

Joint Conference with
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Poster Abstracts

Reconstruction of Causal Networks by Set Covering

Nick Fyson
Bristol

We present a method for the reconstruction of networks, based on the order of nodes visited by a stochastic branching process. Our algorithm reconstructs a network of minimal size that ensures consistency with the data. Crucially, we show that global consistency with the data can be achieved through purely local considerations, inferring the neighbourhood of each node in turn. The optimisation problem solved for each individual node can be reduced to a Set Covering Problem, which is known to be NP-hard but can be approximated well in practice. We then extend our approach to account for noisy data, based on the Minimum Description Length principle. We demonstrate our algorithms on synthetic data, generated by an SIR-like epidemiological model.

Computational Evolution of Dynamical Networks

Thomas Gorochofski
Bristol

We present a computational framework that allows for the study of evolving dynamical networks. We hope this can provide support when attempting to understand the influence of dynamics, topology and evolution on the system-level dynamics exhibited by a complex system.

Causal State Models of FRET Spectra

David Kelly

Bristol

We present methods for inferring Hidden Markov Models from Fluorescence Resonance Energy Transfer (FRET) spectra without the necessity of assuming a model architecture, thus alleviating a potential source of subjectivity. These methods are applicable not only to FRET spectra but to any continuous data which clusters around discrete values. The algorithms developed have been shown to perform well on simulated data, demonstrating the ability to recover the model used to generate the data under high noise, sparse data conditions. The methods are based on the techniques of computational mechanics, developed by Crutchfield et al., and generate so called causal state models, which are informative regarding the statistical complexity of the process under study.

A frequency multiplier, multi-functional synthetic genetic regulatory network

Oliver Purcell
Bristol

Will be given soon

Transcription factor regulation during cell development

Owen Rackham
Bristol

Transcription factors regulate gene expression patterns which are different across cell types. The topology of the transcription factor interaction network at the promoter level is believed to hold important information that defines cell

type. We have developed network analysis techniques to identify important topological changes between cell types and tested these on the THP1 cell line using CAGE data from the RIKEN centre in Japan.

The dynamics of sexual contact networks: effects on disease spread and control

Katy Robinson
Bristol

Sexually transmitted infections continue to pose a challenge for public health despite the use of interventions such as screening programmes, vaccination, drug therapies and efforts to reduce high risk behaviors. In part this ongoing challenge is due to the heterogeneous and dynamic partnership networks over which such pathogens are spread. This has motivated the use of network-based models to study sexually transmitted infections, though most of the literature has focused on static networks. However, edges within the network will appear and disappear over time as partnerships are formed and dissolved, so that some individuals will be inaccessible to a particular pathogen even if located in the same network component.

Using a model for the formation of dynamic sexual contact networks based on partnership data from the National Survey of Sexual Attitudes and Lifestyles (Natsal 2000), we investigate the different effective networks available to pathogens with different durations of infectiousness or of differing transmissibilities. We find that the complex interactions between pathogen characteristics and the behavioral network affect which types of individuals are most at risk of becoming infected. We use our modeling framework to identify high- and low-risk groups and to efficiently select and direct interventions within the population as a whole.

SCORER 2.0: improving the prediction of coiled-coil oligomeric state

Thomas Vincent

Bristol

We present SCORER 2.0, a rigorous mathematical implementation of the original SCORER algorithm to predict coiled-coil oligomeric state from protein sequence information alone. By carefully assessing the relationship between predictive power and redundancy within our training set, we have developed a reference dataset of dimeric and trimeric coiled coils. Using this new dataset and the SCORER 2.0 algorithm, we achieved a marked improvement in our ability to differentiate dimeric and trimeric coiled-coil sequences. Future implementation of our approach will include multi-state prediction as well as coiled-coil region prediction.

Rapidly learned stimulus expectations alter perception of motion

Matthew Chalk
Edinburgh Neuroinformatics

Expectations broadly influence our experience of the world. However, the process by which they are acquired and then shape our sensory experiences is not well understood. Here, we examined whether expectations of simple stimulus features can be developed implicitly through a fast statistical learning procedure. We found that participants quickly and automatically developed expectations for the most frequently presented directions of motion, and that this altered their perception of new motion directions, inducing attractive biases in the perceived direction as well as visual hallucinations in the absence of a stimulus. Further, the biases in motion direction estimation that we observed were well explained by a model that accounted for participants' behaviour using a Bayesian strategy, combining a learned prior of the stimulus statistics (the expectation) with their sensory evidence (the actual stimulus) in a probabilistically optimal manner. Our results demonstrate that stimulus expectations are rapidly learned and can powerfully influence perception of simple visual features.

Receptive fields for EM image alignment and

neural reconstruction

Seymour Knowles-Barley
Edinburgh Neuroinformatics

Serial section TEM can produce very high resolution reconstructions of neural morphology, including synaptic detail and in some cases protein localisation. Alignment and reconstruction of 2D TEM images is currently performed manually or semi-automatically, with the aid of computer software, to generate a 3D model of the imaged neural circuitry. In some cases approximate alignment can be achieved automatically but high quality circuit reconstructions still require many hours of manual annotation.

Here 2D receptive fields, similar to those found in biological vision systems are applied to TEM images to automatically annotate neuronal membrane, synaptic connections, and organelles such as mitochondria.

Similarity based extraction of individual anatomical networks from gray matter MRI scans.

Betty Marije
Edinburgh Neuroinformatics

Previous studies have shown that the structure of the human brain can be represented as an anatomical network (Bullmore & Sporns, 2009), offering the advantage to describe the brain with statistics from graph theory. We present a new method based on similarity of gray matter structure to extract networks from individual gray matter MRI data.

Briefly, the method divides gray matter into small regions of interest that keep the three dimensional cortical structure intact, and computed the statistical similarity between all these cubes. Then networks were constructed, based on the resulting similarity matrix, of which we computed standard statistical properties. To study the robustness of the method, we applied it to a sample of 14 healthy subjects, who where scanned twice in two different scanners. The method successfully constructed networks from individual MRI scans, with highly stable statistical properties. To conclude, the presented method describe gray matter MRI data from individuals, offering a complimentary approach to existing methods that derive individual networks from white matter data and

functional data. For future research we propose to explore how changes in gray matter networks can relate to changes in functional networks.

A model of complex visual hallucinations in the cortex

David Reichert
Edinburgh Neuroinformatics

Predictive coding theories hypothesize that the brain (specifically, the cortex) learns about the world by predicting or generating sensory information. Deep Boltzmann machine models, although mostly used in machine learning, could capture such aspects of cortical learning and perception. Here, we show that when deprived of sensory input, the model can form 'hallucinations' induced by homeostatic mechanisms. We thus present a novel computational model of the Charles Bonnet syndrome, where patients suffering from eye disease but no mental disorders form complex visual hallucinations.

Learning Nullspace Policies

Chris Towell
Edinburgh Neuroinformatics

Many everyday tasks performed by people, such as reaching, pointing or drawing, resolve redundant degrees of freedom in the arm in a similar way. In this poster we present a novel method for learning the strategy used to resolve redundancy by exploiting the variability in multiple observations of different tasks. We demonstrate the effectiveness of this method on three simulated plants: a toy example, a three link planar arm, and the KUKA lightweight arm.

Investigating Rab Prenylation in Choroideremia: A Chemical Proteomics Approach

Alexandra Berry
Imperial College

The retinal degenerative disease Choroideremia results in deficient prenylation of an as yet poorly-defined subset of Rab proteins, causing blindness by middle age. By making use of the enzymatic reaction that attaches geranylgeranyl groups to Rabs, we have developed a novel chemical approach to labelling these proteins. This allows rapid and highly sensitive identification of mis-prenylated Rabs.

We report here a system that exploits a combination of enzyme-ligand engineering and bioorthogonal ligation chemistry to attach labels to Rab proteins, such that their functions can be probed. An azide-tagged geranylgeranyl pyrophosphate analogue (AzGGpp) has been successfully synthesised and shown to be a substrate of the enzyme Rab geranylgeranyl transferase. Once Rabs are azide-tagged, a wide range of secondary labels then allows visualisation of the proteins by in-gel fluorescence and purification by streptavidin-coated magnetic beads. Rab proteins have been prenylated and imaged in mammalian cell lines and in tissue from animal disease models.

