The 4th Annual CBS Research Symposium

Thursday 30th May, Coates Road Auditorium, University Park, University of Nottingham

10.00-10.05	Opening address	Peter Fischer
10.05-10.25	Synthetic biology approach for enhanced lignocellulose degradation and industrial gas fermentation for low-carbon fuel and chemical production by solventogenic and acetogenic *Clostridium* species.	Katalin Kovács
10.25-10.45	A novel role for DEF6: Regulation of Translation in T Cells at the Immune Synapse?	Eleanor Mollett
10.45-11.05	*Introducing Yasuhiko Irie*: Biofilm Extracellular Polysaccharides are Novel Signalling Molecules.	Yasuhiko Irie
11.05-11.30	Coffee break	
11.30-11.40	*Future directions in CBS…* A high-throughput, automated platform for determining the impact of dietary nutritional perturbations on epigenetic regulation in human embryonic stem cells.	Bill Harris
11.40-11.50	*Future directions in CBS…* Computational modelling of bacterial systems.	Steven Higgins
11.50-12.40	*Invited talk*: Innovation and Intellectual Property in Biomolecular Sciences Research.	Alan Burbidge, Business Engagement and Innovation Services
12.40-13.50	Lunch and poster viewing. BBQ ticket collection 12.40-12.55 in the Auditorium.	
13.50-14.10	The ecological implications of synergistically acting quorum sensing signal molecules and the significance for cross-species interactions.	James Gurney
14.10-14.30	Pathogenesis of *Clostridium difficile* – sporulation, germination, toxicity and you.	Daniela Heeg
14.30-14.50	Design, Synthesis and evaluation of a New Series of Bis-Triazoles as G-Quadruplex Ligands.	Maysaa Saleh
14.50-15.00	*Future directions in CBS…* DNA origami: the combination of art and science.	Davy Chang
15.00-15.25	*Introducing Jing Yang:* 3D printing of vascularised hepatic tissues.	Jing Yang
15.25-15.50	*Introducing Pavel Gershkovich*: The Role of Lipids in the Pharmacokinetics and Pharmacodynamics of Lipophilic Drugs.	Pavel Gershkovich
15.50-15.55	Closing remarks and presentation of poster prizes.	Jody Winter
16.00	Meeting ends. BBQ ticket collection 16.00-16.15 in the Auditorium.	
17.00 onwards	Post-Symposium BBQ at the Johnson Arms, Dunkirk.	

Presentation Abstracts

Synthetic biology approach for enhanced lignocellulose degradation and industrial gas fermentation for low-carbon fuel and chemical production by solventogenic and acetogenic *Clostridium* species.

Katalin Kovacs, Katrin Schwarz, Wouter Kuit, Krzysztof Gizynski, Jonathan Baker, Kelly Davidge, Anne Henstra, Ehsaan Muhammad, Gareth Little, Benjamin Willson, Sarah Mastrangelo, Lili Sheng, Hengzheng Wang, Fung Liew, Carlo Rotta, Alexander Grosse-Honebrink, Bean Zak, Carlos Da Silva Granja, Wan Ho, James Millard, Louise Sewell, Ying Zhang, Klaus Winzer and Nigel Minton[1]

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The genus *Clostridium* comprise an extremely diverse group of bacteria that inhabit a wide variety of habitats and therefore are able to utilise a broad range of substrates. These substrates range from simple carbon sources like CO and CO_2, to more complex molecules such as glucose, maltose, sucrose, mannitol, arabinose, starch, cellobiose and cellulose. Several Clostridia species are best known for their ability use these substrates to produce biofuels, such as ethanol and butanol.

In recent years, there has been a renewed interest from both academic and industrial institutions for sustainable production of chemical commodities and biofuels. Our groups is focusing on establishing clostridia as a chassis for the production of chemical commodities and biofuels from renewable resources such as lignocellulose and waste gas using synthetic biology approaches.

Several saccharolytic *Clostridium* species are known to produce butanol, a superior biofuel to ethanol, however these species are un-able to use the most abundant renewable polymer, lignocellulose. Other saccharolytic species are able to degrade cellulose, however they produce predominantly ethanol. We use synthetic and systems biology approaches to create Clostridia strains with improved cellulosic substrate utilization properties to produce butanol.

