing the entire phage into *L. lactis*. Both methods could be potentially coupled with a synthetic hybrid receptor (which was developed by Collins' group) able to sense *V. cholerae* signaling molecules. Six tail fiber protein variants from two bacteriophages

VP3 and ICP3 (which specifically target *V. cholera*) were designed and constructed using PCR. The final result showed successful expression of all protein variants, two of which were able to bind to *V. cholera* . The next step is to fuse two of the variants with *V. cholera* specific AMPs and test for increased *V. cholera* targeting/killing.
During this project I gained confidence using multiple techniques including PCR, western blotting as well as FACS (fluorescence-activated cell sorting).

Reengineering the red blood cell

Joe Hawksworth

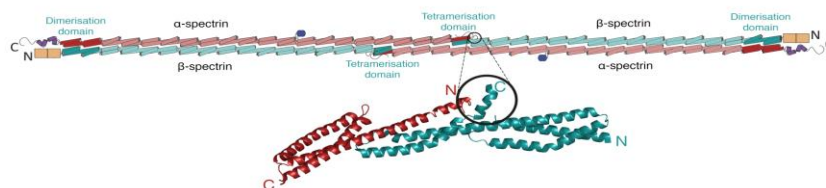
Supervisor: Ashley Toye

The erythrocyte (red blood cell) has a well-characterised life cycle and attractive biophysical properties that make it an ideal candidate for reengineering as a drug delivery vehicle or as a circulating bioreactor for therapeutic use. In order to repurpose the erythrocyte, an efficient and precise genome engineering strategy is required. We are currently working on two proof-of-principle projects which will enable us to knock down, alter or induce gene expression permanently in a newly developed immortalized erythroblast cell line. These projects will generate useful cell lines, whilst laying the foundations for future work - engineering novel functionalities into erythrocytes.

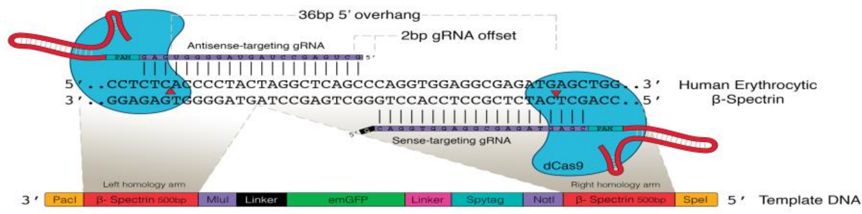
The first project uses CRISPR-Cas9 nickases to introduce a point mutation into the transcription factor KLF1 to mimic the disease phenotype responsible for the clinical condition dyserythropoietic anemia (CDA) type IV. Transcription factors are essential for the maturation of erythrocyte precursor cells. KLF1 encodes Erythroid-Krüppel-like factor which plays an essential role in erythropoiesis, participating in the switch from embryonic γ to adult β globin. This project will demonstrate the ability of the CRISPR system to induce point mutations in an immortalized erythroblast cell line and generate a novel cellular model for studying the CDA type IV phenotype.

The second project aims to create an immortalized erythroblast cell line containing a spectrin cytoskeleton tagged with a fluorescent protein and Spytag. The cytoskeleton is an essential component of the erythrocyte membrane which confers essential properties to red cell functioning such as its mechanical strength and deformability. This project will demonstrate the ability of the CRISPR-Cas9 nickase system to insert large genetic sequences into an immortalized erythroblast cell line, produce a useful study tool for monitoring cytoskeletal assembly and remodeling, and enable novel proteins to be tethered to the cytoskeleton for use in future applications.

These projects are now at the exciting stage of screening for mutations. If successful, we will have developed a cellular model for disease and a useful cell line for studying the erythrocyte membrane structure. The foundational work presented here will enable future applications in red blood cell engineering, including: the reactivation of arrays of genes in erythroblasts which are currently switched off during erythropoiesis. the production of cellular models for human erythroid disorders, systems for the detection of disease, the ability to tether novel proteins to the cytoskeleton, and activation of new metabolic pathways for chemical synthesis.



