The 5th Annual CBS Research Symposium

May 29th 2014, Coates Road Auditorium, University Park, University of Nottingham

10:00-10:05	Opening	Peter Fisher
10:05-10:25	Systems Biology of the pH-induced Metabolic Shift in Clostridia	Thomas Millat
10:25-10:45	Genome editing of human embryonic stem cells for drug toxicity testing in vitro	Viola Borgdorff
10:45-11:00	Synthetic Biology Research Centre (SBRC): Something From Nothing!	Ying Zhang
11:00-11:30	*Coffee break*	
11:30-11:50	Cell aggregation of *Yersinia pseudotuberculosis* is dependent on type-III-secretion and quorum sensing	Anja Wiechmann
11:50-12:45	The Wellcome Trust: a view from the inside	Anne Taylor
12:45-14:00	*Lunch and poster session*	
14:00-14:15	Novel applications for whole animal imaging: more than just finding tumours!	Jeni Luckett
14:15-14:30	Communication between *Serratia plymuthica* and its host plants through cross-kingdom signalling	Xiaoguang Liu
14:30-14:45	Apoferritin: a versatile multifunctional protein nanoparticle for deep-tissue imaging and drug delivery	Philip Bardelang
14:45-15:00	The role of fructose-1, 6-bisphosphate aldolase (FBA) in the pathogenesis of *Neisseria meningitidis*	Fariza Shams
15:00-15:30	*Coffee Break*	
15:30-15:45	Determination of the ancestral genomic sequence of *Pseudomonas aeruginosa* PAO	Hardeep Naghra
15:45-16:00	Proteins concentration gradients in compartmental diffusion model	Hoda M. Eltaher
16:00-16:15	Role of Fibroblast Growth Factor 1 in interaction of *Neisseria meningitidis* with Human Brain Microvascular Endothelial Cells	Sheyda Azimi
16:15-16:25	Closing remarks	
17:00	Barbecue at The Johnson Arms	

Abstracts from speakers

Systems Biology of the pH-induced Metabolic Shift in Clostridia

Thomas Millat[1], Ralf-Jörg Fischer[2], Hubert Bahl[2], Klaus Winzer[1], Nigel P. Minton[1]

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After several decades of declining interest, clostridial acetone-butanol-ethanol (ABE) fermentations are gaining renewed attention in both academia and industry as an attractive resource for sustainable production of bioenergy, biofuels, and bulk chemicals. In a classical ABE fermentation, bacteria first produce organic acids but then shift their metabolism toward formation of solvents such as acetone, butanol, and ethanol. Importantly, this shift strongly depends on several environmental parameters. Among these, the external pH takes a special position. Unable to maintain a constant internal pH, clostridial cells allow it to change in accordance with the external pH and couple their metabolism to a constant transmembrane pH gradient instead. Accordingly, a detailed knowledge of pH-dependent properties of cellular components and regulatory processes is a crucial prerequisite for purposeful engineering of these organisms.

In recent years, the transnational COSMIC consortium, including several German, Dutch, and British groups, investigated the pH-induced metabolic shift of *C. acetobutylicum* using a systems biology approach. Here, systematic experiments in continuous culture proved that the shift of glucose-grown acid forming cells at a pH>5.2, to solvent formation at a pH<5.1, is accompanied by radical changes in transcriptome, proteome, and metabolome. However, a mathematical model of the underlying metabolic network revealed that these experimentally observed changes are insufficient to explain the time courses of the fermentation products measured in shift experiments. Indeed, an improved model suggested that kinetic regulations, in particular pH-dependent specific enzyme activities, are an integral part of the pH-induced metabolic shift. These kinetic regulations have the potential to trigger pH-dependent population growth, which results in an acid-forming subpopulation and a solvent-forming subpopulation. Our mathematical model predicts that the experimentally observed shift from acidogenesis to solventogensis is most likely the consequence of a heterogeneous transition between these two subpopulations rather than a homogeneous intracellular adaption to the changing pH levels.

Based on model analyses, several newly created mutants were investigated to further elucidate the role of the corresponding enzymes in ABE fermentation and their potential for an improved solvent formation. Here, the CoA-transferase CtfA/B is of particular interest, because it is believed that this heterodimeric enzyme is crucial for acetone formation and acid re-assimilation. In contrast to this commonly accepted view, experimental results and mathematical models using a *ctfA*-knockout mutant suggest that butyrate re-assimilation occurs via another, not yet characterized, pathway.