Measuring the Non-specific Binding Properties and Synthesis of Novel Drug Molecules

Chloe Child
Imperial College

Non-specific binding, NSB, is the non-saturable binding of a ligand to the surrounding area around a target receptor.¹ A proportion of a drug will never reach its target site for various reasons but will reside elsewhere in the surrounding area. This non-saturable binding of a labelled molecule to an unrelated site can obscure the visualisation of biological processes in positron emission tomography, PET. There are several factors which affect non-specific binding which include the lipophilicity and hydrophobic nature of the drug, rate of drug metabolism and the affinity of the drug to the receptor target. This phenomenon is commonly measured using radiolabelled ligands in binding assays and is detected and quantified in vivo using positron emission tomography, PET.

Non-specific binding is a poorly understood process but is believed to be related to the interaction of labelled molecules with tissue membranes and a recent hypothesis predicts that molecules with low NSB are able to hydrolyse the lipid bilayer rapidly. The aims of this work include the synthesis of novel non-specific binding properties to prove or disprove hypothesis set out in previous work. The understanding of non-specific binding, the drug-membrane hydrolysis translocation process and how they are related will be investigated leading to the determination of a set of rules for applicable in novel drug design.

Using Protein-Ligand Engineering To Explore Histone Deacetylase Enzyme Function

Robert Felstead
Imperial College

Inhibitors of histone deacetylases (HDACs) are currently under investigation as novel therapeutics for the treatment of neurodegenerative diseases and cancer. As most of the discovered compounds inhibit all 11 human HDAC isoforms, the discovery of isoform selective inhibitors would be a useful tool in dissecting their individual function and possibly provide novel drug targets. As well as discovering selective inhibitors, a chemical genetics approach may be used to dissect protein function and the consequences of isoform selective inhibition without selective inhibitors as selectivity can be engineered into the protein. Attempts to apply chemical genetics to HDACs are reported including the development of active mutant HDACs.

Dielectrophoretic routes towards time-resolved analysis of cellular membrane dynamics

Fabrice Gielen
Imperial College

The project focuses on the development of novel tools for probing cell

membrane dynamics. Here we demonstrate dielectrophoretic trapping of single Jurkat cells (typically 15µm in diameter) as a means to facilitate time-resolved studies on living cell membranes.

Microfluidic networks consisting of integrated micro-electrodes have been fabricated. These devices have been used to trap single-cells near a defined surface within a flowing stream. Once trapped, the live mammalian cells are assayed for minutes timescales.

We use the fluorescent lipid analogs DiO and DiD known to homogeneously partition within a mammalian cell membrane. Addition of the acceptor dye within the microfluidic network allows for real time observation of FRET events from the cellular membrane using scanning confocal lifetime imaging. Such microfluidic devices will be used to study both lipid organization dynamics as well as lipid diffusion on the membrane surface.

The synthesis of linear poly(alkylenimine)s to determine the effects upon cellular membranes

Bryn Monnery
Imperial College

The synthesis of linear poly(alkylenimine)s to determine the effects upon cellular membranes.

Cationic polymers are used in the delivery of macromolecules such as DNA, siRNA and peptides into cells and organisms. However, they have been found to be cytotoxic. In order to elucidate the relationship of the molecular weight of the most commonly used transfection polymer, linear poly(ethylenimine)^{1, 2} upon the interactions with cellular membranes and subsequent cytotoxicity it is necessary to produce well defined polymers whose molecular weight distribution is exceptionally tight. The conventional route to this polymer is the cationic ring opening polymerisation (CROP) of 2-ethyl-2-oxazoline followed by acid catalysed hydrolysis to l-PEI. This is the route used in the manufacture of the current “gold standard” in-vivo-jetPEI.³ This methodology leads to a wide distribution of molecular weight which is the result of side-reactions that alter the molecular weight.

In order to elucidate the structure-activity relationships tight molecular distributions are required. The existing polymerisation methodologies have proven to be insufficient for this purpose. An understanding of the nature of the

side-reactions has allowed for the development of improved methods of synthesising poly(oxazoline)s and their daughter linear poly(ethylenimine)s. These materials will allow for determination of the effect of polymer molecular weight and cationic charge density upon cellular membranes.

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2. Brissault, B. et al. Synthesis of Linear Polyethylenimine Derivatives for DNA Transfection. *Bioconjugate Chemistry* 14, 581-587 (2003).
3. In-vivo jetPEI manufacturing specification;
<http://www.wipo.int/pctdb/en/wo.jsp?WO=2009016507&IA=IB2008002339&DISPLAY=DOCS>

Selective Molecular Networks to Unravel Cell Signalling

Chirag Patel
Imperial College

In this project we investigate new avenues for the improvement of phosphorylation analysis by utilising polymeric materials that incorporate synthetic receptors known to bind phosphorylated species. This has enabled us to physically isolate phosphoproteins from complex protein mixtures via an affinity separation process.

By incorporating such receptors (Mn-PhosTag AcrylamideTM)₂ in a polymeric matrix (SDS-PAGE gels) selective recognition (mobility shift) of phosphorylated proteins was achieved in model mixtures (p27, MBP) and more importantly, in highly complex cell lysate samples (p27) for the first time.

Using optical trapping methods to investigate membrane localisation of Protein Kinase C ϵ in single cells

John Phillips
Imperial College

Optical traps provide a sterile, versatile toolset to manipulate single cells, and have been used here to force cells into contact. Such forced contact is used to characterise the behaviour of the second messenger protein kinase C (PKC), under a variety of conditions. Such studies are important given that PKC is an oncoprotein. Particular attention is given to the role of free calcium in facilitating the recruitment of PKC, and its effect on cell-to-cell adhesion strength. This work shows the potential of using optical tweezers to study membrane proteins, and lays the foundations for the more complex quantitative work to come.

Chemical genetic approaches to understanding AAA+ proteins.

Lucy Rayner
Imperial College

AAA+ proteins (ATPases Associated with various cellular Activities) are a superfamily of ATPases capable of converting chemical energy into physical motion to perform a multitude of functions. The AAA protein phage shock protein F (PspF) is a bacterial enhancer binding protein (bEBP) for the prokaryotic transcription factor λ which is unique in the strict regulation of its associated genes. λ first forms a transcriptionally silent closed holoenzyme complex with promoter DNA, until PspF binds to and remodels the complex with concurrent ATP hydrolysis to give the transcriptionally competent open complex. Conformational changes in the PspF ATP binding site upon hydrolysis are communicated through a pathway of internal conformational changes, resulting in the reorganisation of two mobile loops – termed Loop 1 and Loop 2 [1]. Located at the tip of Loop 1 is the GAFTGA motif, which is highly conserved across bEBPs and is required for interaction with λ [2].

A sub-sequence of PspF, known as Fragment 4, was previously expressed as a maltose binding protein (MBP) fusion and shown to form a stable interaction with promoter bound λ [4]. This work uses synthetic peptides as tools for a chemical genetics approach to understanding the interaction between λ and PspF, using a range of biochemical and biological assays. The peptides have initially been modelled on the PspF Loop 1 structure, whilst investigating Fragment 4 as a positive control for interaction. The peptides are

studied for both binding to α 54, and inhibition of its interaction with PspF, using both in vitro and in vivo techniques. In vitro assays have focussed on binding using isothermal calorimetry (ITC) whilst α -galactosidase assays have investigated the inhibitory effect of the peptides in vivo.

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Lipids behind bars - High pressure as a route to novel micellar assemblies

Arwen Tyler
Imperial College

Non-bilayer phases are thought to be of considerable biological relevance. Whenever there is a topological change in the membrane, corresponding to events such as membrane fusion, non-bilayer structures are assumed to be adopted locally. Several complex three-dimensional lyotropic liquid crystal phases are already known, such as the bicontinuous cubic phases, but for many years only a single example was found – a cubic phase of spacegroup Fd3m – of a structure based upon a complex close packing of inverse micelles. We have recently reported the discovery (J. Am. Chem. Soc. 131, 1678 (2009)) of a novel lyotropic liquid crystal phase, of spacegroup, P63/mmc, whose structure is based upon a hexagonal close packing of identical quasi-spherical inverse micelles.

