The University of Nottingham

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School of Life Sciences

Third Annual Postgraduate Research Symposium

Thursday 14th- Friday 15th July 2016

Dear Delegate,

Welcome to the School of Life Sciences annual Postgraduate Symposium. We hope that over the course of the two days, the arranged programme will prove to be stimulating and enjoyable.

To those presenting, we wish you the best of luck and hope the opportunity to present your research will prove to be a valuable experience. We would also like to draw attention to this year's keynote lecture, from Professor Sir Salvador Moncada, from the University of Manchester, talk entitled 'Aspirin from cardio protection to cancer prevention: Is there a common mechanism?'. As a pre-eminent research leader, we are sure his talk will be of great interest.

Finally, we would like to extend our gratitude to all those who have made the symposium possible, including the numerous staff members who have kindly volunteered to judge and/or Chair sessions. A special thank you must also go to the symposium organising committee, including Leanne Mitchell, Paddy Tighe and Luisa Martinez-Pomares for all their help.

We hope you enjoy the event!

Alessandra Agostini, Fawaz Alassaf, Clare Martin, Omar Mohammed, Raquel Ribeiro and Shivali Kohli

Postgraduate symposium organising committee representatives 2016

Symposium Sponsors

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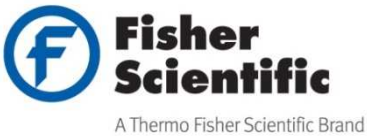

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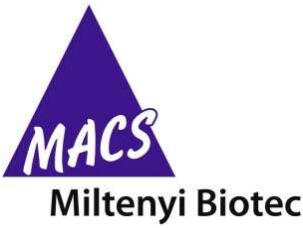

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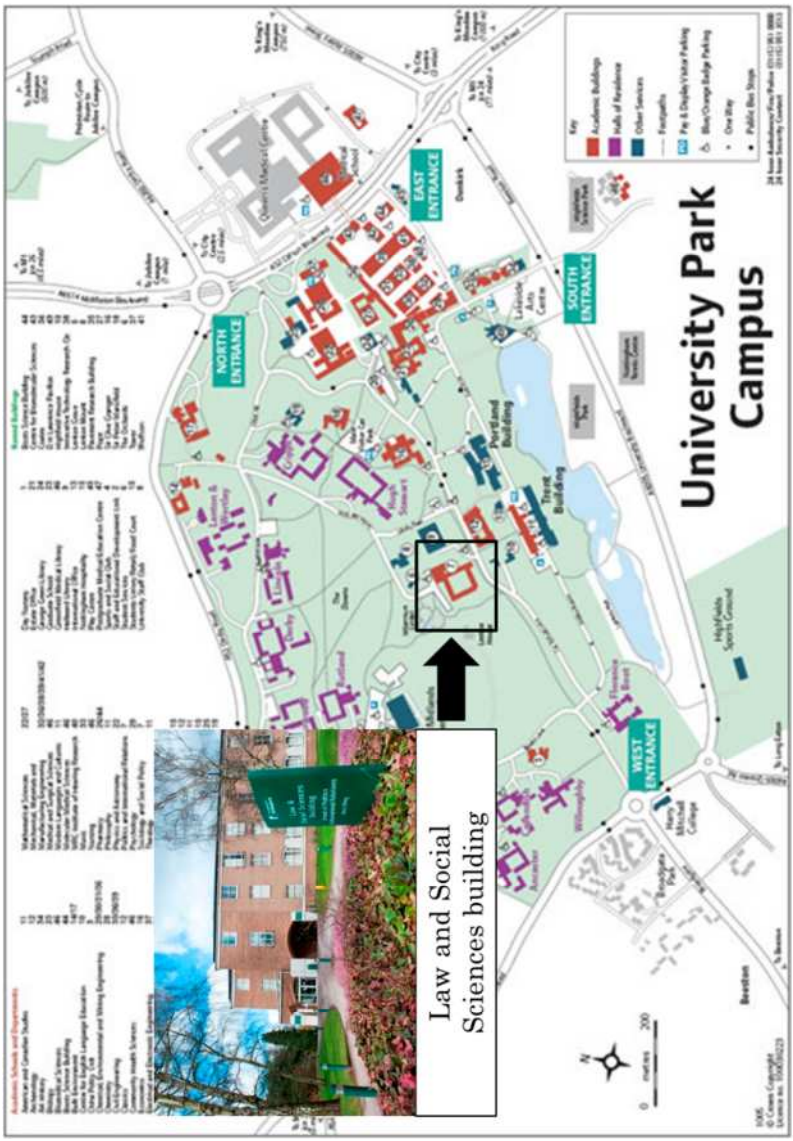
All sessions will be held in the Law and Social Sciences (LASS) building, with the exception of the keynote lecture by Professor Sir Salvador Moncada, our invited speaker, which will take place in Keighton Auditorium.



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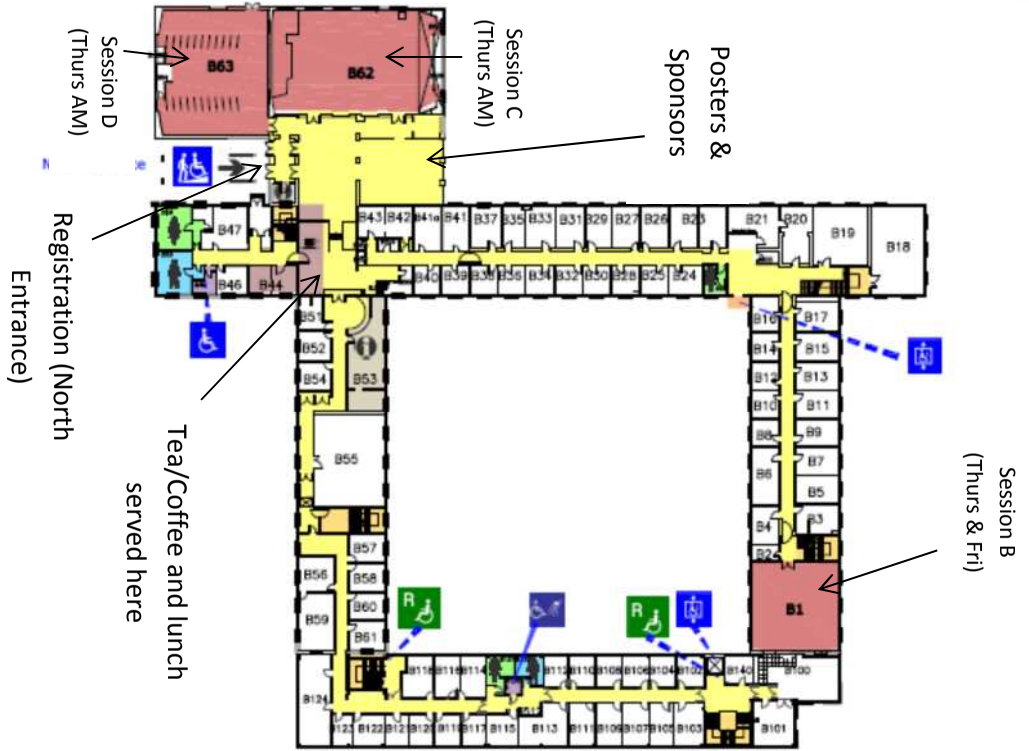

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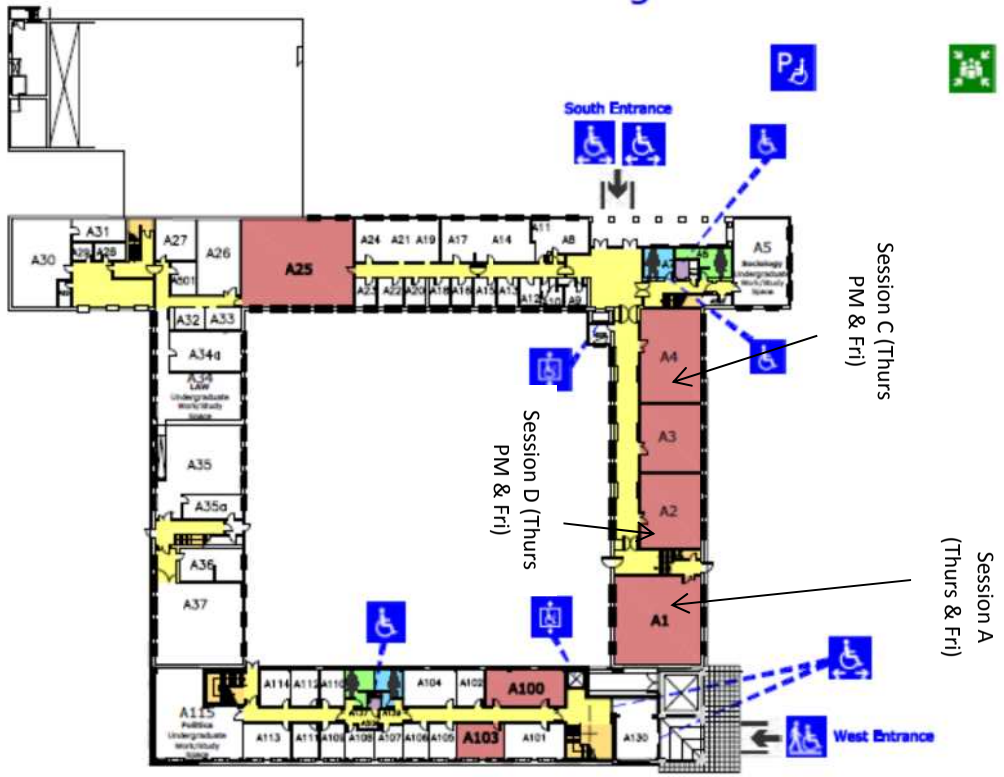

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Oral Presentations: Thursday 14th July

8:30-9:15	**Registration and Poster set-up (Atrium)**			
9:15-9:45	**Welcome Talk: Professor Ian Macdonald (B62)**			
	Microbial Engineering	**Neuroscience**	**Molecular Cell Biology and Development**	**Cell Signalling and Pharmacology**
	SESSION A (A1)	**SESSION B (B1)**	**SESSION C (B62)**	**SESSION D (B63)**
Chair	Thorsten Allers	Nick Holliday	Lynn Bedford	Vincent Wilson
Judge 1	Kim Hardie	Fran Ebling	Rita Tewari	Margaret Pratten
Judge 2	Jean Dubern	John Armour	Andrew Renault	Sebastian Serres
10:00-10:20	Olivier Jack Severn	Andrea Loreto	Natalie Alice Barratt	Abdulla Ahmed
10:20-10:40	Jennifer Spencer	Pongsatorn Meesawatsom	Sirina Muntaka	Raghdan Al-Saad
10:40-11:00	Tom Patrick Wilding-Steele	Peter Gowler		Fawaz Alassaf
11:00-11:30	**Tea/Coffee Break (Atrium)**			
	SESSION A (A1)	**SESSION B (B1)**	**SESSION C (B62)**	**SESSION D (B63)**
Chair	Miguel Camara	Andy Bennett	Alistair Hume	Ian Kerr
Judge 1	Christopher Penfold	Rebecca Trueman	Lucy Fairclough	Paddy Tighe
Judge 2	Frederik Walter	Kim Hardie	Simon Dawson	Angus Brown
11:30-11:50	Elena Geib	Fionn Dunphy-Doherty	Maha Alsayegh	Penny Ensor
11:50-12:10	Alexander Grosse-Honebrink	Shivali Kohli	Huaitao Cheng	Paolo Sanzá
12:10-12:30	Robert Harry Habgood	Matthew Barron	Kim Kenwrick	Mark Soave
12:30-14:00	**Lunch and Poster session A (Atrium)**			

	Microbial Engineering	Neuroscience	Molecular Cell Biology and Development	Genetics, Ecology and Evolution
	SESSION A (A1)	**SESSION B (B1)**	**SESSION C (A4)**	**SESSION D (A2)**
Chair	Katalin Kovacs	Rebecca Trueman	Paddy Tighe	Sara Goodacre
Judge 1	Ying Zhang	Federico Dajas-Bailador	Rob Layfield	Tom Reader
Judge 2	Jess Tyson	Paul Smith	Martin Gering	Will Irving
14:00-14:20	Christian Arenas	Ghayth AbdulRazzaq	Sarah Whipple	Stuart Young
14:20-14:40	Ronja Breitkopf	Kathy Sengmany	James William Chamberlain	Kehinde Olufemi Sowunmi
14:40-15:00	Pawel Piatek	Mei Kee Lee	Amy Louise Slater	James Richard Whiting
15:00-15:20	**Tea/Coffee Break (Atrium)**			
	SESSION A (A1)	**SESSION B (B1)**	**SESSION C (A4)**	**SESSION D (A2)**
Chair	Simon D'archivio	Marie-Christine Pardon	Ian Todd	Paul Scotting
Judge 1	Bill Wickstead	Lynn Bedford	Ian Kerr	Sally Chappell
Judge 2	Katalin Kovacs	Francis Gilbert	Uwe Vinkemeier	Ian MacDonald
15:20-15:40	Robert Patrick William Mansfield	Mohd Fadly Mohd Noor	Deniz Akdeniz	Anne Caroline Barbosa
15:40-16:00	Georgina Hazel Phelan	Noraihan Mat Harun	Mahab Al Jannat	Nzar Ali Ameen
16:00-16:20	Peter Rowe	Fatima Abukunna	Asmaa Sulaiman Al-Bayati	Xiao Xu
16:30-17:30	**Keynote Speaker: Professor Sir Salvador Moncada, University of Manchester (Keighton Auditorium)** **'**Aspirin from cardio protection to cancer prevention: Is there a common mechanism?'			

Poster Presentations: Session A: Thursday 14th July 2016

Name	Poster	Judge
Amanda Elizabeth Walker	1	William Brown
Aisling Burns	2	William Brown
David Stephen Foster	3	William Brown
Elena Moreno	4	Mohammad Ehsaan
Shih-Hung Hseih	5	Mohammad Ehsaan
Amelie Savers	6	Mohammad Ehsaan
George Ashton	7	Paul Williams
Terry Bilverstone	8	Paul Williams
Lorenzo Brusini	9	Paul Williams
Matthew Simon Harold Lau	10	Paul Williams
Johnathon Humphreys	11	Alan Cockayne
Zeenat Bashir	12	Alan Cockayne
Christopher Stead	13	Alan Cockayne
Kazeem Adeyinka Adeboyejo	14	Alan Cockayne
Charlotte Prattley	15	Robert Layfield
Eduard Vico Oton	16	Robert Layfield
Shivam Bhatt	17	Robert Layfield
Brenda Ivonne Canales Coutino	18	Robert Layfield
Ana da Silva	19	Natalie Mack
Sophie Vaud	20	Natalie Mack
Mosaab Elsheikh	21	Natalie Mack
Emily Walker	22	Natalie Mack
Philemon Gyasi-Antwi	23	Catarina Gadelha
Yemisi Oluwatomi Olufemi	24	Catarina Gadelha
Anna Wilsdon	25	Catarina Gadelha
Paolo Pantalone	26	Catarina Gadelha
Nicola Humphry	27	Andy Bennett
Bashir Rumah	28	Alan Cockayne

Oral presentation: Friday 15th July 2016

8:30-9:45	**Registration and Poster set-up (Atrium)**			
	Microbial Engineering	**Neuroscience**	**Molecular Cell Biology and Development**	**Genetics, Ecology and Evolution**
	SESSION A (A1)	**SESSION B (B1)**	**SESSION C (A4)**	**SESSION D (A2)**
Chair	Kim Hardie	Marie-Christine Pardon	Ian Kerr	Tom Reader
Judge 1	Paddy Tighe	Richard Roberts	Ying Zhang	Tamsin Majerus
Judge 2	Sheyda Azimi	Andrew Bennett	Blessing Mukonoweshuro	Stephan Heeb
10:00-10:20	Patrick Samuel Ingle	Valeria Lasio	Hannah May Marriott	Talib Chitheer
10:20-10:40	Michaella Whittle	Ayoub Ali Hussein Al-Bayti	Darren Crowley	Naomi Clement
10:40-11:00	Craig Woods	Entedhar Rabiaa	Alexander Day	Raman Lawal
11:00-11:30	**Tea/Coffee Break (Atrium)**			
	SESSION A (A1)	**SESSION B (B1)**	**SESSION C (A4)**	**SESSION D (A2)**
Chair	Federico Dajas-Bailador	Fran Ebling	Thorsten Allers	Sally Chappell
Judge 1	Neil Oldfield	David Brook	Steve Atkinson	Amir Ghaemmaghami
Judge 2	Karl Wooldridge	Paul Smith	Uwe Vinkemeier	Angus Davidson
11:30-11:50	Lorna Finch	Fatimah Almahasneh	Christopher Mason	Rayan Alansari
11:50-12:10	Florence Jessica Annan	Samuel Bestall	Jennifer McDonald	Emad Dawood Abbas Kaky
12:10-12:30	Ryan John Hope	John William Grzeskowiak	Hala Alhadi Ali Mohamed	
12:30-14:00	**Lunch and Poster session B (Atrium)**			

