WISB
WARWICK INTEGRATIVE SYNTHETIC BIOLOGY CENTRE

2nd Annual Review meeting

June 6-8, 2017

BBSRC bioscience for the future

EPSRC Engineering and Physical Sciences Research Council

WARWICK THE UNIVERSITY OF WARWICK

WISB SAB Visit June 6-8, 2017

June 6

Check in to **Arden Conference Centre** on University of Warwick campus

19.00	Dinner in Scarman Conference Centre, location to be advised

June 7

9.00 – 17.50	Presentations from WISB PDRAs and RCDFs The Oculus, room OC0.04
12.45 -14.15	Posters from SynBioCDT students Area outside Oculus lecture theatre.
19.00	Dinner in Lakeview Restaurant, Scarman Conference Centre

June 8

8.30 – 9.00	Tour of WISB Research Technology Facility (meet in Reception of Life Sciences building)
9.00 – 12.00	SAB review meeting, BSR6 Life Sciences

Research presentations June 7, 2017 — The Oculus, OC0.04

9.00	**The Provost, Chris Ennew**	**Welcome**
9.05	**John McCarthy**	**Introduction and WISB Overview**
9.25	**Sarah Bennett**	WISB Research Technology Facility
9.35	**Graham Jones**	WISB Computer Cluster
9.40	**Nick Lee** **Lauren Swiney (WISB RCDF)**	**ELSA (Theme 5) Overview**
10.05	**Declan Bates**	**Theme 1 Overview**
10.15	**Byron Carpenter (WISB RCDF)**	Engineering GPCR signalling pathways
10.30	**Nathan Archer**	Transcriptional noise in mammalian cells
10.45	**Jamie Luo**	Filamentation enables bacteria to spread more efficiently
11.00 – 11.30	**Break**	
11.30	**Estelle Dacheux**	Optimising synthetic circuitry functionality at the translation level
11.45	**Manish Kushwaha**	A genetically-encoded *in vivo* computing platform to play Tic-Tac-Toe
12.00	**Alexander Nikitin**	Cellular decision making by genetic logic gates
12.15	**Xiang Meng**	Rate control and noise in yeast gene expression systems
12.30	**Emmanuel de los Santos (WISB RCDF)**	Tools for the development of microbial cell factories
12.45 – 14.15	**Lunch and Posters [Presented by SynBio CDT students]**	
14.15	**Alfonso Jaramillo**	**iGEM Overview (2014-2017)**
14.25	**Christophe Corre**	**Theme 2 Overview**
14.35	**Binuraj Menon (WISB RCDF)**	Novel tools for bio-orthogonal chemistry via chemo-enzymatic reactions
14.50	**Chuan Huang**	An efficient platform for entire gene cluster cloning and refactoring
15.05	**Miriam Rodriguez Garcia**	An *E.coli* inducible expression system based on *Streptomyces* signalling molecules

15.20	**Orkun Soyer**	**Theme 3 Overview**
15.30	**Eleanor Jameson (WISB RCDF)**	Application of phage therapy to treat microbiome-related disease
15.45	**Christian Zerfass**	Synthetic microbial co-culture to recycle Mn/MnO
16.00	**James Stratford**	Towards an electrophysiological interface for synthetic biology
16.15 – 16.40	**Break**	
16.40	**Vardis Ntoukakis**	**Theme 4 Overview**
16.50	**Silke Lehmann**	Engineering novel activation of plant signalling pathways
17.05	**Ana Domingues-Ferreras** **Wei-Jie Huang**	Exploring microbial toolkits for manipulation of plant signalling
17.20	**Mathias Foo**	Perturbation-mitigation strategies in plants
17.35	**John McCarthy**	Concluding comments/discussion
17.50	**Programme concludes**	

Abstracts

Engineering G protein-coupled receptor signalling pathways

Byron Carpenter

G protein-coupled receptors (GPCRs) are a diverse family of eukaryotic cell surface receptors that recognise thousands of different extracellular signalling molecules, including hormones and neurotransmitters. GPCRs regulate a wide variety of cellular responses in physiological and pathophysiological states, and are the single largest therapeutic target for the pharmaceutical industry. GPCRs elicit cellular responses by stimulating cytoplasmic signalling through heterotrimeric G proteins. These signalling pathways have evolved a high degree of complexity, however, in their simplest form, signal transduction from GPCRs to G proteins is mediated solely by conformational changes. Since no additional cellular machinery is required for signal propagation, this pathway represents an interesting template for the development of a novel synthetic signalling system. We are using protein engineering approaches to develop a simplified GPCR signalling pathway that will allow non-transcriptional regulation of cellular responses under the tight control of extracellular ligands.