Although a plethora of equilibrium phase diagrams have been published, there is a scarcity of knowledge regarding the kinetics and mechanisms of lyotropic phase transitions. If we are to further our knowledge of events such as membrane fusion then a comprehensive understanding of the processes governing phase transitions, the type of intermediates formed and the mechanism by which a transition occurs are vital.

A superb technique for monitoring and initiating the structural evolution of such systems, in the millisecond regime, is time resolved X-ray diffraction, using pressure as the trigger mechanism. We have employed this technique to investigate lamellar – non-lamellar (P63/mmc phase) transition kinetics in cholesterol/ phospholipid/ diacylglycerol model membrane systems. Equilibrium pressure - temperature composition diagrams have been constructed, allowing us to choose appropriate pressure-jump parameters (temperature, initial and final pressures) for the kinetic studies.

The hair cycle oscillator

Yusur Al-Nuaimi
Manchester

Abstract not submitted.

Designing and building synthetic signal transduction circuitry in yeast

Stephen Checkley
Manchester

The yeast *Saccharomyces cerevisiae* possesses an intracellular signal transduction pathway enabling this organism to initiate a developmental response upon detection of a peptide mating pheromone. This developmental response is the focus of this study. The project aims to modify the natural mating response such that it activates an orthogonal, tuneable synthetic gene circuit designed to produce a quantifiable marker of activity. The organism can then be used as a synthetic biosensor to detect a range of compounds with biotechnology and pharmaceutical application. The project utilizes a combination of standard molecular biology wet lab techniques to construct the interacting genetic components, coupled with dry mathematical modelling techniques to design and optimise the genetic circuitry. The project utilizes an iterative process of design, implementation, and optimization to facilitate a synthetic biology approach to building gene circuits.

Adaptive evolution in the *Pseudomonas fluorescens* Wsp signalling pathway

Sam Farrell

Manchester

Experimental evolution can be a powerful tool for addressing questions on the genetic basis of evolutionary adaptation, allowing us to study evolution directly on manageable timescales in a controlled environment. The repeatable evolution of *Pseudomonas fluorescens* in a spatially structured environment provides just such a tool. *P. fluorescens* diversifies rapidly into niche specialists, one of which is a class of biofilm-forming strains named “wrinkly spreaders” (WS). Around half of WS strains studied previously contained loss-of-function mutations in the *wspF* gene, part of the Wsp signalling pathway. The Wsp pathway is analogous to the Che chemotaxis system in *Escherichia coli*. Such mutations cause downstream overproduction of a cellulose-like polymer, which in turn is essential in WS biofilm formation. Mutations in *wspF* do not simply destroy the protein's effectiveness; instead, different mutations in *wspF* lead to different WS fitnesses. Previous research was unable to establish clear causal links from different genetic changes in *wspF*, via the Wsp pathway, to different effects on fitness. We are addressing this question both using *in silico* models of the Wsp pathway and further characterising *wspF* mutations *in vivo*. We have evolved large numbers of novel WS strains, with a view to building a comprehensive collection of beneficial WS-causing *wspF* mutations. We have constructed ODE-based models of the Wsp pathway. Analysing a large number of samples of possible steady-state fluxes gives a broad view of the system's behavioural capabilities. These approaches, combined with assays to measure Wsp pathway output and strain fitness, will help bridge the gap between genotypic and phenotypic evolution in this model system.

Decisive Noise: Noisy intercellular signalling analysed and enforced through synthetic biology

Victoria Jackson
Manchester

Noise is inherent in most biological processes, for example in gene expression due partly to the low number of molecules involved and the probabilistic nature of the interactions. This can lead to a heterogenous population. However, populations of cells are still able to orchestrate their behaviour.

Components of the Lux quorum-sensing system (*Vibrio fischeri*) are used to engineer “noisy” sender cells and double-fluorescent protein receiver cells to monitor the response to the noisy signal.

Stochastic simulations of mathematical models of the synthetic system, and systems with different network structures (e.g. feedback loops), are conducted to analyse their noise propagation properties.

TBC

Sabrina Khaliq
Manchester

Abstract not submitted.

Experimental and Theoretical Modelling of the MAPK Pathway

Louise Maddison
Manchester

The MAPK pathway plays a crucial role in regulating the cellular response to external stimuli. Binding of growth factors and other mitogenic signals to cell surface receptors initiates a phosphorylation-dependent relay of protein activation, resulting in altered transcription, regulating cell proliferation and differentiation. Signalling through this pathway is regulated by the coordinated function of specific protein kinases and protein phosphatases. A perturbation of this signalling pathway is often associated with diseases such as cancer, modelling is a useful means to help understand the outcomes that may result following changes in component levels or activity. Expansion of current models of the MAPK pathway is necessary to facilitate accurate modelling by providing quantitative information on both total protein levels and stoichiometry of phosphorylation of protein components.

Mass spectrometry is being used to provide absolute quantitative data for ~30 protein kinases, phosphatases, scaffolds and substrates involved in MAPK signalling. We are applying the QConCAT methodology for absolute protein quantification, and have designed and expressed constructs comprising

selected proteotypic tryptic peptides for each of the proteins of interest. Multiple Reaction Monitoring assays are being introduced to quantify the proteins from mek inhibited and mutated raf and ras colon cancer cells

A systems biology approach to the production of red and white biotechnological products through systematic in silico studies

Olusegun Oshota
Manchester

Strain improvement for production of metabolites such as amino acids which previously was based on classical mutagenesis and screening procedures, an approach with limited usefulness, is now being achieved by rational metabolic engineering and in silico methods. However, modern rational metabolic engineering requires powerful theoretical methods such as pathway analysis, allowing for the consideration of the metabolic network topology. Elementary flux mode (EFM) analysis is one of the metabolic pathway analysis tools, which allows for the calculation of a solution space containing all possible steady-state flux distributions of a network. In this study, EFMs were determined for *Saccharomyces cerevisiae* grown on glucose, for the possibility of using the resultant modes in a gene deletion phenotype analysis and channelling carbon flux to a desired pathway, ultimately towards improved production of various target metabolites, including amino acids. The starting point for EFM analysis in Copasi software was the network of 136 reactions for production of amino acids from glucose in *Saccharomyces cerevisiae* obtained from the literature [Cakir et al, 2004] and pathway databases, comprising the reactions of glycolysis, the pentose phosphate pathway, the TCA cycle, the glyoxylate shunt, and the amino acid pathways encompassing the reactions for the amino acids with direct flux values greater than 2 [Gombert et al, 2001]. Computational and statistical tools are being developed for processing and classification of the large numbers of EFMs obtained, representing synthetic pathways leading to various amino acids and other metabolites, thereby enabling in silico manipulations for identifying how best to change the network to increase amino acid yields from glucose. We anticipate that such alterations will consist of a series of knockouts and also introduction of new genes to divert flux away from other parts of the network.

Towards a metabolic model of *Candida albicans*

Michael Rosefield
Manchester

Candida albicans is the primary human fungal pathogen, and can cause infections ranging from superficial to life-threatening.

In order to investigate the metabolic effects of a key virulence factor, the cAMP pathway, a genome-scale metabolic model is being constructed from publicly available sources.

Evolution directed small molecule combinatorial inhibition of IL-1 β expression

Ben Small
Manchester

Objectives: Regulation of pro-inflammatory gene expression such as Interleukin-1 β (IL-1 β) [1, 2] involves complex interactions between signalling cascades (e.g. MAPK, NF- κ B). Our aim is to use selective inhibitors of proteins within these cascades in combination and to identify those combinations that optimally modify nodes in the IL-1 β expression network using an evolutionary algorithm (EA). **Methods:** Identifying unique combinations of inhibitor affecting IL-1 β expression is a multi-objective optimization problem [3, 4]. These objectives inter alia are; inhibition of IL-1 β expression and combination effects on cell viability. J774 macrophages were pre-incubated with inhibitor cocktails prior to stimulation with lipopolysaccharide (1 μ g / mL) or DMSO (0.1%). Intracellular IL-1 β concentration was measured in cell lysates by ELISA, these data in conjunction with measurements of cell viability were fed into the EA and a new population of combinations generated. The iterative 'wet-dry' loop continuing until optimal combinations were located. **Results:** Successive generations of combinations have revealed their synergistic inhibition of LPS stimulated IL-1 β expression. **Conclusions:** Poly-combinatorial mixtures of compounds have the scope to reveal appreciable coalistic activity for exquisite combinations of inhibitor. Future work will focus on the assay of inhibitor combinations via flow cytometric measurements and the integration of this data into an in silico model. We thank the BBSRC/EPSRC for support.

