Fluid flow around the colonial alga Volvox carteri held by a micropipette, imaged with tracer particles. Image courtesy of Sujoy Ganguly and Raymond E. Goldstein, DAMTP, University of Cambridge.

Physics Meets Biology

12–14 September 2016
Clare College, Cambridge, UK

Organised by the IOP Biological Physics Group

http://pmb2016.iopconfs.org
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Scientific programme

Monday 12 September

09:30  Registration and refreshments
       Conference Office, The Gillespie Centre

10:20  Introduction
       Andrew Turberfield, University of Oxford, UK
       Riley Auditorium, The Gillespie Centre

10:30  Regulation of collective cell migration by mechano-biology
       Joachim Spatz, Max Planck Institute for Intelligent Systems, Germany

11:20  Frontiers in single molecule manipulation and imaging of DNA-protein transactions
       Gijs Wuite, VU University Amsterdam, The Netherlands

11:50  Reconstituting biological self-organisation in confinement
       Michael Juniper, The Francis Crick Institute, UK

12:20  DNA origami as a single molecule fluorescence tool to quantify DNA damage
       Helen Miller, University of York, UK

12:40  Lunch
       The Garden Room, The Gillespie Centre

14:00  Homeostasis and dynamic phase transition in a simple model of cells with chemical signalling
       Ramin Golestanian, University of Oxford, UK
       Riley Auditorium, The Gillespie Centre

14:30  Bugs on a slippery plane: advection by microswimmers in thin films
       Mitya Pushkin, University of York, UK

14:50  Assessing collective dynamics of motile cilia in human airway cells
       Luigi Feriani, University of Cambridge, UK

15:10  Working towards conformational switching in a synthetic peptide-based molecular motor
       Lara Small, Durham University, UK

15:30  Poster session and refreshments
       The Garden Room and the Elton/Bowring Room, The Gillespie Centre

18:30  Welcome Drinks Reception
       JCR College Bar, Old Court

19:30  Dinner
       The Buttery, Old Court
Tuesday 13 September

08:30  **Registration**  
*Conference Office, The Gillespie Centre*

09:00  **Talk title tbc**  
Naama Barkai, Weizmann Institute of Science, Israel  
*Riley Auditorium, The Gillespie Centre*

09:50  **Control of gene expression and biological circuits**  
Pascal Hersen, CNRS & Université Paris Diderot, France

10:20  **Dynamics of Escherichia coli’s passive response to a sudden decrease in external osmolarity**  
Teuta Pilizota, University of Edinburgh, UK

10:50  **Morning refreshments**  
*The Garden Room, The Gillespie Centre*

11:20  **Microfluidic technologies for the bottom-up construction of artificial cells**  
Oscar Ces, Imperial College London, UK

11:50  **Title tbc**  
Mark Wallace, King’s College London, UK

12:20  **Sticking pins into lipid membranes**  
Rebecca Green, University of Reading, UK

12:40  **Lunch**  
*The Garden Room, The Gillespie Centre*

14:00  **Tom Duke Lecture - Building with biomolecules: synthetic biology from the bottom up**  
Andrew Turberfield, University of Oxford, UK

15:00  **Algal-bacterial interactions at a distance**  
Otti Croze, University of Cambridge, UK

15:20  **3D modelling of the biogenesis and dynamics of nuclear bodies and transcription factories**  
Davide Marenduzzo, University of Edinburgh, UK

15:50  **Poster session and refreshments**  
*The Garden Room and the Elton/Bowring Room, The Gillespie Centre*

18:00  **IoP Biological Physics Group AGM**  
*Riley Auditorium, The Gillespie Centre*

19:00  **Conference Drinks Reception**  
*Scholar’s Garden or JCR College Bar*
19:30  Conference Dinner  
      Great Hall, Old Court

Wednesday 14 September

08:30  Registration  
      Conference Office, The Gillespie Centre

09:00  Optogenetics: lighting up the brain  
      Gero Miesenböck, University of Oxford, UK  
      Riley Auditorium, The Gillespie Centre

09:50  Talk title tbc  
      Niek F van Hulst, ICFO – The Institute of Photonic Sciences, Spain

10:20  Single-molecule biosensing using plasmonic nanoparticles  
      Peter Zijlstra, Eindhoven University of Technology, The Netherlands

10:40  Morning refreshments  
      The Garden Room, The Gillespie Centre

11:10  Using evolutionary sequence variation to build predictive models of protein structure and function  
      Lucy Colwell, University of Cambridge, UK

11:40  A simple genotype-phenotype map for protein-DNA binding, sequence entropy and its role in speeding up speciation  
      Bhavin Khatri, University College London, UK

12:10  Systematic investigation of bias in Jarzynski-derived free energy landscapes using coarse-grained simulations  
      Megan Engel, University of Oxford, UK

12:40  Lunch  
      The Garden Room, The Gillespie Centre

14:00  The physics and biology of sprouting angiogenesis  
      Rui Travasso, University of Coimbra, Portugal  
      Riley Auditorium, The Gillespie Centre

14:20  Biaxial nematic order of liver transport networks  
      Jens Karschau, TU Dresden, Germany

14:40  Molecular and cellular mechanisms for folding sheets into tubes  
      Katja Röper, MRC Laboratory of Molecular Biology, UK

15:10  Closing remarks  
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Poster programme

Poster Session 1 - Monday 12 September

P:01 Quantum enhanced sensing: using non-linear optics to beat the shot-noise in absorption measurements
E Allen, University of Bristol, UK

P:02 Mechanics of cell division: effects of spontaneous curvature, surface tension, and osmotic pressure
E Beltrán de Heredia Rodríguez, Universidad Complutense de Madrid, Spain

P:03 Controlling lipid bilayer properties by organic electrolytes: the room temperature ionic liquids route
A Benedetto, University College Dublin, Ireland

P:04 Mechanical properties of single supramolecular polymers from correlative AFM and fluorescence microscopy
M Beuwer, Eindhoven University of Technology, The Netherlands

P:05 Regulating mesenchymal stem cell phenotype and morphology via biomimetic nanopatterned surfaces
O Brazil, Trinity College Dublin, Ireland

P:06 Growth kinetics of Escherichia coli in the presence of cell wall targeting antibiotics
R Brouwers, University of Edinburgh, UK

P:07 Development of new experimental platforms for studying respiratory tissue trauma and infection
K Brown, University of Cambridge, UK

P:08 Physics of a purely Brownian ratchet molecular motor
F J Cao-García, Universidad Complutense de Madrid, Spain

P:09 Excitons meet plasmonic antennae: a multiscale approach
S Caprasecca, Università di Pisa, Italy

P:10 Membrane crowding and anomalous diffusion in artificial lipid bilayers
M Cheetham, University of Oxford, UK

P:11 Motile cilia in human airways: the importance of beating in synchrony
M Chioccioli, Cavendish Laboratory University of Cambridge, UK

P:12 Investigation of the molecular interactions relevant to receptor-mediated virus entry using HIV receptors as a model
D Valter Conca, University College London, UK

P:13 The self-assembly of amphiphilic peptide I3K
H Cox, University of Manchester, UK

P:14 Quantitative Raman spectroscopy discrimination of mesenchymal stromal cell lines for regenerative medicine applications
R de Almeida Rocha Ponzoni, University of York, UK
Raman spectroscopy for quantitative assessment of human intervention (cooking evidence) in archaeological marine shells  
A L de Lima Ponzoni, University of York, UK

Purification of 50-base DNA-AuNP mono conjugates using gel-electrophoresis  
A De, TU Eindhoven, The Netherlands

Single-molecule imaging of electroporated chemotaxis proteins in live bacteria  
D Di Paolo, University of Oxford, UK

A patch clamp study of ionic Coulomb blockade in biological ion channels  
O Fedorenko, Lancaster University, UK

Overcoming biological barriers: nanoparticles for targeted nucleic acid delivery  
A Garcia Guerra, University of Oxford, UK

Kinetic model of selectivity in the KcsA potassium ion channel  
W Gibby, Lancaster University, UK

Characterizing the NaChBac channel through MD simulations  
C Guardiani, University of Warwick, UK

Computational design of hepatitis C vaccines using empirical fitness landscapes and population dynamics  
G Hart, University of Illinois at Urbana-Champaign, USA

Widefield microwave imaging using atoms and diamond NV centres  
A Horsley, University of Basel, Switzerland

From invasion to egress: analysis of the blood-stage malaria cycle  
V Introini, University of Cambridge, UK

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W Shi, Peking University, China

Electronic nano-biosensor for the investigation of Alzheimer’s disease induced by Amyloid-beta Oligomers  
W Chen, University of Cambridge, UK

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Y Yuan, Peking University, China
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       J Jarillo, Universidad Complutense de Madrid, Spain

P:30  Chlamydomonas in an air-lift photobioreactor under gyrotactic effects
       D Jin, University of Cambridge, UK

P:31  Clathrin-inspired DNA structure on lipid bilayer
       C Journot, University of Oxford, UK

P:32  Coulomb blockade oscillations and AMFE in Calcium/Sodium ion channels
       I Kaufman, Lancaster University, UK

P:33  Study of the influence of shear stress on the intermediate filament elasticity of alveolar epithelial cells
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P:34  Measuring cell's free energy components on the single cell level; the challenges
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P:35  Synthesis and characterization of bacterial cellulose-nanostructured materials hybrid heterostructures
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       I Maryshev, University of Edinburgh, UK

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P:38  Growth and gene expression dynamics in Escherichia coli under non-equilibrium environmental conditions
       M Panlilio, University of Cambridge, UK

P:39  PC12 differentiation on aligned electrospun polymeric fibres
       A Patharagulpong, University of Cambridge, UK

P:40  Implementation of sense on gpu and multicore cpu using pre-scan and eigen-value sensitivity profiles
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Regulation of collective cell migration by Mechano-Biology

J P Spatz
Max Planck Institute for Intelligent Systems, Germany

The collective movement of epithelial cells drives essential multicellular organization during various fundamental physiological processes like embryonic morphogenesis, cancer, and wound healing. Two hallmarks of collective behavior in migrating cohesive epithelial cell sheets is the emergence of so-called leader cells and the communication between adjacent cells to move correlated to each other. Here we discuss these two phenomena:

(i) The geometry-based cue imposed by the matrix environment like local curvature of the collective's perimeter is capable of triggering leader cell formation and promoting enhanced motility at defined positions. Cytoskeletal tension was found to be important for geometry-induced leader cell formation. Together our findings suggest that high curvature leads to locally increased stress accumulation, mediated via cell-substrate interaction as well as via cytoskeleton tension. The stress accumulation in turn enhances the probability of leader cell formation as well as cell motility.

(ii) Within this cohesive group each individual cell correlates its movement with that of its neighbours. We investigate the distinct molecular mechanism that links intercellular forces to collective cell movements in migrating epithelia. More specifically, we identified the molecular mechanism whereby Merlin, a tumor suppressor protein and Hippo pathway regulator that functions as a mechanochemical transducer, coordinates collective migration of tens of hundreds of cells. In the context of collective cell migration, the transmission and mediation of cellular tension is of major importance.

Nature Cell Biology 2015, 17, 276-287.

Biointerphases 2013, 8, 32.
Frontiers in single molecule manipulation and imaging of DNA-protein transactions

G Wuite
VU University Amsterdam, The Netherlands

The genetic information of an organism is encoded in the base pair sequence of its DNA. Many specialized proteins are involved in organizing, preserving and processing the vast amounts of information on the DNA. In order to do this swiftly and correctly these proteins have to move quickly and accurately along and/or around the DNA constantly rearranging it. In order to elucidate these kind of processes we perform single-molecule experiments on model systems such as RNA polymerases, DNA polymerases and repair proteins. The data we use to extract forces, energies and mechanochemistry driving these dynamic transactions. The results obtained from these model systems are then generalized and thought to be applicable to many DNA-protein interactions. In particular, I will report experiments that use a combination of (super-resolution) fluorescence microscopy and optical tweezers to investigate DNA organisation and DNA overstretching.
Reconstituting biological self-organisation in confinement

M P N Juniper, J C Hannabuss and T Surrey
The Francis Crick Institute, UK

Devices which assemble themselves from simple components are a much sought-after technology. Living cells already demonstrate a mastery of self-organisation at the micron scale, and have much to teach us about such systems. A prominent example is the internal scaffolding or ‘cytoskeleton’, which both contributes to the mechanical properties of cells, and facilitates their internal organisation. One part of the cytoskeleton is composed of filamentous protein polymers called microtubules, which spontaneously grow and shrink by adding or losing subunits.

The size of microtubules is regulated by a dizzying array of molecular partners, which may stabilise, depolymerise, and even sever the filaments. Furthermore, molecular motors called ‘kinesins’ can move whole microtubules (as well as other cargos) around the cell – using other microtubules as tracks. These complimentary and antagonistic activities combine to allow the constant remodelling of the cytoskeleton, allowing cells to grow and divide.

Here, we show how asters – structures with a focused pole and radiating filaments – may be produced in the confinement of artificial droplets (Figure 1). Confinement is vital to both biology and technology, as it isolates a structure or device from its surroundings. In this case, it allows asters to form independently, rather than as part of a percolating and/or collapsing contractile network. We reconstitute this dynamic active structure from minimal components; using just tubulin (the monomer of microtubules) and a unidirectional molecular motor. By understanding the behaviour of this simple system, we aim to uncover fundamental principles, applicable to a broad range of micro-scale self-assembling devices.

Figure 1: A surfactant-stabilised droplet, containing tubulin monomers and molecular motors, forms a stable isolated aster. GTP is required to form microtubules, and ATP fuels the motors.
DNA origami as a single molecule fluorescence tool to quantify DNA damage?

H L Miller\textsuperscript{1}, A M Hirst\textsuperscript{1}, A J M Wollman\textsuperscript{1}, S Antoranz Contera\textsuperscript{2}, D O’Connell\textsuperscript{1} and M C Leake\textsuperscript{1}

\textsuperscript{1}University of York, UK, \textsuperscript{2}University of Oxford, UK

DNA damage is a problem of medical relevance, for instance novel treatments being developed for prostate cancer [1] act by causing double strand breaks to a level that cells cannot tolerate. To develop these treatments a quantitative measure of DNA damage must be used to calculate required doses. Existing methods broadly fall into two categories; in which DNA is extracted from the cells and analysed, for example gel electrophoresis or qPCR, losing any spatial information; or methods in which the cells are stained in situ, for example in the comet assay [2] or immunofluorescence labelling [3]; these methods suffer from subjectivity in analysis, often due to overlapping signals. All of these methods are ensemble and reflect only the average behaviour.

There is a need to develop a quantitative DNA damage probe at the single molecule level to gain further insight to the mechanism of DNA damage. For this, linear DNA is unsuitable as the damaged fragments cannot be linked back to the original molecule. We are using a DNA origami rectangle adapted from earlier work [4], but with improvements of additional T loops to prevent stacking, plus four longer biotinylated staples for surface immobilisation, and we inflict DNA damage with a radio-frequency plasma source. The tightly-packed structure of the DNA origami may be more similar to chromatin-packed DNA in a cell than a linear substrate, yet we have found that damage occurs on similar timescales to both DNA origami and linear DNA. As the DNA is progressively damaged the original molecules can still be clearly identified in single-molecule fluorescence microscopy. We investigate the total initial fluorescence of a DNA origami tile labelled with the fluorescent dye YOYO-1 as a function of the plasma treatment dose duration by tracking with the super-resolution software ADEMS code [5].


Homeostasis and dynamic phase transition in a simple model of cells with chemical signalling

R Golestanian and A Gelimson

University of Oxford, UK

The question of stability of a living system made of active components is addressed and a fundamentally new mechanism is proposed in which a competition between chemical signalling and cell division can determine the homeostatic conditions at the systemic level. A stochastic field theory is proposed to study the large scale behaviour of a population of cells that grow and interact through the concentration field of the chemicals they secrete, and analysed using the method of Dynamical Renormalization Group, which is the standard method to study phase transitions. The combination of the effective long-range chemotactic interaction and lack of number conservation leads to a rich variety of phase behaviour in the system, which includes a sharp transition from a phase that has moderate (or controlled) growth/death and regulated chemical interactions to a phase with strong (or uncontrolled) growth/death and no chemical interactions. The transition point is characterized by nontrivial critical behaviour and power law exponents. The observed stable fixed-point with a large basin of attraction could describe a homeostatic state where the competing dynamical processes are regulated, so far as the parameters lie in the basin of attraction. This work helps to shed light on the interplay between chemical signalling and growth in tissues and colonies, and in particular on the challenging problem of cancer metastasis.

Bugs on a slippery plane: Advection by microswimmers in thin fluid films

D O Pushkin and M A Bees
University of York, UK

Many pathogenic microorganisms live in close association with surfaces, typically in thin films that either arise naturally or that they themselves create. In response to this constrained environment, the cells adjust their behaviour and morphology, invoking communication channels and inducing physical phenomena that allow for rapid colonization of biomedically relevant surfaces. Thus, it is very important to measure and theoretically understand the key mechanisms for the apparent advantage obtained from swimming in thin films.