Choosing a target site for GFP tagging in the erythroid spectrin cytoskeleton. A schematic shows a spectrin heterodimer composed of α (red) and β (blue) spectrin. Dimerisation begins at the α and β dimerisation domains, head-to-head interactions drive tetramerization which stems from the tetramerization domains. A crystal structure of the α (red) and β (blue) spectrin tetramerization domain is shown below. A short flexible tail (encircled) at the β C-terminal domain which will be tagged with GFP and Spytag. Figure adapted from Ipsaro *et al.* (2010).



CRISPR-Cas9 nickase strategy for spectrin tagging. Two Cas9 nickases generate nicks in complementary DNA strands. Single nicks are repaired by high fidelity endogenous mechanisms. Double nicks, occurring in close proximity, can result in double strand breaks which are repaired by homologous recombination using template DNA. The result is the incorporation of template sequence into genomic DNA at a region specified by the template DNA homologous arms.

References

Ipsaro, J. J., Harper, S. L., Messick, T. E., Marmorstein, R., & Mondragón, A. E. Al. (2010). Crystal structure and functional interpretation of the erythocyte spectrin tetramerization domain complex. Blood, 115(23), 4843–52. http://doi.org/10.1182/blood-2010-01-261396.

Coiled-coil directed synthetic protein assemblies

Juntai Liu

Synthetic biology is about applying engineering principle to biolog system, in which most efforts were on engineering at genetic le However, my interest is the engineering at protein level. The goa PhD project is to develop new types of link coupling photovoltaic proteins in controllable means, based on the recent development coiled-coil [1] and spy system [2]. We also aim to explore novel protein assemblies with those synthetic linkers with computation aids.

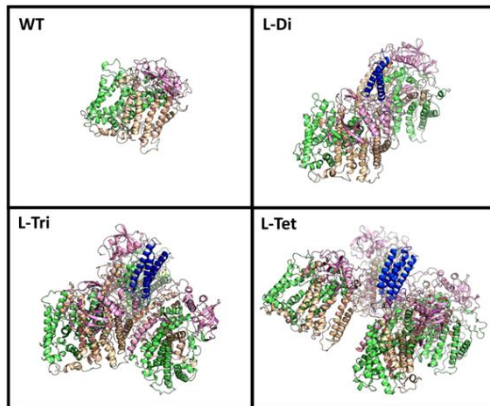


Figure 1 snapshot- of helices endowing specific interactions. The upper left showed structure of wild ty (WT) bacteria RC; upper right showed dimerization of RC by coiled-coil; lower left showed trimer of RC mediated by homo trimeric helices; lower right showe tetramer of RC formed by helices with homo-tetrameric specificity.

Dr. Jones' group has demonstrat reaction centre (RC) oligomers using coiled-coil motifs [3]. Molecular dynamic simulation of this work was shown in fig. 1 as RC formed specific oligomers with short helices created by Prof. Woolfso initial plan is to couple spy-system developed in Oxford with coiled-coil for covalent connecting RC in stable oligomer-states in specific fashion and further assemble those RCs into array-like configuration. Another attempt is to couple plant light harvesting complex (LHII) with bacterial RC in vitro with a switchable manner using coiled coils. In nature, bacteria RC has an absorption gap in the range of because of the adaptation to environment, while plant LHCII complexes provide good coverage over this range. Therefore, coupling of RC and LHCII will yield a photovoltaic system with no obvious energy waste, which could be used in RC based solar cell with enhanced quantum efficiency. To the end, I intend to extend the designed linkers to other membrane proteins for generation of more complicat protein assemblies that can carry out sophisticated chains of reactions.

Supervisor(s): Dr. Mike Jones

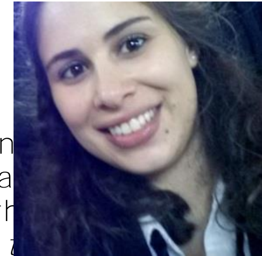
Co-supervisor: Prof. Ian collinson

[1] Woolfson DN, Bartlett GJ, Burton AJ, Heal JW, Niitsu A, Thomson AR, et al. De novo protein design: how do we expand into the universe of possible protein structures? Current opinion in structural biology 2015;33:16-26

[2] Zakeri B, Fierer JO, Celik E, Chittock EC, Schwarz-Linek U, Moy VT, et al. Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. Proceedings of the National Academy of Sciences of the United States of America 2012;109:E690-7

[3] Fletcher JM, Harniman RL, Barnes FRH, Boyle AL, Collins A, Mantell J, et al. Self-Assembling Cages from Coiled-Coil Peptide Modules. SCIENCE 2012;340.