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3. Kell, D. B. (2006). *FEBS J.* 273, 873-94.
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Blood, Sweat and Tregs: Immunological Regulation in the Tumour Microenvironment

Helen Angell
Nottingham

BACKGROUND

Several studies in colorectal cancer (CRC) patients appear to indicate a relationship between tumour immune infiltrate and clinical outcome. The aim of our study was to analyse levels of regulatory T cells (Tregs) in peripheral blood (PB) of CRC patients and to correlate this to infiltrating FOXP3+ Tregs in tumour tissue. We then investigate the ability of Tregs to suppress natural killer (NK) cell anti-tumour activity, via a natural killer group 2 member D (NKG2D) and transforming growth factor (TGF)- β mechanism.

METHODS

Matched patient PB/tumour tissue was analysed for FOXP3+ Tregs. Patient Treg suppression of effector cell proliferation was measured by ³H-Thymidine incorporation. Peripheral blood mononuclear cells (PBMCs) and Tregs were isolated from a healthy volunteer and co-cultured at different ratios with target tumour cells (e.g. K562). NKG2D, TGF- β ; RII, ULBP-2 and MICA expression was analysed on PBMCs and a variety of tumour cell lines.

RESULTS

Treg numbers were significantly higher ($P < 0.0001$) in PB of CRC patients ($n=11$) compared to healthy volunteers ($n=17$). CRC patients with higher levels of peripheral Tregs had higher Treg infiltrates. The suppressive ability of CRC derived Tregs was also demonstrated. A significant reduction in the percentage of K562 cell killing was observed when Tregs were introduced at a 1:30:60 K562:PBMC:Treg cell ratio ($P < 0.05$). We demonstrated that NK cells express NKG2D and TGF- β ; RII and have shown the expression of NKG2D ligands MICA and ULBP2 on various tumour cell lines.

CONCLUSIONS

We demonstrate an increase in PB Tregs in CRC compared to healthy donors. Accumulating evidence points to a critical interaction between Tregs and NK cells in tumour progression. Further investigation using a neutralizing

TGF- β ; RII and NKG2D antibody will aid in determining the role of Tregs in modulating NK cell tumour killing.

Environmentally activated drug delivery systems for osteoarthritis

Matthew Freddi
Nottingham

Intra-articular delivery is an attractive delivery route for osteoarthritis due to the nature of this disease where only individual joints are affected. This means that local delivery to only affected joints can reduce the systemic side effects and hence improve the long term tolerance of treatment. However the synovial cavity is subject to a rapid clearance and so an injectable hydrogel is being developed to increase the residence time of a nanoparticle delivery system within the joint.

Intracellular delivery of neutralising single chain antibodies specific for the anti-apoptotic proteins Bcl-2 and Bcl-XL

Gavin Hackett
Nottingham

Intracellular delivery of neutralising single chain antibodies specific for Bcl-2 and Bcl-xL

G.S.Hackett¹, R.Jackson², P.M.Fischer¹ and W.C.Chan¹

School of Pharmacy, Centre for Biomolecular Sciences, University of Nottingham NG7 2RD, UK
Research Technology, MedImmune, Milstein Building, Granta Park, Cambridge CB21 6GH, U.K.

Antibodies are attractive therapeutic agents due to their high specificity and affinity for target molecules. Therapeutic antibodies are however, almost exclusively limited to extracellular targets, their large size rendering them incapable of passing cell membranes.

The aim of this project is to modify antibodies that are specific to an intracellular target (Transbody) to enable them to penetrate cells and elicit a therapeutic response. The anti-apoptotic protein Bcl-2 is used as a model target, over expression of which occurs in a wide range of cancers and constitutes a critical step in tumour development.

The major goal of this project is to define pharmacokinetic (PK) and pharmacodynamic (PD) properties of a transbody using cell penetrating peptides (CPPs). Subsequent modification to the CPP to optimise PK and PD properties will be investigated. This will provide insight into the possible use of transbodies as therapeutic agents and potential ways to modify CPPs towards more efficient in vivo delivery of macromolecules.

Neutralising single chain antibodies (scFv) have been isolated that are specific for Bcl-2 or its homologue Bcl-xL, using antibody phage display. Synthesis of HIV Tat and Antp has been carried out with the view to conjugate these to scFv, specifically to a C-terminal cysteine. This will be followed by cellular apoptosis assays to compare scFv potency with that of known small molecule Bcl-2 and Bcl-xL inhibitors.

Characterisation of ATP-binding cassette (ABC) transporters in the

Victoria Hutter
Nottingham

Expression and functionality of ATP-binding cassette (ABC) transporters were assessed in Calu-3 cell monolayers cultured at an air-liquid interface.

Transporter gene expression was in agreement with published data in human lungs with the exception of BSEP which was over-expressed in Calu-3 cells.

Net secretory transport of the ABC substrate 3H-digoxin was reduced in presence of the inhibitors verapamil, PSC833 and MK571. However, the transporter(s) involved could not be identified.

Taking off the tumour immunity 'handbrake'

Adnan Khan
Nottingham

The immune system can play a vital role in not only tumour growth and development, but also tumour clearance. Here, we aim to demonstrate the pharmacological inhibition of the migration and activity of regulatory T cells which could be conducive to the propagation of tumour immunity.

Bioenergetic changes in tumour spheroids

Katarzyna Bloch
Oxford LSI

The tumour microenvironment is acidic, hypoxic and low in nutrients. Changes in metabolic pathways involved in energy production are crucial adaptations for tumour survival. Shifting from oxidative phosphorylation to reliance on glycolysis and increased flux through glutaminolysis and pentose phosphate pathways are strategies used to maintain high rates of growth and proliferation. Tumour spheroids (TS) are three dimensional aggregates of cancer cells that replicate characteristics of avascular solid tumours in vitro.

The aim of this study is to examine metabolic changes in TS under the stress condition. The stress condition used in this research is lack of media replacement that replicates nutrient deprivation and variation in growth factors present in microenvironment of avascular solid tumours.

Growth-induced mass-flows in fungal networks

Luke Heaton
Oxford LSI

Fungi form efficient, adaptive, transport networks. Mammals have cardio-vascular systems, but how do fungi distribute essential resources throughout the organism? Water is incompressible, so we propose that within

fungi, fluid flows from the sites of water uptake to the sites of growth. To assess the scale of growth-induced mass flows, we imaged growing fungal networks, calculating currents that are consistent with observed changes in volume while minimising viscous drag. Predicted speeds were in reasonable agreement with experimental data. Furthermore, cords that were predicted to carry fast-moving currents were significantly more likely to increase in size than cords with slower movement.

New Hydrogen-Bonding Motifs for Macromolecular Synthesis

Jem Pearson
Oxford LSI

I will present the design and synthesis of four organic molecules which are designed to hydrogen-bond to each other in an orthogonal manner.

Statistical methods for estimating the rate of de novo mutation at FGFR3 in sperm samples from healthy men using high-throughput sequencing

Susanne Pfeifer
Oxford LSI

Some rare congenital disorders (such as Apert, Crouzon, Pfeiffer, Muenke, Costello and Noonan syndromes and achondroplasia) originate from spontaneous mutations in the germline of healthy fathers which are older than the average (paternal age effect). The prevalence of these specific mutations may reflect a protein-driven, positive selection of mutant cells according to the functional consequences of the encoded amino acid substitution. We aimed to use new sequencing technologies to quantify all

possible point mutations at codon K650 (AAG) of the fibroblast growth factor receptor 3 (FGFR3) leading to thanatophoric dysplasia. 78 sperm and 8 blood samples were sequenced using the Illumina sequencing technology. An attempt to use

Illumina's inbuilt quality scores to estimate the rate of de novo mutations could not account for the underlying error structure. Therefore, a Bayesian approach was used to fit a model to the observed counts of each codon in the sequencing data to account for errors and bias derived from the rounds of PCR and digestion during the sample preparation, and the sequencing process. Titration data were analysed together with biological samples to validate our method down to the level of 10^{-5} . Whilst mutation rates in blood were low, 73% of the total mutations quantified in the sperm samples were caused by a 1948A>G mutation. It reached high mutation levels (with a maximum of 2.1×10^{-4}) in sperm samples which were significantly correlated with donor age (Spearman rank $r=0.34$, $P=0.002$). Several other substitutions attained levels $>10^{-5}$ in a minority of sperm samples. These results show the utility of advanced statistical methods to estimate mutation rates in human sperm from high-throughput sequencing data down to the level of 10^{-5} , whilst capturing subtle features of the machine and run dependent error structure.