Carpenter, B. & Tate, C.G. Active state structures of G protein-coupled receptors highlight the similarities and differences in the G protein and arrestin coupling interfaces. *Current opinions in structural biology* (2017) 45, 124-132.

Carpenter, B. & Tate, C.G. Expression, purification and crystallisation of the adenosine A2A receptor bound to an engineered mini G protein. *Bio-protocol*, (2017) 7 (8): e2234.

Carpenter, B. & Tate, C.G. Expression and purification of mini G proteins from *Escherichia coli*. *Bio-protocol* (2017) 7 (8): e2235.

Nehmé, R., Carpenter, B., Singhal, A., Strege, A., Edwards, P.C., White, C.F., Du, H., Grisshammer, R. & Tate, C.G. Mini-G proteins: novel tools for studying GPCRs in their active conformation. *PLoS One* (2017) 12 (4): e0175642.

Carpenter, B., Nehmé, R., Warne, T., Leslie, A.G. & Tate, C.G. Structure of the adenosine A_{2A} receptor bound to an engineered G protein. *Nature* (2016) 536, 104-7.

Carpenter, B. & Tate, C.G. Engineering a minimal G protein to facilitate crystallisation of G protein-coupled receptors in their active conformation. *Protein engineering, design and selection* (2016) 29 (12), 583-594.

Transcriptional noise in mammalian cells

Nathan Archer

The understanding of cell morphology, developmental decisions and disease have been significantly furthered by the advancement of single cell technologies and “multiomics”. Biological and technical noise hamper the use of this information in synthetic biology and other fields. Improvements to the understanding of these sources of noise, and advances in the technologies used to tackle them will enable improved genetic circuit design to exploit and control this phenomenon as well as a more detailed view of the underlying biological mechanisms.

Here we define and unravel some of the technical noise currently inherent in the study of very low input RNA, particularly in single-cell RNA sequencing – which involves inherently stochastic processes - showing that RNA-seq data coverage lacks uniformity across the transcript; delivering the first approach that allows *in silico* disassembly and reverse engineering of sample preparation; and offering bias correction, improved quantification, and new insights into scRNA-seq methods

development. Ongoing work seeks to exploit this improved knowledge of the technical noise to tackle biological questions related to stochastic transcriptional noise in mammalian cells.

Archer, N., Walsh, M.D., Shahrezaei, V., and Hebenstreit, D. Modeling Enzyme Processivity Reveals that RNA-Seq Libraries Are Biased in Characteristic and Correctable Ways. *Cell Systems* (2016) 3(5): 467-479 DOI:10.1016/j.cels.2016.10.012

Haussmann, I.U., Bodi, Z., Sanchez-Moran, E., Mongan, N.P., Archer, N., Fray, R.G., Soller, M. m6A potentiates Sxl alternative pre-mRNA splicing for robust Drosophila sex determination. *Nature* (2016) 540: 301–304 DOI:10.1038/nature20577

Filamentation enables bacterial colonies to spread more efficiently

Jamie Luo

Long chained filament formation is a characteristic feature of many biofilms, however the effect of filamentous cells on biofilm spread is an open question. To tackle this question we built a simulation of biofilm spread that models the formation of long filaments. By controlling the rate at which filaments separate into smaller filaments, we investigate directly the effects of filamentation on the spread of the biofilm. Using this simulation and experimental comparisons, we demonstrate that filamentation allows colonies to cover more area using less biomass, thus improving the efficiency with which biofilms spread. Guided by the model and validating its predictions experimentally using the model organism *B. subtilis*, we establish in principle procedures to guide and control the spread of these biofilms by the manipulation of the external environment.

