Physics of Life 2023

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HARROGATE CONVENTION CENTRE, UK

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Emanuele Colombo[1,2], Matteo Mazzocca[1], Alessia Loffreda[1], Daniela Gnani[1], Paola Falletta[1,2], Samuel Zambrano[2,3], Davide Mazza[1,2]

P1.49 **Building computers with genetically engineered cells, which can compute, add and subtract numbers and solve maze problems**
Prof. Sangram Bagh[1]

P1.50 **Bio-orthogonal conjugation for "wiring" redox active proteins to any conducting surface**
Nicholas Yates[1], Alison Parkin[1], Martin Fascione[1]

P1.51 **Bioelectric control of locomotor gaits in the walking ciliate Euplotes**
Hannah Laeverenz-Schlogelhofer[1], Kirsty Wan[1]

P1.52 **Accurate dynamics from memory in chemical reactions with small copy numbers**
Moshir Harsh[1], Prof. Dr. Peter Sollich[1,2]

P1.53 **The pioneer Transcription Factor Oct4 can interpret and enhance nucleosome dynamics**
Jan Huertas[1], Caitlin MacCarthy[2], Hans Schöler[2], Vlad Cojocaru[3]

P1.54 **Sticky proteins and moving genomes: a single-molecule perspective of pluripotent cell differentiation**
Srinjan Basu[1]

P1.55 **Quantifying the fitness effects of stochastic gene expression**
Paul Piho[1]

P1.56 **Nucleosome repositioning in cell state transitions**
Dr Vlad Teif[1]

P1.57 **Modulation of DNA entanglements by a Nucleoid Associated Protein**
Yair Augusto Gutierrez Fosado[1], Jamieson Howard[2], Simon Weir[1], Agnes Noy[2], Mark C Leake[2,3], Davide Michieletto[1,4]

P1.58 **Investigating the role of SMC proteins and CTCF in gene expression by HiP-HoP simulations of degron experiments**
Cleis Battaglia[1], Michael Chiang[1], Chris A. Brackley[1], Nick Gilbert[2], Davide Marenduzzo[1]

P1.59 **Exploring the roles of myosin proteins in shaping nuclear organisation**
Chris Toseland[1]

P1.60 **Dissecting a transcription-coupled chromatin silencing mechanism through mathematical modelling**
Govind Menon[1], Eduardo Mateo-Bonmatí[2], Svenja Reeck[1,2], Rea Antoniou-Kourounioti[1], Anis Meschichi[3], Stefanie Rosa[3], Caroline Dean[2], Martin Howard[1]

P1.61 **4D chromatin domains: temporal and spatial resolution of topologically associated domains**
Alonso Pardal[1], Dr Filipe Fernandes Duarte, Dr Andrew Bowman

P1.62 **Response of bacterial regulatory networks under dynamic perturbations imaged with single cell resolution**
Aske Petersen[1], Sine Lo Svenningsen[3], Marco Cosentino Lagomarsino[2], Pietro Cicuta[1]

P1.63 **Polymer Simulations Predict Gene Structural Heterogeneity and Transcription**
Dr Michael Chiang[1], Dr Chris A. Brackley[1], Dr Catherine Naughton[2], Dr Ryu-Suke Nozawa[3], Ms Cleis Battaglia[1], Prof Davide Marenduzzo[1], Prof Nick Gilbert[2]

P1.64 **On the inference of transcriptional burst kinetics from scRNA-seq data**
Dr. Wenhao Tang[1], Dr. Andreas Jørgensen[1], Dr. Samuel Marguerat[2], Dr. Philipp Thomas[1], Dr. Vahid Shahrezaei[1]

P1.65 **Melts of loop-extruded polymers**
Filippo Conforto, Davide Michieletto

P1.66 **Biophysical Characterisation of NANOG-DNA interactions**
Amandine Hong-Minh[1], Nicholas Mullin[1], Davide Michieletto[1], Ian Chambers[1]

P1.67 **Allosteric topological modulation of toehold-mediated strand displacement**
Dr Andrew Stannard[1,2], Dr Aditya Sengar[3], Dr Alexander Clowsley[4,5], Prof Christian Soeller[4,5], Dr Thomas Ouldridge[3], Dr Lorenzo Di Michele[1,2]

P1.68 **Understanding the relationship between mechanical and morphological changes in cells subjected to vibrational stimulation**
Ms Olivia Johnson-Love[1], Dr Peter Childs[1], Prof Stuart Reid[1], Prof Matt Dalby[2]

P1.69 **Towards mimicking the physical bone marrow microenvironment of hematopoietic stem cells**
Juan Rubio Lara[1], Daniel Bode[1], Lilia Cabrera-Cosme[1], Grace Vasey[1], Andrew Hodgson[2], Kevin Chalut[2], David Kent[1]

P1.70 **Circular RNA as a platform for gene expression control in synthetic biology and therapeutics**
Ms. Lisa Doetsch[1], Dr. Wooli Bae[2], Dr. Thomas Ouldridge[1], Prof. Guy-Bart Stan[1]

P1.71 **A geometry-driven organoid model for the investigation of the role of mechanical cues in pancreatic cancer**
Sophie Kurzbach[1], Maximilian Reichert[2], Carsten Grashoff[3], Andreas R. Bausch[1]

P1.72 **Knitting with DNA**
Catherine Fan[1,2], Jonathan Bath[1,2], Andrew Turberfield[1,2]

P1.73 **Enhancing the spectral range of bacterial and plant Light-Harvesting antenna complexes using synthetic chromophores incorporated into lipid membranes**
Dr. Ashley Hancock[1], Dr. Sophie Meredith[1], Dr. David Swainsbury[2], Dr. Kenichi Morigaki[3], Prof. Neil Hunter[4], Dr. Peter Adams[1]

P1.74 **Protein-Functionalized DNA Hydrogels**
Giorgia Palombo[1], Yair A. Fosado[1], Davide Michieletto[1]

P1.75 **Novel Infrared Glucose Biosensors Optimised for in vivo Imaging**
Katherine Sanders[1], Dr Nordine Helassa[2], Dr Adam Wollman[1]

P1.76 **Frost spreading and pattern formation on microstructured surfaces**
Lukas Hauer[1,2]

P1.77 **Video analysis of ciliated epithelia**
Ricardo Fradique[1], Erika Causa[1], Clara Delahousse[1], Laetitia Pinte[2], Marta Vila-Gonzalez[2], Pietro Cicuta[1]

P1.78 **Rotary motor powered gliding motility of a filamentous cyanobacterium as a possible driver of dynamic pattern formation within a biofilm**
Jerko Rosko[1], Sarah Duxbury[1], Kelsey Cremin[1], Mary Coates[1], Marco Polin[1,2], Orkun Soyer[1]

P1.79 **Motile cilia induce velocity and diffusion within the Periciliary Layer**
Ms Erika Causa[1], Mr Morten Kals[1], Dr Luigi Feriani[1], Dr Jurij Kotar[1], Prof Pietro Cicuta[1]

P1.80 **Mathematical modelling of trichome pattern formation in Arabidopsis thaliana**
Toquinha-Oerlia Bergmann[1]

P1.81 **Initially trapped individuals use wrinkles to escape from the centre in Bacillus subtilis biofilms during spatial expansions**
Dr. Nikhil Krishnan[1], Mr. Joseph Knight[1], Dr. Diana Fusco[1]

P1.82 **Light controlled biohybrid microbots**
Nicola Pellicciotta[2,3], Ojus Satish Bagal[2], Ms. Viridiana Carmona Sosa[1,2], Giacomo Frangipane[2,3], Roberto Di Leonardo[2,3]

P1.83 **Towards robust Turing patterns in bacterial colonies**
Martina Oliver Huidobro[1], Dr. Jure Tica[1], Dr. Roozbeh H. Pazuki[1], Prof. Robert G. Endres[1], Prof. Mark Isalan[1]

P1.84 **Spontaneous Flow of Active Biofluids through Heterogeneous Environments**
Tyler Shendruk[1], Benjamin Loewe[1], Ryan Keogh[1], Timofey Kozhukhov[1]

P1.85 **Heads or tails, SNP or error, mobile or not; Bayesian inference in the detection of mobile mRNA**

Franziska Hoerbst[1], Melissa Tomkins[1], Saurabh Gupta[2], Federico Apelt[2], Julia Kehr[3], Friedrich Kragler[2], Richard J. Morris[1]

P1.86 **Emergent surface tension drives self-organised patterning in Dictyostelium group migration**
Giulia Celora[1], Hugh Ford[1], Mohit Dalwadi[1], Benjamin Walker[2], Jonathan Chubb[1], Philip Pearce[1]

P1.87 **Directing and quantifying Min protein surface waves**
Sabrina Meindlhumer[1], Dr. Fridtjof Brauns[2,3], Jernej Rudi Finžgar[2], Dr. Jacob Kerssemakers[1], Prof. Dr. Erwin Frey[2,4], Prof. Dr. Cees Dekker[1]

P1.88 **A Minimal Tissue Model: The Cell as a Physical Object**
Iain Muntz[1], Doris Houtzager[1], Rick Rodrigues de Mercado[2], Thomas Schmidt[2], Gijsje H. Koenderink[1]

P1.89 **Vertex model for the turnover of squamous epithelial tissues**
Joel Hochstetter[1,2], Professor Benjamin D Simons[1,2]

P1.90 **Multiscale measurements of mechanical stress in 3D co-cultures using a deformable micro-device**
Hiba Belkadi[1,2], Shreyansh Jain[1,2], Arthur Michaut[3], Martin Genet[4], Jérôme Gros[3], Charles Baroud[1,2]

P1.91 **Migratory role of extra embryonic tissue in during early avian embryo development**
Dr Lakshmi Balasubramaniam[1,2], Dr Fengzhu Xiong[1,2]

P1.92 **Mechanosensitivity and deformability of mechanically stimulated bone cells**
Rui Sousa[1], Dr Melanie Jimenez[1], Dr Stuart Reid[1], Dr Peter Childs[1]

P1.93 **Light- controlled actomyosin contractility induces cell shape- changes**
Mallica Pandya[1], Dr. Emma Ferber[1], Prof. Dr. Guillaume Charras[1]

P1.94 **Laser ablation informed mechanical state of an early-stage chick embryo**
KVS Chaithanya[1], Molly J. Fitches[1], Guillaume T. Charras[2], Julia M. Yeomans[3], Cornelis J. Weijer[1], Rastko Sknepnek[1]

P1.95 **How do we build brains? Investigating actomyosin contractility in hollowing epithelial tubes**
Millie Race[1], Alexander Fleet, Dr Clare Buckley

P1.96 **Emergence of division of labor in tissues through cell interactions and spatial cues**
Miri Adler[1,2], Noa Moriel[3], Aleksandrina Goeva[1], Inbal Avraham-Davidi[1], Simon Mages[1,4], Taylor S. Adams[5], Naftali Kaminski[5], Evan Z. Macosko[1,6], Aviv Regev[1,7,8], Ruslan Medzhitov[2,9], Mor Nitzan[3,10,11]

P1.97 **Data-driven modelling of tissue growth in Drosophila abdomen**
Andrea Cairoli[1,3], Dr John Davis[1,4], Dr Ana Ferreira[1], Dr John J Williamson, Dr Matthew B Smith[1,2], Dr Anna Ainslie[6], Dr Marcus Michel[5], Prof Christian Dahmann[5], Dr Nic Tapon[1], Prof Guillaume Salbreux[2]

P1.98 **Bio-inspired ultrathin broadband sound absorber metamaterials**
Prof Marc Holderied[1]

P1.99 **Cell density as a negative feedback mechanism to ensure robustness of the body axis elongation process**
Joana M. N. Vidigueira[1], Changqing Lu[1], Cristopher Chan Jin Jie[1], Alicja Maksymiuk[1], Fengzhu Xiong[1,2]

P1.100 **Active gel theory description of actomyosin pulsations in epithelial cells**
Mr Euan Mackay[1], Dr Jens Januschke[1], Dr Rastko Sknepnek[1]

P1.101 **Applying Atomic Force Microscopy in Investigation of Structural Changes in Tomato Fruits Cell Wall**
Dr Lazar Novakovic[1], Dr Richa Yeshvekar[1], Ms. Naomi Simmons[1], Dr Yoselin Benitez-Alfonso[1]

P1.102 **A cell-based model for passive and active tissue rheology**
Fikret Basar[1], Joel Jennings, Richard Adams[2], Alexandre Kabla[1]

P1.103 **Tyssue: modelling morphogenesis from molecular to tissue scales**
Sophie Theis[1], Guillaume Gay[2], Magali Suzanne[3], Timothy Saunders[1]

P1.104 **Three-dimensional soft active matter modelling of corneal epithelial cell migration in vivo**
Prof Jon Martin Collinson[1], Dr Kaja Kostanjevec[1], Dr Rastko Sknepnek[3], Dr Silke Henkes[2]

P1.105 **Shape-tension coupling produces nematic order in an epithelium vertex model**
Dr Jan Rozman[1], Dr Rastko Sknepnek[2], Professor Julia Yeomans[1]

P1.106 **Root angle is controlled by EGT1 in cereals employing a novel anti-gravitropic mechanism**
Jacob Pattem[1], Ricardo Fusi[2], Gleb Yakubov[1], Malcolm Bennett[2]

P1.107 **Nanoscale rheology via quantum photonic interference measurements of molecular rotor lifetimes**
Ashley Lyons[1], Vytautas Zickus[1,2], Raul Alvarez-Mendoza[1], Manlio Tassieri[3], Daniele Faccio[1]

P1.108 **Generating active T1 transitions through mechanochemical feedback**
Dr Rastko Sknepnek[1], Dr Ilyas Djafer-Cherif[2], Prof Cornelis J. Weijer[1], Prof Silke Henkes[3]

P1.109 **Friction when changing neighbours : adhesion-regulated junction slippage controls cell intercalation dynamics in living tissue**
Jocelyn Etienne[1], Alexander Nestor-Bergmann[2], Guy Blanchard[2], Nathan Hervieux[2], Alexander Fletcher[3], Bénédicte Sanson[2]

P1.110 **Extracellular matrix plasticity enables a pro-invasive mechanical cross-talk between cancer cells and cancer-associated fibroblasts**
Hamid Mohammadi[1], Erik Sahai[1]

P1.111 **Engineering covalently crosslinked protein hydrogels for precision medicine**
Rossana Boni[1], Professor David Hay, Professor Lynne Regan

P1.112 **Crosstalk between physical and biochemical cellular heterogeneity dictate collective cell migration during epithelial wound closure.**
Ms Sindhu Muthukrishnan[1], Medhavi Vishwakarma[2]

P1.113 **Cellular Cruise Control: Mechanical energy dissipation regulates collective migration in epithelia**
Mr Simon Martina-Perez[1]

P1.114 **An imaging and FEM study into the mechanics of biological valves – how plants regulate photosynthesis in grasses**
Dr. Clinton Durney[1], Dr. Matthew Wilson[2], Dr. Shauni McGregor[2], Ms. Jodie Armand[2], Dr. Richard Smith[1], Dr. Julie Gray[2], Dr. Richard Morris[1], Dr. Andrew Fleming[2]

P1.115 **Measuring diffusion constant and concentration of planer core polarity proteins molecules using fluorescence confocal microscopy**
Dr. Manoj Prasad[1], Hongyu Shao[1], prof. Ashley Cadby[2], prof. David Strutt[1]

P1.116 **Tracking and tracing complex DNA structures**
Libby Holmes[1], Alice Pyne[1], James Provan[2], Sean Colloms[2]

P1.117 **Untwisting the Torsional Constraints on Processive DNA Replication; Decoding Genome Stability**
Dr Jack Shepherd[1], Dr Zhaokun Zhou[2], Dr Sebastien Guilbaud[1], Prof Mark Leake[1,3], Dr. Jamieson Howard[1]

P1.118 **Single molecule mechanical manipulation of tandem repeat proteins.**
Mohsin M. Naqvi[1], Maria Zacharopoulou

P1.119 **Mechanical Biomolecule Encapsulation inside DNA Origami Boxes**
Mr Matteo Marozzi[1], Mr Elliot Chan[1], Mr Daniel Rollins[2], Dr Alice Pyne[2], Dr Agnes Noy[1], Prof Deborah O'Connell[3], Prof Mark Leake[1]

P1.120 **Live-cell super resolution imaging of actin using LifeAct-14 with a PAINT-based approach**
Haresh Bhaskar[1]

P1.121 **Label-free identification of type III CRISPR-Cas second-messengers, one molecule at a time**
David Fuentenebro[1]

P1.122 **Exploring electric field sensing for solid state nanopore based DNA and Protein sequencing applications**
Mr Muhammad Sajeer P[1], Prof Manoj Varma[1]

P1.123 **Exploration of single-molecule dielectrophoresis by means of trapping and actuation**
Janike Bolter[1], Jacco Ton[2], Dmytro Shavlovskyi[1], Daniel Wijnperle[1], Michel Orrit[2], Sergii Pud[1]

P1.124 **Generating, imaging, and characterising DNA plectonemes with combined transverse magneto-optical tweezers, fluorescence microscopy, and all-atom molecular dynamics simulation**
Dr Jack W Shepherd[1,2], Sebastien Guilbaud[1], Zhaokun Zhou[3], Jamieson AL Howard[1], Matthew Burman[1], Agnes Noy[1], Mark C Leake[1,2]

P1.125 **Visualising NDP52 shape DNA Confirmation**
Daniel Rollins[1], Mingxue Du[1], Dr Ália dos Santos[2], Faeeza Lorgat[2], Dr Christopher Toseland[2], Dr Alice Pyne[1]

P1.126 **Unpicking DNA Translocation in Nanopores with Simultaneous Single-Molecule Fluorescence and Optical Single Channel Recording**
Mark Wallace[1], Daisy Rogers-Simmonds, Steven Vanuytsel

P1.127 **Tractor beams and single molecules: How to visualize and manipulate single biomolecules in real-time**
Emma Verver[1], Artur Kaczmarczyk[1], Jack O'Sullivan[1], Aida Llauro[1], Andrea Candelli[1]

P1.128 **Tracking single molecules on fluorous coated surfaces: New toolkit to study biomolecule interactions**
Carlos J Bueno Alejo[1], Marina Santana Vega[3], Alexander Axe[5], Glenn A. Burley[5], Cyril Dominguez[2,4], Hesna Kara[2,4], Vasileios Paschalis[2,4], Sumera Tubasum[2,4], Ian C. Eperon[2,4], Alasdair W. Clark[3], Andrew J. Hudson[1,2]

P1.129 **Structural Conversion of alpha-Synuclein at the Mitochondria Induces Neuronal Toxicity**
Dr Minee Choi[2], Mr Alexandre Chappard[1], Dr Bhanu P. Singh[1], Prof Andrey Y. Abramov[2], Prof Sonia Gandhi[2], Dr Mathew Horrocks[1]

P1.130 **Single molecule experiments and theory of the bending and looping dynamics of DNA at the scale of its persistence length.**
Dr Bhavin Khatri[1,2], Dr George Pobegalov[1], Dr Maxim Molodtsov[1]

P1.131 **Single cohesin molecules generate force by two distinct mechanisms**
Dr. Maxim Molodtsov[1]

P1.132 **Rapid and Reversible Conformational Switching of Single DNA Hairpins**
Miss Sarah Graham[1], Francisca D'Rozario[1], Steven Johnson[1], Mark Leake[1,2,3], Steven Quinn[1,2]

Poster Session 2

P2. 01 **Investigating the evolution of developmental strategies using spatiotemporally patterned telencephalic organoids.**
Miss Taniya Mandal[1,2], Afnan Azizi[1,2], Zena Hadjivasiliou[1,3], Corinne Houart[1,2]

P2.02 **Novel mathematical models for fate selection in neural crest stem cells**
Karol Bacik[1], Saeed Farjami[3], Karen Camargo Sosa[2], Jonathan Dawes[1], Andrea Rocco[3,4], Vsevolod Makeev[5], Robert Kelsh[2]

P2.03 **Capillary forces functionally remodel membrane-bound organelles and condensates inside cells**
Roland L. Knorr[1]

P2.04 **Gene expression dynamics during cell fate decisions in the retina**
Cerys Manning[1]

P2.05 **A study on the spatiotemporal dynamics and fitness landscape of bacteriophages**
Hassan Alam[1], Dr. Diana Fusco[1]

P2.06 **The interplay of size and pattern during evolution**
Amy Bowen[1,2], Zena Hadjivasiliou[1,2,3]

P2.07 **Curve registration – an approach for comparing gene expression dynamics over different developmental timescales**
Ruth Kristianingsih[1], Alexander Calderwood[1], Gurpinder Singh Sidhu[1], Shannon Woodhouse[1], Hugh Woolfenden[1], Rachel Wells[1], Richard J. Morris[1]

P2.08 **Exploring the design principles of Arabidopsis in response to temperature changes**
Dr Paula Avello[1], Seth J Davis[2], James Ronald[3], Jonathan W Pitchford[2]

P2.09 **Blastoid-on-a-chip: development of a microfluidic platform for dynamic visualisation of pre-implantation embryogenesis**
Dr Georgina Glover[1], Dr Ge Guo[1], Prof Austin Smith[1]

P2.10 **YAP levels and dynamics control cell fate and proliferation**
Kirstin Meyer

P2.11 **Dynamic Sigma Factor Patterning in Bacillus Subtilis Biofilms**
Alexander Mckinnon[1], Chris Schwall[1], James Locke[1]

P2.12 **Fast fingerprint of insulin structure and stability assessment with A-TEEM (Absorbance-Transmission Excitation Emission Matrix) spectroscopy.**
Dr Giorgia Marucci[1]

P2.13 **Advances in Localization Atomic Force Microscopy**
George Heath[1], Tabitha Storer[1]

P2.14 **Role of Sam68 in phase separation and fibre formation**
Dr Cyril Dominguez[1]

P2.15 **The Cellular Electrome: The Extracellular Significance of Potassium**
Ms Oreoluwa Griffiths[1], Dr Fatima Labeed[1], Dr Rebecca Lewis[2], Professor Michael Hughes[3], Mr Federico Bertagna[2], Dr Kamalan Jeevaratnam[2]

P1.16 **The Antagonistic Effect Of Oxysterols In ClyA Pore Formation Pathway**
Mr. Samlesh Choudhury[1], Aditya Upasani[1], Diksha Parwana[1], K. G. Ayappa[1], Rahul Roy[1,2]

P2.17 **Heterogeneous endosomal dynamics within eukaryotic cells**
Nickolay Korabel[1]

P2.18 **Self-quenching behaviour of fluorescent probes incorporated within lipid membranes explored using electrophoresis and fluorescence lifetime imaging microscopy**
Dr Sophie Meredith[1], Yuka Kusunoki[2], Dr Simon Connell[1], Dr Kenichi Morigaki[2], Prof. Stephen Evans[1], Dr Peter Adams[1]

P2.19 **Quantitative Microbiology with Microscopy: Effects of Projection and Diffraction**
Mr Georgeos Hardo[1]

P2.20 **The importance of water in membrane receptor function**
Professsor Anthony Watts[1]

P2.21 **Experimental investigation of non-classical excited-states energy transfer dynamics in green fluorescent protein tandem assemblies using time-resolved fluorescence anisotropy**
Mr. Alejandro Sanchez-pedreno Jimenez[1], Dr. Youngchan Kim[1]

P2.22 **A neutron diffraction study finds that Trimethylamine-N-oxide drives urea out of a β-turn's solvation shell**
Mr Mazin Nasralla[1], Dr Harrison Laurent[1], Dr Oliver Alderman[2], Dr Tom Headen[2], Professor Lorna Dougan[1]

P2.23 **Viral RNA Conformation Analysis via Nanotechnology at Single Molecule Resolution**
Dr Chalmers Chau[1], Dr Andrew Tuplin[1], Dr Paolo Actis[1]

P2.24 **Uncovering conserved mechanisms in the assembly and activity of eukaryotic and archaeal minichromosome maintenance proteins**
Oliver Noble[1], Clement Degut[1], Michael Hodgkinson[1], James Chong[1], Michael Plevin[1,2,3]

P2.25 **Single-molecule imaging of Botulinum Neurotoxin translocation**
Mrs Joanne Carniello[1], Dr. Jason Sengel[1], Miss Daisy Roger-Simmonds[2], Prof Mark Wallace[1]

P2.26 **Probing the Redox Chemistry and Structure Function Relationship of LPMO's via Electrochemistry**
Mr Connor Miles[1], Miss Ella Reid[1], Mr Alexander Ascham[1], Dr Nicholas Yates[1], Dr Glyn Hemsworth[2], Dr Alison Parkin[1]

P2.27 **A new twist on drug design: AdhE spirosomes as cross species anti-virulence targets**
Ester Serrano[1], Andrew Roe[1], Olwyn Byron[1]

P2.28 **Ultrasensitive fluorescence detection of conformational changes in single lipid vesicles**
Lara Dresser[1], Sarah Graham[1], Dr Donato Conteduca[1], Dr Jack Shepherd[1], Dr Lisa Miller[1], Dr Charley Schaefer[1], Prof Steven Johnson[1], Prof Mark Leake[1], Dr Steven Quinn[1]

P2.29 **Interaction between the chlamydia effector protein TarP and the SH2 domain of Vav2**
Dr Tharin M. A. Blumenschein[1], Karen Chau[1], James Tolchard[2], Caragh Scull[1], Jairah R. Lubay[1]

P2.30 **A novel RNA thermosensor element regulating teichoic acid biosynthesis in obligate human pathogen Streptococcus pneumoniae (SPN)**
Mr Kuldeep Sharma, Prof Anirban Banerjee

P2.31 **Transient structural dynamics during allosteric regulation of glycogen phosphorylase**
Ms Monika Kish[1], Dr Jonathan Phillips[1]

P.32 **Tackling topology with TopoStats**
Max Gamill[1]

P2.33 **A toolkit of customised protein sensors for interrogating mechanical forces in the cell**
Dr Maria Zacharopoulou[1], Dr Marie Synakewicz, Dr Janet R Kumita, Dr Mohsin Mubarak Naqvi, Prof Laura S Itzhaki

P2.34 **A novel sliding interaction between the extracellular matrix polysaccharide hyaluronan and its lymphatic vessel endothelial receptor LYVE-1 that regulates immune cell trafficking**
Dr Fouzia Bano[1], Dr Suneale Banerji[2], Dr Tao Ni[2], Dixy Green[3], Prof Paul DeAngelis[3], Dr Martin Lepsik[5], Dr Emanuele Paci[4], Prof Robert Gilbert[2], Dr Ralf Richter[1], Prof David Jackson[2]

P2.35 **Structural dynamics of membrane-associated proteins at microsecond timescales and sub-nanometre resolution with High-Speed AFM**
Tabitha Storer[1,3], George Heath[1,3], Neil Thomson[1,2,3]

P2.36 **Deciphering the structure of integration host factor with supercoiled DNA minivectors**
Ms Neha Ramteke[1], Professor Mark Leake[2], Dr. Agnes Noy[3]

P2.37 **Single-molecule and super-resolved imaging deciphers membrane behavior of onco-immunogenic CCR5**
Mr Patrick Hunter[1], Dr Alex Payne-Dwyer[1], Dr Michael Shaw[2], Dr Nathalie Signoret[1], Prof Mark Leake[1]

P2.38 **Passive microfluidics for the characterisation of neuronal signals in live nematodes**
Dr Nino Läubli[1], Prof. Gabriele Kaminski Schierle[1]

P2.39 **Biofilm Water Channel Network Model for Bacterial Communication**
Yanahan Paramalingam[1], Dr Hamidreza Arjmandi[1], Dr Adam Noel[1]

P2.40 **Modeling the Growth of Kidney Organoids subject to optogenetically-induced BMP4 Morphogens**
Jonas Pleyer[1]

P2.41 **Decoherence and Energy Transfer Dynamics of Green Fluorescent Proteins**
Anna Cusick[1], Marian Florescu, Adam Burgess

P2.42 **DNA origami with fluorescent proteins**
Callum McKeaveney[1], Wooli Bae, Catxere Andrade Casacio, Alejandro Sanchez-Pedreno Jimenez

P2.43 **How to tune the tempo of embryonic development across species: a mathematical toolkit**
Charlotte Manser[1], Dr Ruben Perez-Carrasco[1]

P2.44 **Using Shape Fluctuations to Probe the Mechanics of Stress Granules**
Dr Jack Law[1], Dr Carl Jones[1], Mr Tom Stevenson[1], Mr Thomas Williamson[2], Prof Matthew Turner[3], Prof Halim Kusumaatmaja[2], Prof Sushma Grellscheid[1]

P2.45 **Multimodal quantum sensors for detecting nanoscale dynamics in C. elegans**
Sophia Belser[1], David Jordan[2,3], Louise Shanahan[1], Jack Hart[1], Qiushi Gu[1], Eric Miska[2,3], Mete Atatüre[1], Helena Knowles[1]

P2.46 **Modelling DNA in Complex Topologies: The Role of Gyrase**
Katy Hollands[1], Mark Leake[1,2], Anthony Maxwell[3], Agnes Noy[1]

P2.47 **Correlative light electron microscopy using small gold nanoparticles as single probes**
Professor Paola Borri[1], Dr Iestyn Pope[1], Hugh Tunner[2], Dr Francesco Masia[1], Dr Lukas Payne[1], Kenton Paul Arkill[2], Judith Mantell[2], Professor Wolfgang Langbein[1], Professor Paul Verkade[2]

P2.48**. Broadband Cavity Enhanced UV-VIS Absorption Spectroscopy for Picolitre Liquid Samples**
Ms Imogen Fermor-Worth[1], Dr Catalin Chimerel[1,2]
[1]University of Exeter, Exeter, United Kingdom, [2]Transilvania University of Brașov, Brașov, Romania

P2.49**. A view to a kill: using 3D holographic microscopy to study the motility behaviour of predatory bacteria**
Dr Emma Brock[1,2], Dr Laurence Wilson[2], Dr Christoph Baumann[2]

P2.50 **A fluorescence, microfluidic microscope built for microgravity and extreme Earth environments**
Miss Katrina Crompton[1], Miss Koren Murphy[2], Mr Thomas Wareing[2], Mr Alexander Stokes[2], Mr Jack Dawson[2], Mr Yusuf Ugurluoglu[2], Mr Connor Richardson[2], Dr Adam Wollman[1]

P2.51 **Using light to control cellular energetics in Escherichia coli**
Tommy Schmidlechner[1], Jochen Arlt[1], Luke McNally[1], Teuta Pilizota[1]

P2.52 **Selective manipulation of mitochondria function and cell viability in cancer cells through blue light and photosensitizer agent**
Emily Skates[1], Munehiro Asally[1], Orkun Soyer[1]

P2.53 **Investigating the processes of life in the cold: high resolution imaging of Antarctic fish cells**
Anne-pia Marty[1,2], Professor Clemens Kaminski[1], Professor Melody Clark[2]

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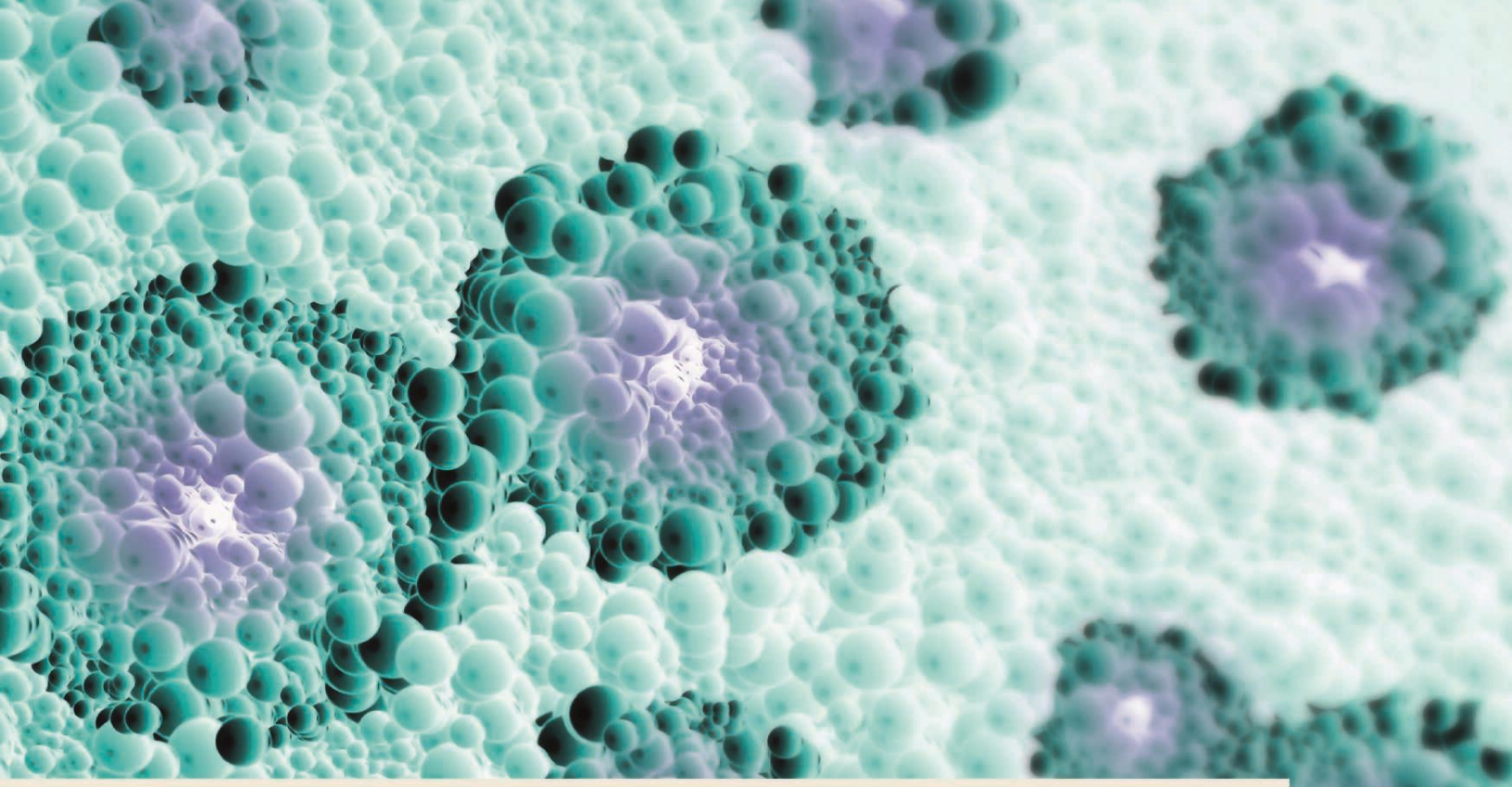

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Biomimetic actin cortices shape cell-sized lipid vesicles
Lucia Baldauf

Animal cell membranes are shaped by the actin cortex, a thin layer of cytoskeletal filaments. This cortex serves two seemingly opposing functions: on the one hand, it provides mechanical stability by stiffening the cell surface and opposing external deformations, but on the other hand it is highly dynamic, turning over constantly and exerting pushing forces that can deform the membrane. How these two factors play out to shape the cell surface remains poorly understood. Here we use bottom-up reconstitution to build dynamic actin cortices in vitro that turn over within around 60 s, comparable to the actin dynamics in living cell cortices. We show that thin branched actin cortices are stiff enough to trap the shapes of giant unilamellar vesicles (GUVs) far from equilibrium, and impart a shape memory on the vesicles that remains far beyond the actin turnover time. Using both photoablation and treatment with an actin depolymerizing drug, we demonstrate the reversibility of these deformations. We show that the same cortices can produce finger-like protrusions in GUVs without requiring actin bundling proteins. Combining theoretical modeling and experiments to explore under what conditions such protrusions form, we find that the concentration of nucleators is crucial, as locally concentrated actin polymerization forces can drive a positive feedback loop between recruitment of actin and its nucleators, and membrane deformation. Actin polymerization speed, by contrast, is found to be less important. Finally, we combine our findings to propose a working model for how actin polymerization forces shape synthetic cell membranes.

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Structural analysis of the influenza genome by high-throughput single virion DNA-PAINT
Christof Hepp

Christof Hepp studied at the University of Tuebingen, where he graduated in Biochemistry (Diplom) in 2011. Subsequently, he studied the mechanism outer membrane DNA transport in Gram negative bacteria using fluorescence microscopy an optical tweezers setup in the lab of Berenike Maier, University of Cologne, where he received his DPhil (Dr rer nat) in Biophysics in 2017. Since 2018, he is a postdoctoral research associate in the lab of Achillefs Kapanidis at the Kavli Insitute for Nanoscience Discovery, Oxford, where he studies the mechanism of transcription, replication and selective packaging of influenza virus, and FISH-based detection of RNA viruses using hybridization techniques, DNA-PAINT and single-molecule FRET.

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Mapping nanostructural changes in E.coli Peptidoglycan

Dr Abimbola Feyisara Olulana

E.coli is a rod-shaped Gram-negative bacterium whose shape is maintained by a biopolymer known as Peptidoglycan (PG). The chemical composition of PG is well understood but the following questions remain unanswered; 1) what is the detailed molecular organization of the PG; 2) In the case of antibiotic-induced shape change and death, what happens to the PG organization? To this end, we utilized high-resolution atomic force microscopy (AFM) to map the changes in the PG organization from the pole to the cylindrical section of the rod. We extend this location-dependent imaging to interrogate different areas of the PG under different antibiotic treatment times.

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Protein-RNA condensates: complementary or competing interactions in ALS progression?
Dr Mark Driver

Membraneless organelles within cells provide organization of the intracellular environment through the process of liquid liquid phase separation (LLPS). The Fused in Sarcoma (FUS) protein is an RNA binding protein that undergoes LLPS with RNA as part of normal activity in healthy cells. PolyPR and polyGR, dipeptide repeat proteins (DPRs) caused by C9orf72 repeat expansion disorders, have been shown to promote protein aggregation of RNA binding proteins, including FUS. This aggregation contributes to the progression of the neurodegenerative diseases ALS (amyotrophic lateral sclerosis) and FTD (frontotemporal dementia). The pathway from stable condensates towards protein aggregation is poorly understood, but has been linked to changes in condensate behavior induced by the addition of DPRs. Here we investigate the effect of DPR and RNA on the stability of FUS condensates through molecular dynamics simulations.

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Experimental investigation of non-classical excited-states energy transfer dynamics in green fluorescent protein tandem assemblies using time-resolved fluorescence anisotropy

Mr. Alejandro Sanchez-pedreno Jimenez

It is implicitly assumed that any quantum mechanical effects (e.g., quantum superposition, tunneling, entanglement) created in a biological system are instantaneously dissipated due to random molecular interactions in the warm, wet, and disordered biological environment. However, owing to advances in sensing and molecular biology technologies, mounting evidence indicates that biological molecules and biomolecular systems can give rise to the quantum effects in such complex biological environments. Notably, a recent study suggests that the dimeric fluorescent proteins exhibit room-temperature exciton coupling when they dimerise (Kim et al., Biophysical Journal, 2019). Here, we use enhanced green fluorescent protein (eGFP) tandem dimers and oligomers to investigate the excited-states energy transfer dynamics in eGFP oligomers. We employ time-resolved fluorescence anisotropy that is used to measure the dynamics of energy transfer between fluorescent molecules. Our results indicate the presence of anomalous ultrafast dynamics in the energy transfer between multiple fluorescent proteins upon oligomerization.

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Bioelectric control of locomotor gaits in the walking ciliate Euplotes

Hannah Laeverenz-Schlogelhofer

Control and coordination of motile appendages is essential for unicellular organisms to successfully navigate their surroundings and respond to environmental cues. The role of physiological mechanisms in this process is poorly understood. Euplotes is a unicellular organism that uses leg-like appendages called cirri (bundles of about 40 cilia) to achieve a variety of gaits including walking along surfaces and helical swimming through the water column. To reorient itself, Euplotes performs a side-stepping reaction, which involves a transient backwards motion followed by a turn. This is among the most sophisticated manoeuvres ever observed in a single-celled organism. By combining high-speed imaging with simultaneous time-resolved electrophysiological recordings, we show that this complex coordinated motion is regulated by spontaneous membrane depolarisation events that control the activity of the cirri. Using machine learning and computer vision techniques we map for the first time the observed cirri dynamics to the spontaneous bioelectric activity of the cell. Our results reveal how Euplotes, an organism without a nervous system, achieves real-time control over the activity of its multiple cirri in order to achieve a highly coordinated gait.

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A neutron diffraction study finds that Trimethylamine-N-oxide drives urea out of a β-turn's solvation shell.

Mr Mazin Nasralla[1], Dr Harrison Laurent[1], Dr Oliver Alderman[2], Dr Tom Headen[2], Professor Lorna Dougan[1]
[1]University Of Leeds, Manchester, United Kingdom, [2]ISIS neutron and muon source, Didcot, United Kingdom

TMAO (Trimethylamine-N-oxide) and urea have opposing effects on the structural stability of proteins. Urea is a denaturant, and TMAO is a protective osmolyte i.e., a small biological molecule expressed by marine life to protect proteins against denaturation by urea and the effects of high pressure. Urea is believed to exhibit a preferential interaction towards the protein's surface, vis a vis water, and once there it binds to the peptide's amide groups, inhibiting the formation of stabilising intra-peptide bonds. The mechanism by which TMAO counteracts urea is uncertain although molecular dynamics (MD) simulations suggest it breaks urea's affinity for the peptide surface. Previously, using NMR spectroscopy to study aqueous urea and TMAO, we found evidence for a urea-TMAO complex and in the presence of TMAO strengthened hydrogen bonding involving water. Motivated by previous studies of the β-turn GPG.NH_2 in aqueous urea, we present here a neutron diffraction study of GPG.NH_2 in TMAO, urea and water in the molecular ratio 1:3:6:58. We find that TMAO's interacts with the β-turn solely through its nitrogen groups. We also find that TMAO generates a differential effect on hydrogen bonding in the bulk solution where it hydrogen bonds to urea, tightens the urea--water network and weakens water-water hydrogen bonding. We suggest that by enhancing hydrogen bonding involving urea, and curtailing the extent of the water network, TMAO simultaneously drives urea out of, and water into the β-turn's solvation shell.

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Exploring the shared membrane tension responsive machinery which controls cell migration and division

Dr Joseph Hetmanski

Migration and division are key cellular functions which underpin mammalian life: in development, health and disease, cells need to be able to move, divide and seamlessly switch between the two. Little is known however about how cells control transition between migration and division, or what signalling and biophysical mechanisms are shared between the processes. We found previously that membrane tension is a key biophysical parameter that drives rear retraction in 3D environments (Hetmanski et al., Dev Cell 2019; Hetmanski et al., Plos Comp Biol 2021): in non-uniform 3D or stiffness gradient environments, cells exhibit heterogeneous membrane tension where tension in lower specifically at the contractile rear. This local lower tension is sensed by mechanosensitive membrane invaginations caveolae, which activate RhoA via the GEF Ect2 to drive forward rear movement, which leads to a local decrease in membrane tension thus perpetuating a self-generated positive-feedback mechanism. Ect-2 was traditionally described as a cell division related RhoA activator, and informed by predictive mathematical models we have found surprisingly that other 'division machinery' proteins/pathways are crucially involved in motility such as the master regulator CDK1 and associated binding partners cyclins A2, B1 and B2. Using different mathematical modelling approaches alongside cutting edge imaging techniques, in particular measuring membrane tension by fluorescent lifetime imaging (FLIM), we now propose that (i) membrane tension is crucially embedded in feedback loops which drives cell division, in particular mitotic rounding, as well as rear retraction; and (ii) the machinery which drives contractility based migration and mitotic rounding is shared and requires exquisite levels of control of repositioning throughout the transition between migration and division.

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Identification of the source of extrinsic noise from the stochastic dynamics of gene expression

Marta Biondo

Cell-to-cell variability in the protein concentration is strongly affected by extrinsic noise, especially for highly expressed genes. Extrinsic noise can be due to fluctuations of several possible cellular factors, such as cell growth and volume or the level of key enzymes in the expression process. However, how to identify the specific predominant sources of extrinsic noise in empirical systems is still an open question. This work considers a general stochastic model of gene expression with extrinsic noise represented as coloured fluctuations of model parameters and focuses on the out-of-equilibrium expression dynamics. Combining analytical calculations with extensive stochastic simulations, we characterize the role of extrinsic noise in shaping the protein variability in the dynamics of gene activation or inactivation depending on the prevailing source of extrinsic variability, on its intensity and timescale. In particular, we show that qualitatively different noise trends can be identified depending on the specific fluctuating parameters. This result indicates an experimentally accessible way to pinpoint the dominant sources of extrinsic noise using time-coarse measurements.

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Direct, nano-rheological studies of in-plane lipid dynamics in model and native membranes
Dr William Trewby

Lipids are now known to play an active part in roles related to protein function, oncogenesis and disease signalling, in contrast to their previously assumed passive character. Their assemblies in organelles such as lipid droplets are recognised as being crucial to the proper storage and transport of fats, and dysregulation can lead to severe pathologies including cardiovascular disease. The complex functions of lipids within the body are largely dependent on their in-plane motion, which governs the ability of biomembranes to restructure, as well as the transport of small molecules along and across the lipid layers.

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CellPhe: a toolkit for cell phenotyping using time-lapse imaging and pattern recognition
Laura Wiggins

With phenotypic heterogeneity in whole cell populations widely recognised, the demand for quantitative and temporal analysis approaches to characterise single cell morphology and dynamics has increased. We present CellPhe, a pattern recognition toolkit for the characterisation of cellular phenotypes within time-lapse videos. To maximise data quality for downstream analysis, our toolkit includes automated recognition and removal of erroneous cell boundaries induced by inaccurate tracking and segmentation. We provide an extensive list of features extracted from individual cell time series, with custom feature selection to identify variables that provide greatest discrimination for the analysis in question. We demonstrate the use of ensemble classification for accurate prediction of cellular phenotype and clustering algorithms for the characterisation of heterogeneous subsets, validating and proving adaptability using different cell types and experimental conditions. Furthermore, we provide an example application for CellPhe to characterise response to chemotherapy, quantifying a population's response to varying concentrations of drug and identifying a subset of "non-conforming" treated cells that resist treatment. Our methods extend to other imaging modalities, such as fluorescence, and would be suitable for all time-lapse studies including clinical applications and drug screening.