Experiment-Guided Analysis and Modelling of Bacterial Taxis

Gabriel Rosser
Oxford LSI

TBC

Systematic Network Analysis

Sumeet Agarwal
Oxford Systems Biology

Many real-world systems are most naturally represented as networks, and a

variety of measures exist for their analysis. However, studies of networks typically employ only a small, largely arbitrary subset of these, and the lack of a systematic comparison makes it unclear which metrics are redundant or complementary. We present a framework for systematic analysis of networks and network metrics, and use it to analyse a large variety of real networks, several synthetic network models, and hundreds of metrics or summary statistics thereof. We demonstrate the utility of the framework for finding redundant metrics, fitting models to real networks, classification of networks, studying evolving networks, and determining the robustness of metrics to network damage and sampling effects.

TBC

Maria Rosa Domingo Sananes
Oxford Systems Biology

Abstract not submitted.

TBC

Guido Klingbeil
Oxford Systems Biology

Abstract not submitted.

The Function of Communities in Protein

Interaction Networks at Multiple Scales

Anna Lewis
Oxford Systems Biology

Background: If biology is modular then clusters, or communities, of proteins derived using only protein interaction network structure should define protein modules with similar biological roles. We investigate the link between biological modules and network communities in yeast and its relationship to the scale at which we probe the network.

Results: Our results demonstrate that the functional homogeneity of communities depends on the scale selected, and that almost all proteins lie in a functionally homogeneous community at some scale. We judge functional homogeneity using a novel test and three independent characterizations of protein function, and find a high degree of overlap between these measures. We show that a high mean clustering coefficient of a community can be used to identify those that are functionally homogeneous. By tracing the community membership of a protein through multiple scales we demonstrate how our approach could be useful to biologists focusing on a particular protein.

Conclusions: We show that there is no one scale of interest in the community structure of the yeast protein interaction network, but we can identify the range of resolution parameters that yield the most functionally coherent communities, and predict which communities are most likely to be functionally homogeneous.

Protein adducts at critical protein sites as markers of toxicological risks

Paul Getty
RASOR Proteomics

The formation of conjugates between the electrophilic reactive metabolites of drugs and nucleophilic protein sites is known to be associated with toxicological risk. At present there is no low cost and high throughput means of reliably detecting the presence of drug-protein adducts in vitro or in vivo. Current methodologies require the expensive and time consuming synthesis of radiolabelled NCE /drug(new chemical entity) analogues and subsequent

proteomic analysis. The development of a reliable high throughput methodology would facilitate the study of underlying mechanisms of toxicity and prove useful in early screening of potential drug molecules.

Assays using trapping agents such as glutathione or more recently the tripeptide GSK (gamma-glutamylcysteinylsine) are successfully used for detecting a wide range of drug reactive metabolites. Trapped metabolites can then be characterised by mass spectrometry and profiles can be associated with particular drugs or NCEs. Glutathione plays an important role in phase II metabolism of xenobiotics via an enzyme catalysed reaction in which it is conjugated with metabolites. In contrast to this, the modification of proteins by means of electrophilic attack on nucleophilic centres often occurs in an enzyme independent manner and is unlikely to be analogous to the glutathione model. In order to create a more suitable model system 3 short polypeptides were designed and synthesised. Peptides were designed to include a cysteine residue (nucleophilic target site) as well as an N-terminal biotin tag for affinity purification. These peptides were incubated with Clozapine, cytochrome P450 1A2, cytochrome c reductase and NADPH in an appropriate buffer. Samples were taken and analysed by nanoLC-MS using a triple quadrupole LIT machine. Using results from previous metabolite trapping experiments a precursor ion scan was designed in order to identify peptides modified by drug metabolites. MS/MS was carried out on these ions in order to identify false positives. It was found that a precursor ion scan for a mass of 359 Da could successfully identify modification of all 3 synthetic peptides.

Absolute quantification of protein isoforms

Robert Kelly
RASOR Proteomics

Since the publication of the human genome, understanding its functional complexities has become a primary goal of high-throughput experimental research. From a proteomic point of view it is alternative splicing which contributes to the complexity of the proteome and possibly even the phenotype. Through the alternative splicing of a single gene it is possible to generate multiple protein isoforms with diverse and even opposing regulatory functions. In addition alternative splicing has been linked to a wide variety of medical conditions including bipolar disorder, schizophrenia, cancer, diabetes, multiple sclerosis, cystic fibrosis and asthma.

While the high sequence similarity with which some of these isoforms present prevents their differentiation on a 2D gel and the raising of specific antibodies can be difficult, their detection and quantification is still possible through the use of labelled synthetic peptides and mass spectrometry. Utilising a technique

termed AQUA, in which a labelled (^{13}C & ^{15}N) peptide is synthesised analogue to a specific proteolytic peptide from a target isoform, this project deals with analysing the effect which different cellular states have on isoform quantities and expression ratios.

TBC

Dominic Ketley
RASOR Proteomics

Abstract not submitted.

Discovery of the MDM2 Interactome Using Label-Free Proteomic Mass Spectrometry

Jude Nicholson
RASOR Proteomics

To develop proteomic methods which can be used to identify the interactome of the p53 E3 ligases MDM2, the well characterised MDM2 inhibitor Nutlin-3 which mimics the BOX-I region of p53 to block the p53 interaction with the MDM2 N-terminal domain was used to treat MCF7 cells over a time course. Data-independent shotgun mass spectrometric analysis of the cell lysates identified 151 proteins which fluctuate after treatment with Nutlin-3, a subset of which are altered two-fold after a 2 hour Nutlin-3 treatment, and a number of proteins which are known to interact with MDM2 were amongst the results. A motif with similarity to an MDM2 binding sequence in p53, BOX-I, was found in a subset of the proteins which fluctuate in stability after Nutlin treatment, and these motifs were validated for MDM2 binding by ELISA.

Development of a reproducible wound to create an in vitro model of spinal cord injury

Lewis Ross
RASOR Proteomics

Damage to the central nervous system (CNS) causes highly debilitating injuries to victims due to the loss of motor control and sensory data from, potentially, large areas of the body. The lack of growth factors and the formation of a scar consisting of densely packed glial cells prevents severed spinal axons, whose cell bodies survive, from re-growing past the site of injury thus almost always making the damage permanent.

One factor critical to producing an in vitro model of spinal cord injury (SCI) is the creation of a reproducible wound. Excessive damage caused to substrates during wounding could lead to distortion of topography and irregular wounds and re-growth.

A novel method of characterising mechanical properties of collagen based scaffolds for tissue engineering

Grahame Busby
Strathclyde Medical Devices

Collagen, the most abundant structural protein in the human body, has become the most widely used matrix for tissue engineering. However, collagen that has been implanted in the body undergoes a constant process of remodelling, and the gradual degradation of the collagen within the matrix must be kept in balance with collagen synthesis if the scaffold is to maintain its strength and integrity. To better understand and assess this process, it is necessary to be able to characterise the mechanical properties of collagen gels over a period of time.

Current protocols tend to be destructive, “one-shot” events or are highly complex processes. We have developed a non-destructive technique that may facilitate the mechanical characterisation of a hydrogel over a period of time, whilst maintaining sterility. Biphasic theory has often been used to characterise

cartilaginous tissues, yet to our knowledge it has not been applied to collagen-based scaffolds. The initial validation of the theory for this biomaterial, and the testing of the device, is reported.

Mechanical HIFU and it's BioEffects

Steven DGLISH
Strathclyde Medical Devices

HIFU shows increasing promise as a non-invasive treatment for various types of cancer. The aim of this project is to determine the biological affects of mechanical HIFU and differentiate it from the biological affects of thermal HIFU. The project will also determine the affects of blood flow on mechanical HIFU.