Luo, J.X., Sirec, T., Asally, M., and Kalvala, S. Filamentation Grants Bacterial Colonies the Ability to Spread More Efficiently, submitted to PLoS Computational Biology

Optimising synthetic circuitry functionality at the translation level

Estelle Dacheux

Yeast is an important host for synthetic circuitry. Up to now, such circuitry has been predominantly transcriptional, comprising components including promoters, activators/ repressors, terminators etc. We have started work on a strategy and a toolset for building translational circuitry; this work comprises ***two*** main areas. ***1. Translational noise.*** Gene expression noise undermines the predictability of synthetic circuits. Previous research has focused on transcriptional mechanisms of noise generation, whereby the role of translation has been inferred or ignored. In a recent detailed study*, we have found that two types of structural element, a stem-loop and a poly(G) motif, generate noise at the translational level when inserted into a mRNA 5'UTR. This has broad significance, since these elements are known to modulate the expression of a diversity of eukaryotic genes. This new knowledge will help guide the design of predictable and reliable synthetic circuitry. ***2. Next-generation synthetic circuitry.*** In an attempt to create a new layer of synthetic circuitry components, and thereby to provide alternatives to the generally slow response times and often limited dynamic range of transcriptionally-regulated synthetic circuitries, we are building new tools based on translational regulators (e.g. Iron Regulatory Protein), specific protein-RNA interactions, protein degrons and signalling pathways.

* Dacheux, E., Malys, N., Meng, X., Ramachandran, V., Mendes, P. and McCarthy, JEG. Translation initiation events on structured eukaryotic mRNAs generate gene expression noise. *Nucleic Acids Res.* (2017) doi: 10.1093/nar/gkx430.

A genetically-encoded in vivo computing platform to play Tic-Tac-Toe

Manish Kushwaha

We propose a biological computation paradigm based on parallel computing across cellular consortia. Parallel computation requires more data intensive communication channels, which is not possible with diffusible small molecules as done before. We use DNA as signalling molecule, which is packaged into a phagemid. We have developed a genetic platform relying on phagemid particles and regulatory circuits based on CRISPR single guide RNAs (sgRNAs) to encode logic operations in bacterial populations. This relies on a carefully fine-tuned system of orthogonal sgRNAs, dCas9, inteins, split T7 RNA polymerase, M13 phagemid and fluorescent reporters. Boolean functions are implemented by decomposing them into elementary logic gates encoded in different cells, creating consortia of bacterial strains. Parallel computing offers distinct advantages of division of labour, insulation, modularity, scalability, diversity, and fault tolerance. Our computing paradigm is demonstrated by implementing in live bacteria an algorithm for playing the game of Tic-Tac-Toe. This system is composed of 9 cell types that report user moves through a red fluorescence signal, and another 24 cell types that process user moves and respond to indicate the counter-move of the bacterial automaton through a green fluorescence signal.

Kushwaha, M., Rostain, W., Prakash, S., Duncan, J.N., and Jaramillo, A. Using RNA as molecular code for programming cellular function. *ACS synthetic biology* (2016) 5 (8), 795-809. DOI: 10.1021/acssynbio.5b00297

Rodrigo, G., Prakash, S., Cordero, T., Kushwaha, M., and Jaramillo, A. Functionalization of an antisense small RNA. *Journal of molecular biology* (2016) 428 (5), 889-892. DOI: 10.1016/j.jmb.2015.12.022

Cellular decision making by genetic logic gates

Alexander Nikitin

Potential applications of engineered biosensory systems could be very broad, for examples for identification of specific cancer cells or/and detection of heavy metals like lead and mercury in the environment. In many cases, the biosensors must produce logical computations over their multiple inputs. However, modern biosensors have a limited robustness to noise and low accuracy in computations. In addition, a computation module based on gene expression with combinatorial promoters cannot produce true logical operations. Indeed, instead of a binary response, the output of the computation module takes the form of multiple levels, the total number of which depends on the number of different combinations of digital inputs. Another problem stems from the non-digital nature of the input signals. Even an ideal computation module is not able to produce correct logical operations over its non-digital inputs. Therefore, there is a risk of misinterpretation of the biosensor response. We introduce an improved design of a biosensor that holds bistable analogue-to-digital converters in its input circuits, and a double-inversion module that converts the response of the computation module to logical (binary) levels. By computer simulations we show that the improved design leads to significantly improved biosensor characteristics.