The presented systems biology project shows that close collaboration of experimentalists and modellers using such a systematic approach has the potential to improve our knowledge of complex biological processes and to guide the development of target-oriented biotechnological application of microorganisms.

Genome editing of human embryonic stem cells for drug toxicity testing *in vitro*

Viola Borgdorff[1], Maria Barbadillo-Muñoz[1], Manousos Koutsourakis[2], Barry Rosen[2], Lorraine Young[1], William C. Skarnes[2], Chris Denning[1]

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Drug development is a time, labour and cost-intensive process. Final drug approval by the FDA or EMA is preceded by screening of millions of compounds by the pharmaceutical industry and in the rare event of the identification of a suitable compound, this is followed by more than a decade of compound optimisation, validation and (pre-) clinical trials. Despite the intensive pre-marketing toxicity testing process, severe drug-induced toxicity may go undetected, only surfacing when the drug is starting to be used by the general population. Drug-induced toxicity has a strong idiosyncratic component associated with the presence of particular single nucleotide polymorphisms (SNPs) in genes essential for the functioning of vital organs such as the heart and the liver. An *in vitro* cellular model harbouring these SNPs would allow for the pre-clinical detection of organ-specific drug-induced toxicity. Here, we describe the engineering of human embryonic stem cells (hESCs) harbouring a G1681A mutation in the potassium channel encoded by KCNH2, which is associated with drug-induced cardiotoxicity. Using the Cas9/CRISPR system for genome editing, we have successfully introduced the mutation into Hues7 hESCs, paving the way for differentiation of these cells into $KCNH2^{G1681A}$ cardiomyocytes that can be used for toxicity screening.

Cell aggregation of *Yersinia pseudotuberculosis* is dependent on type-III-secretion and quorum sensing

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Yersinia pseudotuberculosis is an enteropathogenic gamma-proteobacterium causing food borne gastroenteritis in humans and is considered to be a highly related ancestor of *Yersinia pestis*, the causative agent of plague (Black Death) for which it is regarded as an appropriate model. *Y. pseudotuberculosis* and *Y. pestis* harbour a virulence plasmid (pYV) encoding genes for a type-III-secretion system (T3SS). This temperature and calcium dependent virulence system consists of an injectisome (a molecular hypodermic needle) and the associated export apparatus which together export effector proteins (Yops) into host cells where they trigger apoptosis.

We have shown that *Y. pseudotuberculosis* regulates key virulence determinants in a cell density dependent manner by communicating with other members of the population using chemical signal molecules, a process known as quorum sensing (QS). Recently we have shown that *Y. pseudotuberculosis* aggregation in liquid culture and T3S are QS dependent and that there exists a link between these two apparently unrelated phenotypes. QS mutants promote aggregation but QS mutants which also include a mutation in components of the T3S injectisome (*yscJ*) restore aggregation back to the levels seen in the parent. This is supported by the fact that the removal of pYV from the parent (and QS mutants) also prevents the formation of aggregates. To investigate in detail the mechanisms which underpin these observations we performed transposon mutagenesis on pYV in our QS signal synthase mutant and looked for reversion to the none aggregative mode of growth. Several insertions were in components of the injectisome export apparatus, namely *yscR, yscT, yscU* and *yscV* indicating that the injectisome plays an important role for liquid culture aggregation of *Y. pseudotuberculosis*. Investigations into the timing of aggregate formation revealed that the bacterial cells accumulate throughout the entire growth period but cells which are cured of pYV do not aggregate at any point. Similar results were achieved by constructing single mutants in components of the injectisome such as *yscF* (the structural component of the needle) or yscV (the outer part of the export apparatus).

Taken together these observations reveal that there are clearly links between QS, T3S and cell aggregation which provide us with a platform from where we can further examine the combined roles of these three key virulence determinants in infection models.

Novel applications for whole animal imaging: more than just finding tumours!

Dr Jeni Luckett

The development of whole animal imaging using PET/CT, SPECT/CT and optical platforms has allowed us to follow disease progression or biological responses to treatments within the same animal throughout the time-course of an experiment. The technology can be exploited to look at many areas of biology and preclinical medicine including de novo gene expression, efficacy of drugs, drug targets, and structural changes within the animal. The ability to analyse the same animal at multiple time-points also greatly reduces the number of animals required for in vivo work. Here at Nottingham we have developed imaging strategies for a wide range of models, including the novel markers for detection of bacteria, lung fibrosis, amino acid transport and trafficking of labeled cells in vivo.