Physical and Mathematical Modeling of the Synovial Joint and Cartilage Repair Products

Anthony Herbert
Strathclyde Medical Devices

There is an urgent need to improve the way in which young patients with bone and joint disease are treated. Surgical procedures, such as autologous cell implantation and a new generation of cartilage repair products implementing the patient's own cartilage in conjunction with synthetic, degradable biomaterials and gels, promise to transform the manner in which patients are treated. In order to establish the functional properties required for successful engraftment of these products into native cartilage, we aim to develop realistic physical and mathematical models of the native cartilage environment together with the cartilage repair products.

Effect of High-Intensity Narrow-Spectrum light on Osteoblast Function

Richard McDonald
Strathclyde Medical Devices

A significant portion of medical devices fail due to acquired infection, and infection rates after arthroplasty surgery are between 1-4%, with considerably higher rates after revision surgery. To reduce the associated costs with treatment of infection and revision surgery, a new preventative method is required. High intensity narrow spectrum (HINS) blue light (405 nm) is a new technology shown to have bactericidal effects on a range of medically important bacteria. The effects of HINS light on osteoblasts were investigated to determine the suitability of this technology for infection prevention in operating theatres, during surgery and post-operative dressing changes.

Disposable biocompatible pH sensor for monitoring wound healing

Stephen Milne
Strathclyde Medical Devices

Wound healing research has discovered, mainly in cell or animal models, that there are many states in the wound that can be optimised for healing including moisture and pH. However, the application of this knowledge in clinical practice has been limited by the fact that there are few ways to monitor wounds in real time for patients. The project will design and produce a prototype of a disposable pH sensor for monitoring the pH of wounds during healing. This sensor will enable a better understanding of the complex problem of chronic wound healing and understanding of how pH varies with respect to healing.

Modelling the Role of Aneuploidy in Tumour

Evolution

Arturo Araujo
UCL

The role of aneuploidy (the cellular state of having an abnormal number of chromosomes) in cancer is not well understood. A recent and compelling theory suggests that aneuploidy may be the initial step towards the generation of variation in cancer. This theory however is very difficult to test in a biological experiment. To address this theory and explore the role that aneuploidy has on the development of cancer, a computational model has been developed. Results show that, under certain conditions, aneuploidy creates a pathway for the generation of novel genotypes that may lead to emergent cancer-like behaviour.

TBC

Gwenan Knight
UCL

Abstract not submitted.

TBC

Julija Krupic
UCL

Abstract not submitted.

Actin Dynamics and Cell Death in a Model Epithelium

Dorothy Kuipers
UCL

My project employs molecular, imaging and modeling techniques to investigate actin dynamics at sites of cell death in simple epithelia. I aim to quantify and model the forces that drive epithelial repair, with particular interest in whether they are generated in the dying or surrounding cells.

Fluid-Structure Interaction Modelling of the Mitral Valve

Kevin Lau
UCL

Abstract not submitted.

THE IMPACT OF BLOOD FLOW CHANGE ON INTERORGAN AMMONIA METABOLISM IN CIRRHOSIS.

Noiret Lorette
UCL

Although, urea synthetic capacity in cirrhosis is reduced, the severity of hyperammonemia is contributed to by the development of porto-systemic collaterals. As multiple organs are involved in the regulation of ammonia

metabolism, changes in organ perfusion and porto-systemic shunting was hypothesized to be an important regulator of ammonia levels. To test this hypothesis we are developing a mathematical model which couple blood flow changes and ammonia/glutamine fluxes across organs. The first component of the model provides the distribution of cardiac output for different scenarios of collaterals.

Targeted Magnetic Delivery And Tracking Of Cells Using A Magnetic Resonance Imaging System

Johannes Riegler
UCL

The success of cell therapies depends on the ability to deliver the cells to the site of injury. Targeted magnetic cell delivery is an emergent technique for localised cell transplantation therapy. The use of permanent magnets limits such a treatment to organs close to the body surface or an implanted magnetic source. A possible alternative method for magnetic cell delivery is magnetic resonance targeting (MRT), which uses magnetic field gradients inherent to all magnetic resonance imaging system, to steer ferromagnetic particles to their target region. In this study we have assessed the feasibility of such an approach for cell targeting, using a range of flow rates and different super paramagnetic iron oxide particles in a vascular bifurcation phantom. Using MRT we have demonstrated that 75% of labelled cells could be guided within the vascular bifurcation. Furthermore we have demonstrated the ability to image the labelled cells before and after magnetic targeting, which may enable interactive manipulation and assessment of the distribution of cellular therapy. This is the first demonstration of cellular MRT and these initial findings support the potential value of MRT for improved targeting of intravascular cell therapies

TBC

David Schulz
UCL

Abstract not submitted.

Finite size effects in a stochastic condensation model

Paul Chleboun
Warwick Complexity

We study finite size effects on the condensation transition in a driven diffusive system known as the zero-range process. The condensation transition is already well understood in the thermodynamic limit, however this is at best an idealisation of a large finite system. It is observed that even on relatively large finite systems these effects can be significant and counter intuitive. We observe a large current overshoot above the limiting critical value and an abrupt change between a putative fluid and condensed phases (reminiscent of a first order transition although the phase transition is known to be continuous in the thermodynamic limit). Close to the abrupt transition we also observe metastable type switching between the two 'phases'. We can define an effective free energy landscape and thus predict the scaling of the lifetime of the two phases. We formulate approximations for the fluid and condensed 'phases' and use these to derive the leading order finite size effects.

Dynamic Bayesian analysis of protein signalling connectivity reveals heterogeneity within single breast cancer subtype

Steven Hill Warwick Complexity

Intracellular protein signalling plays an important role in the control of cell function. Aberrations in signalling behaviour play a key role in the biology of cancer. However, we remain limited in our understanding of cancer-specific changes to signalling networks. Here, we utilise stochastic models known as dynamic Bayesian networks to infer protein signalling connectivity using time-varying data from breast cancer cell lines. We take a Bayesian approach, incorporating existing biological knowledge into inference by means of an informative prior on network structure. Instead of resorting to approximate schemes, such as MCMC, we show how biochemically-motivated sparsity constraints permit exact inference to determine posterior probabilities of interest. We apply these approaches to high-throughput proteomic data from individual breast cancer cell lines. The cell lines we study are not only both breast cancers but furthermore belong to the same, well-characterized breast cancer subtype. Yet we find striking evidence of heterogeneity between these cancers with respect to signalling behaviour. Independent experiments validate some of these differences, suggesting that there may be considerable heterogeneity even within recently characterized cancer subtypes.

Determination of Cluster-Cluster Aggregation Kernels Using Inverse Methods

Peter Jones Warwick Complexity

We seek to determine the extent to which the existence of noise and spatial correlations in a real aggregation process can be 'folded back' into an estimation of a kernel function that could then be used within a mean-field phenomenological surrogate. Such a surrogate model might then prove useful for enhancing the accuracy of some models of larger processes that include aggregation processes that are presently weakly modelled. It might also allow us to deduce key properties of the real aggregation processes in question.

Functionality and Speciation in Boolean Networks

Jamie Luo
Warwick Complexity

We investigate the effect of increased complexity on the neutral evolution metagraph and its connectedness.

Out-of-Equilibrium Exchange

James Porter
Warwick Complexity

Will present an out-of-equilibrium model of trading both from an analytical angle, deriving asymptotic results, and numerical angle. The key distinctive features of the model to be presented are:

“Zero” information: contrary to most economic models we assume agents do not know much about other agents. In particular they do not know other agent’s utility functions or bundles; they make and receive offers.

Cautious Trading: Given agent’s lack of information they do not act in anticipation of uncertain future utility, they will only make trades which immediately improve their situation.

Graded firing patterns in attractor networks

Tristan Webb
Warwick Complexity

Experimental evidence suggests that the brain represents information by some neurons firing at a faster rate than others. We show how this can improve the performance of an decision making attractor network. We hypothesize that increased synaptic variation is the cause for the decreased reaction time.

TBC

marina diakonova
Warwick Complexity

Abstract not submitted.