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Root angle is controlled by EGT1 in cereals employing a novel anti-gravitropic mechanism

Jacob Pattem

Root-angle represents a key trait for efficient capture of soil resources by plants. Root angle is determined by competing gravitropic versus anti-gravitropic offset (AGO) mechanisms. Despite its agronomic importance, few regulatory genes have been identified in crops. A new root-angle regulatory gene termed ENHANCED GRAVITROPISM1 (EGT1) has been recently discovered. EGT1 encodes a putative AGO component, whose loss enhances root gravitropism. To investigate biophysical changes in cell-wall properties Atomic Force Microscopy (AFM) nanoindentation approach has been utilised. We analysed 50 µm thick longitudinal cross-sections of 4-day-old barely seminal root tips of Morex (wildtype) and TM194 (mutant) using force-spectroscopy under plasmolysed but hydrated conditions. Nine independent areas within specific root tip tissues where characterised for stiffness (pN/nm), performing n > 100 indentation curves for each biological replicate (n = 4 - 5). Results revealed a significant reduction of 26% in cell-wall stiffness in elongating cells of the mutant compared to wildtype. Moreover, when sub-dividing analysed data into inner versus outer root tissues, mutant roots showed a significant reduction of 36% in root cortical tissues, while we observed no significant difference for stele tissues. Measuring such biophysical properties using AFM, revealed crucial to prove that this reduction in cell-wall stiffness in mutant roots disrupts the ability of the root outer tissues to counteract gravitropic bending, causing them to grow steeper along a gravity vector. Analogous EGT1-dependent regulation of root-angle in barley demonstrates broad significance of EGT1 for trait improvement in cereal crops.

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Investigating the role of SMC proteins and CTCF in gene expression by HiP-HoP simulations of degron experiments

Cleis Battaglia

The three-dimensional organization of chromatin within the nucleus is highly interconnected with gene expression and crucial for cell function. It has been observed that structural maintenance of chromosomes (SMC) complexes play a key role in organizing the genome. Indeed, cohesin is able to extrude loops that stop at convergent occupied binding sites for CCCTC-binding factor (CTCF). However, the effect of cohesin and other loop extrusion regulatory factors on the transcriptional regulatory network of the cell has not yet been completely understood.

In this work, we used molecular dynamics simulations to investigate the roles played by loop extrusion driven by SMC proteins and regulatory factors such as CTCF and WAPL in shaping chromatin architecture. We also studied their effects on gene expression on a chromosomal scale. To obtain the results, we employed the highly predictive heteromorphic polymer (HiP-HoP) framework, which integrates polymer physics with bioinformatic data, to predict the effect of degrading each of these proteins in turn.

Consistently with previous experimental results, we observe that the average transcriptional activity is not strongly impacted by loop extrusion by SMC proteins. Strikingly, the transcriptional noise (measuring the variability of gene expression in the cell population) is instead strongly affected by the removal of these regulatory factors. From our simulations, we are also able to relate these changes in the transcriptional pattern to the ones in 3D chromosomal and gene structure.

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Optimal Control Theory in Cancer Chronotherapy

Mr Byron Tzamarias

The circadian clock is a biochemical oscillator in every cell that is synchronized to the day night cycle and regulates processes in the human body including cell division. Cancer cells typically lose synchronisation of cell division with the diurnal rhythm, with full decoupling from the circadian clock in mature tumours. Cancer chronotherapy exploits this loss of synchronization, utilising drugs whos activity is dependent on the circadian clock and timing drug infusion in order to maximize anti-tumor efficacy while limiting toxicity to healthy cells. We use optimal control theory to optimise chronotherapy for cancer cells that have totally lost synchronization with the circadian clock, minimizing a cost function with a periodically driven running cost. We analyse drugs that increase cell death rates (cytotoxic drugs) and drugs that target cell division. By applying the Pontryagin Minimum Principle we show that in both case studies optimal solutions are bang-bang whilst aperiodic optimal drug infusion schedules are typical, with the duration of the daily drug administration interval increasing as the treatment progresses.

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The Antagonistic Effect Of Oxysterols In ClyA Pore Formation Pathway

Mr. Samlesh Choudhury

Pore-forming toxins are a class of proteins secreted by various bacteria that form nanopores on target cell membranes, causing leakage of cellular components and resulting in cell death. Previous experimental and all-atom MD simulations have emphasized the intricate and supportive role of cholesterols in increasing the pore-formation activity of Cytolysin A (ClyA). On the contrary, oxidized cholesterol derivatives such as 25-hydroxycholesterol produced naturally from cholesterol by the cell have shown antiviral effects and innate immunity against bacterial infections by altering the accessible cholesterol content in the cell. Dye leakage experiments carried out with oxysterols-treated small unilamellar vesicles demonstrate reduced pore-forming activity in a concentration-dependent manner. We observe that the replacement of about one-third of cholesterol by 25-hydroxycholesterol negates the enhancement in ClyA activity observed in the presence of cholesterol. Using all-atom MD simulations, we observe a distinct tendency for oxysterols to replace cholesterols which bind in the β-tongue pockets to stabilize the membrane-inserted oligomeric complex of ClyA. Oxysterols also induce large structural deviations in the membrane-inserted N-terminus protein domain compared to ClyA in purely cholesterol membranes. Unlike cholesterols, an uphill free energy profile is also observed in the presence of oxysterols between the initial monomer and final protomer states, indicating a non-spontaneous conversion from monomeric to protomeric states of ClyA. Our study reveals the mitigating influence of oxysterols in the prevention of pore formation by bacterial toxins and has implications for understanding bacterial infection pathways in aged and senescent cells where oxidative stress levels are elevated.

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Broadband Cavity Enhanced UV-VIS Absorption Spectroscopy for Picolitre Liquid Samples

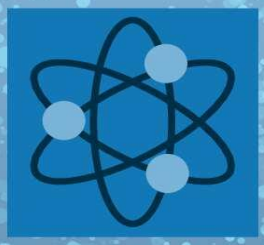
Ms Imogen Fermor-Worth

Absorption spectroscopy is a widely used analytical technique due to its label-free nature. However, its application to small liquid samples is hampered by the associated short absorption pathlengths, limiting sensitivity. A concept for the development of an ultrasensitive broadband absorption spectrometer optimised for thin liquid films is presented. We implemented an optical cavity within a fibre-based absorption spectrometer, to enhance sensitivity of the absorbance measurements. In the setup, light propagates multiple times through the sample of interest resulting in greatly increased sensitivity. The bandwidth of the instrument is determined by the choice of two dielectric mirrors forming the optical cavity and, in this implementation, has been set to be optimised for UV detection (250-450 nm). The sensing volume of the spectroscope is prescribed by the choice of optical fibres employed to deliver light to the sample, here we employed 400 µm diameter fibres, giving a sensing volume of 630 picolitres for a thin film of 5 µm in thickness. As a proof-of-concept, we have used our platform for the ultrasensitive detection of the antifungal drug Amphotericin B. Cavity enhancement factors, the equivalent pathlength increase over classical absorption spectroscopy, in the range of 200X have been achieved across a broad wavelength range. Taking advantage of the extended path length the limit of detection for Amphotericin B in a 5 µm thick aqueous film has been dropped from ~125 µg/ml to ~20 µg/ml. We envision multiple applications of our technology ranging from low concentration nucleic acid quantification to label-free cellular drug uptake.

Key note I: Biophysical models for 3D epithelia in vertebrate embryonic development

Lisa Manning (USA)

In vertebrates, the formation and maintenance of complex three-dimensional shapes in epithelia drives several important developmental processes. In addition, a collection of recent discoveries both in vivo and in vitro suggests that the collective mechanical response of a tissue (its fluidity or rheology) helps to control morphogenetic events. Therefore, there is a need to develop fully 3D biophysical models for epithelia that can predict or validate how global tissue mechanics impacts the structure and function of epithelia. In this talk, I will discuss several recent projects by our group and others to develop such models for specific model systems, including the left-right organizer in zebrafish and stratification and placode formation in mouse skin, where there are interesting interactions between cell shapes, epithelial architecture, and tissue fluidity. I will highlight how an interplay between models and experiments can help to drive a better mechanistic understanding of the processes that drive 3D tissue structure and function, especially when multiple mechanisms are operating at the same time.



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Mitochondrial dynamics as a new mechanics-to-biology transduction module
Sirio Dupont

Recent findings in our laboratory indicate that extracellular matrix stiffness regulates the morphology and dynamics of mitochondria, which triggers a transcriptional and metabolic antioxidant response. How general is such mitochondrial remodelling? How do forces and actomyosin tension regulate mitochondrial fission? What other mitochondrial functions, and what other mitochondrial-dependent signalling events, are regulated by extracellular forces? I will discuss evidence that mitochondria represent a mechanotransduction “information processing system” that coordinates complementary gene expression programs important for metabolism, proliferation and cell fate choices, and the underlying molecular mechanism.

15

Mechanosensitive polyol pathway regulates biomolecular condensates
Stéphanie Torrino

Condensed droplets of protein regulate many cellular functions. Yet, the physiologic conditions regulating their formation remain largely unexplored. Recently, we demonstrated that mechanical forces rewire cell metabolism, which in turn impacts virtually every other cellular process. Yet, whether cell mechanics and mechano-controlled metabolic pathways regulate condensate assembly remains unknown. Here, we found that matrix stiffening increases the intracellular concentration of sorbitol to promote biomolecular condensate formation. We demonstrate in vitro that a physiological range of sorbitol concentrations regulate biomolecular condensate assembly. Furthermore, we show that manipulation of either cell mechanics or the polyol pathway modulate biomolecular condensate formation in vivo. Together, these findings unveil mechanosensitive polyol metabolism as a tightly regulated and tunable pathway controlling protein condensate formation.

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Simultaneous Intracellular Nanorheology and Nanothermometry Using Diamond Quantum Sensing

Louise Shanahan[1], Dr Qiushi Gu[1], Dr Jack Hart[1], Sophia Belser[1], Dr Helena Knowles[1], Prof Mete Atature[1]
[1]*University of Cambeidge, Cambridge, United Kingdom*

Temperature plays a critical role in all chemical reactions in cells and understanding intracellular temperature variations is crucial for biochemical energetics. Yet intracellular thermometry remains challenging, often obscured by the noise due to local biochemical environments. On the other hand, rheology plays an important role in cell morphology, division and transport. It is actively regulated by cells in response to temperature. Understanding temperature, rheology and their interdependence uncovers new territories for studying metabolism, development and diagnostics.
Fluorescent nanodiamonds (FNDs) with negatively-charged nitrogen-vacancy (NV) centers, are versatile sensors that can be used to measure different modalities including magnetic field, temperature and quantum nuclear magnetic resonance. [1] Unlike dye based thermometry systems, FNDs have consistency in measurements across environmental factors such as pH, ion concentration and refractive index. NV centers have high optical stability and do not undergo photo-bleaching. We have previously shown FNDs to have both cellular uptake and low cytotoxicity making them a suitable biocompatible sensor for invitro measurements. [2]
We demonstrate a dual-modal quantum sensor capable of simultaneously sensing nanoscale temperature and rheology in a dynamic cellular environment. We study the intracellular temperature response to external thermal modulations and observe different rheological regimes inside HeLa cells, including evidence of active transport.

1. Nanoscale NMR Spectroscopy Using Nanodiamond Quantum Sensors. Physical Review Applied 13, 044004 (2020);
2. Graphitic and Oxidised High Pressure High Temperature (HPHT) Nanodiamonds Induce Differential Biological Responses in Breast Cancer Cell Lines. Nanoscale 10, 12169 (2018).

93

FRETzel: a new software package for single-cell glucose uptake measurements within heterogeneous populations

Dimitrios Kioumourtzoglou[2], Rebecca Ward[2], Gywn Gould[3], Nia Bryant[2], Adam Wollman[1]
[1]Newcastle University Biosciences Institute, Newcastle-upon-Tyne, United Kingdom, [2]University of York, York, United Kingdom, [3]University of Strathclyde, Glasgow, United Kingdom

Fluorescent biosensors are powerful tools to measure the concentration of metabolites and small molecules, as well as other properties such as pH and molecular crowding inside single living cells. The technology has been hampered by lack of simple software to identify cells and quantify the intracellular biosensor signal. We have developed a new software package, FRETzel [1], to address this and demonstrate its use by measuring insulin-stimulated glucose uptake in different sized individual fat cells for the first time. Our results support the long-standing hypothesis that larger fat cells are less sensitive to insulin than smaller ones, a finding that has important implications for the battle against type 2 diabetes. FRETzel was developed for the messy and crowded environment of cultured adipocytes but we also demonstrate its utility for quantification of FRET biosensors in a range of other cell types, including fibroblasts and yeast via a simple user-friendly quantitative interface. We also showcase our latest developments, imaging FRET-based glucose uptake alongside a glucose regulated transcription factor, Mig1, in yeast and using fluorescent glucose analog, 2-NDBG, to measure uptake rapidly, in seconds.

[1] Adam J. M. Wollman, Dimitrios Kioumourtzoglou, Rebecca Ward, Gwyn W. Gould, and Nia J. Bryant. 2022. 'Large Scale, Single-Cell FRET-Based Glucose Uptake Measurements within Heterogeneous Populations'. IScience 25 (4): 104023. https://doi.org/10.1016/j.isci.2022.104023.

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Metabolism, nonequilibrium thermodynamics and system dynamics

Orkun Soyer[1]
[1]*University of Warwick, , United Kingdom*

Metabolism is the flux of metabolites and ions through membrane-bound and intracellular biochemical reactions. This flux maintains the cellular energy and redox states – crucially encoded in the ratio of key co-substrate pairs, such as ADP/ATP and NAD(P)+/NAD(P)H - and enables other, key cellular process such as cell division, motility, and differentiation. For cells to survive, metabolism need to remain as a nonequilibrium system, and as such, it can display nonlinear system dynamics, including abrupt switching of steady state fluxes, bistability and oscillations.

In this talk, I will summarise our ongoing work focusing on understanding the mechanistic basis of bistability and oscillations in metabolic reaction systems and the impact of specific reaction motifs on system dynamics. Specifically, I will describe how binding of multi-substrate binding enzymes can act as a source of bistability and oscillations, and how co-substrate cycling within and across pathways can result in flux limitations in those pathways. I will discuss how these studies relate to known experimental findings and how they provide new avenues for experimental study and manipulation of metabolic dynamics.

The talk will emphasize the need for further involvement of approaches grounded in nonequilibrium thermodynamics and systems dynamics for the better understanding of metabolism and closely linked processes of division and differentiation.

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Modelling oscillatory dynamics in cell energy metabolism

Joe Rowland Adams[1], Professor Aneta Stefanovska[1]
[1]*Lancaster University, Lancaster, United Kingdom*

The production of the energy carrying molecule, ATP, at the cellular level is an essential, life-sustaining process, and the malfunction of this process has been linked to multiple diseases and disorders [1, 2]. However, how cells maintain this process in a highly perturbative, thermodynamically open environment, and in what circumstances it cannot, is far from completely answered.

We focus on the modelled dynamics of the cell metabolic processes, in particular under which parameters they are or are not stable. From this analysis, we are able to infer under which physical conditions the cell processes enter a healthy or pathological state [3, 4].

This dynamical model is based in the oscillatory nature of the processes responsible for the production of ATP, chiefly oxidative phosphorylation and glycolysis. We will present observations and analysis of these oscillations, and the advantages of representing them in modelling.

This will all be presented in the context of a cellular metabolic model consisting of interacting weighted networks of Kuramoto oscillators, representing the different constituent processes of ATP production [3].

[1] L. A. Demetrius, J. Driver, Biogerontology. 14(6), 641-9 (2013).
[2] T. N. Seyfried, et al., Carcinogenesis. 35(3), 515-527 (2014).
[3] J. Rowland Adams, A. Stefanovska, Front. Physiol. 11, 1845 (2021).
[4] G. Lancaster, et al., Sci. Rep. 6, 29584 (2016).

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Navigating the Waddington Landscape: Geometric models of cell fate decisions
James Briscoe

Abstract currently unavailable

414

Spermatogenesis: a paradigm of stem cell regulation

Ben Simons

To replenish cells lost through exhaustion or damage, tissue stem cells must achieve a perfect balance between renewal and differentiation. To study the factors that control such fate asymmetry, emphasis has been placed on mechanisms in which stem cell competence relies on signals from a discrete anatomical niche. However, in many tissues, stem cell maintenance takes place in a “facultative” niche, where stem cells disperse among their differentiating progenies. Using mouse spermatogenesis as a model, we present evidence that stem cell density regulation relies on a feedback mechanism, reminiscent of “quorum sensing” in bacterial populations, in which cells transition reversibly between states biased for renewal and primed for differentiation. Using a modelling-based approach, we show that this mechanism provides predictive insights into stem cell dynamics during steady-state, as well as under perturbed and transplantation conditions. We discuss the potential implications of these findings for the regulation of stem cell density in other epithelial contexts, as well as their ramifications for the elucidation of dynamic information from single-cell gene expression profiling data.

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Spatial mechano-transcriptomics of the early mouse embryo

Dr. Adrien Hallou[1,2,3], Mr Ruiyang He[1], Professor Benjamin Simons[1,2,3,4], Professor Bianca Dumitrascu[5]
[1]Wellcome / CRUK Gurdon Institute, Cambridge, United Kingdom, [2]Wellcome / MRC Cambridge Stem Cell Institute, Cambridge, United Kingdom , [3]Cavendish Laboratory - University of Cambridge, Cambridge, United Kingdom , [4]DAMTP - University of Cambridge, Cambridge, United Kingdom, [5]Herbert and Florence Institute for Cancer Dynamics - Columbia University, New York City, USA

Cell morphology, mechanical forces, and gene expression act together to orchestrate cell fate decisions and tissue morphogenesis during embryonic development. Analysis into the nature of this cooperation is therefore required to fully understand the nature of the highly complex mechanisms which sculpt the developing embryo in space and time. Here, we present a new and unique computational approach combining image-based mechanical force inference and spatial transcriptomics, and demonstrate its applicability to derive at both single cell and tissue level mechanical and morphometric information alongside gene expression levels. Using a seqFISH dataset of the E8.5 mouse embryo, we show that an integrated analysis of these modalities enables resolution of functional cell sub-types that cannot be distinguished by gene expression alone, as well as identification of gene modules predictive of the mechanical and morphological state of a cell, and of the formation of boundaries between tissue compartments. Our method can be applied to any spatial transcriptomics dataset with sufficient cell membrane segmentation quality, enabling further analysis into the complex interactions between cell morphology, mechanical forces and gene expression.

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Folding oneself into shape: Apical actomyosin contraction is sufficient to drive a furrow-shaped buckling in a curved embryonic epithelium

Jocelyn Etienne[1], Julien Fierling[1], Alphy John[2], Barthélémy Delorme[2], Guy Blanchard[3], Claire Lye[3], Anna Popkova[2], Grégoire Malandain[4], Bénédicte Sanson[3], Philippe Marmottant[1], Catherine Quilliet[1], Matteo Rauzi[2]
[1]Univ Grenoble Alpes – CNRS, Liphy, Grenoble, France, [2]Univ. Côte d'Azur – CNRS, IBV, Nice, France, [3]Univ. Cambridge, PDN, Cambridge, United Kingdom, [4]Inria, Morpheme, Sophia-Antipolis, France

Cell apical constriction driven by actomyosin contraction forces is a conserved mechanism during tissue folding in embryo development. While much is now understood of the molecular mechanism responsible for apical constriction and of the tissue-scale integration of the ensuing in-plane deformations, it is still not clear if apical actomyosin contraction forces are necessary or sufficient per se to drive tissue folding.

To tackle this question, we use the Drosophila embryo model system that forms a furrow on the ventral side, initiating mesoderm internalization. Past computational models support the idea that cell apical contraction forces may not be sufficient and that active or passive cell apico-basal forces may be necessary to drive cell wedging leading to tissue furrowing. By using 3D computational modelling and in toto embryo image analysis and manipulation, we now challenge this idea and show that embryo-scale force balance at the tissue surface, rather than cell-autonomous shape changes, is necessary and sufficient to drive the folding of the epithelial surface and form a furrow which propagates and initiates embryo gastrulation.

Specifically, we show how actomyosin contractility at the embryo surface works like a 'cheese-cutter wire' indenting the surface in a rectilinear fold after a buckling event. Contrary to previously documented buckling dynamics in morphogenesis, this is not due to compressive forces resulting from confinement but rather to a strong tensional anisotropy that emerges due to the embryo geometry and the mechanical balance.

Fierling et al, Embryo-scale epithelial buckling forms a propagating furrow that initiates gastrulation Nature Comms. 13:3348, 2022.

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Feedback mechanisms for morphogen scaling and their evolution

Zena Hadjivasiliou[1,2], Maria Romanova-Michailidi[3], Frank Julicher[4], Marcos Gonzalez-Gaitan[3]
[1]University College London, London, United Kingdom, [2]The Francis Crick Institute , London, United Kingdom , [3]University of Geneva , Geneva, Switzerland , [4]MPI-PKS, Dresden, Germany

Morphogens are secreted molecules that form spatial concentration profiles and control growth and patterning during development. Morphogen gradients often scale to remain proportional to the size of growing organs. A similar expansion or scaling is observed across evolution where the same molecules form gradients of strikingly different length- and timescales. As such, understanding the mechanisms that drive morphogen scaling is central to our study of patterning during developmental growth and evolution. A scaling mechanism that has gained support in the last decade postulates a morphogen and an additional diffusible molecule, the expander, whose expression is repressed by morphogen signalling and can expand the morphogen range by facilitating diffusion or inhibiting degradation. Crucially, this model requires that the expander to form a spatially uniform concentration profile. Experimental support for this mechanism has been found in flies and fish, where a diffusible molecule Pentagone whose production is repressed by the morphogen Dpp plays a role in expanding the Dpp gradient in a dose dependent manner. Recent work however has found that that expression of Pentagone itself is graded, and so cannot be the expander as initially proposed. I will present data and a theoretical model that supports a modified mechanism of morphogen scaling through feedback on the morphogen kinetics that is position dependent. Here both the morphogen and expander scale through advection, dilution, and mutual feedback. I will finally discuss the evolvability of different scaling mechanisms and other features, such as robustness, emerge when scaling is driven by position dependent feedback.

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YAP levels and dynamics control cell fate and proliferation

Kirstin Meyer[1], Nicholas Lammers[2], Lukasz Bugaj[3], Hernan Garcia[2], Orion Weiner[1]
[1]University of California San Francisco, San Francisco, United States, [2]University of California at Berkeley, Berkeley, United States, [3]University of Pennsylvania, Philadelphia, United States

Transcription factors transmit extracellular information to gene expression programs to drive important developmental processes such as pluripotency, proliferation and differentiation. How individual transcription factors transmit signals to control different sets of genes and cellular behaviors remains largely unclear. Importantly, cells make use of time-varying signaling features such as the duration, amplitude and frequency to encode information. Here, we probe how concentrations and temporal dynamics of YAP, a key developmental regulator, direct pluripotency gene activation and developmental decision making. Using an optogenetic approach to control nuclear YAP dynamics with light, we find differential control of Oct4 and Nanog through YAP levels and dynamics. While offset repression thresholds provide differential control of both genes under steady-state YAP levels, dynamic YAP inputs preferentially activate Oct4 at frequencies mimicking the dynamics of the endogenous system. Live imaging of transcription and computational-theoretical analysis of transcriptional regulation demonstrates that Oct4 decodes dynamics by acting as an adaptive change sensor. Using cell fate and proliferation readouts, we identify a similar decoding logic directing cellular decision-making: germ layer fates respond to YAP steady-state levels in a dose dependent manner, while cell proliferation is preferentially activated by oscillatory YAP inputs. Together, we reveal how YAP concentrations and dynamics enable multiplexing of information transmission for the control of developmental gene activation and cellular decision making.

Using Critical Slowing Down to suggest statistical indicators of disease emergence and elimination

Louise Dyson

Abstract currently unavailable.

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Eco-Evolutionary Dynamics of Fluctuating Populations

Mauro Mobilia

Environmental variability greatly influences how the size and composition of a population evolve. The variations of the composition and size of microbial communities, their eco-evolutionary dynamics, are crucial to understand the mechanisms of antimicrobial resistance, and may lead to population bottlenecks, where new colonies consisting of few individuals are prone to fluctuations. How the composition and size of these communities evolve is often interdependent, and demographic fluctuations is coupled to the environmental variability, which can result in feedback loops and cooperative behaviour.

In this talk, we focus on a class of simple and insightful models of populations of fluctuating size whose growth is limited by a binary carrying capacity that endlessly switches between values corresponding to abundant and scarce resources. In these models, the population consists of two strains, one slightly faster than the other, that compete under various scenarios: in the basic model, the competition is only for the same resources, and we will then consider the case where the slow strain produces public goods. Using tools and ideas from statistical physics, we show how the population size distribution and the fixation probability of the slow/cooperative strain are dramatically influenced by the coupling of demographic fluctuations and environmental variability. In addition to random binary switching, the case of periodic and different forms of environmental noise will be commented. If time permits, we will outline how these ideas and techniques are being generalized to study the eco-evolutionary dynamics of cooperative antimicrobial resistance.

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Modelling patterns and coexistence in large ecological communities

Sandro Azaele[1]
[1]*University Of Padua (Italy), , Italy*

In recent years there has been evidence that simple demographic mechanisms at individual level are usually sufficient to explain patterns at macroscales in a wide range of ecological systems. Starting from this basic observation, I will first discuss a stochastic and spatially-explicit model, which can be treated analytically and is able to make several predictions in agreement with empirical datasets. Even though this model can connect patterns across scales, it does not tell us much about the origin of coexistence in large communities. I will then move on to a different model, which explicitly addresses the problem of coexisting species and provides an explanation on general grounds. This is an effective resource competition model which allows many species to live in an environment with very few resources. From this latter model one can derive realistic features of the distribution of species' populations. This talk will be based on the following recent joint work:

[1] F. Peruzzo et al., Physical Review X, 10, 011032 (2020).
[2] D. Gupta et al., Physical Review Letters, 127, 208101 (2021).
[3] E. Pigani et al., Proceedings of the National Academy of Sciences of the USA, 119, 45 e2211449119 (2022).

41

Buckling instabilities in chaining bacterial colonies

Mr Rory Claydon[1], Mr Aidan T. Brown[1]
[1]*University of Edinburgh, Edinburgh, United Kingdom*

Bacteria in the natural environment frequently grow as structured communities known as bacterial biofilms. The morphology of the colony is an emergent property, driven in part by the growth and activity of the constituents. Here, we investigate the effect of pole-pole adhesion between constituents on the resulting colony dynamics and properties, in an effort to understand more about colonies of chaining bacteria such as \textit{Bacillus subtilis}. We have developed a 2D discrete element simulation of a growing bacterial colony composed of non-motile rod-shaped bacteria where daughter cells are able to `chain' together with springs. Despite the simplicity of the model, the emergent dynamics and morphology of the colony are drastically altered by the chaining. At small chain lengths, the classic mosaic of micro-domains is recovered where the colony is isotropic on large length-scales but locally is heterogeneous and composed of domains of aligned cells. As we increase the chain length, there is a crossover to a regime where the colony is able to collectively buckle, characterised by an oscillatory-type morphology and a peak in some observable order parameters e.g. colony aspect ratio, density, micro-domain area etc.. Continuing to increase the chain length gives rise to the possibility of individual chains buckling due to active stresses within the chain overcoming the restoring elastic force of the links, leading to a winding, ribbon-like appearance of the colony and a collapse in these observable parameters.

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Structure and function of MicA, a novel ClpC adaptor for metabolic shutdown

Dr James Torpey[1], Dr Nicola Evans[1], Prof. Amy Camp[2], Dr Rivka Isaacson[1]

[1]King's College London, London, United Kingdom, [2]Mount Holyoke College, South Hadley, United States of America

In the race against antimicrobial resistance, an emerging approach is to hijack bacteria's own degradation pathways as a means of destroying them. The chaperone-protease complex, ClpCP, is an important component of the bacterial degradation machinery and we have discovered MicA, a novel adaptor protein for ClpCP in Bacillus subtilis, that is required for metabolic shutdown in sporulation - the process by which bacteria can form long-lived dormant spores to survive stress conditions. If MicA is knocked out then metabolism in the forespore remains active and MicA is toxic if aberrantly expressed during vegetative growth. I will present our results, obtained using a range of biophysics methodology (X-ray crystallography, cryo-electron microscopy, SAXS, spectroscopic & computational methods) characterising the structure, functions and interactions of MicA & ClpCP with a view to understanding metabolic shutdown in the developing spore and how to harness this system to combat 'hospital superbugs'.

Keynote II: **Symmetry Breaking and Crypt Morphogenesis in Intestinal Organoids (sponsored by Cambridge Centre for Physical Biology)**

Prisca Liberali (Switzerland)

Abstract currently unavailable

On the role of cell rearrangements in pattern formation

Berta Verd

During development, molecular patterns are often established in tissues undergoing morphogenesis and where extensive cell rearrangements are taking place. In order to understand the formation of molecular patterns and their evolvability, it is important we take the role of cell movements explicitly into account. Data-driven mathematical modelling of gene regulatory networks (GRNs) has been very successful at uncovering the mechanisms underlying the formation of complex dynamic molecular patterns in development. However, our efforts have often centred around systems where the timescales of gene expression and morphogenesis could be separated and hence, where pattern could be understood as a function of GRN dynamics alone. While useful, this has greatly restricted the types of developmental patterning systems that could be studied.

We have developed a methodology for reverse-engineering the GRNs driving pattern formation that allows us to take cell movements explicitly into account. We can now fit dynamical models to approximated gene expression trajectories (AGETs) constructed by combining quantitative spatial gene expression data with cell tracking data from live-imaging the developing tissue. The resulting models recapitulate pattern formation on a growing tissue when simulated directly on cell tracks (Live-modelling). By taking this approach to study Tbox patterning in the elongating zebrafish tailbud we have addressed how cell rearrangements and specific modes of cell movements drive patterning as a function of temporal morphogen exposure. We are now applying this framework to axial elongation in cichlid fishes to investigate how developmental patterns evolve.

The alternative sigma factor RpoD4 pulses at division, linking the clock and the cell cycle in cyanobacteria

Chao Ye[1], Dr Arijit Das[2], Dr Teresa Saez[3], Dr Bruno Martins[1], Dr James Locke[3]
[1]School of Life Science, University of Warwick, Coventry, United Kingdom, [2]Abcam, Cambridge, United Kingdom, [3]Sainsbury Laboratory, University of Cambridge, Cambridge, United Kingdom

The cyanobacterial circadian clock is an extensive regulator of gene expression, generating a 24-hour rhythm in the majority of genes in Synechococcus elongatus 7942 (S. elongatus). This raises the question of how the cyanobacterial clock regulates, and receives input from, the diverse cellular processes it controls. Alternative sigma factors, which bind to the core RNA polymerase enzyme and direct the synthesis of specific sets of proteins, are one mechanism for the clock to set the time of its outputs. In this work, we use single-cell time-lapse microscopy to reveal the transcriptional dynamics of RpoD4, an alternative sigma factor that was previously reported as either arrhythmic or circadian. To better follow the joint dynamics of RpoD4 and other genes in individual cells and offset the limitations of strong auto-fluorescence in cyanobacteria, we developed a library of new fluorescent reporters for S. elongatus. We find at the single-cell level that RpoD4 pulses at cell division, dynamics missed by previous bulk averaging. The circadian clock modulates the amplitude of RpoD4 expression pulses, as well as the frequency through its control of division timing. In turn, an RpoD4 mutation causes a reduction in clock period. Further, an RpoD4 mutant results in smaller cell size, and increasing expression of RpoD4 results in larger cell sizes. Thus, our single-cell analysis has revealed pulsing gene expression dynamics in cyanobacteria, linking the clock, RpoD4, and the cell cycle.

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Optical synchronization of synthetic genetic clocks

Maria Cristina Cannarsa[1], Filippo Liguori[1], Giacomo Frangipane[1], Roberto Di Leonardo[1]
[1]*Department of Physics, Sapienza University, Rome, Italy*

Biological noise poses some fundamental limits on the accuracy of synthetic genetic circuits. These shortcomings are observed even in very simple networks, with a small number of interacting genes. A notable example is the repressilator, a bacterial genetic oscillator composed of three mutually inhibiting repressor genes. Although this network has been previously engineered to achieve smooth and robust oscillations, phase drifts lead to rapid de-synchronization of the population signal. We revisited the repressilator by expressing one of the repressor genes under the control of a light-inducible optogenetic system. With our system, called the optorepressilator, we demonstrate the robust synchronization of a population of genetic clocks by an external transient optical signal. Furthermore, we show that periodic stimulation with short pulses of light can lock the phases of individual oscillators and entrain the entire population to oscillate in synchrony. Our results suggest that light-inducible transcription systems can be integrated into synthetic gene networks to achieve greater precision and control.

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Bilateral Feedback in Oscillator Model Is Required to Explain the Coupling Dynamics of Hes1 with the Cell Cycle

Dr Andrew Rowntree[1], Professor Nancy Papalopulu[1], Dr Nitin Sabherwal[2]
[1]*University of Manchester, Manchester, United Kingdom,* [2]*Imagen Therapeutics, Manchester, United Kingdom*

Biological processes are governed by the expression of proteins, and for some proteins, their level of expression can fluctuate periodically over time (i.e., they oscillate). Many oscillatory proteins (e.g., cell cycle proteins and those from the HES family of transcription factors) are connected in complex ways, often within large networks. This complexity can be elucidated by developing intuitive mathematical models that describe the underlying critical aspects of the relationships between these processes. Here, we provide a mathematical explanation of a recently discovered biological phenomenon: the phasic position of the gene Hes1's oscillatory expression at the beginning of the cell cycle of an individual human breast cancer stem cell can have a predictive value on how long that cell will take to complete a cell cycle. We use a two-component model of coupled oscillators to represent Hes1 and the cell cycle in the same cell with minimal assumptions. Inputting only the initial phase angles, we show that this model is capable of predicting the dynamic mitosis to mitosis behaviour of Hes1 and predicting cell cycle length patterns as found in real-world experimental data. Moreover, we discover that bidirectional coupling between Hes1 and the cell cycle is critical within the system for the data to be reproduced and that nonfixed asymmetry in the interactions between the oscillators is required. The phase dynamics we present here capture the complex interplay between Hes1 and the cell cycle, helping to explain nongenetic cell cycle variability, which has critical implications in cancer treatment contexts.

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Cancers Exploit Diverse Mechanical Features of the Stroma
Erik Sahai

The spread of cancer depends on many factors, including complex biophysical interactions between cancer cells, stromal fibroblasts, and the extracellular matrix. The presentation will be split into two parts. The first part will describe how stromal fibroblasts promote the long-range directed migration of cancer cells through dynamic physical re-modelling of the extracellular matrix. The differing roles of matrix plasticity and elasticity will be discussed. The second part will describe how stromal fibroblasts can unexpectedly reduce the migration of cancer cells, and why cancer cells overcome this suppression of migration.

Cancer-associated fibroblasts actively compress cancer cells modulating their drug resistance and invasion
Danijela Vignjevic

During tumor progression, cancer-associated fibroblasts (CAFs) accumulate in tumors. Due to their spindle shape, they spontaneously align along their long axis into nematic ordering, forming a capsule that enwraps cancer cells. This capsule is a barrier that restricts tumor growth leading to the buildup of intratumoral pressure. Combining genetic and physical manipulations in vivo with microfabrication and force measurements in vitro, we found that the CAFs capsule is not a passive barrier but instead actively compresses cancer cells using actomyosin contractility. Cancer cells sense CAF compression, resulting in reduced proliferation and resistance of cancer cells to chemoradiotherapy. We further show that whereas nematically ordered CAFs prevent tumor expansion, defects in the nematic ordering represent weak points where cancer cells escape and invade surrounding tissue.

Insights into cancer from machine learning: translation to clinic.

Peter Weightman[1], Barnaby Ellis[1], Conor Whitley[1], Safaa Al Jedani, James Ingham[1], Caroline Smith[1], Peter Gardner[2], Richard Shaw[1], Stephen Barrett[1], Asterios Triantafyllou[1], Janet Risk[1]
[1]University of Liverpool, , United Kingdom, [2] Manchester Institute of Biotechnology, University of Manchester., , United Kingdom

We have recently shown that the application of a patented machine learning algorithm to infrared spectral images of oral lesions is able to predict which lesions will develop into cancer with an accuracy of ~ 80%. This has the potential to become a major step forward in managing such lesions since the accuracy of current histological approaches in predicting malignancy is 40% or less. This is worse than tossing a coin and fails 60% of patients. The new approach would avoid the need for unnecessary regular painful biopsies for the majority of patients with lesions that will not progress and avoid delays in necessary surgery for patients with lesion that will become malignant.

Clearly it is imperative to translate this advance into clinical as soon as possible and to this end the National Institute of Health Care Research has funded the development of a prototype of the Liverpool Diagnostic Infrared Wand (LDIR Wand) which is a low-cost-hand-held device that will initially be installed in histopathology laboratories. In the longer term the LDIR Wand will be developed in situ analysis of lesions without the need for biopsies and eventually for use in operating theatres. It is anticipated the LDIR Wand methodology would facilitate the early diagnosis of a wide range of cancers.

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Fluctuating methylations clocks allow for the quantification of the evolutionary dynamics in blood cancers

Dr Calum Gabbutt[1], Dr Martí Duran-Ferrer[1], Dr Alison Berner[1], Professor Darryl Shibata[1], Professor Jude Fitzgibbon[1], Professor José Ignacio Martín-Subero[1], Professor Trevor Graham[1]
[1]Institute Of Cancer Research, , United Kingdom, [2]Institut d'Investigacions Biomèdiques August Pi i Sunyer, , Spain, [3]Barts Cancer Institute, , United Kingdom, [4]University of Southern California, , United States of America

Abstract currently unavailable.

Far from equilibrium approach to complex neurovascular dynamics in healthy ageing and dementia

Juliane Bjerkan[1], Dr Gemma Lancaster[1], Dr Bernard Meglič[2], Dr Jan Kobal[2], Mr Karol Budohoski[4], Mr Peter Kirkpatrick[5], Professor Trevor J. Crawford[3], Professor Peter V. E. McClintock[1], Professor Aneta Stefanovska[1]
[1]Department of Physics, Lancaster University, Lancaster, United Kingdom, [2]Department of Neurology, University Medical Centre, Ljubljana, Slovenia, [3]Department of Psychology, Lancaster University, Lancaster, United Kingdom, [4]Department of Neurosurgery, Cambridge University Hospitals NHS Trust,, Cambridge, United Kingdom, [5]Addenbrooke's Hospital, University of Cambridge, Cambridge, United Kingdom

The brain is dependent on glucose and oxygen to function properly. Nutrients are transported by the vasculature, which cooperates with various cells to maintain a balance between energy supply and demand. These cell types include astrocytes, neurons, and pericytes, and, with the microvasculature, they constitute the neurovascular unit (NVU). Impairment of the NVU has adverse consequences for brain health. During ageing the brain and cardiovascular system undergo structural and functional changes. In Alzheimer's disease (AD) vascular changes are increasingly thought to be a very early event in the development of the disease. Similarly, vascular changes have been observed in Huntington's disease (HD). We investigated interactions between the vasculature and cells, based on measurements of near infra-red spectroscopy to evaluate oxygen concentration, and electroencephalography to evaluate brain electrical activity. The ECG and respiratory effort were also simultaneously recorded. Treating the neurovascular system as coupled oscillators away from equilibrium, we analysed data using methods for finite-time dynamics. Instantaneous phases were extracted and the level of phase coherence between them was evaluated. Using surrogate data, we show that effective phase coherence around 0.1Hz is significantly reduced in older participants compared to younger participants, and is further reduced in both AD and HD participants compared to aged-matched controls. Changes in power and coherence, specific to ageing, AD and/or HD, are also revealed. The non-invasive recordings in combination with the finite-time dynamics analysis methods may provide a new avenue for quantitative evaluation of the efficiency of the NVU in both ageing and dementia.

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Direct observation of Shelterin dynamics and T-loop formation at telomeres

Matthew Newton[1], Elizabeth Holmes[2], Jacob Lewis[3], Roopesh Anand[1], Alessandro Costa[3], Alice Pyne[2], Simon Boulton[1]

[1]DSB Repair Metabolism Laboratory, Francis Crick Institute, London, United Kingdom, [2]Department of Materials Science and Engineering, University of Sheffield, Sheffield, United Kingdom, [3]Macromolecular Machines Laboratory, The Francis Crick Institute, London, United Kingdom

Telomeres protect the ends of linear chromosomes, solving the end-replication problem and preventing activation of DNA damage responses which would otherwise result in chromosome fusions. At the core of telomere function is a striking dichotomy: protection and compaction of telomeric ends is crucial for chromosomal stability, however telomeric ends must also be replicated and accessed by telomerase to prevent excessive shortening. Telomere length regulation is vital to human health, telomeres shorten as we age, with rapid shortening resulting in premature ageing, but this also prevents uncontrolled cell replication, acting as a key barrier to cancer development. The telomere bound Shelterin complex is essential for end-protection, through T-loop formation, and for telomere maintenance, via telomerase recruitment. However, how Shelterin orchestrates these two processes remains an unanswered question central to telomere homeostasis.

Using a reconstituted telomere system we are able to study the mechanisms of Shelterin recruitment to telomeric DNA and T-loop formation in real-time at single-molecule resolution. Using optical-tweezers combined with confocal microscopy we can directly observe recruitment of Shelterin complexes to telomeric repeats. We observe very high specificity of Shelterin for telomeric sequences, with no interaction observed at non-telomeric sites. We can monitor capture of the telomeric end, allowing us for the first time to dissect the mechanistic basis of Shelterin mediated T-loop formation which is vital for end-protection. To further understand how Shelterin remodels the telomeric DNA we have used a combination of high-resolution atomic force microscopy (AFM) and electron microscopy (EM) and observe the formation of striking higher order structures within long Shelterin bound telomeric repeats. Our directly observations of Shelterin and T-loop dynamics and new structural information is giving us novel insights into the mechanism of telomere activity.

Community-driven super-resolution microscopy

Dylan Owen

Abstract currently unavailable.

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Cell shape control: from molecules to cellular forces

Dr Ruby Peters

Animal cell shape is primarily controlled by the mechanical properties of the actin cortex; a thin (~150 nm thick) network of actin filaments, myosin motors and accessory proteins. Myosin motors generate contractile forces in the cortex on the nanoscale that gives rise to tension, which is key to maintaining cell shape. Gradients in cortical tension at the cell surface directly drive many cell shape changes including cleavage furrow ingression during cytokinesis and cell rear contraction during migration. Thus, cortical tension must be very precisely regulated, yet how gradients in tension are established and maintained on the nanoscale and how this in turn gives rise to cellular level shape changes, remains poorly understood.

Deciphering the nanoscale organisation of actomyosin structures in the cortex is key to understanding cortical tension regulation, and thus cell shape control. Using super-resolution microscopy approaches, we demonstrate that the organisation of myosin is an important determinant of cortical tension regulation. We demonstrate that myosin does not always overlap with actin in the cortex but remains restricted to the cytoplasmic side of the cortical actin network in low-tension cells. We further show that the degree of myosin cortical penetration can be tuned by perturbing actin network architecture and demonstrate that changing the overlap of cortical actin and myosin directly affects tension.

We are currently investigating how the organisation of the cortical actin network and the dynamics of myosin motors change at the onset of tension gradient formation in the cortex of dividing cells, at the single molecule level.

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Molecular rotors as tools to image non-classical mechanical behaviour of lipid membranes under pathogenic stress

Dr Miguel Paez Perez[1], Dr Nicholas J Brooks[1], Prof Marina K Kuimova[1]
[1]*Imperial College London, London, United Kingdom*

Cellular membranes play a central role in cellular physiology and disease, and therefore are of high relevance in biology. In particular, the biophysical properties of these lipid bilayers are critical to maintain their activity, and this has motivated an increasing effort to understand the structural and biophysical changes of lipid membranes subjected to a range of both physical and chemical stresses – representative of pathogenic indicators.

In this work, we explore the relationship between the membrane's molecular structure and its mechanical behaviour by combining the unique capabilities of molecular rotors – fluorescent molecules capable of reporting on their microviscosity – with fluorescence lifetime imaging microscopy (FLIM), together with X-Ray diffraction (ChemRxiv, 2023). These results suggest there is an analytical relationship between the membrane's rheological and structural properties, and so molecular rotors could be exploited to map changes in the molecular organization of the lipid bilayer.

By using this approach, we examined how the mechanical behaviour of lipid membranes changes during drug treatment (JBO, 2020) under mechanical load, (Chem. Sci. 2021), under oxidative stress, (Comm. Chem. 2023) or in the presence of atherogenic trans-fatty acids. Our results evidence the presence of highly ordered lipid clusters is crucial in the non-classical mechanical response of membranes, including pressure-induced softening, viscoelastic uncoupling, or lipid clustering between domains of comparable thickness. The emergence of such non-classical behaviour can be linked to an increased resilience of the cell membrane to pathogenic stress, emphasizing the importance of the membrane's mechanics in disease development.

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A Label Free Method to Measure Lipid Membrane Dynamics of Giant Unilamellar Vesicles

Freya Turley[1], David Regan[2], Paola Borri[2], Wolfgang Langbein[1]
[1]1.School of Physics and Astronomy, Cardiff University, Cardiff, United Kingdom, [2]2. School of Biosciences, Cardiff University, Cardiff, United Kingdom

The interaction between proteins and lipid membranes is a fundamental process underpinning key functions in cell biology. Despite the importance of such system, many questions are still unanswered concerning the mechanism of protein organisation and function within the cell membrane, and how this is modulated by the lipid environment, due to the lack of suitable measurement techniques.

We are developing a novel optical microscopy technique called “interferometric gated off-axis reflectometry” (iGOR) which promises to be a step-change, enabling us to monitor the motion of single membrane proteins with high sensitivity (~10kDa estimated smallest size limit) rapidly (~1ms) in 3D label-free with ~10nm localisation precision, simultaneously with local membrane properties at the protein site, such as membrane curvature and thickness changes.

As lipid membrane model systems, we are using giant unilamellar vesicles (GUVs) of about 30-50µm diameter. This is a versatile system where we can control the lipid composition and the osmolarity of the internal and external solutions.

We have optimised GUV stability using sucrose concentration differences, characterised via a quantitative differential interference contrast microscopy (qDIC) method developed in house. We found that using a concentration difference between the internal and external sucrose solution >0.2mM establishes an internal pressure too great for a unilamellar vesicle to withstand. We developed a numerical simulation model fitting the experimental qDIC data to accurately determine the lamellarity. An overview of these qDIC results and our progress on characterising lipid membrane dynamics and the insertion of pore forming proteins using iGOR will be presented.