Acetogenic *Clostridium* species, such as *Clostridium ljungdahlii*, are able to capture carbon (CO or CO_2) through anaerobic gas fermentation. They grow on a spectrum of waste gases from industry (*eg.*, steel manufacturing, oil refining, coal and natural gas) as well as 'synthesis gas' (CO & H_2) produced from renewable and sustainable resources, such as biomass and domestic/ agricultural wastes. This enables low carbon fuels and chemicals to be produced in any industrialized geography without consumption of valuable food or land resources. We use metabolic engineering to better understand, optimize and extend product streams through a combination of systems and synthetic biology approaches.

A novel role for DEF6: Regulation of Translation in T Cells at the Immune Synapse?

Eleanor Mollett (Searle Group)

DEF6 is a Rho-Guanine Nucleotide Exchange Factor (GEF) with a poorly characterised role in T cell receptor signalling. Its importance in T cell signalling has been highlighted by observations that DEF6 deficient T cells display defective actin polymerization and polarization, have a decreased susceptibility to CD3-induced apoptosis, and exhibit aberrant expression of the inflammatory cytokine IL-17. DEF6 deficient mice develop a disease similar to systemic lupus erythematosus, and rheumatoid arthritis. Mice that lacked DEF6 were also resistant to the development of a murine model of multiple sclerosis. The molecular mechanism behind these phenotypes remains to be identified.

DEF6 is a 631 amino acid protein whose structure is unknown but can be divided into a number of domains. The N-terminal domain (aa1-109) of unknown function, followed by a Pleckstrin Homology (PH) domain (aa205-315) and a Dbl Homology-like (DHL) domain (aa310-631), with Rho GEF activity but unlike any other Rho-family DH domain. Unlike other Rho-GEFs, DEF6 and its B cell homolog SWAP70 both exhibit a unique reversal of the N-C terminal orientation of the DH and PH domains. Our work aims to elucidate the functions and interactions of these domains using biophysical and cell techniques.

We have found that DEF6 forms cytoplasmic granules under conditions that activate global arrest of translation which co-localize with DCP1, a marker for P bodies, suggesting an unanticipated role in RNA translation regulation in T cells. We have shown that the DHL domain forms a Q/N rich coiled coil, a motif common in aggregating domains. Although the DHL domain is capable of spontaneously forming granules, we have demonstrated that that it is not sufficient for localisation with DCP1. As our proteomics data indicated that the N-terminus of DEF6 interacted with the components of the translational machinery of T cells a GFP tagged N terminal construct was made of this region to investigate its association with P bodies. This localized to DCP1 granules, demonstrating that DEF6 has an aggregating domain and a separate P body-targeting domain. Models that link these functions to signalling downstream of T-cell receptor signalling will be discussed.

I*ntroducing Yasuhiko Irie*: Biofilm Extracellular Polysaccharides are Novel Signalling Molecules.

Yasuhiko Irie (Diggle group)

Bacteria have a tendency to attach to surfaces and grow as structured communities called biofilms. Chronic biofilm infections are a problem because they tend to resist antibiotic treatment and are difficult to eradicate. Bacterial biofilms have an extracellular matrix that is usually composed of a mixture of polysaccharides, proteins, and nucleic acids. This matrix has long been assumed to play a passive structural and protective role for resident biofilm cells. Here we show that this view is an oversimplification and that the biofilm matrix can play an active role in stimulating its own synthesis. Working with the model biofilm bacterium *Pseudomonas aeruginosa*, we found that PSL, a major biofilm matrix polysaccharide for this species, acts as a signal to stimulate two diguanylate cyclases, SiaD and SadC, to produce the intracellular secondary messenger molecule c-di-GMP. Elevated intracellular concentrations of c-di-GMP then lead to the increased production of PSL and other components of the biofilm. This mechanism represents a unique positive feedback regulatory circuit, where the expression of an extracellular polysaccharide promotes biofilm growth in a manner analogous to autocrine signalling in eukaryotes.