	Microbial Engineering	Immunology	Molecular Cell Biology and Development
	SESSION A (A1)	**SESSION B (B1)**	**SESSION C (A4)**
Chair	Rebecca Lowry	Ian Todd	Uwe Vinkemeier
Judge 1	Miguel Camara	Lucy Fairclough	Ian MacDonald
Judge 2	Fred Sablitzky	Martin Gering	Alexander Tarr
14:00-14:20	Andrew Dempster	Su Su Htwe	Okechukwu Onianwa
14:20-14:40	Natasha Kinsmore	Asha Hassan	Daniella Spencer
14:40-15:00	Christopher James Hannes Millard	Theocharis Tsoleridis	Carmen Tong
15:00-15:20	**Tea/Coffee Break (Atrium)**		
	Metabolic and Molecular Physiology	**Immunology**	
	SESSION A (A1)	**SESSION B (B1)**	
Chair	Karl Wooldridge	Sonali Singh	
Judge 1	Paul Greenhaff	Luisa Martinez-Pomares	
Judge 2	Simon Dawson	Ola Negm	
15:20-15:40	Hind Alzahrani	Abeer Sharaf	
15:40-16:00	Seyedah Amenah Madjd Jabari	Siti Raudzah Mohamed Kamal	
16:15-17:30	**Prizes/Closing speeches (B62). Social Event (Atrium)**		

Poster Presentations: Session B: Friday 15th July 2016

Name	Poster	Judge
Marta Lopez Morato	1	Andrew MacColl
Gunnar De Winter	2	Andrew MacColl
Michelle Strickland	3	Andrew MacColl
Abdulfatai Tijjani	4	Andrew MacColl
Johnathon Fenn	5	Naglis Malys
Miles Flitton	6	Naglis Malys
Laura Armstrong	7	Naglis Malys
Christopher John Heward	8	Naglis Malys
Tulsi Patel	9	Naglis Malys
Nahed Alharthi	10	Maria Toledo-Rodriguez
Razan Ahmad Moh'd Al-Momani	11	Maria Toledo-Rodriguez
Nuha Anajirih	12	Maria Toledo-Rodriguez
Nada Mahmood	13	Maria Toledo-Rodriguez
Tara Elizabeth Stirland	14	Lilian Nwosu
Hazulin Mohd Radzuan	15	Lilian Nwosu
Jala Alahmed	16	Lilian Nwosu
Mohammed Shahzad Saleem	17	Lilian Nwosu
Nuria Casanova Vallve	18	Simon Dawson
Diego De Medeiros Costa	19	Simon Dawson
Raquel Ribeiro	20	Simon Dawson
Clare Martin	21	Simon Dawson
Charles Greenspon	22	Federico Dajas-Bailador
Amer Imraish	23	Federico Dajas-Bailador
Neville Mvo Ngum	24	Federico Dajas-Bailador
Alessandra Agostini	25	Federico Dajas-Bailador
Helen Taylor	26	Federico Dajas-Bailador
Yousef Erfaida	27	Rudolf Billeter-Clark
Diana Carvalheira Alcobia	28	Rudolf Billeter-Clark
Nickolaj Johannes Angelo Groenewoud	29	Rudolf Billeter-Clark
Aaron Horsey	30	Rudolf Billeter-Clark
Megan Cox	31	Gill Coburn
Afrakoma Afriyie-Asante	32	Gill Coburn
Dennis Kwadwo Kyeremeh Awuah	33	Gill Coburn
Reem Alzahri	34	Gill Coburn
James Bennett	35	John Armour
Marylka Griffiths	36	John Armour
Luke Simpson	37	John Armour
Jessica Stock	38	Ian Kerr
Joseph Lloyd	39	Lilian Nwosu

Oral Presentations: Abstracts - Thursday 14th July

Session A (A1) - Microbial Engineering

Time: 10:00-11:00 am

Olivier Jack Severn

Quorum sensing in Clostridium acetobutylicum: Talking to Bacteria, and producing Biofuel!

The petrochemical industry is no longer fit for purpose. I hope to jump on the sustainability band wagon in order to fund what I love to do, have conversations with bacteria! Bacteria are capable of communicating with one another, one such way is quorum sensing, the ability of bacteria to sense how many of them are present at any time. My project aims to play around with the way Clostridium acetobutylicum senses the number of cells present in order to coerce it into producing more butanol.

C. acetobutylicum has two phases of its growth, the production of acids, which are then re-assimilated and converted to the solvents Acetone, Butanol and Ethanol. For this reason it is called the ABE pathway. The control of this switch from acid to solvent production is poorly understood, and our work aims to show the role quorum sensing plays in it.

Jennifer Spencer

BIOLOGICAL ENGINEERING OF THE THERMOPHILE GEOBACILLUS TO PRODUCE THE ADVANCED BIOFUEL N-BUTANOL

Increased use of renewable energies is of vital importance for the world's climate. Further use of biofuels is required to mitigate the effects of greenhouse gasses and as an alternative to finite fossil fuels. As a waste product of the agricultural industry, rice straw offers an abundant substrate for microbial fermentation. Utilisation of lignocellulosic biomass for biofuel production is an alternative to burning fossil fuels. Agricultural wastes, such as rice straw, can be converted to high value products such as butanol. Butanol is a biofuel and platform chemical. Butanol is an advanced fuel with high energy content and is compatible with existing infrastructure.

Geobacillus thermoglucosidasius is a thermophilic bacterium. Geobacillus with its natural ability to ferment various substrates, including both hexose and pentose sugars, offers advantages as a process organism. As a thermophile, Geobacillus is an industrially favoured organism. Development of new molecular tools for working with thermophiles is required to advance progress for this group of promising industrial process organisms.

Butanol metabolic pathways will be introduced, with the aim of producing Geobacillus strains capable of efficiently generating butanol as a major fermentation product. Metabolic engineering techniques to improve pathway flux will then be employed to optimise fuel production.

Tom Patrick Wilding-Steele

Enhancing the cellulosic capability of biofuel producing clostridia through the creation of designer cellulosomes using synthetic biology.

There is an urgent need for alternatives to using fossil fuels for the production of fuels and chemicals. Large amounts of lignocellulose waste are produced annually, converting lignocellulose to biofuels is an attractive alternative. However, lignocellulose is inherently recalcitrant and currently expensive pre-treatments are needed to break it down, resulting in it being economically inviable. C. acetobutylicum is a potential candidate to convert lignocellulose to biofuels as it is used industrially to produce acetone and butanol and is able to grow on pentose and hexose sugars found in lignocellulose. Previous attempts at engineering C. acetobutylicum to degrade lignocellulose have involved expressing cellulases from C. cellulolyticum. However, the engineered strains were unable to grow on lignocellulose biomass. This was mainly due to the low expression and secretion of several key cellulases in particular the GH48 cellulase. GH48 cellulases are processive cellobiohydrolases and have been shown to be essential for several organisms to grow on crystalline cellulose, including C. cellulolyticum. In this study several different approaches were taken to increase the expression of GH48 cellulases in C. acetobutylicum including; the use of a novel lactose inducible system, screening of GH48 enzymes for expression and activity in C. acetobutylicum, increasing mRNA stability by adding 5' and 3' UTR's and overexpression of several chaperones which assist in protein folding.

Time: 11:30-12:30

Elena Geib

Biosynthesis of an unusual pigment in Aspergillus terreus conidia

Melanins are ubiquitous pigments found in all kingdoms of life. Most organisms use them for protection from environmental stress, although some fungi employ melanins as virulence determinants. Conidia of the human pathogenic fungus Aspergillus fumigatus and of related Ascomycetes produce dihydroxynaphthalene-(DHN) melanin. This melanin protects from UV radiation and inhibits acidification of phagolysosomes after phagocytosis. However, in Aspergillus terreus the biosynthetic origin of the melanin in conidia has remained a mystery because A. terreus lacks genes for synthesis of DHN-melanin. This presentation shows the identification of genes coding for an unusual NRPS-like enzyme (MelA) and a tyrosinase (TyrP) that A. terreus expressed under conidiation conditions. Further studies revealed that MelA produces aspulvinone E, which is activated for polymerisation by TyrP. Functional studies demonstrate that this new pigment, Asp-melanin, confers resistance against UV light and hampers phagocytosis by soil amoeba. Unexpectedly, Asp-melanin does not inhibit acidification of phagolysosomes, which is in agreement with increased survival of conidia under acidic conditions. This supports a long-term persistence of conidia after phagocytosis.

Alexander Grosse-Honebrink

Increasing DNA Transfer Efficiency and Employing Molecular Tools to Increase Solvent Production of Clostridium Pasteurianum

The price of crude glycerol, a waste product of the biodiesel industry, has dropped dramatically in the past years. Today it constitutes a promising and cheap alternative to other substrates used in biotechnology. However, commonly used production organisms are not well suited to produce valuable biocommodities from this substrate. We are, therefore, establishing Clostridium pasteurianum as a novel production strain. It naturally has the ability to produce the solvents butanol and ethanol and the bioplastic precursor 1,3 propanediol from glycerol. Despite increased interest in using C. pasteurianum for glycerol fermentation, until recently no targeted genetic modification techniques were available for this organism due to a lack of adequate and efficient methods of transformation.

Here we present a high efficient transformable mutant which was found by directed screening employing a method which to our knowledge is unreported. We show that one single nucleotide polymorphism is responsible for this phenotype. This hypertransformable strain laid the foundation for employing synthetic biology tools in C. pasteurianum.

We used the strain to produce clean knock-outs of three key enzymes involved in balancing redox levels. Amongst other differences to the wild type two of the mutant strains showed increased butanol yield of up to 5-fold and one showed abolished 1,3-propanediol production.

Robert Harry Habgood

Engineering the thermophile Geobacillus thermoglucosidasius to produce advanced alkane biofuels

The microbial biosynthesis of alkanes from a renewable feedstock, such as agricultural waste, is a promising solution to the problems raised by conventional transportation fuels. Alkane biosynthesis was first discovered in cyanobacteria and has been characterised as the subsequent reactions of two enzymes: a fatty acyl-acyl carrier protein (ACP) reductase (AAR), and an aldehyde deformylating oxygenase (ADO). Several studies have shown the incorporation of a heterologous alkane biosynthesis pathway in mesophilic organisms but significant yields have not been forthcoming. However, an alkane biosynthesis pathway has yet to be introduced into a thermophilic organism, which possess several inherent benefits to mesophilic organisms in biofuel production.

We have demonstrated the thermostability of an AAR and ADO from Thermosynechococcus elongatus BP-1 strain, and have observed the production of large amounts of long-chain fatty alcohol when coexpressing these genes in our plasmid-based expression system for Geobacillus thermoglucosidasius. Smaller amounts of C17 alkene were also observed. The relatively low activity of ADO compared to native oxidoreductases is a constraint to alkane production. Therefore, the identification of the enzyme, or enzymes, responsible for the reduction of fatty aldehyde to long-chain alcohols, and its subsequent knock-out is essential for achieving higher yields of alkane in engineered G. thermoglucosidasius strains.

Time: 14:00-15:00

Christian Arenas

Engineering the Production of 3-Hydroxypropionic Acid Via Malonyl-CoA Pathway in Cupriavidus necator H16

Since efficient and cost-effective conversion of lignocellulosic waste materials remains problematic, gasification of biomass is increasingly considered an attractive alternative, as the resulting gaseous substrates can be utilised by a number of different bacteria as a source of carbon and energy and converted into interesting products. Here, we focus on the well-studied and genetically amenable 'Knallgas bacterium' Cupriavidus necator, which was chosen as a C1-chassis for the production of 3-hydroxypropionic acid (3-HP) from CO2 and H2.

The first step in 3-HP synthesis is the carboxylation of acetyl-CoA to malonyl-CoA, a reaction that is catalysed by the enzyme acetyl-CoA carboxylase (ACC) and tightly controlled at various levels. The second step is the reduction of malonyl-CoA to 3-HP, a conversion catalysed by the bifunctional enzyme malonyl-CoA reductase (MCR) or, in some archaea, by the combination of two monofunctional enzymes which reduce malonyl-CoA first to malonate semialdehyde and then further to 3-HP. However, the generation of efficient and robust production strains remains a major challenge for metabolic engineering.

Genes encoding ACC subunits and MCRs from different bacteria and archaea were codon-optimised, assembled into functional operons and screened for efficient expression in C. necator. Strategies for establishing high-level 3-HP production and the resulting physiological and metabolic consequences for the host are currently being investigated.

Ronja Breitkopf

Factors affecting growth and metabolic pathways in Clostridium autoethanogenum with the main focus on the reductive TCA cycle

There is an increased need to generate fuels and platform chemicals in a more sustainable manner. One of the chemicals believed to have potential in a bio-based, circular economy is succinic acid. Already used in food and pharmaceutical market, it also functions as C4 building block and can therefore supply the basis for high value-added derivatives with applications in the technical and chemical industry.

Using the acetogenic bacterium Clostridium autoethanogenum as a microbial chassis, the proposed research aims to combine the utilisation of exhaust and waste fumes with the fermentative production of succinic acid. A prerequisite for this is a thorough understanding of the existing native metabolic route(s) to succinate, which is already generated by the organism in low amounts, as well as interconnecting pathways. This will be achieved through a combination of enzymatic studies, 13C labelling experiments and gene inactivation/overexpression analyses.

Pawel Piatek

Quorum sensing in industrial fermentation: Characterizing solvent producing C. autoethanogenum

Clostridium autoethanogenum is a Gram-positive, motile and anaerobic bacterium. The rod-shaped organism was first discovered and isolated from rabbit faeces and is capable of autotrophic growth by fixing carbon in the form CO and CO2. Acetogenic organisms such as C. autoethanogenum share the ancient Wood-Ljungdahl pathway, which is integral to carbon fixation, and producing a variety of fuel-viable solvents. Quorum sensing (QS) is cell to cell communication is responsible for concerted, population-wide changes in gene expression and behaviour in response to cell population density. QS is not fully understood in C. autoethanogenum, and two signal peptide components of the agr system have been deleted. Using in-frame gene deletion, three mutants were created; One from each signal peptide gene, and another with both genes knocked out. Phenotypic studies of these mutants have shown distinct solventogenic profiles. Relative to the WT control, the double knock-out mutants have shown a 4.5-fold increase in ethanol, 40-60% reduction in acetate concentration, and full utilisation of fructose. Single knockout mutants exhibit similar solvent concentrations to WT. Further differences in double knock-out mutants are that CO2 gas pressure is 5-fold higher in serum bottles when compared to the single mutants and WT control. CO2 re-assimilation is a trait commonly found in the WL pathway, via Acetyl-CoA Synthase. These findings suggest that QS may play a role in manipulating the WL pathway.

Time: 15:20-16:20

Robert Patrick William Mansfield

The Road Trip to Industrial Application

Carbon monoxide (CO), carbon dioxide (CO2), methane (CH4) and other single carbon (C1) compounds are attractive feedstocks for the production of fuels and platform chemicals. C1 compounds can be 'fixed' through fermentation processes driven by microbial catalysis to produce these desirable products. Development of industrially applicable microorganisms is an essential part of maximising the utility and economic viability of these processes. The present project centres on the implementation of a 'road map' for development of acetogenic bacterial strains towards industrial application. We investigate methods to improve the amenability of strains to genetic manipulation, establish tools for genome engineering and expand strains natural capacity for solvent production. Specifically, we explore novel methods of overcoming barriers to transformation and conjugation in C. carboxidivorans, and introduce synthetic metabolic pathways to E. limosum to introduce the iso-propanol production capabilities.