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PolyScope: a 3D-printed minimal microscopic system for microswimmer tracking

Wesley Shao[1], Dr. Hermes Gadelha

[1]*University Of Bristol, Bristol, United Kingdom*

Additive manufacturing technology ('3D printing') brings up opportunities for the technology of microscopy to be democratized. However, due to separation between the innovating and applying communities, this paradigm is not fulfilling its full potential. Here, we present the PolyScope: a 3D-printed bright field microscopic system for non-fluorescent observation of microswimmers. Applied to static, motile and living targets, the system shows capability for educational and researching usage. Based on its simple but effective design, we discuss practicalities of a universal method of purpose-guided device development, aiming to bridge the gap between developers and users, and thus maximize the power of innovation in microscopy.

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Correlative atomic force microscopy with structured illumination microscopy for the investigation of nanoscale features of tuneable bacterial outer membrane models

Karan Bali, Zainab Mohamed, Anna Scheeder, Professor Clemens Kaminski, Professor Suzan Daniel, Professor Roisin Owens, Dr Ioanna Mela[1]

[1]*Department Of Pharmacology, University Of Cambridge, Cambridge, United Kingdom*

The rise of antibiotic resistance is a growing worldwide human health issue, with major socio-economic implications. Understanding of the interactions occurring at the bacterial membrane is crucial in the generation of new antibiotics. Supported lipid bilayers (SLBs) made from reconstituted lipid vesicles have been used to mimic these membranes but their utility has been restricted by the simplistic nature of these systems. A breakthrough in the field has come with the use of outer membrane vesicles derived from Gram-negative bacteria to form SLBs, thus providing a more physiologically relevant system. These complex bilayer systems hold promise but have not yet been fully characterised in terms of their composition, ratio of natural to synthetic component and membrane protein content. Here, we use correlative atomic force microscopy (AFM) with structured illumination microscopy (SIM) for the accurate mapping of complex lipid bilayers that consist of a synthetic fraction and a fraction of lipids derived from Escherichia coli outer membrane vesicles (OMVs). We exploit the high resolution and molecular specificity that SIM can offer to identify areas of interest in these bilayers and the enhanced resolution that the AFM provides to create detailed topography maps of the bilayers. We are thus able to understand the way in which the two different lipid fractions (natural and synthetic) mix within the bilayers and we can quantify the amount of bacterial membrane incorporated in the bilayer. We prove the system's tuneability by generating bilayers made using OMVs engineered to contain a Green Fluorescent Protein (GFP) binding nanobody fused with the porin OmpA. We are able to directly visualise protein-protein interactions between GFP and the nanobody complex. Our work sets the foundation for accurately understanding the composition and properties of OMV-derived SLBs to generate a high-resolution platform for investigating bacterial membrane interactions for the development of next generation antibiotics.

Keynote III: **Epithelial mechanobiology from the bottom up**
Xavier Trepat (Spain)

Abstract currently unavailable

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Understanding the link between 3D gene structure and transcription by computer simulations
Davide Marenduzzo

Classical experiments suggest a link between 3D gene structure and transcription, but this idea has been challenging to directly test quantitatively in the lab. I will discuss a different approach using computer simulations based on biophysical principles of genome organisation to simultaneously predict 3D structure and transcriptional output of human chromatin genome wide. We will see that these simulations allow us to identify a functional role for intranuclear microphase separation in transcription, and to propose a molecular mechanism underlying transcriptional noise.

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Approaches to Deconstruct Transcription - from high resolution techniques to mathematical modelling
Jane Mellor

Transcription of eukaryotic genes is a complex process with many regulated steps. These include not only the production of the nascent transcripts, but also their co-transcriptional processing, nuclear export and stability in the cytoplasm which are all related to how a gene is transcribed. The challenge is to understand how these steps are regulated and the nature of the regulators.

We model the "shapes" produced by our data. These shapes can be histograms of the distribution of transcripts numbers in the nucleus and cytoplasm of single cells detected using single molecule RNA-FISH and exploring how these distributions change in mutants or on conditional changes. We have successfully applied this sort of approach to understand more about the role of antisense transcription in sense transcription and transcript dynamics (Brown et al. Antisense transcription-dependent chromatin signature modulates sense transcript dynamics Mol Syst Biol 2018 e8007).

Alternatively, we model the shapes produced by the position of engaged RNA polymerase at base pair resolution over yeast and mammalian genes. We use a variety of techniques (NET-seq, SNU-seq, TT-seq, PROseq) to assess levels of nascent transcription on mammalian and yeast genes with strand-specific base pair resolution. Some of these techniques, such as NET-seq capture elongating, paused, terminating and backtracked RNA polymerase II, produce complex transcription density heatmaps and metagene profiles that can be further deconstructed using semi-supervised k-means clustering. This shows that groups of genes are
transcribed in very different ways, some with a marked promoter proximal pause and other without the pause. Our aim is to understand the molecular basis of these transcription profiles using stochastic mathematical
models and simulations. We have successfully applied this sort of approach to understand how elongation factors influence transcription (Uzun et al. Spt4 facilitates the movement of RNA polymerase II through the +2 nucleosomal barrier Cell Rep (2021) 36:109755.doi: 10.1016/j.celrep.2021.109755). We suggest that any "shape" produced by biological data can be subject to similar analysis to gain insights beyond that obvious in the experimental data

Gene silencing regulation by heterochromatin compaction

Ander Movilla Miangolarra[1], Martin Howard[1]
[1]*John Innes Centre, Norwich, United Kingdom*

We explore mathematically the effects of chromatin compaction in epigenetic silencing. To that end, we consider the case of the HMR locus in S. cerevisiae, which is maintained in a silent state by the effect of the Sir family of proteins. However, in the absence of Sir1, HMR is known to be bistable and spontaneously switch between a state that expresses the gene encoded in the HMR locus and a silent phenotype. We guide the model construction with recent biochemical data and we find a potential mechanism to recapitulate the robust bistability of the system: A locus with variable three-dimensional size, which is more compact when HMR is silenced but it expands as the gene becomes expressed. In the absence of this dynamic locus behaviour, we fail to obtain the bistability observed in experiments. The variability in locus size can be explained by the acetylation status of the H4 histone tails, linking locus size and epigenetic state as H4K16 is deacetylated by Sir2 which, in turn, favours compaction and further deacetylation. Upon parametrisation of the model, we can reproduce several quantitative experimental observations, such as the rates of switching between epigenetic states as a function of the number of nucleosomes within the HMR locus. In addition, the model captures the cooperativity between the silencers (DNA regions at the edge of the HMR locus) and the bulk of the nucleosomes within HMR, highlighting the importance of the physical conformation of the locus at a regulatory level.

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Simulating 3D chromatin structure at genomic rearrangements in cancer

Chris Brackley[1], Daniel Rico[2], Lisa Russell[2]
[1]*University Of Edinburgh, , United Kingdom,* [2]*Newcastle University, , United Kingdom*

Many cancers occur as a result of genome rearrangements, including chromosome translocations, which can alter the coding sequence of genes (e.g., generating gene-fusion oncogenes). Alternatively, rearrangements can leave coding regions intact, but reposition regulatory elements such as enhancers, leading to gene mis-regulation. An example is during B-cell development where the genome undergoes V(D)J recombination: programmed DNA breaking and repair generates genetic diversity at the immunoglobulin (IG) genes. Errors in this process can lead to translocations which reposition the powerful IG enhancers; in their new locations these can drive over-expression of nearby genes, converting them to oncogenes. The mechanisms through which this occurs are poorly understood. I will present new polymer physics simulations shedding light on how rearrangements lead to changes in 3D chromosome structure and gene expression.

Using the highly-predictive heteromorphic polymer (HiP-HoP) model for chromosome spatial organisation, we studied several common IG-rearrangements involving a specific cancer-driving gene, validating the results using qPCR and microscopy experiments. The simulations predict extensive changes to enhancer-promoter interactions within a chromatin domain structure that remains largely intact. Overall, our results suggest that it is downstream chromatin remodelling which gives rise to the oncogene activation, rather than the presence of the enhancer per se. In other words, the genomic rearrangement leads to an "epigenomic rearrangement", which in turn leads to gene de-regulation. The model also identifies sites within the gene and enhancers which strongly interact, and we are now targeting these experimentally with a view to understanding their potential to be therapeutic targets.

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Atomistic Simulations of Supercoiled DNA under Tension

Mr Matthew Burman[1], Dr Agnes Noy[1]
[1]*School of Physics, Engineering and Technology, University Of York, York, United Kingdom*

The reading of biological information in DNA is not simply the process of reading the chemical sequence, but also in understanding the mechanical properties of the strands, which strongly influence biochemical processes involving its readout. DNA supercoiling is the process of adding or removing turns from double-helical DNA, and through the formation of three-dimensional structures called plectonemes, is vital for large-scale genome organisation. Supercoiling can also give rise to denaturation bubbles, a process that is thought to play a role in the initiation of transcription.

Here we present atomistic MD simulations of supercoiled, linear DNA at a range of tensile forces. These simulations allow us to study the formation of, and interactions between, bubbles and plectonemes, with their unprecedented detail allowing for an in-depth examination of the contrasting behaviours of negatively and positively supercoiled systems, revealing the varying roles of both bubbles and plectonemes between the two. In particular, we observe never-before-seen bubble formation in strongly positively supercoiled systems across a range of forces, suggesting that they may be seen much more universally than previously predicted. Additionally, we are able to study the behaviour of so-called 'tip-bubbles', where clear co-localisation of the two structures is seen, and attribute these to both sequence dependence and supercoiling density.
Our simulations also re-create the experimental 'hat-curve', and allow for the testing of statistical models aiming to predict both the formation of bubbles and plectonemes, giving a unique insight into the sequence-dependent nature of both structures.

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Single-molecule imaging of nucleosome dynamics during DNA replication

Dr Dominika Gruszka[1,2], Dr Sherry Xie[2], Prof. Hiroshi Kimura[3], Dr Hasan Yardimci[2]

[1]University of Oxford, Oxford, United Kingdom, [2]The Francis Crick Institute, London, United Kingdom, [3]Tokyo Institute of Technology, Tokyo, Japan

Faithful replication of DNA and maintenance of its correct organisation into chromatin is fundamental to development in eukaryotes and the avoidance of diseases such as cancer. The basic unit of chromatin is a nucleosome, which consists of ~147 base pairs of DNA wrapped around an octameric histone protein core. During DNA replication, nucleosomes are disrupted ahead of the replication fork, followed by their rapid reassembly on daughter strands from recycled parental and newly synthesised histones. Replication-coupled histone recycling is central to chromatin maintenance but the molecular mechanisms underlying this process are poorly understood. Here, through real-time single-molecule visualisation of replication fork progression in Xenopus egg extracts, we determine explicitly the outcome of fork collisions with nucleosomes. We show that most of the parental histones are evicted from the DNA, with histone recycling, nucleosome sliding, and replication fork stalling also occurring but at lower frequencies. Critically, we find that local histone recycling becomes dominant upon depletion of endogenous histones from extracts, revealing that soluble histone concentration is a key modulator of parental histone dynamics at the replication fork. The mechanistic details revealed by these studies have major implications for our understanding of epigenetic inheritance.

Fluid Mechanics of Mosaic Ciliated Tissues

Ray Goldstein

In tissues as diverse as amphibian skin and the human airway, the cilia that propel fluid are grouped in sparsely distributed multiciliated cells (MCCs). We investigate fluid transport in this “mosaic” architecture, with emphasis on the trade-offs that may have been responsible for its evolutionary selection. Live imaging of MCCs in embryos of the frog Xenopus laevis shows that cilia bundles behave as active vortices that produce a flow field accurately represented by a local force applied to the fluid. A coarse-grained model that self-consistently couples bundles to the ambient flow reveals that hydrodynamic interactions between MCCs limit their rate of work so that they best shear the tissue at a finite but low area coverage, a result that mirrors findings for other sparse distributions such as cell receptors and leaf stomata.

Towards controlling bacterial cell behaviours using electricity and light

Munehiro Asally

Bacteria are an attractive model system for investigating emergent dynamics, owing in part to their relative ease of experimentation and wide broad biomedical and biotechnological applications. Recent studies have revealed that changes in membrane potential are used for signalling in bacteria. The discovery of bacterial electrical signalling raised the possibility of controlling bacterial cellular behaviours via the modulation of membrane potential.

In my talk, I will present our recent efforts to modulate bacterial membrane potential through the use of electricity and light. We have developed a custom-built device that allow us to electrically modulate bacterial membrane potential, and in doing so, we have uncovered the link between membrane potential dynamics and cellular proliferative capacity. Intriguingly, our data have suggested that this link is conserved in budding yeast, Gram+ and Gram- bacteria. We have also explored the use of light to modulate the membrane potential and have succeeded in inducing a hyperpolarisation using an azobenzene molecule called Ziapin2. We believe that these tools will set the ground for facilitating more basic research on bacterial electrophysiology and developing novel bioelectrical approaches for controlling microbial cellular behaviours. Finally, I will discuss several unique aspects of bacterial electrophysiology because of their small sizes.

Odd dynamics of living chiral crystals

Alexander Mietke[1,2], Tzer Han Tan[3,4,5], Junang Li[3], Yuchao Chen[3], Hugh Higinbotham[3], Peter J. Foster[3], Shreyas Gokhale[3], Jörn Dunkel[2], Nikta Fakhri[3]

[1]School of Mathematics, University Of Bristol, , United Kingdom, [2]Department of Mathematics, Massachusetts Institute of Technology, , USA, [3]Department of Physics, Massachusetts Institute of Technology, , USA, [4]Quantitative Biology Initiative, Harvard University, , USA, [5]Center for Systems Biology Dresden, , Germany

The emergent dynamics exhibited by collections of living organisms often shows signatures of symmetries that are broken at the single-organism level. At the same time, organism development itself encompasses a well-coordinated sequence of symmetry breaking events that successively transform a single, nearly isotropic cell into an animal with well-defined body axis and various anatomical asymmetries. Combining these key aspects of collective phenomena and embryonic development, we describe here the spontaneous formation of hydrodynamically stabilized active crystals made of hundreds of starfish embryos that gather during early development near fluid surfaces. We describe a minimal hydrodynamic theory that is fully parameterized by experimental measurements of microscopic interactions among embryos. Using this theory, we can quantitatively describe the stability, formation and rotation of crystals and rationalize the emergence of mechanical properties that carry signatures of an odd elastic material. Our work thereby quantitatively connects developmental symmetry breaking events on the single-embryo level with remarkable macroscopic material properties of a novel living chiral crystal system.

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Tuneable metachronal coordination in Platynereis dumerilii larvae

Rebecca Poon[1], Milena Marinkovic[1], Gaspar Jekely[1], Kirsty Wan[1]
[1]*University Of Exeter, , United Kingdom*

Metachronal coordination is a widespread phenomenon in ciliary arrays. The precise mechanism of coordination, and particularly how biological control and hydrodynamic forces interplay to give rise to robust wave behaviour, is an ongoing and unresolved question. It is also unclear what sets the wave propagation direction and why such wave parameters are so organism-dependent. Here we introduce the ciliated larvae of the marine ragworm Platynereis dumerilii as a novel animal model system with which to address this question from a combined experimental-theoretical perspective. Larvae possess multiple cilia, which are arranged into a densely ciliated ring or band where they perform a robust one-dimensional metachronal wave. We develop an objective method for quantifying noisy metachronal coordination in ciliary arrays. By characterising ciliary coordination over long timescales and at high spatiotemporal resolution, we resolve detailed wave parameters including defects and transient loss of metachrony associated with ciliary closures. We compare the timescale for establishment of coordination with existing models. Finally, we apply pharmacological and physical perturbations to the wave, to investigate the interaction of biological and physical mechanisms of ciliary coordination.

We acknowledge funding from the European Research Council (grant 853560 EvoMotion, to KW), and from a Wellcome Trust Investigator Award (214337/Z/18/Z to GJ).

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The interplay of photosynthesis and phototaxis in a model microswimmer

Dr Stephen Williams[1], Dr Marco Polin[1,2], Dr Shilpa Khatri[3]
[1]University Of Warwick, kenilworth, United Kingdom, [2]Mediterranean Institute for Advanced Studies, Esporles , Spain, [3]University California Merced, Merced, United States

Photosynthetic microorganisms are fundamental to the global ecosystem. They form a large portion of the base of major food webs, including oceanic, and contribute about half of all global oxygen production. These organisms also present possible exciting solutions to many of the most pressing global issues, from the production of fuel alternatives to sustainable agriculture.

Light management is essential to the function of such organisms. Specifically, spatio-temporal variations provide information critical to biological regulation and impart energy to fuel intracellular processes. Crucially, these organisms must avoid excess light, as even small deviations can cause serious photooxidative cellular damage and death. Motile organisms deploy two strategies to mitigate intense exposure. Firstly, by using light as information, phototaxis (PT) mechanisms redirect organisms towards/away from light. Secondly, by using light as energy, photosynthesis (PS) systems regulate or dissipate excesses, non-photochemical quenching (NPQ). Observations suggest a fundamental link between PS, PT and NPQ, which has not been studied in quantitative detail. However, the links between these strategies is poorly understood.

In this work we aim to bridge this gap, combining experimental measurements of the model microswimmer Chlamydomonas reinhardtii and a modelling approach. This is aimed at linking the intracellular level (SP, NPQ) and the extracellular level (PT) to understand of how light is managed by motile photosynthetic microorganisms, and further to enable predictions of the responses at the individual and collective levels.

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How precise is patterning with noisy morphogen gradients?

Roman Vetter[1,2], Dagmar Iber[1,2]
[1]ETH Zurich, Basel, Switzerland, [2]Swiss Institute of Bioinformatics, Basel, Switzerland

The precision at which Nature manages to build complex organisms from a single cell is a fundamental problem in developmental biology. By forming concentration gradients, morphogens can instruct cells about their position in a patterned tissue, allowing them to assume location-dependent fates. Despite decades of research on the foundations of this patterning mechanism, a key question keeps puzzling the field: How can the resulting tissue pattern be as robust and precise as it is found to be, given that the morphogen gradients are inevitably noisy and variable between different embryos?

Here I show how it has recently become clear that the mystery of high patterning precision can be explained quantitatively, without requiring precision-enhancing mechanisms such as spatial averaging, cell sorting, or the simultaneous readout of multiple morphogen gradients. A simple reaction-diffusion model predicts the spatial accuracy that morphogen gradients can convey, based on molecular noise in the morphogen production, decay and transport kinetics. I discuss several striking results of this new perspective on gradient-based tissue patterning, such as the role of cell size, cell shape, tissue geometry, and self-enhanced morphogen degradation, with implications on several developmental systems such as the Drosophila wing disc and the vertebrate neural tube.

Patterning DNA-based artificial cells with reaction-diffusion

Lorenzo Di Michele

Bottom-up synthetic biology aims to engineer artificial systems that exhibit biomimetic structure and functionality from the rational combination of molecular and nanoscale elements. These systems often take the form of artificial cells (ACs), micro-robots constructed de novo to replicate behaviours typically associated with biological cellular life. If endowed with sufficiently advanced cell-like responses, ACs could underpin game-changing solutions, e.g in healthcare and biosynthesis. Similar to their biological counterparts, ACs require a micro-compartmentalised architecture to regulate transport and establish internal heterogeneity, as typically necessary to sustain biomimetic molecular pathways. AC compartments often rely on lipid, polymer or protein membranes, but membrane-less implementations based on coacervates or hydrogels are gaining traction, driven by enhanced robustness, easy manufacturing and the renewed interest for biomolecular condensates in cell biology. While internal compartmentalisation can be robustly achieved in membrane- based platforms, e.g. through nesting or sequential assembly, no general platform has been proposed to program local composition in membrane-less scaffolds. Here we leverage the structural and dynamic programmability afforded by DNA nanotechnology to construct membrane-less condensates of DNA nanostructures, which can be “patterned” thanks to a reaction-diffusion scheme. The latter can generate up to five to chemically addressable, distinct micro-environments whose features can be rationalised through numerical modelling. As a proof-of-concept, we use the platform to create model ACs with compartmentalised functionality, namely where a fluorescent RNA aptemer is synthesised in prototypical “nucleus” and accumulates in an outer shell.

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Life-like motility in droplet systems
Nathalie Katsonis

Six hundred years ago Leonardo Da Vinci claimed that “movement is the cause of all life”[1]. Purposeful movement is indeed a defining characteristic of life, from morphogenetic shape transformation, to the beating of a heart. Yet, the fundamental chemical and physical mechanisms that underpin the functional movement of life remain largely unknown. The overarching aim of my work is to unravel the chemical origins of purposeful motion - both in space (from the molecular scale upwards) [2] and in time (during prebiotic evolution) [3]. In both cases, I argue that life-like motile behavior can emerge spontaneously when supramolecular entities are coupled with fluctuations of interfacial tension [4].

In this presentation, I will focus on our recent efforts to uncover the fundamental physical and chemical rules that govern the motile behavior of droplets in lipid systems. I also show that in the presence of artificial molecular machines, these systems demonstrate sophisticated responsiveness to irradiation with light.

[1] “Il moto è causa d'ogni vita” extract from the Codex Trivulzianus, Leonardo da Vinci (1487).
[2] F. Lancia, A. Ryabchun, N. Katsonis. Life-like motion driven by artificial molecular machines, Nature Reviews Chemistry 2019, 3, 536–551.
[3] D. Babu, N. Katsonis, F. Lancia, R. Plamont, A. Ryabchun. Motile behavior of droplets in lipid systems, Nature Reviews Chemistry 2022, 6, 377-388.
[4] N. Katsonis. From molecules to motile chemical systems – setting chemistry in motion, Proceedings of the Solvay Conference 2023, in press.

Membrane transport processes control the kinetics of enzymatic pH clock reactions confined within lipid compartments

Darcey Ridgway-Brown[1], Anna Leathard[2], Oliver France[1], Dr Stephen Muench[1], Dr Michael Webb[1], Professor Lars Jeuken[3], Professor Peter Henderson[1], Professor Annette Taylor[2], Professor Paul Beales[1]
[1]University Of Leeds, Leeds, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom, [3]Leiden University, Leiden, Netherlands

Biochemical feedback mechanisms regulate a wide-range of molecular scale processes within living cells, facilitating biochemical switches, toggles and oscillators. As the field of artificial cells and organelles engineers systems with ever more complex functionality, harnessing feedback mechanisms to regulate their outputs will be required to ensure their predictable and reproducible behaviour. To this end, we combine experiments with reaction-diffusion modelling to study an enzymatic pH clock reaction confined within lipid vesicle compartments. The urea-urease reaction is regulated by an autocatalytic base-catalysed feedback mechanism; in an initially acidic environment the enzyme kinetics are slow but the enzymatic activity accelerates as the reaction proceeds yielding a basic product. The reaction kinetic profile displays a lag time, known as the clock time, that is determined by the initial conditions of the system, before a rapid switch to high pH. This “simple” hydrolysis reaction yields surprisingly complex behaviour when confined within vesicles. We demonstrate synchronisation of reactions between different vesicle compartments where communication occurs through fast ammonia transport. We also find that the reaction kinetics are strongly dependent on membrane transport processes in non-intuitive ways. This is explored by changing the membrane thickness and utilising ionophores and protonophores that enhance the transport of specific solutes across the membrane. Insights into the dominant mechanisms regulating the reaction dynamics are gained from the modelling studies. These experiments exemplify how complex behaviour can be engineered in relatively simple systems and pave the way towards the development of artificial cells and organelles encapsulating feedback-controlled chemical processes.

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A Universal Method for Analysing Copolymer Growth

Mr Benjamin Qureshi[1], Mr Jordan Juritz[1], Dr Jenny Poulton[2], Dr Thomas Ouldridge[1]
[1]Imperial College London, Buckhurst Hill, United Kingdom, [2]AMOLF, Amsterdam, Netherlands

DNA, RNA and proteins are natural examples of copolymers: polymers consisting of more than one type of monomer unit. Information is stored in the sequence of monomers of the copolymer; this information is transferred through templated copolymerisation, and used to direct molecular folding, in the vital processes of the central dogma of molecular biology. Deeper understanding of copolymer assembly mechanisms - which are often sophisticated, in order to ensure sequence accuracy - is central both to our appreciation of natural systems and the engineering of synthetic analogues. In this work, we consider a general class of copolymerisation models in which monomers are added to, or removed from, the end of a growing polymer via an arbitrary reaction network. Hitherto, analysis of these models has required simulation; we introduce a methodology that allows for properties such as the sequence distribution, polymerisation speed and entropy production to be obtained either analytically or via a simple numerical calculation. We demonstrate the power of the approach by analysing a family of Hopfield-style kinetic proofreading mechanisms realised in a copolymerisation setting.

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Bioinspired cytoskeletal active networks

Vahid Nasirimarekani[2], Olinka Ramirez-Soto[2], Stefan Karpitschka[2], Isabella Guido[1]
[1]University Of Surrey, Guildford, United Kingdom, [2]Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany

Cytoskeletal assemblies such as microtubule networks and motor proteins drive vital cellular processes that, together with cargo delivery and cell division, also include providing mechanical stability when cells are exposed to external stresses. The dynamics of these self-organising structures is driven by the continuous supply of energy at the molecular level, which enables the active components to generate internal stresses for spontaneous motion. However, how the cytoskeleton can orchestrate its components to respond to the external environment is not yet well understood.

In this study, we develop a bioinspired system resembling intracellular cytoskeletal networks and characterise its activity under the influence of external mechanical stimulation. To this end, we confine an active network of microtubules and kinesin motors in an evaporating aqueous droplet. The flow field generated by the Marangoni and capillary flow couples with the active stress of the microtubule-motor-protein network. We observe that this coupling influences the spatio-temporal distribution of the driving forces and the emergent behaviour of the system, which shows contraction and relaxation. Thus, this setup turns out to be a bioreactor that mimics the natural environment of biological active networks and allows forces to be exerted on them. By analysing such non-equilibrium systems, our study can contribute to understanding the response of biological structures to cues from the external environment.

Two-dimensional positioning and patterning with a molecular printer made from DNA

Dr Rafael Carrascosa Marzo[1,2], Dr Erik Benson[1,2,3], Mr Sonji Kurishita[1,2], Dr Jonathan Bath[1,2], Professor Andrew Turberfield[1,2]

[1]University of Oxford, Oxford, United Kingdom, [2]The Kavli Institute for Nanoscience Discovery, Oxford, United Kingdom, [3]KTH Royal Institute of Technology, Stockholm, Sweden

A principal focus of molecular nanotechnology is the construction of synthetic machinery that can manipulate matter at the nanoscale. We exploit the programmability of biomolecular interactions to build and control nanoscale molecular printers based on linear actuators made using the DNA origami technique. Our two-axis positioning mechanism comprises a moveable gantry, running on parallel rails, threading a mobile sleeve: this allows the sleeve to be positioned over a DNA origami "canvas" using a cartesian-coordinate system. We have also developed a dial-like device that allows for positioning in a polar-coordinate system. These machines can be locked at and released from programmed locations by the introduction of signalling oligonucleotides. We introduce a "write" functionality by adding pixels, comprising modifiable oligonucleotide extensions, to the canvas surface and a catalyst on the sleeve. The latter, acting as a write-head, catalyzes the deposition of "ink" strands on the canvas. Ink strands carry DNA-PAINT docking sites, enabling the printed pattern to be imaged by TIRF microscopy.

This first-generation DNA-origami-printer demonstrates the functionality of nanoscale robotic components created by programmed biomolecular assembly. Future printers could incorporate alternative modes of actuation and control and other modes of catalysis to find applications in position-directed chemistry and biophysical studies.

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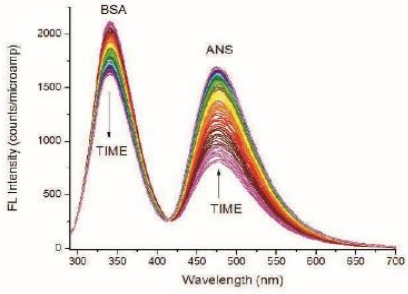
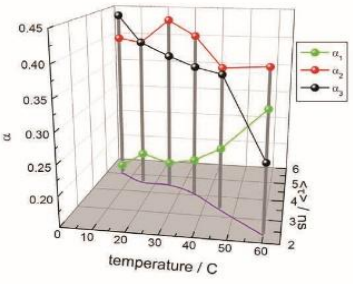


Kinetic spectral scans of native BSA protein with added ANS every 100 ms.

DeltaFlex

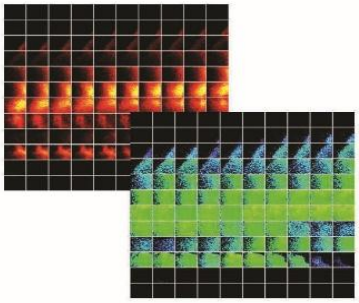
Flexible and easy to use fluorescence lifetime spectrometer



Effect of temperature on the time-resolved decay of tryptophan in HSA, with exponential analysis showing normalised pre-exponentials (αi, 1 relates to shortest-lived, 3 to longest) and average lifetime.

FLIMera

Wide field FLIM camera for video rate imaging (30fps)



TCSPC intensity (left) and Fluorescence life (right) time frames of tumor mimic PpIX-intralipid in optically dense intralipid medium. 250 ms frames were extracted. Total acquisition time was 20s (80 frames).

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Keynote IV: **Designing Biological Circuits for Multicellularity**
Michael Elowitz (USA)

Abstract currently unavailable

The Tom McLeish Lecture
Keynote V: **How would a biophysicist design a cell division machinery?**
Petra Schwille (Germany)

Abstract currently unavailable

How do Biomolecules Walk?

Sarah Harris

Experimental tools such as cryo-electron microscopy and tomography (cryo-EM/ET) are revealing new regimes of biology at the mesoscale that have not yet been seen, such as the organization of protein complexes into subcellular architectures, and the action of molecular motors. We have developed the Fluctuating Finite Element Analysis (FFEA) software1 for modelling mesoscale biomolecular dynamics, which we are using to understand the walking mechanism of molecular machines such as dynein and myosin based on cryo-EM/ET images.

1. Solernou A, Hanson BS, Richardson RA, Welch R, Read DJ, Harlen OG, et al. (2018) Fluctuating Finite Element Analysis (FFEA): A continuum mechanics software tool for mesoscale simulation of biomolecules. PLoS Comput Biol 14(3): e1005897. https://doi.org/10.1371/journal.pcbi.1005897

Minutes-long single molecule tracking in live bacteria reveals that molecular motor tug-of-war regulates elongasome dynamics and bacterial cell shape in Bacillus subtilis

Séamus Holden

Almost all bacteria are surrounded by a mesh-like peptidoglycan cell wall essential for their survival. Bacterial cell wall synthesis proteins remain one of the best targets for antibiotics as defects in cell wall structure cause bacteria to burst and die. How nanometre sized cell wall synthesis proteins coordinate to build a micron sized cell wall is a major unsolved puzzle bridging physics and bacteriology.

Many rod-shaped bacteria, including major antibiotic resistant pathogens, elongate by adding new material to the sides of their cell wall. In these organisms, an essential multi-protein synthesis complex called the elongasome inserts glycan strands around the circumference of the cell, elongating and reinforcing the cell wall and giving cells their rod shape. It is likely that the length of new circumferential glycan strands has substantial effect on key cell wall properties including vulnerability to lysis upon antibiotic treatment or changing environmental conditions. How cells regulate the length of circumferential glycan strands and the effect of circumferential glycan strand length on cell physiology is largely unknown.

We used a novel single molecule tracking method to determine the processivity of the Bacillus subtilis elongasome, which likely determines the initial length of new glycan strands. We found that elongasomes are highly processive, and that B. subtilis elongasome dynamics and processivity are determined by a balance between processive synthesis and molecular motor tug-of-war, where multiple synthases pull individual MreB filaments in opposite directions. We found evidence that elongasome processivity and tug-of-war regulate B. subtilis cell size and shape.

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Mechanisms of action of the DNA binding protein HU towards DNA repair and biofilm stability

Mr Elliot Chan[1], Dr Jamieson Howard[1], Ms Mingxue Du[2], Mr Daniel Rollins[2], Dr Alice Pyne[2], Prof Mark Leake[1,3], Dr Agnes Noy[1]

[1]School of Physics, Engineering and Technology, University of York, York, United Kingdom, [2]Department of Materials Science and Engineering, University of Sheffield, Sheffield, United Kingdom, [3]Department of Biology, University of York, York, United Kingdom

Unconstrained, the genomic DNA of bacteria would form structures over 1000 times larger than the cell. Yet in cells, the DNA is localised to a small region called the nucleoid. This compaction occurs due to the interplay between DNA supercoiling and a group of DNA-binding proteins known as nucleoid-associated proteins (NAPs). One of the most abundant NAPs is HU, which specifically binds to sites of DNA damage and creates sharp bends to aid in DNA repair, but also has non-specific binding which participates in nucleoid condensation. It is also known to act as a "molecular glue" in biofilms, stabilising the extracellular DNA meshes. However, the way in which HU plays these roles is unknown.

By combining all-atom molecular dynamics with in-liquid atomic force microscopy we show, with unprecedented detail, how the protein diffuses along and between strands of DNA towards finding a binding site. Once a site of damage is found, we show a clear multimodality in the binding of HU to DNA, observed in both MD and AFM. Enhanced sampling techniques were applied to characterise the underlying free energies between the distinct binding modes, providing insight into the transitions between metastable states. AFM imaging also shows aggregation of DNA by HU, which simulations show to be highly energetically favourable, explaining both how HU condenses the nucleoid, and why it is key to biofilm stability. The observed structural multiplicity may be a general mechanism used by other NAPs and eukaryotic chromatin-binding proteins towards the physical organisation of cells.

Complementary approaches to obtaining thermodynamic parameters from protein ligand systems: challenges and opportunities and a case for neutrons

Mona Sarter[1], Doreen Niether[2], Simone Wiegand[2,3], Joerg Fitter[4,5], Andreas Stadler[6,7]
[1]ISIS Neutron and Muon Facility- STFC, , United Kingdom, [2]Institut of Biological Information Processing (IBI-4&JARA-SOFT) Forschungszentrum Juelich, , Germany, [3] Department fuer Chemie - Physikalische Chemie, Universitaet zu Koeln, , Germany, [4]I. Physikalisches Institut (IA) AG Biophysik RWTH Aachen, , Germany, [5]Institut of Biological InformationProcessing (IBI-6: Molecular Biophysics) Forschungszentrum Juelich, , Germany, [6]Juelich Centre for Neutron Science (JCNS-1) and Institut of Biological Information Processing (IBI-8: Neutron Scattering and Soft Matter) Forschuengszentrum Juelich, , Germany, [7]Institut of Physical Chemistry RWTH Aachen University, , Germany

Protein ligand interactions play an important role in biology and in order to influence this process in a targeted way increased understanding is necessary. The binding process is heavily influenced by its thermodynamic parameters. While the overall change in enthalpy can be easily measured using isothermal titration calorimetry (ITC) and the change in entropy and Gibbs free enthalpy then calculated this does not provide information about the individual components of these contributions. This presentation aims to discuss how the different components that are responsible for the total change in entropy can be isolated using different complementary techniques, as well as what the challenges faced for each method are and how they might be overcome or mitigated.
All discussions will be based on the system of streptavidin and biotin which will be used as a model system. Upon protein ligand binding, changes of conformational entropy occur in protein and hydration layer, as well as internal dynamics. In this study the binding of biotin to the tetramer streptavidin was investigated using quasi-elastic neutron scattering (QENS), as well as Thermal Diffusion Forced Rayleigh Scattering (TDFRS) and ITC. This specific interaction is enthalpy driven, with an opposing entropic component. An experimental investigation of the components of the entropy change, specifically the change in conformational entropy, indicates a change in conformational entropy strongly opposed to the binding. The adverse change in entropy therefore has to be compensated, with the strongest candidate being a supportive change in the entropy of the surrounding hydration layer. It is also of note that while the change in conformational entropy upon saturation with biotin is on the same order of magnitude as that of protein folding, no significant structural changes take place during the binding process.

Self-assembly and hydration of a β-hairpin through integrated small and wide-angle neutron scattering

Harrison Laurent[1]

[1]*University Of Leeds, Leeds, United Kingdom*

Biological organisms are inherently hierarchically structured, for example where macroscopically observable properties such as muscle elasticity are ultimately controlled by the breaking and forming of by hydrogen bonds involving just a few atoms. For this reason, multiscale and integrative approaches that combine all-atom and course grained simulations with experimental techniques to bridge these vastly different length scales are becoming increasingly vital. Neutron scattering is particularly powerful for the investigation of biological systems as its non-damaging, deeply penetrating, and sensitive to H/D substitution. Here we employ small angle and wide angle neutron scattering (SANS and WANS) using both the Zoom and NIMROD instruments at the ISIS neutron and muon source (Q range of 0.0003 – 0.15 Å-1 and 0.02 – 50 Å-1 respectively) to investigate the self-assembly and hydration of the model β hairpin CLN025 (YYDPETGTWY). This 'miniprotein' is also an important benchmark for protein folding modelling due to its thermodynamic behavior and rapid folding. The SANS data demonstrate that in concentrated solution (150 mg/mL) the peptides self-assemble to form stacks of ~7 β-hairpins. Informed by this insight, we constrain the integrative WANS analysis approach empirical potential structure refinement (EPSR) to provide an all-atom visualisation of the self-assembled β-hairpin stack in explicit water, including hydrogen bonding and hydrophobicity. Such atomistic detail will provide key information for future studies of ligand binding and drug design, the design of new forcefields through techniques such as iterative Boltzmann inversion, and biological self-assembly to create novel smart materials.

Emergence of cell tissue shape during development
Tim Saunders

Abstract currently unavailable.

Mechanical impact of cell delamination on tissue dynamics, in developmental and tumoral contexts

Magali Suzanne

How mechanical forces drive morphogenesis is a fundamental question in the field of biomechanics. Combining imaging, genetics, biophysical and modeling approaches, we found that apoptotic cells, far from being eliminated passively, exert a force before dying and thus actively participate in tissue remodeling. This transient force, generated in the depth of the epithelium, constitute a mechanical signal involved in tissue folding. Comparing apoptotic cell dynamics to cells undergoing EMT, we found that a very similar apico-basal force is generated at the onset of EMT.

To decipher how these forces are transmitted at the tissue scale, we developed a new method that offers the opportunity to extract automatically, in strongly deformed epithelia, a precise characterization of the spatial organization of a given cytoskeletal network combined to morphological quantifications in highly remodeled 3D epithelial tissues.

In parallel, we turned to cancer mechanics, focusing particularly in tumor progression. Cancer is a largely widespread pathology that corresponds to an overproliferation of cells that could finally invade others tissues. Tumors develop through three increasingly aggressive steps: (1) hyperplasia, which corresponds to cells overproliferation; (2) dysplasia, during which cells can acquire a more mesenchymal phenotype, and finally (3) metastasis. Tumor development can be influenced by mutations but also by external factors, such as extracellular matrix rigidity. However, a comprehensive understanding of the intrinsic factors driving tumor evolution is still lacking. Our recent unpublished work identifies unexpected factors that could influence tumor development, and more specifically the hyperplasia/dysplasia transition, a critical step in tumor aggressiveness.

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Patterns of contractility within a tissue modulate force-balance dynamics

Dr John Robert Davis[1], Dr Josephine Solowiej-Wedderburn[3], Dr Carina Dunlop[3], Dr Nic Tapon[2]
[1]University of Manchester, Manchester, United Kingdom, [2]The Francis Crick Institute, London, United Kingdom, [3]University of Surrey, Guildford, United Kingdom

For a tissue to develop and maintain its function, its constituent cells need to alter their contractile forces to produce the appropriate tissue architecture and ensure homeostasis. Previous work in cell doublets has highlighted that cells transmit forces across cell-cell junctions to balance traction forces, with higher traction forces leading to higher intercellular tension. This has been extrapolated to whole tissues with stresses across cell-cell junctions being predicted based on traction forces. However recent work in Drosophila amnioserosa cells and histoblasts have hinted at the opposite relationship, with strong extracellular matrix attachment leading to lower intercellular tension. By combining traction force microscopy and multiphoton annular ablations, we found that increasing substrate stiffness led to higher traction forces but lower intercellular tension across epithelial clusters. This inverse relationship is driven by spatial changes in myosin II contractility, with increasing substrate stiffness leading to higher myosin II activity at the periphery compared to the centre of the epithelial cluster. Furthermore, we found that the pattern of myosin II across the cluster was dependent on focal adhesion distribution. This supports findings in Drosophila and further suggests that the pattern of focal adhesions and contractility are as important factors as matrix stiffness in determining how forces are sensed and transmitted across cell-cell junctions within a tissue. As force transmission is essential for tissues to acquire their desired form and function, this work highlights an overlooked mechanism of adhesion and contractility patterns in influencing how far forces can act as a signal.

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The physics of root bending under gravity

Doctor Sujit Kumar Nath[1,2,3], Doctor Marta Del Bianco[2], Doctor Rob Thomas[2], Mr. Moritz Zilian[1], Professor Stefan Kepinski[2], Professor Netta Cohen[1]

[1]School of Computing, University of Leeds, Leeds, United Kingdom, [2]Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom, [3]Division of Cardiovascular Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester , United Kingdom

How do the roots of plants know to grow down and to bend downward after reorientation? While many of the puzzle pieces of this age-old question are being unravelled, the role of physics in this process is poorly understood. We combined experiments and modelling to study the dynamics of plant roots under gravity (gravitropism). We collected a high-throughput, high-resolution experimental dataset of root bending dynamics, for a wide variety of root orientation conditions, and asked whether a single integrated model could link sensing (which occurs at the tip of the root) and bending/directed growth (some distance up the root). We derive a compact mathematical model that combines angle-sensing, signal transduction and a biomechanical response, and show, for the first time, how sensing-bending feedback loops predict experimentally observed transient dynamics upon sudden reorientation, as well as long-term bending dynamics across our entire dataset. We demonstrate our results in the mean-field to capture population dynamics as well as in a stochastic individual root setting. Combining our model with additional experimental data indicates important roles for sensing, signalling and biomechanical modulation, with overlapping timescales, which we begin to disentangle with additional experiments using both wild type and genetically perturbed strains.

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Modelling forces in confluent cell layers

Mehrana Raeisian Nejad[1], Gaunming Zhang[1], Professor Julia M Yeomans[1]
[1]*University Of Oxford, Oxford, United Kingdom*

The motion of epithelial cells is key to many life processes from morphogenesis to wound healing and cancer metastasis. Despite its importance, and considerable recent attention, much remains unknown about the mechanisms that lead to collective cell motility, both at the mechanistic level of understanding the underlying biochemistry, and at a more coarse-grained level of identifying the primary forces involved.

Therefore we map a coarse-grained model of a confluent cell layer that includes several physical forces – traction forces, intercellular interactions, cell-cell adhesion - that may be important drivers for collective cell motility onto continuum equations which are then solved numerically. We compare our results to phase-field models of cell layers and suggest experiments that may help to distinguish the forces underlying collective cell motility.

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Revealing the missing parameters in tissue folding - Out-of-plane stresses and patterned elasticity

Dr. Steph Höhn[1], Dr. Pierre Haas[2], Dr, Elia Benito-Gutierrez[3]

[1]University of Cambridge, Department of Applied Mathematics and Theoretical Physics, Cambridge, United Kingdom, [2]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany, [3]Genentech, San Francisco, USA

Folding of cellular monolayers is a universal theme of embryonic development and disease. Cell-wedging through apical constriction can drive local changes in preferred curvature, but is not sufficient to explain dramatic global tissue folding. Moreover, the associated out-of-plane forces have remained a mystery. Here, we combine novel experimental approaches with quantitative modelling to uncover the global elastic behaviour of folding cell sheets. During both, inversion in Volvox globator (Chlorophyta) and gastrulation in Amphioxus (Cephalocordata) the posterior hemisphere of the initially spherical embryos moves into the anterior. This tissue invagination is driven by waves of active cell shape changes [1-3]. Using orthogonal imaging during laser ablation enables the quantification of out-of-plane elastic responses. From these we infer preferred curvatures using a 3D elastic model. We find that residual torques result from the incompatibility of local cellular and global tissue geometry. Even small torques significantly affect tissue mechanics [4]. Another limitation of current models of tissue folding is the assumption of homogeneous mechanical properties. We present the first tissue-scale micropipette aspiration experiments on cellular monolayers. We reveal differences in visco-elastic properties in the invaginating area vs. adjacent tissue. Patterned mechanical properties might foster the dramatic folding occurring at the boundary of the invagination [5].

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Genetic Circuits that Adapt: Design Principles and Genetic Implementation

Mustafa Khammash

Billions of years of evolution have yielded genetic regulatory mechanisms that precisely and robustly control biological processes, even in noisy and uncertain environments. Understanding such naturally existing mechanisms not only contributes to our understanding of biological complexity but also informs the design of novel genetically-engineered control circuits, with application in various fields such as biotechnology and personalized medicine. In this talk, I will present a theory for genetic controller circuit design, with a focus on circuits that achieve robust perfect adaptation. These circuits adapt completely to perturbations in their parameters and topologies, enabling tight and robust regulation without requiring specific knowledge of the network they control. I will then show how such adaptive circuits can be genetically engineered using RNA or protein components in various cell types. This work highlights the fundamental design principles of robust genetic control circuits and their potential impact on a range of applications.

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Mechanisms for functional noise in gene regulation

James Locke

Gene expression in individual cells can be surprisingly noisy. In unicellular organisms this noise can be functional. For example, it may allow a few cells in a bacterial population to enter a stress prepared state that allows survival of a sudden environmental stress. The role of gene expression noise in multicellular organisms is less clear. I will first discuss our work using alternative sigma factor circuits in bacteria as model systems for understanding noisy output from genetic circuits. Using a combination of single-cell time-lapse microscopy, synthetic biology techniques and mathematical modelling, we have investigated the circuit properties required for functional noise in gene regulation. I will then look at the role of noise in a multicellular system. Our work is revealing an unexpected level of variability in gene expression between and within genetically identical plants. This noise in gene expression may act as a mechanism for generating functional phenotypic diversity in plants, similar to how it does in bacteria.

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Multiscale, dynamic and spatially-periodic coordination of gene expression in the developing spinal cord

Dr Veronica Biga[1], Mr Joshua Hawley, Dr Cerys Manning, Prof Nancy Papalopulu, Prof Paul Glendinning
[1]*The University Of Manchester, Manchester, United Kingdom*

We investigated the pattern of expression of Hairy and enhancer of split 5 (HES5) in the ex vivo spinal cord [1]. We found evidence of organization of gene expression into a dynamic and spatially-periodic pattern consisting of clusters of few cells with correlated HES5 levels repeated along the dorsal to ventral axis. The behaviour of pairs of cells in a microcluster showed coordination of gene expression at different timescales: (i) mean HES5 levels were highly correlated between cell pairs and their persistence time matched that of microclusters, approx. 6-8h; (ii) on top of slow-varying changes in mean HES5, cell pairs showed local (but not global) in-phase coordination of small amplitude ultradian oscillations (3-4h) [2].