Communication between *Serratia plymuthica* and its host plants through cross-kingdom signalling

Dr Xiaoguang Liu

The plant endophytic strain G3 of *Serratia plymuthica* displays a wide range of antimicrobial activities, as well as plant growth promoting capacity. Within the genome of G3, we have identified three *luxI*/*luxR* *N*-acylhomoserine lactone (AHL)-mediated quorum sensing (QS) homologues, suggesting a high level of complexity of QS in this organism. We have also identified two copies of the *ipdC* genes responsible for the biosynthesis of the plant auxin Indole-3-acetic acid (IAA). We have found that both *S. plymuthica* and its host plants can sense and respond to both AHLs and IAA produced by bacteria modulating their physiology, as well as their beneficial plant-microbe interactions. Future studies and better understanding of the underlying mechanisms behind this interaction may help us to exploit them for the development of novel strategies with a view to improve plant defences.

Apoferritin: a versatile multifunctional protein nanoparticle for deep-tissue imaging and drug delivery

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Developing strategies to deliver drugs and imaging agents to specific tissues is currently a significant area of biomedical research and multiple strategies for specific *in vivo* tissue imaging and drug delivery are being pursued. Some of these strategies are nanoparticle-based and comprise of natural lipoproteins, viruses or ferritins as nanoscaffolds to encapsulate the drug/imaging agent[1] and improve its biocompatibility or tissue delivery and targeting. The attraction of these natural scaffolds are numerous: (1) detailed information on their atomic structure and biological functions are usually available (2) the methods and potential for labelling are often diverse and well established permitting multivariant ligand display (3) there is a relative ease in scaffold preparation and purification (4) and all show good intrinsic biodegradation.

Previously we demonstrated that PbS semi conductor nanocrystals (quantum dots) which emit within the near infrared '*optical window*' (700-1100 nm) for *in vivo* imaging could be encapsulated within the cavity of horse spleen apoferritin[2+3]. Our recent progress expressing genetically modified human ferritin genes in bacteria and their subsequent use for the production of novel human apoferritin nanoscaffolds will be described. In brief, an overview of three different routes to producing novel apoferritins with new properties will be presented; (1) the conjugation of agents to 2-azidobiotin by *E.coli* biotin ligase (BirA) at an Avitag™ sequence engineered into apoferritin[4]; (2) the conjugation, via click chemistry, of small molecules to the unnatural amino acid azidohomoalanine site-specifically incorporated into apoferritin[5]; (3) the use of gene fusions to localise enzymes (e.g. luciferase) on the outer surface of apoferritin. In principle by utilising a combination of routes a variety of tailored multifunctional apoferritin nanoparticles can be produced. Our progress towards a bioluminescent apoferritin nanoparticle highlights the potential of the apoferritin nanoscaffold to become a deep-tissue imaging probe.

References

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The role of fructose-1, 6-bisphosphate aldolase (FBA) in the pathogenesis of *Neisseria meningitidis*

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Neisseria meningitidis resides harmlessly in the human nasopharynx, but may sometimes cause fatal sepsis and meningitis. Fructose 1,6 bisphosphate aldolase (FBA), a glycolytic pathway enzyme, has been described as having moonlighting functions in *N. meningitidis.* This project aims to explore the moonlighting functions of FBA in the pathogenesis of meningococcal disease. Constructs were generated to express rFBA with mutations in the active (cation-binding) site (D83A and H81A/H84A). A coupled assay confirmed the aldolase enzyme activity of wild-type rFBA. The kinetic parameters of purified wild-type rFBA for the cleavage of FBP were calculated as K*m*=0.027 mM and k*cat*=420 min^{-1}. In contrast, the two mutated FBA enzymes had no detectable enzymatic activity. Plasminogen was found to bind to intact meningococcal cells and to purified wild type rFBA and mutated rFBA lacking aldolase activity. This can be inhibited by the lysine analogue ε-aminocaproic acid indicating the involvement of lysine residue(s) of rFBA in this interaction. Interestingly, a truncated FBA spanning the C-terminal 134 amino acids of FBA and containing most of its lysine residues was also able to bind plasminogen. Substitution of the terminal lysine residue with alanine significantly reduced the binding of plasminogen binding. In addition, our preliminary data also suggest that FBA might be required for optimal adhesion of *N. meningitidis* to human brain endothelial (HBME) but not to human pharyngeal epithelial (Detroit -562) cells.