Nikitin, A.P., Ojalvo, J.G., and Stocks, N.G., Cellular Decision Making by Genetic Logic Gates, In preparation

Rate control and noise in yeast gene expression systems

Xiang Meng

If we are to incorporate predictability and robustness into synthetic circuitry, we need to elucidate the principles governing the relationship between control and stochasticity in the gene expression machineries of the cell. For example, noise in the synthesis of components of the translation machinery will not only exert a direct impact on organism fitness, adaptability and evolution but also on the stochasticity of production of all other proteins in the cell. Protein synthesis is estimated to use approximately 76% of the energy budget in a yeast cell, and thus noise management in this machinery is of critical importance to both organism competitiveness and to the success of system engineering projects in yeast. In order to enhance understanding of the relationship between rate control and noise, we have focused on a translation machinery component called eIF4G that acts as a 'scaffold' for pathway-critical complexes and also exerts strong control over protein synthesis*. We have discovered that engineered changes in eIF4G abundance amplify noise, demonstrating that minimum stochasticity coincides with physiological abundance of this factor. In further work, we have explored how the growth environment affects gene expression noise, and have characterised the relationship between noise and the available carbon source.

* Meng, X., Firczuk, H., Pietroni, P., Westbrook, R., Dacheux, E., Mendes, P. and McCarthy, JEG. Minimum-noise production of translation factor eIF4G maps to a mechanistically determined optimal rate control window for protein synthesis. *Nucleic Acids Res.* (2016) doi: 10.1093/nar/gkw1194.

Tools for the development of microbial cell factories

Emmanuel de los Santos

I have been responsible for the bioinformatics needs of the Theme 2 research. This involves the assembly and functional annotation of bacterial genomes and organizing the large amount of bacterial genomic sequence information to identify biosynthetic gene clusters of interest for experimental characterization. To identify gene clusters of interest, we look for functional modules that are responsible for the biosynthesis of chemical moieties of interest. These moieties are of interest for their applications or because of the chemistry involved in their biosynthesis. Clusters of interest are determined, first by conducting genome wide searches for the different components necessary for the biosynthesis of the chemical moiety. Regions of the DNA that contain all the functional genes required within close physical proximity are identified for further analysis and functional annotation. I used this method to identify putative biosynthetic gene clusters that produce biosynthetic gene clusters for polyketide alkaloids and to predict the gene clusters responsible for the production of known polyketide alkaloids and other natural products of interest to the PIs in the group.

Awodi, U. R., Ronan, J. L., Masschelein, J., de los Santos, E.L.C., Challis, G.L. Thioester reduction and aldehyde transamination are universal steps in actinobacterial polyketide alkaloid biosynthesis. *Chemical Science* (2017) 8 (1), pp. 411-415

Zhou, S., Alkhalaf, L.M., de los Santos, E.L.C., Challis, G.L. Mechanistic insights into class B radical-S-adenosylmethionine methylases : ubiquitous tailoring enzymes in natural product biosynthesis. *Current Opinion in Chemical Biology* (2016) 35, pp. 73-79

My ***RCDF*** research is focused on natural product biosynthesis and the ways which bacteria can create complex small molecules. To this end, I plan to focus on designing tools, both computational and experimental, to enable the rapid engineering of biosynthetic gene circuits. On the computational side, I'm focused on designing frameworks and workflows to analyze the ever-growing collection of genomic data. I'm developing software that identifies regions in the DNA that

contain functional units that produce chemical moieties of interest based on biosynthetic hypotheses. This can be used by non-programmers to identify clusters they are interested in, or be used to mine genomes for functional units that can be packaged into synthetic parts. In collaboration with other universities, I'm also contributing to software used to functionally annotate and dereplicate biosynthetic gene clusters (antiSMASH and BiG-SCAPE). The experimental tools I'm developing focus on packaging the functional units identified through computational analysis for use in synthetic gene circuits. I plan to do this through the use of synthetic docking domains and *in vitro* transcription-translation systems. I plan to develop a workflow where one can rapidly identify, package, and test different biosynthetic modules to ensure that a synthetic circuit is producing the desired small molecule before transferring the system to a host organism and optimizing its production.

de los Santos, E.L.C., Challis, G.L. clusterTools : proximity searches for functional elements to identify putative biosynthetic gene clusters (2017) (pre-print, not peer-reviewed)