We discuss experimental measurements of flows around a peritrichously flagellated bacterium constrained in a thin film, present a simplified mathematical theory and Green’s functions for flows in a thin film with general slip boundary conditions, and establish connections between theoretical and experimental results [1]. Our theory extends and corrects previously obtained results [3] and explains the dependence of fluid flows generated by motile cells on their morphology.

Next, we apply our mathematical theory to discuss advection of small passive particles by microswimmers in thin films. We show that this process occurs qualitatively and quantitatively differently in films and in bulk [4]. Our results shed light on the mechanisms of biomixing enhancement in fluid films observed experimentally but remaining unexplained [5].

Fig. 1: The flow field around a stuck cell of Bacillus subtilis (with permission [2]).


Assessing collective dynamics of motile cilia in human airway cells

L Feriani¹, M Juenet¹, M Chioccioli¹, N Bruot¹, C J Fowler²,³, S M Holland², C E Bryant³ and P Cicuta¹

¹Cavendish Laboratory, UK, ²National Institutes of Health, USA, ³University of Cambridge, UK

In mammals, airway cilia beat cooperatively, exhibiting metachronal waves, which play essential roles in mucus clearance. It is not currently well understood how this collective dynamics emerges out of the behaviour of individual motile cilia. In various genetic and infectious diseases, the cilia motility is compromised.

The assessment of the cilia beating frequency (CBF) is the first test in diagnosing diseases related to the airways, and specifically primary ciliary dyskinesia (PCD), which is an umbrella of genetic conditions leading to cilia malfunction. The tools used in clinical settings are manual evaluation of cilia wavebeat, and analysis by Fourier Transform of the intensity level over time of a 4-by-4 pixel region selected by the user, in order to extract a dominant frequency.

In this work we suggest an alternative based on differential dynamic microscopy (DDM), a technique recently developed by Cerbino and Trappe to extract wavelength-dependent dynamics in a video [1]. As opposed to previous modes of analysis, this video analysis approach is completely automated and requires no user input, thus removing a possible source of bias, and massively reducing the time required for the analysis.

Our new approach is tested on a video library of clinically relevant conditions, collected by C. J. Fowler and S. Holland at NIH. The results are compared with CBF analysis carried out by C. J. Fowler using the standard software, showing good agreement.

In addition to providing a robust and accurate estimate of the ciliary beating frequency, DDM has other unique advantages, as it can explore parameters of the collective motion of cilia that are not investigated by the standard approach. We have developed DDM to give an estimate of a lengthscale over which cilia movement is synchronised. In model data, we show that DDM can also reliably estimate the angular direction of ciliary beating, detect metachronal waves, and measure their physical parameters.

Given the advantages in term of automation, speed, and completeness of the information assessed, we suggest DDM as a serious alternative to the established video analysis techniques (based on pixel fluctuation frequency analysis, segmentation & tracking, or Particle Image Velocimetry) for the analysis of in-vitro ciliated cells in research and in the clinical environment.

Working towards conformational switching in a synthetic peptide-based molecular motor

L S R Small¹, M J Zuckermann², P M G Curmi³, N R Forde², D N Woolfson⁴, H Linke⁵, R B Sessions⁴ and E H C Bromley¹

¹Durham University, UK, ²Simon Fraser University, Canada, ³University of New South Wales, Australia, ⁴University of Bristol, UK, ⁵Lund University, Sweden

Molecular motors are responsible for the successful execution of an array of biological processes. These molecular machines, involved in many fundamental cellular activities, from muscle contraction to cell division, are protein-based. The variety of interactions between the amino acids which make up these proteins make them both the preferred building materials of nature, and difficult systems to fully explore and predict. In addition to improving our knowledge of how these natural proteins perform their individual tasks, understanding and replicating their impressive abilities is highly attractive for bionanotechnology applications.

One means of understanding such systems is through bottom-up design of synthetic molecular motors. The coiled coil is a peptide motif commonly found in nature. Coiled coils appear in tropomyosin, transcription factors and the motor proteins which we have a particular interest in. Previous studies of natural, mutant and designed coiled coils have revealed some ‘rules’ for coiled-coil design (though not without caveats); this, combined with their stability, make them an attractive motif to use in synthetic constructs.

Our aim is to combine coiled-coil peptide designs with other molecular components to devise and produce synthetic motors. These include the Tumbleweed, a synthetic molecular motor designed to progress via rectified diffusion along a DNA track, and the Bar motor, through which we are trying to create a synthetic peptide-based motor which undergoes conformational changes as part of its walking mechanism. Both systems are reliant on the self-assembly of orthogonal coiled-coil dimers. I will discuss the requirements of such systems, the biophysical characterisation (DLS, CD, AUC, disulphide exchange) of designed peptides with the ability to form suitable structures, and our work thus far on inducing conformational changes in a potential coiled-coil system for the Bar motor, using azobenzene, a compound with UV light-induced conformational switching properties.

Figure 1: We aim to induce end-to-end separation changes in a central, staple-like moiety, in order to switch between rigid and flexible (bar- and v-like) states of a coiled-coil peptide-based system. Combining this with ligand-gated track binding should encourage directional motor-like progressive motion.

Bromley et al., The Tumbleweed: Towards a Synthetic Protein Motor, HFSP Journal. 2009, 3(3): 204–212
Tuesday 13 September

Talk title tbc
N Barkai
Weizmann Institute of Science, Israel

Control of gene expression and biological circuits
P Hersen
CNRS & Université Paris Diderot, France

Gene expression plays a central role in the orchestration of cellular processes. We recently developed an experimental platform for real-time, closed-loop control of gene expression that integrates microscopy for monitoring gene expression in live cells, microfluidics to manipulate the cells environment, and dedicated software for automated imaging, quantification and model predictive control strategy. This method implements a dynamic interaction between cells and a computer, making it possible to control precisely the level of expression of a gene for both time-constant and time-varying target profiles, at the population level, and even at the single-cell level. I will discuss recent developments of this method and the relevance of control theory for systems and synthetic biology.

Dynamics of Escherichia coli's passive response to a sudden decrease in external osmolarity
T Pilizota
University of Edinburgh, UK

For most cells, a sudden decrease in external osmolarity results in fast water influx, which can burst the cell. To survive, cells rely on the passive response of mechanosensitive channels, which open under increased membrane tension and allow the release of cytoplasmic solutes and water. Although the gating and the molecular structure of mechanosensitive channels found in Escherichia coli have been extensively studied, the overall dynamics of the whole cellular response remain poorly understood.

I will present single-cell characterization of E. coli’s passive response to a sudden hyposmotic shock (downshock) and show that initial fast volume expansion is followed by a slow volume recovery that can end below the initial value. I will also show similar response patterns observed at downshocks of a wide range of magnitudes. Apart from the response of the wild type E.coli cells, I will characterise the response of a double mutant strain that lacks two major mechanosensitive channels, Delta MscL and Delta MscS. While the wild type adapted to osmotic downshocks and resumed growing, cells of a double mutant expanded, but failed to fully recover, often lysing or not resuming growth at high osmotic downshocks.

Finally, I will present a theoretical model to explain the observations by simulating mechanosensitive channels opening, solute efflux, and water flux. The model illustrates how solute efflux, driven by mechanical pressure and solute chemical potential, competes with water influx to reduce cellular osmotic pressure and allow volume recovery. It includes the stress stiffening characteristics of the cell wall and makes several predictions, which I will discuss. The work highlights the vital role of mechanosensation in bacterial survival.
Microfluidic technologies for the bottom-up construction of artificial cells

O Ces
Imperial College London, UK

This talk will outline microfluidic strategies for bottom-up synthetic biology that are being used to construct multi-compartment artificial cells where the contents and connectivity of each compartment can be controlled. These compartments are separated by biological functional membranes that can facilitate transport between the compartments themselves and between the compartments and external environment. These technologies have enabled us to engineer multi-step enzymatic signalling cascades into the cells leading to in-situ chemical synthesis and systems that are capable of sensing and responding to their environment. Finally, we have developed printing strategies for translating these enzymatic pathways into microfluidic flow reactors that have the potential to be scaled-up for industrial usage.

Title tbc

M Wallace
King’s College London, UK

Abstract to follow.
Sticking pins into lipid membranes

R J Green¹, M R Sanders¹, L A Clifton² and R A Frazier¹

¹University of Reading, UK, ²Science and Technology Facilities Council, UK

Antimicrobial proteins and peptides are able to bind to lipids within biological membranes leading to cell lysis via a number of different mechanisms. They are able to exhibit selectivity to different cell types as a result of the amino acid sequence of the lipid-binding region of the protein or peptide, and due to the lipid composition of the biological membrane. Puroindolines are basic proteins found within wheat. The wild type isoforms of puroindoline, Pin-a and Pin-b contain a hydrophobic tryptophan rich domain that is believed to be responsible for the proteins antimicrobial activity.

We have studied the lipid binding behaviour of both wild type Pins and a naturally occurring Trp44-Arg44 mutant form of Pin-b (Pin-bs), to determine how changes to amino acid composition within the lipid-binding domain impact on biological activity. We have also used a variety of lipid membrane models to investigate the role that lipid composition plays on antimicrobial protein binding. Pin binding to lipid monolayers was investigated using surface pressure measurements, external reflection (ER) FTIR spectroscopy and Brewster angle microscopy. Lipid bilayers have been prepared to enable the Pin-lipid interfacial structure to be determined by neutron reflectometry, and liposomes used to investigate the interaction using isothermal titration calorimetry and differential scanning calorimetry. Figure 1 shows data from surface pressure measurements and ER-FTIR spectroscopy comparing the lipid binding behaviour of Pin-b and Pin-bs to different lipid compositions.¹

Our findings have provided evidence of lipid domain formation and preferential binding of Pin to domains containing DPPG. We have been able to identify the role of lipid charge and fluidity on Pin binding, and observed differences in these trends depending on the amino acid sequence of the Pin tryptophan rich domain. In addition to defining the different lipid binding behaviour of the puroindolines studied, this work has provided an insight into how lipid composition within biological membranes is able to control protein interaction.

[1] MR Sanders, LA Clifton, RA Frazier, RJ Green, Langmuir 32 (8), 2050-2057
Tom Duke Lecture

Building with biomolecules: synthetic biology from the bottom up

A J Turberfield
University of Oxford, UK

Nanofabrication by biomolecular self-assembly can be used to create atomically precise, nanometre-scale structures. The control offered by DNA-self-assembly is spectacular: thousands of oligonucleotides can be designed to form rigid, three-dimensional complexes with defined contours and internal cavities. Each oligonucleotide has a different sequence which defines its unique position in these structures, and chemically modified oligonucleotides can be used to position other molecular components on DNA scaffolds. Synthetic nucleic acids can also form programmable dynamic systems which compute and exhibit complex temporal behaviours. RNA can be programmed to assemble within cells, and devices formed from nucleic acids can couple to and interact with living systems. Peptides and proteins can also be used for molecular-scale construction: they offer chemical diversity and naturally evolved functions at the expense of a less predictable set of assembly rules. I shall survey this rapidly evolving research field and its potential to provide new tools and technologies from biophysics to manufacture to medicine.

Algal-bacterial interactions at a distance

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Complex microbial communities play essential roles in the proper functioning of industrial processes, health and the natural environment. Within these communities, microbial interactions are often predicated on metabolism. For example, auxotrophs depend on nutrients made by other microbes. Many algae are vitamin auxotrophs, with 52% of 326 species requiring exogenous vitamin B12 [1]. This vitamin must be obtained from bacteria, as only they can synthesise it. Laboratory experiments have demonstrated mutualistic interactions between bacteria and B12-dependent algae. Populations of the bacterium Mesorhizobium loti (B12 producer, carbon requirer) and the green alga Lobomonas rostrata (B12 requirer, carbon producer) in co-culture sustain each other and typically stabilise to an algae/bacteria ratio of 1/30, independent of initial inoculum ratio [2]. Similar dynamics are observed in a marine system [3].

Experiments on hard agarose indicate that these mutualistic interactions can persist at a distance. Here, we investigate the interactions between mutualistic microbial populations separated in space. We present a mathematical model capturing the essence of these experiments: growing populations of algae and bacteria coupled by a diffusive channel in which nutrients can be exchanged. Solutions to the model reveal rich dynamics. An experimental set-up allowing nutrients to diffuse between microbial cultures is also presented, together with experimental results. These are compared with the model predictions. Finally, we speculate on the ecological significance of our findings for understanding environmental microbial communities such as biofilms and microbial mats.

3D modelling of the biogenesis and dynamics of nuclear bodies and transcription factories

D Marenduzzo
University of Edinburgh, UK

I will describe in this talk results from Brownian dynamics simulations of chromatin interacting with bivalent of multivalent chromatin-binding proteins. Chromatin binding mediates cooperative interactions between proteins which naturally leads to the creation of clusters. The properties of these clusters are strikingly similar to some of the "nuclear bodies" and transcription factories which are often found inside the nucleus of eukaryotic organisms. Our model therefore suggests a new biophysical mechanism leading to the formation and growth (or arrested coarsening) of such structures, which is profoundly linked to the 3D organisation of chromatin.

Wednesday 14 September

Optogenetics: Lighting up the brain

G Miesenböck
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An emerging set of methods enables an experimental dialogue with biological systems composed of many interacting cell types—in particular, with neural circuits in the brain. These methods are called "optogenetic" because they employ light-responsive proteins ("opto-") encoded in DNA ("-genetic"). Optogenetic devices can be introduced into tissues or whole organisms by genetic manipulation and be expressed in anatomically or functionally defined groups of cells. In a decade and a half, optogenetic control has developed from a far-fetched idea to a widely used technique. My talk will recount how this happened, drawing on the earliest and latest results from my lab. To illustrate what is now possible, I will present recent work on the neural control of sleep.

A bit of Quantum in Photosynthesis? Tracking Lightharvesting on the nm and fs scale

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Nature has developed photosynthesis to power life. Quantum coherences have been observed in the energy transfer of photosynthetic complexes, even at room temperature, hinting to a role of deep physics in natural light harvesting. Does nature exploit quantum concepts? Does the coherence help to find an optimal path for robust or efficient transfer? How are the coherences sustained? What is their spatial extent in a real light-harvesting network?

Specializing on combining femtosecond spectroscopy with nanoscale microscopy, I aim to look ultrafast into the nanoscale, to see biomolecules in action in a real system. Indeed, addressing LH2 of purple bacteria, we revealed the first coherent oscillations of a single photo-synthetic complex at physiological conditions, moreover non-classical photon emission of individual LH2 complexes. These results, pave the way to address photosynthetic networks in real nano-space and on femtosecond timescale. Advances and ideas will be discussed.
Single-molecule biosensing using plasmonic nanoparticles

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Eindhoven University of Technology, The Netherlands

Optical detection of single molecules mostly relies on their fluorescence because of the high contrast of this technique against the background. Since their invention in the early 1990s, single-molecule fluorescence microscopy and spectroscopy have spread to many fields in chemistry, physics, and biology, and have provided unique access to nanometer scales. However, the majority of native bioorganic molecules such as proteins hardly fluoresce at all. Therefore, their detection in native and unmodified state requires a different approach.

We demonstrate the label-free detection of single proteins using plasmonic nanoparticles. We monitor the scattering signal of hundreds of particles simultaneously in a total-internal-reflection microscope. This approach allows for the statistical analysis of single-molecule interactions without requiring any labeling of the analyte. Single-protein binding events are resolved as step-wise changes in the scattered intensity, see Fig. 1. We study an antibody–antigen interaction and find that the waiting time distribution is concentration-dependent and obeys Poisson statistics. The ability to probe hundreds of nanoparticles simultaneously will provide a sensor with a dynamic range of 7 decades in concentration and will enable the study of heterogeneity in molecular interactions.

Figure 1 Illustration of the principle of single-molecule plasmon sensing. (a) Immobilized gold nanoparticles are imaged on a dark-field microscope. Each diffraction-limited spot corresponds to a single nanoparticle. (b) As shown in the inset, the particles are functionalized with receptors at their tips and the sides are blocked with an antifouling coating. Single antibodies that bind induce a shift of the plasmon that is probed at a fixed wavelength. (c) Binding events of single molecules are resolved as step-wise changes in the signal. Statistical distributions of waiting times can be established.

Using evolutionary sequence variation to build predictive models of protein structure and function

L Colwell
University of Cambridge, UK

The evolutionary trajectory of a protein through sequence space is constrained by its function. Collections of sequence homologs record the outcomes of millions of evolutionary experiments in which the protein evolves according to these constraints. The explosive growth in the number of available protein sequences raises the possibility of using the natural variation present in homologous protein sequences to infer these constraints and thus identify residues that control different protein phenotypes. Because in many cases phenotypic changes are controlled by more than one amino acid, the mutations that separate one phenotype from another may not be independent, requiring us to understand the correlation structure of the data.