Future directions in CBS… **A high-throughput, automated platform for determining the impact of dietary nutritional perturbations on epigenetic regulation in human embryonic stem cells.**

William Harris and Ralph Hyde

Wolfson Centre for Stem cells, Tissue Engineering and Modelling (STEM), University of Nottingham, Centre for Biomolecular Sciences, University Park, Nottingham NG7 2RD, UK.

It has been shown in both animal models and human epidemiological studies that gross deviations in dietary protein or calorific intake in pregnant individuals can have significant physiological effects in the pre-implantation embryo and developing foetus. These changes are thought to affect normal embryonic development, but are also associated with later adult life predisposition to chronic diseases such as diabetes, obesity and cardiovascular disease. An emerging concept in this field is that specific nutritional fluctuations impact upon the methionine/folate cycle, thus altering the supply of methyl groups required for histone and DNA methylation: two important mechanisms of epigenetic regulation for gene expression. Epigenetic mechanisms are known to coordinate and maintain specific programs of global gene expression in the regulation of processes such as pluripotency, lineage differentiation and, ultimately, embryonic development.

For this study a panel of normal dietary nutrients will be assessed for their effect on the growth and differentiation capacities of human embryonic stem cells (hESCs). A Design of Experiments (DofE) approach will allow robust testing of multiple nutrients at various concentrations contemporaneously to highlight the most important nutrients and most significant interactions between nutrients, whilst an automated robotic platform will be utilised to carry out experiments in a large-scale, high-throughput, accurate and reliable manner. hESCs will be subsequently analysed for various measures of epigenetic change, including the ratio between methyl donor S-adenosyl methionine and its post-methyl donation product S-adenosyl homocysteine (SAM:SAH), as well as cell viability and capacity to differentiate into the three germ layers. Further, global analysis of the chromatin architecture using methylated DNA immunoprecipitation (MeDIP) or chromatin immunoprecipitation (ChIP) followed by next generation clonal sequencing will likely reveal specific gene loci sensitive to particular nutrient fluctuations which can be investigated mechanistically for a role in normal development. These sensitive loci may also prove useful as biomarkers to assess and inform dietary intake during pregnancy.

Future Directions in CBS... **Computational modelling of bacterial systems.**

Steven Higgins (Williams and ICOS groups)

With the development of functional genomics, biological systems are revealing their complexity and we need novel approaches to understand how and why genetic networks have evolved. Transcriptomic, proteomic or metabolomic experiments usually only cover a few data points over a limited set of conditions. When using single gene reporter fusions it is difficult to identify key elements within large signalling and regulatory networks where multiple interactions are the rule more than the exception. To get around these shortcomings computational modelling of biological systems in combination with wet lab validation is developing as a novel and powerful method of investigating bacterial communication and regulatory networks acting at the cell as well as at the population level.

Here I will present a recent example of a computational model describing the relationship between the las and rhl quorum sensing systems in Pseudomonas aeruginosa. This model was built using novel and dedicated software designed by the ICOS group from the School of Computer Science. This allows biologists to easily describe biological networks and run non-deterministic simulations of living bacterial cells in silico. The data generated from modelling can help to identify logic gates which have specific information processing capabilities or response acceleration properties. Ultimately, modelling can be used to aid in the behavioural prediction of regulatory or metabolic circuits and hence in the design of efficient synthetic systems.

The ecological implications of synergistically acting quorum sensing signal molecules and the significance for cross-species interactions.

James Gurney[1], Roman Popat[2], Luke McNally[2] Dan Cornforth[2], Sam P. Brown[2], Klaus Winzer[1], Stephen P. Diggle[1]

[1] **School of Molecular Medical Sciences, University of Nottingham, Nottingham NG7 2RD, United Kingdom.**

[2] **Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, King's Buildings, Edinburgh EH9 3JT, UK**