Kai, B., Wolf, T, Chevrette, M.G., Lu, X, Schwalen, C.J., Kautsar, S.A., Suarez Duran, H.G., de los Santos, E.L.C., Kim, H.U., Nave, M., Dickschat, J.S., Mitchell, D.A., Shelest, Ekaterina, Breitling, Rainer, Takano, Eriko, Lee, Sang Yup, Weber, Tilmann, Medema, Marnix H... antiSMASH 4.0: improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Research* (2017)

Novel tools for bio-orthogonal chemistry via chemo-enzymatic reactions

Binuraj Menon

Bio-orthogonal chemistry that enables selective modification of a specific moiety in a complex biomolecule provides an exquisite and indispensable selectivity, which is a requisite for most of the *de novo* synthesis. In this post genomic era, it has greatly enhanced our understanding of biomolecular dynamics and functions. This fast emerging interdisciplinary research area provides not only tools for derivatising, conjugating and labeling biological targets, but also involved in synthesizing new non-natural synthetic building blocks for the development of therapeutic peptides and artificial enzymes. These tools are crucial for synthetic biology applications, and for bioengineering and biomaterial research. Here, we are extending these tools via feasible chemo-enzymatic reactions that involve a number of recently explored and engineered halogenases and methyl transferases that perform site-specific halogenation reactions over proteino and non-proteinogenic amino acids. Currently, only chemo-enzymatic approaches could provide reliable and facile methods for the synthesis of these halogenated compounds, which are important in all sectors of chemical and pharmaceutical industry. It was demonstrated that by combining halogenases with Pd-catalysed cross-coupling chemistry, it is possible to create several functionally and chemically divergent amino acid analogous that could expand the available amino acid chemical space. The RCDF research plan is to develop and investigate these chemo-enzymatic tools to synthesize a number of important non-natural derivatives and synthons by exploiting various biosynthetic pathways.

Latham, J., Brandenburger, E., Shepherd, S., Menon, B. R. K., Micklefield, J. Development of Halogenase Enzymes for Use in Synthesis. *Chemical Reviews* (2017) DOI : 10.1021/acs.chemrev.7b00032

Menon, B. R. K., Latham, J., Dunstan, M.S., Brandenburger, E., Klemstein, U., Leys, D., Karthikeyan, C., Greaney, M., Shepherd, S., & Micklefield, J. Structure and biocatalytic scope of thermophilic flavin-dependent halogenase and flavin reductase enzymes. *Org Biomol Chem* (2016) DOI: 10.1039/C6OB01775D

Ortmayer, M., Lafite, P., Menon, B. R. K., Tralau, T., Fisher, K., Denkhous, L., Scrutton, N. S., Rigby, S. E. J., Munro, A. W., Hay, S., & Leys, D. From ubiquitous sensor to enzyme: characterization of a heme-dependent PAS oxidative N-demethylase. *Nature* (2016) 539, 593-597 DOI:10.1038/nature20159.

Latham, J., Henry, J-M., Sharif, H., Menon, B. R. K., Shepherd, S., Greaney, M., & Micklefield, J. Integrated catalysis opens new arylation pathways via regiodivergent enzymatic C-H activation. *Nature Communications* (2016) 7, [11873]. DOI:10.1038/ncomms11873.

An efficient platform for entire gene cluster cloning and refactoring

Chuan Huang

Fast development of DNA sequencing and bioinformatics has revealed that microorganisms possess enormous potential to produce structurally-complex specialized metabolites. However, only a minority of the specialized metabolite biosynthetic gene clusters (BGCs) from microorganisms are well expressed under laboratory growth conditions and many of these microorganisms are genetically intractable. To tackle such problems, heterologous expression of BGCs in a well-established host is a common strategy and this requires effective methods for BGC cloning and refactoring. Developing a platform that combines highly efficient gene cluster cloning via transformation-associated recombination (TAR) or de novo synthesis, with pathway refactoring has the potential to greatly accelerate research into specialized metabolite BGCs, as well as the applications of the metabolites they produce. As a proof of concept, we have applied these strategies to the eponemycin, TMC-86A and epoxomicin BGCs, which direct the biosynthesis of epoxyketone proteasome inhibitors that inspired the development of the multiple myeloma drug carfilzomib. We attempted to directly clone these BGCs and rebuild them in refactored form from synthetic DNA for expression in heterologous hosts. By comparing the yield of products from the native and refactored BGCs, we would hope to better understand the rules for effective gene cluster heterologous expression.