Georgina Hazel Phelan

The use of forward and reverse genetics to select Clostridium strains more tolerant to bio-butanol and cellulosic feedstock inhibitors

The focus of this research, to make the production of the biofuel (n-butanol (1)) viable through increasing the tolerance of Clostridium strains to both butanol and feedstock inhibitors, will lead to much needed alternatives to fossil fuels and their negative effects. Solvent-producing Clostridia have always been the most important species when producing butanol and indeed the first industrial cultures to be isolated and patented for large scale production. This research focuses on improving the molecular tools used in the manipulation of the chosen Clostridial strain, Clostridium sacchrobutylicum (NCP262, the type I strain and NCP258, the type II strain).

Methylation to overcome type I restriction systems, conjugational methods, developing electroporation, the use of ClosTron technology and novel assembly of plasmids all have formed important parts of this task. Further work will utilise ACE and CRISPR knockouts and the TraDIS system to build a comprehensive gene library from which screening can take place and selection for better tolerance.

Peter Rowe

Acetogenic engineering

Due to their ability to convert C1 gases to commodity chemicals, acetogenic bacteria have increasingly become the subject of interest both academically and industrially. Naturally the main objectives of studying these gas-utilizing bacteria is to understand and improve the mechanisms governing chemical production, for which metabolic and genetic engineering techniques are vital.

In the case of this project, Clostridium autoethanogenum is used as a chassis to produce ethanol, 2,3-butanediol and acetate. Despite the potential of this chassis, strain development has been limited due to poor DNA transfer and genome editing tools. Conventional tools for generation of knock-outs in the Clostridial genus are ClosTron™ and ACE (Allele-Coupled Exchange). However, both have significant draw-backs, namely the inability to knockout multiple genes for the former, and the length of time for mutant generation with the latter.

The paradigm shift in genome editing caused by CRISPR-Cas9 has led to huge advancement in the speed and accuracy of mutant generation across a broad-range of organisms. Here we demonstrate how we have modified this technology for use in C. autoethanogenum, and outline how we plan to use it for the increase of ethanol production.

Session B (B1) - Neuroscience

Time: 10:00-11:00 am

Andrea Loreto

NAD-precursor NMN and axon degeneration: downstream mechanisms and in vivo relevance

Wallerian degeneration (the degeneration of the axon after an injury) is a widely used model to comprehend mechanisms underlying axon degeneration. Axotomy leads to rapid depletion of the axonal NAD-biosynthetic enzyme NMNAT2 and high levels of its substrate NMN, followed by axonal fragmentation. We proposed a key role for NMN in Wallerian degeneration supported by pharmacological and genetic data showing that reduction of NMN levels, by inhibition of NMN-synthesising enzyme NAMPT or expression of the bacterial NMN-scavenging enzyme NMN deamidase, robustly delays Wallerian degeneration. Here we show that downstream events of NMN accumulation include a late increase in intra-axonal Ca2+. NMN requires the presence of the recently identified pro-degenerative protein SARM1, a Toll-like receptor adapter, to induce Ca2+ rise and axon degeneration. Despite preserving axonal integrity, inhibition of NMN synthesis and SARM1 deletion fail to prevent early mitochondrial dynamic changes. Furthermore, depolarizing mitochondria does not alter the rate of Wallerian degeneration. We also show that scavenging NMN strongly delays Wallerian degeneration in vivo. These data corroborate the key role for NMN in promoting axon degeneration in vivo. They reveal that NMN and SARM1 act in a common pathway culminating in intra-axonal Ca2+ rise and fragmentation. Finally, they indicate that mitochondrial dysfunction plays a negligible role in the NMN-SARM1 pathway controlling Wallerian degeneration.

Pongsatorn Meesawatsom

Differential Modulation of Spinal Nociceptive Processing by Aspirin-triggered Resolvin D1 in Rat Pain Models

Aspirin-triggered resolvin D1 (AT-RvD1) has robust analgesic effects in behavioural models of pain, however the potential underlying spinal neurophysiological mechanisms contributing to these effects in vivo are yet to be determined. This study investigated the effects of spinal AT-RvD1 on evoked responses of deep dorsal horn wide dynamic range (WDR) neurones in vivo in carrageenan-induced inflammatory or monosodium iodoacetate (MIA)-induced osteoarthritic pain. At timepoints of established pain behaviour, neuronal responses to transcutaneous electrical stimulation of the hindpaw were recorded pre and post direct spinal administration of AT-RvD1. AT-RvD1 (15 ng/50□l) significantly inhibited neuronal responses at C-, A□-fibre intensities, wind up and post-discharge responses in carrageenan-treated animals, compared to pre-drug response ($p<0.05$). AT-RvD1 did not alter evoked neuronal responses in non-inflamed or MIA-treated rats. Electrophysiological effects in carrageenan-inflamed rats were accompanied by a significant increase in mRNA for chemerin receptor and 5-lipoxygenase activating protein (FLAP) and a decrease in 15-lipoxygenase mRNA in spinal cord of the carrageenan group. Our data suggest that peripheral inflammation mediated changes in spinal FLAP expression may contribute to the novel inhibitory effects of spinal AT-RvD1 on WDR neuronal excitability. Inflammatory driven changes in this pathway may offer novel targets for inflammatory pain treatment.

Peter Gowler

Preclinical evidence for a role of BDNF in a model of OA pain

There is a clear need to understand the mechanisms behind chronic joint pain, and to identify novel therapeutic targets. Recent studies have explored the contribution of brain derived neurotrophin (BDNF) to osteoarthritis (OA) related joint pain.

Naive rats received intra-articular (IA) injection of either 10ug, 1ug, or 100ng BDNF, or 0.9% saline. Pain behaviour was measured for 7 days post injection. OA was induced in rats via IA injection of 1mg MIA. Once OA pathology had been established animals received IA injections of either 10ug BDNF, 10ug NGF or 0.9% saline. Pain behaviour was then measured for up to 2 weeks. Rats with MIA induced joint pain received IA injections of either trkBFc chimera or IgG control, and pain behaviour was measured for up to 7 days.

IA injection of either 1ug or 10ug BDNF in naive rats did not change pain behaviour when compared to the saline control. However, in rats with MIA induced OA joint pain, IA injection of 1ug BDNF significantly increased weight-bearing asymmetry and decreased hindpaw withdrawal threshold 4 days post injection compared to saline controls. IA injection of 100ng trkBFc chimera also reversed MIA induced OA joint pain.

Rats with MIA induced OA showed a greater behavioural response to BDNF than naive animals. Localised anti-BDNF treatment also reversed chronic joint pain as induced by MIA injection. This suggests that there may be an upregulation of BDNF's receptor TrkB in the periphery in the osteoarthritis disease.

Time: 11:30–12:30

Fionn Dunphy-Doherty

Does social isolation from weaning result in 'depressive-like' behaviour in the rat.

It is well established that exposure to unpredictable early-life stress is a risk factor in the aetiology of several common psychiatric conditions including anxiety-related disorders, depression, schizophrenia and PTSD. Post weaning social isolation (SI) of rat pups produces robust neurodevelopmental changes in cognition and hippocampal function akin to schizophrenia and depression, making this is a useful model to examine the pathophysiological basis of psychiatric disorders. This study used a behavioural task battery to record SI-induced changes in anxiety, cognition and physiological response to restraint stress together with immunohistochemical markers of hippocampal neurogenesis shown to decrease in depression. Our previous research has shown antipsychotics reverse several components of the isolation syndrome in rats but few studies have investigated sensitivity to antidepressants. This study determined whether chronic administration of the selective serotonin reuptake inhibitor, fluoxetine (FLX), or acute treatment with the NMDA receptor antagonist, ketamine, altered development of the isolation syndrome and neurogenesis, to further characterise the predictive validity of this neurodevelopmental model to abnormalities relevant to depression. SI produced cognitive deficits some of which were reversed by treatment with chronic FLX or acute ketamine. However the drugs had differential effects on neurogenesis suggesting this was unrelated to reversal of cognitive deficits.

Shivali Kohli

Reversing social deficits in a two-hit neurodevelopmental rat model of schizophrenia

N-Methyl-D-Aspartate (NMDA)-receptor hypofunction may contribute to the social deficits seen in schizophrenia. Injection of NMDA-receptor antagonist, phencyclidine (PCP), in rat pups, followed by isolation-rearing (SI) from weaning induces behavioural changes in adults akin to symptoms seen in schizophrenia. This study determined if chronic administration of the Glycine-Transporter (GlyT1)-inhibitor RO4993850, could influence social deficits in this model.

Sixty-eight male Lister-hooded rat pups received 10mg/kg PCP or 2ml/kg saline s.c. on post-natal days (PND) 7, 9 and 11, before weaning PND 23 into single (PCP-SI) or group (SAL-GH) housing for 5 weeks. Development of 'behavioural syndrome' was assessed by novel arena locomotor activity (LMA) before injection of RO4993850 (3mg/kg) or vehicle 4ml/kg i.p. for 14 days. Rats were paired for social interaction, during which ultrasonic vocalisations (USVs) were recorded (a marker of communicative interaction). Post-trial, hippocampal parvalbumin-positive GABA interneurons (which are reduced in schizophrenia) were quantified.

Early-life interventions induced social impairments, altered communication and reduced the number of hippocampal parvalbumin-positive interneurons in the adult rat. Chronic treatment with RO4993850 attenuated some of these social deficits but did not influence parvalbumin-immunoreactivity. GlyT1 inhibitors therefore, are potential therapeutics for social deficits seen in disorders such as schizophrenia.

Matthew Barron

An acute systemic immune challenge rapidly reduces tau phosphorylation in hTau mice expressing murine tau

Human tau (hTau) mice, void of murine tau (mTau), express all 6 human tau isoforms. hTau mice, exhibiting tau pathology akin to Alzheimer's disease (AD), show an isoform ratio imbalance. To restore the isoform imbalance to further resemble AD, mice were bred on mTau+/- background. Consequently, the impact of isoform ratio on lipopolysaccharide (LPS)-induced tau phosphorylation was assessed.

Food burrowing was assessed in 3-month male wild-type, mTau+/-, mTau-/-, hTau/mTau+/- and hTau/mTau-/- mice prior to LPS administration: 0, 100, 250 or 330µg/kg (i.v., n=8-9). Susceptibility to LPS-induced "sickness" behaviour and spatial working memory was assessed in the spontaneous alternation task 4 hours following. Pathological tau was quantified through immunoblotting (n=6).

Both hTau genotypes possessed comparable, impaired, food burrowing behaviour. LPS inhibited locomotor activity, indicative of LPS-induced "sickness" syndrome. hTau/mTau+/- mice exhibited an improved isoform ratio and increased tau phosphorylation compared to hTau/mTau-/- mice. Surprisingly, LPS decreased tau phosphorylation in hTau/mTau+/- mice at 250 and 330µg/kg.

hTau/mTau+/- mice exhibit similar behavioural impairments, a greater AD-relevant isoform ratio with increased tau phosphorylation, of which LPS reduced, again providing a dual role of inflammation in AD.

Time: 14:00–15:00

Ghayth Muyassar AbdulRazzaq

N-arachidonoyl glycine (NAGly) and GPR18 as a novel ligand: orphan GPCR pairing

Background. GPR18 is a candidate cannabinoid receptor with potential as novel therapeutic target. NAGly had been suggested by many studies as an endogenous ligand of GPR18. Yet some studies have reported a lack of activation of GPR18 by NAGly.

Aims. To study the pharmacology and signalling routes of GPR18 in a recombinant system. To define the involvement of GPR18 in NAGly-evoked responses in INS-1 832/13 β cells.

Methods. SNAP-tagged human GPR18 receptor under the control of a tetracycline regulated expression system was heterologously expressed in HEK293TR cells. The effect of NAGly and Δ9THC on calcium mobilization was assessed in transfected cells and in β-cells loaded with fluo-4 AM dye using a FlexStation. NAGly induced ERK phosphorylation was quantified by immunoblotting.

Key Results. NAGly and THC did not induce noticeable calcium mobilization or ERK1/2 phosphorylation comparing to the positive controls in GPR18-transfected HEK293TR cells. NAGly increased total calcium mobilization in β cells.

Conclusion. In this study, recombinant GPR18 was not activated by NAGly, which suggests that NAGly may not be the direct agonist or that the activation of GPR18 may involve other signalling pathway not examined in the current study. Screening of β-cell line for the presence of GPR18 mRNA and further investigation for cells endogenously expressing GPR18 may provide a better understanding of the elements involved in GPR18 signalling.

Kathy Sengmany

Biased allosteric agonism and modulation of metabotropic glutamate receptor 5: implications for optimising preclinical neuroscience drug discovery

Emerging evidence for CNS disorders with glutamatergic dysfunction suggests the metabotropic glutamate receptor subtype 5 (mGlu5) is a promising target. Current mGlu5 allosteric modulators have been classified based solely on modulation of intracellular calcium (iCa2+) responses to orthosteric agonists, resulting in a narrow classification of ligands, and potential unappreciated signalling bias. We assessed seven diverse mGlu5 allosteric modulators across iCa2+, inositol phosphate (IP1) accumulation and phosphorylation of extracellular signal-regulated kinases (pERK1/2), to explore their potential for engendering biased agonism and/or modulation. Relative to the reference orthosteric agonist, DHPG, all seven allosteric ligands exhibited biased agonism in HEK293A cells stably expressing mGlu5, coupling more strongly to IP1 accumulation and pERK1/2 over iCa2+. These bias profiles were generally recapitulated in cortical neuron cultures. Relative to DHPG, VU0424465 showed significantly greater potency at IP1 accumulation, as well as pERK1/2. Interestingly, VU0360172, which showed no agonism for iCa2+ mobilisation in recombinant cells, showed robust agonism for both IP1 and pERK1/2.Application of an operational model of agonism allowed quantification of biased agonism across mGlu5 signalling pathways relative to DHPG. Unappreciated biased agonism and modulation may contribute to unanticipated effects when translating from recombinant systems to preclinical models.

Mei Kee Lee

Tiger Milk Mushroom relaxes airway

Lignosus rhinocerotis, or Tiger Milk Mushroom, is a medicinal mushroom which has been used in folk medicine in Southeast Asia to treat asthma and chronic cough for centuries. Whilst emerging studies have demonstrated Lignosus rhinocerotis's safety profile in animals to date, there is limited scientific data available to support the most popular traditional use in asthma and cough. This study aims to investigate the direct relaxant effects of the mushroom cold water extract on the rat isolated airways.

Cold water extract was subjected to organ bath experiment using tracheal and bronchial rings from male Sprague-Dawley rats. The extract caused marked relaxation in rat airway segments pre-contracted by carbachol, concentration- and time-dependently. In subsequent experiments, pre-incubation of the extract was found to significantly reduce the Emax of both carbachol and 5-hydroxytryptamine cumulative concentration response curves. Results revealed that the contraction responses of both drugs were mainly dependent on extracellular calcium ion. In addition, pre-incubation of cold water extract in rat airway has significantly reduced the contraction induced by reintroduction of calcium ion.

We are the first group to report that Lignosus rhinocerotis relaxes rat airway smooth muscle in concentration- and time-dependent manner. Altogether, the results suggest that the airway relaxation effect is mainly mediated by effect on the entry of extracellular calcium ion.

Time: 15:20 –16:20

Mohd Fadly Mohd Noor

Effects of Cannabinoid CB1 Receptor Activation upon Exercise-induced Insulin Stimulated Glucose Uptake in Human Primary Skeletal Muscle Myotubes

Endocannabinoid system (ECS) plays an important role in the regulation of energy balance and metabolism. Studies showed that the activation of cannabinoid receptor type-1 (CB1) may lead to increase in food intake, weight gain, obesity and impairment in glucose tolerance. The aim of this study is to examine the effects of CB1 activation upon exercise-induced insulin stimulated glucose uptake in human primary skeletal muscle myotubes.

We have developed a contractile myotubes model using electrical pulse stimulation (EPS) which demonstrates elevation in glucose transporter 4 (GLUT4), together with increased in insulin stimulated glucose uptake by the cells after EPS. Activation of CB1 by Arachidonoyl 2'-Chloroethylamide (ACEA) resulted in significant reduction in insulin stimulated glucose uptake and this effect was reverted with pre-treatment with CB1 antagonist: Rimonabant (RIM). Protein expression by western blotting revealed the activity of Akt/protein kinase B in insulin signalling pathway was inhibited by ACEA and this may suggest the mechanism of the inhibition of glucose uptake.