We generated a multicellular coupled model with delayed intercellular repression to show that microclusters of local in-phase activity can emerge from cell:cell interactions at weak coupling strength [2]. Using an extended coupled model including long-range interactions and perturbations from differentiating cells, we explained the emergence of spatially periodic and dynamic microclusters resembling the data[3].

Our coupled model indicated that increasing values of coupling strength can switch the pattern from local in-phase to global synchrony to alternating on-off and this was associated with an increase in rate of differentiation. Indeed, we found that the spatial patterns of the interneuron (microclusters) vs motorneuron (alternating) domains varied predictably with changes in rate of differentiation controlled by coupling strength in our model [2].

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Effect propagation through gene regulatory networks

Natalia Ruzickova[1], Gasper Tkacik[1]
[1]*Institute of Science and Technology Austria, Klosterneuburg, Austria*

Genetic variation leads to differences in measurable traits implicated in health and disease. Finding genetic variants associated with relevant traits is at the forefront of medical genetics and is spearheaded by "genome-wide association studies" (GWAS) - statistically rigorous procedures whose power has grown with the number of sequenced genomes. Nevertheless, GWAS have a substantial shortcoming: they are ill-equipped to detect the causal basis and reveal the complex systemic mechanisms of polygenic diseases, such as cancer. As the well-known Omnigenic model proposed, even a single genetic change can propagate throughout the entire genetic regulatory network, causing a myriad of spurious non-causal GWAS detections which drastically limits GWAS usefulness. We aim to address this shortcoming with a novel statistical framework: to predict gene expression levels, we explicitly model the propagation of genetic effects through an experimentally reconstructed transcriptional regulatory network. Describing indirect (trans) genetic effects as a result of the propagation of direct (cis) effects has the potential to uncover causal regulatory mechanisms while reducing the number of parameters by orders of magnitude compared to GWAS. In a yeast model, we reached performance comparable to traditional GWAS-like models, while obtaining interpretable results, thereby supporting the omnigenic hypotheses. Furthermore, our approach can be extended to include the propagation of environmental effects through regulatory networks, bridging statistical genomics with systems biology.

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A dynamical systems approach to design function of Gene Regulatory Networks

Dr Ruben Perez-Carrasco[1]

[1]*Imperial College London, LONDON, United Kingdom*

Traditional descriptions of cellular states involve static pictures of gene expression. Nevertheless, during embryo development cells are under constant transformation. Similarly, synthetic circuits used for biocomputation require a dynamical response. This limitation of our static picture can be understood using bifurcation theory, providing information of the transient behaviours and linking them with the different steady states of gene expression. In this talk I will show how we can use this knowledge together with Bayesian computation to design experimentally and computationally circuits with new custom dynamical functionalities including a synthetic spatial bistable switch, the AC/DC circuit, and the mushroom bifurcation.

Perception and propagation of activity through the cortical hierarchy is determined by neural variability

Dr. James Rowland[1,5], Mr. Thijs van der Plas[1,5], Mr. Matthias Loidolt[1,2,4,5], Dr. Robert Lees[1], Mr. Joshua Keeling[2], Mr. Jonas Dehning[2], Dr. Thomas Akam[3], Dr. Viola Priesemann[2], Dr. Adam Packer[1]
[1]Department of Physiology, Anatomy and Genetics; University of Oxford, Oxford, United Kingdom, [2]Max-Planck-Institute for Dynamics and Self-Organization, Goettingen, Germany, [3]Department of Experimental Psychology; University of Oxford, Oxford, United Kingdom, [4]Laboratory for Molecular Cell Biology; University College London, London, United Kingdom, [5]These authors contributed equally., ,

The brains of higher organisms are composed of anatomically and functionally distinct regions performing specialised tasks; but regions do not operate in isolation. Orchestration of complex behaviours requires communication between brain regions, but how neural activity dynamics are organised to facilitate reliable transmission is not well understood. We studied this process directly by generating neural activity that propagates between brain regions and drives behaviour, allowing us to assess how populations of neurons in sensory cortex cooperate to transmit information. We achieved this by imaging two hierarchically organised and densely interconnected regions, the primary and secondary somatosensory cortex (S1 and S2) in mice while performing two-photon photostimulation of S1 neurons and assigning behavioural salience to the photostimulation. We found that the probability of perception is determined not only by the strength of the photostimulation signal, but also by the variability of S1 neural activity. Therefore, maximising the signal-to-noise ratio of the stimulus representation in cortex relative to the noise or variability in cortex is critical to facilitate activity propagation and perception. Further, we show that propagated, behaviourally salient activity elicits balanced, persistent, and generalised activation of the downstream region. Hence, our work adds to existing understanding of cortical function by identifying how population activity is formatted to ensure robust transmission of information, allowing specialised brain regions to communicate and coordinate behaviour.

Location and concentration of aromatic-rich segments dictates the percolating inter-molecular network and viscoelastic properties of ageing condensates

Jorge Rene Espinosa

Maturation of functional liquid-like biomolecular condensates into solid-like aggregates has been linked to the onset of several neurodegenerative disorders [1]. Low-complexity aromatic-rich kinked segments (LARKS) contained in numerous RNA-binding proteins can promote the aggregation process by forming inter-protein □-sheet fibrils that accumulate over time—ultimately driving the liquid-to-solid transition of the condensates [2]. However, key open questions such as how the behaviour of individual molecules within condensates, and their intermolecular interactions, change over time; or if we can rationally design protein sequence modifications to prevent ageing, remain open [3]. In this study, we combine atomistic Molecular Dynamics simulations with sequence-dependent coarse-grained models of various resolutions [4,5] to investigate the role of LARKS abundance and position within the amino acid sequence in the maturation of biomolecular condensates. We find that the location of the LARKS motifs along the protein sequence crucially determines the rate of cross-□-sheet transitions and the associated loss of liquid-like behaviour over time. Our simulations show that shifting the location of the LARKS-containing domain in fused in sarcoma (FUS) protein towards its centre slows down condensate aggregation. More strikingly, our simulations further predict that adding RNA to FUS with re-located LARKS fully inhibits the accumulation of □-sheet fibrils, maintaining functional liquid-like behaviour without ageing [6]. Taken together, this work sheds light on a key area of research aiming to advance our molecular understanding of condensate ageing, which is needed to design novel therapies to prevent age-related diseases caused by condensate dysregulation.

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Multiscale modelling of liquid-like chromatin organisation
Rosana Collepardo Guevara

The three-dimensional organisation of the DNA is one of the great marvels of physical biology. By winding around a special class of proteins, the metre-long DNA manages to compress enormously to fit inside tiny (6 μm) nuclei, avoid entanglement and, moreover, maintain exquisite control over the accessibility of the information it carries. The structure of this remarkable complex of DNA and proteins, known as chromatin, determines how easily the DNA can be accessed and, thus, it is intimately linked to gene expression regulation. In this talk, I will present our multiscale modelling techniques designed to investigate the structure of chromatin in conditions that mimic those inside cells (Farr et al, Nature Communications, 2021). I will discuss why nucleosomes, the building blocks of chromatin, should be viewed as highly plastic particles that foster multivalent interactions and promote chromatin's liquid-liquid phase separation.

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Counting the cold: do flowering plants encode winter memory with large nuclear assemblies?

Dr Alex Payne-Dwyer[1], Dr Govind Menon[2], Dr Geng-Jen Jang[2], Dame Professor Caroline Dean[2], Professor Martin Howard[2], Professor Mark Leake[1]

[1]University of York, York, United Kingdom, [2]John Innes Centre, Norwich, United Kingdom

Canonical epigenetic regulation involves addition of chemical marks, such as trimethylation, to histones. These marks encode silenced genetic states and maintain functional memory and state switching in cells. However, the small number of marks at each locus means this process alone is insufficient to retain memory over multiple cell cycles. We consider a complementary framework [1] in which large protein assemblies can provide the robust feedback needed to avoid degradation of memory, and demonstrate rapid single-molecule imaging to support this model.

Silencing of the gene FLOWERING LOCUS C (FLC) in Arabidopsis thaliana directly regulates timing of flowering after winter [2]. We adapt the single-molecule microscopy technique, Slimfield [3] to image live root tips. We then track the in vivo dynamics of the Polycomb-interacting protein, VRN5, and its counterpart VIN3, which is induced at low temperatures [4].

We find that these proteins form striking, dynamic assemblies of tens of molecules in the nucleus in response to prolonged cold exposure. These assemblies are sufficiently large and persistent to act as strong candidates in our model for initiating and maintaining the silenced state of the FLC gene, leading to vernal flowering. Our model indicates that the robust statistics of self-assembly could have important, general implications for epigenetic processes in eukaryotes.

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A model system reveals how different linker histones direct chromatin to liquid-like or fibre-like condensed states

Katherine Stott[1], Matthew Watson[1]
[1]*University of Cambridge, , United Kingdom*

In all organisms, metre-length DNA is packaged to a micrometre scale through charge neutralisation by basic polymers. In eukaryotes, the main condensing polymers are the histone proteins and the resulting protein/DNA complex is called chromatin. Chromatin organisation is hierarchical: the basic unit is the nucleosome, arrays of which form “beads on a string” that make up a 10 nm fibre. The second stage of condensation is achieved through binding of a different set of histones, the linker histones, which ultimately dictates DNA accessibility. This stage is more enigmatic and results in condensed states that range from dynamic and liquid-like to the more traditional textbook 30-nm fibres, depending on many factors, including the type of linker histone. The growing appreciation of dynamics in chromatin packaging has paralleled developments by us and others in our “bottom-up” understanding of the linker histone proteins themselves. We have developed an in-vitro model system of linker histone and linker DNA, which although very minimal, displays surprisingly complex behaviour, and is sufficient to model the known states of H1-condensed chromatin: disordered complexes (“open” chromatin), dense liquid-like assemblies (dynamic condensates) and higher-order structures (organised 30-nm fibres). A crucial advantage of such a simple model is that it allows the study of the various states by NMR, CD and scattering methods. Moreover, it allows capture of the thermodynamics underpinning the transitions between states through calorimetry. With these methods we can rationalise the distinct condensing properties of linker histone variants across species that are encoded by their amino acid sequence.

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How the condensation behaviour of the RNA binding protein TDP-43 influences RNA binding and regulation

Martina Hallegger[1], Anob M Chakrabarti[1], Flora CY Lee[1], Bo Lim Lee[4], Klara Kuret[5], Aram Amalietti[5], James Shorter[4], Nicholas M Luscombe[6], Jernej Ule[2]

[1]The Francis Crick Institute, London, UK, [2]UK Dementia Research Institute at King's College London, , UK, [3]Department of Genetics, Environment and Evolution, UCL , , UK, [4]Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Pennsylvania, US, [5]National Institute of Chemistry, Ljubljana, Slovenia, [6]Okinawa Institute of Science & Technology Graduate University, Okinawa, Japan

The condensation of RNA binding proteins (RBPs) is fundamentally important to organise our cells by forming membranless organelles. The impairment of these RBP condensate to form has been linked to several neurological disease. Mutations in the intrinsically disordered regions (IDRs) of several RBPs were linked to motor neuron disease and Frontotemporal dementia and the aggregation of the TDP-43 and FUS RBPs are a hallmark of these pathologies. To investigate the function of RBP condensates in our cells and how they become impaired in diseases novel technologies are necessary. Here we describe how UV crosslinking and immunoprecipitation (iCLIP) allows us to monitor the multivalent interaction of RBPs with RNA on a transcriptome-wide level and how condensation changes alter RNA binding. iCLIP exploits zero-length covalent protein-RNA crosslinking induced by UV-light in living cells to map protein-RNA contacts. Thereby we purify short RNA fragments that are crosslinked to a specific RBP and we then identify these fragments by sequencing.

By integrating iCLIP and 3' mRNA-Seq, we show that TDP-43 condensation promotes the efficient assembly on a subset of RNAs binding sites and promotes their 3' end processing (Hallegger et al; 2021; doi.org/10.1016/j.cell.2021.07.018). TDP-43 condensation is required for efficient assembly on RNA regions with long clusters with specific motifs in a dispersed conformation. These 'binding-site condensates' are promoted by homomeric interactions through a conserved peptide element in the IDR that adapts a helical confirmation and this homomeric assembly is required for efficient regulation of a subset of bound transcripts, including autoregulation of TDP-43 mRNA.

Our findings establish that the manipulation of TDP-43 condensation through biophysically informed IDR mutations can selectively fine tune its RNA-regulatory networks by modulating its RNA interactions. We speculate that disease associated changes in condensation would interfere with this network regulation, particularly early in disease and would have profound effects on TDP-43 autoregulation.

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Coarse-grained simulations of biomolecular condensates with the Martini forcefield

Dr Chris Brasnett[1], Mr Liguo Wang[1], Dr Maria Tsanai[1], Mr Fabian Grünewald[1], Professor Dr Siewert-Jan Marrink[1]

[1]*Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institute for Advanced Materials, University of Groningen, Groningen, , The Netherlands*

Molecular dynamics simulations can provide insights into the physical behaviour of biological systems, on length and time scales inaccessible to experiments. While atomistic simulations can provide a very high level of resolution into biomolecular processes, they are limited by both the time and length scales they can reasonably probe. To overcome this, the latest version of the Martini coarse-grained force field represents several atoms as a single particle called a bead, which interact via effective potentials.

It is increasingly evident that biomolecular condensates are formed by molecules on a multitude of scales, from peptides with sticker-and-spacer motifs up to intrinsically disordered proteins. Using coarse-grained simulations with the Martini force field, we have investigated peptides and disordered proteins known to form both simple and complex coacervates, and demonstrate that the Martini force field can reproduce experimental phenomena. The generic coarse-grained nature of the Martini force field can be further used to highlight the power of simulation methods in understanding the physical properties of these condensates. To this end, we have investigated phenomena such as the partitioning of small molecules, the calculation of upper critical solution temperatures, and the dynamic internal structure of coacervates, to further understand the physico-chemical of liquid-liquid phase separation in peptide-based systems.

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Does evolution have an inbuilt Occam's razor?

Ard Louis

Why does evolution favour symmetric structures when they only represent a minute subset of all possible forms? One argument might be natural selection. Here we provide a different explanation based on biases in the arrival of variation. Just as monkeys randomly typing into a computer language will preferentially produce outputs that can be generated by shorter algorithms, so the coding theorem from algorithmic information theory predicts that random mutations, when decoded by the process of development, preferentially produce phenotypes with shorter algorithmic descriptions. Since symmetric structures need less information to encode, they are much more likely to appear as potential variation. Combined with an arrival-of-the-frequent population genetics mechanism, this evolutionary Occam's razor predicts a much higher prevalence of low-complexity (high-symmetry) phenotypes than follows from natural selection alone. It explains striking patterns observed in protein complexes[1], RNA secondary structures[2], a gene regulatory network and Richard Dawkins' biomorphs model of development [3].

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[2] Phenotype bias determines how RNA structures occupy the morphospace of all possible shapes, Kamaludin Dingle, et al., Molecular Biology and Evolution, 39, msab280 (2021)
[3] Bias in the arrival of variation can dominate over natural selection in Richard Dawkins' biomorphs, Nora Martin et al., arxiv (2023)

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Quantitatively Understand Microbial Community Traits During Evolution

Wenying Shou

Using a synthetic yeast community as a case study, I will illustrate how quantitative measurements and mathematical modelling together can allow us to understand community-level traits in terms of individual-level phenotypes. I will also demonstrate how rapid evolution can quickly alter community-level traits.

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A theory of the evolution of multi-site population resistance/rescue and how the genetic diversity of the population significantly increases its probability.

Dr Bhavin Khatri[1], Prof Austin Burt[1], Mr Kieran Chopra[1]
[1]*Imperial College London, Ascot, United Kingdom*

CRISPR-based gene drives — a genetic modification where a deleterious trait is introduced, to force super-Mendelian inheritance, "driving" it into the population despite its harmful effects — have the potential for controlling natural populations of disease vectors, such as malaria-carrying mosquitoes in sub-Saharan Africa. If successful, they hold promise of significantly reducing the burden of disease and death from malaria and many other vector-borne diseases. A significant challenge to success, is the evolution of resistance: can the mosquito populations develop resistance before are eliminated? This is a classic population rescue problem and of great importance to the evolution of bacterial resistance and immune escape of viruses. A potential anti-resistance strategy is to design a gene drive which makes multiple modifications, which all must develop resistance to stop a population crash. Exact theory is difficult as it needs solution of the multi-allelic diffusion equation of population genetics, in a situation when detailed balance is not obeyed. Using a simple heuristic approach, we find the probability of population resistance/rescue — unlike for resistance from a single site — is very sensitive to genetic diversity that already exists in the population, increasing the probability of resistance by orders of magnitude (PNAS, Khatri et al). However, using simulations where mosquitos are able to migrate, populations undergo a novel non-equilibrium spatial phenomenon of "chasing", with indefinite persistence, as the wild-type and drive play a form of spatial rock-paper-scissors game with empty space — the question then becomes when not if resistance will arise...

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Phenotypic noise as an evolutionary trait in bacteriophage populations

Diana Fusco[1], Wolfram Moebius[2], Somenath Bakshi[1], Michael Hunter[1], Temur Yunusov[1], Charlie Wedd[1]
[1]University of Cambridge, Cambridge, United Kingdom, [2]University of Exeter, Exeter, United Kingdom

The life history parameters of lytic phages is typically summarized by three quantities -adsorption rate, lysis time and bust size- which are traditionally measured as bulk averages from liquid culture experiments. Directed evolution experiments in different bacterial conditions have been shown to select for different optimal sets of these parameters in qualitative agreement with predictions from simple models of phage infection. In a variation of such experiments, we have recently evolved lines of phage T7 that spread 5 times faster than the ancestor on two-dimensional bacterial lawns. Surprisingly, in contrast to model expectations and previous similar experiments that predict a decrease in adsorption rate, our evolved lines fail to show any significant or consistent change in life history parameters bringing into questions both the assumptions underneath current models and the experimental methods we use to assess phage-bacteria interactions.

In an attempt to provide and test a hypothesis for our surprising experiment, I will present simulation results revealing that a slightly wider distribution of lysis time can confer 5-6% fitness advantage to a phage population, while going undetected in traditional phenotypic assays. To bypass this problem and precisely quantify the amount of stochasticity in phage-bacteria interactions, I will introduce a novel platform that leverages CRISPR gene editing of phages and mother-machine microfluidic experiments to monitor phage infection dynamics at single-cell resolution over thousands of cells simultaneously.

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Is the variation of intragenic DNA methylation in Arabidopsis natural populations governed by genetic or epigenetic inheritance?

Dr Amy Briffa[1], Dr Elizabeth Hollwey[1,2], Dr Zaigham Shahzad[1,3], Dr Jonathan Moore[1], Dr David Lyons[1], Professor Martin Howard[1], Professor Daniel Zilberman[1,2]

[1]John Innes Centre, Norwich, United Kingdom, [2]Institute of Science and Technology, , Austria, [3]Lahore University of Management Sciences, , Pakistan

Gene body methylation (gbM) in Arabidopsis shows stable transgenerational inheritance, despite the imperfect fidelity of semiconservative maintenance by Dnmt1/MET1, thus providing an ideal system to study the fundamental dynamics of CG methylation at both short and evolutionary timescales.

Through an integrated experimental and computational approach, we find substantial MET1 de novo activity, which is strongly enhanced by existing proximate methylation (i.e. cooperative feedback). This cooperative de novo MET1 activity both seeds and stably propagates gbM, demonstrating that intragenic mCG establishment and inheritance constitute a unified epigenetic process. It is suppressed by the histone variant H2A.Z and active demethylation at gene-ends to produce localized mCG patterns.

It is an open question whether mCG variation in natural populations is governed by genetic or epigenetic inheritance. We develop a mathematical model that precisely reproduces the spatial mCG inheritance dynamics, and can predict intragenic steady-state mCG patterns from zero initial methylation (given only the CG site spacing within methylatable regions as input).

Remarkably, even in the absence of any selection pressure, our simple stochastic model of purely epigenetic inheritance can explain the majority of the observed population-scale variation, providing strong evidence that gbM is a stochastic phenomenon. We find that intragenic mCG undergoes large, millennia-long epigenetic fluctuations, and can therefore mediate evolution on this timescale.

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Mechanical interactions affect the growth and evolution of bacterial colonies

Dr Bartlomiej Waclaw[1]

[1]*The University Of Edinburgh/dioscuri Centre Ipc Warsaw, , United Kingdom*

Biofilms are not only ubiquitous in nature but also play a major role in industry and medicine, where biofilm growth on implants and catheters, and the evolution of antibiotic resistance is a concern.

Thanks to the work of researchers from the soft-matter and statistical physics community (including our group), we now know that mechanical interactions between bacterial cells and the surface on which they grow affect the shape of bacterial colonies and population dynamics of novel genetic variants in such colonies. This has been mostly investigated in bacterial colonies grown on agarose gel. When a colony is initiated with a mixture of two different fluorescent microbial strains, radial expansion and random fluctuations in the number of bacteria at the edge of the colony lead to the formation of "sectors" of genetically related bacteria.

Here I will discuss our work that shows how cell shape, cell-cell and cell-substrate adhesion and friction affect the probability that a faster-growing mutant 'surfs' at the colony's frontier and creates a macroscopic sector. Although all these physical factors contribute to the surfing probability in seemingly different ways, I will show that they do it by affecting two key parameters that have a causal relationship on the surfing probability: front roughness and cell alignment.

Our work suggests a possible new approach to fighting persistent biofilms: the physical properties of bacterial cells could be targeted alongside standard antimicrobial therapy to kill the bacteria while at the same time reducing the probability of evolving resistance to antibiotics.

Title to be confirmed
Pietro Lio

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Combinatorial approaches for understanding morphogenesis in 3D embryos

Dr Salvish Goomanee

Cells in tissues are inherently linked to their neighbours along their common interfaces which gives rise to mechanical forces being exerted onto each other and on their environment. These are complex interactions that allow for morphogenetic events to occur. Such interactions can be studied via vertex models that have been initially developed to study foams. For example, epithelial tissue can be viewed as a 2D network where one describes the epithelia by a set of vertices and interactions among them as edges which capture the mechanical constraints externally imposed. This is well documented in the literature. We are particularly interested in cell fate analysis as a result of such interactions in 3D embryos. We leverage techniques from discrete and computational geometry to construct 3D meshes from which we extract we equivalent graph with structured data. We employ state of the art machine learning techniques to study the resulting graphs in order to predict pertinent physical quantities such as line tensions between embryos and pressures within each embryo.

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CellPhe: a toolkit for cell phenotyping using time-lapse imaging and pattern recognition

Laura Wiggins

With phenotypic heterogeneity in whole cell populations widely recognised, the demand for quantitative and temporal analysis approaches to characterise single cell morphology and dynamics has increased. We present CellPhe, a pattern recognition toolkit for the characterisation of cellular phenotypes within time-lapse videos. To maximise data quality for downstream analysis, our toolkit includes automated recognition and removal of erroneous cell boundaries induced by inaccurate tracking and segmentation. We provide an extensive list of features extracted from individual cell time series, with custom feature selection to identify variables that provide greatest discrimination for the analysis in question. We demonstrate the use of ensemble classification for accurate prediction of cellular phenotype and clustering algorithms for the characterisation of heterogeneous subsets, validating and proving adaptability using different cell types and experimental conditions. Furthermore, we provide an example application for CellPhe to characterise response to chemotherapy, quantifying a population's response to varying concentrations of drug and identifying a subset of "non-conforming" treated cells that resist treatment. Our methods extend to other imaging modalities, such as fluorescence, and would be suitable for all time-lapse studies including clinical applications and drug screening.

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Probing the rules of cell coordination in live tissues by interpretable machine learning based on graph neural networks

Takaki Yamamoto[1], Katie Cockburn[2,3], Valentina Greco[2,4], Kyogo Kawaguchi[1,5,6]

[1]Nonequilibrium Physics of Living Matter RIKEN Hakubi Research Team, RIKEN Center for Biosystems Dynamics Research, Kobe, Japan, [2]Department of Genetics, Yale School of Medicine, New Haven, United States of America, [3]Department of Biochemistry and Rosalind & Morris Goodman Cancer Institute, McGill University, Montreal, Canada, [4]Departments of Cell Biology and Dermatology, Yale Stem Cell Center, Yale Cancer Center, Yale School of Medicine, New Haven, United States of America, [5]RIKEN Cluster for Pioneering Research, Kobe, Japan, [6]Universal Biology Institute, The University of Tokyo, Tokyo, Japan

During homeostasis and development, cells interact biochemically and mechanically with each other to maintain, grow, and deform the tissues. What mechanisms underly the robustness of multicellular systems is a fundamental question in biology, and whether there is universality in the rules governing multicellular dynamics is also an interesting problem from the point of view of physics. However, even with modern high-throughput cell profiling technologies and high-resolution microscopy, it is still challenging to identify the rules of cell-to-cell interactions due to the complexity of the problem and the limited methods to perform perturbation experiments. In order to seek and compare the different rules enforced in multicellular dynamics, it will be desirable to construct a systematic computational method that extracts the rules from live images and cell tracks. We here demonstrate that graph neural network (GNN) models are suited for this purpose, by showing how they can predict cell fate and expose the hidden rules that possibly govern the dynamics. Analyzing the mammalian epidermis data, where spatiotemporal graphs constructed from cell tracks and cell contacts are given as inputs, GNN discovers distinct neighbor cell fate coordination rules that depend on the region of the body. This approach demonstrates how the GNN framework is powerful in inferring general cell interaction rules from live data without prior knowledge of the signaling involved.

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Topic Modeling to analyze spatial transcriptomic data from Allen Human Brain Atlas

Letizia Pizzini[1]

[1]*University of Turin, Turin, Italy,* [2]*INFN Turin, Turin, Italy*

Human brain is a complex interconnected structure controlling all elementary and high-level cognitive tasks. The availability of dataset with vastly enhanced structural coverage allows an explicit approach aimed at identifying genetic networks common across individuals which is related
to structural and functional organization of the entire brain. Allen Human Brain Atlas consists of microarray data in 3702 spatially distinct samples taken from six neurotypical adult brains, quantifying the expression levels of more than 20,000 genes. To understand large-scale transcriptome organization, we apply a Topic Modeling tool called hierarchical Stochastic Block Modeling (hSBM) to a set of highly variable genes. Topic models are a group of algorithms developed to infer the latent topical structure of a collection of documents. hSBM is a generative model and differs
from other methods describing the problem as a community detection problem on a bipartite network, where one layer has samples as nodes, the other one has genes and links are weighted accordingly to the expression of the gene in the sample. The goal would then be to cluster similar brain samples and to identify the sets of genes (topics) that characterize each subtype, in order to compare samples organization into clusters with the
anatomical structure and functional connectivity.

One of the key results of the work is that different levels of clustering
present clusters composed quite homogeneously of samples coming from six donor brains. This is proof that the algorithm does not focus on inter-individual differences in gene expression but captures characteristics of the dataset that can be considered conserved between subjects.

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Physics-informed neural networks for solving the inverse Turing problem

Antonio Matas Gil[1], Prof. Robert Endres[1], Dr. Roozbeh Pazuki[1]
[1]*Imperial College London, London, United Kingdom*

Morphological patterns are ubiquitous in everyday life and in a wide range of scales. A chemical basis for this process was proposed by Alan Turing in his 1952 paper using diffusing activator and inhibitor molecules [1]. Turing models have been replicated in chemical systems, but even after 70 years it has not been possible to robustly engineer these patterns in biological organisms. In our research, we aim to use least-squares minimisation and physics-informed neural networks [2] to solve the inverse Turing problem: given a Turing pattern, we want to recover the model parameters that can generate this pattern – a task made difficult due to sensitivity of the patterns to the initial conditions. In particular, we investigate the dependence of the solution on the number of image pixels and level of noise. Solving the inverse Turing problem will ultimately aid the robust engineering of Turing patterns using synthetic circuits.

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Decoding cellular identities from single-cell data

Zoe Piran[1], Mor Nitzan[1]
[1]*The Hebrew University of Jerusalem, Jerusalem, Israel*

Uncovering the underlying biological processes in single-cell data is a challenging problem since a cell's gene expression profile contains multiple, overlapping signals arising from its internal state or from external interventions. Consequently, focusing on a single signal (measured or recovered) may miss important cellular attributes. To this end, we present two data-driven methods for filtering and disentangling single-cell data. First, we present SiFT (Signal Filtering), a flexible and robust framework for filtering signals induced by different biological processes in single-cell data. For the problem of disentangling cellular factors of variation, we present bioLORD, a deep learning framework for disentangling and reconstructing observed and hidden attributes in single-cell data. The disentangled representation of the data exposes the underlying structure of different cellular identities. The reconstructed expression, based on a generative model, allows for the prediction of unseen states and the identification of gene expression trends. As we demonstrate over a diversity of tasks these methods allow for uncovering and studying different facets of cellular identities. To recover underlying biological structure we leverage prior knowledge regarding liver zonation to filter the spatial signal from single-cell liver data thereby enhancing the temporal circadian signal. Next, considering a case-control setting, we expose disease-related dynamics in COVID-19 data and highlight disease-informative cells and their underlying disease response pathways by filtering reference samples from healthy donors. At last, we show the applicability of the generative framework for accurate out-of-distribution predictions of drug perturbation response and the recovery of gene expression trends in response to infection.

Keynote VI: **Super-resolution imaging of transcription in living cells**

Ibrahim Cisse (Germany)

We will discuss the latest efforts in our laboratory to develop highly sensitive methods of microscopy, to go directly inside living cells and uncover the behavior of single biomolecules as they effect their function in transcription.

Transcription is the first step in gene expression
regulation, during which genetic information on DNA is decoded into RNA transcripts.

Methodologically, the so-called live cell single molecule and super resolution techniques –that break the optical diffraction limit– are revealing with unprecedented spatial and temporal resolutions, novel emergent phenomena inside the living cells.

We will discuss our recent discoveries on highly dynamic biomolecular clustering, and phase transitions in vivo.

These discoveries are challenging the 'textbook view' on how our genome (DNA) is decoded in living cells

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Control of cortical mechanics by signalling

Guillaume Charras

As they enter mitosis, cells undergo profound shape changes that are controlled by mechanical changes in the submembranous actin cortex, a thin meshwork of actin filaments, myosin motors and actin-binding proteins. These mechanical changes are controlled by RhoGTPases, key regulators of the cytoskeleton and contractility. In turn, two classes of proteins regulate RhoGTPase activity: RhoGEFs activate them, while RhoGAPs inactivate them. Despite their central role in cell morphogenesis, we know little about how RhoGEF recruitment leads to the changes in cell mechanics that drive shape change. This is because we do not know how to link RhoGEF localisation, RhoGTPase activity, actomyosin force generation, and mechanics. Here, we use optogenetics to relocalise the DH-PH domain of RhoGEFs to the membrane to examine the dynamics of the mechanical changes induced by RhoA activation. We find that relocalisation of optogenetic actuators to the membrane in response to a single pulse of blue light lead to recruitment of myosin and a ~2-fold increase in cortical tension as well as changes in cell elasticity and viscosity. Using a coarse grained model of the signalling cascade downstream of optogenetic activation, we show that the different steps in the cascade in response to a single pulse of light can be linked by a delay and a rescaling with upregulation of tension occurring ~50s after activation. Our approach paves the way for a quantitative understanding of the effect of signalling on cortex mechanics.

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Mechanical control of immune synapse organisation and function

Katelyn Spillane

Abstract currently unavailable.

Friction forces determine cytoplasmic reorganization and shape changes of ascidian oocytes upon fertilization

Silvia Caballero Mancebo[1,2], Andrew Callan-Jones[3], Raphaël Voituriez[4,5], Carl-Philipp Heisenberg[2]
[1]London Centre for Nanotechnology, University College London, London, United Kingdom, [2]Institute of Science and Technology Austria, Klosterneuburg, Austria, [3]Laboratoire de Matière et Systèmes Complexes, Université de Paris, Paris, France, [4]Laboratoire Jean Merrin, Sorbonne Université and CNRS, Paris, France, [5]Laboratoire de Physique Théorique de la Matière Condensée, Sorbonne Université and CNRS, Paris, France

Contraction and flow of the actin cell cortex have emerged as a common principle by which cells reorganize their cytoplasm and take shape. This is crucial in large cells like oocytes in which cytoplasmic (ooplasmic) reorganizations direct patterning of the oocyte for later stages of embryonic development and morphogenesis. However, how these cortical flows interact with adjacent cytoplasmic components, changing their form and localization, and how this affects cytoplasmic organization and cell shape remains unclear. Here, we show that in ascidian oocytes, a well-established model of oogenesis, the coordinated activities of cortical actomyosin flows and deformation of the adjacent mitochondria-rich myoplasm drive oocyte cytoplasmic reorganization and shape changes following fertilization. By combining biophysical experimentation and theory, we show that fertilisation induces an increase in cortical tension that triggers vegetal-directed cortical actomyosin flows. These flows, resisted by friction with the subcortical myoplasm, lead to the accumulation of cortical actin at the vegetal pole of the zygote and compression and local buckling of the adjacent elastic solid-like myoplasm layer. Once cortical flows have ceased, the myoplasm straightens and resolves its multiple buckles into one larger buckle, which again drives the formation of the contraction pole, a protuberance of the zygote's vegetal pole where maternal mRNAs accumulate. Thus, our findings reveal a novel mechanism of cortical actomyosin network flows that determine cytoplasmic reorganization and cell shape by deforming adjacent cytoplasmic components through friction forces.

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Study of E-cadherin and actin dynamics during cell-cell adhesion using a cell - lipid bilayer assay

Sayantika Ghosh[1], Helena Coker[2], Marco Fritzsche[2], Darius Koester[1]

[1]University Of Warwick, Warwick Medical School, , United Kingdom, [2]University of Oxford, Kennedy Institute of Rheumatology, , United Kingdom

The formation and regulation of connections between cells play a crucial role in maintaining tissue homeostasis and tumour repression. In many tissues, particular importance comes to adherens junctions that are formed by the binding of E-cadherin proteins at the surface of adjacent cells, followed by their clustering and recruitment of catenins and other adaptor proteins on the intracellular site linking the protein complex to the actomyosin cytoskeleton. This process depends on multiple parameters such as the mobility, density, type of cadherins, and the mechanical forces acting on the cadherin-catenin complexes and can change dramatically during developmental stages or in diseases such as cancer.
To study the initial steps of adherens junction formation in a controlled environment in cancer cells, we have established a reconstituted system of cells adhering to supported lipid bilayers decorated with extracellular domains of E-cadherin proteins. This reconstituted system allowed us to capture the initiation and dynamics of cadherin interactions, the real-time localisation and clustering of adaptor proteins and the maturation of adherens junction in cancer cells over time using live cell imaging with confocal and total internal reflection microscopy. To understand the importance of the different parameters and to mimic the cell/tissue abnormalities occurring in cancer scenarios, we then perturb the controlled system by varying cadherin density, membrane mobility and inducing mechanical stress by confining the cell system over time. We find, for example, that reduced E-cadherin mobility leads to the formation of stable regions showing actin enrichment and that confinement increases the steady state adhesion zone. This approach provides new insights about cell surface dynamics during cell-cell adhesion and can improve our understanding of the role of cell mechanics and E-cadherin organisation patterns in cancer progression.

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Using AFM and automated image analysis to study the correlation between bacterial shape and Peptidoglycan orientation in B. subtilis

Dr Laia Pasquina-Lemonche[1], Dr Oliver Meacock[3], Dr Abimobla Feyisara-Olulana[1], Prof Simon Foster[1], Prof Jamie Hobbs[1], Prof Ethan Garner[2]

[1]University of Sheffield, Sheffield, United Kingdom, [2]Harvard University, Boston, USA, [3]University of Lausanne, Lausanne, Switzerland

The current antibiotics do not work to combat common bacterial infections because the cells are developing resistance mechanisms. One of the most common antibiotic targets is the bacterial cell wall. We need to use biophysical approaches to understand the synthesis and organisation of the cell wall to find better solutions against AMR bacterial strains.

After years of research using atomic force microscopy (AFM), the conclusion is that the cell wall, composed mainly of peptidoglycan is a highly porous heterogeneous hydrogel with four different architectures [1]. In this project, we deciphered the molecular architecture (on the order of 1 nm) of Bacillus subtilis cell wall. Then, we applied the same methods to study genetically modified strains where their two main synthesis mechanisms were controlled with inducer conditions. B. subtilis is a rod and it has two types of synthesis machineries with different trajectories: the mreB protein moves in helical movements along the short axis of the cell and the pbp2 protein moves randomnly distributed along the cell [2]. The mutant strains where the activity of mreB or pbp2 proteins can be overexpressed or underexpressed were imaged with AFM. The peptidoglycan fibres forming their internal architecture were detected and analysed with a custom-made automated image analysis routine [3]. The aim of this work is to understand the relationship between the proteins trajectories, the cell shape and the peptidoglycan organisation at nanometric level.

[1] L. Pasquina-Lemonche*, J. Burns*, et al. Nature, 582, 294-297 (2020).
[2] Dion MF, Kapoor M, Sun Y, et al. Nat Microbiol. 4(8):1294-1305. (2019)
[3] O. Meacock*, L. Pasquina-Lemonche*, A. Feyisara-Ouluana, W. M Durham, E. Garner, S. J Foster, J. K Hobbs (in preparation).

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Model of inverse bleb growth explains giant vacuole dynamics during cell mechanoadaptation

Andrea Cairoli[1,2], Mrs Alice Spenlehauer, Prof Darryl Overby[1], Dr Chiu Fan Lee[1]
[1]Imperial College London, London, United Kingdom, [2]University of Cambridge, Cambridge, United Kingdom

Cells can withstand hostile environmental conditions manifest as large mechanical forces such as pressure gradients and/or shear stresses by dynamically changing their shape. Such conditions are realized in the Schlemm's canal of the eye where endothelial cells that cover the inner vessel wall are subjected to the hydrodynamic pressure gradients exerted by the aqueous humor outflow. These cells form fluid-filled dynamic outpouchings of their basal membrane called giant vacuoles. The inverses of giant vacuoles are reminiscent of cellular blebs, extracellular cytoplasmic protrusions triggered by local temporary disruption of the contractile actomyosin cortex. Inverse blebbing has been first observed experimentally during sprouting angiogenesis, but its underlying physical mechanisms are poorly understood. Here, we hypothesize that giant vacuole formation can be described as inverse blebbing and formulate a biophysical model of this process. Our model elucidates how cell membrane mechanical properties affect the morphology and dynamics of giant vacuoles and predicts coarsening akin to Ostwald ripening between multiple invaginating vacuoles. Our results are in qualitative agreement with observations from the formation of giant vacuoles during perfusion experiments. Our model not only elucidates the biophysical mechanisms driving inverse blebbing and giant vacuole dynamics, but also identifies universal features of the cellular response to pressure loads that are relevant to many experimental contexts.

How do bacteria interact with cells?

Clare Bryant

How bacteria interact with host cells determines how successfully the pathogen infects the host and modification of this process might be an important way in which infection can be prevented. The bacteria-cell interaction appears to be simple yet it is not and successful cellular infection depends upon multiple factors. We have focussed on understanding how the zoonotic pathogen Salmonella entericia serovar Typhimurium (S. Typhimurium) interacts with phagocytes (macrophages). Using mathematical models we defined the dynamics of how Salmonella might successfully infect a macrophage and then tested the models by live imaging of infection events. Surprisingly despite the fact that macrophages readily phagocytose particles, like beads, they relatively rarely take up Salmonella such that successful infection events are quite rare. The factors that facilitate successful bacterial infection include bacterial motility, the duration of bacterial contact with cells, the mechanical properties of macrophages and the activity of host bacterial sensing immune mechanisms. Our work has defined bacterial and host factors that important for cellular infection, but also demonstrated macrophage heterogeneity in infection susceptibility and intracellular control of Salmonella. Determining the molecular basis for how these processes occur should help to identify novel therapeutic targets for targeting bacterial infection.

Environmental conditions define the energetics of bacterial dormancy and its antibiotic susceptibility
Teuta Plizota

The global surge of antibiotic resistance calls for a better understanding of bacterial survival strategies. Bacteria that cause hard-to-treat infections are commonly characterised as resistant, persistent, or tolerant, depending on whether they owe their survival to changes in their DNA or physiological features. Regardless of their DNA makeup, stopping growth is often linked to survival. Microbes can enter such dormancy for a number of reasons, but the mechanisms that lead to it and maintain it, and how dormancy leads to survival are multi-faceted and often poorly understood. Despite this, it is attractive to investigate if there is a dominant feature of dormancy that underpins much of its protective effects. One of the leading hypotheses is that dormancy reduces the activity of antibiotic targets, but several studies have attempted to establish a link between cellular energy and the survival capabilities of dormant cells. Results are seemingly contradictory, likely because of the assumption that all dormant states should be energetically equivalent. Instead, here we initiate a typologisation of different dormant states based on their energy profiles.

We have found that the energetic states of cells that entered dormancy due to different environments are not equivalent. In addition, we found that the energy profile and levels associated with a given dormancies influence survival under certain antibiotics and not others. Our work portrays dormancy as a phenotypically complex state, a notion that shows the knowledge of the environmental conditions present at the infection site is important for the design of accurate treatment strategies.

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Learning the differences: a transfer-learning approach to predict antigen immunogenicity and T-cell receptor specificity

Barbara Bravi[1], Andrea Di Gioacchino[2], Jorge Fernandez-de-Cossio-Diaz[2], Aleksandra M. Walczak[2], Thierry Mora[2], Simona Cocco[2], Rémi Monasson[2]

[1]Imperial College London, , United Kingdom, [2]Ecole Normale Supérieure Paris, , France

Antigen immunogenicity and the specificity of binding of T-cell receptors to antigens are key properties underlying effective immune responses. Here we propose diffRBM, an approach based on transfer learning and Restricted Boltzmann Machines, to build sequence-based predictive models of these properties. DiffRBM is designed to learn the distinctive patterns in amino acid composition that, one the one hand, underlie the antigen's probability of triggering a response, and on the other hand the T-cell receptor's ability to bind to a given antigen. We show that the patterns learnt by diffRBM allow us to predict putative contact sites of the antigen-receptor complex. We also discriminate immunogenic and non-immunogenic antigens, antigen-specific and generic receptors, reaching performances that compare favorably to existing sequence-based predictors of antigen immunogenicity and T-cell receptor specificity. More broadly, diffRBM provides a general framework to detect, interpret and leverage selected features in biological data.

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New tools to study cell-cell interactions: Using optical tweezers and microfluidics to study malaria parasites invading human erythrocytes

Miss Emma Jones[1], He/Him Morten Kals[1], She/her Viola Introini[1,2], He/Him Jurij Kotar[1], He/Him Boyko Vodenicharski[1], He/Him Pietro Cicuta[1], He/Him Julian Rayner[1]
[1]University Of Cambridge, Cambridge , United Kingdom, [2]EMBL Barcelona , Barcelona, Spain

The blood stage of the Plasmodium falciparum parasite lifecycle is responsible for all the clinical symptoms of malaria. For Plasmodium parasites to multiply within the blood they must invade human erythrocytes, a complex process involving multiple receptor-ligand interactions. Historically invasion was primarily assessed using end-point assays in which the rate of invasion in a static culture is quantitated using flow cytometry. However, these assays do not completely mimic the conditions of in vivo invasion in blood circulation. To gain a better biophysical understanding of invasion, we used optical tweezers to directly manipulate recently egressed parasites and erythrocytes, to quantify the strength of attachment between these cells and the frequency with which attachments occur. Using a range of inhibitors, antibodies, and genetically modified strains with deletions in known invasion-associated genes, we quantitated the contribution of a range of proteins to erythrocyte attachment. This did not identify a clear correlation between attachment measurements and bulk invasion rates. We hypothesised that strength of attachment might be more important for invasion in blood circulation. We therefore compared the invasion rate of our knock-out lines between static and shaking culture and discovered clear differences. To assess the effect of flow more directly, we have built a microfluidic device in which we monitor invasion under flow conditions by video microscopy. Automated image analysis tracks the movement of parasites and erythrocytes. This data yields new insight into the molecular basis of invasion, particularly in families of invasion ligands that were previously thought to play redundant roles.

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Physiological dynamics of individual microbes under complex changing conditions

Dr. Somenath Bakshi[1]

[1]*University Of Cambridge, Cambridge, United Kingdom*

In nature, most of the microbes are lying in dormant states, waiting for the conditions to improve. When conditions do improve, cells need to rapidly wake up and consume the nutrients to outcompete others in the same ecological niche. The metabolic requirements for quickly and accurately sensing the changes in the environment is at odds with the requirement to stay metabolically inactive and survive long periods of stressful conditions. How do unicellular microbes resolve this conflict?

To explore this question, we have recently developed the microCOSM platform, which enables us to track the physiology and metabolism of individual microbes in a microfluidic device which acts as the microcosm of a connected suspension culture. This method enables us to track individual cells as they enter and exit dormant states under complex changing conditions. Using this method and a set of reporters for cellular metabolism, we have found that microbial populations exploit population heterogeneity to hedge their bet for an unforeseen future. For short intervals of starvation, this population heterogeneity is of no consequence, as the benefits of entering and exiting dormant states early is cancelled out by the increased number of progenies of cells that continue to replicate until late commitment to dormancy. However, for an extended period of dormancy, this heterogeneity ensures population survival under stressful conditions (such as antibiotic treatments) and efficient resumption of population-growth under improved conditions. The talk will cover the relevant method developments, findings, and a discussion of the results in the context of antibiotic persistence.

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Use of Shigella-septin interactions to explore biophysical determinants in cell-autonomous immunity

Gizem Ozbaykal-Guler[1], Serge Mostowy[1]
[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Shigella is an important human pathogen and paradigm of cellular microbiology whose investigation has enabled landmark discoveries in infection and cell biology. Septins, a poorly understood component of the cytoskeleton recognised for their roles in cell division, can entrap cytosolic Shigella in cage-like structures for targeting to destruction by autophagy. Recent work showed micron-scale curvature presented by growing bacterial cells is recognized by septins for cage entrapment, suggesting biophysical determinants play key roles in Shigella-septin interactions and cell-autonomous immunity.