Determination of the ancestral genomic sequence of *Pseudomonas aeruginosa* PAO

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The most commonly used strain in the study of the opportunistic pathogen *Pseudomonas aeruginosa* is strain PAO1 - a spontaneous chloramphenicol-resistant mutant of the original *Pseudomonas aeruginosa* PAO which was isolated from an infected wound in Australia in 1954. This original isolate, from which the PAO1, PAO2 and PAO3 strains were derived, is no longer available. PAO1 exists in laboratories all over the world as sublines in which varying genotypes have emerged over time.

The current reference genome for *Pseudomonas aeruginosa* PAO1 is based on a laboratory subline from the University of Washington, which has been found to contain genetic differences compared to other PAO1 sublines. The most extreme example of this is a 2.2 Mb inversion between two ribosomal operons, *rrnA* and *rrnB*, which has so far been found to be unique to the reference strain. There are also an array of single nucleotide polymorphisms (SNPs) and small insertions and deletions which are found in PAO1-UW and not in any other PAO1 subline.

It is for this reason that we have built a more complete repertoire of PAO derivatives, by carrying out whole genome sequencing and optical mapping of various laboratory PAO1 sublines, PAO2 and PAO3, in order to deduce the consensus sequence of the ancestral *Pseudomonas aeruginosa* PAO genome. This sequence could be used as a universal reference sequence for PAO1, and would be of particular importance given that whole genome and transcriptome resequencing is becoming a more routine practice in the genetic analysis of *Pseudomonas aeruginosa* PAO1.

Proteins concentration gradients in compartmental diffusion model

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Vital cellular processes like migration, angiogenesis and differentiation are guided by gradients of different physical and chemical cues. Depending on the local concentration of signalling molecules, different cellular responses could be elicited favouring distinct tissue fate over another. The ability to control the spatiotemporal profile of signalling molecules such as growth factors within matrices is therefore important for inducing desirable differentiation of stem cells. In this current study, We have developed a highly efficient system we term Glycosaminoglycan (GAG)-binding enhanced transduction or GET and wanted to explore if GET protein delivery could be controlled spatiotemporally and ultimately allow us to direct cell responses.

Hydrogels (5x5x15 mm) with or without NIH3T3 mouse fibroblasts ($2x10^6$ cells/mL) were cast within a modified diffusion chamber to create a 3 compartmental diffusion assembly of source-gel-sink. Different GET - monomeric red fluorescent proteins (GET-mRFP) were allowed to diffuse from the source compartment to the hydrogel matrix. Gradient profiles at different time points were defined as function of distance inside the hydrogel at 20 µm resolution by serial slicing of the scaffolds perpendicular to the direction of protein diffusion using a Leica CM1100 cryostat at -20 ºC.

Our system emphasized on the transduction capabilities of the GET proteins in comparison to the non-transducing mRFP which diffused to equilibrium throughout the hydrogel volume. Cellular uptake of GET mRFP completely depleted the hydrogel of free diffusible protein and demonstrated that the cells themselves acted as sink that retained the GET-mRFP. The compartmentalized diffusion model presented herein is capable of generating gradients of functional proteins which could potentially be used to control stem cell fate and direct differentiation to specific tissue phenotype in 3D systems for regenerative medicine and tissue engineering applications.

Role of Fibroblast Growth Factor 1 in interaction of *Neisseria meningitidis* with Human Brain Microvascular Endothelial Cells

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Neisseria meningitidis (meningococcus) is an obligate human commensal bacterium that can cause meningitis and sepsis. Crossing the Blood-Brain Barrier (BBB) is a crucial step in the development of meningitis, but the mechanisms used by the meningococcus to achieve this are not fully understood. The aim of this study was to investigate the role of the Fibroblast Growth Factor1-IIIc isoform (FGFR1-IIIc) in the attachment to, and invasion of, Human Brain Microvascular endothelial cells (HBMECs) by *N. meningitidis*. Confocal microscopy showed that micro-colonies of adhered *N. meningitidis* recruit activated FGFR1. Direct interaction between meningococci and the extracellular domain of FGFR1-IIIc was demonstrated by ELISA confirming the ability of this bacterium to bind FGFR1-IIIc. Other bacterial meningeal pathogens, including *Streptococcus pneumoniae* and *Haemophilus influenzae*, were unable to bind to this receptor confirmed specificity. This study identified a novel receptor for meningococci, FGFR1, which may play an important role in the pathogenesis of this pathogen, and may constitute a new therapeutic and prevention target for disease caused by these bacteria.