In conclusion, activation of CB1 by ACEA inhibit exercise-induced insulin stimulated glucose uptake in human primary skeletal muscle myotubes via inhibition of Akt/protein kinase B activity and the effects was blunted by pre-treatment with RIM.

Noraihan Mat Harun

Role of Fatty Acid Binding Proteins in Pain Signalling in Rat Dorsal Root Ganglia

The main objective of this study is to investigate the interaction of FABPs with TRPV1 and PPARs in modulating nociceptive effects. The subpopulation distribution of these different types of FABPs and PPARs in rat DRG sections was determined by in situ hybridization (ISH) together with Taqman qPCR. In addition, protein-protein interactions of FABPs and PPARs in response to FABP activators and inhibitors need to be defined by Bimolecular fluorescence complementation (BiFC) assays. Furthermore, the differential effects of PPAR agonists on inflammatory genes induced by lipopolysaccharides or flagellin in rat DRG cultures were investigated by TaqMan Low Density Array (TLDA).

ISH revealed that TRPV1 mRNA was mainly expressed in small-fiber neurons. However, the expression of FABPs and PPARs in different population of cells in DRG was not conclusive. Therefore, separation of neuronal and glial cells from mixed rat DRG culture was done to study the mRNA expression of FABPs and PPARs in this different type of cells. Meanwhile, TLDA showed PPAR agonists downregulate various inflammatory mediators after inflammatory induction via activation of TLR4 or TLR5 by LPS or flagellin, respectively. Most of the inflammatory genes were equally inhibited by all three PPAR agonists in LPS-induced group but unequally inhibited in flagellin-induced group which suggest the anti-inflammatory effects of PPAR agonists might be influenced by specific inflammatory signalling pathway.

Fatima Abukunna

Human Stem Cell-derived Hepatocytes (an in vitro model for Human Liver)

The liver has a remarkable ability to regenerate to repair tissue damage; in severe liver injury hepatic progenitor cells (HPCs) are activated giving rise to both hepatocytes and biliary epithelial cells.
HPCs hold great promise as a proliferating and genetically stable source of cholangiocytes and hepatocyte-like cells for medical use.
Their potential to be utilized in cell-based therapies, in vitro drug testing and disease modelling underlines the need to produce high quality, homogeneous and functional populations of human HPCs.
Our objectives are production of physiologically relevant human liver models from HPC-derived human liver organoids and to scale-up production for use in both in-vitro drug testing and disease modelling.
Ethical approval was obtained from the University of Nottingham Medical School Research Ethics Committee. Primary human liver tissues were obtained with informed consent from patients undertaking partial hepatectomy. IHC, q-PCR, FACS and were used to identify, isolate and characterise HPCs and mature liver cell populations.
HPC cells formed an expandable human liver organoids, expressing CD133, CD24, Lgr5, TROP2, EpCAM, CK19 and HNF4α.
Differentiation of organoids resulted in the loss of progenitor cell marker expression whilst the expression of differentiated hepatocyte- specific genes was evident.
Further investigation will involve the detailed analysis of differentiated organoids at gene expression and metabolomic levels.

Session C (B62) - Molecular Cell Biology and Development

Time: 10:00-11:00

Natalie Alice Barratt

Unraveling the relationship between quorum sensing, type III secretion and auto-aggregation.

The Ysc-Yop type III secretion (T3S) system is an important feature of Yersinia pseudotuberculosis, which is a hypodermic-needle-like injectisome that translocates Yop effector proteins directly into eukaryotic cells triggering apoptosis. This secretion system is encoded on the pYV virulence plasmid and controlled by quorum sensing (QS). QS is the population density dependent regulation of gene expression through detecting autoinducer signal molecules.

The T3S system, pYV and QS have been linked to a form of auto-aggregation. The wild type and QS negative strains containing pYV auto-aggregate, with the QS mutant aggregating more. However, both strains lose this phenotype when cured of pYV.

This project aimed to investigate the relationship between these inter-linking systems, the auto-aggregation phenotype was analysed in wild type and QS negative strains, with and without pYV, as well as strains with mutations in genes of the secretion system.

Using different mutations, some of the required elements of the injectisome for auto-aggregation have been identified, including the proteins associated with needle formation. Combinations of mutations of QS and the T3S system had slightly increased auto-aggregation ability.

Sirina Muntaka

IFN-γ and IL-17A establish the balance between P. aeruginosa clearance and inflammatory potential during infection of human macrophage-neutrophil co-cultures.

Neutrophils are essential for protection against extracellular bacteria but neutrophil-dominated inflammation can also cause tissue damage. Optimal anti-bacterial immunity should harness the microbicidal activity of neutrophils while minimising their potential for causing injury. Neutrophils do not act in isolation, during inflammation, they respond to cues from other cells such as macrophages that influence their activation and life span. In vivo models have dominated the study of anti-bacterial immunity, hence the need of human models for the dissection of phagocytes communication during bacterial infection. This work describes the use of a human macrophage-neutrophil infection assay to model Th1 (IFN-γ) and Th17 (IL-17A)-driven microbicidal activity and inflammatory potential. Results show that; bacterial killing was reduced by IFN-γ and promoted by IL-17A. Also, macrophages and neutrophils specifically collaborated for the production of IL-1β and IL-1α and IFN-γ-treated co-cultures generated significantly less IL-1β and IL-1α compared with IL-17A treated. This effect was not observed with other cytokines such as TNF-α, IL-6, MIP-1α and MCP-1. Thus phagocyte co-cultures provide a suitable model of human anti-microbial immunity and unveil an unappreciated collaboration between macrophages and neutrophils in the promotion of IL-1-mediated inflammation which is quenched in the presence of IFN-γ.

Time: 11:30-12:30

Maha Alsayegh

Understanding molecular mechanisms of DEF6 function in immunological synapse formation and its role in mRNA translation control

DEF6 is a guanine nucleotide exchange factor for Rho GTPases that is predominantly expressed in T cells. DEF6 has been shown to regulate cell adhesion, Ca2+ flux and transcription factor activity upon T cell receptor-mediated signalling thereby orchestrating T cell-mediated immunity. While in resting Jurkat T cells, DEF6 associates with polysomes, a phospho-mimic mutant forms cytoplasmic granules co-localising with mRNA decapping enzyme subunit 1 a marker of P-bodies implicating DEF6 in the control of mRNA translation, stalling and/or degradation. N-terminal truncation mutants as well as the corresponding phospho-mimic mutants of DEF6 lacking either the C-terminal coiled coil domain and/or the pleckstrin homology domain also co-localise with DCP1 suggesting that the N-terminal end of DEF6 is sufficient for P-body association. Furthermore, we establish that upon TCR-mediated activation, DEF6 co-localises with eukaryotic translation initiation factor 4E as well as polyA-binding protein but not with the eIF4E-binding protein 4E-T that is a component of the mRNA decay machinery in the immunological synapse. Co-immunoprecipitation experiments suggest however, that DEF6 might not interact directly with either eIF4E or the Cap 5' mRNA but might be in close proximity to members of the translation machinery. Together these results suggest that DEF6 can shuttle between polysomes and P-bodies and might be involved in the regulation of mRNA translation in the immunological synapse.

Huaitao Cheng

Structure Analysis of DEF6 P-body formation

DEF6 is a multidomain protein that acts as guanine nucleotide exchange factor activating Rho GTPases thereby regulating actin polymerisation. DEF6 is highly expressed in T cells where it is indispensable for TCR-mediated signaling and down-stream transcriptional activation of specific immune response genes. Upon activation, DEF6 is recruited through phosphorylation by LCK on Tyr133/144 to the immunological synapse (IS). In contrast, phosphorylation of Try210/222 through ITK appears to result in DEF6 co-localising with mRNA decapping enzyme subunit 1 (DCP1), a marker of P-bodies that are formed in the cytoplasm either result in stalling of mRNA translation and/or mRNA degradation. To further dissect the role of the various DEF6 domains in cellular localisation and function, wild type and mutant DEF6 proteins were generated by site-directed mutagenesis and expressed as GFP fusion proteins in COS7 or Jurkat T cells. We show that the N-terminal 45 amino acids of DEF6 are sufficient to target to P-bodies. The immunoreceptor tyrosine-based activation motif (ITAM) or the pleckstrin homology (PH) domain are diffusely localised in the cytoplasm. The C-terminal coiled coil domain facilitates formation of large DEF6 aggregates that seem to attract P-body marker, DCP1. Under stress conditions however, both ITAM and coiled coil domains seem to facilitate P-body localisation. Further studies are underway to determine which domains are required for IS localisation and actin polymerisation.

Kim Kenwrick

Quantifying the effective range of in vivo signals regulating cell migration.

In order to understand the effects of extracellular signalling molecules it is important to have quantitative information about their range of influence. However, this can be difficult to assess in the dynamic three-dimensional environment in vivo, where cells can encounter multiple, concurrent signals. In Drosophila, the migration of primordial germ cells during gonadogenesis requires spatial information provided by the diffusible signals of two separate pathways: Wunen and HMG-CoA reductase (HMGCR). While the Wunen pathway effectively repels the germ cells by rendering their local environment unfavourable for survival, the HMGCR signal is thought to be one of attraction. However, previous HMGCR misexpression studies produced a germ cell mismigration phenotype that showed only limited evidence of attraction. Here, we examined the requirement of HMGCR expression for germ cell attraction and determined the effective range of its signal in vivo. To do this, we expressed GFP tagged HMGCR in embryos otherwise null for somatic HMGCR. Expression was restricted to a parasegment positioned anterior to the migrating germ cells, which was not on the migratory route. By using germ cell positioning relative to the ectopic domain we observed that HMGCR does produce germ cell attraction and were able to quantify the effective range of its signal in vivo.

Time: 14:00-15:00

Sarah Whipple

Unravelling the host-parasite interface of African trypanosomes

To survive in the host bloodstream, the extracellular parasite Trypanosoma brucei must perform critical surface functions such as nutrient uptake and secretion whilst evading the host immune response. To achieve this, the parasite surface is compartmentalised so that all endocytosis and its invariant receptors are restricted to a specialised invagination of the plasma membrane defined by the flagellum – the flagellar pocket (FP). The overall requirement of the FP for virulence, and the essentiality of the only two receptors characterised to date, make other components of the FP attractive therapeutic targets. Our lab has identified novel proteins that localise to the FP and their topologies resemble those of receptors and transporters. Here we begin to functionally characterise these molecules by RNA interference. Without pressure from the host immune system, parasite survival was not affected by ablation of individual FP proteins, and we are now developing an in vivo genome-wide lethality screen to determine their essentiality in the host. To perform their functions in the secluded environment of the FP, these receptors and transporters access host macromolecules through a 'channel' that connects the FP lumen to the extracellular space. This route of entry requires a molecular motor to transport membrane-associated material across the channel. We have identified a novel kinesin implicated in this process and its role in FP function and parasite fitness will be discussed.

James William Chamberlain

The trypanosome telomeric expression site one ESAG at a time

Trypanosoma brucei lives exclusively extracellularly in the mammalian host bloodstream, in full view of the immune system. To evade the host humoral response the parasite is covered by a major surface antigen called variant surface glycoprotein (VSG), which is periodically switched to an immunologically distinct variant. VSG is transcribed solely from one of around 15 subtelomeric bloodstream expression sites (BES), which also encode a group of expression site associated genes (ESAGs), the functions for most of which remain unknown. Given that these genes are co-transcribed and switch along with VSG, they are thought to be important for parasite survival. ESAGs form 14 distinct families, encompassing the BES ESAGs and non-BES genes related to ESAGs (GRESAGs). Most ESAGs localise to the cell surface, where they may play roles in host-parasite interactions. Here we use endogenous-locus tagging, RNA interference and gene knockout to investigate the essentiality of individual ESAGs and also whole families. I show that ablation of the BES ESAG does not impair parasite proliferation, while silencing whole families results in cell growth impairment. These findings suggest a redundancy mechanism between the BES ESAGs and their GRESAG counterparts, and that, as a family, they are essential to the survival of the parasite in vitro. It remains the question as to why the parasite evolved to contain non-essential genes in such a privileged genomic location.

Amy Louise Slater

Uncovering the regulatory mechanisms underpinning cell-to-cell signalling and type three secretion in Yersinia spp.

Yersinia species uses AHL Quorum sensing (QS) to regulate gene expression in response to population density. A two part system of diffusible signal molecules (AHLs) working in conjunction with signal transducers results in changes in gene expression upon reaching a population-dependent threshold concentration. The virulence genes of the pathogen Yersinia pseudotuberculosis, located on the pYV plasmid, represent one system under the control of QS. These genes encode the components of the type 3 secretion (T3S) system, a hypodermic needle-like structure which translocates Yersinia outer proteins (Yops) into the host cell where they trigger apoptosis. Thermo dependent regulation of the T3S system requires a chromosomally encoded histone-like protein YmoA that prevents expression below 37oC and pYV-encoded LcrF, a transcriptional activator of many pYV-associated genes.

To further understand the relationship between T3S-dependent virulence and QS, the expression of lcrF and ymoA was monitored through promoter fusions using a lux-based reporter cassette in mutants of the QS genes ytbI, ypsI, ypsR and ytbR, where I genes are AHL synthases and R genes are signal transducers. ymoA expression was greatly upregulated by all QS genes whereas lcrF is downregulated by the ypsIR QS system and upregulated by the ytbIR system. These data illustrate that QS is a key player in the complex thermo-regulation of virulence and that cell density is an important trigger for activating virulence.

Time: 15:20-16:20

Deniz Akdeniz

Investigating the roles of DEF6a and SWAP70b in zebrafish embryonic development and the role of DEF6 in T cells

Def6 and Swap70 are guanine nucleotide exchange factors (GEFs) for Rho GTPases. Mammalian Def6 and Swap70, predominantly expressed in mature T and B cells, respectively, have been shown to play a role in cell activation, differentiation, migration and cell responses in the immune system. Def6 is recruited to the immunological synapse (IS) upon T cell receptor (TCR)-mediated activation reorganising the actin cytoskeleton and cell polarity. However, function and interacting partners of Def6 during TCR signalling are poorly understood. Therefore, in an attempt to establish a Def6 interactome in T cells, the BioID-mediated biotinylation of proximal and interacting proteins, is being applied. The promiscuous biotinylation of interacting and proximal proteins of full-length-Def6 in T cells was carried out for identification of biotinylated proteins by mass spectrometry. In addition, zebrafish orthologous, Def6a and Swap70b, have been shown to act downstream of Wnt5a or Wnt11 signalling pathway, respectively, regulating cell movements during gastrulation. To further elucidate the function of Def6a and Swap70b underlying the non-canonical Wnt signalling pathway, Transcription Activator-Like Effector Nucleases (TALENs)-induced mutagenesis was employed to establish mutant zebrafish lacking either Def6a or Swap70b. Heterozygous qmc811 Def6a and qmc809 Swap70b mutant lines were established for further phenotypic analysis.

Mahab Al Jannat

Exploring the moonlighting activities of meningococcal enolase, peroxiredoxin and DnaK

Neisseria meningitidis colonizes the human nasopharynx and occasionally spreads via the bloodstream to the meninges, causing life-threatening meningitis and sepsis. Enolase, peroxiredoxin and DnaK, were previously described as moonlighting proteins that are expressed on the surface of meningococci, where they can bind human plasminogen (Plg). To further explore the role of these proteins in the pathogenesis of meningococcal disease, the genes encoding enolase, peroxiredoxin and DnaK were amplified from meningococcal strain MC58 and cloned into the expression vector pQE30. Recombinant proteins were expressed in E. coli and affinity-purified. Recombinant enolase (rEno) was shown to bind Plg more than recombinant DnaK (rDnaK) or peroxiredoxin (rPrx). In all cases, binding was inhibited by the lysine analogue, ε-aminocaproic acid. Whilst mutation of various sub-terminal lysine residues in rEno, rDnaK and rPrx had no impact on the binding of each protein to Plg, mutation of the C-terminal lysine residue in each protein significantly reduced, but did not completely abolish, Plg binding. Rabbit antisera raised against the purified recombinant proteins were utilized to examine the localisation of these proteins on the surface of N. meningitidis. An N. meningitidis strain MC58 peroxiredoxin mutant and its complemented derivative were also constructed. Data relating to the phenotypic characterisation of these stains will be presented.