Research into quorum sensing (QS) has traditionally focused on understanding mechanism, with less attention paid to evolutionary considerations of QS. Many bacterial species produce more than one QS signal and a key question that receives little attention is why make multiple QS signal molecules? Do molecules have overlapping roles in gene regulation in a non-additive manner? We test this and other questions using the opportunistic pathogen Pseudomonas aeruginosa, which produces two major N-acylhomoserine lactone (AHL) signals. We show that interactions of two signal molecules at the level of gene transcription can lead to synergistic interactions, resulting in (1) drastic regulation of QS-controlled genes and (2) 14 distinct QS regulons. These 14 regulons appear to be a finer control of regulation depending on the ratios of signals that a bacteria encounter lending support to the notion that bacteria can use production of signals molecules to sense population density and the environment factors such as mass transfer. Synergy can also be activated by non-cognate signal molecules leading to complex cross-species interactions which might have implications in the fitness of bacteria during infection.

Pathogenesis of *Clostridium difficile* – sporulation, germination, toxicity and you.

Daniela Heeg, Sarah Kuehne, Mark Collery, Emma Butt, Alan Cockayne and Nigel Minton

Clostridia Research Group, NIHR Biomedical Research Unit in GI Disease, Centre for Biomolecular Sciences, School of Life Sciences, University of Nottingham, UK

Clostridium difficile is an anaerobic, spore forming bacillus and the underlying cause of antibiotic-associated diarrhoea. In recent years, *C. difficile* -associated disease has become one of the major nosocomial infections and causes greater fatality rates than MRSA. Endospores, formed during sporulation represent the principle means of transmission. These dormant spores are highly resistant to adverse environmental conditions such as antibiotics, and hospital cleaning products. In order to cause disease, however, spores need to abjure dormancy and return to vegetative cell growth in a process termed germination. Vegetative cells of *C. difficile* can then produce the characteristic toxins A and B, which action disrupts the intestinal epithelial barrier leading to severe inflammation and diarrhoea. Tools developed for the genetic manipulation of *Clostridium difficile* in the Clostridia Research Group (CRG) have allowed us to target genes involved in spore formation and spore germination pathways, those involved in toxin production and regulation of expression. The outputs of our studies have continued to challenge current perceptions in the field. This talk will outline the past and current work performed in the CRG aimed at unravelling the molecular mechanisms of *C. difficile* –associated disease.

Design, Synthesis and evaluation of a New Series of Bis-Triazoles as G-Quadruplex Ligands.

Maysaa Saleh[1,2], Christopher Moody[2], and Charlie Laughton[1]

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The unlimited proliferation of cancer cells depends on a telomere maintainance mechanism which is most commonly provided by the telomerase enzyme[1]. The telomeric ends form structures called G-quadruplexes. Stabilization of these structures by small binding molecules called G4 ligands inhibits telomere elongation, and targets telomere maintainance mechanisms, resulting ultimately in delayed cell death and abrogation of tumourigenicy *in vivo*[2]. In this project, we are developing a new series of triazoles which are designed to bind to and stabilize G-quadruplex structures selectively, and which may therefore have potential as anti-cancer drugs. These triazoles are synthesized via 1,3-dipolar cycloaddition reactions of organic azides with quinone derivatives which afford two possible bis-triazole products: centrosymmetric and noncentrosymmetric regioisomers. The different regioisomers are expected to have different DNA-binding characteristics. Quantum mechanics calculations have been used to investigate these reactions, and predict that the centrosymmetric regioisomer is the most stable geometry and favoured product. The target ligands have been produced through multi-step synthesis in moderate to good yields, as the QM-predicted regioisomers. Their selectivity for G-quadruplex DNA over duplex DNA was investigated using a FRET-based assay. This showed four compounds to be moderately effective G4 binders over the concentration rage examined, with particular affinity for the quadruplex formed by the Hsp90a promoter sequence. Encouragingly, good selectivity for G-quadruplex DNA vs. duplex DNA was displayed by all the ligands over the concentration range used. Molecular modelling studies were performed to rationalize the affinity and selectivity of the ligands in binding to G4-DNA. The study showed a moderate correlation between Glide docking scores and the experimental FRET results.

