

SESSION 1A

Methods for identification of methylated arginine residues in nuclear protein complexes.

Helen Phillips

Arginine methylation is a common post-translational modification of mainly nuclear proteins in eukaryotic cells. Arginine methylation is of interest as it is implicated in many cellular processes including transcription, protein-protein interactions, RNA processing and transport, cellular localisation, translation, signal transduction, cellular signalling, DNA repair, and apoptosis. The identification and quantification of post-translational modifications is one of the major challenges in the field of proteomics as they are often present in very low abundances. PSF (polypyrimidine tract-binding protein-associated splicing factor) is a multifunctional nuclear protein which has been shown to contain arginine methylation, and is believed to participate in a range of nuclear reactions. Sites of arginine methylation in PSF have been identified using Mass Spectrometry. The effects of the modifications have also been studied by mutation of the modified residues. Methods to identify sites of modifications are desirable as they allow site directed studies to further elucidate the role of methylation.

Investigation of paralogous metallothioneins in a coastal cyanobacterium.

Jie Chu

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].

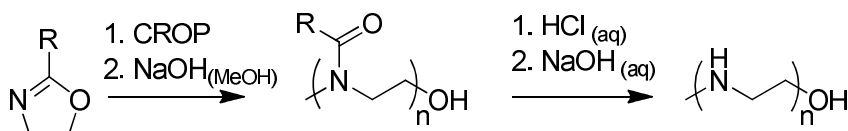
Bacterial MT, SmtA found in fresh water Cyanobacteria *Synechococcus* PCC7942 is the only fully characterised prokaryotic MT[2]. Now, other cyanobacteria have been found to contain similar proteins; the *Synechococcus* CC9311 is particularly special one. This strain was found to contain no less than 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.

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2. C.A.Blindauer, M.D. Harrison, J.A.Parkinson, A.K.Robinson, J.S.Cavet, N.J.Robinson, P.J.Sadler, A metallothionein containing a zinc finger within a four-metal cluster protects a bacterium from zinc toxicity. *Proc. Natl. Acad. Sci. U.S.A* 98(2001)9593-9598.

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

Bryn Monnery



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.

1. Boussif, O. *et al.* A Versatile Vector for Gene and Oligonucleotide Transfer into Cells in Culture and in vivo: Polyethylenimine. *Proceedings of the National Academy of Sciences* **92**, 7297-7301, (1995).
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>

SESSION 1B

Optimisation of Culture Conditions for a 3D Human Bone Model

J Edwards, G Reilly, University of Sheffield

Bone is a dynamic tissue, undergoing constant remodelling over time. It is this remodelling which allows the structure of bone to be adapted throughout life in response to external stimuli. During

remodelling, an imbalance between bone deposition and resorption can lead to changes in the overall tissue structure. This may be beneficial, such as an increase in bone mass due to exercise, or detrimental, such as the bone deterioration associated with osteoporosis. However, the mechanisms which regulate these processes are poorly understood. An in vitro bone model would allow investigation into the cell signalling occurring between cell types within bone, without the need for animal experiments. Such a model would be beneficial in terms of cost and animal welfare, whilst also allowing the collection of data relating to human cells.

For a model representative of human bone tissue, there are several factors which are thought to be important. A suitable scaffold should be used to provide a 3D structure for cell attachment and growth. The cell types used in such a model (primary cells or cell lines, introduction of co-culture) are also important, as this will affect the production of bone tissue and cellular interactions during remodelling. Introduction of mechanical stimulation during culture (such as compression or fluid flow) may also be used to improve the quality of the constructs produced. This may be due to improvements in cell viability within constructs, or an increase in bone deposition.

Work on the project to date has involved the use of an open cell polyurethane foam scaffold, tested with several cell types and has studied the cell attachment and response to compressive mechanical stimulation. More recent work has involved use of a secondary scaffold type, as well investigating the use of high frequency vibrations as an alternative to direct mechanical compression.

Using meta-dynamics to accelerate molecular dynamics simulations of bio-mineralisation

Matthew Bano

Biological processes are capable of producing mineral structures (bones, shells, teeth etc.) with unique properties, often with astonishing rapidity. Such materials often contain several polymorphs of the base mineral in patterns and structures which cannot currently be recreated in the lab.