The challenge is to distinguish true interactions from the noisy and under-sampled set of observed correlations in a large multiple sequence alignment. We show that maximum entropy models of the protein sequence, constrained by the statistics of the multiple sequence alignment, are capable of predicting key aspects of protein function. These include (i) the inference of residue pair interactions that are accurate enough to predict all atom 3D structural models; (ii) accurate predictions of binding partners between different proteins; (iii) accurate prediction of binding between protein receptors and their target ligands. We will discuss how a mathematical framework based on random matrix theory bounds which sequence alignments contain sufficient information to build accurate predictive models. Finally, we will pose questions about the physics of binding interactions in an example from the immune system where large sets of evolutionarily related sequences are not available.
A simple genotype-phenotype map for protein-DNA binding, sequence entropy and its role in speeding up speciation

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Natural selection acts on organismal function, whilst variation arises from mutations; understanding the mapping between the two, between genotype and phenotype, represents a major outstanding challenge for evolutionary theory. Here we present a simple biophysical model of the map from genotype to phenotype for protein-DNA binding that controls gene expression and its consequences for the process of speciation. Using both simulation (Khatri et al. Genetics, 2015) and theory (Khatri et al., Journal of Theoretical Biology, 2015), we find that a quantity analogous to Boltzmann entropy from statistical mechanics, called sequence entropy (Iwasa, JTB, 1988 & Sella & Hirsh, PNAS, 2005), plays a key role in the dynamics of speciation; at small populations stochastic effects due to genetic drift dominate and those phenotypes with the greatest number of sequences (high sequence entropy) dominate – simply as a consequence of the fact that there are many more sequence pairs that bind poorly than well, means the protein-DNA binding is on average less well adapted for small populations, but still co-evolved to maintain function. When a lineage splits into two, the protein-DNA pair on each lineage now co-evolves independently and speciation arises once the hybrid DNA-binding becomes non-functional. Since the common ancestor pair is less well adapted at small population sizes, a smaller number of mutations is needed before hybrids become non-functional and speciation arises more quickly. In contrast to founder effect models, this model represents a new robust mechanism for more rapid speciation at small population sizes, consistent with large species diversity in small habitats such as Cichlids in the East African Great Lakes, and contrasted with the lower diversity of marine animals, which have large ranges and population sizes.

Figure showing a biophysical model of protein binding DNA, where the pair of sequences have co-evolved with each other for functional binding with few energetically unfavourable interactions (indicated in red at the protein-DNA binding interface). The population splits, causing geographic isolation between lineages. After a period of divergence, independent co-evolution on each lineage causes hybrids to become non-functional. This work uses the tools of stochastic dynamics to examine this process and finds speciation arises more quickly for small populations as they are poised near non-functionality, as there are many more sequences with worse binding than good.
Systematic investigation of bias in Jarzynski-derived free energy landscapes using coarse-grained simulations

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Understanding the self-assembly of biological molecules has attracted the attention of scientists from diverse disciplines for decades. Knowledge of the mechanism by which sequences of monomer building blocks fold into three-dimensional protein and nucleic acid structures is crucial to our ability to design and predict their biological functionality. Further, comprehension of the pathways by which folding occurs has broad ramifications, and can be applied to study mechanisms of viral operation¹; neurodegenerative diseases like Parkinson’s, caused when proteins misfold²; and optimization of engineered structures designed for uses ranging from drug delivery³, to biological computation⁴, to artificial light harvesting⁵. The folding problem is thus supremely relevant.

Currently, the best routes to probing molecular folding processes in the laboratory are single-molecule force spectroscopy (SMFS) experiments⁶, in which measurements are taken of a single molecule unfolding and refolding repeatedly under applied tension. SMFS experiments enable the reconstruction of molecular free energy landscapes, which contain all the useful information about the folding process⁷. Unfortunately, for many molecules, these experiments necessarily occur out of equilibrium⁸, and the theoretical equality underpinning non-equilibrium data extraction – the Jarzynski equality⁹ of non-equilibrium statistical physics – is known to introduce sampling biases¹⁰.

While phenomenological attempts to describe and correct for these biases exist¹¹, a systematic study of how bias varies with number of trials, experimental protocol, and structure of interest is lacking. Further, systematic experimental bias characterization is intractable: firstly, because experimentalists often do not have access to the true, intrinsic free energies for comparison, and secondly, because obtaining the required volume of trials in the laboratory is unfeasible. Using oxDNA, a nucleotide-level, coarse-grained model of nucleic acids¹², we have performed a systematic investigation of the effect of bias on Jarzynski-based landscape reconstructions. We present the results of thousands SMFS simulations on DNA hairpin systems, deriving equilibrium free energy landscapes and comparing them with Jarzynski-derived landscapes to explore the behavior of bias and propose general strategies to help experimentalists and other computational scientists maximize the reliability of their results.

The physics and biology of sprouting angiogenesis

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Sprouting angiogenesis, where new blood vessels grow from pre-existing ones, is a complex process where biochemical and physical mechanisms regulate endothelial cell proliferation and movement. The formation of a functional blood vessel network, which is essential for nutrient delivery to tissues, often depends on the regulatory mechanisms in sprouting angiogenesis. In this work, we introduce a model of sprouting angiogenesis capable of predicting sprout morphology as a function of the elastic properties of the tissues, and of the traction forces exerted by the cells [1]. We explore how different types of endothelial cell proliferation regulation are able to determine the shape of the growing sprout. The largest region in parameter space with well-formed long and straight sprouts is obtained always when the proliferation is triggered by endothelial cell strain and its rate grows with angiogenic factor concentration. We conclude that in this scenario the tip cell has the role of creating a tension in the cells that follow its lead. On those first stalk cells, this tension produces strain and/or empty spaces, inevitably triggering cell proliferation. The new cells occupy the space behind the tip, the tension decreases, and the process restarts. We also use this model to predict the evolution of large groups of cells that re-organize into vascular networks (see figure below). We compare vascular morphology of two-dimensional and three-dimensional networks, assessing the role that tissue cells have in directing the growth of the new vessels. To obtain a functional vasculature, the new vessel sprouts have to meet each other (anastomosis), thus forming loops capable of carrying blood flow. We demonstrate that the cells in the tissue have a pivotal role in directing the anastomosis of the sprouts in 3D, and participate actively in determining the branch density and vessel diameter of the resulting network.

Left: Confocal imaging of vascular formations in an aortic ring assay: In this figure the endothelial cells’ nucleus are marked in red by DAPI and endothelial cells’ membranes are stained in blue by lectin. Lines in red and in cyan were added to better visualise the processes simulated in this work.

Right: Simulation of the evolution of an endothelial cell spheroid. Several cells acquire the tip cell phenotype (marked in red) and, given the right conditions, rearrange themselves into a vascular network.

Biaxial nematic order of liver transport networks

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The liver represents a "chemical factory" that is characterized by intertwined transport networks for toxins and metabolites. Each hepatocyte cell interacts with two space-filling networks which either transport bile or blood through bile canaliculi or sinusoids. How these networks grow and establish their distinct geometry to supply all cells whilst providing robustness with respect to perturbations remains elusive.

Here, we elucidate design principles of liver architecture and network self-organisation to ultimately understand bile and blood transport in the liver — pursuing a two-fold approach:

We analyse high-resolution imaging data of cell membrane compartmentalisation in mouse liver tissue. We propose a nematic tensor formalism to characterise an unconventional type of hepatic cell polarity. Our analysis reveals global patterns of aligned cell polarity on the scale of the liver lobule, the functional unit of the liver, thereby characterizing three-dimensional liver tissue as a biaxial nematic liquid crystal. We quantify co-alignment of hepatocyte cell polarity and transport network anisotropy. (ii) We then ascertain the functional relevance of this network geometry for robust transport, seeking to elucidate the relationship between local network properties and global transport properties. For that aim, we perform flow simulations in reconstructed bile canaliculi networks (Fig. 1) as well as in synthetic networks simulated by sets of simple local rules (Fig. 2). With this approach that bridges physics and biology, we seek to link structure and function of transport networks in liver tissue.

Figure 1 Section of a liver lobule. Experimentally determined links of two networks (green and magenta lines) connect cells in the liver tissue and transport substances from the portal (left) to the central (right) vein.

Figure 2 An example output of synthetic networks derived by interaction rules for either network type (coloured lines). Those links must provide each cell (grey cubes) in such a synthetic liver slice.
Molecular and cellular mechanisms for folding sheets into tubes
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How sheets of epithelial cells give rise to complex three-dimensional tissue structures such as tubes is an exciting open question in morphogenesis. Many previous studies have oversimplified the problem by reducing a highly three-dimensional process to two-dimensions, focussing on the apical junctional domain of epithelial cell, where actomyosin and adherens junctions are located and exert their effects. Thus, the 3D cell behaviours underlying tubulogenesis have been ignored and their contributions have been neglected.

We have developed a combination of live imaging and computational tools to identify and characterize 3D cell behaviours involved in tubular morphogenesis. We are using the formation of the salivary glands in the fly embryo as a model process to understand how cell shape changes are controlled by the cytoskeleton and by interactions between cells in the epithelial sheet that is forming the glands. Our preliminary morphometric quantification of changes at the tissue and cell level suggests that in addition to apical constriction/cell wedging, other cell behaviours clearly contribute to tissue bending and tube invagination when the 3D context is taken into account. In addition, the anisotropic distribution of contractile actomyosin structures plays a crucial role in guiding cell behaviours during the tissue folding process.
Poster abstracts

Poster session 1

P:01 Quantum enhanced sensing: Using non-linear optics to beat the shot-noise limit in precision measurement

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Optical measurements are of fundamental importance to many fields within science, including biology and biophysics. Examples include: the use of optical coherence tomography for medical diagnosis of the eye and retina [1], particle tracking in optical tweezers for bio-motor force measurements [2], and phase-contrast microscopy for cell imaging [3]. The light sources of the majority of these techniques are laser light, L.E.D.’s or lamp structures. These types of emitters are all subject to a fundamental source of noise called the shot-noise. In some cases, this can be the limiting source of noise for the measurement in question [4,5].

Quantum sensing (often called quantum imaging or quantum metrology) is a new paradigm of measurement, using the unique properties of quantum mechanics to beat the shot-noise limit. This is achieved by generating new sources of light that can be used as a probe in a variety of applications. There has seen significant activity and progress in quantum sensing over the last five years, with a number of ‘proof-of-principle’ experiments showing the practical viability of the proposed sensing schemes with biological systems [6].

We will present our efforts to improve absorption spectroscopy measurements. This can be done by taking advantage of two non-linear optical phenomena: self-phase modulation and spontaneous parametric down conversion. We are engineering systems that take advantage of both of these effects [7]. I will report how to assess the advantage of using such a scheme in absorption spectroscopy, paying specific attention to the practicalities of implementing this source in a real experiment. I will show that the schemes can produce significant advantages (more than doubling sensitivity) with minimal experimental overhead, even reaching near-optimal sensitivity for more costly setups. This is the critical starting point for the practical implementation of a new technology, currently in the process of being constructed and implemented in practical experiments.

This work is made possible by the EPSRC national quantum technologies programme, the EPSRC quantum engineering doctoral training centre and the Quantum Imaging Hub.

Mechanics of cell division: effects of spontaneous curvature, surface tension, and osmotic pressure

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Cell constriction is an important cytokinetic phase preceding division. Before splitting in two daughters, symmetrically dividing cells accommodate their duplicated contents into spatially separated compartments defined by a stable fission site located at midcell (see Fig. 1). We found analytical expressions for the symmetric constriction of a vesicle by computing the energy of the deformed membrane. These analytical expressions are obtained for the constriction force, constriction energy, volume and area of the vesicle. They are expressed in terms of the spontaneous curvature of the membrane, the surface tension, and the osmotic pressure difference between internal and external environments. They describe membranes curved in their equilibrium state due to the compositional inhomogeneity between the two monolayers, membrane trafficking, and external, hypo, iso, or hypertonic medium.

FIG.1. Shapes during a symmetric constriction process.

Changing the shape of a vesicle from its equilibrium non-constricted configuration requires an input of energy. Under the osmotic pressure $\Delta \rho$ (the inner pressure minus the outer one) and under the action of the surface tension $\Sigma$, the total energy of a vesicle is given by

$$E = \frac{\kappa}{2} \int_{\Omega} (C_1 + C_2 - C_0)^2 dA + \int_{\Omega} \Sigma dA + \Delta \rho V,$$

where $\kappa$ is the bending modulus of the vesicle, $\Omega$ the surface, $dA$ its element of area, $V$ its volume, $C_1$ and $C_2$ its principal curvatures, and $C_0$ the spontaneous curvature.

We see that the constriction force is inversely proportional to the scale parameter of the vesicle. This indicates that smaller vesicles are harder to constrict. For cell-sized vesicles, with a flexible membrane $K \approx 10 \ k_B \ T$, we calculate constriction forces in the range of piconewton. Additionally, we analyze the effects of the spontaneous curvature, the surface tension and the osmotic pressure in all magnitudes and determine under which conditions vesicles constrict more easily (with smaller constriction forces). We obtain that smaller forces are needed for positive spontaneous curvatures, low surface tensions and hypertonic mediums.

Our analytical and numerical results can be useful for the design of synthetic divisomes and can serve to get insight on other biological processes involving membrane bending, such as exocytosis and endocytosis.

Controlling lipid bilayer properties by organic electrolytes: the room temperature ionic liquids route

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The lipid bilayers in water and in water-electrolyte solutions represent paradigmatic self-assembled soft matter systems, and display a wide variety of phases and behaviours. Together with proteins and saccharides, they form the protective envelop of eukaryote cells, and represent basic components of cellular organelles. Crucial characteristics of lipid bilayers include their elastic properties, their permeability, and their stability with respect to temperature and to chemical attack. Controlling these properties has wide implication in bio-medicine, and might also open new opportunities in nanotechnology.

Electrolyte species dissolved in the water environment in which bilayers reside provide a first suitable way to control these properties, whose flexibility and power has been greatly expanded by the recent introduction of organic ionic compounds of the room temperature ionic liquid family. The size, shape and hydrophilic or hydrophobic character of these compounds represent additional parameters to tune the system properties, based on the interplay of Coulomb and dispersion interactions, and on their different ability to form hydrogen bonds [1].

In my contribution I briefly review a recent work on the structure [2,3] and dynamics [3,4] of lipid bilayers interacting with RTILs, carried out by neutron reflectometry [2], elastic neutron scattering [4], and by computer simulation [3]. The results point to the incorporation of cations into the bilayers, affecting their basic properties, eventually leading to the bilayer destabilisation at high electrolyte concentration. I will discuss, in particular, the prospects of extending these investigations to other phases of lipids in water systems such as vesicles and micelles, and to broader classes of organic ionic liquids, focusing on bio-compatible compounds based on amino acid anions [5]. If time allows, I shall outline work in progress on the stability and properties of nearly-dry lipid bilayer stacks intercalated by organic ionic liquids, investigated for their potential interest as innovative dielectric materials.

P:04 Mechanical properties of single supramolecular polymers from correlative AFM and fluorescence microscopy

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Supramolecular polymers are aggregates of monomers that self-assemble through non-covalent interactions. They are promising as biocompatible agents in drug delivery and as scaffolds for tissue engineering. Although the physical properties of the polymer are in principle determined by the chemical structure of the monomer, simulations [1] and experiments [2] have revealed large differences that are likely caused by stacking faults and defects. The origin of these imperfections remain elusive but are expected to impact the structure and dynamics of the polymer. It is therefore crucial to study the morphology of these structures experimentally.

We correlated fluorescence measurements of single supramolecular polymers (benzene-1,3,5-tricarboxamide, Fig.1a) to atomic force microscopy (AFM) images of one-and-the-same molecule (Fig. 1b,c). We find a small fraction of polymers are clustered and/or entangled due to inter- and intrachain interactions. We determine the distribution of apparent persistence lengths for the single and unentangled fibers using cosine correlation analysis (Fig. 2). We demonstrate the added value of analyzing the distribution of single-molecule persistence lengths instead of the mere average by comparing the measured distribution to simulated worm-like chains. We find that the measured distribution is broader than the simulated one, which we attribute to molecule-to-molecule variations of the persistence length and/or to variations in local substrate-molecule interactions.

Fig. 1: (a) BTA structure. (b) Fluorescence and (c) AFM image of the same polymer. Scale bars are 1 µm.

Fig. 2: Distribution of apparent persistence lengths determined from experiments (left, blue) and simulated polymers (right, green).


In recent years the role of nanotopography in controlling the differential fate of mesenchymal stem cells (MSCs) has gathered a great deal of interest [1]. In their native environment, MSCs are surrounded by the extracellular matrix (ECM), a fibrous network composed largely of collagen, which supplies mechanical and chemical information to the cell, as well as providing structural support. The ECM displays feature sizes and spacings on the nanoscale, and as such any attempt to identify the mechanical factors that influence cell phenotype requires the ability to deterministically control the nanoscale landscape an MSC resides in.

Despite a great deal of progress in the past decade, a rigorous framework describing the role of the various nanoscale cues (geometry, size, spacing, etc.) in the macroscopic behaviour of the cell has yet to be arrived at. In this work we examine the theory that more compliant substrates lead to softer cell types [2]. By introducing dense arrays of nanopillars of varying heights, but with the same inter-pillar spacing and diameter, we are able to study in isolation the role of local substrate stiffness, with taller pillars being more compliant than shorter pillars. Substrates are produced via the thermal nanoimprint of porous anodic imprint molds of varying thicknesses into PMMA.