Asmaa Mohammed Sulaiman Al-Bayati

Investigating DAP/lysine pathway genes in the predatory bacterium Bdellovibrio bacteriovorus

Bdellovibrio bacteriovorus is a small deltaproteobacterium which invades the periplasm of other Gram negative bacteria replicating within them. Predatory invasion requires modification of the prey cell wall to prevent premature lysis during predator growth. Invasion inside another bacterium also causes osmotic changes which must be tolerated by the invading predator, possibly involving self-wall modifications. Early research by Michael Thomashow detected an increase in diaminopimelate (DAP) content in prey cell walls during predation.

DAP is an amino-acid component of the peptide cross-links of Gram negative bacterial wall peptidoglycan. Cross-linking changes can affect cell wall flexibility. DAP is synthesised by a metabolic pathway shared also by lysine synthesis. A key enzyme at the start of this pathway, and in the synthesis of other amino-acids is aspartokinase. Bioinformatic studies by Lo and co-workers showed that a lateral gene transfer (LGT) event caused a secondary aspartokinase gene (bd0134) to be present in the B. bacteriovorus HD100 genome in addition to the ancestral one (bd0528).

These two genes and other genes involved in DAP/Lysine pathway have been investigated using RT- PCR, gene deletions and fluorescent tagging. Also we investigated a surprising linking between the DAP/Lysine pathway and diguanylate cyclase DgcB, deletion of which affects predation.

Session D (B63) - Cell Signalling and Pharmacology

Time: 10:00-11:00

Abdulla Ahmad

Sex differences in the regulation of porcine coronary artery tone by perivascular adipose tissue: role of adiponectin.

Perivascular adipose tissue (PVAT) exerts complex effects on vascular tone with more than one factor released from PVAT to alter blood vessel tone. The aim of present study was to determine whether there are sex differences in PVAT- mediated regulation of the porcine coronary artery (PCA) tone. Changes in tone in isolated coronary arteries with or without PVAT were recorded in an isometric tension recording system in the absence and presence of a range of putative inhibitors. Western blot was performed to examine the expression of adiponectin in PVAT. The level of adiponectin release from PVAT was measured using ELISA. In the presence of adherent PVAT, contractions to the thromboxane mimetic (U46619) and endothelin-1 were significantly reduced in PCAs from females, but not males. In PCAs pre-contracted with U46619, re-addition of PVAT caused relaxation in PCAs from females, but not males. This relaxant response was inhibited by a combination of both NO synthase inhibitor (L-NAME) and the cyclooxygenase inhibitor indomethacin. Both adiponectin expression in PVAT and its release from PVAT did not differ in both sexes. PCAs from females were more sensitive to adipoRon, an adiponectin receptor agonist, than PCAs from males. These findings have found a clear sex differences in PVAT function and highlighted the adiponectin as mechanistic link between PVAT and its anticontractile effects in PCAs.

Raghdan Al-Saad

The development of Rab27-effector protein interaction inhibitors for treatment of cancer cell invasion and proliferation

INTRODUCTION: Rab27a/b proteins family are GTPases that interact with their effector proteins preferentially in their GTP-bound state. In the recent years, Rab27 has attracted the attention of scientists due to the possible relationship between cancer metastasis and Rab27 overexpression. The aim of this project is to identify the key residues of the Rab27-effector protein interaction in order to design inhibitors for these interactions.

METHODOLOGY: To achieve these goals m.Cherry-Rab27b, GFP-Slp1 and GFP-Slp2 proteins were used to measure the binding affinity of Slp1 or Slp2 effector proteins using a novel FRET assay. In addition, an organelle distribution assay was used as a read-out of Rab27-effector protein interaction in cells.

RESULTS AND DISCUSSION: The results of the FRET assay showed that there are variable affinities of these Rab27-effctor proteins. Accordingly, the FRET assay can be used in the future experiments to test the effect of different inhibitors. The in vitro crystal structure data of Slp2 do not correspond with the results of the melanosome clustering assay; therefore, larger fragments of Slp2 were used in order to identify the key residues for interaction in cells.

CONCLUSIONS:These observations can be used to develop Rab27-effector protein interaction inhibitors. Accordingly, these inhibitors can be tested further in cancer cell lines in terms of reducing cancer cell metastasis.

Fawaz Alassaf

Vascular actions of the Pseudomonas aeruginosa quorum sensing molecule, N-3-(oxododecanoyl)-L-homoserine lactone

N-3-(oxododecanoyl)-L-homoserine lactone (3OC12-HSL), is an important quorum-sensing molecule for Pseudomonas aeruginosa and is known to play a central role in the development of both chronic infections and generalised sepsis. We examined the effect of 3OC12-HSL with a mechanistic view and compared the effect of prolonged exposure to Pseudomonas aeruginosa (PAO1-L) and the mutant bacteria LasI (which do not produce 3OC12-HSL), on vasoconstrictor responses in PCAs. Contraction-based studies using isometric tension recording was used throughout. 3OC12-HSL caused reproducible, slow-developing relaxations in PCAs that were completely reversible on removal of 3OC12-HSL. Both endothelial denudation of the arterial segment and the inclusion of the nitric oxide synthase inhibitor caused a similar degree of enhancement of 3OC12-HSL-induced relaxations, suggestive a minor role for endothelium-derived factors in limiting responses. The vascular effects of 3OC12-HSL did not involve prostacyclin, a hyperpolarising mechanisms, inhibition of calcium influx, PPAR-gamma receptor activator or inhibition of mitochondrial function. Overnight exposure of the PCAs to Pseudomonas aeruginosa produced a selective reduction in the magnitude of contractions to KCl. The vasorelaxant effect of 3OC12-HSL occurs at concentrations known to be present in biofilms of Pseudomonas aeruginosa and is likely to influence the vasculature in vivo, and therefore the supply of nutrients, via a novel mechanism.

Time: 11:30-12:30

Penny Ensor

Investigating the interaction between the dopamine D2 receptor (D2R) and the dopamine transporter (DAT)

Disruption of synaptic dopamine (DA) levels is implicated in a number of neurological disorders such as schizophrenia. DA levels are regulated by the short isoform of the D2R (D2S) and DAT co-expressed in pre-synaptic DA nerve terminals. Co-expression of D2S with DAT is reported to increase the uptake function of DAT. Here we investigate the effect of co-expressing these proteins on their function.

Using Chinese Hamster Ovary (CHO) cells expressing DAT, D2S or long isoform of D2R (D2L) alone or in combination (DAT-D2S and DAT-D2L), DAT activity was quantified by measuring the uptake of DAT fluorescent substrate (ASP+) and receptor activity determined using [35S]GTPγS radioligand binding.

ASP+ uptake was inhibited by DAT inhibitors with the same order of potency in DAT, DAT-D2S and DAT-D2L cells (indatraline > GBR-12909 ≥ JHW007 > bupropion). The D2R full agonist quinpirole (QP) had no effect on ASP+ uptake in any cell line. Similarly, pEC50 values of D2R agonists were not altered with DAT co-expression in the [35S]GTPγS assay. However bromocriptine (BC) efficacy at theD2S/L receptor was reduced with DAT co-expression (Rmax values: D2S=76±9, DAT-D2S=51±3 and D2L=70±11, DAT-D2L=34±9% (% 10μM QP response; $P<0.05$, unpaired t-test, Welch's correction).

These data suggest that co-expression of either isoform of D2R with DAT does not affect DAT uptake function; however reduced BC efficacy upon DAT expression could indicate an alteration in receptor function.

Paolo Sanzá

Regulation of organelle transport by cell cycle

Rab proteins are key regulators in the trafficking of vesicles in the cell. Rab27a has been identified as a pivotal regulator of vesicular transport. Rab27a is regulated by other proteins, which control its activity and its localization to specific compartment membranes e.g. melanosomes. Rab27a is GTPase, the switching from GDP to GTP is supported by guanine exchange factor (Rab3GEP). In melanocytes, Rab27a in the active form is able to bind its effector (melanophilin) and myosin V which enable melanosomes trafficking from the perinuclear area to the periphery. Observation of Rab3GEP knockout melanocytes has revealed that the deletion of the Rab3GEP causes a mixed phenotypes with different proportions of clustered and dispersed cells. The number of clustered cells is positively correlated with the rate of cell growth suggesting temporary deactivation of the Rab27a machinery during cell division. Moreover, cells treated with phorbol 12-myristate 13-acetate (PMA), a key growth factor for melanocytes, increase the number of cells with melanosomes clustered in the perinuclear area, which is positively correlated with the amount of PMA. PMA has been suggested to act through PKC and Src families. To determine the involvement of Src family in the deactivation of the trafficking machinery and the activation of the cell cycle we used PP2, an inhibitor of Src family. It has revealed delays in the melanosomes clustering as well as inhibition of cell growth.

Mark Soave

The Molecular Pharmacology of a Monoclonal Antibody raised against a Thermostabilised β1-Adrenoceptor

Autoimmune antibodies raised against extracellular loop 2 (ECL2) of the β1adrenoceptor (β1AR) play a role in idiopathic dilated cardiomyopathy1. A monoclonal antibody (mAb3) was raised against the ECL2 of a thermostabilised turkey β1AR (β1AR StaR)2. Here, we have characterised the pharmacological properties of mAb3 in Chinese Hamster Ovary cells (CHO) expressing turkey β1ARs.

CHO cells expressing the turkey β1AR (tβtrunc)3 or a thermostabilised mutant (tβ6-m23)3 were studied using 3H-CGP12177 radioligand binding, 3H-cAMP accumulation and a cAMP response element (CRE) reporter gene (secreted placental alkaline phosphatase; SPAP). Quantitative analysis of mAb3 binding to turkey β1ARs was also performed using wide field imaging (Image Xpress Micro).

MAb3 bound purified β1AR StaR and inhibited 3H-CGP12177 binding to CHO tβtrunc or tβ6-m23 cells (pIC50 7.9±0.1; 7.8±0.1 n=7, respectively). Wide field imaging showed mAb3 specifically bound to tβ-ARs expressed on the cell surface. MAb3 inhibited isoprenaline-induced 3H-cAMP accumulation and CRE-SPAP production in tβ6-m23 cells. MAb3 alone was unable to elicit any agonist response at either receptor.

This study demonstrates the ability of an antibody directed against ECL2 to bind selectively to turkey β1ARs and inhibit isoprenaline-mediated cAMP and CRE-SPAP responses.

1- Deubner N et al., 2010, Eur J Heart Fail, 12, 753-62
2- Hutchings CJ et al., 2014, mAbs, 6, 1-16
3- Baker JG et al., 2011, Naunyn Schmiedebergs Arch Pharmacol

Session D (A2) - Genetics, Ecology & Evolution
Time: 14:00-15:00

Stuart Young

The causes and consequences of immune variation in wild mice

Decades of study using lab-reared mice, Mus musculus – maintained in controlled conditions and often genetically modified – have provided exquisite mechanistic detail on the operation of the immune system. These studies, however, fail to address the fact that animals living in the wild are often infected by multiple pathogens while coping with various environmental stresses. This leads to variation in the immune response: some individuals may be resistant, where the immune response clears infection whereas others may be tolerant, the damage caused by infection is modulated. Further, wild animals are unable to expend unlimited resources on immunity: food is limited, the climate may be harsh and reproduction may draw on reserves. So, a host in poor condition may make a reduced or different type of response. Using a wild population of house mice on the Isle of May, I've been able to investigate this variation in the immune phenotype. Immune response was measured using qPCR of a suite of immune genes and multiplex bead assay of circulating cytokines. I have found that immune response is strongly influenced by body condition – those individuals in better condition elicit a stronger immune response. Using stable isotope analysis, I aim to explore the influence of diet in driving body condition and ultimately immune phenotype. Here, I will present data from a pilot study showing spatial variation in diet across the Isle of May mice, link this to parasite load and immune response.

Kehinde Olukemi Sowunmi

Investigations of the Copper/Zinc superoxide dismutase 1 gene as a marker for resistance/susceptibility in Biomphalaria glabrata snails

Biomphalaria glabrata snails vary in immune response to infection with the larval stages of the trematode parasite, Schistosoma mansoni. Development of either a resistant or a susceptible phenotype in B. glabrata snails interacting with S. mansoni is partially determined by various genes including those related to the stress response initiated by parasite invasion. Specifically, one allele of such gene, the Cu/Zn superoxide dismutase 1 (SOD1), has been connected to the resistant phenotype. This study investigates resistance/susceptibility-associated alleles of the Cu/Zn superoxide dismutase 1 (SOD1) gene in three laboratory maintained populations of B. glabrata varying in susceptibility to the PR-1 strain of S. mansoni. Four alleles were identified, two unique to this study and with slight differences from reported alleles. A link between resistant snails and the resistant SOD1 allele across other B. glabrata snails is however corroborated.

James Richard Whiting

The Evolutionary Consequences of Genetic Adaptation to Parasitism

Pampered laboratory models have driven current immunology understanding. However, in the wild organisms are subject to stresses and trade-offs that mediate immunity and create a diverse array of immune phenotypes. The immune system is a costly trait both in terms of resources and the potential for autoimmunity. This project investigates how the diversity observed in immune phenotypes affects the various costs associated with its evolution and application. Over time the immune system can cause host deterioration, senescence, through cumulative autoimmunity or increased parasitism, immunosenescence. I investigated this relationship in male reproducing stickleback, which decline in condition over the breeding season. I used qPCR to examine the expression of immune genes in breeding and non-breeding males within 2 lakes, and between breeding males from 5 lakes. Breeders show higher expression of inflammatory-linked genes, suggesting a possible association between autoimmunity and senescence. A link between fish condition and inflammatory expression differences between lakes further supports this. These differences may be environmentally plastic, or genetically determined. Ongoing work includes a GWAS approach to evaluate the genetic basis for the observed expression differences to better understand how evolved immunity may interact with complex life history traits such as senescence. We are also examining the role of immune genes in wild hybrid inviability.

Time: 15:20-16:20

Anne Caroline Barbosa

Using chromosome engineering on unusual natural isolate of the fission yeast Schizosaccharomyces pombe to investigate epigenetic inheritance of the kinetochore

Kinetochores are the protein components of the centromere that bind the chromosome to the microtubule spindle and direct chromosome segregation at cell division. In any one species kinetochores can be associated with a variety of unrelated centromeric sequences. This has led to the idea that kinetochore location is determined by an epigenetic process. In humans cytogenetic data suggests that the number of centromere-specific CENP-A nucleosomes at each centromere is uniform regardless of the nature and quantity of the centromeric DNA. This observation suggests that the number of these centromere-specific nucleosomes is tightly regulated. The "kinetochore feedback loop" is a model that explains this observation and suggests a mechanism for the epigenetic process itself. The key observation prompting the model was the cytogenetic data derived from human so we need to confirm this in a tractable model system. One way to do this is to increase the amount of centromeric DNA at one centromere and confirm that the number of the centromeric nucleosomes remains unaltered. A second prediction of the model is that if a candidate sequence is placed adjacent to the native centromere then centromeric nucleosomes will form over the candidate sequence and if the native centromere is then removed the kinetochore will efficiently reform over the candidate. The S. pombe strain CBS2777 has re-arranged chromosomes that allow us to test these predictions and this is the aim of my work.

Nzar Ali Ameen Shwan

Characterising human amylase gene CNVs and application to association studies

The human amylase gene family is highly copy number variable (CNV), and the salivary (AMY1) and pancreatic (AMY2A and AMY2B) amylase genes encode the starch-digesting enzyme expressed in the salivary gland and pancreas, respectively. High AMY1 copy number (CN) has been shown to be correlated with dietary starch intake, and low AMY1 CN reported to be a predisposition factor to obesity. These findings have not been replicated independently, and reliable measurement methods and accurate structural characterisation of the region are important to address such findings. In this study high-resolution measurement assays were developed to define the full range of amylase variation in different populations, and test the hypothesis of BMI association with amylase CNV in the Finnish Biobank cohort (1000 samples) and the British 1958 Birth Cohort (1481 samples). The results showed multiple expansion of a unit containing one copy each of AMY1, AMY2A and AMY2B in sub-Saharan Africans. High quality data were obtained from both European cohorts and the data analyses for the association study are in progress. Our data suggest that the pancreatic amylase genes should be taken into account when evaluating the adaptive significance of variation in this gene cluster. Furthermore, our results suggest that the developed CNV typing methods constitute an accurate, reliable and high throughput method for measuring amylase CN in a large set of samples.