A popular hypothesis is that the formation of these materials begins with nanoparticles of just a few hundred atoms, which may exist in several different phases, with proteins and other biomolecules facilitating transitions between these phases. These nanoparticles then serve as nucleation sites for growth into much larger structures. We have been studying nanoparticles of the mineral tricalcium phosphate, which is biocompatible with bone and is found in teeth. Our initial goal is to explore which phases are available to these nanoparticles and which are the most energetically favourable.

Using conventional molecular dynamics we can simulate such nanoparticles for a few nanoseconds, but the transitions we are interested in are likely to occur on timescales which are many orders of magnitude longer. We have been using a technique called meta-dynamics, which introduces a biasing force to drive the nanoparticle to explore the energy landscape much more rapidly.

The talk will give an overview of the techniques involved, and a description of how the phases of the nanoparticles will be identified with the phases of the bulk mineral.

Using Optical Trapping Methods to Study Membrane Proteins in Single Cells

John Phillips

In order to understand the development and spread of cancer, the methods by which cells communicate and disseminate information must be closely examined. As such, membrane proteins are an important target in modern cancer research due to their role in cellular signalling cascades. Disruption of these cascades can lead quickly to abnormal cell growth and the onset of cancer.

Therefore, characterising the recruitment and action of such membrane proteins, and in particular oncoproteins is a key facet of the Single Cell Proteomics (SCP) initiative.

Here, work is presented on a project to use optical trapping methods to study membrane proteins on single cancer cells. 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 ϵ (PKC ϵ), under a variety of conditions. Particular attention is given to the role of free calcium in facilitating the recruitment of PKC ϵ , 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 later in this project.

SESSION 1C

Measuring the Non-Specific binding Properties and Synthesis of Novel Drug Molecules

Chloe Child

Non-specific binding, NSB, is the non-saturable binding of a ligand to tissue devoid of the target receptor or enzyme of interest.¹ This non-saturable binding of a labelled molecule to an non-specific sites can obscure the visualisation of biological processes at the target site of interest when using in vivo imaging modalities such as positron emission tomography (PET). There are several factors which affect non-specific binding including the lipophilicity and hydrophobic nature of the molecule, rate of parent molecule metabolism and the affinity of the molecule 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.

A recent hypothesis predicts that molecules with low NSB are able to hydrolyse the lipid bilayer of tissue membranes rapidly. The aims of this work include the synthesis of a novel molecular series to assess their non-specific binding properties and further examine the validity of this hypothesis set out in previous work.² 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.

Reference List

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2. M. Baciú, S. C. Sebai, O. Ces, X. Mulet, J. A. Clarke, G. C. Shearman, R. V. Law, R. H. Templer, C. Plisson, C. A. Parker, and A. D. Gee, *Philosophical Transactions of the Royal Society A*, 2006, **364**, 2597.

New chemical approaches towards the treatment of Methicillin-resistant Staphylococcus aureus (MRSA)

Simon England, University of Sheffield

Supervisors: Dr. Simon Jones, Prof. Simon Foster

Research recently carried out by the Foster group and others has identified a new compound that is effective in killing and incapacitating MRSA^{i ii}. cis-6-Hexadecenoic acid is a component of human sebum, the skin's natural antimicrobial defense. Human sebum consists of a number of compounds, most notably lipids. C-6-H is derived from sebaceous triglycerides and has a MIC of 2.5 µg/ml against wild-type SH1000 *S. aureus*:ⁱ

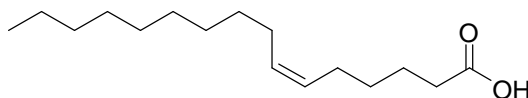


FIGURE 1-STRUCTURE OF C-6-H

C-6-H is capable of killing *Staphylococcus aureus* and suppressing key virulence factors that can lead to drug resistance.

C-6-H's simplicity and ability to kill *Staphylococcus aureus* and also to inhibit its ability to gain antibiotic resistance makes it a very appealing compound to synthesize.