In this work, we reconstitute Shigella-septin cages in vitro using purified protein complexes to investigate biophysical determinants underlying the recognition of bacterial cell surfaces by septins. By combining microfluidics and live cell microscopy, we monitored the kinetics of septin binding to bacterial surfaces and showed the rate of binding depends on the amphipathic helix motif in SEPT6. Next, we hypothesized the surface composition of bacteria can also impact the rate of septin binding. Consistent with this, measurements using mycobacteria (surface composition significantly deviates from that of Shigella) revealed septins bind mycobacteria faster than Shigella. Considering this, we propose variation in bacterial surface composition significantly impacts the binding affinity of septins to a wide variety of bacterial pathogens.

Using our in vitro reconstitution system, we are next focused on biophysical determinants, such as bacterial shape, and how they act as a danger signal to stimulate cell-autonomous immunity through septin-cage assembly. These outcomes are expected to reveal novel concepts in infection biology and contribute to our fundamental understanding of cell-autonomous immunity.

Tackling Topology using TopoStats

Dr Alice Pyne[1]
[1]*University of Sheffield, Sheffield, United Kingdom*

Nearly all processes that act on DNA alter its topology, producing knotted, catenated, and supercoiled forms. Determining how these variations in DNA topology affect fundamental DNA interactions is challenging because of the length scale at which they occur, 100x less than the wavelength of light. High-resolution atomic force microscopy (AFM) is unique in its ability to visualise DNA structure and interactions in liquid with sub-molecular resolution without the need for labelling or averaging. The rate-limiting step of this technique is now fast becoming the analysis of these information-rich datasets. We have developed an open-source Python tool TopoStats, capable of processing AFM data and quantifying molecular structure within each image.

We use our AFM pipeline to determine the effect of supercoiling on DNA structure, demonstrating that DNA under superhelical stress is far richer in structure, e.g., containing kinks and defects, than can be observed in short linear sequences. We build on this to demonstrate that we can accurately and automatically pinpoint crossings in complex DNA structures such as knots and catenanes. We develop new image analysis routines which can almost unambiguously automatically identify under- and over-passing segments of DNA at each DNA crossing, thus allowing full identification of the knot/catenane type and chirality. Finally, we expand our toolkit beyond single DNA molecules, to show that the DNA-binding protein NDP52 binds specifically and with high affinity to double-stranded DNA and that this interaction leads to changes in DNA structure. This, together with proteomics data indicating enrichment for interactions with nucleosome remodelling proteins and DNA structure regulators, suggests a possible function for NDP52 in chromatin regulation.

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The NEOtrap, DyeCycling, NEO-FRET & Co: expanding the single-molecule toolbox.
Sonja Schmid

We develop new single-molecule methods based on nanopores [1] and FRET [2], to learn how biomolecular function arises at the nanoscale. Thereby, we focus on time domain information to reveal the nanodynamics of chaperone proteins and other biomolecular systems.

Recently, we invented the Nanopore Electro-Osmotic trap (NEOtrap) [3], to trap and electrically sense single unmodified proteins for hours with sub-millisecond resolution. We demonstrated that this new label-free technique can sensitively distinguish single proteins, and even distinct conformations of a protein. The latest generation of the NEOtrap allows us to geometrically control electro-osmosis at the nanoscale, by which we could significantly increase the trapping performance, sensitivity, and applicable range of the technique [4]. This opens exciting new avenues to study single-molecule enzymology, protein folding, or protein fingerprinting based on size and shape, which we now pursue in the lab.

In addition, we develop DyeCycling [5], a new measurement scheme to overcome the photobleaching limit in single-molecule FRET. As DyeCycling decouples smFRET recordings from photobleaching, it can vastly expand the timescales that can be observed in one single molecule by bridging from milliseconds to the hour range. Thanks to this gain in information-per-single-molecule, we aim to investigate so far inaccessible effects, like ergodicity breaking, and the interplay of fast and slow dynamics in proteins and ribozymes.

I will present the latest results of our electrical and optical single-molecule studies of biomolecular nanodynamics.

[1] Fragasso, Schmid, Dekker (2020) ACS Nano
[2] Hellenkamp, Schmid, et al. (2018) Nature Methods
[3] Schmid, Stömmer, Dietz, Dekker (2021) Nature Nanotechnology
[4] Wen, et al. (2022) Nano Letters
[5] Vermeer, Schmid (2022) Nano Research

Watching bacterial cell division one molecule at a time using vertical cells

Dr. Kevin Whitley[1], Dr. James Grimshaw[1], Prof. Séamus Holden[2]
[1]Newcastle University, Newcastle Upon Tyne, United Kingdom, [2]University of Warwick, Coventry, United Kingdom

Cell division is a fundamental need for bacteria and a key antibiotic target. Central to this process in most bacteria are the cytoskeletal protein FtsZ that forms a ring of motile filaments at the division site and the peptidoglycan (PG) synthases that build the cell wall inward. Despite their centrality, exactly how these molecules contribute to cell division—and coordinate their activities with one another—is unclear. In my talk, I will show how we use single-molecule microscopy to understand the mechanism of cell division in the model Gram-positive bacterium Bacillus subtilis. I will introduce an imaging method we recently developed where we force rod-shaped cells to stand vertically in nanofabricated arrays of bacteria-shaped holes, forcing their division rings into a single microscope imaging plane. I will show how we use this new method to watch single molecules of the PG synthase PBP2B as they move around the division ring to build the cell wall inward. Finally, I will show how we use division-specific antibiotics to understand how PBP2B coordinates its activity with FtsZ. Our results reveal key new information about the molecular mechanism of cell division in Gram-positive bacteria while demonstrating broadly applicable new methods for bacterial microscopy.

Dimers are forever: single-molecule techniques shine light on single-protein exchange within synaptic complexes – a potential mechanism for long-term memory storage

Katie Morris[1], Noboru Komiyama[2], Edita Bulovaite[2], Anoushka Handa[3], Steven F. Lee[3], Seth G.N. Grant[2], Mathew H. Horrocks[1]

[1]School of Chemistry, University Of Edinburgh, Edinburgh, United Kingdom, [2]Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, United Kingdom, [3]Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge, United Kingdom

Little is known about the life – and death – of single synaptic proteins on the nanometre length scale. More than 1000 different types of proteins have been identified below the post-synaptic membrane of excitatory neurons alone, although their spatial organisation is not well studied. We utilised photoactivatable localisation microscopy (PALM) and photobleaching step counting to probe the distribution of one of the most prolific proteins in the post-synaptic density – PSD95. PSD95 is a scaffolding protein responsible for the recruitment of ion channels, receptors and other functional proteins into macromolecular clusters within the post-synaptic density. Mutation of PSD95 reduces the ordered nature of these functional protein clusters, which can impede normal brain function and manifest as psychiatric and neurological conditions such as autism spectrum disorder and schizophrenia. Using PALM, we confirmed that PSD95 exists in a dimeric form in extracts of protein clusters from the brains of mice, as previously reported in the literature. We imaged more than 10000 dimers and showed that the average separation distance between PSD95 proteins was 38 ± 2 nm. We also showed that old copies of PSD95 are replaced within some dimers after 7 days, independently of whole-complex turnover. This turnover rate is faster in young mice, and varies across brain regions, potentially relating to function. Independent protein turnover could provide a mechanism for stabilising functional protein assemblies and carrying forward molecular alterations, thus preserving long-term memory which exists on a timescale significantly longer than protein lifetimes.

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Super-resolution single-molecule detection of biomolecules using a nanopore

Dr Kaikai Chen[1,2], Ulrich F. Keyser[2]

[1]University of Chinese Academy of Sciences, Beijing, China, [2]University of Cambridge, Cambridge, United Kingdom

High-resolution analysis of biomolecules has brought unprecedented insights into fundamental biological processes and dramatically advanced biosensing. Notwithstanding the ongoing resolution revolution in electron microscopy and optical imaging, only a few methods are presently available for high-resolution analysis of unlabelled single molecules in their native states. Here, we demonstrate label-free electrical sensing of structured single molecules with a spatial resolution down to single-digit nanometres. Using a narrow solid-state nanopore, we detect the passage of a series of nanostructures attached to a freely translocating DNA molecule, resolving individual nanostructures placed as close as 7 nm apart and with a surface-to-surface gap distance of only 2 nm. We attribute such super-resolution ability to the nanostructure-induced enhancement of the electric field at the tip of the nanopore. Our work demonstrates a general approach to improving the resolution of single-molecule nanopore sensing and presents a critical advance towards label-free, high-resolution DNA sequence mapping and digital information storage independent of molecular motors.

Revealing the mechanism of supercoil relaxation by the Human Topoisomerase3α-RMI1-RM2 complex

Graeme King[3], Dian Spakman[1], Andreas Biebricher[1], Nikolai Born[1], Anna Bizard[2], Ian Hickson[2], Erwin Peterman[1], Gijs Wuite[1]

[1]Department of Physics and Astronomy, and LaserLaB, Vrije Universiteit Amsterdam, , The Netherlands, [2]Nordea Center for Healthy Aging, University of Copenhagen, , Denmark, [3]Department of Structural and Molecular Biology, University College London, , United Kingdom

Topoisomerase enzymes are essential for regulating DNA topology in the cell. The Human topoisomerase complex Topo3α-RMI1-RMI2 (TRR) is important for unlinking intertwined DNA structures (UFBs) present between chromatids during mitosis. However, the mechanism underpinning this is still not well understood. It has recently been proposed that TRR can facilitate the resolution of UFBs by relaxing underwound (negatively supercoiled) loops of DNA generated by the protein PICH. Here, we reveal the mechanism of supercoil relaxation by TRR using a novel single-molecule assay. Our assay exploits a recently developed approach called Optical DNA Supercoiling (ODS), which enables the generation and manipulation of negatively supercoiled DNA using dual-trap optical tweezers. ODS is advantageous for two key reasons. First, it enables the supercoiled substrate to be moved freely between different buffer/protein solutions. Second, it can be readily combined with fluorescence microscopy, allowing visualization of TRR interactions with the supercoiled DNA. By applying our novel ODS-based assay, we demonstrate that TRR relaxes negatively supercoiled DNA in a highly processive manner, and can perform thousands of catalytic cycles without unbinding. We also reveal that TRR remains bound to the DNA long after supercoil relaxation has completed. Furthermore, our results indicate that the rate of supercoil relaxation by TRR is sufficiently high that it likely provides an efficient means to relax the transient supercoiled loops generated by PICH. Taken together, our findings uncover the mechanistic basis for supercoil relaxation by TRR, and suggest that unbinding of TRR from DNA likely requires the action of accessory proteins.

Keynote VII: **Our archaeal ancestry**
Buzz Baum (UK)

Abstract currently unavailable

P1.01

A force-spectroscopy assay for studying binding of DNA repair proteins at individual DNA double-strand breaks

Nicholas Bell[1,2], Justin Molloy[2]

[1]University College London, London, United Kingdom, [2]Francis Crick Institute, London, United Kingdom

DNA double-strand breaks (DSBs) are a highly toxic form of DNA damage which can lead to genome instability and cell death. DSBs are repaired by biochemical pathways which involve detection, processing and ligation by a large number of DNA repair proteins. We present a magnetic tweezers assay which enables us to study the dynamics of this process in real-time at the single-molecule level with control of DNA tension, topology and end chemistry. We use our assay to study PARP2 – a signalling enzyme which plays key roles in eukaryotic DNA damage sensing. We find that PARP2 forms a remarkably stable mechanical link across blunt-end 5'-phosphorylated DNA ends and switches between stable binding and bridging modes depending on DNA phosphorylation state and the 5' or 3' direction of DNA overhangs. Our works gives new insights into the role of PARP2 in detecting DNA damage and provides a platform for studying other proteins involved in DSB repair.

P1.02

How plants avoid 'bursting the bubble'

Jared Carpenter[1,2], Mark Blyth[2], Richard Morris[1]
[1]John Innes Centre, Norwich, United Kingdom, [2]University of East Anglia, Norwich, United Kingdom

Plants transmit nutrients and signaling molecules via their vascular system. To successfully carry out such transport processes, plants must overcome a number of physical challenges. For instance, the transpiration-driven xylem system, transporting water from root to shoot, operates under absolute negative pressure. This negative absolute pressure is a metastable state which can induce nanobubble formation that is detrimental to the plant. Nanobubbles are bubbles that are usually 50-200 nanometres in diameter that have the potential to form an embolism in the plant. Plants thus need to somehow balance physically dangerous conditions with their needs for nutrient acquisition, water transport and signaling. How plants meet these conflicting demands is currently unknown.

I will present current ideas and approaches for addressing this fascinating problem. My current research involves modelling a bubble in infinite space using Stokes flow and deforming its shape by perturbing the radius by a function which varies in time. We can then determine under what conditions the bubble remains in a stable state. The calculations I will present assume that the leading-order bubble radius is steady and that surface tension does not vary over the bubble surface, that is there are no surfactants present. However, the effect of surfactants will be modelled as part of my future work.

P1.03

A novel tensioned explant human skin platform for clinically relevant applications across medicine and the aesthetic and pharmaceutical industries

Dr Paul Campbell[1], Dr Michael Conneely, Dr Robyn Hickerson
[1]*Ten-Bio, Dundee, United Kingdom*

Human skin models are a widely accepted approach for studying skin biology. Such models include organotypic systems reconstructed from human cells as well as models based on discarded surgical tissue. Cell-based systems are time-consuming to set up and maintain, are unable to fully recapitulate the differentiated architecture of the skin, and typically lack minor skin cell populations. Traditional full-thickness skin models prepared from excised skin tissue collected during surgery quickly lose viability and the ability to respond to stimuli once off the body. Traction-force balance is essential for maintaining skin homeostasis and is, therefore, an important factor in regulating both tissue structure and physiological function. Here we describe a tension-based skin explant model to address the unmet need for a human skin model that reliably mimics in vivo skin even in complex biological processes such as wound response and healing. This tension-based model mimics an in vivo-like response to
laser ablative wounding, with keratin 17 observed throughout the wounded skin sample, whereas only minimal expression is observed in skin cultured without tension. Additionally, observation of the wound sites for up to 3 weeks shows that reformation of the basement membrane is highly dependent on the presence of tension. qPCR analysis of wound healing markers also showed a delayed and diminished response to wounding in non-tensioned skin compared to skin cultured in tension. Application of optimized tension can therefore restore skin's inherent mechanobiology, enabling a more in vivo-like behavior, greatly increasing the utility of full-thickness ex vivo skin models.

P1.04

A nanomaterials approach to functional tissue substrates and cellular stimulation

Dr Alice King[1], Christopher Brown[1], Professor Giorgios Giamas[1], Dr Lisa Woodbine[1]
[1]*Nanobiotechnology group, Department of Physics, University Of Sussex, Brighton, United Kingdom*

The 2D layered nanomaterials offer a unique palette of specific chemistry, structure and mechanical properties that can be used to develop cell substrates and tissue scaffolds with controlled nanoscale architectures. The physical interface of the cells with the substrates interrogates different pathways and offers novel imaging and stimulation opportunities. We have high levels of control and tunability over the physicochemical properties of the nanomaterials as well as organisational control for structure in 2 and 3 dimensions, enabling improved cell compatibility and functional interaction. Examples include improved spontaneous in vitro vascularisation of GBM tissue on graphene-based scaffolds, collagen expression in chondrocytes grown on woven carbon nanotube structures and localisation and stimulation of the endoplasmic reticulum in cancerous cells with 2D MoS2. These pathways also offer the chance to image live systems using Raman spectroscopy and identify changes in cell structure and cell fate, without evidence of toxicity.

By controlling nanoscale properties of our systems, including the nanomaterial, but also the fabrication process, we are able to offer new approaches to understanding physical cell mechanisms, interfaces and cellular interactions with external stimuli, as well as fine tuning for a biocompatible and process compatible scaffold that can be tailored to a specific mechanobiology application.

P1.05

LifeHack: An open-source, modular microscope for live & fixed cell single molecule imaging with high performance sample stabilisation

Mr Josh Edwards[1,2], Dr Kevin Whitley[2], Mr Suheer Peneti[2], Dr Yann Cesbron[2], Dr Seamus Holden[1]
[1]University Of Warwick, Coventry, United Kingdom, [2]Newcastle University, Newcastle Upon Tyne, United Kingdom

Single molecule microscopy can reveal both sub diffraction limited structures and molecular dynamics directly in living cells but requires advanced microscopes to do so. Commercial microscopes can provide these capabilities but are expensive and inflexible. In contrast, home-built systems are highly modifiable but require a significant level of expertise and time to create.

LifeHack is an open-source microscope capable of live & fixed-cell single molecule imaging beyond current commercial and open-source microscopes. It also functions as a development platform without the setup hurdles of a home-built system. The microscope features: ring-TIRF illumination for artifact free imaging, A custom incubation box providing thermal stability for living samples and reduced microscope drift, and open-beam pathways providing the efficiency needed for SMLM.
Comprehensive microscope designs have been released through a dedicated website (https://holdenlab.github.io/LifeHackWebsite/). This includes complete CAD models and detailed instructions for construction, alignment, and control of the system. The designs are split into three separately adaptable modules to streamline future upgrades. The system is almost exclusively made from either 3D printed or commercially available parts purchasable for approximately £150,000.

The practical resolution of single-molecule microscopes is frequently limited by sample drift. To combat this the LifeHack microscope is also features ImLock. ImLock is an infrared, image-based, 3D sample-stabilisation tool that is capable of providing <10nm stability for long periods at or away from the cover-slip. It can also handle multi-FOV experiments, z-stacks, and rapidly growing samples. Minimal hardware requirements and no need for fiducial markers make it easily incorporated into existing systems.

P1.06

Within-host modelling of T cell memory generation

Yiping Zhang[1], Matt Keeling[2], Nigel Burroughs[2]
[1]*University of Warwick, Coventry, United Kingdom,* [2]*Zeeman Institute: SBIDER (Systems Biology & Infectious Disease Epidemiology Research), University of Warwick, Coventry, United Kingdom*

Establishing a strong T cell memory is essential for protection against future exposure to the same, or related pathogens. However, an understanding of what determines immune system dynamics and what is needed to create a strong immunological memory remains unclear. We developed mathematical (ordinary differential equation) compartment models incorporating various mechanisms for T-cell dynamics and the development of T-cell memory. We examined memory generation under infection and vaccination protocols, determining for instance which models give better memory after 2 doses of vaccine. We also examined the identifiability of the models from longitudinal (synthetic) data. Parameter fitting was achieved by maximum likelihood methods; even with high resolution (daily measurements) longitudinal data on viral load, activated T-cell counts and T-cell memory, all the models remain unidentifiable with around ½ of the approximately 20 parameters being inferable from such data.

P1.07

In vitro spatiotemporal characterization of immune-tumor interactions reveals impairment of T cell cytotoxicity by mutation of adenomatous polyposis coli.

Valentin Bonnet[1], Vincenzo Di Bartolo[2], Erik Maikranz[1], Marianne Madec[2], Nadia Vertti-Quintero[1], Andrés Alcover[2], Charles N. Baroud[1]
[1]*Institut Pasteur / Ecole Polytechnique - Physical Microfluidics and Bioengineering, Paris, France,* [2]*Institut Pasteur - Cellular biology of lymphocytes, Paris, France*

Familial adenomatous polyposis is a disease caused by germinal mutations of adenomatous polyposis coli (APC) gene. Patients develop hundreds of colorectal tumors. In addition to favoring cancer development, recent publications show that APC mutations can also affect the migration and cytotoxicity of T lymphocytes. The immune system being a key actor in tumor regulation and elimination, the impact of APC on T cell behavior affects the long-term cancer development. However, the complexity of the in vivo system hinders the understanding of mechanisms by which the APC mutation reduces the ability of the immune cells to control tumorigenesis.

Here, we use a recently published droplet microfluidic approach to coculture tumor spheroids with primary cytotoxic T lymphocytes (CTLs) coming from healthy or mutant APCMin/+ mice. The method relies on simultaneously following migration and killing dynamics of hundreds of individual CTLs on tens of spheroids in parallel, by combining confocal and time-lapse microscopy. The measurements show that APC mutation does not affect CTL accumulation on the tumor spheroid. Once on the spheroid however, APC mutant cells have a reduced ability to kill cancer cells. Close inspection of the migration patterns on the spheroids reveals that the APC mutants have shorter periods of arrest than the control cells, and different collective dynamics. These arrest periods are crucial in the formation of the immunological synapse that leads to the killing events. Biophysical mechanisms of these observations are explored by comparing and modulating expression of adhesion molecules.

P1.08

Understanding polymicrobial communities via direct microscopical observation in microfluidics

Dr Leonardo Mancini[1]

[1]*University of Cambridge, Cambridge, United Kingdom*

Polymicrobial infections of the respiratory tract are infamously known for their chronicity and some of their worst manifestations occur in patients affected by cystic fibrosis. Their lungs accumulate thick mucus that becomes a perfect niche for microbes, which cause relapsing infections that progressively acquire antimicrobial resistance and eventually impair respiratory function. For reasons not yet understood, disease progression and antimicrobial resistance are associated with microbial diversity, suggesting that the discoveries necessary to develop better treatments may lie behind the understanding of their ecology.

Polymicrobial communities have thus far mostly been studied using genomic approaches which only provide information on species abundance, from which it is difficult to draw mechanistic conclusions. Direct observation is necessary to gain in-depth understanding of the interactions and mechanisms that drive the evolution of polymicrobial communities, but it has thus far been limited by the low tractability of these microbial consortia.

Overtaking these experimental hurdles, we co-culture, in a bioreactor, a community of P. aeruginosa, S. aureus and C. albicans - microbes that are often found in the sputum of patients with cystic fibrosis. Deploying microfluidics we gain exquisite control over an array of experimental parameters, while being able to directly measure the growth of microbes and the efficacy of antibiotics. This allows us to study spatial organisation and its functional consequences. With the understanding generated, we will be able to pinpoint the conditions that render the community most fragile and devise strategies to cause its extinction.

P1.09

The effect of the spatial distribution of control agents on the spatial spread of resistance

Wolfram Moebius[1,2], Thomas Tunstall[1,2], Dibyendu Dutta[1,2], Tim Rogers[3]
[1]Living Systems Institute, Faculty of Health and Life Sciences, University of Exeter, Exeter, United Kingdom, [2]Physics and Astronomy, Faculty of Environment, Science and Economy, University of Exeter, , United Kingdom, [3]Center for Networks and Collective Behaviour, Department of Mathematical Sciences, University of Bath, Bath, United Kingdom

Pesticides, antimicrobials, and cancer drugs are designed to control populations of pests, microbes and tumour cells, respectively. In all scenarios, the emergence of mutants resistant to the control agent is a major concern. While strategies to suppress the emergence of these mutant populations are widely studied, we know relatively little about what determines their success in spatially heterogeneous environments.

To address this question, we consider two-dimensional structured environments, where we study the dynamics of wildtype population that can spread everywhere except in patches protected by the control agent. In this process, mutants resistant to the control agent can arise and spread uninhibited.

A combination of lattice-based simulations and geometrical arguments allows us to characterise the success of mutant populations as a function of mutant rate, mutation fitness and patch distribution, extending prior work on the dynamical phase transition in environments without protected patches. We find that the mutant can become dominant quickly even if it enjoys unrivalled spread in a relatively small proportion of the environment. Moreover, we characterise the effect of spatial structure and mutation rate on genetic diversity.

Our findings are a first step towards optimising the spatial distribution of control agents to suppress not only the emergence of resistant subpopulations but also their spatial spread.

P1.10

The Characterisation of Xog1 a β-1,3-glucanase from Candida albicans for Potential Biotechnological Applications

Jennifer Littlechild, Daniel E Mitchell[1], Neil A R Gow[1]
[1]*University of Exeter, , United Kingdom*

The exo-β-1,3-glucanase from Candida albicans, is a key enzyme in cell wall remodelling and is a potential target for biosensing and drug design. Additionally, its ability to break down polymeric sugars into individual glucose units makes it of interest for biocatalysis applications. Xog1 was found to be stable up to 60 ^{o}C in a range of different buffering systems, while also retaining its activity when immobilized onto gold nanoparticles, suggesting that it would be suitable for biotechnological applications. Additionally the interaction between the human antimicrobial peptide LL-37 and Xog1 was investigated demonstrating an enhancement in glucanase activity but a reduction in thermal stability with increasing concentration of LL-37. The LL-37 peptide can also be used in a Xog1 pull down assay showing potential for developing a sensor for the detection of Candida species.

P1.11

Star-shape: investigating astrocytes' mechanobiology and shape-function dynamics

Ludovica Guetta[1], Andrea Serio[1]
[1]*The Francis Crick Institute , London, United Kingdom*

Astrocytes are active players in brain homeostasis and their dysfunction is implicated in several diseases.

One central aspect to astrocytic function is their morphology. They have a complex shape which undergoes extensive remodeling to allow astrocytes to contact their targets and respond to stimuli.

While the significance of these dynamic morphological changes is recognized, we are lacking a systematic understanding of the biophysical factors that control them.

We aim to explore how intracellular mechanics and external forces participate in determining astrocytic shape and its regulation.
To address this, we have optimized the generation of iPSC-derived astrocytes with distinct morphologies using protocols that employ developmentally relevant cytokines. We use these to understand the role of internal cytoskeletal processes, membrane tension and adhesion dynamics in the acquisition and maintenance of astrocyte shape.

We are also fabricating a range of bioengineered substrates that have tunable mechanical properties, including hydrogels and micro-post arrays. Using these, we can study force transduction in our cells and dissect how, in vitro, mechanical forces affect the biophysical properties of astrocytes and, consequently, modify their shape and behavior in physiological and pathological conditions. This allows us to uncover the role of cell mechanics in modulating astrocyte motility, reactivity, and response to injury.

Our systematic investigation sheds light on the tight interplay between astrocyte mechanobiology, morphology and function and will be instrumental to devise bioengineering strategies to control astrocyte behavior during processes, like injury and neurodegeneration, which are characterized by alterations in tissue mechanics and see astrocytes as major players.

P1.12

Resolving the membrane attack complex on live bacteria with atomic force microscopy

Christian Bortolini[1,2], Bart Hoogenboom[2]
[1]King's College London, London, United Kingdom, [2]University College London, London, United Kingdom

Complement is a crucial component of our innate immune system. Its activation, induced by pathogens like bacteria, leads to an irreversible enzymatic cascade, ending with the formation of membrane attack complex (MAC) pores on microbial membranes. This process leads to cell death.
To shed light on the mechanism of bacteria killing by the MAC, we developed protocols capable of imaging E. coli at the nanoscale and monitor cell viability. By combining Atomic Force Microscopy (AFM) and live-dead fluorescent staining, we can assess MAC formation on live bacteria and relate subsequent cell death to changes at the bacterial surface as induced by MAC pores.
The precise mechanism underlying anti-bacterial killing remains elusive and more experiments are needed to understand the correlation between bacterial outer-membrane integrity and cell death.

P1.13

Identifying Immune Status to COVID-19 Using Dielectrophoresis

Ms Krista Clarke[1], Mr Alexander Stewart[1], Ms Emma Sinclair[1], Dr Fatima Labeed[1], Professor Michael Hughes[2], Professor Deborah Dunn-Walters[1]

[1]University of Surrey, Centre for Biomedical Engineering, Guildford, United Kingdom, [2]Khalifa University, Department for Biomedical Engineering, Abu Dhabi Emirate, United Arab Emirates

During times of hospitalization, isolation and lockdown, the necessity for a quick, easily adoptable, and relatively inexpensive tool to determine a person's immune status to COVID-19 became increasingly urgent and relevant to everyday decisions. The 3DEP device, developed at the University of Surrey, is a machine which characterises the electrophysiological properties of cells using the phenomenon dielectrophoresis.

In this pilot study, we investigated whether the electrical properties of white blood cells, characterised by the 3DEP, could be used to differentiate individuals who had not been exposed to COVID-19 with those who had, either through infection or by vaccination.

White blood cells were isolated from whole blood from a total of 22 donors from four donor cohorts: never had COVID, recovered from COVID, one vaccine dose and two vaccine doses. Isolated cell samples were stimulated with the SARS-CoV-2 spike protein for three hours, after which their electrical properties were characterised.

Individuals who had recovered from COVID-19 could be differentiated from those who had not been exposed to COVID-19 with a sensitivity of 85.7% and specificity of 100%. Furthermore, membrane conductance was significantly higher in individuals who had received two vaccine doses than recovered from COVID-19 donors ($p<0.05$), and the cytoplasmic conductivity of white blood cells from one vaccine donors was significantly greater ($p<0.05$) than those who had received two doses. However, limitations include whether differences in electrophysiological properties were due to immune responses to the spike protein specifically, or non-specific interactions with a molecule in the buffer.

P1.14

Measurement of cell cortical tension using AFM parallel plate compression

Andreas Weber[1], Fabrice Delbary[2], Hervé Turlier[2], Guillaume Charras[1]
[1]London Centre For Nanotechnology, University College London, London, United Kingdom, [2]Center for Interdisciplinary Research in Biology (CIRB), Collège de France, CNRS, INSERM, Université PSL, Paris, France

The cell surface is lined by the actomyosin cortex, a thin network made of actin and myosin that defines cell mechanics. Cells can be modelled as liquid drop surrounded by an elastic shell with a tension which can be derived by Laplace law. Gradients in tension are known to lead to changes in cell and tissue shapes. By parallel plate compression with AFM, deformations at given forces can be used to approximate cell shape to calculate surface tensions.

Methods to date use simplifications to determine cortical tension, like assuming zero contact angles, no adhesion, or spherical arc deformation. These methods rely on combination of AFM with an optical method to approximate cell shapes. We propose a novel solution to derive cortical tension from multiple step AFM compressions.
We perform stepwise parallel plate compression via AFM on rounded cells and use a novel approach to determine the deformation shapes, contact angles and the surface tension using three pairs of forces and compressions This provides minimal errors compared to the exact solution and overall performs better than routinely applied approximations. In addition, it enables tension determination without combination of an AFM with an optical microscope. We verify the applicability with measurements of water drops in oil and chemical interventions on cells changing actomyosin based contractility. Overall, our approach provides a novel tool for exact determination of cortical tension with simple instrumentation that will enable further testing of the model of cortical tension to describe cell mechanics.

P1.15

Membrane Targeted Azobenzene Drives Optical Modulation of Bacterial Membrane Potential

Dr Tailise De Souza G. Rodrigues[1], Dr Gaia Bondelli[2], Dr Iago Grobas[3], Dr Stefano Donini[2], Dr Valentina Sesti[4], Prof Chiara Bertarelli[4], Prof Guglielmo Lanzani[2,5], Dr Munehiro Asally[1], Dr Giuseppe Maria Paternò[2,5]
[1]School of Life Sciences, University of Warwick, , United Kingdom, [2]Center for Nanoscience and Technology, Istituto Italiano di Teconologia, , Italy, [3]Physical and Theoretical Chemistry Laboratory, University of Oxford, , United Kingdom, [4]Department of Chemistry. Materials and Chemical Engineering "Giulio Natta" Politecnico di Milano, , Italy, [5]Department of Physics, Politecnico di Milano, , Italy

The membrane potential, the electrical potential difference across the membrane, is pervasive in biological cells. Recent studies have demonstrated that bacteria can use the membrane potential for cellular signalling. However, our understanding of the bacterial electrical signalling is still largely limited. In this project, we used a membrane-targeted photoswitch molecule to modulate the membrane potential of the Gram-positive model bacterium Bacillus subtilis. We found that a 470 nm light stimulation can induce a hyperpolarisation of the membrane, which occurs in a chloride channel and potassium transporter dependent manner. Intriguingly, stimulations can be applied in a repeated manner. These results provide proof of concept that bacterial membrane potential can be modulated using light. We believe that this new optical tool can contribute to advancing our understanding of bacterial electrical signalling and provide novel optical approaches to control cellular behaviours.

P1.16

Innovation in the assessment of antimicrobial properties of biogenic nanomaterials

Dr Tailise De Souza G. Rodrigues[1], Debarati Bhowmik[2], Prof Ljubica Tasic[3], Dr Munehiro Asally[1]
[1]School of Life Sciecnes, University of Warwick, , United Kingdom, [2]University of Birmingham, , United Kingdom, [3]Department of Chemistry, University of Campinas, , Brasil

Antimicrobial resistance (AMR) is on the rapid rise globally, which has led the World Health Organization (WHO) to call for urgent interdisciplinary action. Early and rapid detection of vital microbes is crucial to aid in correct decisions on treatments for the elimination of contaminations and infections.

Our group previously showed that the dynamics of the electrical potential across the membrane (i.e. membrane potential) relate to the proliferative capacity of cells when stimulated by an externally applied electric field (EEF). However, it was unclear whether such a relationship can be applied more universally to eukaryotic cells and with silver nanoparticles -a promising new class of antimicrobial compound.

In this project, we monitored the membrane-potential dynamics of the budding yeast Saccharomyces cerevisiae in response to EEF to assess the microbial inhibition level of different compounds. We observed that fungal bioelectricity dynamics can differentiate inhibited from proliferative cells, as well as be used to assess growth rate. Using the Gram-positive bacterium Bacillus subtilis, we also applied this technology to investigate the antimicrobial properties of biogenic silver nanoparticles (Bio-AgNPs). We observed that Bio-AgNPs induce a severe depolarization even when bacterial cells were exposed to below minimum inhibitory concentrations. Additionally, preliminary data of Bio-AgNPs-inhibited cells exposed to EEF show a dose-dependent membrane potential dynamics. This suggests that this technology has the potential to differentiate between microbicidal and microbiostatic effects of different compounds, including nanoparticles, on both bacteria and yeast.

P1.17

Evolution of populations in fluctuating environments

Mr Matthew Asker[1]

[1]*University Of Leeds, Leeds, United Kingdom*

Dynamics of bacteria where the ecological and evolutionary processes are linked occur widely in nature and are not well understood. The development of antimicrobial resistance (AMR) is one particularly important example of this, since AMR is responsible for ~1 million deaths per year with this reaching 10 million by 2050. Thus, we look to develop a mathematical model that can investigate this problem.

We consider a stochastic system of varying size containing resistant and sensitive bacteria with a switching environment. This environment either impacts the birth rates (toxin levels) or the death rates (food availability) or both. Depending on the setup of the system we either see dominance of the sensitive / resistant strain or coexistence of the two strains. Upon finding coexistence, it is also possible to accurately predict the fraction of resistant fraction present.

We will demonstrate the theoretical framework developed and that this accurately reproduces the results we find from simulation, alongside future plans to investigate these results experimentally.

P1.18

Understanding how antigen mobility influences B cell activation: from early signalling to antigen internalisation

Mrs Hannah McArthur[1,2], Dr Anna Bajur[1,2], Miss Maro Iliopoulou[1], Dr Katelyn Spillane[1,2]
[1]Department of Physics, King's College London, London, United Kingdom, [2]Randall Centre for Cell & Molecular Biophysics, King's College London, London, United Kingdom

B cells are essential to the adaptive branch of the immune response and are responsible for the production of protective antibodies directed against invasive pathogens. B cells activate following specific binding events between the B cell receptor (BCR) and antigens tethered to the surfaces of antigen-presenting cells. B cells sense physical properties of the antigen-presenting cell, such as the mobility of antigens, through forces loaded onto BCR-antigen bonds. In contrast to the biochemical mechanisms governing B cell activation, the role of physical factors remains poorly understood. Here, we systematically explore the role of antigen mobility on B cell activation. We control forces loaded onto BCR-antigen bonds by manipulating substrate viscosity and antigen density.

Using a combination of high-resolution fluorescence microscopy, DNA tension sensors, and quantitative image analysis, we show that reduced antigen mobility dampens early B cell signalling yet promotes cell spreading and antigen internalisation. Preliminary data also suggest that low mobility substrates promote B cell discrimination of antigen affinities, which is important for the generation of high affinity antibodies during an immune response. Together, this work highlights how B cells adapt their responses to the physical properties of the antigen-presenting membrane, as well as emphasises the importance of considering antigen mobility in experimental designs.

P1.19

Biophysics of Mammalian Primordial Germ Cell Migration

William Foster[1], Malgorzata Borkowska[2], Harry Leitch[2], Ewa Paluch[1]
[1]University Of Cambridge, Cambridge, United Kingdom, [2]MRC London Institute of Medical Sciences, London, United Kingdom

Primordial germ cells (PGCs) are the precursor cells to gametes (sperm and egg). PGCs are specified early in development and in mammals, as with many other species, are specified at a distant location with respect to PGCs' eventual location, the developing gonad. PGCs therefore migrate to the gonad, and this migration represents a critical feature of normal embryonic development.

We investigate the biological and biophysical mechanisms of PGC migration. In particular, we investigate whether mammalian PGCs display amoeboid or mesenchymal migration. PGCs in the mammal traverse different environments on their path to the gonad, including the gut endoderm and the dorsal mesentery, and it is therefore possible that changes (mechanical and chemical) in the local environment along the migratory route could lead to changes in migratory mechanism.

We are currently investigating migratory mouse PGC morphology during migration in vivo, and the migration of isolated PGCs in in vitro microchannels mimicking in vivo confinement. Such microfluidic-based systems where single cells can migrate and be exposed to various extracellular cues (including chemokine gradients, extracellular matrix proteins, and different levels of confinement) allows us to interrogate the mechanisms underlying PGC migration. We aim to establish which migratory mechanisms are used by mouse PGCs and decipher the effects of changes in the extracellular environment on this process.

P1.20

A molecular and mechanical study of cell shape changes and cortex reorganisation during post-mitotic spreading

Jeanne Lefévère-Laoide[1], Professor Ewa Paluch[1]
[1]*Department of Physiology, Development and Neuroscience, University of Cambridge, , United Kingdom*

Cell division is an essential physiological process in multicellular organisms, underlying growth and development, yet its dysregulation is one of the hallmarks of cancer. In healthy cells, accurate division requires precise coordination of cytoskeletal structure changes, as cells undergo large-scale deformations throughout mitosis. At the onset of mitosis, most cells display characteristic mitotic rounding, driven by reduced adhesions to the substrate and an increase in cell surface tension and stiffness. At cytokinesis, the two daughter cells typically spread out. The mechanical changes underlying mitotic rounding have been extensively studied; in contrast, post-mitotic spreading is poorly understood.

To investigate molecular and mechanical aspects of the cell shape changes and the underlying actomyosin cortex reorganisation occurring during post-mitotic spreading, we are undertaking a quantitative description of shape change dynamics during post-mitotic spreading. To this aim, we use live-cell imaging to analyse cell shape and identify key phases of spreading during mitotic exit. We also show early evidence of actin and myosin reorganisation at the cortex at the onset of spreading, and investigate the actin interactors that may play a role in regulation of spreading. Altogether, we show macro- and micro-scale changes underlying post-mitotic spreading.

P1.21

Study of cell-cell adhesion formation and dynamics in cancer cells using a hybrid cell - lipid bilayer system

Sayantika Ghosh[1], Prof Marco Fritzsche[2], Dr Darius Koester[1]

[1]Centre Of Mechanochemical Cell Biology, Warwick Medical School, University of Warwick, Gibbet Hill Campus, Coventry, United Kingdom, [2]Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom

Formation and regulation of connections between cells play a crucial role in maintaining tissue homeostasis and tumour repression. In many tissues, particular importance is given to adherens junctions formed by the binding of E-cadherin proteins at the surface of adjacent cells, followed by recruitment of catenins and other adaptor proteins on the intracellular site linking the protein complex to the actomyosin cytoskeleton. This process depends on mobility, density, type of cadherins, and the mechanical forces acting on the cadherin-catenin complexes that can change dramatically during developmental stages or in diseases like cancer.

To study the adherens junction formation in a controlled environment in cancer cells, we have established a reconstituted system of cells adhering to supported lipid bilayers decorated with extracellular domains of E-cadherin proteins. This system allowed us to capture the initiation and dynamics of cadherin interactions, localisation and clustering of adaptor proteins and adherens junction maturation in cancer cells in real time using live cell imaging with confocal and total internal reflection microscopy.

To understand the importance of the different parameters and to mimic the cell/tissue abnormalities in cancer scenarios, we perturb the controlled system by varying cadherin density, membrane mobility and inducing mechanical stress by confining the cell system over time. We find that reduced E-cadherin mobility leads to the formation of stable regions showing actin enrichment, and confinement increases the steady-state adhesion zone. This approach provides new insights about cell surface dynamics during cell-cell adhesion and can improve understanding of E-cadherin organisation patterns in cancer progression.

P1.22

Scale-invariance in coarse-grained models for red blood cells

Mr Paul Appshaw[1], Prof Annela Seddon[1], Dr Simon Hanna[1]
[1]*University Of Bristol, Bristol, United Kingdom*

Accurate coarse-grained molecular dynamics representations of the morphology and elasticity of human red blood cells (RBCs) require an extremely detailed particle-based model with emphasis placed on the structure of the membrane. The length-scale appropriate to the coarse-graining is determined by the bilayer thickness of the cell, which sets the number of particles required to simulate the entire cell. Such models tend to be computationally expensive so that it is generally only possible to simulate "miniature" RBCs, with the implicit assumption that the properties of these cells are scale-invariant and provide realistic models of their full-sized counterparts. In this paper we examine the coarse-grained RBC model of Fu et al. [Comput. Phys. Commun., 210, 193–203 (2017)]. RBCs were constructed with diameters in the range 0.25 – 0.75 µm, corresponding to between 34,944 and 896,703 coarse-grained particles, respectively. The shape evolution of the cells was followed, indicating a lower size limit governing the appearance of the characteristic biconcave shape. Cells of diameter 0.5µm or greater were biconcave, while cells with smaller diameter equilibrated to bowl-shaped stomatocytes. A quantitative analysis of the thermal fluctuations of the model cell membranes, analogous to the experimental video-microscopic analysis of real cells, demonstrated that the bending rigidity of the cells was constant over all cell sizes simulated, and consistent with measurements on whole cells and sections of bilayer. Therefore it was confirmed that the computer model under examination is a good representation of a full-size RBC, but only when the model diameter is 0.5µm or more.

P1.23

Patterning of membrane adhesion under hydraulic stress

Céline Dinet[1], Alejandro Torres-Sánchez[2], Lorenzo Di Michele[3], Roberta Lanfranco[3], Marino Arroyo[2], Margarita Staykova[1]

[1]Durham University, , United Kingdom, [2]Universitat Politecnica de Catalunya-BarcelonaTech, Barcelona, Spain, [3]University of Cambridge , Cambridge, UK

Hydraulic fracturing plays a major role in the formation of biological lumens during embryonic development, when the accumulation of pressurized fluid fracture the cell-cell contacts and leads to the formation of microlumens that later evolve into a single large lumen. However, the physical principles underpinning the formation of a pattern of microlumens from a pristine adhesion and their subsequent coarsening are poorly understood. We have used artificial membrane systems, together with theoretical modelling and numerical simulations, to provide a mechanistic understanding of the nucleation of hydraulic cracks, their spatial patterns and their coarsening dynamics. Besides coarsening, we show that microlumens can irreversibly bud out of the membrane, reminiscent of endocytic vesicles in cell-cell adhesion. By establishing the physics of patterning and dynamics of hydraulic cracks, our work unveils the mechanical constraints for the biological regulation of hydraulically-driven adhesion remodeling

P1.24

Multiple Intermediates in the Detergent-Induced Fusion of Single Lipid Vesicles

Miss Lara Dresser[1], Dr Casper Kunstmann[1], Professor Steven Johnson[1,2], Dr Jamie Blaza[3], Professor Carlos Penedo[4], Professor Mark Leake[1,2,5], Dr Steven Quinn[1,2]

[1]School of Physics, Engineering and Technology, University of York, York, United Kingdom, [2]York Biomedical Research Institute, University of York, York, United Kingdom, [3]York Structural Biology Laboratory, Department of Chemistry, University of York, York, United Kingdom, [4]School of Physics and Astronomy, University of St Andrews, St Andrews, United Kingdom, [5]Department of Biology, University of York, York, United Kingdom

Vesicle biophysics is heavily influenced by a range of physical forces, local microenvironmental effects and interactions with perturbative molecules, including detergents. Detergent-induced vesicle solubilization - critical for biotechnological applications including virus inactivation and protein extraction – varies in magnitude according to the detergent type and membrane composition, but the underlying mechanistic details remain largely under explored. In one prevailing hypothesis, detergent-induced vesicle solubilization is modelled as a three step-process, whereby saturation of the membrane by detergent triggers the formation and release of mixed detergent-lipid micelles to solution. Here, we identify the presence of a fourth intermediate and dynamic transition towards the solubilized state: vesicle fusion. By using highly-curved sub-micron sized model-membrane vesicles, and combinations of single-vesicle characterization approaches, including measurement of the extent of Förster resonance energy transfer (FRET) between lipophilic membrane probes, we demonstrate that the widely-used non-ionic detergent Triton-X 100 induces vesicle fusion comprising an initial docking event, hemi-fusion and full lipid mixing. Our approaches reveal that fusion occurs in detergent concentrations below the critical micellar concentration and that the kinetics and mechanism are regulated by environmental factors including membrane composition and phase. We expect the developed approaches to be applicable beyond the sub-micron sized vesicles studied here, have implications for unmasking complex detergent-vesicle interactions, and lead to applications including controllable drug delivery and release.

P1.25

A biophysical model of phagocytosis

Mr Peyman Shadmani[1]

[1]*University Of Exeter, Exeter, United Kingdom*

Phagocytosis is a key part of the immune system in which cells identify, ingest, and break down foreign particles such as bacteria. Understanding the underlying biophysics of this process is relevant to improving the immune response, tackling various medical conditions, and designing micron-sized drug-delivery systems.

The details of phagocytosis are still poorly understood, particularly how cells capture and internalise particles. Cell shape change, membrane remodelling, and the actin cytoskeleton are fundamental parts of the process, and yet their facilitators and coordinators are still to be elucidated. Further, important questions such as how long internalisation takes, and the effect of target size and shape are at best only partially understood.

In this talk, I will describe my recent work to develop a computer simulation of the internalisation stage of phagocytosis. This is a type of vertex model, where biophysical forces act on each vertex. After discussing previous models in this area, I will cover the underlying biophysics of my model, and explain how it was developed and implemented. I will then introduce some new results, including the dependence on target size, the effect of target stiffness and the role of the actin cytoskeleton. Finally, I will discuss possible model extensions and what these will allow us to learn in the future.