Xiao Xu

Human Alpha defensin CNV diversity: discovery of a Neanderthal-derived haplotype under positive selection

The human alpha defensin genes encode an important class of innate immunity effectors. Alpha defensin 1 (DEFA1) and 3 (DEFA3) are interchangeable gene variants located in a mixed multiallelic copy number variable region (CNV) on 8p23.1. Modern humans have between 3 and 16 copies of DEFA1/DEFA3. Recent GWAS studies of IgA nephropathy patients identified two SNPs associated with the disease in the LD block of the DEFA1A3 CNV. To better understand the origin of modern human diversity at the DEFA1A3 CNV, a phylogenetic analysis of the telomeric and centromeric sequence flanking the CNV was carried out. We found there are both frequency and haplotype class differences between African, European and Asian populations. Our analysis identified two haplotypes associated with two reported IgA nephropathy GWAS SNPs in European and Asian populations. We also identified a putative Neanderthal introgressed haplotype in present-day European populations. We found evidence of introgression by molecular clock, S* statistics, geographic distribution and CNV internal variants. We further evaluated natural selection in this region, and found evidence of positive selection on the Neanderthal-derived haplotype from the integrated Haplotype Score (iHS). The potential role of these variants in IgA nephropathy needs to be further evaluated.

Oral Presentations: Abstracts - Friday 15th July

Session A (A1) - Microbial Engineering

Time: 10:00-11:00

Patrick Samuel Ingle

Investigating the role of the spoVA operon in Clostridium difficile endospores

Bacterial spores are among the most resilient forms of life, capable of surviving exposure to UV radiation, extreme temperatures and disinfectants. Such properties enable endospores of the pathogenic bacterium Clostridium difficile to persist in healthcare facilities and act as the infectious agents of C. difficile associated disease (CDAD). Upon ingestion by susceptible individuals, these spores arrive in the colon where they germinate to form toxin-producing vegetative cells. The resulting CDAD is responsible for over 14,000 deaths annually in the US and is a substantial economic burden on healthcare facilities worldwide.

Associated with spore resilience is the presence of dipicolinic acid (DPA) in the spore core. Previous studies in other Firmicutes have shown that DPA entry into the forming spore during sporulation, and subsequent release during germination, is mediated by the SpoVA proteins. These proteins are thought to bind DPA and form a channel in the spore inner membrane through which DPA translocates. Allelic exchange has been used to create a C. difficile spoVA in-frame deletion mutant and also to complement the mutation in the chromosome. Using the created strains, the role of the spoVA operon in C. difficile has been investigated via characterisation of C. difficile spores lacking the SpoVA proteins.

Michaella Whittle

Isolation and characterisation of four novel bacteriophages infecting clinically relevant isolates of Clostridium difficile

Clostridium difficile is a Gram positive, anaerobic, endospore-forming bacterium and one of the leading causes of hospital-acquired diarrhoea, causing a substantial financial burden on health systems. Routinely used treatments comprise the broad-spectrum antibiotic metronidazole and the 'last-line-of-defence' antibiotic vancomycin. Both cause collateral damage to the GI-microflora which pre-disposes patients to C. difficile infection (CDI). There is clearly a need for alternative and more targeted therapies. One such possibility is bacteriophage therapy as the high specificity of phages (viruses which kill bacteria) will eliminate the damaging effect on the gut microbiota, likely leading to reduced incidence of relapse. Four phages (phiCD08011, phiCD2301, phiCD418 and phiCD1801) which infect clinically relevant C. difficile ribotypes (002, 014, 023 and 078) have been isolated. The genomes of the phages have been determined using Illumina MiSeq and key genes identified. Phage morphology has been assigned using transmission electron microscopy (TEM) suggesting the phages belong to Myoviridae (3) and Siphoviridae (1). The host range of each phage has been determined and showed phiCD2301 and phiCD418 are very narrow spectrum phages, only able to infect their propagating host. In comparison, phiCD1801 and phiCD08011 have a broader spectrum of activity with phiCD1801 able to infect and lyse 87.5 % of isolates tested and phiCD08011 able to infect and lyse 65.2 % of isolates tested.

Craig Woods

Forward Genetic Studies in Clostridium autoethanogenum

Clostridium autoethanogenum is the organism of choice for Lanzatech, the world's leading gas-to-biofuels developer. Forward genetics studies in Clostridium autoethanogenum aim to elucidate mechanisms of product formation and tolerance. A pool of transposon mutants can be screened for useful phenotypic traits to provide candidate genes for directed strain production. Initial product targets for this work are ethanol, 2,3-butanediol and isobutanol but the system can be applied to a variety of products of an industrial strain. The transposon library can also be used to undertake transposon directed insertion-site sequencing (TraDIS). TraDIS involves sequencing a transposon mutant library, using the transposon integration site to prime a sequencing reaction into the adjacent interrupted gene. Genes essential for growth will be unrepresented or highly-under represented and will therefore represent candidate essential genes. This will be of use for directed methods of strain production, to avoid wasteful attempts at knocking out essential genes. With a large enough mutant pool TraDIS can also be used to generate lists of genes advantageous or disadvantageous to a given growth condition.

Time: 11:30-12:30

Lorna Finch

Germination and Resistance of Clostridium difficile Spores

Clostridium difficile spores represent the principal transmission route of C. difficile associated disease (CDAD). CDAD is not only characterised by debilitating symptoms but is also complicated with a high incidence of recurrence and is increasingly found in the community.

Preventing and reducing recurrent infection is pivotal to reducing both the economic and morbidity burden it poses on the healthcare system. The RAPID clinical trial aims to assess whether rifaximin can reduce the relapse rate of CDAD, following clinical resolution of CDAD with standard therapy (metronidazole or vancomycin). During this talk I will highlight some of the techniques employed to characterise clinical isolates, specifically their sporulation and germination efficiency and their contribution to recurrent infection.

CDI and re-infection, of cohabiting individuals arises due to the inadvertent ingestion of highly resistant spores from contaminated surfaces. Much of the resistant features owe to the presence of α/β-type small acid soluble proteins (SASPs) which bind to and 'protect' spore DNA. During this talk I will also discuss my research targeting the association and degradation of SASPs and how this remains important in understanding both the resistant properties and germination characteristics of C. difficile spores. Thus, havi direct applications for disease prevention in the healthcare system.

Florence Jessica Annan

Improving the Performance of Two Gas Fermenting Clostridial Species Using Synthetic Biology to Fix the Pantothenate Pathway

Clostridium autoethanogenum and *Clostridium ljungdahlii* are industrially relevant gas fermenting anaerobic acetogens, which use the wood-ljungdahl and mixed acid fermentation pathway to convert carbon monoxide and carbon dioxide into useful bulk and fine chemicals. To increase the industrial attractiveness of producing chemicals via this route, the process has to be as economic as possible. One way to increase the economic viability of this process is to reduce the input costs of the media. It is important to characterise the essential vitamins required for growth as they are expensive. Three vitamins which have been suggested to be essential are thiamine, biotin and pantothenate. Pantothenate (B5) is an essential nutrient from the B class of vitamins used in the synthesis of CoA. Using a mix of classical microbiological techniques we show that the two species are auxotrophic for the production of pantothenate and that pantothenate is an essential vitamin for the growth and viability of these two species. Using synthetic biology we attempt to fix this problem by determining which genes are essential in the pathway and introducing three missing genes into the two species in order to create two autotrophic strains. The more efficient the media, the more economical the process will be, and therefore, more attractive as a mechanism for the sustainable production of the platform chemical than from fossil fuels leading us one step closer to decoupling our civilisation from oil.

Ryan John Hope

The Acetone-Butanol-Ethanol (ABE) Fermentation of Saccharolytic Clostridia: Rational Routes to Improvements

Clostridium acetobutylicum has a long established fermentation process for the production of 1-butanol, with wild type batch fermentations capable of producing approximately 15g L-1 along with acetone and small amounts of ethanol - which is known commonly as the ABE fermentation. 1-butanol is an attractive candidate as a liquid transport fuel as it can be used with existing infrastructure and its high energy density.

Butanol is a saturated alcohol and its metabolic pathway is dependant on the supply of reduction equivalents from NADH. Conservation of these reduction equivalents by subverting other reduction reactions such as the reduction of ferredoxin results in improved butanol specific productivity.

Time: 14:00-15:00

Andrew Dempster

Bile be there for you: The protective role of Clostridium scindens

The largest reservoir of microorganisms in the human body resides in the gut. Although made up of eukaryotes, archea and viruses, bacterial species make up a diverse and significant component of the gut microbiota. The combined metabolic effect of this multifarious bacterial community and the complex homeostatic interactions with the host are responsible for many specific functions integral to maintaining the optimal health and well-being of an individual. The multitude of functions performed by this virtual organ includes metabolising indigestible compounds, essential nutrient and vitamin provision, shaping the architecture of the intestine, immune system development and resistance to colonisation of pathogenic bacteria. Pharmaceuticals, such as broad spectrum antibiotics, exert a devastating effect upon the microbial population of the human gastrointestinal tract. The resulting dysbiosis of the commensal population in the gut lumen is a key risk factor for colonisation by the enteric pathogen, Clostridium difficile. Clostridium scindens is an integral member of the healthy human gut microbiota, thought to be a key organism in the maintenance of colonisation resistance against C. difficile through the biotransformation of bile acids. Work to characterise and study C. scindens and the bile acid inducible operon will be presented and the proposed mechanism by which C. difficile is inhibited.

Natasha Kinsmore

Understanding certain Clostridium difficile Virulence and Antibiotic Resistance Factors and how these relate to Clinical Outcome

Clostridium difficile is a Gram-positive, anaerobic, spore-forming bacillus, known for causing the nosocomial diarrhoeal disease, C. difficile infection (CDI). One important feature of CDI is the high relapse rate (19-35%) through either reinfection or reactivation of an original infection, which is debilitating for patients and extremely costly for healthcare systems. It has been hypothesised that the presence of certain C. difficile virulence factors, such as C. difficile binary toxin (CDT) and spores, as well as antibiotic resistance, is associated with increased disease severity and/or relapse.

This research has been done in conjunction with the Nottingham Digestive Diseases Biomedical Research Unit (NDDBRU) with the aim being to characterise certain virulence and antibiotic resistance factors and relate these findings to clinical outcome.

C. difficile was isolated from patient stool samples and ribotyped. A CDT PCR has been established to amplify and sequence the CDT locus from patient isolates and then compare it to known strains. The different loci were investigated further through single nucleotide polymorphism (SNP) analysis. Sporulation assays were also completed on patient isolates with results compared to a known strain. Lastly, a cohort of isolates from relapse patients were analysed for Rifaximin and Fidaxomicin antibiotic resistance with positive samples sequenced and examined for SNPs.

Christopher James Hannes Millard

Mimicking the native methylation patterns of Clostridia

The genus Clostridium contains several bacterial species of potential industrial interest. One such species, Clostridium carboxidivorans, is distinguished by its ability to use carbon monoxide as sole carbon and energy source. It produces several acids and alcohols which are of use as platform chemicals, including ethanol and butanol.

To improve the product profile of C. carboxidivorans, it is necessary to make directed genetic changes. This requires the establishment of DNA transfer. However, C. carboxidivorans possesses a large number of restriction endonuclease systems, which serve to destroy incoming foreign DNA. These restriction systems possess corresponding methyltransferases that protect native DNA from self-restriction.

In the present work, a novel system is being assembled in which C. carboxidivorans' native methylation systems are cloned into artificial operons for expression in E. coli. The E. coli host strain being employed possesses no methylation systems of its own, and constitutes a "blank slate" for heterologous expression of clostridial methyltransferases. The methylation operons are under the control of an inducible system as a measure to minimise toxicity issues in E. coli. Because the methyltransferase operons are assembled in vitro prior to integration into E. coli, this system is species-agnostic and can be used to generate E. coli strains that mimic the methylation profiles of various species of industrial interest.

Session A (A1) - Metabolic & Molecular Physiology

Time: 15:20-16:20

Hind Alzahrani

Continuous Beat to Beat Monitoring the Cardiovascular Parameters in Response to Autonomic Stress Tests

Study objective: The aim was to develop a method of assessing cardiovascular function (heart rate (HR), cardiac output (CO), blood pressure (BP) in response to deep breathing, standing maneuver and handgrip exercise using a Finometer. Subjects: Twenty subjects (10 males and 10 females) were healthy, young mean age 24.4years males and 26.7years females, non-obese mean±SD of BMI was 23.4±7.4, 22.8±4.5 males and females respectively.Methods: Beat to beat Heat rate and blood pressure variability were monitored during deep breathing test, posture change to standing position and handgrip exercise using a Finometer. This involves a finger cuff pressure which placed in the middle left finger and arm cuff pressure on the upper left arm. Then, automatic calibration was made followed by recording of baseline measurements for 3 minutes, and then physiological manoeuvres were performed starting with deep breathing (2minutes), standing (2mniutes) and handgrip exercise at 60% of MVC (1 minute). This event separated by time for recovery. Results: A significant increased and decreased in cardiovascular parameter during inspiration and expiration in both gender respectively ($p<0.05$). Orthostatic manoeuvre caused a significant reduction of SBP and CO whereas HR ($p<0.05$) and DBP ($P>0.05$) increased in both genders. Cardiovascular parameters showed a significant increased during handgrip exercise at 40% and 60% of MVC whereas 20% of MVC had no significant changes of cardiovascular parameters.

Seyedah Amenah Madjd Jabari

Beneficial effects of replacing diet beverages with water on Type 2 diabetic obese women following a hypo-energetic diet - a randomized, 24 week clinical trial

Aims: To compare the effect of replacing diet beverages (DBs) with water or continuing to drink DBs, on weight loss in Type 2 diabetes during a 24 week weight loss program.

Materials and Methods: 81 Overweight and obese women with type 2 diabetes, who usually consumed DBs in their diet, were asked to either substitute water for DBs or continue drinking DBs five times per week after their lunch for 24 weeks (DBs group), while they were on a weight loss program.

Results: Compared with the DBs group, the Water group had a greater decrease in weight (Water: -6.40 ± 2.42 kg; DBs: -5.25 ± 1.60 kg; P =0.017), BMI (Water: -2.49 ± 0.92 kg/m2; DBs: -2.06 ± 0.62 kg/m2; P =0.018), FPG(Water: -1.63 ± 0.54 mmol/l; DBs: - 1.29 ± 0.48 mmol/l, P=0.003), Hb A1C (Water: -1.16 ± 1.09%; DBs: -0.42 ± 0.21%, P<0.001),Fasting Insulin (Water: -5.71 ± 2.30 m lU/ml; DBs: -4.16 ± 1.74 m lU/ml, P=0.001), HOMA IR (Water:-3.20 ± 1.17; DBs: -2.48 ± 0.99, P=003) and 2h post prandial glucose (Water: -1.67 ± 0.62 mmol/l; DBs: -1.35 ± 0.39 mmol/l; P=0.009) over the 24 weeks. However, there was no significant group * time interaction for waist circumference or lipid profiles within both groups over 24 weeks.

Conclusion: Replacement of DBs with water after the main meal in patients with type 2 diabetes may lead to more weight reduction during a weight loss program. It offers the clinical benefits of improved plasma glucose and insulin sensitivity.

Session B (B1) – Neuroscience

Time: 10:00-11:00

Valeria Lasio

Chronic exposure to chemotherapy impairs neurogenesis in Sox1-GFP transgenic mice: potential protective effect of indomethacin

Purpose: Patient studies show an association between chemotherapy treatment and cognitive impairment. Previously we showed that the chemotherapy agent 5-FU, reduces hippocampal neurogenesis and cognition in rodents. Here Sox1-GFP mice were tested for the effects of 5-FU given with and without indomethacin on cell proliferation (Ki67) and stem cell subpopulations (GFP and GFAP) in the subgranular zone (SGZ). In these animals neural stem cells can be identified by expression of GFP and divided into early (radial) or late (horizontal) stem cells by morphology.

Methods: Indomethacin was administrated one week prior and during 5-FU treatment. Mice were injected with 5-FU or saline every second day for two weeks.