We report on the effect of squat, medium, and long nanopillars of diameter 125 nm and centre to centre spacing 250 nm on the morphology and mature phenotype of porcine mesenchymal stem cells. Furthermore, we introduce secondary topography in the form of line grating and crosshatch patterns of several 10’s of microns via SU8 imprint molds to simulate the microenvironment of cartilage and muscle tissue. Finally, the effect of the surface modification on the stiffness of the substrate is experimentally quantified via nanoindentation using a 2 µm diameter flat punch.


P:06 Growth kinetics of *Escherichia coli* in the presence of cell wall targeting antibiotics

R Brouwers

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We examine the growth kinetics of *E. coli* MG1655 at varying concentrations of three cell wall targeting antibiotics: Cefotaxime, Fosfomycin and Mecillinam. Our aim is to build a physiological model for the effect of cell wall targeting antibiotics, in a similar way to recent work on ribosome targeting antibiotics [1].

The three antibiotics chosen work on different parts of the cell wall synthesis machinery [2,3,4]. Growth curves for *E. coli* MG1655 in the presence of these antibiotics were measured using 96 well plates and a plate reader. Phase contrast microscopy has also been carried out for the early time growth in the presence of Cefotaxime.

We have so far found very different growth curves for the three antibiotics, a reflection of their different targets and modes of action. In particular, we see unexpected features in the growth curves such as sharp decrease in optical density that are probably caused by cell lysis. Microscopy analysis indicates the presence of cell filaments. Further work will characterise in detail the filamentation behaviour upon exposure to the antibiotic, and build models to describe how these antibiotics act on the cell.

Acute injury to respiratory tissues following trauma remains a significant source of morbidity and mortality, and in many cases can be accompanied by infections leading to pneumonia. A considerable body of literature exists describing clinical observations of respiratory trauma-associated infections, but few experimental models exist to study the combined effects of mechanical damage and infection of tissue integrity and function.

Here we describe two experimental platforms using a split-Hopkinson pressure bar (SHPB) and a shock tube to study respiratory trauma. Each platform has been adapted to perform dynamic pressure loading tests on freshly harvested larynx and trachea tissue specimens. The SHPB is a system where the impact of a striker on a cylindrical rod produces stress pulses, which are transmitted onto a sample. The stress pulse is partially transmitted through, and partially reflected from, the sample: the shape and intensity of the waves is captured using strain gauges mounted on the rods. The SHPB and quasi-static Instron systems were used to characterize the material properties of these tissues over a range of loading rates representative of injury conditions. The data obtained reveal show distinct and reproducible material responses, and represent an important step towards developing biofidelic numerical models for mitigation and biomedical applications.

Shock tubes, in comparison, generate blast overpressures that are thought to closely resemble free-field blast. We have specifically adapted an air-driven shock tube system to deliver controlled mechanical damage to whole trachea rings and ex vivo respiratory tissues cultures maintained in Petrie dishes. Dose-responses that correlate pressures with mechanical damage have been established using imaging data obtained from optical, EM and microCT techniques and functional assays of cilia motion in tissues. EM data also reveal considerable detail about the nature of mechanical damage to the ciliated surfaces of respiratory tissues and their susceptibility to bacterial colonization. These observations are consistent with clinical observations of pneumonia and tracheobronchitis that develop following trauma and damage to respiratory tissues. Future studies will focus on the identification of molecular components in bacteria and respiratory tissues responsible for damage-induced colonization. A key aim of this work is to use these models to develop new therapeutic strategies to improve the outcome of patients with respiratory trauma arising from military-related blast injuries, ventilator damage, and chronic infections associated with cystic fibrosis.
Physics Meets Biology

P:08 Physics of a purely Brownian ratchet molecular motor

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We have determined that φ29 polymerase in the DNA replication uses a purely Brownian ratchet mechanism [3], in contrast with other molecular motors that use a power stroke mechanism. In power stroke molecular motors the advance of the motor takes place during a strongly energetically favorable process that pushes the motor forward. In Brownian ratchets molecular motors the advance could not be energetically favorable; however it is fixed by a subsequent favorable process that fixes the advance. For φ29 polymerase we have determined the stepping process and the energetics of the replication cycle combining single-molecule experiments at different forces and nucleotide concentrations with previous biochemical information. This has allowed us to determine that the stepping process is energetically unfavorable, however it is fixed by the subsequent processes of nucleotide binding to the polymerase that is strongly energetically favorable. Therefore it is a good example of a purely Brownian ratchet molecular motor. In this presentation we will discuss Brownian ratchet and power stroke mechanisms, and the φ29 polymerase case [1-3].

FIG. 1. **Left diagram** shows experimental configuration, in this configuration the force acts directly on the polymerase aiding or opposing the displacement associated with replication. **Right plot** shows the free energy profile of the pre to post-translocation process and how its activation and transition energies are affected by an aiding force.


P:09 Excitons meet plasmonic antennae: a multiscale approach
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In photosynthetic complexes, multichromophoric systems are responsible for the light absorption and transfer, which eventually lead to charge separation in the reaction centres. Both absorption and energy transfer processes are extremely efficient, and great effort has been devoted in the last years to achieve a detailed understanding of their functioning at a molecular level. It has also been observed that the optical properties of such complexes can be greatly affected by the presence of nearby plasmonic devices, which may induce fluorescence enhancements of several orders of magnitude.

From a computational perspective, the modelling of these processes is particularly challenging, because of the large size of the systems under study, which may contain thousands of atoms. Multiscale approaches must therefore be devised. In particular, quantum-mechanical (QM) methods are necessary to describe the interaction with light, while a classical representation is used for the protein environment, which does not actively take part in the process, but rather acts as a perturbation.

We present our results on the effect that gold nanoparticles (NPs) have on the optical properties of the LH2 light-harvesting system of bacteria, shown in the Figure. This is a perfect excitonic system characterised by a high symmetry. We employ a multiscale approach whereby time-dependent density functional theory is used for the description of the 27 bacteriochlorophylls (BChls), later combined in an excitonic scheme. The effect of the surrounding protein is described with a discrete polarisable approach, in terms of fixed charges and induced dipoles [1], which allows to account for the structured interactions between QM and classical subsystems. The gold NP is instead described using a continuum model [2].

We analyse the absorption, quantum yield and fluorescence enhancements when the NP is placed at various distances and orientations from the LH2. Our study reproduces the experimental findings [3], and is able to interpret and rationalise them in terms of the different effects of the metal on the excitonic system.

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P:10 Membrane crowding and anomalous diffusion in artificial lipid bilayers

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In contrast to membrane protein diffusion in artificial bilayers, the diffusion of proteins in cells is both slower and non-ergodic. Anomalous diffusion in cells has been attributed to cytoskeletal-membrane interactions and nanoscopic heterogeneity in membrane protein and lipid composition. Using a combination of single-molecule fluorescence tracking and interferometric scattering microscopy we quantify how these factors affect anomalous diffusion over a range of time-scales in artificial lipid membranes. By varying the membrane composition and nature and density of pinning sites, we examine the onset of anomalous diffusion and its dependence on these properties. We observe a reduction in tracer diffusion rates as membrane crowding is increased, and the diffusion appears to become increasingly anomalous as membrane components are immobilised.
Motile cilia in human airways: the importance of beating in synchrony

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The coordinated action of large numbers of motile cilia is essential to mediate proper mucociliary clearance in mammalian respiratory airways, (1, 2). Dense carpets of cilia extend over the airway surfaces and beat together in a synchronous pattern that results in a phenomenon known as metachronal waves (3). This movement propels the mucosal fluid into the upper airways where it is expelled, keeping the airways clear of inhaled particles and pathogens. Synchrony is essential for the generation of metachronal waves, and impaired ciliary motion or defects in cilia structure underlie diseases in the brain, reproductive tracts and airways, and are associated with inherited disorders such as cystic fibrosis (CF), primary cilia dyskinesia (PCD), and acquired disorders such as chronic obstructive pulmonary disease (COPD) and asthma (4). The physical forces that generate and sustain cilia synchronization are still poorly understood (5). In particular, little attention has focused on understanding ciliary motion at the single-cell level, as opposed to the entire population of beating cilia. It is therefore unclear how parameters such as cilium beating frequency, amplitude, power and recovery stroke velocity, as well as cilium motion pattern and synchronisation converge to generate metachronal waves in the entire population of beating cilia. Quantification of these parameters is of fundamental importance for a functional classification of complex ciliopathies such as PCD and CF and, in turn, for our ability to understand their effect on mucociliary clearance (6). In this study, we describe an experimental approach that combines high-speed video microscopy with video analysis to study ciliary motion. We investigate how multiple parameters such as beating frequency, amplitude, power and recovery stroke velocities, ciliary motion pattern, and synchronization play a common role in mediating mucosal clearance in healthy human airway epithelial cells. This analysis is performed in a physiologically relevant culture system that closely mimics the in vivo environment, using the air liquid interface (ALI) culture system. We then compare healthy tissue with samples taken from patients with CF, asthma and COPD, and show how these different conditions are characterised by significant differences in ciliary motion. Taken together, these data provide an in-depth characterisation of the physical parameters associated with motile cilia dynamics in healthy and diseased human airway tissue. These data can be used to improve the diagnosis of different ciliopathies based on the novel biophysical parameters described herein and, importantly, to better understand how malfunctions in cilia beating and synchronisation contribute to impaired mucosal clearance across a range of ciliopathies.

Investigation of the molecular interactions relevant to receptor-mediated virus entry using HIV receptors as a model

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The plasma membrane physically separates the interior of the cell from its surrounding environment and stands as the main barrier to prevent virus entry. However, viruses have evolved to exploit fundamental cellular processes in order to overcome this obstacle and deliver their genetic cargo. In this way, new viral particles can be generated by the host cell, spreading infection and causing disease. Here we focus on the interaction with specific cell-surface receptor proteins that allows viruses to bind to the cell membrane and trigger their uptake by new host cells.

The Human Immunodeficiency Virus (HIV) is a good model to investigate receptor-mediated virus entry since it is well established that transmembrane receptor proteins CD4 and CCR5 and/or CXCR4 are exploited by the virus as specific anchoring sites. However, despite the intensive studies carried out on HIV in the last three decades, little is known about the dynamic properties of these viral receptors, their anchoring to the actin cortex or how these might influence the outcome of individual HIV-entry events. The actin cortex is a mesh of actin filaments and actin-binding proteins which lies beneath the cellular membrane regulating cell shape and stiffness.

Focusing on CD4, our aim is to precisely quantify the diffusive mobility of the CD4 receptor on the cell surface and to investigate the role of putative attachments of CD4 to the cellular actin cortex. These attachments can modify receptor mobility, enable the clustering of receptors and/or stabilise the virus-receptor interactions required for virus entry. The overall goal is to investigating the baseline physical properties of the HIV receptors in absence of viruses and how these may change upon virus binding, to elucidate their relevance to virus entry.

For this purpose, we have designed and built a multifunctional fluorescence-force microscope that combines dual colour, light-sheet fluorescence imaging with optical tweezers for single molecule force sensing. Here we present the details of our set-up as well as the single molecule force spectroscopy procedures we are currently employing to test the attachment of the CD4 receptor to the cytoskeleton.

The self-assembly of amphiphilic peptide I3K

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Materials made from self-assembling materials, such as synthetic peptides, are a topic of active research due to potential therapeutic applications like tissue engineering or drug delivery. I3K is a small synthetic peptide which can self-assemble to form a hydrogel constructed from a network of self-assembled nanotubes. Passive particle tracking micro-rheology is used to determine the properties of I3K solutions and gels. The technique relates the thermal forces felt by microscopic beads embedded in a medium with the physical properties of the medium itself. Preliminary results suggest that a phase change occurs between 1 mM and 10 mM concentrations in pure water. Moreover, a nematic liquid crystalline phase may exist above 20 mM concentrations. The physical sizes of I3K aggregates have been imaged with STORM super resolution fluorescence microscopy. The results confirm that twisted cylindrical structures are formed with lengths of the order of many microns and diameters of tens of nanometres. Furthermore, the fibres remain stable under dilution and incubation for weeks at room temperature.
Quantitative Raman spectroscopy discrimination of mesenchymal stromal cell lines for regenerative medicine applications

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Raman spectroscopy (RS) is a vibrational spectroscopy method based on the inelastic scattering of laser-light. RS is a label-free technique, and with careful application, is able to access molecular-scale information from biological samples without altering cell morphology or inducing cell death [1,2]. In this work, we explore the potential of RS to discriminate clonal mesenchymal stromal cells (MSCs) that have different differentiation capacities to form bone, cartilage and fat [3]. The application of these cells in regenerative medicine is compromised by the inability to reliably characterise and identify appropriate MSC subtypes for therapy.

Four immortalised MSC cell lines (Y101, Y201, Y102 and Y202) were investigated in this study. The ‘01 MSCs have tri-lineage differentiation potential, whereas the ‘02s are differentiation-incompetent. To characterise each cell line, a minimum of 70 averaged Raman spectra were collected per experimental replicate using a 532nm laser spot size of ~1 µm from the nucleus of randomly chosen air-dried cells (5 random spectra/cell). In total, 560 spectra were obtained for Y101 (over 6 replicates) and 200 spectra were obtained for each of the other cell lines (over 2 replicates). Spatially resolved, single-cell Raman maps were also acquired with ~400 spectra/map. A comparison with three primary MSCs randomly chosen from a heterogeneous population and measured at 36 random spectra/cell was also made.

Raman markers were determined from the set of two-peak intensity ratios (PIRs) in the averaged cell spectra. The most discriminatory PIRs involved the 932 (proteins), 970 (proteins and DNA/RNA), 1060 (lipids, carbohydrates and DNA/RNA), 1085 (lipids, carbohydrates and DNA/RNA), 1549 (proteins) and 1615 (proteins) cm\(^{-1}\) peak assignments ($\pm$ 1–3 cm\(^{-1}\)). Twenty-seven PIRs in the >999 cm\(^{-1}\) range (aside from those relative to the 970 cm\(^{-1}\) peak) were found to be separated for the 01 and 02 cells, thus showing RS to clearly distinguish the Y101/201 and Y102/202 MSCs. Twelve PIRs against 970 cm\(^{-1}\) and the 1656/932 PIR were found to fully discriminate all cell lines with the results separated outside of any uncertainties measured. The population converged %SE uncertainties associated with the PIRs ranged from 3–7%. Spatially resolved cell-line discrimination was achieved using PIR maps, which revealed rich protein structures (nucleoli) within the nuclei of the 02 cells. PIRs against the 1085 cm\(^{-1}\) peak determined two of the primary MSCs to be closely-related to the 01 group, whereas the third cell was comparable to the 02 group. In summary, we demonstrate through rigorous testing the potential for RS to discriminate MSCs for regenerative medicine applications.

P:15 Raman spectroscopy for quantitative assessment of human intervention (cooking evidence) in archaeological marine shells

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Studies of archaeological marine shells using isotope analyses can reveal the behaviour of people in the past (e.g., seasonality) as well as characteristics of the paleoenvironment, such as salinity and water temperature [1,2]. However, such analyses are only trustworthy if the shells have not extensively changed (i.e., undergone diagenesis) and if human intervention (e.g., cooking), has been quantitatively assessed [2,3]. Quantification of diagenesis and cooking evidence have proven difficult with current methods being not only limited but also destructive in accessing this information [4].

In this work, we propose a quantitative method for the assessment of extensive heating/cooking in archaeological Conomurex fasciatus shells using Raman spectroscopy (RS). RS provides a detailed molecular-scale fingerprint of matter giving it immense potential for application in assessing archaeology artefacts [5,6]. Conomurex fasciatus shells are aragonitic and comprise the dominant species in the archaeological shell middens of the Farasan Islands in the Red Sea [7]. To simulate cooking, modern shells that were collected from this region were subjected to furnace heat-treatment at 100°C, 180°C, 250°C, 325°C, 400°C and 500°C, and at time periods of 3 min, 7 min, 15 min, 30 min, 1h, 2h, 4 and 8h at each temperature point. Two hundred spectra were randomly collected and analysed for each heat-treated sample as well as for non-heat-treated samples. These experiments were repeated for three biological (shell) replicates.

Raman spectra of the heated shells showed an aragonite-calcite transition between 1-2h at 400 ºC and at 500°C between 15-30 min. No transition was detected below 400 ºC. The peak intensity ratio of the lattice mode at 153 ± 1 cm⁻¹ (L₁) to the main carbonate peak at 1083 ± 1 cm⁻¹ (C) decreased by 50% ± 3% during the transition for both temperatures, thus representing a relative change to the percentage compositions of aragonite and calcite. The full width at half maximum of both the L₁ and C peaks decreased by 35% ± 1% and 15% ± 0.5%, respectively at 400°C, and 30% ± 2% and 10% ± 0.5%, respectively at 500°C over the total time measured. These changes show that the calcium carbonate becomes more crystalline with increasing heat treatment. Thermal degradation of the organic macromolecules that support the strained mineral lattice lead the calcium carbonate to relax and transform from aragonite to calcite [8]. Our results show the sensitivity of quantitative RS for being able to detect molecular-scale changes specific to heating at different temperatures and times, thus providing potential quantitative markers for open fire cooking in archaeological sea shells.