An *E. coli* inducible expression system based on *Streptomyces* signalling molecules.

Miriam Rodríguez García

Inducible systems for expression of genes of interest in bacteria have been used for decades with many applications, like industrial production of recombinant proteins. We have been implementing an inducible expression system in *E. coli* based on a natural system that triggers methylenomycin production in *Streptomyces coelicolor* [1]. The methylenomycin biosynthetic pathway is regulated by the TetR family member MmfR that, like other TetR family members, is a homodimeric protein with a C-terminal DNA binding region and a N-terminal ligand-binding domain[2]. In the absence of the inducer, MmfR is bound to the methylenomycin autoregulatory response element (MARE) and represses transcription. This repression is removed upon the addition of a small molecule furan ligand (MMFs), that causes a conformation change in the repressor and its release from DNA, thereby allowing transcription[3] (Figure 1).

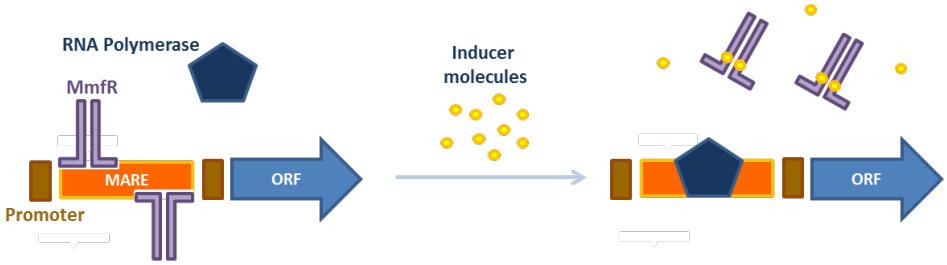

Figure 1. MmfR and MMF regulation in methylenomycin antibiotic biosynthesis.

We have studied and implemented the effect of MmfR and MMF on the native and new synthetic operators and the results showed that the MmfR/MMF/MARE system has a great potential as a novel inducible expression system in *E. coli*. Moreover, in that system the expression of gene(s) of interest could be achieved by feeding the microorganism with the synthetic ligand molecule, which is stable, cheap, and easy to synthesise.

1. O'Rourke S., Wietzorrek A., Fowler K., Corre C., Challis G. and Charter K. (2009) *Mol.Microbial*. 71,763.

2. Cuthbertson L. and Nodwell J.(2013). *Mircrobiol. Mol.Biol.Rev*. 77,440.

3. Corre C., Song L., O'Rourke S., Chater K. and Challis G. (2008). *Proc. Natl. Acad. Sci*. 105,17510.

González A, Rodríguez M, Braña AF, Méndez C, Salas JA, Olano O. New insights into paulomycin biosynthesis pathway in Streptomyces albus J1074 and generation of novel derivatives by combinatorial biosynthesis. *Microb Cell Fact.* (2016) Mar 21:15(1):56. doi: 10.1186/s12934-016-0452-4.

Application of phage therapy to treat microbiome-related disease

Eleanor Jameson

Phage therapy has the potential to control bacterial populations responsible for diseases linked to the gut microbiome. The gut microbiome plays an important role in disease including; ulcerative colitis, obesity, autism, autoimmune diseases, outcome of premature birth and cardiovascular disease. It has been shown that gut microbiota are responsible for trimethylamine (TMA) production, which is linked to cardiovascular disease. My previous work has significantly advanced our understanding of novel enzymes responsible for the formation of TMA from human gut microbiota, including choline-TMA lyase, CutC[1], and carnitine monooxygenase, CntAB[2]. By carrying out data-mining analyses on human gut metagenomes it is clear that *Enterobacteriaceae* are prevalent in the gut and contain numerous TMA production genes[3]. The data-mining work provides bacterial targets in the *Enterobacteriaceae* to understand TMA production in the gut. These *Enterobacteriaceae* will be tested for TMA production in culture and in the context of a wider microbial community, to test the potential for phage therapy to reduce TMA production. Gut-associated bacteriophage have received relatively little research attention, yet previous studies have shown they play a role in inflammatory bowel disease, where bacterial communities are out of balance. Lytic phage specific to the identified TMA-producing *Enterobacteriaceae* will provide a vital tool to engineer the microbiome and offer an alternative approach to combat cardiovascular disease.