P1.26

Mitotic Spindle Remodelling in Response to Severe Metabolic Stress

Jessica Tin Wai Ng[1,2,3], Guillaume Charras[2,3], Shiladitya Banerjee[4]

[1]Department of Physics and Astronomy, University College London, London, United Kingdom, [2]London Centre of Nanotechnology, London, United Kingdom, [3]Institute for the Physics of Living Systems, University College London, London, United Kingdom, [4]Department of Physics, Carnegie Mellon University, Pittsburg, United States

In response to severe metabolic stress, some organisms enter a reversible state known as suspended animation (SA), in which all observable activities, such as growth and motility, come to a halt. Remarkably, following SA, organisms can resume their activities, unharmed. How cells switch off their activities upon SA and preserve memory of their pre-SA states are little understood. Here we investigate how cell division, a highly dynamic process, is affected by SA. We treated mitotic HeLa cells with metabolic inhibitors and observed that spindles shrink significantly, indicating that their size control involves energy-dependent processes. After restoration of metabolism, most spindles recovered their size and cells completed division. To understand this phenomenon, we developed a mathematical model to explore how changes in microtubule dynamics and motor proteins during energy depletion affect spindle size. Model predictions suggest that differential decreases in motor activities, MT polymerization, MT flux and spindle stiffness lead to the observed spindle shrinkage. The model also predicts the lengthening of the overlap between microtubules emanating from opposite spindle poles. Overall, the spindle can be viewed as a composite material consisting of components with different sensitivities to energy and whose size is controlled by the opposing forces generated by these components.

P1.27

Less is more: Elucidating cellular transport using simplified cell models

Dr. Ran Tivony[1], Marcus Fletcher[1], Prof. Ulrich Keyser[1]
[1]*University Of Cambridge, Cambridge, United Kingdom*

Cells carefully regulate the movement of solutes across their membrane using an intricate array of interconnected transport pathways. While beneficial for mediating essential cellular activities, the abundance of complex transport pathways severely limits the elucidation of particular translocation mechanisms in live-cell studies. We alleviate this impediment by taking a reductionist approach to incorporate specific transport pathways (e.g., transport proteins) in simplified artificial cell models, using giant unilamellar vesicles (GUVs) as a biologically-relevant chassis. To gain maximal control over the bioengineering process, we developed an integrated microfluidic platform capable of high-throughput production and purification of monodispersed GUV-based cell models. Using single-vesicle fluorescence analysis, we quantified the passive permeation rate of two biologically important electrolytes, protons (H+) and potassium ions (K+), and correlated their flux with electrochemical gradient buildup across the GUV lipid bilayer. Applying similar analysis principles, we also determined the H+/K+ selectively of two archetypal ion channels, gramicidin A and outer membrane porin F (OmpF). Altogether, our results provide an insight into the transport mechanism of ions across lipid bilayers and set a framework for elucidating protein-based transport in artificial cell models.

P1.28

Exploring the shared membrane tension responsive machinery which controls cell migration and division

Dr Joseph Hetmanski[1], Dr Matthew Jones[2], Dr Patrick Caswell[1]

[1]University Of Manchester, , United Kingdom, [2]University of Plymouth, , United Kingdom

Migration and division are key cellular functions which underpin mammalian life: in development, health and disease, cells need to be able to move, divide and seamlessly switch between the two. Little is known however about how cells control transition between migration and division, or what signalling and biophysical mechanisms are shared between the processes. We found previously that membrane tension is a key biophysical parameter that drives rear retraction in 3D environments (Hetmanski et al., Dev Cell 2019; Hetmanski et al., Plos Comp Biol 2021): in non-uniform 3D or stiffness gradient environments, cells exhibit heterogeneous membrane tension where tension in lower specifically at the contractile rear. This local lower tension is sensed by mechanosensitive membrane invaginations caveolae, which activate RhoA via the GEF Ect2 to drive forward rear movement, which leads to a local decrease in membrane tension thus perpetuating a self-generated positive-feedback mechanism. Ect-2 was traditionally described as a cell division related RhoA activator, and informed by predictive mathematical models we have found surprisingly that other 'division machinery' proteins/pathways are crucially involved in motility such as the master regulator CDK1 and associated binding partners cyclins A2, B1 and B2. Using different mathematical modelling approaches alongside cutting edge imaging techniques, in particular measuring membrane tension by fluorescent lifetime imaging (FLIM), we now propose that (i) membrane tension is crucially embedded in feedback loops which drives cell division, in particular mitotic rounding, as well as rear retraction; and (ii) the machinery which drives contractility based migration and mitotic rounding is shared and requires exquisite levels of control of repositioning throughout the transition between migration and division.

P1.29

Direct, nano-rheological studies of in-plane lipid dynamics in model and native membranes

Dr William Trewby[1], Professor Kislon Voitchovsky[1]
[1]*Durham University, Durham, United Kingdom*

Lipids are now known to play an active part in roles related to protein function, oncogenesis and disease signalling, in contrast to their previously assumed passive character. Their assemblies in organelles such as lipid droplets are recognised as being crucial to the proper storage and transport of fats, and dysregulation can lead to severe pathologies including cardiovascular disease. The complex functions of lipids within the body are largely dependent on their in-plane motion, which governs the ability of biomembranes to restructure, as well as the transport of small molecules along and across the lipid layers.

Despite the significance of a holistic understanding of lipid dynamics, experimental techniques rarely have access to parameters such as friction or diffusive coefficients at a local level and over a broad range of timescales. Instead, the reliance on either equilibrium fluctuations or experimentally friendly velocities results in timescales that are typically two orders of magnitude too small for modelling real-life applications.

Here, we develop a novel high-frequency shearing device that can be used to provide dynamical information about supported lipid membranes. Crucially, the technique has contact areas of order ~nm2, allowing for direct, local energetic measurements on the scale of single proteins. We demonstrate that such bilayers have non-linear diffusive behaviour that depends sensitively on frequency, confinement and composition. Finally, we combine the technique with high-resolution imaging of a bovine eye lens to uncover the heterogeneous dynamics of crowded membranes.

P1.30

Biomimetic actin cortices shape cell-sized lipid vesicles

Lucia Baldauf[1,2], Dr. Felix Frey[2,4], Dr. Marcos Arribas Perez[2], Miroslav Mladenov[3], Prof. Michael Way[3,5], Prof. Timon Idema[2], Prof. Gijsje Koenderink[2]

[1]London Centre for Nanotechnology, University College London, London, United Kingdom, [2]Department of Bionanoscience, Delft University of Technology, Delft, The Netherlands, [3]The Francis Crick Institute, London, United Kingdom, [4]Institute of Science and Technology Austria, Klosterneuburg, Austria, [5]Department of Infectious Disease, Imperial College London, London, United Kingdom

Animal cell membranes are shaped by the actin cortex, a thin layer of cytoskeletal filaments. This cortex serves two seemingly opposing functions: on the one hand, it provides mechanical stability by stiffening the cell surface and opposing external deformations, but on the other hand it is highly dynamic, turning over constantly and exerting pushing forces that can deform the membrane. How these two factors play out to shape the cell surface remains poorly understood. Here we use bottom-up reconstitution to build dynamic actin cortices in vitro that turn over within around 60 s, comparable to the actin dynamics in living cell cortices. We show that thin branched actin cortices are stiff enough to trap the shapes of giant unilamellar vesicles (GUVs) far from equilibrium, and impart a shape memory on the vesicles that remains far beyond the actin turnover time. Using both photoablation and treatment with an actin depolymerizing drug, we demonstrate the reversibility of these deformations. We show that the same cortices can produce finger-like protrusions in GUVs without requiring actin bundling proteins. Combining theoretical modeling and experiments to explore under what conditions such protrusions form, we find that the concentration of nucleators is crucial, as locally concentrated actin polymerization forces can drive a positive feedback loop between recruitment of actin and its nucleators, and membrane deformation. Actin polymerization speed, by contrast, is found to be less important. Finally, we combine our findings to propose a working model for how actin polymerization forces shape synthetic cell membranes.

P1.31

APC-driven actin nucleation controls collective cell remodelling and motility in colorectal cancer cells

Lautaro Baro[1,2], Asifa Islam[1,2], Hannah Brown[1,2], Zoë Bell[1,2], Maria Angeles Juanes[1,2,3]

[1]National Horizons Centre, Darlington, United Kingdom, [2]Teesside University, Middlesbrough, United Kingdom, [3]Centro de Investigación Príncipe Felipe, Valencia, Spain

Cell remodelling relies on dynamic rearrangements of cell contacts powered by the actin cytoskeleton. The tumour suppressor Adenomatous Polyposis Coli (APC) nucleates actin filaments (F-actin) and localizes at cell junctions. Whether APC-driven actin nucleation acts in cell junction remodelling remains unknown. To explore that possibility, we have combined bioimaging and genetic tools with artificial intelligence algorithms applied to colorectal cancer cell monolayers. We found that the APC-dependent actin pool contributes to sustaining levels of F-actin and adhesive components at cell junctions. Moreover, this activity preserved cell junction length, angle and motion, as well as vertex motion and integrity. Finally, the loss of this F-actin pool led to larger cells with slow and random cell movement within a sheet. Our findings suggest that APC-driven actin nucleation promotes cell junction integrity and dynamics to facilitate collective cell remodelling and consequent motility. Our study offers a new perspective to explore the relevance of APC-driven cytoskeletal function in gut morphogenesis.

P1.32

A computational model for the transit of cancer cells through a constricted microchannel

Prof Yi Sui[1], Zhen Wang
[1]*Queen Mary University of London, LONDON, United Kingdom*

The dynamics of cancer cells flowing in microchannels is a fundamental problem that lies in the heart of numerous biomedical applications. In this presentation, I will summarise our recent work concerning the development of a three-dimensional computational framework to simulate the transient deformation of suspended cancer cells flowing through a constricted microchannel. We model cancer cells as a liquid droplet enclosed by a viscoelastic membrane, and its nucleus as a smaller stiffer capsule. The cell deformation and its interaction with the suspending fluid are solved through an immersed boundary lattice Boltzmann method. To identify a minimal mechanical model that can quantitatively predict the cell deformation, we conduct extensive parametric studies of the effects of the rheology of the cell membrane, cytoplasm and nucleus, and compare the results with a recent experiment conducted on human leukaemia cells. We find that the classical Skalak's law can accurately predict the steady deformation of the cancer cell in the straight channel, however, for cell transient deformation in the constriction region, excellent agreement with the experiment can only be achieved by employing a viscoelastic cell membrane model with the membrane viscosity depending on its mode of deformation (shear versus elongation). The cell nucleus limits the overall deformation of the whole cell, and its effect increases with the nucleus size. The proposed computational model will be varied on different types of cancer cells in our future work. It has the potential to serve as a general model to guide the design of microfluidic devices to sort cancer cells, or to inversely infer cell mechanical properties from their flow-induced deformation.

P1.33

2D Vertex model of early mammalian embryogenesis

Ms Alaina Cockerell[1], Dr David Richards[1]
[1]*University of Exeter , Exeter, United Kingdom*

Despite decades of research, the earliest stages of mammalian embryogenesis remain poorly understood. The first few days of development, before blastocyst implantation, give rise to a host of fascinating, unsolved questions, including the physical principles that govern cellular self-organisation, how system robustness is achieved, and ways of quantifying embryo quality. These questions are crucial for understanding human fertility, improving IVF treatment (where the majority of human embryos fail to develop beyond implantation) and tackling global food security (via economically-important animals such as pigs, cows and sheep).

Here, I will discuss my recent work to create a novel vertex model to simulate the very first few days of development. After describing the key biophysical forces involved, I will start by explaining how the model was developed and implemented. I will then present my key initial findings, including the factors that drive cell compaction and internalisation, the role of membrane tension and cell-to-cell adhesion, and key ways in which early embryogenesis can fail. I will finish by discussing future plans including experimental tests and how the model can be extended to later stages of development.

P1.34

“The very beautiful principles of natural philosophy”: Paper marbling and the physics of organic forms

Professor Robert Pepperell[1]

[1]*Cardiff Metropolitan University, Cardiff, United Kingdom*

When writing to thank an author who had sent him a book on paper marbling, Michael Faraday referred to “the very beautiful principles of natural philosophy” that the process of dropping ink on thickened water involved. What are these “beautiful principles” to which Faraday, who had apprenticed as a bookbinder, referred? Like many natural processes, paper marbling depends on the minimisation of free energy, in this case from surface tension and chemical reactions. Over time the attractive and repulsive forces acting on the inks and the water reach a state of equilibrium where the entropy of the system is maximised. This produces forms and patterns in the ink that look remarkably like cellular structures, or indeed the lines of force that Faraday observed in his own experimental work on electricity and magnetism, which he often described as “beautiful”. In this poster I will present examples of marbled images I have made that exemplify these “beautiful principles” and which seem to underlie the creation of many natural patterns. I will discuss how the physics that produces them might play a role in the organisation of living systems.

P1.35

Functional Resilience of Mutually Repressing Motifs Embedded in Larger Networks

Mr. Pradyumna Harlapur[1], Mr. Atchuta Duddu[1], Mr. Kishore Hari[1], Mr. Mohit Jolly[1]
[1]*Cancer Systems Biology Lab, BSSE, IISc Bengaluru, Bengaluru, India*

Elucidating the design principles of regulatory networks driving cellular decision-making has important implications for understanding cell differentiation and guiding the design of synthetic circuits. Mutually repressing feedback loops between 'master regulators' of cell fates can exhibit multistable dynamics enabling "single-positive" phenotypes: (high A, low B) and (low A, high B) for a toggle switch, and (high A, low B, low C), (low A, high B, low C) and (low A, low B, high C) for a toggle triad. However, the dynamics of these two motifs have been interrogated in isolation in silico, but in vitro and in vivo, they often operate while embedded in larger regulatory networks. Here, we embed these motifs in complex larger networks of varying sizes and connectivity to identify hallmarks under which these motifs maintain their canonical dynamical behavior. We show that an increased number of incoming edges onto a motif leads to a decay in their canonical stand-alone behaviors. We also show that this decay can be exacerbated by adding self-inhibition but not self-activation loops on the 'master regulators'. These observations offer insights into the design principles of biological networks containing these motifs and can help devise optimal strategies for the integration of these motifs into larger synthetic networks.

P1.36

Characterizing bacterial genes with bimodal distributions of single-cell protein levels

Ines Baptista[1], Vinodh Kandavalli[1,2], Andre Ribeiro[1]

[1]Laboratory of Biosystem Dynamics, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland, [2]Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden

Genes with the ability to express at two distinct levels, under the same set of conditions, can produce bimodal single-cell distributions of gene expression levels. This ability is believed to be evolutionarily advantageous, by allowing isogenic populations to occupy two separate regions of the state space of the phenotype, particularly in fluctuating environments. Presently, we lack knowledge of bacterial genes with this expression profile. Here, we report the finding of a few native genes of Escherichia coli which we observed to have a bimodal expression in optimal growth conditions. Next, we propose a stochastic model of bistable gene expression that allows mimicking the observed distributions. Moreover, the model provides potential intervals for the underlying rate constants that allow for the bimodal property. We expect these genes, assisted by the model, to become promising building blocks for future synthetic circuits.

P1.37

A microfluidic platform to develop continously-operating nucleic acid based systems

Kate Collins[1]

[1]*Imperial College London, London, United Kingdom*

Nucleic acid strands can be designed to assemble into arbitrary chemical reaction networks. This poster will describe a microfluidic platform developed to study continuously transcribed RNA-based reaction networks. The device contains microchambers in which DNA templates have been immobilised so that transcription reagents and waste products can be continuously added and removed from the system. As part of this work, a new method to pattern and immobilise DNA inside microfluidic devices will be described. Continuously transcribed RNA reaction networks can be monitored by fluorescence microscopy using sequence-specific fluorescent probes. Finally, the potential applications of this platform will be discussed, with a focus on investigating the long-term behaviour of RNA-based reaction networks under time-dependant control, providing a model system to explore far from equilibrium molecular processes.

P1.38

To be or not to be: Uncovering the Interplay between Phenotypic Robustness and Plasticity in Gene Regulatory Networks.

Mr Anantha Samrajya Shri Kishore Hari[1], Dr Mohit Kumar Jolly[1]
[1]*Indian Institute Of Science,bengaluru, India, Bengaluru, India*

The ability of cells to switch between different states, known as phenotypic plasticity, and the ability to maintain a stable state, known as phenotypic robustness, are crucial processes in the development of organisms and the pathogenesis of diseases like cancer. While previous studies have explored the emergence of these properties from gene regulatory networks, the balance between them remains unclear. In this study, we delve deeper into the complex gene-regulatory networks underlying Epithelial Mesenchymal Plasticity (EMP) to understand the interplay between phenotypic plasticity and robustness. Our findings reveal that the topological traits of these networks hold key information for explaining these emergent properties. We discover that while plasticity and robustness have an antagonistic relationship, common topological features such as positive feedback loops support both. These features are uniquely enriched in biological networks, hinting at their evolutionary importance. Our research opens new avenues for therapeutic targets that can control the plasticity and reduce the robustness of hybrid E/M phenotypes of cancer cells, properties that contribute greatly to their metastatic potential.

P1.39

Stochastic modelling of heterogeneity in cell populations

Francesco Puccioni[1]
[1]*Imperial College of London, London, Italy*

Quantifying cellular growth is crucial to understand the dynamics of cell populations such as microbes and cancer cells. The standard behaviour of batch cultures is well known and a delay usually characterises it before the start of exponential growth, an exponential phase, and a steady phase; however, at the single-cell level, growth varies drastically from cell to cell due to the fluctuations in the cell cycle duration, variability caused by changing environments, and cells interactions.

At the present time, understanding how the cell-to-cell variability affects the evolution of the entire population is still a open challenge; de facto, there are still lacking solid theoretical and simulation methods to forecast the effects of cell heterogeneity on the population dynamics.

We propose a novel stochastic model where the cells are represented by agents who divide, die, convert to other species, rejuvenate in response to an internal continuous state which increases with time.
While such models are usually only amenable to simulations, we show that the population structure can be characterized by a functional master equation which can be manipulated to obtain a novel integral renewal equation.

Compared to the classic results about renewal theory, as the Bellman Harris branching process and the Galton-Watson theory, the latter equation takes a step further. In fact, it provides a solid and compact stochastic description of the role played by cell heterogeneity on the population dynamics.

The analytical framework allowed us to fully describe the population size distribution, population growth rate, ancestor and division times distributions; it also enables to understand the role played by heterogeneity in the initial conditions. Moreover, we provide an analytical and numerical characterization of the extinction probability and first extinction times distribution for any cell-to-cell heterogeneity range. We also propose a novel way to simulate the evolution of cell populations affected by the variability of the individuals. Such computational tool allowed us to substantiate the analytical and numerical results obtained during this investigation.

Our last results also provide novel methods to address the role of cell-to-cell variability in time-dependent environments. We showed that the stochastic description of agent-based population dynamics can be obtained in scenarios where the reaction network rates depend explicitly on time in addition to the internal traits of the cells.

In conclusion, the following research project proposes a novel methodology to describe the stochastic behaviour of structured cell population with numerical, computational and analytical methods. Our results open a new theoretical path to understanding stochastic mechanisms underlying fluctuations in various biological and medical applications as the extinction of cancer cell populations under treatment, cell population growth in adverse environments, dormancy-awakening transition in breast cancer and microbial quiescence.

P1.40

Sparse mathematical modelling of genetic networks

Dr Andreas Jørgensen[1], Dr Mark Sturrock[2], Dr Atiyo Ghosh[1], Dr Vahid Shahrezaei[1]
[1]Department of Mathematics, Imperial College London, London, United Kingdom, [2]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland

Many biological systems, such as genetic regulatory networks, are highly complex. As a result, mathematical models of such systems often involve a large number of free parameters. These parameters must be constrained by real-world data, which can become an intractable task. Indeed, without suitable experimental constraints, it is often even impossible to pin down the relevant mechanisms to include in the model a priori, which further increases the number of model parameters. Our work presents a hierarchical approximate Bayesian computation sampling scheme using sparsity-inducing priors to alleviate these issues. Our simulation-based method is flexible and can be adapted to a wide range of mathematical models, ranging from differential equations over stochastic agent-based simulations to emulation-based machine-learning approaches. We have thus applied our sampling scheme to different biological systems, such as genetic networks, and shown that we correctly identify the underlying network topologies. Furthermore, we demonstrate the utility of our approach in discovering biochemical network topologies that can exhibit an adaptive response.

P1.41

Physical model of supercoiling mediated regulation in synthetic gene circuits

Victor Manuel Velasco Berrelleza, Sarah Harris, Susan Stepney, Penny Faulkner Rainford, Carsten Kröger, Charlie Dorman, Aalap Mogre

[1]University of Leeds, Leeds, United Kingdom, [2]University of Leeds, Leeds, United Kingdom, [3]University of York, York, United Kingdom, [4]University of York, York, United Kingdom, [5]Trinity College Dublin , Dublin, Ireland, [6]Trinity College Dublin , Dublin, Ireland, [7]Trinity College Dublin , Dublin, Ireland

Current synthetic gene circuits are designed to perform logical functions, mimicking those in electronic circuits. These designs do not include DNA supercoiling, which is intimately related to transcription as it can both up- and down-regulate the expression of distal genes. Information transfer is mediated through local DNA torsional stress, where the expression of one gene alters the local superhelical level that can either promote or repress the expression of adjacent genes.

Here, we present a physical model of supercoiling mediated regulation, which takes as an input a gene circuit (plasmid DNA sequence) under certain biological conditions and computes the output of the given system. Preliminary results show that the expression profiles directly depend on the gene circuit design (e.g gene orientation, topological barriers, promoter sequence). The model also highlights the interplay between transcription machinery and genomic architecture and can be used to predict how the system changes when varying biological conditions. Results are to be tested against wet-lab experiments of synthetized gene circuits, which are currently under preparation. Our model and experimental results will aid in the development of a computational toolkit (called TORC), which will be able to describe and design genetic circuits that exploit transcription dependent supercoiling, introducing a novel component in the new generation of synthetic biology.

P1.42

Optimal prediction in a noisy environment

Dr Jenny Poulton[1]
[1]*AMOLF, Utrecht, Netherlands*

Autonomous or self-perpetuating systems typically exist in dynamic environments. These systems include cells, robots, or even a sufficiently complex chemical network. A general requirement for self-perpetuating systems to thrive in such environments is the ability to respond to changing conditions. Ideally, a system would make an instantaneous change to respond to an environmental change. In reality, mounting a response takes time. Given this, an optimal response requires systems to predict an environmental change. In this talk, we consider the optimal prediction of time-varying signals.

For many signals, the trajectory of the signal in the past will be predictive of its future behaviour. Measuring this trajectory allows a system to predict future values of the signal. In cells, a relevant trajectory is the concentration of ligands in the environment. Here, receptors on the surface of the cell measure the concentrations of ligands in the environment. Ligands bind to these receptors, which transmit information to a downstream system within the cell. This process, like all processes at the cellular scale, is noisy. This talk is interested in understanding how systems might mitigate the effect of this signal noise.

This talk will combine two classic signal processing methods, the Wiener filter and the information bottleneck method, to find optimal signal processing methods for general systems. The results of this general procedure will then be compared to the actual method used by the push-pull motif, a common signal-processing motif in cellular systems.

P1.43

Emergence of ion-channel mediated electrical oscillations in Escherichia coli biofilms.

Mr Emmanuel Akabuogu[1], Mr Victor Martorelli[1], Dr Rok Krasovec[1], Prof Ian Roberts[1], Dr Thomas Waigh[1]
[1]*University Of Manchester, Manchester, United Kingdom*

Bacterial biofilms are communities of bacteria usually attached to solid strata and often differentiated into complex structures. Communication across biofilms has been shown to involve chemical signaling and more recently electrical signaling in Gram-positive biofilms. We report for the first time, community-level synchronized membrane potential dynamics in three-dimensional E. coli biofilms. Two hyperpolarization events are observed in response to light stress. The first requires mechanically sensitive ion channels (MscK, MscL and MscS) and the second needs the Kch-potassium channel. The channels mediated both local spiking of single E. coli biofilms and long-range coordinated electrical signaling in E. coli biofilms. The electrical phenomena are explained using Hodgkin-Huxley and 3D fire-diffuse-fire agent-based models. These data demonstrate that electrical oscillations through potassium waves are a mechanism by which signaling occurs in Gram-negative biofilms and as such may represent a conserved mechanism for communication across biofilms.

P1.44

Identification of the source of extrinsic noise from the stochastic dynamics of gene expression

Marta Biondo[1], Michele Caselle[1], Abhyudai Singh[2], Matteo Osella[1]
[1]University Of Turin and INFN, Turin, Italy, [2]University of Delaware, , USA

Cell-to-cell variability in the protein concentration is strongly affected by extrinsic noise, especially for highly expressed genes. Extrinsic noise can be due to fluctuations of several possible cellular factors, such as cell growth and volume or the level of key enzymes in the expression process. However, how to identify the specific predominant sources of extrinsic noise in empirical systems is still an open question. This work considers a general stochastic model of gene expression with extrinsic noise represented as coloured fluctuations of model parameters and focuses on the out-of-equilibrium expression dynamics. Combining analytical calculations with extensive stochastic simulations, we characterize the role of extrinsic noise in shaping the protein variability in the dynamics of gene activation or inactivation depending on the prevailing source of extrinsic variability, on its intensity and timescale. In particular, we show that qualitatively different noise trends can be identified depending on the specific fluctuating parameters. This result indicates an experimentally accessible way to pinpoint the dominant sources of extrinsic noise using time-coarse measurements.

P1.45

Exploring transcription dynamics along the cell cycle with single-cell RNA-sequencing data

Mr Dimitris Volteras[1], Dr Philipp Thomas[1], Dr Vahid Shahrezaei[1]
[1]*Department of Mathematics, Imperial College London, London, United Kingdom*

The transcriptional activity of a gene can vary significantly from cell to cell and over time. The cell cycle is a key global driver of cell-to-cell variability, causing fluctuations in gene expression levels between different cell cycle stages. At the single gene level, variability is induced by transcriptional bursting and the random timing of mRNA synthesis and degradation. Single-cell transcriptomics (scRNA-seq) technologies are central in profiling cell-to-cell variability in gene expression. However, scRNA-seq data only provides a static snapshot of transcriptomic profiles and does not provide a direct insight into transcription dynamics. Experimental approaches to temporally resolving scRNA-seq data either utilise cell cycle reporters or metabolic labelling protocols. We are focusing on a scRNA-seq dataset that combines cell cycle reporters with metabolic labelling information, providing both layers of temporal resolution (Battich et al., Science 367, 1151–1156 (2020)). We have developed a stochastic model of bursty transcription coupled to cell cycle progression and cell division dynamics, based on moment equations. The estimates of the model allow us to setup a Bayesian inference framework for extracting the cell-cycle dependence of burst frequency, burst size and mRNA degradation rate at a genome-wide scale. As a result, we are able to identify cell-cycle dependent genes, while characterising how their transcription kinetics are regulated by the cell cycle. Our method thereby aims to reveal mechanisms of global bursty transcription coupling to the cell cycle.

P1.46

Effect of receptor clustering on E.coli chemotaxis: Sensingversus adaptation

Mr. Shobhan Dev Mandal[1], Dr. Sakuntala Chatterjee[2]
[1]Senior Research Fellow, S. N. Bose National Centre for Basic Sciences, Kolkata, India, [2]Associate Professor, S. N. Bose National Centre for Basic Sciences, Kolkata, India

The cooperative behaviour of receptor dimers forming densely packed clusters is recently found to be a significant source of fluctuation in E. coli chemotaxis pathway. Highly dense large clusters can sense the nutrient concentration more efficiently, which enhances the chemotactic performance. But large clusters increase the fluctuation also, which causes adaptation module to respond strongly. Therefore, a competition develops between sensing and adaptation. At very large cluster size adaptation wins the competition resulting decrease in the sensitivity of chemoreceptors. Hence chemotactic efficiency deteriorates resulting in a performance peak[1]. To explore the sensing versus adaptation competition further we have observed the methylation dynamics of chemoreceptors during a run[2]. Change of the methylation level in a run depends sensitively on strength of concentration gradient and direction of cell movement. In weak gradient for both uphill and downhill runs, after initial demethylation we see late time methylation and range of this methylation-demethylation gets amplified as cluster size increases. In strong gradient uphill runs also show similar behaviour in methylation dynamics whereas the downhill runs show highly non-trivial dependence of methylation level on cluster size due to sensing and adaptation interplay[2]. In another study we see faster switching rate of receptor activity enhances chemotactic performance. Variation of activity for small and large value of switching rate is significantly different in downhill runs and this asymmetry causes the enhancement[3].

[1] Effect of receptor clustering on chemotactic performance of E. coli: Sensing versus adatation, by Shobhan Dev Mandal and Sakuntala Chatterjee, Phys. Rev. E Letters 103, L030401(2021).
[2] Effect of receptor cooperativity on methylation dynamics in bacterial chemotaxis with weak and strong gradient, by Shobhan Dev Mandal and Sakuntala Chatterjee, Phys. Rev. E 105, 014411(2022).
[3] Effect of switching time scale of receptor activity on chemotactic performance of Escherichia coli, by Shobhan Dev Mandal and Sakuntala Chatterjee, Indian J Phys 96, 2619 (2022) .

P1.47

Dynamical landscape of epithelial–mesenchymal plasticity as an emergent property of coordinated teams in regulatory networks

Mohit Kumar Jolly[1]

[1]*Indian Institute of Science, Bengaluru, India*

Elucidating the design principles of regulatory networks driving cellular decision-making has fundamental implications in mapping and eventually controlling cell-fate decisions. Despite being complex, these regulatory networks often only give rise to a few phenotypes. Previously, we identified two 'teams' of nodes in a small cell lung cancer regulatory network that constrained the phenotypic repertoire and aligned strongly with the dominant phenotypes obtained from corresponding network simulations. However, it remained elusive whether these 'teams' exist in other networks, and how do they shape the phenotypic landscape. Here, we demonstrate that five different networks of varying sizes governing epithelial–mesenchymal plasticity comprised of two 'teams' of players – one comprised of canonical drivers of epithelial phenotype and the other containing the mesenchymal inducers. These 'teams' are specific to the topology of these regulatory networks and orchestrate a bimodal phenotypic landscape with the epithelial and mesenchymal phenotypes being more frequent and dynamically robust to perturbations, relative to the intermediary/hybrid epithelial/mesenchymal ones. Our analysis reveals that network topology alone can contain information about corresponding phenotypic distributions, thus obviating the need to simulate them. We propose 'teams' of nodes as a network design principle that can drive cell-fate canalization in diverse cellular decision-making processes.

P1.48

Characterizing the dynamic crosstalk between p53 and NF-kB and its regulation of gene expression and cancer cell fate

Emanuele Colombo[1,2], Matteo Mazzocca[1], Alessia Loffreda[1], Daniela Gnani[1], Paola Falletta[1,2], Samuel Zambrano[2,3], Davide Mazza[1,2]

[1]San Raffaele Scientific Institute, Experimental Imaging Center, Imaging of gene regulation, Milan, Italy, [2]Vita-Salute San Raffaele University, Milan, Italy, [3]San Raffaele Scientific Institute, Division of Genetics and Cell biology, Chromatin dynamics, Milan, Italy

p53 and NF-kB are two key transcription factors (TFs) with opposite roles in cancer (tumor suppressor and pro-survival, respectively). Their pathways have been previously reported to intersect but how such crosstalk affects gene expression and cell fate remains unclear. Importantly, both TFs are "biological oscillators" and such dynamic dimension must be taken in account in order to fully represent the in-vivo cancer scenario where both TFs are activated. Here, we hypothesize that p53/NF-kB coupled dynamics can directly tune gene expression and cell fate.

We investigated p53 and NF-kB dynamics in single cells via live-cell imaging of a breast cancer cell line (MCF7) carrying fluorescently tagged p53 and NF-kB; we developed an automated AI-assisted pipeline of analysis allowing to extract TFs dynamics and morphological features changes, at single cell level.

We show that NF-kB activation by proinflammatory cytokines boosts the p53 dynamic responses, that are accelerated and reach higher levels. Such boosting is functional, since p53 target genes are transcribed more upon NF-kB co-activation. Preliminary analysis indicates that, contrarily to the expectation, NF-kB mediated boosting of p53 dynamics has a protective role against cell death. Through mathematical modelling, we identify coupling schemes that could be responsible for the observed NF-kB mediated boosting of p53 dynamics, that we are validating experimentally.

Our results highlight the relevance of studying the crosstalk of biological pathways from a dynamic perspective: our single-cell dynamic characterization of the p53/NF-kB system provides novel insights on how treatment and tumor micro-environmental cues co-participate to control cancer cell fate.

P1.49

Building computers with genetically engineered cells, which can compute, add and subtract numbers and solve maze problems

Prof. Sangram Bagh[1]

[1]*Saha Institute Of Nuclear Physics, Kolkata, India*

Abstract: The implementation of synthetic genetic logic circuits in living cells paved the way for human-designed computations to be performed by genetically engineered cells. Such cellular computations have enormous importance in biocomputer technology development at the micron scale, where microprocessor-based computers have limitations due to energy, cost and technological constraints. Here, we have created artificial neural networks (ANNs) with genetically engineered cells, where engineered bacteria worked as artificial 'neuro-synapses'. We adapted the basic concept of artificial neural networks (ANNs) and experimentally demonstrated a broadly applicable single-layer ANN type architecture with molecular-engineered bacteria to perform complex irreversible computing like multiplexing, de-multiplexing, encoding, decoding, majority functions, and reversible computing like Feynman, double Feynman and Fredkin gates. We expanded the capability of bacterial ANN and built bacterial computational devices, which can add and subtract binary numbers. Further, adapting the idea of distributed computing and applying it to the molecular biology regime, we built a biocomputer, that can solve simple maze problems. This work represents a new approach to designing and building complex cellular computation and may have significance in establishing a new platform for cellular computing and in transforming bacterial cells into ANN-enabled hardware.

P1.50

Bio-orthogonal conjugation for "wiring" redox active proteins to any conducting surface

Nicholas Yates[1], Alison Parkin[1], Martin Fascione[1]
[1]*University of York, York, United Kingdom*

Redox proteins are the proteins that facilitate biological electron transfer processes. Without such proteins many biological processes, including photosynthesis, respiration, nitrogen fixation, bioluminescence, nucleic acid biosynthesis and apoptosis, would cease to function. The study of these redox proteins (and thus the study of many fundamental biological processes) often necessitates the immobilisation of these proteins onto electrode surfaces. Currently, most covalent redox protein immobilisation methodologies broadly follow the same "electrode-up" approach – i.e. to first functionalise electrode surfaces with moieties capable of ligating to proteins, then subsequently treat the electrode surface with the protein in the hope that bioconjugation ensures - and require several factors to be considered or controlled (i.e. the thickness and composition of the grafted organic film and the orientation of immobilised redox protein). Consequently, only a small portion of published protein film electrochemistry studies use proteins that have been covalently crosslinked to the electrode, even though covalent immobilisation of redox proteins and enzymes onto electrode surfaces offers several advantages over immobilisation via non-covalent interactions. Development of "protein-down" methods of redox protein immobilisation, whereby a protein is functionalised with a moiety capable of directly forming a covalent bond with a surface, could prove interesting in this regard. Such a method could negate the need to control grafted film thickness and composition (as the steric bulk of the protein would enforce a monolayer regime), and the orientation of the immobilised proteins could be directed via the site of ligation to the surface-ligating moiety. Suitable heterobifunctional probes based on photochemically-labile triazabutadiene groups have the potential to facilitate the direct diazonium electrografting of redox proteins onto electrode surfaces.

P1.51

Bioelectric control of locomotor gaits in the walking ciliate Euplotes

Hannah Laeverenz-Schlogelhofer[1], Kirsty Wan[1]
[1]*Living Systems Institute, University of Exeter, Exeter, United Kingdom*

Control and coordination of motile appendages is essential for unicellular organisms to successfully navigate their surroundings and respond to environmental cues. The role of physiological mechanisms in this process is poorly understood. Euplotes is a unicellular organism that uses leg-like appendages called cirri (bundles of about 40 cilia) to achieve a variety of gaits including walking along surfaces and helical swimming through the water column. To reorient itself, Euplotes performs a side-stepping reaction, which involves a transient backwards motion followed by a turn. This is among the most sophisticated manoeuvres ever observed in a single-celled organism. By combining high-speed imaging with simultaneous time-resolved electrophysiological recordings, we show that this complex coordinated motion is regulated by spontaneous membrane depolarisation events that control the activity of the cirri. Using machine learning and computer vision techniques we map for the first time the observed cirri dynamics to the spontaneous bioelectric activity of the cell. Our results reveal how Euplotes, an organism without a nervous system, achieves real-time control over the activity of its multiple cirri in order to achieve a highly coordinated gait.

P1.52

Accurate dynamics from memory in chemical reactions with small copy numbers

Moshir Harsh[1], Prof. Dr. Peter Sollich[1,2]
[1]Institute for Theoretical Physics, University Of Göttingen, Göttingen, Germany, [2]Department of Mathematics, King's College, London, UK

Chemical reactions in the regime of small copy numbers can occur in gene regulation and some protein interaction networks. Small copy numbers lead to large relative fluctuations, making mean field solutions as given by mass action kinetics unreliable. Accurate calculations of the one and two-time quantities of these stochastic processes remain a challenging problem; numerical solutions of the master equation, for example, rapidly become infeasible as the number of molecular species grows, while stochastic simulations do not allow likelihood inference from dynamical trajectories.

Here, we present a method that captures the fluctuations beyond mean field using self-consistently determined memory: by integrating information from the past we can systematically improve our approximation for the dynamics of chemical reactions. This memory is not added ad-hoc, but can be shown to arise naturally by considering the effective action of the Doi-Peliti field theory of chemical reactions. The effective action is treated perturbatively but we can self-consistently re-sum a very large class of diagrams resulting in a stable expansion.

We can treat any general network of chemical reactions by deriving an analytical expression for the corrections to the mean-field free energy inspired from the diagrammatic perturbation theory calculations. We demonstrate this method and its accuracy on single and multi-species binary reactions across a range of parameter values. We show how this approach also opens a route to making inferences from experimentally measured dynamics.

P1.53

The pioneer Transcription Factor Oct4 can interpret and enhance nucleosome dynamics

Jan Huertas[1], Caitlin MacCarthy[2], Hans Schöler[2], Vlad Cojocaru[3]
[1]University Of Cambridge, Cambridge, United Kingdom, [2]Max Planck Institute for Molecular Biomedicine, Münster, Germany, [3]Babes-Bolyai University , Cluj-Napoca, Romania

Transcription factors are proteins that bind to DNA to regulate gene expression. Historically, DNA accessibility has been considered a prerequisite indispensable for transcription factor function. Nevertheless, in recent years certain transcription factors have been shown to have "pioneering activity", meaning that they can bind their specific binding sites in packed regions of the genome, where the DNA is wrapped around a nucleoprotein complex called the nucleosome.

Oct4 is a pioneer transcription factor, involved in pluripotency maintenance and that can be used for reprogramming of adult cells into stem-cell like cells. Using experimental data, we built models of Oct4 bound to two different native nucleosomes. We then performed over 50 µs of atomistic simulations of the Oct4-nucleosome complexes, which we compared to our already existing library of 25µs of nucleosome alone simulations.

We found that nucleosome flexibility is a requirement for a multidomain transcription factor like Oct4 to bind. Furthermore, we described two different mechanisms in which Oct4 can alter nucleosome dynamics, either by stabilizing naturally occurring nucleosome opening events or inducing large opening events not seen in the nucleosome alone simulations.

Overall, our findings provide a mechanistic description of the effects of a pioneer transcription factor bound to a nucleosome, at an unprecedented resolution. This work allows us to begin understanding the changes of genome architecture that happen upon expression and binding of certain transcription factors, and ultimately gives us the tools to understand cell-fate transitions.

P1.54

Sticky proteins and moving genomes: a single-molecule perspective of pluripotent cell differentiation

Srinjan Basu[1]

[1]*Wellcome-MRC Cambridge Stem Cell Institute, University Of Cambridge, , United Kingdom*

Revealing how pluripotent cells generate distinct cell lineages in the early mammalian embryo is central to uncovering the mechanisms that drive mammalian development and for unlocking the potential of pluripotent cells for regenerative medicine. The objective of my lab is to provide single-molecule insights into nuclear protein complexes that influence the ability of pluripotent cells to differentiate. Although recent studies have characterised the enzymatic activities of some of these protein complexes, little is known about their intra-nuclear dynamics or how they influence spatiotemporal 3D enhancer-promoter relationships. To investigate these dynamics, we have established tools for live-cell 3D tracking of single HaloTag-tagged nuclear proteins and inactive dCas9-labelled genes. We have also developed machine-learning algorithms to infer properties about the resulting trajectories, allowing us to determine the chromatin binding kinetics of key nuclear protein complexes and also how they control chromatin movement at specific enhancers/promoters. Using these approaches, we reveal the chromatin binding kinetics of several protein complexes that regulate differentiation. Furthermore, we show that both the chromatin remodeller NuRD and the histone methyltransferase KMT2B influence the range genes explore within the nucleus. We then use single-cell transcriptomics (scRNAseq) and chromosome conformation capture (Hi-C) to show that these changes in chromatin movement are linked to the length-scale over which enhancers activate transcription at nearby genes. We propose a model linking the movement of enhancers to gene activation as pluripotent cells differentiate. Our results highlight the importance of making dynamic measurements at single-cell and single-molecule resolution to provide insight into transcriptional control during differentiation. We are currently establishing these technologies within live mouse embryos and we believe this will help the field understand the underlying molecular mechanisms of cell-fate decisions in vivo.

P1.55

Quantifying the fitness effects of stochastic gene expression

Paul Piho[1]
[1]*Imperial College London, , United Kingdom*

Chemical master equation models are a well established framework in modelling intracellular gene regulation dynamics. These models account for intrinsic noise in gene expression to model cell-to-cell variation in clonal populations. Recent advances use agent-based modelling of growing and dividing cells to extend the chemical master equation. However, these recent works often assume that the gene expression network of interest does not affect the cell division and growth. Here, we propose a new agent-based framework for gene expression systems coupled with cell division. Thus noisy gene regulation in these systems directly contributes to natural selection when cells compete for growth.

We compare the agent-based framework to effective master equation models where the cell cycle dynamics are modelled implicitly through first-order effective decay or a dilution reaction. We use the theory to show that the effective dilution models can be in qualitative disagreement with the agent-based description due to the effect of selection acting on the stochastic reaction network. Finally, we demonstrate parameter inference for the agent-based models using two different data sets and quantify the effects of selection on the reaction network dynamics.

P1.56

Nucleosome repositioning in cell state transitions

Dr Vlad Teif[1]
[1]*University of Essex, Colchester, United Kingdom*

Genomic nucleosome positions differ from cell to cell and define accessibility of DNA for regulatory molecules, which in turn modulates gene expression. The interplay between nucleosome positioning and architectural chromatin proteins such as CTCF is further shaped by changes in DNA methylation and hydroxylation. In addition, at a larger scale, differences in nucleosome positioning lead to differences in local 3D packing of the genome. In several recent papers we aimed to dissect such interplay in biological systems ranging from stem cell differentiation to cancer development. We showed that CTCF biding can set boundaries for DNA methylation spreading (Wiehle et al., 2019) and regulates changes in the distances between neighbouring nucleosomes during cell differentiation (Clarkson et al., 2019), while a large part of this epigenetic interplay is hardwired in the DNA sequence (Thorn et al., 2022). More recently we investigated how the interplay of nucleosome repositioning and CTCF regulates cancer development and can be utilised for patient diagnostics (Piroeva et al., 2022). In this talk I will give an overview of nucleosome repositioning in cell differentiation and cancer and briefly summarise our ongoing research.

Wiehle et al (2019) Genome Research 29, 750-761.
Clarkson et al (2019) Nucleic Acids Res 47, 11181-11196.
Thorn et al (2022) Nature Communications 13, 1861.
Piroeva et al (2022) bioRxiv 2022.12.20.518743

P1.57

Modulation of DNA entanglements by a Nucleoid Associated Protein

Yair Augusto Gutierrez Fosado[1], Jamieson Howard[2], Simon Weir[1], Agnes Noy[2], Mark C Leake[2,3], Davide Michieletto[1,4]

[1]School of Physics and Astronomy, University of Edinburgh, Edinburgh, United Kingdom, [2]School of Physics, Engineering and Technology, University of York, York, United Kingdom, [3]Department of Biology, University of York, York, United Kingdom, [4]MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom

Bacterial genomes are highly packaged within cells. Their correct folding and organisation is vital as entanglements between DNA segments are detrimental of cell function and may lead to cell death. Despite the nanoscale and single-molecule insights into how nucleoid associated proteins (NAPs) bend, kink, coat, stiffen and bridge bacterial genome, their action to organise bacterial genomes in dense and crowded environments remains elusive. In this talk, I will focus on the Integration Host Factor (IHF), an abundant NAP that kinks DNA at extreme angles. I will report on recent results we obtained using particle tracking microrheology and molecular dynamics simulations of entangled solutions of lambda-DNA and IHF proteins. We show that IHF can strongly reduce entanglements and even remove elastic components, opening up the possibility that IHF may act as a "genomic fluidiser". Our results suggest a previously unappreciated key role of IHF in regulating DNA dynamics and re-organisation in vivo.

P1.58

Investigating the role of SMC proteins and CTCF in gene expression by HiP-HoP simulations of degron experiments

Cleis Battaglia[1], Michael Chiang[1], Chris A. Brackley[1], Nick Gilbert[2], Davide Marenduzzo[1]
[1]SUPA, School of Physics and Astronomy, University of Edinburgh, , United Kingdom, [2]MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, , United Kingdom

The three-dimensional organization of chromatin within the nucleus is highly interconnected with gene expression and crucial for cell function. It has been observed that structural maintenance of chromosomes (SMC) complexes play a key role in organizing the genome. Indeed, cohesin is able to extrude loops that stop at convergent occupied binding sites for CCCTC-binding factor (CTCF). However, the effect of cohesin and other loop extrusion regulatory factors on the transcriptional regulatory network of the cell has not yet been completely understood.

In this work, we used molecular dynamics simulations to investigate the roles played by loop extrusion driven by SMC proteins and regulatory factors such as CTCF and WAPL in shaping chromatin architecture. We also studied their effects on gene expression on a chromosomal scale. To obtain the results, we employed the highly predictive heteromorphic polymer (HiP-HoP) framework, which integrates polymer physics with bioinformatic data, to predict the effect of degrading each of these proteins in turn.

Consistently with previous experimental results, we observe that the average transcriptional activity is not strongly impacted by loop extrusion by SMC proteins. Strikingly, the transcriptional noise (measuring the variability of gene expression in the cell population) is instead strongly affected by the removal of these regulatory factors. From our simulations, we are also able to relate these changes in the transcriptional pattern to the ones in 3D chromosomal and gene structure.

P1.59

Exploring the roles of myosin proteins in shaping nuclear organisation

Chris Toseland[1]
[1]*University Of Sheffield, Sheffield, United Kingdom*

Myosins are well characterised cytoskeletal proteins but a subset also exists within the nucleus. Here we focus on myosin VI, a protein which is regulated by an array of partner proteins that relate to functions ranging from endocytosis, autophagy and cellular homeostasis. Myosin VI is present in the nucleus and contributes to gene expression, DNA damage response and chromosome organisation.