Results: 5FU treatment caused a significant reduction in the number of proliferating (Ki67+) cells in the SGZ. Chemotherapy reduced the number of quiescent (SOX1+/GFAP+) and activated (SOX1+/GFAP-), radial neural stem cells but had no effect on the numbers of horizontally orientated SOX1+ cells. Indomethacin prevents the reduced cell proliferation.

Conclusions: These results show that chronic 5FU has a severe effect on hippocampal neurogenesis by reducing cell proliferation and depleting early neural stem cells, an effect which explains the prolonged reduction in hippocampal neurogenesis and cognitive impairments found in patients and animal models.

Ayoub Ali Hussein Al-Bayti

The deterioration of white matter tracts caused by chemotherapy and their protection by antidepressant and anti-inflammatory drugs

Purpose: Cognitive impairment has been associated with chemotherapy treatment and many studies have shown that white matter (WM) integrity is reduced. This could be due to an inflammatory response to chemotherapy leading to a reduction in the proliferation of oligodendrocyte precursor cells (OPCs) and causing demyelination. This study evaluates the effect of the chemotherapy drug (5FU) on WM. We also evaluated the ability of an antidepressant and anti-inflammatory drugs to protect WM when co-administrated with 5FU.

Methods: Adult rats were chronically administrated 5FU. Some groups were co-administered with either Fluoxetine (FLX) or Indomethacin (INDO) prior to and during 5FU injection. Cells proliferation was quantified in WM. Transmission electron microscopy (TEM) was used to determine the density of myelinated axons and myelin thickness 7 days after treatment.

Results: 5FU treatment caused a significant decrease in cell proliferation in WM. Co-treatment with either FLX or INDO prevented this decline. TEM showed no change in the density of myelinated axons but a decrease in myelin thickness in WM fibers after 5FU treatment, whereas there was no significant difference in groups co-treated with FLX or INDO.

Conclusions: Either FLX or INDO could provide a novel therapeutic approach to reduce cognitive impairment after chemotherapy. However the interaction of FLX with cancer and cancer treatments is unclear while anti-inflammatory drugs are known have anti-cancer properties

Entedhar Rabiaa

Astrocytes protect neural stem cells from damage by chemotherapy

Objectives: Systemic chemotherapy successfully treats cancer; but many patients experience cognitive decline. Hippocampal neurogenesis involves the proliferation of neural stem cells in the sub granular zone (SGZ) and is required for aspects of memory and pattern separation. Animal studies show that chemotherapy causes a reduction in cell proliferation in the SGZ. However dividing cells in contact with blood vessels appear to be spared. This study examines the role of cell types associated with blood vessels, in protecting neural stem cells.

Methods: Neural N2a, astrocytes C6, endothelial and 3T3 cells were tested for sensitivity to the chemotherapy agent 5FU. Following this stained neural N2a cells were co-cultured with other cell types in the presence of 5FU. Cells were either cultured in contact or separated by a porous membrane. Gap junction proteins (CX43) were detected by immunostaining and tested for functionality by dye transfer (calcein-AM). The impact of a gap junction inhibitor (CBX) was tested.

Results: N2a cells were the most sensitive to 5FU. C43 were found in N2a and C6 cells which allowed dye transfer. Co-culture of N2a cells with astrocytes protected them from 5FU when the cells were in contact but not when separated by a membrane or treated with CBX.

Conclusions: Astrocytes, which form part of the blood brain barrier, afford protection to proliferating neural cells by means of gap junctions.

Time: 11:30-12:30

Fatimah Almahasneh

Effects of vascular endothelial growth factor-A165b on pain behaviour in the monosodium iodoacetate model of osteoarthritis in rat.

Osteoarthritis (OA) is the most common joint disease and a major cause of chronic pain, but treatment of OA associated pain is still inadequate. Anti-angiogenic vascular endothelial growth factor-A165b (VEGF-A165b) has anti-nociceptive effects in models of inflammatory arthritis and diabetic neuropathy. This study assessed the effects of VEGF-A165b on pain behaviour in the monosodium iodoacetate (MIA) model of OA.

Thirty-two male Wistar rats were either injected with MIA (1 mg/50 µl saline, n=23) intra-articularly in the right knee or received no injection (n=9). Rats were then given: VEGF-A165b (20 ng/g body weight, days 0-14) followed by PBS vehicle (days 15-28, intra-peritoneal (I.P.) twice weekly) or PBS (days 0-14) followed by VEGF-A165b (days 15-28) in a cross-over design. Weight bearing asymmetry and mechanical withdrawal threshold to von Frey (vF) filaments were measured twice weekly.

Treatment of MIA rats with VEGF-A165b on days 0-14 caused a significant reduction in weight bearing asymmetry compared to the MIA/PBS control group on days 18 ($p<0.05$), 25 ($p<0.05$) and 28 ($p<0.001$), as well as a reversal of mechanical vF withdrawal thresholds to control levels.

Weight bearing and vF thresholds in animals injected with VEGF-A165b on days 15-28 were not different from those of MIA/PBS rats.

These results demonstrate that VEGF-A165b has an anti-nociceptive effect in the MIA model of OA in rat if given early, but not later, in the development of the disease.

Samuel Bestall

A novel mechanism of peripheral sensitization in diabetic sensory neuropathy involving RAGE and TRPV1

Painful neuropathy is a serious diabetic complication that affects up to 20% of diabetics. TRPV1 and TRPA1 have been implicated in neuropathic pain, including diabetic neuropathy. Adult female Sprague Dawley rats were rendered diabetic by streptozotocin injection (STZ, 50mg/kg, i.p), and developed thermal and mechanical behavioural hypersensitivity after 3 weeks. Sensory neurons from these rats exhibited increased agonist-evoked TRPV1 activity in vitro, indicating neuronal sensitisation. DRG neurons from naïve rats incubated for 24 hours in 50mM glucose also showed increased TRPV1 activity compared to basal glucose conditions (10mM). The high glucose-mediated TRPV1 sensitization was blocked by FPS-ZM1 (RAGE antagonist, 1-100nM), in a concentration-dependent manner. These findings suggest that neuronal TRPV1 sensitization in high glucose conditions is stimulated through RAGE activation, and this may contribute to the development of diabetic pain.

John William Grzeskowiak

Characterising the amino acid substitutions (L925I, L925M and, L925V) associated with pyrethroid resistance in Varroa destructor

A significant cause of honey bee population decline is the Varroa mite (Varroa destructor), particularly in the case of the European honey bee Apis mellifera. Varroa infestation is commonly treated with pyrethroid insecticides.

A novel amino acid substitution, L925V, has recently been identified in the domain II S5 helix of the Varroa destructor sodium channel (Nav). Similarly the substitutions, L925I and L925M have been identified in the Navs of field populations of Varroa. All of these mutations correlate well with pyrethroid resistance in the field.

We characterised the mutations L925I, L925M and L925V via two-electrode voltage clamp of Xenopus oocytes expressing a Drosophila para Nav with individual mutations inserted. Voltage protocols were applied to voltage clamped oocytes that enabled values for half activation voltage and half inactivation voltage to be estimated, and to record the properties of tail currents if present. The pyrethroids deltamethrin, flumethrin and, tau-fluvalinate were applied to oocytes at bath concentrations between 1 nM and 10 μM, and their effects on the aforementioned channel properties measured.

We aim to understand how these mutations affect the biophysical properties of Navs, and if changes in these properties may provide a molecular mechanism to explain resistance to pyrethroid compounds that has been identified in the field.

Session B (B1) – Immunology

Time: 14:00-15:00

Su Su Htwe

Investigating the role of ROCK Isoforms in Regulation of Stiffness Induced Myofibroblast Differentiation in Lung Fibrosis

Fibrosis is a major cause for progressive organ dysfunction in most of chronic pulmonary diseases. Rho associated coiled-coil forming kinase (ROCK) has shown to be involved in myofibroblast differentiation driven by the increased matrix stiffness in fibrotic state. There are two known isoforms of ROCK in human, ROCK1 (ROKβ) and ROCK2 (ROKα) which have 65% in amino acids sequence homology and 92% identity in their kinase domains. However, the isoform specific role of ROCK in myofibroblast differentiation in lung fibrosis has not been studied. To evaluate the importance of ROCK isoforms in pulmonary fibrosis, we developed a Gelatin Methacrylate based hydrogel culture system with different stiffness levels which can induce myofibroblast differentiation with high αSMA expression. Interestingly knocking down the expression of either ROCK1 or ROCK2 individually did not result in reduction of αSMA expression in myofibroblast.Paradoxically, absence of just one isoform exaggerated αSMA expression including fibre assembly and colocalisation wit F actin, predominantly in the absence of ROCK2, indicating their counter regulatory role in myofibroblast differentiation. Moreover complete loss of αSMA fibre assembly was seen only in the absence of both ROCK isoforms without effecting αSMA protein level suggesting that both isoforms are mainly implicated in the assembly of αSMA fibre. Overall our results indicated that ROCK isoform balance is important in myofibroblast differentiation.

Asha Hassan

The immune regulatory properties of Necator americanus and development of a disease model for Necatoriasis.

We studied the effects of N.americanus larvae (L3) on the phenotype and function of human dendritic cells (DC). DC incubated with viable axenic larvae exhibited an immature phenotype as evidenced by no up-regulation in maturation markers CD80, CD83, CD86, CD40, and HLA-DR. In addition there was a down regulation observed in CD206 expression. However, DC maintained their ability to acquire a mature phenotype in response to LPS. DC co-stimulated with LPS and N.americanus larvae exhibited an overall suppression of both anti and pro-inflammatory cytokines (e.g. IL-12, IL-10, IL-8 and IL-6) compared to DC stimulated with LPS only. In the presence of DC, we observed exsheathing of the larvae; DC formed aggregations around the discarded sheath but did not interact with the emerging larvae, alluding to a disparity between the surface chemistry of the larvae and its cuticle. Our data also suggest that the interaction between DC and larvae is likely to be mediated via C-type lectin receptors (CLRs) as evidenced by an inhibition in the formation of DC aggregates around the larvae cuticle in the presence of DC-SIGN and mannose receptor blocking antibodies. LPS stimulated DC could degrade the L3 larvae intracellularly, followed by a digestion of the cuticle. These data provide novel insights into the early events at the interface of DC and N.americanus larvae which could pave the way for the rational design of new and more efficient intervention strategies against hookworm infection.

Molecular Cell Biology & Development

Theocharis Tsoleridis

Discovery of Novel Alphacoronaviruses in European Rodents and Shrews

Coronaviruses (CoVs) infect a plethora of both animals and birds. The recent emergence of the novel SARS and MERS coronaviruses in humans and porcine epidemic diarrhoea virus (PEDV) in pigs indicates that coronaviruses have significant zoonotic and epi-zoonotic potential. In humans, coronaviruses are associated with respiratory disease. In mammals and birds, coronaviruses have been associated with enteric and respiratory diseases as well as hepatitis and neurological disorders. To date, with the exception of the murine hepatitis virus (MHV), there have been few reports of coronaviruses in European rodents. In this study, 813 European rodents encompassing seven different species were screened for alphacoronaviruses using degenerate PCR. Four novel alphacoronaviruses were detected in the species Rattus norvegicus, Microtus agrestis, Sorex araneus and Myodes glareolus. These, together with the recently described Lucheng virus found in China, form a distinct clade in the coronavirus phylogeny. This study has shown the first evidence of alphacoronaviruses present in European rodents and also highlights that Eurasian rodent alphacoronaviruses described to date form a single clade within this genus. Coronavirus infection of rodents appears widespread, thus these animals may pose a threat for cross-species transmission to humans and/or other animals, especially livestock due to sympatric habitats.

Session B (B1) - Immunology

Time: 15:20-16:00

Abeer Sharaf

The effect of cigarette smoke extract on macrophages generated in vitro as a model to study COPD

Chronic obstructive pulmonary disease (COPD) involves lung function impairment leading to permanent airway obstruction. Cigarette smoking is a major cause of COPD. Thus, studying the effects of cigarette smoke on their function may elucidate their role in the pathogenesis of COPD. This was investigated using PBMCs to model the activity of macrophages. In this study, Peripheral blood mononuclear cells (PBMCs) were separated from buffy coats and monocytes were isolated with anti-CD14 coated-magnetic beads. The CD14+ cells were collected, stimulated with M-CSF and cultured in 24well plates coated with 1ml 1% Hexamethyldisilazane(HMDS) in100% propanol. After seven days, cells were stimulated with lipopolysaccharide (LPS) and Poly-IC, and then exposed to 1% or 3%cigarette smoke extract (CSE), or 50 or 150µg/ml nicotine. Our results showed that Poly-IC increases the expression of CD14 and CD16 slightly, and decreases CD206 and CD11b expression. The expression of CD14, CD16 and CD11b was slightly reduced upon stimulation with LPS, and a greater reduction was seen in CD206 expression. There was a significant suppression of CD14 by nicotine with ($p \leq 0.05$ for 50µg/ml and $p \leq 0.001$ for 100µg/ml nicotine) or without ($p \leq 0.05$ for both) Poly-IC (but not LPS), and a significant suppression of CD206 by nicotine alone ($p \leq 0.05$ for 150µg/ml nicotine). Accordingly, further research is being undertaken to study the effects of CSE and nicotine on inflammatory signalling pathways in macrophages

Siti Raudzah Mohamed Kamal

The role of NOD-like receptors (NLRs) on human dendritic cells in regulation of immune responses to allergens.

Dendritic cells (DCs) proved pivotal in sensing the presence of foreign antigens, infectious agents and in initiating allergic responses. Nucleotide-binding oligomerisation domain (NOD)-like receptors (NLRs) are expressed by human DCs and epithelial cells with entrenched function in regulating DC response to pathogens. Thymic stromal lymphopoietin (TSLP) as the master switch for allergic inflammation are expressed by epithelial and immune cells onsite of allergen entry in the airways. How NLRs modulate TSLP receptor (TSLP(R)) complex expression following their ligand dependent and independent activation is yet to be investigated. NOD2 ligation via muramyl dipeptide (MDP) significantly upregulate the expression of both TSLP(R) subunits on DCs. Der p 1 induced the expression of TSLP(R) complex albeit at lower level than MDP. Additive effect of MDP and Der p 1 were seen on TSLP(R) complex expression when DCs were co-stimulated with both stimuli. This data suggests that ligand dependent stimulation of DC via NOD2 using MDP could significantly increase the sensitivity of human DCs to TSLP and enhanced in presence of allergens and allergen extracts. Further experiments should elucidate intracellular pathways mediating augmentation effects of NOD2 ligation with or without allergen exposure and the impact on TSLP(R) subunits, DC maturation and T cell activation as well as differentiation. It is also interesting to see whether NOD2 ligation affects airway epithelial cells congruently.

Session C (A4) - Molecular Cell Biology & Development
Time: 10:00-11:00

Hannah May Marriott
Chromosome Architecture and DNA Replication in Haloferax volcanii

Haloferax volcanii is a halophillic ('salt loving') archaeon used to study DNA replication and repair, it is unique amongst cellular organisms in that it can thrive without replication origins.

There are four replication origins on the main circular chromosome (including the integrated megaplasmid pHV4) and one each on the megaplasmids pHV1 and pHV3. Origins are required to initiate replication but H. volcanii has been found to grow 7% faster when all chromosomal origins are deleted. This has led to the suggestion of an alternative form of DNA replication by recombination-dependent replication (RDR).

The chromosome architecture of H. volcanii can be manipulated via the forced integration of pHV3 onto the main chromosome. When combined with the deletion of the pHV1 megaplasmid, this generates a strain with only one chromosome. Deleting all the origins of replication from this single chromosome could lead to even faster growth, and simplify the investigation of DNA replication with and without origins.

The link between chromosome architecture in H. volcanii and how this relates to DNA replication, origins of replication and RDR, will help us to understand how life is possible without origins.

Darren Crowley

The evolution of Pluripotency: Hijacking an ancient network

Cells in the developmental ground state of pluripotency have the potential to produce any somatic cell type or the primordial germ cells (PGCs), which form the germ line. This naïve state can be recreated by direct reprogramming. The homeodomain-containing transcription factor Nanog is essential to the establishment of pluripotency, and this is conserved from urodele amphibians to the primitive ectoderm of mammals. Pluripotency is fundamental to vertebrate development, so it is surprising that the pluripotency GRN is not conserved outside of vertebrates.