References

1. Moorhouse, A. D.; Haider, S.; Gunaratnam, M.; Munnur, D.; Neidle, S.; Moses, J. E., *Mol. BioSyst.*, *4*, 629-642, **2008**.
2. Campbell, N. H.; Patel, M.; Tofa, A. B.; Ghosh, R.; Parkinson, G. N.; Neidle, S., *Biochemistry*, *48*, 1675-1680, **2009**.

***Future directions in CBS*… DNA origami: the combination of art and science.**

Chien-Yi Chang

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Synthetic biology is a new discipline comprising biology, computer science, chemistry, engineering, genetic engineering and biophysics. Through design and engineering, highly complex bio-systems can be constructed, standardised, created and re-structured by utilising natural biological systems or components in order to understand and provide new solutions for public health, energy and environmental challenges. Construction of nano-stuctures for useful functions such as disease diagnosis, cancer cell targeting and drug delivery is particularly challenging in Synthetic Biology. DNA origami has been proven as a powerful strategy to design and construct such nano-structures. By folding a long single-stranded DNA molecule (scaffold) with several short oligonucleotides (staples), different patterns or structures can be generated. Here I present the preliminary data of a DNA origami structure, which has been designed, constructed and observed using atomic force microscopy. Furthermore several ongoing synthetic biology projects within the group will also be mentioned.

Acknowledgement: EPSRC's project Towards a Universal Biological-Cell Operating System (AUdACiOuS) - EP/J004111/1

Introducing Jing Yang: **3D printing of vascularised hepatic tissues.**

Jing Yang

My previous research concerned the development of novel biomaterials for stem cell and bacteria related applications. I embarked on a project in which thousands of new materials were screened simultaneously for their ability to support the growth of human embryonic stem cells. A sample format called polymer microarray was employed to facilitate the high throughput screening of a large number of materials. New 100% synthetic materials that supported robust growth of stem cells have been discovered and patented. These materials have potential to replace gold standard animal tumour derived materials used in current stem cell culture that are not safe. In particular, I contributed to the understanding of how stem cells responded to these new materials' surface properties. Using the same sample format, we also identified new materials that resisted bacterial adhesion. These materials could be applied as coatings on biomedical devices such as urinary catheters to reduce infection rate. For my Nottingham Research Fellowship, I will lead a new research direction in 3D printing of human tissues. These tissues can potentially serve a wide range of applications such as drug screening, eventually implants.

Introducing Pavel Gershkovich: **The Role of Lipids in the Pharmacokinetics and Pharmacodynamics of Lipophilic Drugs.**

Pavel Gershkovich

Biography:

I have completed BScPharm, MSc in Clinical Pharmacy and PhD in Pharmacokinetics in The Hebrew University of Jerusalem, Israel. I have then spent 5 years in the University of British Columbia, Vancouver, Canada doing postdoctoral research in the field of Oral Drug Delivery. Currently, I am a Lecturer in Pharmacokinetics in the School of Pharmacy, University of Nottingham. To date I have published 31 peer-reviewed papers in leading pharmaceutical journals.

Expertise:

Biopharmaceutics, Pharmacokinetics, Pharmacodynamics, Bioanalytical Techniques, Oral Drug Delivery.

Research Interests:

Intestinal absorption of drugs; Lymphatic transport and targeting; Effect of disease states on pharmacokinetics and pharmacodynamics; Effect of food and its composition on absorption, disposition, activity and toxicity of drugs; Translational research.