This work outlines the design of an effective multi-gram route to cis-6-hexadecenoic acid. The synthesis needs to be capable of producing cis-hexadecenoic acid in an isomerically pure fashion. Once the synthetic route has been optimized analogues of C-6-H were prepared.

Three separate routes towards cis-6-hexadecenoic acid have been studied so far with varying degrees of success. Firstly, Wittig chemistry furnished the target compound over 4 steps in an 80:20 cis:trans ratio. Secondly, 9-methyl anthracene was used as a chemical scaffold to set-up a masked alkene which could be used to furnish both cis and trans double bonds. The key step for this synthetic route was a copper mediated cross coupling reaction that proved to be extremely capricious. The final synthetic route employed alkyne coupling and subsequent selective alkyne reduction (Lindlar reduction) to yield the cis alkene. The final steps for this route have proved extremely difficult as the cis alkene is very susceptible to double bond isomerisation under oxidative and mildly acidic conditions.

i Clarke, S. R., *Cell Host & Microbe*, 2007, 1, 199–212

ii Drake, D. D., Brogden, K. A., Dawson, D. V., Wertz, P.W., J. *Lipid Res.*, 2008, 49, 4–11

Polymersome penetration into human skin

Carla Pegoraro¹, Giuseppe Battaglia² and Sheila MacNeil¹

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The aim of this research is to investigate the 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) ethyl methacrylate) (PMPC-PDPA), fluorescently labelled with Rhodamine) that self-assemble when exposed to an aqueous environment into vesicles. These vesicles can enter the cell

via endocytosis where they are exposed to a low pH which causes the polymersome to lose stability and to dissociate into free copolymer chains releasing its upload inside the cytoplasm.

Skin is a highly stratified and impermeable tissue with specific size, charge, hydrophilic and lipophilic restraints on the kind of molecule that can diffuse across it. However by using a perfusion system developed in our laboratory we've demonstrated that polymersomes are capable of crossing this biological barrier successfully. Polymersome diffusion has been performed across ex-vivo skin, tissue engineered skin models and synthetic porous polycarbonate membranes with an average pore size of 50 nm. 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. We prepared 400 nm, 200 nm and 100 nm diameter sized vesicles and placed them on top of 50 nm pore sized membranes inside the perfusion system. We reproduced the same flow and ambient conditions for each experiment and we found that very large vesicles diffused more easily through small pores (approximately 70-90% of the initial polymersome solution diffused through the membrane) compared to vesicles with approximately the same size as the pore (diffusion is only around 20-30% of the initial amount). Most importantly we found that polymersomes maintained structural stability and did not defragment during diffusion (as shown by Dynamic-light-scattering analysis, DLS), thereby proving that they possess mechanical properties that allow them to deform and squeeze in the gaps between the tightly packed dead cells of the top skin layers. This was further confirmed by measuring their ability to carry proteins across the barrier without significant loss and by analysing diffusion across ex vivo skin and tissue engineered skin models via confocal microscopy.

Using Bayesian hierarchical clustering on time-series microarray data to identify genes that are co-regulated during a stress response

Emma Cooke

Microarrays can be used to measure the levels of gene expression over a whole genome. Microarray data can be collected at several points over a time-series to capture how the genome responds over time to a stress such as pathogen infection.

We can identify genes that are likely to be co-regulated by the same transcription factor by grouping together genes which exhibit similar variations in expression over time. This information can be used to infer topology in the gene network involved in the stress response. A greater understanding of this network enables us to target specific genes which could result in more effective resistance against disease.

The talk will focus on the use of a Bayesian hierarchical clustering algorithm to group genes based on their time-series expression profiles and the measures used to quantify the biological homogeneity of these gene clusters.

‘Under Pressure’: Static and Time-Resolved X-ray Studies of Inverse Phases in Cholesterol / Lipid Mixtures

Arwen II Tyler, Gemma C Shearman, Nicholas J Brooks, Richard H. Templer, Oscar Ces, Robert V Law and John M Seddon

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.

SESSION 2B

Selective Molecular Networks to Unravel Cell Signalling

Chirag Patel, Joachim Steinke, Ramon Vilar, David Mann and Lucy Elphick.