1. Jameson*, E. Fu*, T., Brown, I.R., Paszkiewicz, K., Purdy, K.J., Frank, S. and Chen, Y. Anaerobic choline metabolism in microcompartments promotes growth and swarming of Proteus mirabilis. *Environmental Microbiology* (2016). 18: 2886-2898. *joint first authors

2. Zhu*, Y., Jameson*, E., Crosatti*, M., Schäfer, H., Rajakumar, K., Bugg, T. D. H. and Chen, Y. Carnitine metabolism to trimethylamine by an unusual Rieske-type oxygenase from human microbiota. *PNAS* (2014), 111: 4268–4273. *joint first authors

3. Jameson, E., Doxey, A.C., Airs, R., Purdy, K.J., Murrell, J.C., and Chen, Y.. Metagenomic data-mining reveals contrasting microbial populations responsible for trimethylamine formation in human gut and marine ecosystems. *Microbial Genomics* (2016) 2:9.

Jameson, E., Taubert, M., Coyotzi, S., Chen, Y., Eyice, Ö., Schäfer, H., Murrell, J. C., Neufeld, J. D., Dumont, M. G. (2017). DNA-, RNA-, and Protein-Based Stable-Isotope Probing for High-Throughput Biomarker Analysis of Active Microorganisms. In: Wolfgang S, Rolf D (eds). *Metagenomics: Methods and Protocols, Methods in Molecular Biology*. Springer Science and Business Media: New york. In press.

Synthetic microbial co-culture to recycle Mn/MnO.

Christian Zerfass

We developed a microbial bi-culture of a manganese-oxidising and -reducing bacterium to facilitate the cycling of manganese. This strategy offers the possibility to continuously regenerate *de novo* precipitated biogenic manganese oxide, a promising material for e.g. water treatment or battery applications due to its high-surface reactive state as a powerful oxidant.

By rigorously analysing the growth characteristics of the individual bacteria we identified a bi-culture medium accommodating both bacteria of interest, which are a marine and a freshwater species. Furthermore, we established a cross-feeding interaction to couple the respective organism's growth. While both manganese-oxidation, and -reduction are present in a globally aerobic culture condition, the bi-culture is most efficient in converting soluble (reduced) manganese into its insoluble (mostly oxide) state when compared to the respective monocultures. Improvements on the respective reaction rates are currently under investigation.

Christian Zerfass, Joseph Christie-Oleza, Orkun S. Soyer, A synthetic co-culture enabling manganese / manganese oxide cycling, *in preparation*
Christian Zerfass, Joseph Christie-Oleza, Orkun S. Soyer, Bio-manganese oxide relaxes nitrite-toxicity on Roseobacter sp. AzwK-3b, *in preparation*
Christian Zerfass, Orkun S. Soyer, Joseph Christie-Oleza, Marine cyanobacteria can deal with oxidative stress through the secretion of α-keto acids, *in preparation*

Towards an electrophysiological interface for synthetic biology

James P Stratford

There is growing awareness of electrical signalling in bacterial communities, however there is not yet any way of hacking into these electrical communication systems to control microbial physiology. The ultimate goal of this project is to provide a new electrical-microbial interface for regulation of cell physiology and gene expression for synthetic biology applications. We have now constructed a device which allows the control of bacterial membrane potential via bursts of low frequency AC signal. The application of electrical signals is regulated through a programmable interface which can time pulses with the cadence of microscope imaging. This allows changes in cell physiology to be precisely timed to coincide with time lapse imaging sequences. Fluorescent dyes allow measurements to be made of cell membrane potential with single cell resolution while fluorescent reporter genes are used to determine gene expression response or other key cell physiological changes. Using this novel tool we're now exploring the space of possible electrical signals and associated cellular responses.

Patent application: James Stratford and Munehiro Asally. Electrical signalling system for control of microbial physiology and gene expression. Disclosure stage.