P:16 Purification of 50-base DNA-AuNP mono conjugates using gel-electrophoresis

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Monovalent DNA–gold nanoparticle (mDNA–AuNP) conjugates are important as building blocks for plasmonic molecular architecture both for ensemble and single molecule measurements. Most widespread method of synthesizing such mono-DNA-AuNP conjugates involve use of thiolated DNA, together with ligand exchange of the gold nanoparticles to make them stable in high ionic strength buffers. Use of DNA with single thiol modifiers is most prevalent. However, use of single-thiol modified DNA in conjugating mDNA-AuNP conjugates is not very stable and does not provide a “cookie in a jar” approach to functionalize these building units for storage. There have been reports of using tri-thiolated DNA modifier [1], long poly-A tail [2] in addition to DNA sequence of interest as well as double-thiol DNA [3] modifiers to improve on the long-term shelf life of the mDNA-AuNP conjugates. Here, we report a facile method to prepare mDNA-AuNPs using commercially available double-thiol DNA modifier and also able to purify the mDNA-AuNP conjugates with 50 bases of DNA using agarose gel-electrophoresis and PEG4 [4] coating with ease using electro-elution techniques [3]. The yields of mDNA-AuNPs were sensitive to the stoichiometric ratio between DNA and AuNPs, and the mDNA-AuNPs purified exhibited high efficiency for sequence-specific hybridization and dimer formation. In Fig 1 we can see the comparison of the bands of DNA-AuNP conjugates which is enhanced with PEG4 coating using 100-bases of DNA and improvement of band resolution is clearer in the gel with both 100 and 50 bases DNA and PEG4 coating as in Fig 2.

Many species of motile bacteria use rotating extracellular filaments to propel themselves through liquid media. Each filament is driven by a membrane spanning rotary nano-machine called the bacterial flagellar motor. In *Escherichia coli* and *Rhodobacter sphaeroides* the motor is powered by a transmembrane flux of H⁺ and the chemical energy is converted into work through a ring of stator units pushing on a central rotor.

Chemotaxis is the biasing of movement towards regions that contain higher concentrations of beneficial, or lower concentrations of toxic, chemicals and is one of the most well-understood bacterial sensory pathways. Upon phosphorylation, the response regulator protein CheY transduces changes of environmental chemical gradients detected by specific transmembrane chemoreceptors to the flagellar motors: it binds to the N-terminus of the FliM proteins in the C-ring part of the motor inducing a cascade of conformational changes that modulate the direction of rotation (in *E. coli*) or the motor stopping (in *R. sphaeroides*).

In this work, a novel technique for protein internalisation in live bacteria based on electroporation and single-molecule imaging using a custom-built microscope are combined to perform an in-depth investigation of the interactions between wild type and mutant chemotaxis proteins, chemoreceptors and the motor complex *in vivo*.

Chemotaxis proteins are purified, labelled with organic dyes and inserted into live *E. coli* and *R. sphaeroides* by electroporation. In typical experiments exploiting this new capability, video fluorescence microscopy shows single molecules diffusing within cells, interacting with the sensory clusters and individual flagellar motors.

This approach allows for the first time imaging and tracking of single dye-labelled chemotaxis proteins performing their function as response regulators in real time. Diffusion as well as relevant binding constants and dwell times at each end of their journey can be measured, and a comparison of such quantities across different protein mutants, genetic backgrounds and environmental conditions can be made.
A patch clamp study of ionic Coulomb blockade in biological ion channels

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Ion channels play an essential role in all living creatures, from bacteria to humans, and their dysfunction leads to numerous diseases. A major functional feature of ion channels is their ability to select between ions of different size and/or charge. A new Coulomb blockade-based physical model of ionic permeation¹ connects this ability with the value Q of the fixed charge in the channel’s selectivity filter (SF).

Our experiments aimed to define the role played by the fixed charge associated with the amino acid residues in the SF of NaChBac, a bacterial voltage-gated sodium channel. The whole-cell patch clamp technique was employed to investigate permeability for mono- (Li⁺, Na⁺, K⁺, Rb⁺ and Cs⁺) and divalent (Mg²⁺, Ca²⁺, Sr²⁺ and Ba²⁺) cations, and in mixed Na⁺/Ca²⁺ solutions. Wild type NaChBac (Qf=-4e) is highly selective for Na⁺, but it can be converted to become Ca²⁺-permeable through mutagenesis²⁻⁵ in its SF (which consists of six amino acid residues: leucine, glutamate, serine, tryptophan, alanine and serine (LESWAS))³. Key to sodium selectivity is the negatively charged amino acid residue, glutamate (E).

Using site-directed mutagenesis we have generated mutant channels whose SFs have “balanced charge” Qf=0 (LEKWAS, where the neutral serine is replaced with positively charged lysine), “deleted charge” Qf=0 (LASWAS, in which the glutamate is replaced with electrically neutral alanine) and “added charge” Qf=-8e (LEDWAS and LEEWAS, in which the serine is replaced by negatively charged aspartate or glutamate respectively).

Our experiments confirm that wild type NaChBac is a highly selective Na⁺ channel with negligible permeability for all other cations tested, except Li⁺. The LEKWAS and LASWAS mutant channels exhibited negligible cation permeability and no significant NaChBac mediated ion currents could be measured. The “additional charge” mutants (LEDWAS and LEEWAS) conducted not only Na⁺ and Li⁺, but also K⁺ and divalent cations. Furthermore, LEDWAS exhibited a strong anomalous mole fraction effect (AMFE) with minima at 1 mM Ca²⁺ solution.

Our results confirm the importance of Q, as a determinant of NaChBac ion selectivity. Deletion or neutralisation of the negatively charged glutamate residue leads to loss of the channel’s permeability to both mono- and divalent cations. However, the addition of negatively charged amino acid residues to the SF results in permeability for divalent cations and leads to such phenomena as divalent blockade of Na⁺ current and AMFE. These results are being used to validate and refine the ionic Coulomb blockade model of ion permeation through Na⁺ and Ca²⁺ selective channels¹.

Overcoming biological barriers: nanoparticles for targeted nucleic acid delivery

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Nucleic acids are versatile molecules with tremendous potential for therapeutic, nanofabrication, and applications at the interface with biology. However, therapeutic oligos, and nucleic acid devices are fragile under physiological conditions1. Furthermore, they are not readily taken up by cells, and cannot access the cytosol under normal circumstances. To overcome the numerous biological barriers present in vivo, from clearance by the immune system, to escape from the endosomal compartments, they require the assistance of bio-compatible and responsive carriers. We present two delivery systems for enhanced nucleic acid delivery: Polymeric Spherical Nucleic Acids (PSNAs), and Cell-Derived Vesicles (CDVs). PSNAs are formed from DNA-conjugated di-block copolymers2 with the ability to reversibly assemble into vesicles depending on the pH. These polymeric nanoparticles exhibit internal and external DNA coronas that can be used as steric shields against nucleases and as sequence-specific docking sites for DNA functionalized-cargoes. CDVs are bio-inspired3 nanovesicles produced from cell membranes. CDVs retain the membrane proteins characteristic of their cell source, resulting in different functionalities.

Figure 1. A. Formation of a PSNA. PMPC-PDPA diblock co-polymer is conjugated to a DNA oligo. The PDPA directs the reversible self-assembly into vesicles at pH greater than its pKa. B. Endosomal escape mechanism: PSNAs dissolve within acidic endosomes resulting in pores due to an osmotic shock. C. Cell-Derived Vesicles. Bar 100 nm.

P:20 Kinetic model of selectivity in the KcsA potassium ion channel

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Ion channels conduct physiologically important ions through the membranes of all biological cells. Paradoxically their charged selectivity filters allow fast conduction (nearly at the rate of free diffusion ∼ 10^8 s^{-1}) to coexist with high selectivity. To resolve this conundrum, we use a statistical mechanics approach and apply a multi-species kinetic model in the optimal transport regime of the KcsA potassium channel to investigate selectivity and conduction of K^+ and Na^+.

Conduction in this open system is discussed in terms of ion transitions between neighbouring energy levels in the filter. These energy levels arise due to ion discreteness [1,2,3], analogous to discrete electron transport in quantum dots [4].

Solving our kinetic model in steady state gives binding probabilities in the filter as a function of transition rates. By varying the fixed charge numerically we find: a Coulomb staircase of occupancy; and conduction peaks for each individual species (see Fig:1a). The peaks maximise at different values of n_f, and are vastly different in order of magnitude, indicating strong selectivity. These results display ionic Coulomb blockade analogous to its electronic counterpart [2,4] and in agreement with Brownian dynamics simulations of Ca^{++} channels [1] and single-species kinetic theory [3]. We also show a preliminary I-V curve (see Fig:1b), fitted against experimental data taken from [5].

Resonant transport conditions are derived analytically in the linear response regime, and we verify that these match the kinetic scheme when it is close to equilibrium. We find that K^+ matches the equilibrium barrier-less knock on condition [6].

Figure 1: Calculated resonant conduction currents vs. the fixed charge n_f and preliminary I-V curve. (a) K^+ and Na^+ current, with: a voltage drop of 50mV and symmetric concentrations 0.5mM for both species. (b) I-V curve plotted with data taken from [5], with: n_f = −2.5, filter diffusion coefficient of 0.15 · D where D is the bulk value and symmetric concentrations of 0.2mM.

In summary we have resolved the long standing problem of how high selectivity and fast conduction are combined in the potassium channel. The research was supported by the Engineer- ing and Physical Sciences Research Council UK (grant No. EP/M015831/1 and 1331678).


Characterizing the NaChBac channel through MD simulations

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The *Bacillus halodurans* voltage-gated sodium-selective channel NaChBac is an orthologue of mammalian voltage-gated Ca$^{2+}$ (Cav) and Na$^+$ (Nav) channels. Each of the 4 identical subunits of NaChBac comprises 6 transmembrane helices, the first four forming the voltage sensor domain and the last two the channel domain. An homology model built using the NavMs template, was embedded in a fully solvated POPC bilayer and submitted to a 100 ns simulation in the NPT ensemble. Overall, the structure appears to be very stable with RMSD oscillating in a narrow band between 2.0 and 2.5 Å. The analysis of the RMSF profile highlights the rigidity of the selectivity filter (SF) since the four corresponding TLESWAS stretches correspond to minima of the profile. The maxima of the RMSF are typically mapped to the initial fragment of helix S5 and the final fragment of helix S6 as well as to the turret loop and the final part of helix P2. The most flexible regions are thus partially unstructured fragments acting as linkers between secondary structure elements. After 5 ns the filter is already occupied by two ions at the level of the EEEE-ring and the LLLL-carbonyl ring respectively. The trajectory then shows several transient entering events of a third ion that are normally short-lived unless the first two ions move to a lower position. The Potential of Mean Force (PMF) as a function of the axial position reveals the existence of four minima, three of which correspond to the EEEE, LLLL, and TTTT rings respectively, while the fourth one, S4, identifies a binding site at the level of the serines above the EEEE ring. The first three minima thus correspond to the HFS, CEN and IN binding sites predicted by Catterall (Nature 475, 353-358, 2011) for NavAb and whose existence was later confirmed by a number of studies (J. Am. Chem. Soc. 134, 1840-1846, 2012, J. Phys. Chem. B, 117, 3782-3789, 2013). When sodium occupies the IN or CEN minima it interacts with a single threonine or leucine residue only in 5% of the frames while it is normally surrounded by six water molecules arranged at the vertices of an octahedron, thus occupying an on-axis position. By contrast, in the HFS site, sodium interacts with a glutamate and a serine belonging to the same subunit as well as with four water molecules. As clearly revealed by the 2D-PMF, this results in an off-axis placement. A permeation mechanism could be derived by using a Markov State Model (MSM) in conjunction with Transition Path Theory (TPT). The states involved in the MSM transitions were chosen to be all the possible ion occupancy configurations of sites IN, CEN, HFS, S4 and of two regions immediately below (CC) and above (EX) the selectivity filter. The dominant paths connecting a typical configuration before and after a permeation event, were identified through TPT, as the pathways with the largest bottleneck flux. The most probable path (28-27-20-17) reveals a knock-on mechanism. In this pathway, in fact, the ion in the EX position advances by one position occupying the S4 site. This pushes forward the ions in CEN and HFS that move to sites IN and CEN respectively. Finally, the ion in site IN leaves the SF and enters into the central cavity of the channel. In order to attain better converged free energy surfaces both 1D and 2D-metadynamics simulations were also run. Interestingly enough, the PMF that we derived from a 1D-metadynamics simulation biasing a single ion in the SF, showed a single very deep minimum instead of the four ones exhibited by the unbiased PMF. This disagreement may reflect the existence of an high energy barrier in the SF so that conductance requires the presence of at least two ions. This scenario was confirmed by a 2D-metadynamics run with axial bias on two ions in the SF, that yielded a PMF in better agreement with the unbiased one.
Hepatitis C virus (HCV) afflicts 170 million people worldwide, 2-3% of the global population, and kills 350,000 each year. Prophylactic vaccination offers the most realistic and cost effective hope of controlling this epidemic in the developing world where expensive drug therapies are not available. Despite 20 years of research, no vaccine is yet available. A major obstacle to vaccine design is the extreme genetic variability of the virus and its rapid mutational escape from host immune pressure. Empirical precepts to guide and accelerate vaccine design efforts are urgently required.

Coupling data mining of sequence databases with spin glass models and maximum entropy inference techniques from statistical physics, we have developed a computational approach to translate clinical sequence databases into empirical fitness landscapes quantifying the replicative capacity of the virus as a function of its amino acid sequence. These landscapes explicitly connect viral genotype to phenotypic fitness, and reveal vulnerable immunological targets within the viral proteome that can be exploited to rationally design vaccine immunogens. Viewing these landscapes as the mutational "playing field" over which the virus is constrained to evolve, we have integrated these fitness landscapes with agent-based models of the coupled dynamics of viral mutation and the host immune response to establish a data-driven multi-scale immune simulator of HCV infection. We have employed this simulator to perform high-throughput in silico screening of HCV vaccine immunogens to rationally design vaccines to both cripple viral fitness and block mutational escape. By systematically identifying a small number of promising vaccine candidates, these computational models can massively reduce the search space for experimental vaccine development, guiding and accelerating the search for a vaccine.
P:23 Widefield microwave imaging using atoms and diamond NV centres

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Alkali vapor cells and nitrogen vacancy (NV) centres in diamond are among the best sensors for electromagnetic fields, with applications in physics, medicine, and biology. Among their uses, vapor cell magnetometers have been used for imaging the magnetic fields of the human heart and brain, and NV centres in nanodiamonds can be implanted as sensors into living cells. Both have been used for NMR and MRI. Vapor cells and NVs have generally been used for detecting dc and low frequency fields. Our groups have developed techniques using both vapor cells [1,2] and NV centres [3,4] for imaging magnetic fields at microwave frequencies (GHz to tens of GHz), detecting the fields through coherent Rabi oscillations driven on transitions within the atoms or NV centres. In this presentation, we aim to stimulate discussion on how our techniques might be able to benefit the biophysics community.

The focus of our microwave imaging to-date has been in the context of microwave devices, which form the backbone of many scientific and technological applications, from quantum devices (atom chips, ion traps, atomic clocks, qubits…) to telecommunications (wifi, mobile phones…). There is great interest in techniques to image the microwave near-fields close to such devices, which promise to transform device development, characterisation, and debugging. Using atomic vapor cells and a camera-based widefield imaging system, we can record 2D images of microwave magnetic near fields over a 6x6 mm$^2$ field of view, with a voxel size of 50x50x140 µm$^3$ (see Fig. 1) [2].

Figure 1: Images of the microwave magnetic field, $|B_{mw}|$, at several positions above a microwave circuit, obtained using a Rb vapor cell. The central signal line of the circuit is shown in red, and the ground planes are in orange.

Data for all voxels is taken simultaneously, and our recently upgraded imaging system allows for sub-millisecond temporal resolution over an image. The current sensitivity is $\sim 1 \mu$T.Hz$^{-1/2}$, and we expect an improvement of 2-3 orders of magnitude in our new setup. Using scanning probe NV centres, we have performed near field microwave imaging with $\sim 20$ nm spatial resolution and a tens of micrometre field of view [3]. We are building a widefield NV imaging system which will provide micrometer-order spatial resolution in a $\sim 100$ µm field of view, with a sensitivity of tens of nT.Hz$^{1/2}$ per voxel, and again sub-millisecond temporal resolution over an image.

With the aid of a tesla-order dc magnetic field, we are able to detect microwaves of any frequency, from GHz to tens of GHz [1]. Our microwave imaging techniques represent a fundamentally new approach to microwave sensing, providing intrinsically calibrated measurements with high spatial and temporal resolution. In addition to already realised applications in microwave device characterisation, we anticipate application in medical imaging, and hope to inspire further applications in biophysics.