With regard to transcription, using a combination of super resolution imaging and 3D single molecule tracking we will show how myosin VI can support transcription hub formation. The hubs bring together several genes and multiple RNA Polymerase molecules to enable simultaneous gene expression.

For the DNA damage response, we will reveal how myosin VI contributes to DNA damage signalling following treatment with cancer therapeutics. We will also explore how chromosome rearrangements occur during these processes.

Overall, there are multiple nuclear functions of myosin VI, similar to its diverse activity in the cytoplasm. Myosin VI has roles in cancer and neurodegeneration, further studies will reveal if the nuclear activity contributes to disease.

P1.60

Dissecting a transcription-coupled chromatin silencing mechanism through mathematical modelling

Govind Menon[1], Eduardo Mateo-Bonmatí[2], Svenja Reeck[1,2], Rea Antoniou-Kourounioti[1], Anis Meschichi[3], Stefanie Rosa[3], Caroline Dean[2], Martin Howard[1]

[1]Computational and Systems Biology, John Innes Centre, Norwich, United Kingdom, [2]Cell and Developmental Biology, John Innes Centre, Norwich, United Kingdom, [3]Swedish University of Agricultural Sciences, Uppsala, Sweden

Our understanding of chromatin mediated epigenetic memory and maintenance of transcriptional states is mainly driven by bioinformatic analysis of histone modifications and transcription at the genome level. Mechanistic mathematical models, describing histone modification dynamics at individual genomic loci, offer an alternative approach, going beyond networks of correlational relationships. In conjunction with quantitative experimental data, this allows us to dissect causal molecular mechanisms that drive switching/maintenance of transcriptional states. Here we use mathematical modelling to understand a transcription-coupled mechanism for chromatin silencing at the FLC gene locus in Arabidopsis, a model system for epigenetic regulation.

The FLC locus can be epigenetically silenced in early development by the highly conserved Polycomb repressive complexes, which work by adding repressive histone modifications and read-write maintenance of these modifications. Regulation by the Polycomb system is a core mechanism for switching gene expression programmes during differentiation and development in plants and animals. Polycomb mediated repression is antagonised by transcriptional activity, which promotes removal of repressive modifications. However, Polycomb silencing at the FLC locus requires a transcription-coupled mechanism, mediated by alternative termination of non-coding antisense transcription from the 3' end of the locus. Here we develop a mathematical model of this transcription-coupled regulation, describing the dynamics of sense and antisense transcription, alternative use of transcription termination sites, and the interplay of these processes with dynamically varying levels of activating (H3K4me1) and silencing (H3K27me3) histone modifications. The model allows us to capture different feedback mechanisms operating at the level of a single gene locus, and make testable predictions, which we then validate through a combination of genetic, molecular, and imaging approaches.

Our mathematical model, combined with experiments, elucidates how a transcription-coupled repression mechanism can set up an epigenetically silenced state during development. The model reveals how such a co-transcriptional mechanism can set the level of productive transcription at a locus, thus dictating the level of transcriptional antagonism to Polycomb silencing, which then determines the probability per unit time of switching to an epigenetically silenced state.

P1.61

4D chromatin domains: temporal and spatial resolution of topologically associated domains

Alonso Pardal[1], Dr Filipe Fernandes Duarte, Dr Andrew Bowman
[1]*University of Warwick, Coventry, United Kingdom*

Chromatin movement is implicit in genomic processes, yet it is only beginning to be understood. Whilst visualisation of individual loci has greatly expanded our knowledge of underlying chromatin dynamics, how chromosomes move at the domain level is relatively unexplored. Here we used a histone pulse-labelling approach (RAPID-release - Apta-Smith et al., 2018) to visualise late replicating TADs/RDs, leveraging lattice light sheet microscopy to analyse domain-level dynamics at extended sampling regimes. Whilst time and ensemble averaged MSD values were comparable to those reported previously for individual loci, we found two discrete populations of exponents, suggesting heterogeneity in domain diffusion. Visualising dynamics for extend periods (up to one hour) revealed rare but significant translocation events. In these events, domains could exceed three microns of translocation and displayed elastic behaviour, often retracing their trajectory and sending out sub-domain extrusions long their vector of movement. We discuss our observations and the implications they have for the topological dynamics of chromatin.

P1.62

Response of bacterial regulatory networks under dynamic perturbations imaged with single cell resolution

Aske Petersen[1], Sine Lo Svenningsen[3], Marco Cosentino Lagomarsino[2], Pietro Cicuta[1]
[1]University Of Cambridge, Cambridge, United Kingdom, [2]Istituto FIRC di Oncologia Molecolare, Milan, Italy, [3]University of Copenhagen, Copenhagen, Denmark

Recent developments in the production of microfluidic devices have inspired and empowered biologists to pursue questions with much higher throughput and resolution. Microfluidics enables control of the extracellular environment while using optical microscopy to characterise individual cells repeatedly within a cell cycle. Such experiments have the power to revolutionize our understanding of how gene expression links to cell growth. I have constructed a prototype device of the so-called mother-machine which can be used to simultaneously follow hundreds of single cell divisions under changing environmental conditions. When downshifting, or starving, bacterial cells various stress responses are activated. In bacterial cells, an efficient way of adjusting gene expression to strong environmental cues is through small RNA (sRNA) regulation. With this mother-machine device the functional role of the small RNA glycine cleavage system B (GcvB) will be investigated under various amino acid fluctuations. The option to switch on and off between sRNA inducing conditions, while following growth rate, will give a clear indication of how sRNAs assist in growth adaptation. Previously, the laboratory used the mother-machine to interrogate dynamic size-growth behavior across carbon upshifts. Building on this work, and well-established “growth laws”, we aim to use bacterial strains reporting on ribosomal and constitutive promoters to investigate how ribosomal levels alter when increasing the nutrient state.

P1.63

Polymer Simulations Predict Gene Structural Heterogeneity and Transcription

Dr Michael Chiang[1], Dr Chris A. Brackley[1], Dr Catherine Naughton[2], Dr Ryu-Suke Nozawa[3], Ms Cleis Battaglia[1], Prof Davide Marenduzzo[1], Prof Nick Gilbert[2]

[1]SUPA, School of Physics and Astronomy, University of Edinburgh, Edinburgh, United Kingdom, [2]MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom, [3]Division of Experimental Pathology, Cancer Institute of the Japanese Foundation for Cancer Research, Tokyo, Japan

A long-standing belief in chromatin biophysics is that the three-dimensional (3D) organisation of chromatin is strongly coupled to gene transcription. Despite significant progress being made in understanding chromatin structure, its connection to gene expression is still elusive, partly because it remains challenging to measure DNA and transcription dynamics simultaneously in experiments. In this work, we consider an orthogonal approach to address this question by performing large-scale simulations using the highly predictive heteromorphic polymer (HiP-HoP) model, a first-principle model that does not require fitting to existing genome structural data. Our simulations predict the 3D conformations of all genes across the human genome and their transcriptional activity with a high level of agreement with empirical data. We then use this pan-genomic data set to infer generic principles linking gene structure and expression. We find that gene transcription activity is highly correlated to the formation of microphase-separated clusters of DNA regulatory elements, while cell-to-cell variation in activity, or transcriptional noise, is driven by structural heterogeneity. We additionally discover that loop extrusion, an important determinant of chromatin architecture, promotes noise but has minimal impact on mean transcription. Our work thus establishes key structural mechanisms driving transcription and its noise, which could be relevant to cell development and differentiation.

P1.64

On the inference of transcriptional burst kinetics from scRNA-seq data

Dr. Wenhao Tang[1], Dr. Andreas Jørgensen[1], Dr. Samuel Marguerat[2], Dr. Philipp Thomas[1], Dr. Vahid Shahrezaei[1]

[1]Imperial College London, London, United Kingdom, [2]UCL Cancer Institute, London, United Kingdom

Gene expression is characterized by stochastic bursts of transcription that occur at brief and random periods of promoter activity. The kinetics of gene expression burstiness is different for different genes in the genome and is dependent on the promoter sequence, among other factors. The gene expression variability is also affected by coupling to other stochastic processes in the cell, such as the cell cycle. Single-cell RNA sequencing (scRNA-seq) has made it possible to quantify the cell-to-cell variability in transcription at a global genome-wide level. However, scRNA-seq data is prone to technical variability, including low and variable capture efficiency of transcripts from individual cells. Here, we propose a novel mathematical theory for the observed variability in scRNA-seq data. Our method captures burst kinetics and variability in cell size and capture efficiency, which allows us to propose several likelihood-based and simulation-based methods for the inference of burst kinetics from scRNA-seq data. Using both synthetic and real data, we show that the simulation-based methods provide an accurate, robust and flexible tool for inferring burst kinetics from scRNA-seq data. In particular, a simulation-based inference method based on neural networks proves to be accurate and useful in application to both allele and non-allele specific scRNA-seq data.

P1.65

Melts of loop-extruded polymers

Filippo Conforto, Davide Michieletto
[1]*University of Edinburgh, Edinburgh, United Kingdom*

Polymer melts are ubiquitous in industry and biology. They display peculiar viscoelastic properties which are dependent on the way polymers are entangled with each other. Inside the nucleus of cells, chromosomes are also heavily entangled and several ATP-consuming proteins perform mechanical and topological operations on them to manage entanglements. Among the most well studied are Topoisomerases and SMCs, also known as loop extruders. To understand their impact on the viscoelastic properties of the genome, in this work we simulate entangled melts of polymers under the action of Topoisomerases and Loop Extruders proteins. We discover that loop extruders drive the formation of transient bottle-brush-like structures which significantly lower the viscosity of the melt by modulating the entanglements between chromosomes; we characterise how this effect depends on the abundance and speed of the loop extruders. Intriguingly, we also discover that Topoisomerases have a weaker impact on the dynamics of the polymers and can, in certain conditions, increase the effective viscosity of the melt when bridging is included.
Our work will inform and guide future in vitro experiments with Condensins and Topoisomerases.

P1.66

Biophysical Characterisation of NANOG-DNA interactions

Amandine Hong-Minh[1], Nicholas Mullin[1], Davide Michieletto[1], Ian Chambers[1]
[1]*University Of Edinburgh, Edinburgh, United Kingdom*

The properties of mammalian stem cells are governed by the action of cell-specific transcription factors. The transcription factor NANOG determines the efficiency of embryonic stem cell self-renewal (Chambers et al., 2007). NANOG contains a tryptophan repeat (WR), an intrinsically disordered region which is characterised by ten tryptophans spaced five residues apart. The WR is necessary for NANOG function (Mullin et al., 2017). However, the role of the WR in the biophysical and structural properties of NANOG-mediated DNA organisation is unknown.

We have performed in vitro assays of wild-type NANOG and functional/non-functional WR mutants. At concentrations below that which causes gel formation by wild-type NANOG, none of these variants self-assemble into gel-like structures. However, in the presence of long, double stranded DNA, functional variants trigger gelation whereas the non-functional mutant does not.

The data suggests that wild-type NANOG binds DNA via the homeodomain and forms short oligomers via the WR that crosslink DNA. In contrast, non-functional WR mutants can bind DNA but cannot oligomerise, and consequently cannot crosslink DNA.

Our results suggest a link between the biophysical properties of the WR and the biological function of NANOG. NANOG-mediated crosslinking of specific DNA sequences (e.g., certain promoters and enhancers) may be necessary for efficient stem cell self-renewal. Future work will investigate the biological relevance of the gel-like properties of DNA-NANOG fluids and their control on gene expression.

P1.67

Allosteric topological modulation of toehold-mediated strand displacement

Dr Andrew Stannard[1,2], Dr Aditya Sengar[3], Dr Alexander Clowsley[4,5], Prof Christian Soeller[4,5], Dr Thomas Ouldridge[3], Dr Lorenzo Di Michele[1,2]

[1]Department of Chemistry, Imperial College London, London, United Kingdom, [2]Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge, United Kingdom, [3]Department of Bioengineering and Centre for Synthetic Biology, Imperial College London, London, United Kingdom, [4]Living Systems Institute and Biomedical Physics, University of Exeter, Exeter, United Kingdom, [5]Institut für Physiologie, Universität Bern, Bern, Switzerland

For over two decades, nucleic acid strand displacement reactions have been employed in a panoply of dynamic DNA nanotechnologies. Ever more elaborate reaction schemes of toehold-mediated strand displacement (TMSD) have been developed to exquisitely control strand displacement rates for the purpose of actuating DNA-controlled kinetic processes. Here we have (i) explored how topological constraints regulate ssDNA hybridisation and, thus, TMSD, and (ii) developed an approach to allosterically modulate the process.

P1.68

Understanding the relationship between mechanical and morphological changes in cells subjected to vibrational stimulation

Ms Olivia Johnson-Love[1], Dr Peter Childs[1], Prof Stuart Reid[1], Prof Matt Dalby[2]
[1]University Of Strathclyde, Glasgow, United Kingdom, [2]University of Glasgow, Glasgow, United Kingdom

Vibrational stimulation has been shown to affect the mechanical and morphological properties of cells. Devices have been developed capable of applying nanoamplitude vibration of 1 kHz at amplitudes of 30 nm. Applying this stimulation to cells has been shown to result in increased cell contractility and actin stress fibre formation, and when applied to mesenchymal stem cells, to direct differentiation toward an osteoblastic phenotype. However, the mechanical changes involved in this process remain poorly understood. Here, murine fibroblast cells (NIH 3T3) were used to investigate the relationship between mechanical and morphological changes in response to 1kHz, 30nm vibrational stimulation for 72 hours. Atomic force microscopy (AFM) was used to further understand cellular stiffness in both the nucleus and cytoplasm. Immunofluorescent staining was then used to investigate changes in nuclear and cell area, and actin and vinculin intensity. Through AFM, the nucleus of 3T3 cells were found to stiffen significantly within 24 hours of vibrational stimulation. Simultaneously, nuclear area also significantly increased, along with actin intensity, although less consistently. The increase in cell stiffness may be due to increased cytoskeletal tension following vibration, also altering nuclear shape. Inhibiting actin polymerisation and contraction would be one method to demonstrate a causal link. By measuring cellular mechanical properties, it may be possible to optimise key vibration parameters which direct mesenchymal stem cell differentiation.

P1.69

Towards mimicking the physical bone marrow microenvironment of hematopoietic stem cells

Juan Rubio Lara[1], Daniel Bode[1], Lilia Cabrera-Cosme[1], Grace Vasey[1], Andrew Hodgson[2], Kevin Chalut[2], David Kent[1]

[1]University of York, York, United Kingdom, [2]Wellcome-MRC Cambridge Stem Cell Institute, Cambridge, United Kingdom

The expansion of haematopoietic stem cells (HSCs) ex vivo is crucial for the manufacture of hematopoietic cells including blood for clinical applications. Current in vitro cultures efforts have focused on supplementing the cell media with specific recombinant growth factors or small molecules, but relatively few have accounted for the role of physical attachment to cells in the bone marrow microenvironment. We have created a more bone-marrow like microenvironment outside the body by utilising STEMBOND hydrogels1 with different mechanical stiffnesses in combination with the ability to functionalise these gels with different extracellular matrix (ECM) proteins. First, we miniaturised the STEMBOND fabrication to make the gels on 96 well plates, allowing bulk and single cell studies comparable to tissue culture plastic. Using short and long-term HSC expansion cultures2,3 we explored the impact on retention and/or expansion of HSCs grown on different hydrogels. When using short term cultures on young murine HSCs (8-12 weeks) we observed an increase of expansion and purity of phenotypic HSCs (pHSCs) in softer gels, that closely resembled the in vivo bone marrow niche stiffness (15-30% purity) and a reduction in HSCs with increased stiffness (5% purity). In the case of middle-aged mice (1 year old), there was an increase in numbers of pHSC positively correlated with a gradual increase of stiffness. Next, we moved to functionalising gels with different ECM molecules where we observed that ECM protein vitronectin supported a 50-fold expansion of phenotypic HSCs (with 30% ELSK) which was accompanied by an increase in transplantation efficiency in vivo compared to other molecules and cells cultured on tissue culture plastic. Using long-term expansion cultures along with proteomic data from TET2 knockout HSCs, a known epigenetic regulator that leads to leukemia, we identify novel ECM proteins that could alter HSC expansion in malignancy. Finally, using alternate hydrogels, we explored 2D versus 3D configurations to determine whether the orientation of ECM protein delivery was also involved. Together, this platform sets the stage for new studies that will further our understanding of HSC:niche interactions.

P1.70

Circular RNA as a platform for gene expression control in synthetic biology and therapeutics

Ms. Lisa Doetsch[1], Dr. Wooli Bae[2], Dr. Thomas Ouldridge[1], Prof. Guy-Bart Stan[1]
[1]Imperial College London, London, United Kingdom, [2]University of Surrey , London, United Kingdom

Ribonucleic Acid (RNA), once just the intriguing intermediary between deoxyribonucleic acid (DNA) and proteins, is now increasingly recognised for its large variety of functions and engineering potential. RNA properties such as its programmability, fast in vivo production and low cell burden lend themselves well for synthetic biology. But, anyone that has worked with RNA knows: RNA is difficult to work with. Apart from the issues rapid degradation generates with lab work, the in vivo consequences are high dynamics, often low concentration levels and high variety in molecule numbers over time and between cells. This poses a problem for their utility in synthetic biology, where reproducibility and control are key.
The 5'- and 3'-ends of RNA mediate the action of many ribonucleases. Circularised RNA, not having any ends, could therefore be reasoned to be comparatively stable and literature does indicate this to be true. However, inefficient production has limited the use of cRNA in engineering so far. A novel method, Tornado, reported by Litke and Jaffrey, produced levels of cRNA in mammalian cells significantly higher than other in vivo production methods, reaching micromolar concentrations that can saturate protein targets. Still, they do not report on other RNA species, such as RNA of longer lengths, or other cell systems.
This project aims to facilitate more control in RNA engineering in two directions: first, circularising short RNA species and implementing them in genetic circuits to regulate gene expression in bacteria, and second, circularising mRNA for translation of proteins directly from the circularised species.

P1.71

A geometry-driven organoid model for the investigation of the role of mechanical cues in pancreatic cancer

Sophie Kurzbach[1], Maximilian Reichert[2], Carsten Grashoff[3], Andreas R. Bausch[1]

[1]Cellular Biophysics, Center for Functional Protein Assemblies (CPA), Technical University of Munich (TUM), Garching b. München, Germany, [2]Klinik und Poliklinik für Innere Medizin II, Klinikum rechts der Isar, Munich, Germany, [3]Quantitative Cell Biology, Institute for Molecular Cell Biology, University of Münster, Münster, Germany

Pancreatic ductal adenocarcinoma (PDAC) remains a particularly aggressive and lethal form of cancer, being mostly resistant to conventional treatments. In decades of cancer research, the primary role of the extracellular matrix (ECM) mechanics was underestimated, which is now known to be crucial for the initiation of malignant phenotype.

Organoids are a promising in vitro model system for the investigation of the underlying mechanisms of the characteristic branching morphogenesis of PDAC under controlled conditions. In comparison to in vivo models, not only the biochemical composition but also the mechanical properties of the ECM like the geometry can be regulated in a controlled manner. The design of geometry-driven organoids offers the possibility of studying the effect of those parameters on the invasive behavior of cancer cells depending on their microenvironment. Here, we present a powerful tool for the measurement of the spatiotemporal distribution of forces in cell-cell and cell-ECM interaction regions during PDAC morphogenesis. We show, that genetically encoded FRET-based tension sensor modules (TSMs) integrated into the protein sequence of vinculin and alpha-catenin can be transferred from cell monolayers to complex multicellular systems. By this quantitative readout, we can better understand the role of ECM mechanics in specifying mechanosensitive pathways, which opens new prospects in therapeutic development.

P1.72

Knitting with DNA

Catherine Fan[1,2], Jonathan Bath[1,2], Andrew Turberfield[1,2]
[1]University of Oxford, Department of Physics, Oxford, United Kingdom, [2]The Kavli Institute for Nanoscience Discovery, Oxford, United Kingdom

Nucleic acid nanostructures can integrate sensing, computation and actuation, enabling creation of molecular robotic devices. Most nucleic acid nanostructures are made from DNA and designed such that the target structure, which is assembled by annealing multiple synthetic oligonucleotides, is the most stable possible product. The ability to create recombinant RNA nanostructures within cells has the potential to advance the field by creating new technologies from functional imaging probes to theranostic devices combining diagnosis and treatment with (sub)cellular resolution. Nanostructure assembly by co-transcriptional folding of a single strand requires new design principles. Structures must not be designed solely (or even primarily) for stability but to control the dynamic assembly process and exploit the kinetic traps created while the polymer folds as it is synthesized. Taking inspiration from knitting, we are designing sequences that form rows of interlocked (pseudoknotted) loops. Each row locks the previous row in place. As proof of principle we are using rolling circle amplification of DNA (not RNA) to investigate the folding during polymerization of a long, periodic strand. We have demonstrated the formation of interlocked hairpin loops and are investigating the formation of knitted DNA ribbons.

P1.73

Enhancing the spectral range of bacterial and plant Light-Harvesting antenna complexes using synthetic chromophores incorporated into lipid membranes

Dr. Ashley Hancock[1], Dr. Sophie Meredith[1], Dr. David Swainsbury[2], Dr. Kenichi Morigaki[3], Prof. Neil Hunter[4], Dr. Peter Adams[1]

[1]University Of Leeds, Leeds, United Kingdom, [2]University of East Anglia, Norwich, United Kingdom, [3]Kobe University, Kobe, Japan, [4]University of Sheffield, Sheffield, United Kingdom

Light-harvesting (LH) protein complexes are found within plants and have the crucial role of absorbing sunlight in the early stages of photosynthesis. LH proteins contain a suite of natural pigments that each absorb light at specific wavelengths, however, the natural combinations of pigments found in any one protein do not cover the full spectral range. We developed a biohybrid model system where the effective absorption range of the plant protein Light-Harvesting Complex II (LHCII) was enhanced by assembling the protein into lipid membrane vesicles alongside synthetic lipid-linked Texas Red (TR) chromophores. Förster resonance energy transfer (FRET) from TR donors to LHCII acceptors was observed with an efficiency of up to 95% by fluorescence spectroscopy. Alternatively, LHCII and TR were organised into lipid nanodiscs to provide an idealised system to probe TR-to-LHCII energy transfer using ultrafast transient absorption spectroscopy. These measurements revealed a ~4 ps timescale for FRET and were correlated with molecular dynamics simulations and theoretical calculations of excitation dynamics. Recently, we demonstrated the modularity of such biohybrid systems with an in-depth comparison of the effectiveness of five different organic chromophores for enhancing the absorption range of two different LH complexes (plant and bacterial). At the highest tested dye-to-protein ratios the absorption strength integrated over the full spectral range was increased to ~180% of its natural level for both LH complexes. In summary, the biohybrid system developed is a platform to quantify the biophysical properties of photosynthetic machinery and demonstrate that complementary synthetic dyes can significantly enhance their spectral range.

P1.74

Protein-Functionalized DNA Hydrogels

Giorgia Palombo[1], Yair A. Fosado[1], Davide Michieletto[1]
[1]*School of Physics and Astronomy, University of Edinburgh, Edinburgh, United Kingdom*

The Watson-Crick base pairing makes DNA an ideal building block for intelligent biomaterials[1]. Recently, there has been an increasing effort to design and characterise hydrogels made by DNA nanostars[2]. Despite this, their functionalisation using proteins or enzymes has been overlooked.

In this poster, I shed new light on this by employing experimental and computational methods. First, I perform the experimental design of active DNA hydrogels with a controlled tuning of their viscoelasticity in time by the action of proteins that cut, bend, and alter the network's topology. Second, I present our attempt to characterise from simulations the geometrical structure of this system and rationalise the observations from experiments. Finally, I introduce (1) a potential biomedical application[3] of DNA nanostar hydrogel minimising bacterial biofilm growth on surfaces and (2) an appealing soft material obtained by hybridising DNA with peptides.

This work encourages further studies on this class of materials and paves the way for drug delivery and tissue regeneration applications.

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P1.75

Novel Infrared Glucose Biosensors Optimised for in vivo Imaging

Katherine Sanders[1], Dr Nordine Helassa[2], Dr Adam Wollman[1]

[1]Newcastle University Biosciences Institute, Newcastle, United Kingdom, [2]Liverpool Centre for Cardiovascular Science, Liverpool University, Liverpool, United Kingdom

Glucose sensing is fundamental in understanding key mechanisms and metabolic pathways implicated in diseases such as diabetes and cancer. The need to understand these mechanisms has led to the development of glucose biosensors that can measure live glucose uptake in cells through the use of Forster Resonance Energy Transfer (FRET). FRET occurs between two fluorescent proteins with overlapping excitation and emission spectra brought within 10 nm of each other. These fluorophores can then be linked to a protein that undergoes a conformational change upon ligand binding. The change in FRET as a result of glucose binding can be used as a fluorescent readout of glucose uptake in cells. However, current glucose biosensors are restricted by the fluorescent proteins only emitting in the visible light spectra, limiting their application to be used alongside other fluorescent tags and within tissues, which are relatively opaque to visible light. Replacing these fluorescent proteins with those in the near infrared spectrum will open the possibility of multiplex imaging and deep tissue imaging. The possible applications of an infrared glucose biosensor include patient glucose sensing or cancer tumour imaging.

Preliminary data of design and characteristics of a novel infrared glucose biosensor will be presented, utilising new infra-red fluorescent proteins based on bacterial phytochromes. Sensors were designed empirically and visualised using structure prediction software AlphaFold2 before construction. Gibson Assembly was used to create sensor plasmid constructs before being assessed via fluorimetry for glucose sensitivity.

P1.76

Frost spreading and pattern formation on microstructured surfaces

Lukas Hauer[1,2]

[1]*Humboldt University, Berlin, Germany,* [2]*Max Planck Insitut for Polymer Research, Mainz, Germany*

Frost is found in nature as a symphony of nucleation and heat and mass transport, cascading from angstroms to several meters. Here, we use laser-induced fluorescence microscopy to investigate the pattern formation of frost growth in experiments which tune the mesoscopic length scale by using microstructured pillar arrays as a frost condenser surface. By controlling the degree of surface supercooling and the amount of condensate, different modes of frost patterning are uncovered, ranging from complete surface coverage to fractal-looking and limited-coverage structures of spiky appearance.

P1.77

Video analysis of ciliated epithelia

Ricardo Fradique[1], Erika Causa[1], Clara Delahousse[1], Laetitia Pinte[2], Marta Vila-Gonzalez[2], Pietro Cicuta[1]
[1]Cavendish Laboratory, University Of Cambridge, Cambridge, United Kingdom, [2]Wellcome - MRC Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, ,

Motile cilia are fundamental to physiology of various human organs, allowing nutrient transport in the brain and mucociliary clearance in the lungs. The coordination of cilia is one of the questions where physics seems to have a large role. Fraction of ciliated cells, cilia density, spatial distribution, and beating frequency (CBF) are all important parameters both in diagnostics, for example in the case of Primary Ciliary Dyskinesia, and in the study and development of in vitro models. As well as physics questions we have been working with samples derived from induced Pluripotent Stem Cells, and there the challenge is to make tissues as physiological as possible, and to quantify the success.

Most of the open source tools available at the moment for video analysis of motile cilia are limited in the type of information they can provide, usually only describing the CBF of very small areas, while requiring human intervention and training for their use. Here, we propose a novel method to fully characterize the cilia beating frequency and the beating cilia coverage over large areas, in an automated function without any required human intervention.

We demonstrate the ability of the method to differentiate between different coverage densities, identify even small patches of cilia in a larger field of view, and fully characterize the cilia beating frequency of all moving areas. We also show that the method can be used to combine multiple fields of view to better describe a sample without relying on small pre-selected regions of interest.

This development allows us to deploy a complete automated open-source software pipeline, with a simple graphical user interface for sample selection, that can fully analyse single fields of view in a few minutes, with a larger sample characterization possible in a few hours.

P1.78

Rotary motor powered gliding motility of a filamentous cyanobacterium as a possible driver of dynamic pattern formation within a biofilm

Jerko Rosko[1], Sarah Duxbury[1], Kelsey Cremin[1], Mary Coates[1], Marco Polin[1,2], Orkun Soyer[1]
[1]University of Warwick, Coventry, United Kingdom, [2]Mediterranean Institute for Advanced Studies, Esporles , Spain

Filamentous cyanobacteria capable of gliding motility play an important role in assembly of some types of structured microbial communities. While motility is hypothesized to be a key driver of structure formation, it is itself poorly understood, and the mechanism may vary between species.

We studied a novel filamentous cyanobacterium, isolated from a local water body, which forms biofilms exhibiting highly dynamical wave patterns. Out of the two mutant strains, non-motile and motile, only the latter gives rise to such biofilms, pointing to a role of motility in pattern formation.

Similar filamentous cyanobacteria were previously thought to glide using type IV pili or to generate displacement as they extrude large quantities of material through secretion complexes. However, in the present case, and several others, such models are unable to capture all aspects of the behavior.

Thus, we combined light microscopy with analytical techniques to elucidate the mechanism behind gliding motility of this species. We found evidence for a different model where, similar to Myxobacteria, molecular motors power axial rotation of the filament, and linear displacement is generated through cell-surface interactions.

P1.79

Motile cilia induce velocity and diffusion within the Periciliary Layer

Ms Erika Causa[1], Mr Morten Kals[1], Dr Luigi Feriani[1], Dr Jurij Kotar[1], Prof Pietro Cicuta[1]
[1]*University of Cambridge, Physics Department, Cambridge, United Kingdom*

The ciliated epithelium of the human respiratory tract is lined by a thin stratified fluid. This airway surface liquid (ASL) serves as a protective barrier and is essential for maintaining respiratory mechanics. However, our understanding on how it is propelled by cilia and how flow is coupled between the two ASL compartments is still fragmentary. Mucus transport can be measured experimentally via various techniques, but the complex and impenetrable structure of the Periciliary Layer (PCL), occupied by cilia-tethered mucins which create a brush with nanometric mesh size, is more challenging as for example it interferes with the conventional use of tracer beads. Earlier studies have measured the average displacement of fluorescent dyes localised in the PCL, but have not managed to extract a clear velocity profile in this layer. In the last decades, great effort has also been put in understanding cilia-driven flows from a theoretical perspective. However, given the complexity of the system, many studies simulated the problem by introducing one or several approximations, commonly producing contrasting results.
We tackled the constrains posed by the PCL structure with the use of caged-fluorescent compound and high-speed imaging of airway epithelium from a side view. Briefly, we photoactivated the dye in a localised region within the PCL and followed its translation and diffusion over time. We believe that activating the compound at different distances from the apical surface of the epithelium and extracting the velocity for each position provides an effective method to experimentally determine the flow profile in the PCL.

P1.80

Mathematical modelling of trichome pattern formation in Arabidopsis thaliana

Toquinha-Oerlia Bergmann[1]

[1]*Freiburg Center for Data Analysis and Modeling, Freiburg im Breisgau, Germany*

How does a spatial pattern occur in a tissue, when every individual cell has the same genetic material? Turing was the first to propose an answer in form of a reaction-diffusion system, which, under certain conditions, forms a heterogeneous pattern through diffusion-induced instability. Many morphogen-mediated developmental mechanisms based on Turing's theories have since been identified.

A common model to study two-dimensional pattern formation is the distribution of trichomes on leaves of the plant Arabidopsis thaliana. By studying modified pattern of mutants, underlying regulatory genes related to trichome development have been identified. In 2020, Balkunde et al. proposed a combined activator-inhibitor (AI) and activator-depletion (AD) model that describes phenotype-genotype relationships, which were not included in the previous AI and AD models alone. However, there are still mutants, such as the TRY/CPC mutant, that the AI-AD model cannot describe.

The aim of our work is to further develop the existing model structure to describe unexplained phenotypes. We incorporate genes and mechanisms into the existing model utilizing our experimental findings. We then simulate our model using various initial conditions and compare the simulation results with experimental observations. Because growth and division were neglected in the previous models, we simulate this system using a growing and dividing tissue to replicate in vivo experiments. With this model, we investigate the effects of growth on the emergent trichome patterning, allowing us to parse the effects of growth and gene regulation on the patterning system.

P1.81

Initially trapped individuals use wrinkles to escape from the centre in Bacillus subtilis biofilms during spatial expansions

Dr. Nikhil Krishnan[1], Mr. Joseph Knight[1], Dr. Diana Fusco[1]
[1]*Cavendish Laboratory, Department of Physics, University of Cambridge, Cambridge, United Kingdom*

In bacterial expansions, upon inoculation of a small radial colony on solid agar that is allowed to expand over time, one expects that individuals that initially lie well within the initial boundary of the colony will remain trapped due to lack of access to nutrients.

In biofilms, a bacterial lifestyle characterized by cell aggregation to surfaces and other individuals as well as division of labor and cooperativity, the onset of complex architecture formation sets it apart from other modes of growth.

Indeed, the observation that initially trapped cells fail to make it to the front of the expansion holds true in a B subtilis biofilm comprised of a mutant that is unable to form fluid filled wrinkles or aerial projections that extent from the centre to the edge of the colony.

However, when wrinkling is allowed to occur, initially trapped cells are found at small quantities outside of their initial boundary within wrinkles and at the biofilm edge. Interestingly, this occurs with or without flagellar expression, which provides B subtilis the ability to swarm and swim.
When the initially trapped cells are given a selective advantage the few cells that are able to traverse the wrinkles out-compete the initially outer cells forming sectors at the tip of the wrinkle.

We show that spatial distributions of sectors follow known population dynamics of spatial expansions with respect to initial inoculum radius and selective advantage. In addition these results are the first known evidence for cellular transport through B subtilis biofilm wrinkles.

P1.82

Light controlled biohybrid microbots

Nicola Pellicciotta[2,3], Ojus Satish Bagal[2], Ms. Viridiana Carmona Sosa[1,2], Giacomo Frangipane[2,3], Roberto Di Leonardo[2,3]

[1]Cavendish Laboratory, University of Cambridge, Cambridge, United Kingdom, [2]Dipartimento di Fisica, Sapienza Università di Roma, Rome, Italy, [3]NANOTEC-CNR, Soft and Living Matter Laboratory, Rome, Italy

Biohybrid microbots integrate biological actuators and sensors into synthetic chassis with the aim of providing the building blocks of next-generation micro-robotics. One of the main challenges is the development of self-assembled systems with consistent behavior and such that they can be controlled independently to perform complex tasks. Here we show that, using light-driven bacteria as propellers we can steer 3-D printed microbots by unbalancing light intensity over different microbot parts. We designed an optimal feedback loop in which a central computer projects a tailor-made light pattern onto each microbot, calculated from its position and orientation. In this way, we can independently guide multiple microbots through a series of spatially distributed checkpoints. By exploiting a natural light-driven proton pump, these bio-hybrid microbots are able to extract mechanical energy from light with such high efficiency that, in principle, hundreds of these systems could be controlled simultaneously with a total optical power of just a few milliwatts.

P1.83

Towards robust Turing patterns in bacterial colonies

Martina Oliver Huidobro[1], Dr. Jure Tica[1], Dr. Roozbeh H. Pazuki[1], Prof. Robert G. Endres[1], Prof. Mark Isalan[1]
[1]*Life Sciences Department, Imperial College London, London , United Kingdom*

How robust spatial patterns are produced in biological systems is still largely an open question. Amongst various hypothesis, Alan Turing proposed a mechanism to explain pattern formation based on reaction-diffusion networks [1]. However, this mechanism is far from biological complexity and requires fine tuning of the parameters to produce such patterns [2]. Furthermore, natural relevant phenomena such as non-linearities, large network sizes, growth and realistic boundary conditions are often not addressed in Turing models. In this study, our overall goal is to investigate the robustness and characteristics of Turing patterns when these realistic phenomena are introduced. In particular, we aim to replicate and scrutinise experimental results where patterning occurs in bacterial colonies with synthetic gene circuits [3]. For this purpose, we combine cellular-automata simulations of colony growth with a PDE solver for non-linear reaction-diffusion models. Our resulting model does not only successfully replicate the experimental patters but also provides insight into the origin of the fine tuning of parameters.

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P1.84

Spontaneous Flow of Active Biofluids through Heterogeneous Environments

Tyler Shendruk[1], Benjamin Loewe[1], Ryan Keogh[1], Timofey Kozhukhov[1]
[1]*University Of Edinburgh, Edinburgh, United Kingdom*

Many biological fluids are intrinsically out-of-equilibrium, containing both biochemical fuels and microscopic biomachinery that actively converts fuel into work. Such active fluids can spontaneously flow, and mathematical models of active fluids provide an understanding of bulk flow states in the simplest environments. However, biological systems are generally characterized by the presence of complexities, including interfaces, inclusions, obstacles and other heterogeneities. While these complications are challenging for hydrodynamic solvers, we perform coarse-grained simulations of active nematic fluids in complex environments using an active-nematic multiparticle collision dynamics algorithm. These simulations examine the dynamics of thin active films in contact with a passive fluid and measure the entrainment of passive fluid due to the active interface. Solid interfaces can also have a substantial impact on active flows. Our simulations explore structured surfaces by considering active fluids confined within wavy channels. In particular, substrate distortions are found to strongly interact with defects in the active fluid, substantially modifying the spontaneous flow state. We extend this study to consider active flows within porous environments. Surprisingly, the disordered environment can both increase and decrease active fluxes. While the presence of obstacles does introduce an additional source of disorder, the pores between obstacles can match the intrinsic size of vorticity within the active fluid and lock coherent flow structures in place. These examples of non-trivial heterogeneous environments demonstrate that coarse-grained simulations of active fluids can model the geometrical aspects of complexity that characterizes biological systems of interest, such as swarming bacteria within porous soil ecosystems.

P1.85

Heads or tails, SNP or error, mobile or not; Bayesian inference in the detection of mobile mRNA

Franziska Hoerbst[1], Melissa Tomkins[1], Saurabh Gupta[2], Federico Apelt[2], Julia Kehr[3], Friedrich Kragler[2], Richard J. Morris[1]

[1]John Innes Centre, Norwich, United Kingdom, [2]Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany, [3]Institute of Plant Science and Microbiology, Universität Hamburg, Hamburg, Germany

Despite their main role being construction manuals for proteins, potentially hundreds of messenger RNAs (mRNAs) leave their cells of origin and travel long distances within plants. This movement of macromolecules is thought to form a communication system and enables plants to adjust their development to changing environmental conditions.

The detection of mobile mRNA is often based on finding point mutations – single nucleotide polymorphisms (SNPs) – in RNA-Sequencing data. However, separating sequencing errors from SNPs presents a challenge, as population numbers of the mobile mRNAs can be very low and sequencing errors cannot be fully eradicated.

We solve this problem using an analogy of tossing a coin: We found exact solutions to a Bayesian formalism for differentiating sequencing errors from actual SNPs. In our new probabilistic framework, we calculate a Bayes factor for each position of interest, where we compare the evidence for expected sequencing errors to the evidence for SNPs in RNA-Seq data. In that way, we can identify the positions where solely sequencing errors cannot explain the variability – we identify the true SNPs, and therefore true mobile RNA.

We tested and validated the performance of this method extensively on simulated data and found an astonishing accuracy. Hence, we are now exploring the application of our exact solutions to a Bayesian framework in other areas. Of course, we also used the method to re-analyse published mRNA-mobility data, which is giving us insights into the determinants for mRNA mobility, and we are now ready to explore their potential involvement in signalling processes.

P1.86

Emergent surface tension drives self-organised patterning in Dictyostelium group migration

Giulia Celora[1], Hugh Ford[1], Mohit Dalwadi[1], Benjamin Walker[2], Jonathan Chubb[1], Philip Pearce[1]
[1]University College London, London, United Kingdom, [2]University of Bath, Bath, United Kingdom

Dictyostelium amoeba are widely studied for their collective cell migration. These cells can aggregate together and move as a cohesive multicellular structure (or swarm) in response to chemical signals, such as cAMP and nutrition. In this work, we use state-of-the-art imaging to visualise the complex spatio-temporal evolution of Dictyostelium cell swarms as they feed on a population of bacteria. Feeding and chemotaxis combine to cause the Dictyostelium swarms to move along a self-generated bacterial concentration gradient. Surprisingly, we observe that as the swarms move collectively, they periodically shed groups of cells at the rear. By representing the cell swarm as an active droplet, we have developed a novel continuum mathematical model to understand the biophysical mechanisms that drive the experimentally observed shedding dynamics. The model suggests that self-organised shedding is driven by the interplay between feeding, active movement and proliferation of cells, and an emergent surface tension. Our work reveals a novel mechanism for self-organised pattern formation in biological systems.

P1.87

Directing and quantifying Min protein surface waves

Sabrina Meindlhumer[1], Dr. Fridtjof Brauns[2,3], Jernej Rudi Finžgar[2], Dr. Jacob Kerssemakers[1], Prof. Dr. Erwin Frey[2,4], Prof. Dr. Cees Dekker[1]

[1]Department Of Bionanoscience, Kavli Institute Of Nanoscience Delft, Delft University Of Technology, Delft, Netherlands, [2]Arnold Sommerfeld Center for Theoretical Physics and Center for NanoScience, Department of Physics, Ludwig-Maximilians-Universität München, Munich, Germany, [3]Present address: Kavli Institute for Theoretical Physics, University of California Santa Barbara, Santa Barbara, USA, [4]Max Planck School Matter to Life, Hofgartenstraße 8, 80539 Munich, , Germany

The Min protein system is arguably the best-studied model system for pattern formation in cell biology. It exhibits pole-to-pole oscillations in E. coli bacteria as well as a variety of surface wave patterns when reconstituted on supported lipid membranes in vitro. Recently, we were able to theoretically predict and experimentally show that the propagation direction of in vitro Min protein patterns can be controlled by a hydrodynamic flow of the bulk solution. We observe downstream propagation of Min surface wave patterns for low MinE:MinD concentration ratios, but upstream propagation for large ratios, and multistability of both propagation directions in between. Our results suggest that flow can be used to probe molecular features of pattern-forming systems, leading to an improved understanding of the inner mechanism that drives the pattern formation.

Notably, this study required us to perform careful quantitative analysis of how Min protein patterns change in wavelength and traveling direction, so we could compare these properties for different MinE:MinD ratios and bulk flow rates. While Min surface wave patterns can be visibly stunning, they pose particular challenges to quantification as they are typically non-stationary and only semi-periodic. Therefore, we developed tools for quantitatively analyzing such Min surface patterns, aiming to make our methodology accessible and easy to use for researchers faced with similar challenges. We developed an analysis pipeline in which we use autocorrelation analysis to extract global parameters such as the average spatial wavelength, and flow-field analysis to extract local properties, such as the wave velocity and propagation direction.

P1.88

A Minimal Tissue Model: The Cell as a Physical Object

Iain Muntz[1], Doris Houtzager[1], Rick Rodrigues de Mercado[2], Thomas Schmidt[2], Gijsje H. Koenderink[1]
[1]TU Delft, Delft, Netherlands, [2]Leiden University, Leiden, Netherlands

Human tissues are complex systems composed of cells embedded in an extracellular matrix (ECM), a network of polymers which confers mechanical and structural integrity on the tissue. In addition, the ECM provides physical cues to the cells that affect their biological activity. Conversely, cells affect tissue mechanics by a volume exclusion effect combined with biochemical remodelling. The biophysics of the ECM-cell interplay in tissue mechanics is challenging to study because of the huge molecular complexity of the ECM and the reciprocal mechanochemical crosstalk of cells and ECM.
To tackle this challenge, we develop a minimal tissue model to understand how cells affect tissue mechanics through purely physical effects, specifically the volume exclusion interaction. To do this we use poly(acryl amide) based microgels as a proxy for the cell. We show that networks of fibrin, which exhibit compression-softening, instead stiffen under compression when cell-mimetic microgels are embedded in the network. This effect is, at least partially, explained through stretching of the network in between the beads due to the inhomogeneities in the system. However, we observe stronger than expected stiffening effects at short times which we postulate arise from poroelastic effects in the system, where fluid flows through the porous network initially dominate the response. These results are important to understand not only native tissue mechanics, but also as a step to producing tissue engineered constructs with properties more closely resembling those of native tissue.

P1.89

Vertex model for the turnover of squamous epithelial tissues

Joel Hochstetter[1,2], Professor Benjamin D Simons[1,2]
[1]Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Cambridge, United Kingdom, [2]Gurdon Institute, University of Cambridge, Cambridge, United Kingdom

Throughout adult life epithelial tissues are constantly turned over. At homeostasis, the stochastic loss of cells must be perfectly compensated by the division and differentiation of stem cells. The mechanisms that regulate stem cell fate choice remains in debate. In squamous tissues, such as the skin interfollicular epidermis and oesophagus, emphasis is becoming placed on how the interplay of mechanical and chemical feedback mechanisms regulate of stable density homeostasis. To address this question, mathematical and computational models will be critical in interpreting in vivo clonal lineage tracing and in vitro live imaging experiments in homeostatic and perturbed conditions. To this end, we developed a vertex model with cell loss and replacement, with stochastic cellular decisions either controlled by mechanical density-dependent feedback or a biochemical competition for niche factors. We use our model to explore qualitative and quantitative differences in clonal dynamics, patterning and response to perturbations in tissues where stem cell decisions are controlled by these different feedback rules. Our vertex model may prove to be a valuable tool in understanding how homeostasis emerges from collective cellular dynamics in two-dimensional epithelial tissues. Additionally, we can address more physical questions, such as how glass-like or fluid-like phases of epithelial tissues are affected by cell loss and replacement.

P1.90

Multiscale measurements of mechanical stress in 3D co-cultures using a deformable micro-device

Hiba Belkadi[1,2], Shreyansh Jain[1,2], Arthur Michaut[3], Martin Genet[4], Jérôme Gros[3], Charles Baroud[1,2]

[1]Physical microfluidics and Bioengineering, Institut Pasteur, Paris, France, [2]LadHyX, CNRS, École polytechnique, Institut polytechnique de Paris, Palaiseau, France, [3]Dynamic regulation of morphogenesis, Institut Pasteur, Paris, France, [4]LMS, CNRS, École polytechnique, Institut polytechnique de Paris, Palaiseau, France

A wide range of methods is available to measure cellular mechanics, with a view of differentiating cancerous vs. normal cells. However, there is a lack of high-throughput methods to measure the mechanics of multicellular aggregates, even though these provide much better models of health and disease. Indeed, understanding the mechanical properties of a tissue must account for cell-cell contacts, the extracellular matrix, geometric factors, etc, in addition to the mechanics of individual cells. Here we present a method to actuate and observe many three-dimensional cellular aggregates, such as spheroids or organoids, within a single deformable micro-device. Measuring the two-dimensional deformation field of an aggregate under compression allows us to detect heterogeneities of the mechanical properties within it, namely in the co-culture of different cell types. Alternatively, confocal microscopy allows us to link the mechanical response of the whole aggregate to local deformations or displacements of its constitutive elements, such as the nuclei or the cell membranes. This multiscale approach is presented using a 3D graph that relates the positions and geometric parameters of individual cells together and that thus identifies topographical changes due to the compression. This platform therefore allows a wide range of actuation and measurement approaches, in order to probe the mechanical properties of 3D tissues. The goal is to build a model of mechano-biological force transfer through a three-dimensional cell aggregate and apply it to diseases like cancer and fibrosis.