The development and optimisation of protocols for the enrichment of phosphorylated proteins or peptides is essential to aid substrate identification. Advances have been made on the enrichment and identification of phosphoproteins, but these processes are still not straightforward and their applications remain limited. (Delom and Chevet 2006)

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 Acrylamide™) (Kinoshita, Kinoshita-Kikuta et al. 2006) 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. Furthermore, investigations into the ability to maintain selectivity within the gels over a variety of conditions (pH, denaturing agent) has revealed that the receptors work best within conditions determined by Laemmli. (Laemmli 1970) Furthermore, other metals (Zn, Cd, Fe) have been investigated for their selective recognition of phosphorylated proteins in gels.

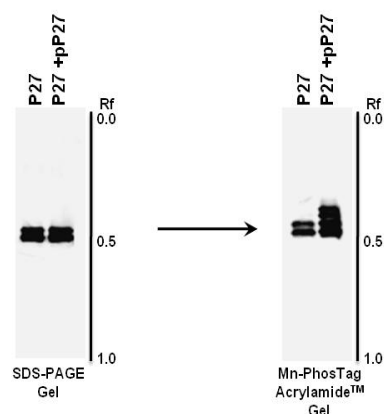


Fig 1: Separation of Phosphoprotein p27 by Mn-PhosTag Acrylamide™ SDS-PAGE gels

1. F. Delom and E. Chevet, *Proteome Science*, 2006, **4**, 15.
2. E. Kinoshita, E. Kinoshita-Kikuta, K. Takiyama and T. Koike, *Mol. Cell Proteomics*, 2006, **5**, 749-757.
3. U. K. Laemmli, *Nature*, 1970, **227**, 680-685.

Parallel synthesis of regulatory module mutant libraries

Max Joseph

Regulatory modules are DNA sequences which affect gene expression of a nearby promoter. Distal (10-50kbp) regulatory modules interact with promoters via transcription factors bound to specific sequences on both. Higher order interactions between regulatory modules are a mechanism whereby cells are able to relate competing

external and internal signaling cues to determine whether a gene should be expressed or not. This decision is particularly critical for master regulatory genes such as myod. Although the signaling cues which activate and repress myod expression in developmental contexts have been investigated by in situ hybridisation (ISH) and transgenic animal studies, genetic ablation has so far failed to identify the mechanism of myod regulation in several important developmental contexts.

A bioinformatic approach has previously identified several regulatory modules and these are the target of this study. Libraries of regulatory module mutants can be used to investigate higher order interactions of regulatory modules and the promoter in cellular transfection studies.

The work presented here is on the development of novel methods for the parallel synthesis of regulatory modules and the testing of their action in an in vitro cell transfection context.

Chemical Genetic dissection of Histone Deacetylase Function

Robert Felstead

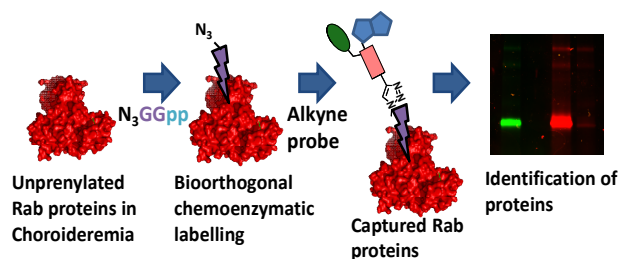
Breast cancer is one of the most commonly diagnosed malignancies and one of the main causes of cancer death among women. It has become apparent that imbalances of histone acetylation play a significant role in cancer development and progression, and HDACs (histone deacetylases) have an important role in tumorigenesis. We aim to mimic isoform specific HDAC inhibition utilising a novel application of protein-ligand engineering. Mutant HDACs will be expressed and introduced into biological systems which will then be probed by specially designed chemical inhibitors. Novel synthetic approaches shall be used to synthesise these mutant and isoform selective inhibitors including the use of Ruthenium catalysed cross-metathesis to create a novel class of cyclic tetrapeptide based 2-amino phenylamide inhibitors. It is hoped that this overall approach will allow the function of particular HDACs to be dissected and allow better understanding of their roles in breast cancer progression.