From invasion to egress: analysis of the blood-stage malaria cycle

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Malaria is a life-threatening disease caused by infection by the protozoan *Plasmodium* parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. Malaria is a devastating health problem in many countries, with one-third of the world’s population at risk of infection and causing half a million deaths every year [1]. With parasite drug resistance rising continually, the search for a viable vaccine is dependent on developing a better understanding of the molecular and physiological processes through which the parasite is able to proliferate inside the blood stream.

In our research we focus on the asexual reproduction of the *Plasmodium falciparum* species during blood stage, which is the agent of the most severe and deadly form of malaria in humans. We study the evolution of the infection throughout an approximately 48-hour gestation period inside host cells, which has not been tried before. During such experiments, we observe the flickering dynamics of the erythrocytes membrane, the phases of the parasite development, and the frequency and timing of egress-invasion processes. Two of the most crucial yet least understood events in the lifecycle of the *Plasmodium* are the egress of parasites from a mature infected red blood cell and the subsequent invasion of healthy erythrocytes. It has been suggested that pre-invasion events, the deformation of the targeted cell membrane and the alignment of the apical end of the parasite, could be relevant in the invasion efficiency [2]. The hypotheses formulated for this mechanism would include the crucial role of elevated intracellular calcium. However, a detailed analysis of recent results shows as if early pre-invasion deformations (Figure 1) occur without any sign of increased calcium in red blood cells [3]. The central question is how does the elevated calcium signal relate to pre-invasion and penetration stages? We are currently investigating these hypotheses by using a fluorescent marker for calcium and performing live-imaging fluorescent microscopy.

![Figure 1. Deformation of red blood cell due to parasite alignment during pre-invasion.](image)

P:25 Network sizes and regulatory rules both contribute to the topologies for perfect adaptation

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The design principles of complex biological networks are of great interest to understand how molecules working together to achieve certain functions. Many studies have focused on different functions but used as small network sizes as possible and mostly involved in one regulatory rule (such as enzymatic or transcriptional regulation). However, biological networks are far more complex than the coarse-gained models and more than one reaction type is evolved in biological functions. While it is still unclear whether different network sizes and regulatory rules in our models affect the topologies for certain functions. In our work, we choose perfect adaptation as a model function to investigate these issues due to the universality and abundance in research of adaptation. Negative feedback loops and incoherent feedforward loops are two families that can achieve perfect adaptation but only in the consideration enzymatic reactions within three-node networks. So we set out to change network size and regulatory rules one by one, and then find their influences compared with the results before and after the change.

For the network size part, we investigate the networks with just more nodes but still focus on enzymatic reactions. We generalize proportional adaptation mechanism by establishing a steady- state proportionality relationship among a subset of nodes in a network. Adaptation can be achieved by using any two proportional nodes in the sub-network to respectively regulate the output node positively and negatively. We first identify basic regulation motifs consisting of two and three nodes that can be used to build small networks with proportional relationships. Larger proportional networks can then be constructed modularly similar to LEGO's. A new “anti-phase” four-node network - a coherent feedforward loop that cannot adapt for three-node condition is found capable of adaptation.

For the regulation rules part, we focus on the networks within three nodes but change from enzymatic reactions to transcriptional regulations, with three different types of gene regulation logics. After computational enumeration and theoretical analysis, we find distinct topological features emerge comparing to enzymatic networks when zooming in the control node. Specifically, for incoherent feed-forward loop, the control node can be either a proportional node or an inversely-proportional node. It’s the mathematical form of regulatory rules that makes the topological differences and different constraints of parameters.

Our studies systematically investigate the influences of network sizes and regulatory rules and find they also work as determining factors of biological network topologies, together with network functions. More nodes can release the constraints that smaller networks have both in architectures and parameters. Different regulatory rules have different topologies and parameters constraints. These results for adaptation may be used for reference for other functions and deepen our understanding of the design principles of biological systems.
P:26 Electronic nano-biosensor for the investigation of Alzheimer's disease induced by Amyloid-Beta oligomers

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The earliest abnormal biological events resulting in neuronal damage in neurodegenerative diseases, e.g. Alzheimer's diseases, can be investigated by novel biophysical methods at the single-cell level. In this work, a nanoscale adenosine triphosphate (ATP) biosensor installed on a homebuilt nanodosing system based on scanning ion conductance microscopy (SICM) was developed to study the toxicity of aggregation of misfolded proteins, i.e. Amyloid-beta peptides (Abeta), which were locally dosed onto a single neuronal cell in vitro.

The ATP biosensor was fabricated at the tip of a double-barrel carbon-filled nanopipette, and it worked as a Hexokinase-cofunctioned electrolyte-gated organic field-effect-transistor (EGOFET), where polypyrrole was adopted as its biocompatible active material.

One of the critical hypotheses for the earliest stage of Alzheimer's disease suggests that holes at the neuronal cell membrane may be formed due to the presence of Abeta oligomers, and they could consequently lead to cell death and cause neurodegenerative diseases. This hypothesis will be verified if cytoplasmic leakage of ATP as the result of induced membrane holes can be examined by our ATP biosensor.

P:27 Design principles for mitochondrial apoptosis network

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Apoptosis is a major process for multicellular organisms to remove superfluous or damaged cells. Proteins participating in apoptosis pathway can be divided into two types: pro-apoptotic proteins such as caspases and anti-apoptotic proteins such as XIAP (X-linked inhibitor of apoptosis protein). XIAP was originally identified as inhibitor of caspases in model organisms. Caspases are central regulators in apoptosis pathway. Initiator caspases such as caspase-9 in mitochondrial apoptotic pathway sense and integrate the apoptosis signal and transmit it to executor caspases. Executor caspases such as caspase-3 make final life-death decisions. Previous studies have showed that the activation of caspase-3 exhibits an all-or-none behavior. Once activated, cells will definitely die. However, what the design principle is for mitochondrial apoptosis remains unknown.

Mitochondrial apoptosis is triggered by MOMP (Mitochondrial Outer Membrane Permeabilization) and the subsequent release of pro-apoptotic proteins into the cytosol. Caspase-9, as an initiator, senses the apoptosis signals and activates its downstream caspase-3. The activated caspase-3 can reversely activate caspase-9, which forms a positive feedback loop. XIAP inhibits caspase-3 and caspase-9 in a competitive manner, which is supposed as an implicit positive feedback. What's more, caspase-3 can inhibit and cleave XIAP in some specific cell type so that a double negative feedback between XIAP and caspase-3 forms. In this study, we constructed an inducible apoptosis cell system and made two important mutants (one mutant breaks the positive feedback loop between caspase-3 and caspase-9, the other knocks out the negative regulation between XIAP and caspase-9. Experimentally, population-level analysis (high-throughput FACS) and single cell-level analysis (confocal spinning disc) were performed on the inducible WT cells and mutants. Combining with mathematical models, we unraveled the functions of the coupled positive feedback and negative regulation related to caspase-3 activation in the process of apoptosis. The positive feedback loop between caspase-9 and caspase-3 guarantees a necessary cell death without any hesitation. The negative regulation between caspase-9 and XIAP set a threshold in the process of apoptosis, which acts as a guardian to protect unwanted cell death.
The disturbance of beating frequency of rice fish gills in the contaminated water with titanium dioxide nanoparticles

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The quasi real-time information on the beating frequency of the Medaka gill (rice fish: *Oryzias latipes*) is extracted using current video – computer system equipped with GPGPU (General Purpose Graphics Processor Unit). This setup allows 30 frame images for every 1 second over 4 days. In Fig. 1 and Fig. 2 basic camera set up and original image are shown. Fig. 3 shows the fundamental algorism to extract the image of the gill out of graphically noisy image using three-dimensional (two dimensional space axis plus time axis) Fast Fourier Low-Pass Filtering Method. The extracted sample with 5 second is shown in Fig. 4. We can find the cylinder with heart shaped cross-section of spatial image and time in the longitudinal direction. The heart shape vibrates about 25 times in 5 second which means about 5 Hz.

Fish gill is directly interface with surrounding environment and the role in fish homeostasis by means of gas exchange, osmoregulation and acid-base regulation. In Fig. 5 the beating frequency of the control and exposed Madakas are presented. The latter was in the water contaminated with nano-particles:100ppb anatase type TiO₂ of 25 nm size (T-25) *in vivo*.

TiO₂ is believed to be non-toxic materials even in nano-particle form. However, when this was introduced into the water, on first two days clear frequency rise was observed in exposed Medakas with significant difference (p < 0.05).

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Fig. 1. Detections system set up
Fig. 2. Image of medaka taken from bottom
Fig. 3. Color contour to define “gill area” (R/G:1.1.1.2.1.3.1.4)
Fig. 4. Gill movement in 5 sec (Contour R/G-1.4)
Fig. 5. Experimental result for 100 ppb exposure
The binding of small ligands to long polymers occurs in different biological processes. For example, TFAM protein binds to DNA, and is essential for mitochondrial DNA compaction [1]; replication protein A (RPA) binds to single-stranded DNA, and plays a critical role in genome replication, recombination and repair [2]; and actinomycin D binds to double-stranded DNA, and is used to treat some highly malignant cancers [3]. Here, we present a theoretical model for the mechanics, the dynamics and the kinetics of such binding processes. For the mechanics, we assume that the polymer can be effectively divided in its naked and ligand-covered regions (see Fig. [1]). Then, we can express the extension per monomer of the polymer when a pulling force $F$ is applied at its ends as the sum of the extensions per monomer of the naked and covered sub-chains, normalized by the fraction of uncovered and covered monomers, respectively. Thus, with this effective decomposition we achieve a force-extension relation that explicitly depends on the coverage fraction.

For the dynamics, using the aforementioned force-extension relation and assuming for the chemical potential of the bound ligands the ideal gas expression, we obtain the Gibbs free energy of the complex, and the steady fraction of covered monomers as the one which minimizes it. Finally, for the binding kinetics we consider different scenarios. If different binding modes exist, the relative number of ligands bound in each mode may change until reaching the steady configuration. This may cause transitory experimentally-observable shortenings or lengthenings of the polymer.

P:30  *Chlamydomonas* in an air-lift photobioreactor under gyrotactic effects

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Photobioreactors are systems for the mass cultivation of algae, agents for CO₂ sequestration, synthesis of high value products, and bioenergy. To improve photobioreactor efficiency, the effects of light regime and mass transfer rate have been characterized theoretically and experimentally [1]. However, past studies have ignored cell motility and considered only turbulent-flow operations, a culprit of high operational expense. Here, we study the collective motion of the model green alga *Chlamydomonas reinhardtii* in a downward Poiseuille flow, a constituent flow in air-lift photobioreactors operated under a laminar regime. Due to a gravitational torque resulting from cell bottom-heaviness and reorientation by flow shear, cells collectively migrate and form a structure known as a plume along the pipe axis. By analyzing microscopic videos of free algal suspensions, motility parameters such as swimming speed and reorientation rate were obtained to parameterize a simulation based on a bio-hydrodynamic theory. An experimental test of this theory is provided by analyzing plume images from a lab-scale air-lift photobioreactor, and the comparison will improve the predictability of cell distribution for given flow rates. During cell division, *C. reinhardtii* resorb their flagella, losing motility: the plume effect diminishes. Therefore, the investigation also leads to a method of characterizing the algal cell cycle at a population level.

Compartmentalisation is essential for cellular function, in which context lipid bilayers divide the cell into organelles and subdivisions. Membrane-binding proteins play important roles in dynamic membrane architectures, many of which are still poorly understood. Clathrin is one such protein involved in endocytosis[1]. The clathrin triskelion has three curved arms that can bind to neighbouring triskelia to form a polyhedral lattice that coats a newly-formed vesicle. We propose to design a similarly-shaped dynamic DNA structure that can actively bend a lipid membrane upon stimulation. The DNA nanostructure is anchored to the bilayer through cholesterol functionalization[2]; sticky ends at the extremities of each arm assemble the structure into arrays[3]. This is a first step toward the development of a bio-inspired system that will help us understand the mechanisms and forces involved in deforming lipid membranes. Here, we show that this new structure can be folded and inserted in the membrane and that polymerisation of the tile is controlled by the addition of the linking staples. These structures are mixed with lipid vesicles and the formation of membrane-nanostructure complexes observed by transmission electron microscopy. We also show that this new DNA structure can be decorated with gold nanoparticles, which simplifies purification and TEM imaging.

Figure: (A) schematic representation of a vesicle coated with polymerised DNA structures. (B) Schematic of one monomeric structure. (C) TEM image of monomers (D) TEM of DNA origami mixed with DOPC lipid vesicles and polymerised. Scales bares 200 nm.

Coulomb blockade oscillations and AMFE in Calcium/Sodium ion channels

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Biological ion channels are protein nanotubes providing for the highly-selective transmembrane transport of physiologically important ions [1]. Reaching an understanding of the selectivity mechanisms of ion channels is a long-standing problem in biophysics.

The conduction and selectivity of calcium/sodium ion channels have recently been described [2] in terms of ionic Coulomb blockade (CB) [3], a phenomenon based on charge discreteness, an electrostatic exclusion principle, and stochastic ion motion through the channel [4].

Here we demonstrate analytically that strong concentration-dependent ionic CB appearing for Ca2+ ions shows itself in divalent blockade and the anomalous mole fraction effect (AMFE), we perform mutation studies of the NaChBac bacterial sodium channel to confirm these predictions.

The generic electrostatic model describes the selectivity filter (SF) of a Ca2+/Na+ ion channel as an axisymmetric, water-filled pore of radius $R = 0.3$nm and length $L = 1.6$ nm through the cellular membrane. A centrally-placed, uniform, rigid ring of negative charge $|Q_f| = 0 - 8e$, embedded in the wall, represents charged residues lining the SF. In what follows $e$ is the proton charge, $z$ the ionic valence, $T$ the temperature, and $k_B$ Boltzmann’s constant.

We approximate the Gibbs free energy $G_n$ as the dielectric self-energy $U_n$ of the excess charge $Q_n = zne + Q_f$ with concentration-dependent shift $\sim TS_n$ using corresponded [Ca] calcium concentrations, SF volume $V_{SF}$ and self-capacitance $C_{SF}$ terms, respectively:

$$G_n = U_n - TS_n = \frac{Q_n^2}{2C_{SF}} - k_B T \ln([Ca]V_{SF})$$

CB as a phenomenon appears in low-capacitance systems from the quantization of (1) at a grid of discrete states, providing a Coulomb energy gap $\Delta G_n$ that is large enough $(\Delta G_n >> k_B T)$ to block thermally-activated transitions between neighbouring $[n]$ states.

The CB model predicts a periodic pattern of stop/conduction bands similar to the electronic CB in quantum dots[5]. We connect selectivity and AMFE with valence-, concentration-, and chargedependent shifts of conduction bands. Namely, the first captured Ca2+ ion provides blockade of channel, while the second provides knock-on fast Ca2+ conduction and AMFE.

The CB model provides a good account of both the experimental (AMFE and valence selectivity) and the simulated (discrete multi-ion conduction and occupancy bands) phenomena observed in Ca2+ and bacterial sodium channels (NaChBac, wild type and mutants), including concentration-related shifts of switching lines and conduction bands. The results should also be applicable to biomimetic nanopores with charged walls.

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P:33 Study of the influence of shear stress on the intermediate filament elasticity of alveolar epithelial cells

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Physical forces are increasingly recognized as an important biological signal. Cells have been shown to be able to respond to forces by, among others, reorganizing their cytoskeleton, thereby changing cellular mechanics and affecting cellular behaviour. The lungs are a good example of tissue that is constantly subject to physical stimuli. Both by stretch due to expansion and contraction during breathing as well as shear stress due to airflow. Changes in this stimuli can cause cell damage f.e. by overstretching by mechanical ventilation or increased shear stress in fluid-filled and atelectatic lungs.

Here we studied the cell lines R3/1 and A549 as model systems for alveolar type I and II cells respectively. Type I cells are very thin and enable gas-exchange between blood and alveoli. Type II cells are thicker and secrete pulmonary surfactant. We applied shear stress to simulate the effect of atelectasis where collapsed, surfactant-depleted alveoli are reopened.

In this study we used microrheology to measure the mechanical properties of the intermediate filament (IF) network around a micron-sized bead and applied fluid flow-induced shear stress (2.5 dyn/cm², overnight).

Results show that the elasticity of both cell types is similar without shear stress. Applying shear stress increases the elasticity of the A549 IF network by ~2-fold at 1.5 Hz, while it did not significantly change the IF network elasticity of R3/1 cells. Their different response is likely due to their distinct function in the lung. Secretion of surfactant by ATII cells have been shown to be regulated by shear stress. Surfactant decreases the surface tension at the air-liquid interface decreasing the pressure needed for inhalation and preventing the collapse of alveoli during exhalation.

P:34 Measuring cell’s free energy components on the single cell level; the challenges.

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All the vital cellular processes such as protein expression, DNA replication, active transport, swimming – require energy consumption. Life stores energy mainly in the form of ATP and electrochemical gradient of the protons, also referred to as proton motive force (PMF). PMF components are pH difference between cytoplasm and the environment (\(\Delta p\text{H}\)) and membrane potential (\(\psi_m\)). In order to understand cell energy coordination, both during cell cycle as well as under different stresses, we are developing tools needed to measure these components. Since cells populations are often heterogenic in terms of the stress responses the measurements should be done on a single cell level and allow us to perform time series of desired duration.