Rational Engineering of Synthetic Microbial Communities



Andrea Martinez Vernon

As part of the Synthetic Biology Centre for Doctoral Trainin semester comprised of the taught component of our training a taught modules. Although I found all modules quite interesting, th that helped expand my skills and knowledge the most were *Introduction t Synthetic and Systems Biology*, *Introduction to Programming*, *Synthetic Biology Across Scales* and *Systems and Control Theory*. Not only did I learn more about programming and mathematical modelling, I was introduced to the ideas, concepts and tools behind rational engineering for bio

= ˙ WUff]YX ˙ ci h ˙ am ˙ Z *3D printing of bacteria* under the supervision of Profs. Hagan Bayley and Kevin Foster (Oxford). Spatial organisation is a crucial aspect of many bacterial functions and interactions, but their study at a microscopic level remains practically challengin The project consisted in developing a new technology that consists of the generation and placement of lipid-stabilised droplets containing bacteria into 3D arrangements to achieve a 3D spatial organisation platform. We demonstrated that our 3D printing technology is robust, comparable to traditiona culturing techniques with a 3D compartment and allows a range of behaviours to emerge. It is foreseeable that this 3D printing droplet technology can be applied to develop disease models and the production of high value chemicals, besides a wide range of other basic research questions.

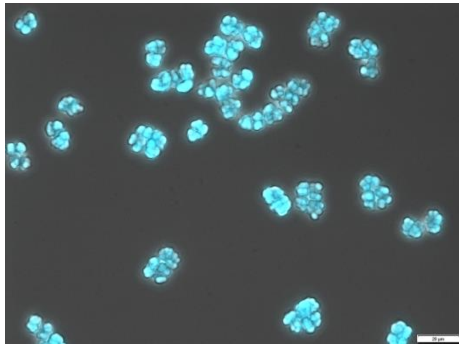


Figure 1 Characteristic growth of *M. barkeri* aggregates and its native autofluorescence [1] shown in the phase contrast and cy fluorescent channel composite image. bar 20 μm.

My ¥Wc bX ˙ dfc ^YWh ˙ *Rational Engineering of Synthetic Microbial Communities* kUg ˙ WUff]YX ˙ i b supervision of Prof. Orkun Soyer (University of Warwick). Synthetic microbial communities have been used to either increase the production yields in industrial bioreactors or to use them as a model system to understand the underlying interactions, motifs and other dynamics. However, our ability to rationally engineer synthetic microbial communities is limited by a lack of experimental and theoretical tools. In this project we took the first step towards developing such tools by using *E. coli* / *M. barkeri* anaerobic co-culture as a model system. To overcome practical limitations of anaerobic culturing monitor, we developed a measure device that uses absorbance df]bW]d`Yg ˙ cZ ˙ 6YYfĐg ˙ @Ukž ˙ h\Y ˙ 7cbh] measurement device. Using the device, we monitored the growth of *E. coli* and *M. barkeri* (Figure 1) in monocultures to extract parameters of interest. We then incorporated these paramete mathematical model to investigate the dynamics of the system. This information could be use understand the environmental and initial condition requirements to successfully engineer a synthetic microbial community.

Following on from my second rotation project, my PhD will focus on further developing tools engineer synthetic microbial communities. Under the supervision of Prof. Orkun Soyer and Dr. M Polin, we will look at coupling electrical interactions with microbial metabolism and physiology, in a similar way to microbial fuel cells, for synthetic biology applications. By manipulating either single cells or microbial communities, a range of applications could be developed, including the control production of high value chemicals.

REFERENCES : [1] Doddema HJ, Vogels GD. Appl Environ Microbiol [Internet]. 1978 Nov [cited 2015 Jul 16];36(5):752

Supervisor(s): Prof. Orkun Soyer
Dr. Marco Polin

Multi-cellular Circuits

James Scott-Brown

Is it better for a synthetic circuit to be implemented within a population of identical cells, or split across two types of cells that can communicate with each other chemically?