Nanog acts at the top of the GRNs that govern both pluripotency and PGC specification, however there is no Nanog gene present before vertebrates evolved. Nanog orthologs have been identified in invertebrates, and we identified the Vent family of transcription factors as the closest relative to Nanog due to the highly conserved amino acid sequence within the DNA binding domain. Our working hypothesis is that Nanog evolved from the Vent gene family of transcription factors that are encoded in the genomes of invertebrate models for development.

Alexander Day

Investigating the Evolution of Bacterial Virulence using an in vivo host model

Despite a substantial body of theory examining the evolution of virulence, there is a surprising paucity of experimental testing. The extant studies have generally focussed on either macroscopic host-parasite systems or bacteria-phage interactions. There are currently few studies investigating the in-host virulence evolution of clinically relevant human pathogens. To investigate this, a selection experiment was developed using Caenorhabditis elegans as the host organism and Pseudomonas aeruginosa as the pathogen. The evolution of virulence under conditions of low host density and high host density were compared. Low host density was achieved using large plates, whereas small plates were used to create a high host density environment. This selection experiment was run for 20 rounds of selection, each lasting three days, and the evolved bacteria tested for virulence towards C. elegans. A general trend to lose virulence at low host density was observed, however this wasn't universal. Additionally, controls suggest this is due to factors independent of the presence of the C. elegans host, as a similar loss of virulence occurred in control populations. This is consistent with the virulence of recent clinical isolates of P. aeruginosa from chronic environments, which also had low virulence towards C. elegans.

Time: 11:30-12:30

Christopher Mason

Effects of Polymorphisms in the FCN2 Gene on the Antiviral Properties of L-Ficolin against Hepatitis C Virus and Ebola Virus Infection

Hepatitis C virus (HCV) is a leading cause of hepatic disease worldwide, and the recent Ebola (EBOV) virus outbreak in West Africa has highlighted the need for widely available treatments. Immune control of HCV and EBOV infection is still poorly understood. L-ficolin, encoded by the FCN2 gene, is a liver-expressed lectin that contributes to the innate immune response against viral infections. L-ficolin neutralises several enveloped viruses through direct blocking of viral entry and complement activation.

Two SNPs in the FCN2 exon 8, encoding T236M and A258S amino acid substitutions, are maintained in human populations at high frequencies. They affect ligand binding and serum L-ficolin concentration, and may have a role in hepatitis C disease outcome. Optimisation of L-ficolin expression using human cell lines was attempted. Using HCV and EBOV pseudotyped viruses, binding interactions and neutralisation of virus entry by the L-ficolin variants was investigated. Genotyping of HCV-infected patient cohorts was performed to investigate association of these variants with HCV infection outcome. Furthermore, the potential of L-ficolin as a scaffold for chimaeric immune lectins was explored, due to its unique oligomeric structural properties.

Understanding the antiviral significance of FCN2 polymorphisms may explain their high prevalence in human populations and inform the use of L-ficolin in the prognosis, prophylaxis and treatment of a wide variety of enveloped virus infections.

Jennifer McDonald

Defining the structure of variant antigen expression sites in the cattle parasite Trypanosoma congolense

Antigenic variation is a mechanism used by many pathogens to evade the host adaptive immune responses. The African trypanosome Trypanosoma brucei is a model for antigenic variation. The parasite periodically switches its major surface protein, VSG. Only 1 of ~3000 VSG genes is expressed at a time from a telomeric expression site.

T. congolense is a relative of T. brucei and a major pathogen of livestock in sub-Saharan Africa. T. congolense displays many differences from T. brucei, indicating that the system for antigenic variation may not use the same underlying mechanism. The T. congolense genome has been sequenced but the chromosome ends are not assembled, leaving the presence or structure of expression sites a mystery. I am investigating the structure and function of VSG expression sites in T. congolense. To define the structure of expression sites in T. congolense, I have used Transformation Associated Recombination cloning of chromosome ends. Data from a number of different baits have allowed optimization of capture and suggests differences in structure to subtelomeric regions between T. congolense and T. brucei. Sequencing of chromosome end clones will reveal these structures and I am also using component tagging to investigate the proteins involved in switching.

Understanding the mechanisms of antigenic variation in this important parasite could be used in future to exploit weaknesses in the system for the development of new drugs.

Hala Alhadi Ali Mohamed

Increased expression of neurofilament proteins, but decreased mRNA, in cortical neurons with 26S proteasome dysfunction

Neurofilament proteins (NFs) are the major component of the cytoskeleton in mature neurons. Accumulation of NFs has been observed in major neurodegenerative diseases, but the mechanisms underlying this are unclear. The ubiquitin proteasome system (UPS) plays an essential role in the degradation of NFs in neurons. This has led to investigations into the relationship between NFs and the UPS in maintaining the neuronal cytoskeleton. Here, we investigate gene expression, protein levels as well as localisation of neurofilament proteins in mouse cortical neurons following 26S proteasome dysfunction (Psmc1fl/fl;CaMIIα-Cre mice). We found NFH (heavy), NFM (middle) and NFL (light) levels significantly increased with progressive neurodegeneration in Psmc1fl/fl;CaMIIα-Cre mice, supporting a role for the 26S proteasome in NF protein turnover. Interestingly, the increase in NF protein levels was associated with down-regulation of their transcripts, which decreased by 45%, possibly reflecting a feedback mechanism to compensate for increased proteins. Further, we also investigate cellular distribution of NF proteins in cortical neurons. We show increased NFs level is associated with morphological changes, including increased nuclear size, dendritic arborisation and apical dendrite length. Taken together, our data contributes to the knowledge on molecular and cellular events underlying neurodegeneration and suggest control of NF expression may help to slow neurodegenerative mechanisms.

Time: 14:00-15:00

Okechukwu Onianwa

Molecular Epidemiology of an Outbreak of Lymphocytic Choriomeningitis Virus Infection in Rodents and Primates at a Zoo in the United Kingdom.

Lymphocytic choriomeningitis mammarenavirus (LCMV) is principally harboured by house mice and causes diseases in humans and non-human primates (NHPs). Outbreaks of LCMV infection among rodents, humans and NHPs have been previously reported globally. In this study we investigated an outbreak of Callitrichid Hepatitis in a zoo in the United Kingdom. Total RNA was extracted from 586 rodents and NHPs tissue samples; reverse transcribed using random hexamers and used as a template in PCR assays using published primers targeting the S-gene and L-gene segments. Novel primers were also designed to amplify the complete LCMV glycoprotein precursor (GPC). Full GPC was aligned with published sequences from Genbank using Molecular Evolutionary Genetics Analysis (MEGA) software. Twenty four mice, 2 Geoffroy's marmoset, 1 black and white colobus and 1 Black crested gibbon were LCMV-positive. Positive samples were sequenced and aligned with published sequences from Genbank. The novel LCMV strains clustered with other published lineage-1 LCMV sequences. Amino acid and nucleotide differences between the novel LCMV GPC sequences and those from lineages II, III and IV were 8.7% to 19.4% and 21.1% to 26.7% respectively. The novel LCMV also formed a separate cluster when aligned with sequences obtained from a similar study carried out between 2006 and 2008 in UK based on a 282bp nucleotide fragment of the GPC gene. This is the first report of LCMV infection in a non-callitrichid species.

Daniella Spencer

Surviving and Thriving in the airway: How the Arginine Specific Autotransporter Aminopeptidase could promote the success of Pseudomonas aeruginosa in the CF lung

Pseudomonas aeruginosa is a prominent pathogen in Cystic Fibrosis (CF) as it can survive challenges from the immune system and antibiotic treatment through biofilm formation. Furthermore, it can thrive under low oxygen conditions. P. aeruginosa possesses an Arginine Specific Autotransporter Aminopeptidase (AaaA) which releases arginine from protein N-terminals, enabling it to utilise this amino acid as an energy source. This should confer a fitness advantage in oxygen limited environments, including areas of the CF lung. To further understand the relevance AaaA plays in chronic P. aeruginosa CF airway infections, we investigated whether aaaA:(i)is controlled by regulators of relevance to the CF environment;(ii)is conserved in CF isolates and (iii)plays a role in biofilm formation. We have found that RpoN, ArgR, NarX/NarL and AaaA itself control aaaA expression and that binding sites for the regulatory proteins are located in the promoter region of aaaA. Correspondingly, exogenous NO3 was found to reduce aaaA expression. Eighteen CF isolates showed the presence of a highly conserved aaaA gene. The levels of AaaA activity in these strains will be presented. Comparison of aaaA promoter sequences from GenBank revealed a 180bp promoter deletion in a LES strain with changes to the ArgR and NarL binding sites but with an intact RpoN binding domain. Finally, the ability of PAO1 ΔaaaA to form biofilms was impaired. These data suggest aaaA is important in CF lung infections.

Carmen Tong

Screening for Inhibitors of Staphylococcal Sortase A as novel anti-infective agents using a Gaussia luciferase cell based reporter assay

Drug-resistant strains of pathogenic bacteria are becoming an increasing issue globally and the need for alternative drug therapies is crucial. In 2014, 80% of S. aureus infections reported in Western Europe were due to Methicillin-resistant Staph. aureus (MRSA). Sortases are a group of bacterial transpeptidases found in many Gram-positive bacteria, including Staphylococcus aureus. Sortase A (SrtA) is responsible for cell wall protein anchoring, through the recognition of a highly conserved LPXTG motif on secreted cell wall proteins 2. In mouse models of infection, S. aureus SrtA mutants displayed a dramatic decrease in virulence3. Consequently, they are of particular interest for alternative antimicrobial drug therapies. We have designed a cell-based assay which allows us to detect the activity of Sortase A using a Gaussia Luciferase photoprotein (GLuc) that has been engineered to possess the LPXTG motif. After the secretion of GLuc in S. aureus, SrtA recognises the LPXTG motif, cleaves and covalently anchors the GLuc onto the cell wall. When SrtA is inhibited or inactive, GLuc is secreted into the supernatant. By separating the cell sample from the supernatant we are able to determine SrtA activity based on bioluminescence levels emitted from GLuc proteins. We have used this assay for High Throughput Screening to identify compounds as possible Sortase Inhibitors.

Session D (A2) - Genetics, Ecology & Evolution

Time 10:00-11:00

Talib Matlob Chitheer

Eco-evolutionary feedbacks in fish-zooplankton communities on the Scottish island of North Uist

Eco-evolutionary feedbacks occur when evolution of organismal traits causes environmental change that drives further evolution. Predator and prey interactions provide good examples of eco-evolutionary feedback. Here we examine the potential for eco-evolutionary feedbacks between three-spined sticklebacks and their zooplankton prey in lochs on the Scottish island of North Uist. Many lochs on the island were colonised by sticklebacks after the last glaciation, 16,000 years ago. We show that sticklebacks diversified greatly in functional foraging traits that determine the efficiency of capturing different prey items. This could strongly affect total primary production and the structure of prey communities. We also examine the effect of predation on prey life-history, by comparing reproductive traits of the zooplankton communities in lochs with and without sticklebacks . The results showed that ancestral sticklebacks populations have adapted according to the habitat type they colonised. Fish feed on benthic prey in shallow lochs, requiring greater effort for a successful foraging strategy, compared with fish that feed on pelagic zooplankton. In turn, zooplankton in lochs with fish have more rapid reproductive cycles and higher fecundity parameters, probably in response to the increased threat of predation. Our results suggest a strong possibility of eco-evolutionary feedbacks in these simple ecosystems.

Naomi Clement

Examining Predicted Splice Variants in Late Onset AD Risk Loci

Late Onset Alzheimer's disease (LOAD) is the commonest cause of dementia affecting one in six individuals over the age of 80. The exact cause of LOAD still remains unknown, however, it is estimated that approximately 60% of the cause may be due to genetics. In 2011, Genome-Wide Association Studies identified 21 disease risk genetic loci, one of which is the ABCA7 gene however; the cause of this association remains unknown.

Potential causal functional variants within this gene were therefore examined, identifying a variant at the very beginning of exon 32, bioinformatically predicted to splice out exon 32 of the ABCA7 protein. This variant was therefore examined through minigene splicing assays as well as total RNA being examined from both brain tissue samples and lymphoblastoma cell lines of individuals carrying both alleles of this variant.

The predominant product from both minigene assays consisted of products with exon 32 spliced out. However, a minor product in the mutant samples did contain exon 32. Upon analysis of RNA from the brain tissue samples and the lymphoblastoid cell lines, both produced only one RNA product, containing the exon.

It can therefore be concluded that this variant actually stabilises the splice site in vitro, forcing it to be included in the mRNA. However, in the more natural environment, exon 32 is included even without the variant, perhaps suggesting secondary structure interactions or alternative regulatory mechanisms.

Raman Akinyanju Lawal

Genetic Introgression Through Selection In Domestic Chickens: Insight From Whole Genome Sequence Analysis

The evolutionary history of domestic chicken has been subjected to debate since Charles Darwin first proposed the red junglefowl Gallus gallus spp as its sole ancestor. However, molecular evidence of introgression from the grey junglefowl G. sonneratii in the form of the yellow skin locus, and success in producing fertile offspring from Gallus spp hybrids, have challenged the single species origin for the domestic chicken. In this project, we analysed the full genomes of 50 birds, including: the four junglefowl species, indigenous chickens from Ethiopia, Sri Lanka and Saudi Arabia, as well as European fancy chicken for evidence of introgression from G. sonneratii, G. lafayetti and/or G. varius. Using the ABBA-BABA and Fst statistics, we identified several candidate regions of introgression from G. sonneratii and G. lafayetti into domestic chicken and vice versa. These regions represent new genomic landmarks of the selection pressures which have shaped the genome of domestic chicken, and may provide us with new insights on the history of the geographical dispersion of domestic chicken populations.

Time 11:30-12:30

Rayan Alansari

Coevolution of an insect-plant interaction: Alkanna orientalis and its pollinator Anthophora pauperata in the Middle East

Intra- or inter-population gene flow is an essential factor to define the structure and evolution of populations and species. The physical landscape structure plays a role in genetic isolation by restricting or enhancing gene flow over a large geographical scale. Dispersal of plant genes can happen in seeds or pollen, by wind, water or by pollinator foraging behaviour.

My project studies coevolution of a mountain-top plant (Alkanna orientalis: Boraginaceae) and its main pollinator, the bee Anthophora pauperata (Anthophoridae), in Sinai and Saudi Arabia. I use molecular, ecological and behavioural methods to look at how plant genes are moved by the bee and its consequences for adaptation and diversification.

Using published primers for a 250-bp non-encoding sequence of chloroplast DNA (trnH-psbA), there was high variability within and between all sites in Alkanna orientalis, while a ~ 500-bp section of nuclear ribosomal DNA (ITS) showed less variability. From ITS perspective, all individuals from western Saudi Arabia and Egypt cluster together, differing in two SNPs from samples from Turkey and Azerbaijan.

Anthophora pauperata was the most common visitor in Saudi Arabia for both pollen and nectar, and was also the most efficient pollinator, transferring pollen to the stigmas at a high rate. These solitary bees can move pollen genes further than we thought: the largest recorded movement was ~ 4000 m.

Emad Dawood Abbas Kaky

Species Distribution Modelling of Egyptian Plants under Climate Change

It is thought that climate change will have a major impact on species distributions by changing the habitat suitability for organisms. Species distribution modelling is a modern approach to assess the potential effect of climate change on biodiversity. In this study, I used the MaxEnt algorithm to model the distributions of 114 Egyptian plant species under current conditions, and then to project these relationships into three different future times under two different climate-change emission scenarios and two hypotheses about the capability of the species of dispersal

I tested the value of Egypt's Protected Areas under climate change by estimating the species richness inside and outside the Protected Areas under each scenario. Species richness inside Protected Areas was significantly higher than outside for all models. Egyptian plants were assessed based on IUCN Red List categories and criteria. Based just on the records, between 75% and 90% of species could be classified as Least Concern, according to the assumptions made. Similarly, based on SDMs all species could be classified as LC at the current time, whilst in the future under climate change, up to 18% of species face the risk of extinction, depending on assumptions. Subsequently, I used the SDMs for conservation planning using Zonation software.

Species distribution models using MaxEnt appear to be extremely useful for IUCN Red List assessments and conservation planning under climate change.