Here we present the combination of fluorescence and back focal plane interferometry techniques for monitoring two components of \(E.\text{coli}\) energetics – PMF and \(\Delta p\text{H}\) – simultaneously and at a single cell level. We consider the challenges that need to be addressed in order to produce reliable results. We also discuss the possibility of expanding our tools to include sensors of the remaining components of free energy.
P:35 Synthesis and characterization of bacterial cellulose-nanostructured materials hybrid heterostructures

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The nanostructured materials, such as graphene, the other 2D materials and colloidal nanocrystals, represent an unique class of materials with intriguing electronic and optical properties. The integration of these novel materials with bio-materials has attracting, in the last years, increasing attention since it provides a novel, sustainable and cheap route to design novel devices. Among the others bio-materials, the bacterial cellulose (BC) provides an interesting platform for this integration. The BC is derived from Komagataeibacter xylinus 5P3 bacteria, isolated and characterized in our lab; The derived cellulose is more pure and sustainable than the wood cellulose and shows exceptional mechanical characteristics. The structure of the BC is made of one-dimensional (1D) fibers with a diameter in nanoscale and a length in microscale and can be used as biomaterial for medical applications, electrical instruments and food industry. However, BC alone has limited capabilities to fulfill current demand on high-performance bio-materials; the functionalization of the BC could enhance their properties through addition of foreign materials. In this study, we present the synthesis and characterization of BC-graphene and BC-dichalcogenides and BC-nanocrystals heterostructures. We show that the integration of the these materials in the bacterial cellulose matrix can be achieved by different synthesis processes and we will discuss how the BC properties are affected by the presence of foreign materials.

P:36 Motor-mediated pattern formation in microtubules

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Microtubules are long and stiff rod-like biopolymers that are found in all eukaryotic cells. Their roles range from keeping cell’s shape and organising directed intercellular transport, to separating sister chromatids during cell division. Spatial distribution of microtubules inside cells is controlled by molecular motors — small molecules that utilise ATP hydrolysis to produce directed motion along microtubular filaments. It is currently believed that microtubule-molecular motor mixtures are the key ingredient of many large-scale patterns inside cells, including mitotic spindle.

This hypothesis is supported by recent experiments in simplified quasi-2D in vitro systems where interactions between stabilised microtubules and molecular motors were demonstrated to lead to spontaneous ordering of an initially homogeneous and isotropic state. The resulting patterns resemble microtubular distribution observed in living cells thus offering a minimal model system to understand in vivo cytoskeleton dynamics.

In this work we develop a theoretical model to describe pattern formation in microtubule-molecular motor mixtures. We extend previous treatments of similar systems and introduce two types of molecular motors that perform directed motion along polar microtubular filaments in either of the two directions. We observe that motors simultaneously associated with two microtubules can reorient them with respect to each other. This information is encoded in a kinetic, Boltzmann-type equation that treats such motor-mediated interaction between microtubules as ‘collisions’. We derive new mean-field evolution equations for the density of microtubules, their orientation, and a nematic Q-tensor, and perform the stability analysis and simulate their evolution numerically. We argue that the two types of motors are essential for spatial separation of the emergent patterns, and discuss a possible mechanism of mitotic spindle formation.
Motility assays and contractile rings through dynamical networking formalism
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Molecular machines lead to a broad range of interesting and extensively studied non-equilibrium phenomena. It is clear that on filaments with polarity motors are not only responsible for the generation of force and motion, but also that they are either temporarily or permanently tethered to other structures, e.g. to transport loads, to other filaments, or to a substrate. We explore the roles of these two effects in dense systems: reversible cross-linking, which tends to cause aggregation, and the active forces that locally tend to separate linked filaments. A collective treatment of the contractile ring leads to an understanding of the distinct roles of the contributions of activity and networking to the ring tension, which must be linked to the phase behavior due to the two types of forces. We also extend an equilibrium networking formalism to non-equilibrium that also permits active cross-linking and is applicable in dense systems. We show how the collective dynamical behavior of contractile rings and of some flexible filament motility assays can be derived from a semi-microscopic perspective.

Growth and gene expression dynamics in Escherichia coli under non-equilibrium environmental conditions
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The survival of a species is largely determined by the ability of its members to respond to dynamic environmental conditions. Bacteria have developed a vast biochemical arsenal to cope with external stressors, from heat to cold to osmotic shock or simply nutritionally altered growth conditions. While these responses are generally well characterized in terms of transcription factor and gene involvement, much is still unknown with respect to the biophysical state of the chromosome. There is a growing body of evidence highlighting the importance of chromosomal dynamics and physical properties such as DNA supercoiling and confinement in the spatiotemporal coordination of cell physiology, especially during such global response programs. We have developed a high throughput microfluidics system to probe single cell E. coli growth physiology under a precisely controlled nutrient upshift, in particular the difference in the activity between a constitutive promoter and supercoiling sensitive ribosomally associated promoters at different points along the chromosome. The configuration of the device enables long term tracking of cells at a high spatiotemporal resolution for observation at both cell cycle and generational timescales. Directly following the nutrient upshift, cell elongation rates increase and division times decrease with significant lag between the two response processes, resulting in an initial overshoot in cell size. Constitutively expressed proteins experience a net dilution after the upshift, with the increased rate of expression unable to keep pace with the cell’s increased elongation rate. In contrast, supercoiling-sensitive proteins increase in concentration from the moment the nutrient shift is induced and maintain elevated concentrations despite non-equilibrium cell size. Our results shed light on the precise timing of growth regulatory processes and implicate supercoiling and local chromosomal properties as fundamental biophysical mechanisms facilitating physiological adaptation.
P:39 PC12 differentiation on aligned electrospun polymeric fibres

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Near-field electrospinning (NFES) is a technique where aligned polymeric fibres could be patterned onto substrates such as Polydimethylsiloxane (PDMS), which is transparent and flexible. As cells could be grown on top of the fibres, this 2D system could serve as a platform to visualize and study the interaction between the cells and the fibres progressively under conventional microscopy, without sophisticated imaging techniques required in the 3D fibrous system. Conceptually, polymeric fibres could mimic the glial cells' aligned projections which act as a track for neural regeneration. In severe Parkinson’s disease, these glial cells fail to grow, therefore, these polymeric fibrous scaffold seeded with PC12, dopaminergic neural cell line that differentiates into neurons, could be transplanted to compensate the neuronal loss, and hence should be studied further. With our system, the physical effects of the fibre sizes, spacing, and stiffness, on PC12 differentiation could be progressively studied. The parameters of PC12 differentiation include neurite length, number of neurites, and MAP2 neuronal marker expression.

The results showed that 1) Aligned PEO fibres were successfully NFES with the spacing of 50, 100, and 150 µm, respectively. The spacing determines the average number of neurites of each cell grown on top of the fibres, which increase in closer spacing as the cells were able to connect to adjacent fibres. However, the fibres with closer spacing promoted greater proliferation, of which too high extent could block the neurite extension. The average intensity of MAP2 increases as the average number of neurite increases, and therefore, cells grew for 8 days on NFES fibres with the spacing of 100 µm, being optimized between the 2 effects, showed the highest average MAP2 intensity. 2) Aligned PEO fibres were NFES with the stiffness ranging from 3-125 kPa measured by AFM and rheometry. As the fibres restricted only single neurite to project on top of it, the effect of stiffness on single neurite had therefore been studied for the first time. The average neurite length was shown to be greater with larger stiffness, however, the difference was decreasing as the cells were growing up until 8 days. 3) Aligned PEO fibres were NFES with different ranges of sizes; small (0.5-2 µm, smaller than the diameter of conventional PC12 neurites), medium (2-7 µm, in the range equal to the diameter of the neurites), large (7-25 µm, larger than the diameter of the neurite). The average number of neurites was greatest in cells grown on large fibres, and smallest in cells grown on small fibres in the 2-4 days, whereas, at 8 days, average neurite length were indistinguishable for all 3 groups, as the higher proliferation blocked the neurite extension in cells grown on large fibres. The neurite projections were found to be squeezed in small fibres, which results in greater average neurite length than the medium and large fibres respectively. Finally, the average MAP2 intensity between cells grew on small and medium fibres were comparable and much higher than the cells grew on large fibres.
Implementation of sense on gpu and multicore cpu using pre-scan and eigen-value sensitivity profiles
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GPUs using CUDA have great potential to reduce the long data acquisition time in MRI by using the inherent parallelism present in its reconstruction algorithms¹,². SENSE³ by Pruessmann is used in this work to perform reconstruction on multicore CPU and GPU using coil sensitivity profiles estimated from two different methods 1) Pre-scan method and 2) Eigen-value method. The results are based on performance comparison of multicore CPU and GPU using both sensitivities. The results elaborate that Eigen Value approach to estimate the coil sensitivities provides good image reconstruction results on CPU and GPU along with the highest performance of GPU in terms of Artifact power (AP)⁴ and time.

P:41 Observing molecular changes in cells using Raman micro-spectroscopy

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A plethora of optical techniques exist for studying single cells, from the huge arsenal of fluorescence spectroscopies to the many variants of phase-contrast microscopy. The availability of these techniques has been crucial for advancing our understanding of the behaviour of single cell systems across the life sciences. However, no single technique is perfect, and many approaches either require large fluorescent labels which can be invasive, lack molecular specificity, or require long measurement times. Raman micro-spectroscopy (RMS) is a mature technique for the non-invasive, label-free measurement of biological samples. This is based on spontaneous Raman scattering, in which the inelastic scattered light from molecular vibrational modes is measured from a sample under laser excitation. RMS is highly compatible with biological samples, as it can be operated in the NIR wavelength regime where optical effects due to water, and sample degradation are minimized. RMS produces spectra rich in biomolecule information, which when combined with confocal laser-scanning can produce diffraction-limited hyperspectral maps. This provides an alternative modality for studying cells, which can be used to answer important biological questions beyond the reach of fluorescence and phase microscopies. We have developed a home-built RMS microscope for studying various cell systems, equipped with an incubator to allow measurement under physiological conditions. Applications have ranged from measuring the synapse of interacting immune cells [1], differentiation of stem cells [2,3], to host-pathogen interactions of various parasites [4,5]. We have also developed novel methods for greatly increasing the speed of RMS acquisition (one of the limitations of RMS) [4], as well as incorporating other modalities such as atomic force microscopy to investigate the concentration of cellular components [6], and isotopic labelling to measure biomolecule exchange [5].

Figure 1. Example of a time-course RMS measurement of the infection of human retinal pigment epithelial cells (ARPE-19) cells with Toxoplasma gondii parasites. Stable-isotope labelled phenylalanine (960cm⁻¹, normal Phenylalanine is 1004cm⁻¹) measured amino acid transport from pathogen to host, as seen in both the mean Raman spectra and Raman hyperspectral maps. Modified from reference [5].

P:42 Hofmeister effect on coenzyme FAD: a time-resolved fluorescence study
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The fluorescence kinetics of a chromophore strongly depends on the structural and dynamic properties of the hosting molecule and its close vicinity. According to the literature [1], the flavin adenine dinucleotide (FAD) coenzyme can take an open and (several) closed conformations, resulting in long or significantly lessened fluorescent lifetimes, as supported also by our previous comparative studies [2, 3]. A novel approach developed in our group for the analysis of the fluorescence lifetime components [2] made possible to characterize the corresponding conformation states by these parameters. The relation between the conformational states and fluorescence lifetimes in the case of FAD enables the study of the Hofmeister effect on this molecule. A group of anions – the chaotrops (eg. ClO₄⁻, SCN⁻) – facilitates the solvation (open conformations) of proteins and other macromolecules, the kosmotropes (SO₄⁻, F⁻) promote their aggregation (closed conformations) [4]. Here we show that the effect also takes place on FAD by monitoring the populations of conformations via fluorescence kinetics data taken in the 100 fs – 10 ns range (Fig. 1). The advanced analysis identified a planar conformation with $\tau_1 = 2.5$ ns and three closed ones corresponding to $\tau_2 = 80$ ps, $\tau_3 = 10$ ps and $\tau_4 = 2$ ps (Fig. 2). The presence of kosmotropic and chaotropic anions did not change these lifetimes, but controlled their relative amplitudes, exactly according to the Hofmeister series. The advantage of FAD is that being a small molecule, its conformational states can be easily modelled by QM/MM methods. Performing such calculations we have found that their results correlate very well with that obtained from fluorescence lifetime measurements.


Biology has been an inspiration throughout history for elegant engineering designs, from the hip-bone inspired Eiffel Tower, to the burr shape of Velcro. Recently, there is increased interest in engineering materials that mimic the dynamic functions of molecular machinery, signalling, and cytoskeletal dynamics.

Symmetry breaking in living systems is often achieved by reaction-diffusion coupling, but recently nonlinearities in material properties have been shown key to achieving morphogenesis. Artificial materials exhibiting symmetry breaking, such as dynamic shape-change behaviour, can be parsimonious, compared to biological systems, based on few components and mechanisms. This elegance allows us to study in greater fundamental detail their mechanisms and potential to control such behaviour. We explore ways to break symmetry from the macroscopic to the molecular level. We have discovered fundamentally novel mechanisms to direct growth, shape change, and multi-functionality, and have a vision to develop them in the fields of artificial muscles, adaptive structures, and for bringing insights in the processes of morphogenesis. An additional benefit of these materials is their bottom-up synthesis, which results in sustainable material-efficient, and scalable processes.

We show examples of engineering the symmetry breaking and dynamics for multiple structures and processes, on multiple length scales – from nanometers to centimeters – by non-linear mechanical stresses in materials. We demonstrate the formation of Janus and other asymmetric particles, which form as a result of coupling of chemical reactions to non-linear mechanical properties of materials[1,2]. We also demonstrate the opposite effects – how mechanical deformations and molecular interactions can help one simplify chemical syntheses[3]. Further, we also demonstrate that even without reactions, the material properties and geometry alone could cause symmetry breaking. Equilibrium deformations in a conical shell also show symmetry breaking, the energetics of which was explained by a finite element model. Upon inversion a circular fold can form 2-, 3-, 4- and 5- sided polygonal shapes.

We combine geometrical approaches with chemistry to achieve multifunctionality.[4] Instead of designing all the desired functions in a single molecule with unpredictable chemistry approach, we use controlled internal phase separation in a material to introduce existing materials with already optimized functions, and interweave them into one. This route to combining existing syntheses results in over 8000 3-function combinatorial possibilities from just 20 different functions. We have demonstrated the incorporation of ionic actuation, shape memory, and electrical conductivity, as well as emerging effects, to create the first bottom up programmable movement material.

Finally, we describe a simple 3-component molecular system we have discovered, which gives geometric shapes to liquid droplets through processes only internal to the droplets. This scalable, bottom-up process generates a number of regular geometric shapes, including octahedra, hexagons, rhomboids, triangles and fibers. We explain how the formation of a surface-templated semi-solid layer of ordered molecules is responsible for the transitions between these shapes and we identify methods to control them. This artificial morphogenesis is a molecularly based method where geometric frustration in a single process is resolved by multiple symmetry breaking on various scales.[5] It has potential insights into mechanical signalling, cytoskeleton dynamics, and morphogenesis development in the evolutionarily simplest organisms. [6] Under special conditions, these particles can mimic the
autonomous movement of flagellated bacteria with potential to reverse (recharge) the jet-like extruding of filaments, yet obtain time-irreversible results. [7]

I will outline implications for further fundamental discoveries and for potential applied explorations we are pursuing in symmetry breaking, manufacturing and nanoscience.


Statistics of discrete motor-driven events in active actin-myosin networks

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Active materials inspired from biological systems are a paradigmatic model to study non-equilibrium statistical mechanics. A common method to access their non-equilibrium statistics is to measure the fluctuation distribution of a tracer particle embedded in the material. Usually, discrete motor-induced events cannot be characterized in this way due to the multitude of active processes taking place simultaneously during measurement.

By decoupling the structural evolution of the system from its fluctuations, we are able to measure the statistics of such discrete events showing the appearance of distinct shoulders in the van Hove correlation function at high motor concentration. We do so by tailoring an experimental model active system based on cytoskeleton proteins to maintain approximately steady-state dynamics over several hours. The shoulders' appearance coincides with a transition from steady-state dynamics to slowly evolving dynamics as the motor concentration increases. The estimated force, extracted from the shoulders appearance is consistence with discrete single motor events. We further demonstrate how these discrete active events accumulate at longer lag times to a broadened Gaussian distribution, and compare it to simple minimal simulations that capture the main features of our experiments.

Van Hove correlation function of probe particles for actin networks with increasing myosin concentrations at two different motor sizes; (a) average of myosin molecules in a motor, \( N_{\text{myo}} = 19 \) and (b) \( N_{\text{myo}} = 32 \). Colors and symbols correspond to different \( [\text{Myosin}]/[\text{Actin}] \) ratios: 0 (blue circles), 0.0017 (red squares), 0.0025 (green triangles), 0.005 (orange diamonds), 0.0083 (violet right triangles), 0.01 (maroon down triangles), 0.12 (magenta stars) and 0.02 (black pluses).