My main thesis work will focus on the question of how, and under what circumstances, splitting a circuit across two cells can improve its performance.

In my first year at the DTC I did two lab rotations:

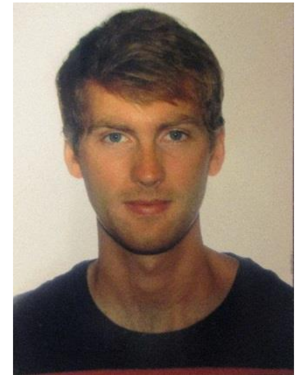
'External Control of Gene Expression', supervised by Prof. Mario di Bernardo (Department of Engineeirng Mathematics, University of Bristol). This involved designing and simulating controllers that would be implemented in software and control living cells in a microfluidic device.

'Applications of cell-cell communication to Synthetic Biology', supervised by Prof. Antonis Papachristodoulou (Department of Engineering Science, University of Oxford). This involved modelling and comparing synthetic oscillator circuits based on quorum sensing systems.

Supervisor(s): Antonis Papachristodoulou and Thomas Prescott

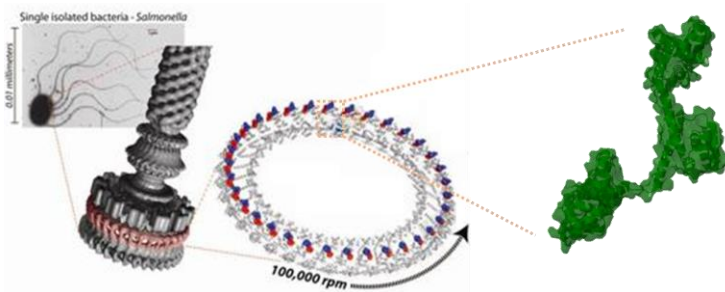
A Look at the Bacterial Flagellar Motor's Torque-Generating Protein

The bacterial flagellar motor is one of the most complex protein machines found in nature and how it self-assembles and produces force are very much open questions. Recent work has sought to study its constituent parts *in vitro*, with much attention being focussed on FliG, a protein which forms the motor's torque-generating ring.



Joel Spratt

To study how the ring forms, one approach has looked to employ a DNA template to direct the *in vitro* formation of the FliG ring, but ensuring a well-characterized and stable connection between the protein and the template has proved to be a weak point in this design. In a recent short project, I implemented a new design, wherein FliG was covalently coupled to an oligonucleotide handle in a site-specific manner, thereby allowing it to bind to complementary strands on the scaffold in a well-defined fashion.



The FliG Protein Forms the Torque-Generating Ring of the Bacterial Flagellar Motor

To do this, I used a novel technique pioneered by Kurt Gothelf[1] which, along with the protein and the oligonucleotide to label it, incorporates another strand of DNA which directs the conjugation reaction to a specific location on the surface of the protein. Thus, using this technique, it was possible to homogeneously label FliG in a region separate from that which is believed to engage in the yet-mysterious ring-forming interactions.

Going forward, this new system with FliG covalently attached to an oligonucleotide label will be used to study how individual FliG monomers come together to form a ring capable of generating the torque necessary to spin a bacterium's flagella. Using a bit of classic biochemistry and some cutting-edge microscopic techniques, the assembly process will be studied and visualized *in vitro* and compared to the current theories[2] of ring-formation. It is my hope that building from FliG, investigations of a larger scope incorporating further components of the motor can then be pursued

Supervisors: Dr. Richard Berry and Prof. Andrew Turberfield

1 Rosen, Christian B., KodalAnne, L. B., Nielsen, Jesper S., Schaffert, David H., Scavenius, Carsten, Okholm, Anders H., . . . Gothelf, Kurt V. Template-directed covalent conjugation of DNA to native antibodies, transferrin and other metal-binding proteins. Nat Chem, 6(9), 804-809. (2014).

2 Lee, Lawrence K., Ginsburg, Michael A., Crovace, Claudia, Donohoe, Mhairi, & Stock, Daniela. Structure of the torque ring of the flagellar motor and the molecular basis for rotational switching. *Nature, 466*(7309), 996-1000. (2010).