Investigation of multiple paralogous metallothioneins of a coastal cyanobacterium

Jie Chu
Warwick MOAC

Metallothionein (MT) is cysteine-rich protein with small molecular weight, usually 5-10 kDa, and occupied with multiple metal ions, e.g. zinc, copper, cadmium etc. MTs were previously thought to be restricted to eukaryotes, recently a large amount of both fresh and marine cyanobacteria are found to contain metallothionein genes [1].

Bacterial MT, SmtA found in fresh water Cyanobacteria *Synechococcus* PCC7942 is the only fully characterised prokaryotic MT[2]. Now, other cyanobacteria have been found to contain similar proteins; the *Synechococcus* CC9311 is particularly special one. This strain was found to contain no less than 4 MTs (usually there's one found in most cyanobacteria). Those proteins are coded by 4 genes: *sync_0853*, *sync_1081*, *sync_2426* and *sync_2379*. So we hypothesise that the different MTs with different metal binding properties are expressed in response to changes in the environment. In this project, we plan to comprehensively characterise the four paralogous proteins, such as the metal specificities and binding affinity, protein folding and structure, metal uptake and release kinetics; and assess the effects in metal toxicity and deficiency on

bacterial growth in defined media with different trace metal elements. Ultimately this project aims to establish the relationship between environmental factors, express patterns, and biophysical properties.

Transcriptional signatures of single cell lineages

Mike Downey
Warwick MOAC

Live cell fluorescence has become a valuable technique for observing gene expression in cells. The goal of the project is to develop tools to study gene expression using a combination of High Throughput Imaging and live cell fluorescence. This will be based on action of the MSX1 regulatory modules and their role in the fate of mesenchymal stem cells, using C2C12 mouse cells as the model cell. Current systems have shortcomings in segmentation and tracking. The challenge is to create a flexible but easy to use system to analyse the cell data. Tools have been developed for statistical analysis and visualisation of the data.

TBC

Nigel Dyer
Warwick MOAC

Abstract not submitted.

Massively parallel combinational mutant
synthesis

Max Joseph
Warwick MOAC

Developing methods for parallel assembly of large numbers of related but unique DNA sequences.

Enzymology of the penicillin-binding proteins

Daz Braddick
Warwick Systems Biology

Studying the enzymology of the *Strep. pneumoniae* penicillin-binding proteins, the primary determinants of resistance in the bacteria to our most important drugs, the penicillins.

Systems analysis of G protein-mediated signalling - spatial regulation of RGS

Wayne Croft
Warwick Systems Biology

G-protein coupled receptor (GPCR) signalling controls all aspects of eukaryotic physiology. GPCRs initiate signalling by promoting exchange of GDP, on the α subunit of heterotrimeric G-proteins, for GTP thereby initiating a cellular signalling cascade. Signalling is terminated by hydrolysis of GTP to GDP through the intrinsic GTPase activity of the α subunit. This activity is accelerated by the regulator of G-protein signalling (RGS) proteins. Through taking a systems biology approach, the RGS protein has been shown to be capable of positive regulation in addition to its more common negative regulatory role.

Recent Biological data implicates a role for an interaction between RGS and the GPCR for RGS catalysed GTP hydrolysis on the α subunit. A

mathematical model of the system predicts that it is the plasma membrane localisation primarily directed by the RGS-GPCR interaction that is required for this regulatory event.

Combinatorial regulatory interactions in myoD1 expression

Polly Downton
Warwick Systems Biology

Cis-regulatory modules (CRMs) are the functional DNA elements that encode the spatial and temporal expression patterns of a gene. Each gene is regulated by multiple CRMs, which may be hundreds of kilobases distant from the promoter. Many mammalian CRMs have been characterised in isolation, but how CRMs act together to regulate complex patterns of expression is unknown.

Five CRMs that regulate expression of the muscle-specifying master gene myoD1 have been identified. Reporter plasmids containing thirty-two combinations of these CRMs were made, and their expression was measured in an immunologically-defined subpopulation of transfected mouse myoblast cells. This demonstrates that the CRMs do not operate independently of each other, supporting the existence of a functional interaction. Conserved transcription factor binding sites have been identified in each CRM, and these are being investigated by ChIP and mutagenesis strategies to identify the functionally important components of each CRM.

Seizure prediction in in vitro models of epileptiform activity

Robert Gardner
Warwick Systems Biology

Epilepsy is one of the most common neurological disorders affecting 1% of the global population, of whom 20 million people are untreatable by current drugs or surgical techniques. Epileptic seizures occur in a variety of forms with the most significant resulting in complete loss of consciousness for the sufferer. The ability to predict an impending seizure would provide a better quality of life for sufferers, in addition to opening up potential time-specific treatments.

In this work, recordings are taken from hippocampal slices subjected to epileptiform activity inducing conditions. This preparation provides an in vitro model of temporal lobe epilepsy, providing data that includes both ictal (seizure) and pre-ictal components. The work presents current progress in attempts to determine precisely when seizures are going to occur. It also highlights potential future avenues of research to reduce the frequency and severity of seizures in epilepsy sufferers.

Discovery of Promoter Motifs in *Arabidopsis thaliana* Stress Response Genes

Richard Hickman
Warwick Systems Biology

Environmental stress is responsible for reduced crop productivity throughout the world. Plant adaptation to stress is dependent upon the initialisation of cascades of molecular networks involved in stress perception, signal transduction and the expression of specific stress-related genes. By examining large, high-resolution microarray datasets we can track gene expression changes over time in response to fungal pathogen infection, bacterial infection, drought, high-light and senescence in the model organism *Arabidopsis thaliana*. Dramatic variations in gene expression are observed at the onset of stress with different groups of genes showing different expression time-courses. This observation must, for a large part, be down to the action of different transcription factors (TFs) or combinations of TFs binding to the promoters of genes in each group. We are combining both expression and sequence data analysis in order to identify promoter motifs that may be responsible for recruiting specific TFs that orchestrate the activation of stress-response genes. Predictions will be tested using experimental techniques.

Transcriptional modules controlling the defence response of *Arabidopsis thaliana* to infection by *Botrytis cinerea*

Steven Kiddle
Warwick Systems Biology

It is already known that the plant defense response involves the regulation of gene expression. By measuring gene expression we are beginning to characterise genome-wide transcriptional regulation and are focusing on transcription factors that have a large impact on susceptibility.

To extract regulatory predictions from this data requires the application of sophisticated and novel computational approaches; such as hypothesis testing, clustering, alignment and inference. For example, I have developed a method called Temporal Clustering by Affinity Propagation that detects time-delayed correlations between groups of genes, which I show to recover known and predict novel gene regulation (Kiddle et al., 2010).

Hypoxia Adaptation And Performance At Altitude

Heather Riley
Warwick Systems Biology

In 2007, over 200 volunteers climbed to Everest Base Camp during the Caudwell Xtreme Everest Expedition (CXE) in the largest ever field study of human adaptation to hypoxia at altitude. In collaboration with the team who lead the CXE Expedition, this project looks at the molecular basis of such adaptation, and aims at predicting how well an individual will perform at altitude, as a measure of hypoxia adaptation.

Cartography of Cell Motion

Richard Tyson
Warwick Systems Biology

QuimP allows the automatic tracking of cell outlines and samples intensities of fluorescently tagged proteins in the cortex of migrating cells. QuimP has been successfully used to study chemotaxis and the dynamics of actin regulation. We want to develop the next-generation software which will allow accurate correlation of local protrusion/retraction activities of the cell outline, their angular distribution and persistence with local fluorescence intensities.

We have development of a novel electrostatic approach for quantifying local deformations of the cell membrane and envisage the release of open source software that will contribute to the building of a standardized library of spatio-temporal maps of protein localisation.

Viscoelastic Properties of Blood Clots

Peter Allan
White Rose

Blood clotting is an essential process of life as it prevents an organism from bleeding to death when tissue is damaged. A clot forms from a biochemical cascade initiated when a cell is ruptured - ultimately the protein fibrinogen is converted into fibrin which then has the ability to self-assemble into a 3 dimensional fibrous mesh. This branched network of fibres traps red blood cells and is anchored by platelets to the site of injury; this arrests the flow of blood while damaged cells are repaired.