Poster Presentations

	Authors	Title
1	Ye Chen*, Stephan Heeb, Paul Williams and Miguel Cámara.	ScdA is responsible for the biosynthesis of *cis*-2-decenoic acid in *Pseudomonas aeruginosa*.
2	Alexander Grosse-Honebrink, Ying Zhang, Nigel P. Minton	A universal *mariner* transposon system for forward genetic studies in any *Clostridium* species.
3	James G Smith, Adam D Celiz, Katarzyna Lis-Slimak, Maria Barbadillo-Munoz, Ralph Hyde, William Harris, Qian Liu, Lorraine Young, Martyn C Davies, Morgan R Alexander, Chris Denning	Synthetic Surfaces for the Automated Manufacture of hPSCs.
4	Tiangong Lu	Evaluation of (E)-Styrylsulfonyl methylpyridine: A novel kinase inhibitor targeting mitotic pathways.
5	Quian Liu	High throughput screening of human nutrient-gene interactions at epigenetic level in hESC-derived hepatocytes hepatocytes.
6	Daniela Heeg[1], Sarah A Kuehne[1], Andrew W. Dempster[1], Christopher Longshaw[2] and Nigel P. Minton[1]	Analysis of germination and outgrowth of six clinically-relevant *Clostridium difficile* polymerase chain reaction (PCR)-ribotypes challenged with fidaxomicin.
7	Sarah A. Kuehne[1], Andrew W. Dempster[1], Daniela Heeg[1], Christopher Longshaw[2] and Nigel P. Minton[1]	Use of allelic exchange to characterize the impact of *rpoB/C* mutations on fitness of *Clostridium difficile* and sensitivity to fidaxomicin.
8	Andrea J Vernall, Leigh A Stoddart, Stephen J Briddon, Stephen J Hill, Barrie Kellam.	Highly potent and selective fluorescent antagonists of the adenosine A3 receptor: Use as an imaging tool.
9	Tina Patel & Felicity Rose	Confocal Raman Microscopy: The future for dermal penetration studies…
10	Eric J.G. Pollitt, Shanika A. Crusz, Stephen P. Diggle	Is *Staphylococcus aureus* actively motile?
11	Alexander A. Popov [1*], George A. F. Roberts [2], David M. Grant [2], Colin A. Scotchford [2] and Virginie Sottile	Porosity in the development of a cellularised construct for osteochondral modelling and repair.
12	K.W. Cook, D.P. Letley, K. Ragunath, J.C. Atherton and K. Robinson	Increased CCL20 and CCR6+ regulatory T-cell responses in the *Helicobacter pylori* infected human gastric mucosa.
13	Anchala Kuruppu	The role of ErbB receptors in breast cancer.
14	Ashawesh Mahmoud, Hardie Kim and Penfold Christopher	Localization of an autotransporter EspC protein secretion.
15	Jayson Bispham	The Design, optimisation and implementation of a multi-parameter pluripotency flow cytometry analysis panel.
16	R. J. M. Ingram, E. Staples, J. C. Atherton and K. Robinson	Luminex assay optimisation: a novel approach to characterising cytokine expression profiles in human gastric biopsies in *Helicobacter pylori* infection.
17	Anne M. Henstra, K. Winzer, N.P. Minton	NEW: The CBS Gas Fermentation Research Facility.
18	Vinoj George	Characterising a novel method for efficient monolayer differentiation of human pluripotent stem cells into cardiomyocytes.
19	Alexander Kondrashov	Establishing cellular models to study the role of gene specific polymorphisms of ß2-adrenergic receptor (ADRB2).
20	James Crutchley, Vinoj George, James Smith and Chris Denning	Automation and scale-up of human induced pluripotent stem cell models of cardiovascular disease for drug screening.

21	Asha K. Patel[1,2], Adam D. Celiz[2], Daniel G. Anderson[3], Robert Langer[3], Martyn C. Davies[2], Morgan R. Alexander[2] and Chris Denning[1]	Chemically diverse polyacrylate and polyacrylamide surfaces for human cardiomyocyte culture and their effect on phenotype.
22	L Flatt, M Barbadillo-Muñoz and L Young	Driving towards Small Molecule induction of Definitive Endoderm, in Automatic.
23	Spandan Kalra, Emily Dick, Mojgan Reza, Rita Barresi, Hanns Lochmuller, Chris Denning	In vitro Modelling of Duchenne Muscular Dystrophy.
24	Alexandra Hughes	Translational Control of Genes involved in Alzheimer's Disease.
25	Ralph Hyde	Automated Platform for Stem Cell Maintenance, Differentiation and Screening.
26	Hao Shao	Synthesis and in vitro evaluation of selective CDK9 inhibitors.
27	Hanna Parker	Dynamic changes of the poly(A) tail during inducible gene expression.
28	Divya Rajamohan	In vitro modelling of the cardiac channelopathies using human induced pluripotent stem cells.

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