Engineering novel activation of plant signalling pathways

Silke Lehmann

Pathogens and beneficial microbes apply highly similar infection strategies that greatly rely on effector-mediated reprogramming of host signaling. However, the outcome of these infections is significantly different resulting in plant diseases and beneficial plant effects, respectively. In collaboration with BASF Plant Science, we analyze the potential of *Piriformospora indica* derived effectors to activate beneficial pathways in plants. Exploiting the evolution of effector development,

we expect to identify plant proteins targeted by effector that play a key role in observed beneficial activities (e.g. biotic and abiotic stress resistance). As a strategy to apply this knowledge we express *P. indica* effectors in barley roots and analyze root resistance against biotic and abiotic stress.

Exploring microbial toolkits for manipulation of plant signalling

Ana Domínguez-Ferreras

Many plant-interacting microbes, either pathogenic or mutualistic, make use of an array of proteins which can be delivered into the host cell during colonization. These proteins, known as effectors, are highly specialized tools used by the microorganism to target the cell metabolism. Some have been long recognized by their ability to modulate the plant innate immune system, while others have been described to affect different physiological pathways. This intrinsic versatility is central for microbes to achieve a successful interaction with their hosts. However, plant-interacting microbes can have very extensive effector batteries and in most cases their function is not fully understood. Here we take a systematic approach to the discovery of effectors targeting specific signaling pathways or transcriptional networks. As a starting point, an array of effectors from the pathogenic bacteria *Pseudomonas syringae* and the oomycete *Hyaloperonospora arabidopsidis* were used in a protoplast-based screening to assess their effect on several pathways including hormonal, abiotic stress-related and defense-related signaling. Then, we made use of an extensive collection of *Arabidopsis thaliana* transcription factors to assess targeting of gene expression networks by effectors from different sources. In the future, effector candidates will be tested for their ability to engineer bespoke pathways in planta.

Perturbation-mitigation strategies in plants

Mathias Foo

External perturbations affecting gene regulatory networks (GRNs), such as pathogen/virus attacks, can lead to multiple adverse effects. In plants, attacks by the common fungus *Botrytis cinerea* can lead to rotting in plant organs such as the fruit and leaves, and this has resulted in considerable losses to farming industries. Using data obtained from a *Botrytis*-infected plant, *Arabidopsis thaliana*, we develop a systematic approach based on control engineering principles that can mitigate the effect of the network perturbation using the tools of synthetic biology. Using time-series data, we employ system identification techniques to build an accurate model of a subnetwork of the infected *Arabidopsis* GRN that contains key genes determining the plant defence response. From the obtained model, a genetic feedback controller designed using a combination of proteases is constructed. We show that the synthetic feedback controller is able to counter the effect of the perturbation effectively. The proposed approach highlights the potential of using feedback control theory with synthetic biology for developing more resilient plants.

M. Foo, J. Kim, R. Sawlekar and D.G. Bates (2017), “Design of an embedded inverse-feedforward biomolecular tracking controller for enzymatic reaction processes”, *Computer and Chemical Engineering,* vol. 99, pp. 145-157. DOI: 10.1016/j.compchemeng.2017.01.027

M. Foo, I. Gherman, K.J. Denby and D.G. Bates (2017), “Control strategies for mitigating the effect for external perturbations on gene regulatory networks” *to appear in Proceedings of IFAC World Congress, 9-14 July, Toulouse, France*.

M. Foo, J. Kim and D.G. Bates, “System identification of gene regulatory networks for perturbation mitigation via feedback control (2017)” *to appear in Proceedings of IEEE International Conference on Networking, Sensing and Control, 16-18 May, Calabria, Italy.*

M. Foo, J. Kim, J. Kim and D.G. Bates (2016), “Proportional-Integral Degradation (PI-Deg) control allows accurate tracking of biomolecular concentrations with fewer chemical reactions”, *IEEE Life Sciences Letters*, vol. 2, no. 4, pp. 55-58. DOI: 10.1109/LLS.2016.2644652

Poster titles

Designing feedback controllers to manage translational limitations
Alexander Darlington

Title tba
Marco Delise

Engineering bacteriophages to enhance their therapeutic use
Aurelija Grigonyte

Constructing the gut microbiota and investigating *Clostridium difficile* infection
Jack Hassall

Carbon nanodots as a tool to examine root-microbe interactions
Peter Morrison

Towards synthetic control of cell and community metabolism
Andrea Martinez Vernon