We can use the Atomic Force Microscope (AFM) to follow this reaction in-situ with sub-molecular resolution, and answer many questions about the detail of this process. Many diseases are associated with blood clotting, e.g. strokes and heart attacks, and result from clots which do not form optimally. This can be related to microscopic changes in structure and therefore mechanical properties of the fibrils. The final mechanical properties of fibrin fibres are extremely important to a clot's function, and we use the AFM to quantitatively measure the elasticity of a single suspended fibre. This will be correlated with measurements of the viscoelastic properties of the final clot determined using a novel magnetic tweezer device.

The action of various blood clotting factors, such as FXIII, will be investigated, along with mutant fibrinogen from patients in order to discover the molecular basis of their disorders. Diabetes and vascular disease are a major cause of human mortality, both of which are linked to blood clots. The study of blood clots physical properties will help to better understand the mechanisms which underpin these diseases and should ultimately translate to better prevention and treatments.

cross linking nucleobases to form constrained substrates to test the activity of a Flap Endonuclease

Amanda Beddows
White Rose

The formation of a disulfide bond between two substituted nucleobases is described along with the objective of producing 'locked' substrates to test the requirement by Flap Endonucleases for resolution of secondary structure for activity

Convergent transcription and nested gene models studied by Atomic Force Microscopy

Daniel Billingsley
White Rose

A nested gene is located within the boundaries of a larger gene, often within an intron and in the opposite orientation. Nested genes were first discovered in *Drosophila* flies [1], but have since been observed in a range of organisms, both prokaryotic and eukaryotic, and also in humans. The presence of nested genes in introns may raise questions about whether they can be transcribed simultaneously or even be co-regulated. Although the existence of nested genes has been established, there has not been extensive research into this

area. The existence of simultaneously-transcribed nested genes would require a process of convergent transcription resulting from the presence of two oppositely aligned promoters and two RNA polymerases (RNAPs) travelling towards each other. These will collide at some point on the DNA leading to a number of different possible situations. Atomic force microscopy (AFM) was used to investigate the collision event between *E. coli* RNAPs on a linear DNA template with two convergently aligned promoters. Complexes representing different stages of the transcription cycle were formed, and by comparing the positions of the RNAPs on the DNA it was possible to examine the results of such collisions. These can involve the backtracking of one polymerase, the expulsion of one or both polymerases from the DNA chain, or the stalling of both polymerases [2].

1. Henikoff S, Keene M.A., Fechtel K., Fristrom J.W., Cell 44 (1986) 33-42
2. Crampton N, Bonass W A, Kirkham J, Rivetti C, Thomson N H, Nucleic Acids 34 (2006) 5416-5425

New Synthetic Chemical Approaches to the Treatment of Methicilin Resistant *Staphylococcus Aureus*

Simon England
White Rose

Abstract not submitted.

The effect of acute rejection on erythrocyte metabolic profiles: A ¹H-NMR study of renal transplantation

Hayley Fenton
White Rose

Hayley Fenton¹ Paul Goldsmith² Rajendra Prasad² Niaz Ahmad² and Julie Fisher¹

¹School of Chemistry, University of Leeds, Leeds, UK.

²Hepatopancreatobiliary and Transplant Unit, St. James's Hospital, Leeds, UK

Background: Acute rejection is associated with an increase in the risk of graft loss in kidney transplantation. Current clinical tests are non-specific and often the only way to accurately diagnose acute rejection is via an invasive biopsy, posing risks to both patient and graft, and potentially leading to delayed detection of acute rejection episodes. As a result the condition may be allowed to progress, with a greater degree of damage inflicted to the graft before intervention is carried out. Hence an early onset biomarker is sought.

Method: A Nuclear Magnetic Resonance (NMR) based approach using lipidic and aqueous extracts from erythrocyte samples from eighteen patients, taken at regular intervals up to 7 days post-transplant has been performed. Integration of characteristic signals was carried out in order to observe differences in the lipid profiles between rejecting and non-rejecting patients. A chemometric approach involving pattern recognition methods and multivariate statistical analysis (primarily Principal Component Analysis) will also be used to analyse the NMR spectra in search of a biomarker indicating acute rejection.

Results: Differences were observed in the relative concentrations of particular lipid classes between rejecting and non-rejecting patients.

Conclusion: These early results suggest that NMR-based metabolic profiling may provide some insight to a biomarker of acute rejection.

Modelling Malaria Metabolism

Thomas Forth
White Rose

Human acute malaria, which is caused by the parasite *Plasmodium falciparum*, kills over a million people every year and is particularly lethal to young children and pregnant women. Rapidly evolving resistance to existing drugs threatens to leave us without a defence against the parasite. Moreover, attempts to develop a vaccine have so far met with only limited success.

I aim to use the existing and proven method of flux-balance analysis (FBA) to advance our understanding of the metabolic network of the erythrocytic life stages of *P. falciparum*. By considering the interaction of all metabolic reactions, rather than considering each reaction in isolation, I hope to suggest drug targets against the parasite. A fuller understanding of malaria metabolism could also allow the design of attenuated strains for use in vaccines.

Although *P. falciparum* has been grown in culture since 1976 it remains difficult to grow large amounts of the parasites or to manipulate them genetically. This limitation has made it difficult to develop a metabolic network model suitable for FBA. I have overcome part of this limitation by experimentally deriving a biomass/drain function for *P. falciparum* when grown in minimal media of known composition.

I have developed MetNetMaker, a simple metabolic network editor based on the KEGG LIGAND database. Using this software I have completed a systems biology markup language (SBML) format metabolic network model bringing together much of the existing but uncollated knowledge on malaria metabolism. The model I have created is unique because it incorporates experimentally measured information on biomass production and nutrient uptake and creates a metabolic model ready for FBA in the COBRA toolbox.

POlymersome penetration onto human skin

Carla Pegoraro
White Rose

The aim of this research is to investigate penetration of polymersomes into the deep layers of human skin to deliver useful payloads. Polymersomes are a novel drug delivery solution based on flexible and tough nano-sized polymeric vesicles formed from high molecular weight amphiphilic pH sensitive block copolymers

(poly(2-(methacryloyloxy)ethyl-phosphorylcholine)-co-poly(2-(diisopropylamino)ethylmethacrylate)(PMPC-PDPA), fluorescently labelled with Rhodamine) that self-assemble when exposed to an aqueous environment into vesicles. The penetration of polymersomes across ex-vivo or tissue engineered skin or synthetic 50nm pore-sized membranes was studied using a perfusion system developed in our laboratory. Results show that polymersome diffusion across the barrier is strongly dependant on concentration, flow and the ratio between vesicle and membrane pore size. With higher concentration gradients and flow rates diffusion across the barrier is increased, confirming their dependence on the osmotic gradient across the barrier. Polymersomes maintain structural stability and do not defragment during diffusion (as shown by Dynamic-light-scattering analysis, DLS). This was further confirmed by measuring their ability to carry proteins across the barrier without significant loss. Diffusion across membranes also increases with polymersome size suggesting that their ability to deform is crucial for penetration. These promising results support using this novel delivery system with potential applications spanning from cosmetics, to dermatology to vaccine technology.

TBC

Helen Phillips
White Rose

Abstract not submitted.

Mechanical Stimulation for an in vitro Human Bone Model

Jennifer Robertson
White Rose

A tissue engineered bone construct has the potential to be used as an in vitro model to further understanding of the interactions between the main cell types responsible for the maintenance of healthy bone matrix. As the structure of bone is known to be improved in vivo by mechanical stimulation, it may be possible to improve matrix deposition and mineralisation in vitro by the application of suitable mechanical forces throughout culture. Direct mechanical compression and low magnitude, high frequency vibration are being investigated during the development of a 3D human bone model.

Using a novel stretching device to study the effects of mechanical strain on tenocytes in vitro

Louise Way
White Rose

Currently, the most successful treatment for tendon injury is the use of autografts but, as well as long healing times, this results in long-term donor site

morbidity. Tissue engineered tendons are mechanically weak and thus need to be mechanically conditioned before use. For this purpose, in collaboration with Bio-manipulation Ltd, we have developed a miniature device that can expose tissue engineered tendons to longitudinal stresses in vitro.

Biomechanical strain is known to influence tendon cell behaviour. However, the mechanisms of mechanotransduction are still not fully understood. If we can study cell responses to unloading and loading regimes we can optimise specific loading cycles as methods of conditioning tissue-engineered tendons or preferably of minimising tendon damage due to immobility or repetitive strain.