SESSION 2C

Rab prenylation in Choroideremia: A chemical proteomics approach

*Alexandra F. H. Berry**, William P. Heal, Abul K. Tarafder, Tanya Tolmachova, Miguel C. Seabra and Edward W. Tate

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.

Convergent transcription and nested gene models studied by Atomic Force Microscopy

D.J. Billingsley^{1,2}, N.H. Thomson^{1,2}, W.A. Bonass², J Kirkham².

¹School of Physics and Astronomy, ²Department of Oral Biology, University of Leeds

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 λ pr 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

Getting more from ChIP-chip and ChIP-seq data

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Chromatin Immunoprecipitation combined with microarray technology (ChIP-chip) is a well established technique for determining the locations on the genome where proteins such as transcription factors bind. This process involves fixing all the proteins to the DNA, breaking the DNA into fragments, using antibodies to select the fragments where a specific protein is bound and then using a microarray to identify the locations of the fragments in the genome.

Recently this has been supplemented with the ChIP-seq process where sequencing of the ends of the DNA fragments to which the target protein were bound, rather than microarrays, is used to locate the binding sites of the target protein.

New techniques are being developed that allow more information to be extracted from the large amounts of raw data produced by both these techniques. These have been used to obtain more detailed information on the interactions between SeqA that is bound to closely spaced binding sites in *E. Coli*, and also the effect on the likelihood of SeqA binding of the DNA sequence adjacent to the binding site.

Existing techniques for analysing ChIP-seq data identify regions within which the target protein binds to the DNA. Some early results will also be presented on techniques for distinguishing between overrepresented sequences within these regions that are associated with the target protein and those that are associated with binding sites for other proteins. These techniques may also provide additional information on the nature of the binding between the DNA and these proteins.

AAA Proteins – Using Chemistry to Understand a Universal Biomolecular Machine

Lucy J. Rayner*, *Martin Buck, Edward W. Tate and Xiaodong Zhang*

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 σ_{54} which is unique in the strict regulation of its associated genes. σ_{54} 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 (Rappas, Schumacher et al. 2006). Located at the tip of Loop 1 is the GAFTGA motif, which is highly conserved across bEBPs and is required for interaction with σ_{54} (Rappas, Schumacher et al. 2005).

This work uses synthetic peptides as tools for a chemical genetics approach to understanding the interaction between σ_{54} and PspF, using a range of biochemical and biological assays. The peptides

are studied for both binding to $\sigma 54$, and inhibition of its interaction with PspF, using both in vitro and in vivo techniques.

1. Rappas, M. et al., J Mol Biol, 2006. **357**(2): p. 481-492; 2. Rappas, M. et al., Science, 2005. **307**(5717): p. 1972-1973

SESSION 3B

Dielectrophoretic routes towards time-resolved analysis of cellular membrane dynamics

Fabrice Gielen

The project focuses on the development of novel tools for probing cell membrane dynamics. Here we demonstrate dielectrophoretic trapping of single Jurkat cells (typically 15 μ m 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.

1. F. Delom, et al., *Proteome Science*, 2006, **4**, 15.
2. E. Kinoshita, et al., *Mol. Cell Proteomics*, 2006, **5**, 749-757.
3. U. K. Laemmli, *Nature*, 1970, **227**, 680-685.
4. M. Rappas, et al., *Journal of Molecular Biology*, 2006, **357**, 481-492.
5. M. Rappas, et al., *Science*, 2005, **307**, 1972-1975.

Transcriptional Signatures of Single Cell Lineages.

Mike Downey

Mesenchymal stem cells are multipotent stem cells which are capable of differentiating into different types of cell including muscle, cartilage, fat or bone cells, depending on their environment. The MSX1 protein prevents differentiation of mesenchymal cells during development.

Cells are fluorescently labelled using Hoechst stain for detection and tracking. Segmentation algorithms have been developed which locate the fluorescent nuclei. Tracking algorithms are used to follow the movement of the cells during the course of an experiment. When cells are modified to express a fluorescent protein, changing level of gene expression can be measured. By including algorithms to detect cell divisions, gene expression over several generations can be followed.