(c) Van Hove correlation functions showing shoulders at large displacement. Different colors correspond to different networks; \( [\text{Myosin}]/[\text{Actin}] = 0.02, N_{\text{myo}} = 19 \) (blue), \( [\text{Myosin}]/[\text{Actin}] = 0.005, N_{\text{myo}} = 32 \) (green), \( [\text{Myosin}]/[\text{Actin}] = 0.01, N_{\text{myo}} = 32 \) (red). Arrows point to displacements of higher probability, i.e., appearance of shoulders. The brown oval highlights that at the same \( N_{\text{myo}} \) some of the shoulders appear at the same displacement. Inset: Using the values of the shoulders displacement, the force applied by the motors at a single active event is estimated, which is close to the force applied by one myosin molecule (~1 pN).
Relating repeat protein dynamics and mechanics to their function

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The HEAT-repeat protein PR65 comprises the scaffolding subunit of the heterotrimeric protein phosphatase 2A (PP2A). It belongs to a class of tandem repeat proteins consisting of simple repeating structural motifs whose relative orientation determines their overall shape. They have been shown to possess high tertiary flexibility where the structure remains intact even under mechanical stress. At higher forces most repeat proteins unfold one repeat at a time and are able to refold with minimal loss of energy. Mechanical disruption may not only affect protein stability, but also a protein’s affinity for binding partners or its catalytic efficiency. Using interdisciplinary techniques, we are interested in investigating whether solenoidal repeat proteins (such as PR65) are just a simple molecular recognition platform or whether their mechanical properties are functionally relevant.

Both MD simulations and coarse-grained Elastic Network Models indicate that the shape of PR65 drives dynamics that could be essential for PP2A function. We are taking different protein engineering strategies to alter the mechanics of PR65: a) by using staples to constrain its dynamics; and b) increasing PR65 by one to four HEAT repeats to alter the frequency of its elastic fluctuations. Currently, we are setting up force spectroscopy experiments to mechanically interrogate PR65, including the full PP2A heterotrimer, using optical tweezers. These will be complemented by biophysical analysis and biochemical assays of phosphatase activity. We hope that by elucidating the importance of PR65 mechanics to PP2A function in vitro, we will be able to contribute to the basic understanding of its biological role in the cell and pathological mechanisms in diseases such as Alzheimer’s and cancer.
Understanding how bacteria respond to antibiotics is important in light of a global antibiotic crisis. Although it is common for bacterial infections to occur in biological niches of small volume, such as eukaryotic cell interiors and biofilms, much of the research into antibiotic action is conducted in large (macroscopic) volumes such as the test tube or multiwall plate.

We aim to investigate how bacterial communities respond to antibiotic changes when confined in a small volume. In particular, we study how confining bacteria within a microenvironment of similar volume to a human cell affects their growth inhibition by ribosome targeting antibiotics such as kanamycin and streptomycin.

We achieve this by encapsulating small bacterial communities within microfluidic droplets which are around 50µm in diameter. Using microfluidic techniques, we can create thousands of droplets each containing an isolated bacterial community. This methodology allows us to study the stochastic nature of bacterial growth and inhibition by antibiotic.

Based on simple computer simulations we hypothesise that when bacteria are loaded into small droplets, at a fixed antibiotic concentration, variation in the number of bacteria loaded into each droplet will result in a greater percentage of bacteria surviving, when compared to an equivalent experiment being conducted in the macroscopic bulk.

We have designed and fabricated a microfluidic device that produces a large array of monodisperse droplets, within which we can image bacterial growth using a microscope. In the near future we plan to measure the effects of exposing these confined communities to antibiotic.

This research could further our understanding of antibiotic resistance when considering biologically relevant systems as opposed to traditional growth media.
P:47 Abuse prevention by controlled release of Opioids from micro to nano-structured silica Xerogel delivery systems

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Background: Analgesics are a multi-billion dollar pharmaceutical industry in the developed world. Studies have proven OxyContin1 to be effective in treating pains associated with arthritis, lower back conditions, injuries, and cancer2. Statistics indicate that sale of OxyContin in the USA topped $1.2 billion in 20032; opioids prescriptions sky-rocketed up to nearly 210 Million by 20133, from around 75 million in 1991, with USA the biggest global consumer accounting for up to 80% and 100% in consumption of oxycodone and hydrocodone, respectively4.

Controlled-release Oxycodone tablets became available in 12 hr time release doses in 19962 and also became a significant source of drug abuse tripling in 20 years5. Deathrelated opioid abuse increased during the 21st century and opioid poisoning was a common cause of death compared to cocaine and heroin6. Measures to curb its illicit use have been unsuccessful so far. Interest in developing abuse-proof alternative controlled-release methods was generated and remains a key issue in resolving the drug abuse problem. Objectives: This study proposes that release concentrations, of opioid, not exceeding therapeutic target levels can be achieved by incorporating the dosage in a nano-structurally optimized delivery material.

The Bacterial Flagellar Motor is a self-assembled, ion-driven rotary motor, capable of rotating at >1000Hz, switching direction within ms, and continually rebuilding itself to tune performance and sensitivity.

Motor self-assembly starts with a membrane-embedded ring of protein FliF, which templates the sequential assembly of rings of FliG, FliM and FliN. FliG/FliM/FliN together make up the cytoplasmic C-ring: the site of torque generation and switching in the motor. The switching of the motor involves a co-operative conformational change of the entire C-ring, regulated by binding of a signalling molecule CheY-P. A subpopulation of FliM/FliN continuously leave and join the working motor. This turnover is believed to play some role in switching regulation. Cryo-EM analysis of purified motors shows a rotational symmetry mismatch between the FliF ring (~26-fold) and C-ring (~34-fold), which remains unexplained.

We recently proposed a domain-swap polymerization model to explain the assembly of FliG on the FliF template, and potentially the symmetry mismatch [1]. Here we describe attempts to template FliG oligomers in vitro by substituting the FliF ring with a variety of rationally-designed DNA templates. Assembly of FliG on the templates is quantified by electrophoresis and single-molecule fluorescence. By relating FliG assembly to template design, we aim to test the FliG domain-swap polymerization model. This is the first step to templating a fully-functional C-ring in vitro, both as a platform for in-vitro study and a tool to study self assembly from the bottom-up. This represents a new approach to the study of large protein complexes.

Structurally well-defined PAMAM dendrimers have been explored as potential drug and gene delivery systems. Cationic dendrimers efficiently internalise genes and drugs into cells; and have shown antibacterial and antiviral activity (in vitro, in vivo and clinical studies). However, PAMAM dendrimers’ therapeutic application is still limited by their toxicity and despite their similar structure the cationic PAMAMs (e.g. G5) are generally reported to be more toxic than anionic PAMAMs (e.g. G4.5). In order to aid the development of clinically safe PAMAM-based therapeutics, this study is aiming for a better understanding of the PAMAM interactions with both eukaryotic and prokaryotic membranes.

Phospholipid monolayers were used as simple, but well-established models for studying the impact of lipid and solution properties on PAMAM binding at the air/water interface. Neutron reflectometry on supported bilayers was used to study PAMAM adsorption on the solid/liquid interface and explore structural effects of the dendrimer binding. The zwitterionic 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and anionic 1,2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DPPG) were chosen as model lipids (predominant in eukaryotes and prokaryotes respectively) to explore the contribution of individual factors such as dendrimer charge or concentration.

Langmuir surface pressure (SP) measurements revealed a concentration-dependent penetration of both, PAMAM G4.5 and PAMAM G5, into the monolayers (see Figure 1). PAMAM G5 showed a binding preference to DPPG indicated by a considerably higher surface pressure change recorded than for binding to DPPC (at 0.06 mg mL⁻¹ max. SP change of 4.2 ± 0.4 mN m⁻¹ vs. 0.9 ± 0.4 mN m⁻¹). In comparison, PAMAM G4.5 displayed similar levels of penetration into both, DPPC and DPPG, monolayers (at 0.06 mg mL⁻¹ max. SP change of 5.6 ± 0.5 mN m⁻¹ and 6.1 ± 1.0 mN m⁻¹, respectively).

Neutron reflectometry studies on lipid bilayers supported the results observed with the monolayer experiments. Furthermore, the fitted model data provided useful structural information of the lipid bilayers and the location of the PAMAM dendrimers in relation to the membrane. The observations for the PAMAM G5 interactions can be explained with strong electrostatic interactions between the cationic PAMAM surface groups and the anionic head group of DPPG, whereas interactions with the lipid tail and changes in lipid packing might be a possible explanation for the penetration behaviour observed for PAMAM G4.5.

Neural plasticity due to repetitive transcranial magnetic stimulation evaluated with neural field theory

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In repetitive transcranial magnetic stimulation (rTMS) neurons in the cortex are stimulated by applying rapid pulses of magnetic field externally; this magnetic field induces an electric field within the outer layers of the brain which in turn triggers cells to fire action potentials along axons. This induced firing can, in some circumstances, have long-term effects such as changing the cell’s excitability, changing the strength of connections between cells (termed ‘plasticity’) or influencing expression of genes. rTMS has been widely used for treatment of stroke, tinnitus and depression, and trialled for other conditions such as Parkinson’s disease. However, results of rTMS are highly variable, and many of its underlying mechanisms are poorly understood. The key experimental measure, the motor evoked potential (the voltage recorded at a muscle due to an applied pulse at the motor cortex) is an indirect measure of the effect. Numerical modelling, alongside experiments, therefore can provide a valuable contribution to uncovering the key mechanisms of rTMS.

Neural field theory is a mean-field approach to the problem. It models the firing rates of neurons and flux rates of neural pulses along axons linking neurons. It allows for population averaged quantities such as mean firing rate of cells to be modelled with time, under various stimuli. It is particularly relevant for considering stimuli such as those of rTMS, which trigger many thousands of neurons, rather than individual neurons. Plasticity effects can be modelled through a volume-averaged calcium-dependent plasticity mechanism. Here, the strengths of synapses (connections) between cells are strongly determined by the concentration of calcium ions in the vicinity; these in turn are modulated by the activity of the pre- and post-synaptic cells.

We have used a neural field model of excitatory and inhibitory neuron populations to evaluate the plasticity due to typical rTMS pulse sequences. In theta-burst stimulation (TBS), pulses are provided as a series of bursts, repeating at around 5 Hz. A further common modification is to make this bursting sequence intermittent – e.g. to provide it for 2 s, then have a silent period of 8 s, before repeating. The model allows us to try out a wide range of possible sequences and make predictions for the results of changing the standard protocols in various ways. We find that the first burst of pulses in a sequence has a particularly strong effect on plasticity, causing significant strengthening of connections, whereas subsequent bursts tend to weaken connections slightly. This suggests that pauses of order a second in time in pulse sequences are important for causing strong growth in synaptic weights, in agreement with the general experimental observation that intermittent TBS has a strong, positive effect. We have also been able to use the model to predict the effect of changes of neural excitability. We find that changing excitability can lead to very similar motor evoked potentials as changing plasticity, making the task of identifying underlying mechanisms particularly difficult.
Untreatable infections caused by antibiotic-resistant bacteria are a recognised global threat and the second-leading cause of death worldwide. Consequently, understanding bacterial behaviour is one of the key objectives of modern medical research.

The molecular mode of action of antibiotics is usually well known but their effect on the cell at a "systems" level (on the regulatory networks, metabolism, etc.) is not quantitatively understood. Population (bulk) assays can be misleading because of intra-population heterogeneity and so to contribute to an integrated picture of bacterial response to antibiotics, it is necessary to deploy single-cell level research methods.

We addressed some of these response phenotypes in *Escherichia coli* as a function of antibiotic class and growth conditions. Specifically, we studied localisation and motility of specific fluorescently-tagged chromosomal loci, which report for the state of locus compaction. We find that sublethal doses of ciprofloxacin and vancomycin relax and compact specific chromosomal loci, respectively. Since the physical organisation of the DNA is central to its function, this finding provides a biologically important insight, potentially connected to broad regulatory effects of antibiotics.

To elucidate effect mechanisms we also studied the motility of fluorescent cytosolic particles, to distinguish the chromosomal effects from effects arising in the cytoplasm (e.g. from changes in macromolecular crowding). We report that the sublethal doses of ciprofloxacin and vancomycin again increase and decrease cytosolic particle motility, respectively, thus providing insight into the crowding effects of antibiotics.

In addition to insights into biophysical effects of antibiotics, we developed improved data treatment procedures that account for marker photo-bleaching and size effects, and are thus necessary to precisely quantify the magnitude of changes to local chromosomal dynamics.

Since the effects we observe are linked to cell volume expansion and protein production, we are now studying how antibiotics affect bacterial growth rates. Furthermore, to gain insight into the regulatory effects, we are probing directly (using a reporter library) the link between modulations in chromosomal loci compaction to changes in the expression of genes controlled by supercoiling-sensitive promoters. Finally, by sequencing bacterial transcriptomes during treatments, we aim to link our insights to the dynamic changes to global gene expression levels.
P:52 Characterising protein adsorption on a stainless steel surface

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Suggested binding model of fibrinogen on the 316L stainless steel surface based on neutron reflectometry and QCM results.

The interaction of prevalent physiological proteins with biomaterial surfaces is of paramount importance to their ultimate biocompatibility. 316L stainless steel is commonly used in surgical applications, but the nature of its surface and interaction with such proteins remains poorly understood. Here, the challenges raised in producing thin film steel samples for neutron reflectometry that are tenable models for a real steel surface are presented, with several techniques used to fully characterise the prepared steel surface, including TOF-SIMS and XPS. As it is commonly proposed in the literature that stainless steel is covered by a chromium oxide layer[1], chromium films were also prepared for comparison.

Previously-developed techniques that have proved successful in characterising layers of organic molecules at the metal/liquid interface²-⁴ to atomic-level detail have been extended to gain an improved understanding of protein layers that form at steel/water and chromium/water interfaces under physiological conditions. In particular, fibrinogen was studied as being of particular interest in establishing whether thrombosis (clotting) occurs upon introduction of the biomaterial into the body. Data from a neutron reflectometry experiment are presented, along with other supporting techniques, such as quartz crystal microbalance (QCM), to give an overall picture of the protein layers formed. To the best of our knowledge, this is the first neutron reflectometry experiment conducted on a stainless steel surface, and demonstrates great potential of the technique for further study of biointerfacial structure.


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The role of the cell cycle in stem-cell fate decisions: a single-cell analysis approach

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Cellular division, proliferation, cycling speeds and duration of cell cycle stages in stem cells has profound influence on cell state, fate decisions and differentiation¹,². We aim to study the cell cycle duration and cycling speeds of mouse embryonic stem (mES) cells at single cell resolution across cell cycle stages (G1, S and G2/M)³ using the Fluorescent FUCCI reporter system⁴. We image mES cells across two culture conditions (‘2i+LIF’: 2 inhibitors with Leukemia inhibitory factor and ‘Serum-LIF’: serum conditioned media with LIF)⁵,⁶ and hypothesize that differences in cycling speeds contribute to heterogeneity and differentiation.

We are currently carrying out systematic time lapse imaging of mES cells enabling capture of hundreds of single cells and analyze expression of oscillating fluorescent-tagged genes (Cdt1-mCherry and Geminin-mVenus)⁴. In previous mES cell studies, cell cycle dynamics during stem cell fate changes have not been directly measured in live single stem cells, and thus no clear understanding of the coordination of cell cycle progression and execution of cell fate choices has been established⁷. We hope to perform semi-automated custom image analysis for segmentation and measurement of intracellular fluorescence to distinguish duration of cell cycle stages and cycling speeds across the different culture conditions.

Investigation of bacterial capsular polysaccharides with super-resolution fluorescent imaging

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One of the keys factors involved in the virulence of urinary tract infections is the capsular polysaccharide which covers *Escherichia coli* bacteria (Uropathogenic *E. coli*, UPEC) (1). This polysaccharide layer makes UPECs more resistant to the immune cells the body uses to eradicate these bacteria. We studied the morphology of bacterial capsules and the proteins used in the capsular polysaccharide pathway with both diffraction limited (~250 nm) and super-resolution (~20 nm) fluorescent microscopy techniques.

The morphology of bacterial capsules was observed using stochastic reconstruction microscopy (STORM) (Figure 1). The bacterial polysaccharide capsule is formed from polysialic acid (a polymer of 2,8 neuraminic acid) which is normally found on healthy mammalian cells, so there are limited choices for antibodies (otherwise there would be a massive autoimmune response). However, using lectin recognition of polysialic chains, the bacterial polysaccharide capsule has been successfully labelled and imaged with high spatial resolution.

In the pathway for the creation of capsular polysaccharides, a membrane protein that functions as a transporter was investigated which showed the localization of the transporter proteins, KpsE (2, 3), in the poles of the UPEC (Figure 2). The early stage of KpsE was also observed, but it was limited by the inefficient translation of the marker protein, which was a genetically modified dsRED fluorescent protein that was expressed in a pBAD plasmid, with an arabinose induction system.


Figure 1. The capsular polysaccharide on *E. coli* measured with STORM

Figure 2. Deconvolved KpsE-dsRED image from conventional fluorescence microscopy.