P1.91

Migratory role of extra embryonic tissue in during early avian embryo development

Dr Lakshmi Balasubramaniam[1,2], Dr Fengzhu Xiong[1,2]

[1]*Wellcome Trust/ Cancer Research UK Gurdon Institute, University of Cambridge, United Kingdom,*

[2]*Department of Physiology, Development and Neuroscience, University of Cambridge, , United Kingdom*

Avian embryo development occurs through the shape transformation of a circular blastodisc into an anisotropically shaped body axis. This shape transformation occurs while cells actively migrate over the vitelline membrane, a glycoprotein membrane. While vitelline membrane tension is key for proper embryonic development, link between the migratory role of extra-embryonic cells and tension is poorly understood. While a tension gradient within the embryonic region is important during development, how the migratory properties of edge cells or extra-embryonic tissue regulate gastrulation is not fully understood. In this work, using a combination of imaging, and drug-based assays we test the role of the migratory properties driven by ECM deposition of the extra-embryonic tissue in ensuring proper positioning of the primitive streak and elongation of the body axis.. We then infer the mechanical forces and stresses using biochemical sensors and tension inference tools to delineate the role of tension heterogeneity across the blastodisc in positioning the streak.

P1.92

Mechanosensitivity and deformability of mechanically stimulated bone cells

Rui Sousa[1], Dr Melanie Jimenez[1], Dr Stuart Reid[1], Dr Peter Childs[1]
[1]*University of Strathclyde, Glasgow, United Kingdom*

Wolff's law states that healthy bone will be able to self-regenerate and adapt to stresses caused by the environment. The bone's ability to regenerate comes from a homeostatic balance between osteoblasts, bone forming cells, and osteoclasts, bone resorbing cells. An imbalance between these two groups can lead to a degenerative bone condition, osteoporosis.

One specific technique, developed to stimulate mesenchymal stem cell differentiation into osteoblasts, is mechanical stimulation via nano-amplitude vibration. Results in vitro have shown that 1 kHz, 30 nm amplitude vibration stimulates osteogenic differentiation of mesenchymal stem cells. These studies have shown that it is possible to stimulate osteoblast formation whilst inhibiting osteoclast creation.
Due to advancements on the cytometry field, we can now visualise how cells deform under compressive forces imposed by a flow of fluid of known viscosity while in suspension. This new technique named Real-Time Deformability cytometry (RT-DC), is allowing an insight of the mechanical properties and mechanosensitivity of the cytoskeleton of vibrationally stimulated cells whilst undergoing osteogenesis. In fact, preliminary results have shown vibrationally stimulated osteogenic cells to be significantly more deformable ($p<0.05$) than the control counterpart. These results contradict previous results using Atomic Force Microscopy (AFM) when cells are adhered. Thus, it is hypothesised than a rearrangement of the cytoskeleton's stress fibres could be the reason that cells change stiffness between suspension and attachment. In future this method could be used to determine if cells from different aged populations react differently to various mechanical stimuli.

P1.93

Light- controlled actomyosin contractility induces cell shape- changes

Mallica Pandya[1], Dr. Emma Ferber[1], Prof. Dr. Guillaume Charras[1]
[1]*University College London, London, United Kingdom*

Tissues acquire their shapes during morphogenesis, a process during which complex tissue shapes arise from collective, individual cell movement. Single cells undergo mechanical changes controlled by actomyosin contractility downstream of signalling. However, the specific roles of different signaling molecules in controlling cell shapes is not well understood. Signaling can be spatiotemporally controlled by combining activators of actomyosin contractility with light sensitive proteins. Here we use optogenetics to recruit signalling domains to the plasma membrane, giving rise to cell shape-changes due to cortical contractility. Shape changes induced in multiple individual cells can bring about tissue-scale shape changes, as a technique to reverse-engineer morphogenesis.

P1.94

Laser ablation informed mechanical state of an early-stage chick embryo

KVS Chaithanya[1], Molly J. Fitches[1], Guillaume T. Charras[2], Julia M. Yeomans[3], Cornelis J. Weijer[1], Rastko Sknepnek[1]

[1]University of Dundee, Dundee DD1 5EH, United Kingdom, [2]University College London, London WC1H 0AH, United Kingdom, [3]University of Oxford, Oxford OX1 3PU, United Kingdom

Morphogenic processes during embryonic development involve a complex interplay of coordinated cell behaviours on length scales significantly larger compared to that of an individual cell. Coordinated cell motion is driven and regulated by an intricate interplay of chemical and mechanical signalling. At present, it has not been fully understood how this interplay leads to the formation of tissue and organ-scale functional structures. Determining the mechanical forces and, thus, the mechanical state of the embryo is, therefore, the key to addressing this problem. In this study, we examine the local mechanical state of the chick embryo using force inference applied to the data obtained from experiments that use laser ablation to measure recoil resulting from a cell-cell junction being cut. Moreover, the force-inference data is used to inform vertex model simulations. The simulations are then used to track the temporal evolution of the mechanical state of the system. Furthermore, we characterize the local variation of tension across the embryo and its correlation with myosin and actin dynamics.

P1.95

How do we build brains? Investigating actomyosin contractility in hollowing epithelial tubes

Millie Race[1], Alexander Fleet, Dr Clare Buckley
[1]*University Of Cambridge, , United Kingdom*

In order to build organs during development, cells organise themselves into complex tissues. Epithelial tubes are essential structures in the formation of multiple organs including the brain, spinal cord, kidney, and salivary glands. They are hollow tubes with epithelial cells polarised towards a central lumen. The neural tube is an epithelial tube that forms the brain and spinal cord during neurulation. In amniotes, it forms in two different ways: in the anterior region, primary neurulation occurs through the folding of an already polarised epithelial sheet; and posteriorly, secondary neurulation occurs through de novo apical-basal cell polarisation in the centre of a solid rod and the tube opens through hollowing. The zebrafish hindbrain forms via a secondary-like mechanism, providing a tractable in vivo model.

Various research has shown that actomyosin contractility is important in neural plate folding in primary neurulation, however, it is not known the role of this in hollowing epithelial tubes. In this study, the role of actomyosin contractility was investigated over the course of polarisation and zebrafish neurulation through immunofluorescence and drug inhibition of non-muscle myosin II (NMYII). Upon inhibition of NMYII contractility, the neural tube still opens but the extent of inflation is affected. However, the main localisation of active NMYII was found to be at the base of primary cilia, not at junctions, and apical constriction does not appear to be required for lumen opening. We are now investigating how myosin contractility could be working through alternative mechanisms to shape and open the neural tube.

P1.96

Emergence of division of labor in tissues through cell interactions and spatial cues

Miri Adler[1,2], Noa Moriel[3], Aleksandrina Goeva[1], Inbal Avraham-Davidi[1], Simon Mages[1,4], Taylor S. Adams[5], Naftali Kaminski[5], Evan Z. Macosko[1,6], Aviv Regev[1,7,8], Ruslan Medzhitov[2,9], Mor Nitzan[3,10,11]
[1]Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, USA, [2]Tananbaum Center for Theoretical and Analytical Human Biology, Yale University School of Medicine, New Haven, USA, [3]School of Computer Science and Engineering, The Hebrew University of Jerusalem, Jerusalem, Israel, [4]Gene Center and Department of Biochemistry, Ludwig-Maximilians-University Munich, Munich, Germany, [5]Section of Pulmonary, Critical Care and Sleep Medicine, Yale University School of Medicine, New Haven, USA, [6]Massachusetts General Hospital, Department of Psychiatry, Boston, USA, [7]Department of Biology, Massachusetts Institute of Technology, Cambridge, USA, [8]Genentech, 1 DNA Way, South San Francisco, USA, [9]Howard Hughes Medical Institute, Department of Immunobiology, Yale University School of Medicine, New Haven, USA, [10]Racah Institute of Physics, The Hebrew University of Jerusalem, Jerusalem, Israel, [11]Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

Most cell types in multicellular organisms can perform multiple functions. However, not all functions can be optimally performed simultaneously by the same cells. Functions incompatible at the level of individual cells can be performed at the cell population level, where cells divide labor and specialize in different functions. Division of labor can arise due to instruction by tissue environment or through self-organization. I will present a computational framework to investigate the contribution of these mechanisms to division-of-labor within a cell-type population. By optimizing collective cellular task performance under trade-offs, we find that distinguishable expression patterns can emerge from cell-cell interactions vs. instructive signals. We propose a method to construct ligand-receptor networks between specialist cells and use it to infer division-of-labor mechanisms from single-cell RNA-seq and spatial transcriptomics data of stromal, epithelial, and immune cells. Our framework can be used to characterize the complexity of cell interactions within tissues.

P1.97

Data-driven modelling of tissue growth in Drosophila abdomen

Andrea Cairoli[1,3], Dr John Davis[1,4], Dr Ana Ferreira[1], Dr John J Williamson, Dr Matthew B Smith[1,2], Dr Anna Ainslie[6], Dr Marcus Michel[5], Prof Christian Dahmann[5], Dr Nic Tapon[1], Prof Guillaume Salbreux[2]
[1]The Francis Crick Institute, London, United Kingdom, [2]University of Geneva, Geneva, Switzerland, [3]University of Cambridge, Cambridge, United Kingdom, [4]University of Manchester, Manchester, United Kingdom, [5]Technische Universit¨at Dresden, Dresden, Germany, [6]The University Medical Center Groningen, Groningen, Germany

During metamorphosis histoblasts in the abdomen of the fruit fly Drosophila Melanogaster invade the larval epidermal tissue, by coordinating the extrusion of larval epithelial cells through apoptosis, and fuse at the dorsal midline to complete the formation of the adult abdominal epidermis. How the histoblasts fine regulate their proliferation, divisions and kinetics to reach the correct tissue size and to perform the fusion process at the dorsal midline while preserving tissue integrity is largely unknown to this day. In this talk, I will study the tissue growth process in the abdomen of Drosophila by reconstructing the spatiotemporal dynamic of cell elongation, area and velocity of the histoblasts and larval cells that constitute the abdominal epithelium of Drosophila. I will recapitulate the experimental observations by formulating a continuum model of the dynamic of the tissue.

P1.98

Bio-inspired ultrathin broadband sound absorber metamaterials

Prof Marc Holderied[1]
[1]*University Of Bristol, Bristol, United Kingdom*

Providing healthy living and working environments in our ever-noisier world necessitates increasing use of noise control technology. Acoustic metamaterials can act as ultrathin subwavelength sound absorbing methods, allowing sound insulation to be thinner and lighter than ever before. We will present our discovery of a natural acoustic metamaterial and show our ongoing work towards bioinspired metamaterial absorbers. Our bioinspiration arises from the 65-million-year acoustic arms race between echolocating bats and their moth prey, which has turned the wing scales of moths into an omnidirectional, ultrathin (1/100 λ) and broadband absorber of ultrasound (peak α = 0.71) that provides acoustic camouflage against bats. Our up-sized moth-inspired panels absorb sound over more than one octave in the human hearing range at a thickness of 1/30 λ. This research paves the way for bioinspired novel broadband sound absorbers that are substantially thinner and lighter than current technical solutions - using clues from nature to inform the design of the next wave of advanced noise control solutions.

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P1.99

Cell density as a negative feedback mechanism to ensure robustness of the body axis elongation process

Joana M. N. Vidigueira[1], Changqing Lu[1], Cristopher Chan Jin Jie[1], Alicja Maksymiuk[1], Fengzhu Xiong[1,2]
[1]Wellcome Trust / CRUK Gurdon Institute, University of Cambridge, Cambridge, United Kingdom, [2]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom

Tissue morphogenesis is a complex process in embryonic development. In vertebrate body axis elongation, the different tissues interact chemically and mechanically to achieve the characteristic morphology for each species. The rate of segmentation is set by an oscillatory gene regulatory network. Although body axis elongation process accompanies segmentation, its speed is set independently, through a mechanism which remains unknown. We propose a model in which a combination of mesenchymal cell density and motility in the presomitic mesoderm (PSM) sets the speed of elongation. Changes in cell density provide a feedback mechanism to ensure elongation speed remains stable over time. Our computational modelling revealed PSM-PSM cell repulsion as a key parameter in determining the rate of elongation. We sought to manipulate this parameter experimentally through changing cell motility and cell density in the PSM. To manipulate cell density, we artificially extended or compressed the body axis of chicken embryos. Our results show that the embryos respond to changes in cell density to ensure robustness of the elongation process. Furthermore, cell motility is necessary but not sufficient for elongation. Our data agrees with the predictions of our model. Overall, our findings indicate that cell density dynamically regulates elongation speed, ensuring a robust morphogenetic process.

P1.100

Active gel theory description of actomyosin pulsations in epithelial cells

Mr Euan Mackay[1], Dr Jens Januschke[1], Dr Rastko Sknepnek[1]
[1]*University Of Dundee, Dundee, United Kingdom*

The actomyosin cortex is a network formed of actin filaments, myosin motors, and actin-binding proteins. It plays an important role in cell mechanics. Many questions, however, remain open about the dynamics of these networks. For example, cellular and tissue processes such as cell interactions and convergence-extension have been accompanied by actomyosin pulsations. Active gel theories have previously been explored as a good candidate to model cytoskeletal actomyosin networks. Thus far, most of the work on active gel theory has been done in simple geometries, e.g. assuming one-dimensional systems with periodic boundaries. In this work, we study a version of the active gel theory for actomyosin networks using the finite element method. This allows us to examine domains of arbitrary shapes, thus making it possible to directly compare to the imaging data of actual cells. We map the phase diagram of the two dimensions active gel as a function of activity and cell's stiffness.

P1.101

Applying Atomic Force Microscopy in Investigation of Structural Changes in Tomato Fruits Cell Wall

Dr Lazar Novakovic[1], Dr Richa Yeshvekar[1], Ms. Naomi Simmons[1], Dr Yoselin Benitez-Alfonso[1]
[1]*University Of Leeds, Leeds, United Kingdom*

Tomato is an important crop with an estimated annual world production of 177 million tons. However, tomato production suffers a lot from post-harvest losses which are estimated to be 5-7% in Europe alone (1 million tons), and between 25-42% in other regions. Understanding the role of tomato fruits' cell wall (CW), in tomato fruit ripening could help us identify new molecular targets and breeding traits which will enable creation of tomato cultivars with extended shelf-life, decreasing economic losses and contributing to food security. CW is a polysaccharide exoskeleton which surrounds all plant cells providing them with mechanical strength and defining their shape. We applied atomic force microscopy to elucidate structural changes of cellulose, a major CW component, in tomato fruit pericarp. High resolution structural imaging of paraformaldehyde fixed and wax embedded tomato CW sections showed changes in diameter of cellulose microfibrils in CW during fruit development. Cellulose microfibrils diameter decreased from 1-week old fruits to 6-week old fruits. Change in cellulose microfibril diameter together with structural changes of other CW polysaccharides could directly affect CW mechanical status. CW mechanical status determines fruit softness and cellular adhesion, main traits that determine shelf-life of tomato fruits.

Key words: cell wall, cellulose, tomato, atomic force microscopy.

P1.102

A cell-based model for passive and active tissue rheology

Fikret Basar[1], Joel Jennings, Richard Adams[2], Alexandre Kabla[1]
[1]Department of Engineering, University of Cambridge, Cambridge, United Kingdom, [2]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom

During embryogenesis, cell and tissues deform significantly under the action of passive and active mechanical forces, either via cellular deformation or rearrangements. These depend on cellular mechanical properties and cell-cell interactions, including force signalling and adhesions. Computational models are valuable tools to study the effect of cellular and subcellular mechanical properties on such processes, at relevant spatial and temporal scales.

We present a cell-based computational model focussing on cell-cell mechanical interactions and dissipation due to cell-cell adhesion to study confluent epithelial tissues in the context of morphogenesis. A finite element method is used to maintain large-scale stress equilibrium and control tissue boundary conditions, enabling precise assessment of tissue rheology. The model inputs are simple mechanical parameters corresponding to cell elastic moduli and adhesion turnover.

As a material, the model tissue resembles a Bingham Plastic, predicting the solid-to-fluid transition exhibited by tissues at large timescales and forces. The model inputs can be mapped to rheological components of the mechanical circuit.

Active behaviours, such as cell protrusions within the tissue, are also implemented and lead to localised cell migration or bulk deformation of the whole tissue. Active cell and tissue behaviour is directly linked to passive rheological parameters, with similar yielding response. Overall, the model allows us to capture the interplay between passive and active behaviours in setting the rheology of active materials.

P1.103

Tyssue: modelling morphogenesis from molecular to tissue scales

Sophie Theis[1], Guillaume Gay[2], Magali Suzanne[3], Timothy Saunders[1]

[1]Warwick Medical School, University of Warwick, Coventry, United Kingdom, [2]UAR BiocAMPUS 3426 SNRS - US 09 INSERM, Montpellier, France, [3]Molecular, Cellular and Developmental Biology unit (MCD), Toulouse, France

Tissue remodelling is a complex process, integrating multiple inputs such as gene expression patterns, cell adherent properties and cell mechanics. It can be difficult to manipulate specific aspects genetically or capture morphogenetic events when the process is rapid. Critically, morphogenesis is inherently a mechanical process. Theoretical modelling of tissue formation can help us to understand how tissues acquire their shape and go beyond the limitation of experimental systems.

Motivated by this, we developed Tyssue, a vertex epithelium simulation library developed in Python. It is multiscale, and can include protein properties that lead to tissue shape change. Thanks to its modularity, we can simulate a wide range of processes. Further, the same mesh can be used to model different processes with different physical properties.

Tyssue was used to characterise the role and importance of different forces in Drosophila epithelial fold formation. In order to integrate myosin dynamics (which is at the heart of force generation), we added a specific parameter that integrates myosin dynamics at the level of junctions. This generated an apical cell tension polarity, which we show is important in ensuring robustness of the Drosophila leg fold. We evolved our model to directly include myosin particle dynamics, enabling them to interact with themselves, and other particles and/or with the mesh. Finally, we extended Tyssue to model 3D epithelium cell shapes under anistropic stress. We used this to study the appearance of scutoid cell shapes within 3D tissues when perpendicular forces are applied to the apical and basal layers.

P1.104

Three-dimensional soft active matter modelling of corneal epithelial cell migration in vivo

Prof Jon Martin Collinson[1], Dr Kaja Kostanjevec[1], Dr Rastko Sknepnek[3], Dr Silke Henkes[2]
[1]University of Aberdeen, Aberdeen, United Kingdom, [2]University of Leiden, Leiden, Netherlands, [3]University of Dundee, Dundee, United Kingdom

The purpose of this study was to apply soft active matter physics to recapitulate and predict cell behaviour in an in vivo biological system: cell migration in the vertebrate corneal epithelium. An in-silico active soft matter migration model was developed and applied to a three-dimensional topology mimicking the mouse cornea. Corneal epithelial cells were treated as soft, self-propelled particles with density-dependent proliferation, apoptosis, alignment, physical interaction with neighbours and biological noise. It was found that in the absence of global guidance cues, patterns of migration were simulated that formed stripes and spirals closely recapitulating those found in vivo. The model was scalable, could predict patterns of recovery from injury and can be used to understand disease processes and mutations. This first fully mechanised in silico cornea demonstrates for the first time a fundamental link between a cell biological system and the physics of swarms on curved surfaces using a soft active matter paradigm.

P1.105

Shape-tension coupling produces nematic order in an epithelium vertex model

Dr Jan Rozman[1], Dr Rastko Sknepnek[2], Professor Julia Yeomans[1]
[1]Rudolf Peierls Centre for Theoretical Physics, University of Oxford, Oxford, United Kingdom, [2]University of Dundee, Dundee, United Kingdom

We study the vertex model for epithelial tissue mechanics extended to include coupling between the cell shapes and tensions in cell-cell junctions [1]. This coupling represents an active force which drives the system out of equilibrium and leads to the formation of nematic order interspersed with prominent, long-lived +1 defects. The defects in the nematic ordering are coupled to the shape of the cell tiling, affecting cell areas and coordinations. This intricate interplay between cell shape, size, and coordination provides a possible mechanism by which tissues could spontaneously develop long-range polarity through local mechanical forces without resorting to long-range chemical patterning.

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P1.106

Root angle is controlled by EGT1 in cereals employing a novel anti-gravitropic mechanism

Jacob Pattem[1], Ricardo Fusi[2], Gleb Yakubov[1], Malcolm Bennett[2]
[1]*Nottingham University, Soft Matter Biomaterials and Biointerfaces, Sutton Bonington, United Kingdom,*
[2]*Nottingham University, Plant Sciences, Sutton Bonington, United Kingdom*

Root-angle represents a key trait for efficient capture of soil resources by plants. Root angle is determined by competing gravitropic versus anti-gravitropic offset (AGO) mechanisms. Despite its agronomic importance, few regulatory genes have been identified in crops. A new root-angle regulatory gene termed ENHANCED GRAVITROPISM1 (EGT1) has been recently discovered. EGT1 encodes a putative AGO component, whose loss enhances root gravitropism. To investigate biophysical changes in cell-wall properties Atomic Force Microscopy (AFM) nanoindentation approach has been utilised. We analysed 50 µm thick longitudinal cross-sections of 4-day-old barely seminal root tips of Morex (wildtype) and TM194 (mutant) using force-spectroscopy under plasmolysed but hydrated conditions. Nine independent areas within specific root tip tissues where characterised for stiffness (pN/nm), performing n > 100 indentation curves for each biological replicate (n = 4 - 5). Results revealed a significant reduction of 26% in cell-wall stiffness in elongating cells of the mutant compared to wildtype. Moreover, when sub-dividing analysed data into inner versus outer root tissues, mutant roots showed a significant reduction of 36% in root cortical tissues, while we observed no significant difference for stele tissues. Measuring such biophysical properties using AFM, revealed crucial to prove that this reduction in cell-wall stiffness in mutant roots disrupts the ability of the root outer tissues to counteract gravitropic bending, causing them to grow steeper along a gravity vector. Analogous EGT1-dependent regulation of root-angle in barley demonstrates broad significance of EGT1 for trait improvement in cereal crops.

P1.107

Nanoscale rheology via quantum photonic interference measurements of molecular rotor lifetimes

Ashley Lyons[1], Vytautas Zickus[1,2], Raul Alvarez-Mendoza[1], Manlio Tassieri[3], Daniele Faccio[1]
[1]University Of Glasgow, Glasgow, United Kingdom, [2]Department of Laser Technologies, Center for Physical Sciences and Technology, Vilnius, Lithuania, [3]James Watt School of Engineering, University of Glasgow, Glasgow, UK

Micro and nanoscale viscosity gradients play a key role in biology, ranging from the dynamics of the microbial environment underpinning biogeochemistry of the oceans to cancer cell motility. However, we still lack a contactless technique for the measurement of micro and nanoscale viscosity that does not mechanically perturb the bio-environment itself.
Molecular rotors are a family of fluorescent markers that can be used to probe both viscosity and flow rates of fluids. Typically, the fluorescent intensity is monitored and as an indicator for the viscosity however this requires careful calibration that can easily be influenced by other factors e.g. molecule concentration. It has previously been shown that the lifetime of these molecular rotors provides a much more robust probe that doesn't require any a priori characterisation. Lifetimes of common molecular rotors can fall into the few-picosecond regime making them nearly impossible to measure using conventional Fluorescence Lifetime Imaging Microscopy (FLIM) microscopes that typically have temporal resolutions on the order of hundreds of picoseconds. We introduce a new technique that relies on the bunching of photons due to quantum interference to directly measure fluorescence lifetimes down to the few picosecond scale with the potential for femtosecond resolution. We demonstrate our method using a range of fluorescent probes reaching lifetimes as short as 3 picoseconds without the need for any numerical deconvolution. We also illustrate applications in microrheology, using the lifetime to determine the viscoelasticity of the 4-DASPI molecular rotor in solution with a sensitivity down to 0.0065 mPa seconds and demonstrating the ability to measure viscosity ranging from the of pure water to relatively viscous solutions of relevance for biosensing.

P1.108

Generating active T1 transitions through mechanochemical feedback

Dr Rastko Sknepnek[1], Dr Ilyas Djafer-Cherif[2], Prof Cornelis J. Weijer[1], Prof Silke Henkes[3]
[1]University of Dundee, Dundee, United Kingdom, [2]Dioscuri Centre for Physics and Chemistry of Bacteria, Warsaw, Poland, [3]Leiden University, Leiden, Netherlands

Convergence-extension in embryos is controlled by chemical and mechanical signalling. A key cellular process is the exchange of neighbours via T1 transitions. We propose and analyse a model with positive feedback between the recruitment of myosin motors and mechanical tension in cell junctions. The model produces active events, which elongate the tissue perpendicular to the main direction of tissue stress external pulling. Using an idealized tissue patch comprising several active cells embedded in a matrix of passive hexagonal cells we identified an optimal range of pulling forces to trigger an active T1 event. We show that directed stresses pulling also generates tension chains in a realistic patch made entirely of active cells of random shapes and leads to convergence-extension over a range of parameters. Our findings show that active intercalations can generate stress that activates T1 events in neighbouring cells resulting in tension-dependent tissue re-organization, in qualitative agreement with experiments on gastrulation in chick embryos.

P1.109

Friction when changing neighbours : adhesion-regulated junction slippage controls cell intercalation dynamics in living tissue

Jocelyn Etienne[1], Alexander Nestor-Bergmann[2], Guy Blanchard[2], Nathan Hervieux[2], Alexander Fletcher[3], Bénédicte Sanson[2]

[1]Univ. Grenoble Alpes – CNRS, Liphy, Grenoble, France, [2]Univ. Cambridge, PDN, , United Kingdom, [3]Univ. Sheffield, Mathematics, Sheffield, United Kingdom

During development tissues undergo dramatic shape changes to build and reshape organs. In many instances, these tissue-level deformations are driven by the active reorganisation of the constituent cells. This intercalation process involves multiple cell neighbour exchanges, where an interface shared between two cells is removed and a new interface is grown. The key molecular players involved in neighbour exchanges, such as contractile motors proteins and adhesion complexes, are now well-known. However, how their physical properties facilitate the process remains poorly understood. For example, how do cells maintain sufficient adhesive contact while actively uncoupling from one another? Then, how does a new interface grow in a contractile environment? Many existing biophysical models cannot answer such questions, due to representing shared cell interfaces as discrete elements that cannot uncouple.

Here, we develop a model where the junctional actomyosin cortex of every cell is modelled as a continuous viscoelastic rope-loop, explicitly representing cortices facing each other at bicellular junctions and the adhesion molecules that couple them. The model parameters relate directly to the properties of the key subcellular players that drive dynamics, providing a multi-scale understanding of cell behaviours. The code is distributed as an open-source free software.

We show that active cell neighbour exchanges can be driven by purely junctional mechanisms. Active contractility and cortical turnover in a single bicellular junction are sufficient to shrink and remove a junction. Next, a new, orthogonal junction extends passively. Our Apposed-Cortex Adhesion Model (ACAM) reveals how the turnover of adhesion molecules regulates tension transmission and junction deformation rates by controlling slippage between apposed cell cortices. The model additionally predicts that rosettes, which form when a vertex becomes common to many cells, are more likely to occur in actively intercalating tissues with strong friction from adhesion molecules.

P1.110

Extracellular matrix plasticity enables a pro-invasive mechanical cross-talk between cancer cells and cancer-associated fibroblasts

Hamid Mohammadi[1], Erik Sahai[1]
[1]*Francis Crick Institute, London, United Kingdom*

The extracellular matrix of tumours undergoes substantial biomechanical alterations during cancer development, including cross-linking events. Crosslinking of the matrix has a pro-invasive role in cancer progression by increasing cancer cell aggressiveness, yet it remains unclear whether these changes affect stromal cells in a manner that promotes or impedes metastasis. To investigate this, we employed intact and lysyl oxidase-induced cross-linked collagen matrices, molecular dynamics simulation, and xenograft mouse models. Our findings demonstrated that the transition from a plastic to an elastic matrix due to crosslinking impaired a pro-invasive mechanical interaction between cancer-associated fibroblasts (CAFs) and cancer cells. Cancer cells migrated in a directed manner in response to the millimetre-scale remodelling caused by CAFs in plastic collagen matrices, which was restricted following cross-linking. Notably, laser ablation of the collagen network disrupted matrix remodelling by CAFs and inhibited the migration of cancer cells, indicating a biomechanical signal. The signal polarised the force between cancer cells and the matrix, favouring the migration of cancer cells towards the origin of the signal. Weakening or strengthening cancer cells' contractility inhibited or enhanced their directed migration, respectively. Further, our results showed that linear and nonlinear elastic properties of the matrix were inadequate to account for force transmission at the millimetre scale. Instead, the plastic deformation enabled long-range transmission of the biomechanical signal by permanently reconfiguring matrix fibres through fibre slippage. Our findings identified a new pro-invasive role for CAFs in the tumour microenvironment that was strictly regulated by extracellular matrix plasticity.

P1.111

Engineering covalently crosslinked protein hydrogels for precision medicine

Rossana Boni[1], Professor David Hay, Professor Lynne Regan
[1]*University of Edinburgh, Edinburgh, United Kingdom*

Protein engineering allows for the programming of specific building blocks to form functional biomaterials with customisable biophysical properties. Biomaterials for tissue regeneration must mimic the biophysical properties of the native physiological environment. A re-engineered approach using functionalised protein hydrogels allows to specify the biophysical properties independently and customise them to suit a particular physiological environment.

Herein, we have successfully designed and programmed engineered proteins to form covalent molecular networks with defined physical characteristics able to sustain mammalian cell culture without loss of viability. Our hydrogel design incorporates the SpyTag (ST) peptide and SpyCatcher (SC) protein to spontaneously form covalent-crosslinks upon mixing. The genetically encoded chemistry allows us to easily incorporate different recombinant proteins in the hydrogels and induce a wide array of controlled biophysical characteristics. By changing the crosslinker protein and ratios of the protein building blocks (ST:SC), we demonstrated that we could alter the viscoelastic properties and gelation speeds in the hydrogels. Fine tuning the rate of gelation of our biocompatible hydrogels allowed us to adapt our design to biofabrication techniques, such as 3D printing. The physical properties of the hydrogel could easily be altered further to suit different environments by tuning the key features in the protein sequence (e.g. functional proteins, numbers of SC repeats, properties of the linkers between ST-ST and SC-SC).

By showing cell viability is maintained in the presence of the hydrogels, our work demonstrates how this functional approach could be applied to tissue engineering creating a level of biomaterial customisation previously inaccessible.

P1.112

Crosstalk between physical and biochemical cellular heterogeneity dictate collective cell migration during epithelial wound closure.

Ms Sindhu Muthukrishnan[1], Medhavi Vishwakarma[2]

[1]Centre for BioSystems Science and Engineering, Indian Institute Of Science, Bangalore, India, [2]Centre for BioSystems Science and Engineering, Indian Institute Of Science, Bangalore, India

Biological tissues display heterogeneity in terms of biochemical processes within the cells. Besides biochemical heterogeneity, recent studies have shown spatiotemporal variations in cellular forces representing physical heterogeneity in epithelial tissues. While the role of deterministic heterogeneity in tissues has been studied, stochastic cellular heterogeneity is often characterized as biological noise and its physiological relevance remains unexplored. This work explores the crosstalk between physical and biochemical heterogeneity within the epithelial monolayer and demonstrates how stochastic heterogeneity within the epithelium regulates collective cell migration during wound closure. We map the spatiotemporal activity of Rac1 and RhoA within the epithelial monolayer using FRET-based biosensors and show that stochastic heterogeneity in Rac-1 and Rho-A activity pre-polarize the edge cells to become leading individuals when the wound arises. The stress landscape of the monolayer shows that the pre-polarization of Rac-1 and RhoA coincides with high-stress regions within the monolayer and that such crosstalk between physical and biochemical heterogeneity is essential for collective cell migration. We show that upon modulating the stiffness of the ECM, the cellular heterogeneity is perturbed, and wound healing is impaired. Lastly, we built a multiscale model integrating physical and biochemical heterogeneity of epithelium in order to predict the efficiency of wound healing in healthy and fibrotic conditions, and on the basis of our model, we are currently testing the effect of fibrosis on stochastic heterogeneity and on wound healing.

P1.113

Cellular Cruise Control: Mechanical energy dissipation regulates collective migration in epithelia

Mr Simon Martina-Perez[1]

[1]*University Of Oxford, Oxford, United Kingdom*

Collective cellular migration is crucial for several key biological processes such as wound healing, morphogenesis, and metastasis. The mechanisms that regulate this dynamic migratory behavior are a complicated consortium of intracellular cytoskeletal mechanics and signaling pathways, intercellular cell-cell adhesion, and extracellular interactions with the environment. Here, we develop a data-driven continuum mechanics model that predicts collective migratory behavior from past energy dissipation alone, considering cell-substrate friction and viscous cell-cell interactions. Using this model, we show that cells self-regulate their migratory behavior by modulating their local force expenditure as a function of their past energy expenditure. Finally, we perform canonical electrotaxis (the guided migration of cells experiencing an electrical current) experiments to show that exogenous migratory cues shift cell self-regulation to different modes of migration while conserving energy dissipation as a dominant regulator of cell response to the field. Our model accurately recapitulates the phenomenon wherein epithelia exhibit a migratory slowdown when undergoing prolonged stimulation and has applications in developing closed-loop control of epithelial migration.

P1.114

An imaging and FEM study into the mechanics of biological valves – how plants regulate photosynthesis in grasses

Dr. Clinton Durney[1], Dr. Matthew Wilson[2], Dr. Shauni McGregor[2], Ms. Jodie Armand[2], Dr. Richard Smith[1], Dr. Julie Gray[2], Dr. Richard Morris[1], Dr. Andrew Fleming[2]
[1]John Innes Centre, Norwich, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom

Stomata are tiny adjustable pores on the surface of plants that balance gas exchange for photosynthesis with water loss. The mechanical interactions of a pair of cells, known as guard cells, are responsible for the reversible opening and closing of each pore, reliably regulating this dynamic process. Grasses, which inhabit dry environments, have evolved specialised stomatal complexes. Grass stomata consist of dumbbell shaped guard cells plus a pair of subsidiary cells, and this is thought to enable them to open and close more rapidly. We study the biomechanics of grass stomata through a combination of microscopy and Finite Element Method (FEM) modelling. We investigated the mechanical interactions of the guard cells with the subsidiary cells and elucidated the role that geometry, cell wall mechanical properties and cell-cell interactions play in the rapid opening and closing process of grass stomata. Our results are contrasted with a FEM model of a two-cell kidney shaped stomata, underscoring the unique properties present in grass stomata that enhance their performance. Overall, this research provides a deeper understanding of the interplay between cellular geometry and material properties for biomechanical mechanisms that enable efficient gas exchange in grasses.

P1.115

Measuring diffusion constant and concentration of planer core polarity proteins molecules using fluorescence confocal microscopy

Dr. Manoj Prasad[1], Hongyu Shao[1], prof. Ashley Cadby[2], prof. David Strutt[1]
[1]School of Biosciences, University of Sheffield, Sheffield, United Kingdom, [2] Department of Physics and Astronomy, University of Sheffield, Sheffield, United Kingdom

The planer core polarity (PCP) refers to symmetry break within the cells and its propagation to the cell located a dozen to hundred cells apart[1, 2]. Deciphering the mechanism of symmetry break is important to understand the mechanism of self-organization in tissue, which plays an essential role in organ formation[2-4]. The planer polarity is often studied in the Drosophila wing and is known establish through six core proteins namely Frizzled, Dishevelled, Diego, Stan, prickle, and Flamingo[5, 6]. The symmetry breaking in the wing results in distal localization of the Frizzled, Dishevelled, and Diego, and proximal localization of the Vang, Prickle, and Flamingo[5, 7]. To decipher the mechanism of PCP formation, using fluorescence confocal spectroscopy, we measure the core protein dynamical properties and their concentrations, and the rate of their complexes formation at various stages of the wing development. This will allow us to understand the role of the protein molecules in global and local molecular signaling and their role in polarity establishment. In my talk, I will present our recent findings.

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P1.116

Tracking and tracing complex DNA structures

Libby Holmes[1], Alice Pyne[1], James Provan[2], Sean Colloms[2]
[1]*Department of Materials Science and Engineering, The University Of Sheffield, Sheffield, United Kingdom,*
[2]*Institute of Molecular, Cell, and Systems Biology, The University Of Glasgow, Glasgow, United Kingdom*

The topology of DNA is tightly regulated due to its profound effect upon DNA replication, transcription, and recombination. DNA topology describes how the two strands of the double helix intertwine intramolecularly, and how intermolecular relationships between multiple molecules occur. Topologically complex DNA conformations can be formed accidentally or intentionally through DNA-protein transactions, such as accidentally during the strand-exchange action of type-II topoisomerases, or intentionally by the reactions of site-specific recombinases.

Despite the long history of DNA topology as a field of study, it remains difficult to determine the exact topology of complex DNA molecules, such as knots and catenanes. Seminal studies of DNA topology used Electron Microscopy (EM) to determine topologies of DNA by coating the DNA strands in proteins (e.g. RecA). Protein coating was required due to the flexibility and small size of DNA, which make it challenging to visualise. To overcome this issue, we use high-resolution Atomic Force Microscopy (AFM), demonstrating that we can visualise the secondary and tertiary structure of topologically complex DNA molecules and determine crossing direction, i.e. chirality at each crossing point.

This resolution allows us to explicitly classify the topology of multiple DNA knots and catenanes. We demonstrate this by successfully characterising 5 node twist-knots and torus-knots, showing that by tracing the molecules by hand and determining the handness of each crossing, we can explicitly determine topology. This work provides a basis for establishing a new high-resolution methodology for determination and classification of the topology of complex DNA structures.

P1.117

Untwisting the Torsional Constraints on Processive DNA Replication; Decoding Genome Stability

Dr Jack Shepherd[1], Dr Zhaokun Zhou[2], Dr Sebastien Guilbaud[1], Prof Mark Leake[1,3], Dr. Jamieson Howard[1]
[1]School of Physics, Engineering & Technology, University of York, York, United Kingdom, [2]Guangdong Provincial Key Lab of Robotics and Intelligent System, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen , China, [3]Department of Biology, University of York, York, United Kingdom

High-fidelity replication of cellular DNA is a complex multi-stage undertaking. This is due to several factors such as the volume of DNA that must be replicated and structural complexities arising from its double-helical nature and topological constraints of unwinding. It is becoming clear that nucleoprotein blocks are one of the foremost reasons for replication fork stalling and as such present a large barrier to genome integrity and stability. Recent studies are elucidating how replisomes are able to navigate the issue of blocks to replication at the single replisome level, however all of these studies are necessarily carried out in the absence of topological constraint owing to the absence of techniques that allow for the tortional control and concurrent imaging of DNA.

It is a long-term goal to setup a single molecule system in York that would allow the interrogation of the interplay between DNA topology and replisome collisions with nucleoprotein complexes. This will be achieved using COMBI-tweez, a cutting-edge microscope that combines optical and magnetic trapping of DNA with fluorescence imaging of the transversely trapped DNA. This alongside quantitative image analysis using bespoke adaptations of PySTACHIO single molecule tracking software will allow for precise measurement of replication procession. Highly tuneable DNA tethers will also be created in order to further the understanding of sequence specific effects on the regulation of DNA replication. The long-term goal of this research will be to provide an in-depth understanding of the kinetics of DNA replication at a single molecule level at near physiological condition.

P1.118

Single molecule mechanical manipulation of tandem repeat proteins.

Mohsin M. Naqvi[1], Maria Zacharopoulou
[1]*University of Cambridge, , United Kingdom*

Using optical tweezers here we are studying how small molecule (SMAPS) binding alters the energy landscape of the large PR65 scaffold protein of PP2A complex. This study will help in confirming the structural changes which PR65 undergoes upon SMAP binding and how these changes are linked to the activation of PP2A in tumor cells. In another study we are exploring the thermodynamics and kinetics of folding and unfolding of de-nuovo designed helical repeat proteins. The data will help in the understanding of how the computationally designed proteins differs from their natural counterparts in terms of structure and functionality. Single molecule experiments showed intermediate states populated by DHR proteins during their folding process and will help in the quantification of intrinsic and interfacial energies of the repeat motifs.

P1.119

Mechanical Biomolecule Encapsulation inside DNA Origami Boxes

Mr Matteo Marozzi[1], Mr Elliot Chan[1], Mr Daniel Rollins[2], Dr Alice Pyne[2], Dr Agnes Noy[1], Prof Deborah O'Connell[3], Prof Mark Leake[1]

[1]The University Of York, York, United Kingdom, [2]The University of Sheffield, Sheffield, United Kingdom, [3]Dublin City University, Dublin, Ireland

DNA origami is one of the most prominent techniques for developing nanotechnologies compatible with biological systems. So far, the technique has been mainly used to produce high complexity structures whose strength mainly relies on a precise functionalisation of the DNA strands that allows a fine target recognition and dynamic structural modifications.

However, less effort has been put into the development of origami structures able to interact with their target molecules by mechanical means, avoiding the use of specific chemical recognition. Such a strategy would present considerable advantages in the fields of drug delivery and surface microscopy: in the former by allowing the development of drug carriers compatible with a larger number of drugs, and in the latter by providing a surface-tethered system for imaging biomolecules in their native state while retaining the advantages of a more controllable size and the option of undergoing dynamic conformational changes over other highly represented comparable technologies such as lipid vesicle tethers.

The goal of the project is to provide a thorough, single molecule characterisation of DNA origami box-like structures to evaluate their potential for the mechanical encapsulation of biomolecules in such applications. A 25 nm DNA box has been modified in multiple instances and analysed through atomic force microscopy, fluorescence microscopy and molecular dynamics simulations to evaluate its suitability as a mechanical container for biomolecules of different sizes. While the project is still a work in progress, promising results have been obtained, hinting towards the mechanical encapsulation of GFP molecules inside the origami structures.

P1.119

Live-cell super resolution imaging of actin using LifeAct-14 with a PAINT-based approach

Haresh Bhaskar[1]

[1]*University Of Edinburgh, , United Kingdom*

We present direct-LIVE-PAINT, an easy-to-implement approach for the nanoscopic imaging of protein structures in live cells using labeled binding peptides. We demonstrate the feasibility of direct-LIVE-PAINT with an actin-binding peptide fused to EGFP, the location of which can be accurately determined as it transiently binds to actin filaments. We show that direct-LIVE-PAINT can be used to image actin structures below the diffraction-limit of light and have used it to observe the dynamic nature of actin in live cells. We envisage a similar approach could be applied to imaging other proteins within live mammalian cells.

P1.120

Label-free identification of type III CRISPR-Cas second-messengers, one molecule at a time

David Fuentenebro[1]

[1]*Wageningen University and Research, Wageningen, Netherlands*

Cyclic oligoadenylates (cOAs) are a group of second messenger molecules of type III CRISPR-Cas systems that mediate signaling pathways in prokaryotes aimed to combat invading viruses and plasmids by the allosteric activation of associated effector proteins. So far, the characterization of these small cOA molecules required expensive equipment for bulk measurements, such as liquid chromatography - mass spectrometers (LC-MS). In addition, an assay with single-molecule resolution that could provide a more detailed picture of the cOA composition of different samples was lacking.

Here we demonstrate the label-free single-molecule detection of cOAs using a simple and affordable nanopore assay that can detect and count single cOA molecules 1 by 1. By first studying monodisperse samples of known composition, we identified the characteristic nanopore translocation events per cOA type, and distinguish cOA stoichiometries at the population level.

In addition, beyond population-wide analysis, we used the single-molecule information provided by our nanopore assays to train a neural network in order to identify single translocation events. This allowed us to distinguish cOA3/cOA4 from cOA5 and those from cOA6, achieving 75% accuracy per single cOA translocation event. Moreover, we demonstrate the utility of our new approach by identifying cOAs and their stoichiometries in enzymatic, biologically relevant samples of unknown composition. This nanopore assay can be easily integrated into handheld portable devices allowing a quick and sensitive cOA identification, one molecule at a time.

P1.121

Exploring electric field sensing for solid state nanopore based DNA and Protein sequencing applications

Mr Muhammad Sajeer P[1], Prof Manoj Varma[1]

[1]*Center for Nanoscience and Engineering, Indian Institute of Science, Bangalore, Bangalore, India*

The solid-state nanopores have received significant attention in the past years due to their simplicity and potential applications expected in genomics, proteomics, and single-molecular sensing. The fundamental technique on which nanopore technology has built-in is the analysis of modulations in the current blockade signal while analytes translocate through the nanopore. This current blockade based sensing is currently well established (eg. DNA sequencing). However, when it comes to applications such as single-molecular protein sequencing, where an inhomogeneous charge distribution is involved, a sensing method that is more sensitive to the diverse charge profiles of the analyte is required. In this work, we explored the potential of electric field (EF) sensing as an alternative to current blockade measurements for nanopore based single molecular sensing applications. This Multiphysics simulation-based study compares the ionic current and EF sensing-based techniques. Further, the EF sensing has potential advantages over current blockade measurements, such as EF measurement does not necessarily require the electrolyte solution, which was a mandatory requirement for the nanopore current blockade measurement setup until today. This technique can be used in extreme environments and can be further developed to use in defence and space-related applications such as future mars, moon, and Europa missions. We hope this work will act as a starting point for developing electric field sensing techniques for exciting nanopore single molecular sensing applications.

P1.122

Exploration of single-molecule dielectrophoresis by means of trapping and actuation

Janike Bolter[1], Jacco Ton[2], Dmytro Shavlovskyi[1], Daniel Wijnperle[1], Michel Orrit[2], Sergii Pud[1]
[1]MESA+ Institute for Nanotechnology, University of Twente, Enschede, Netherlands, [2]Huygens-Kamerlingh Onnes Laboratory, Leiden University, Leiden, Netherlands

Dielectrophoresis (DEP) is an electrokinetic effect often used in lab-on-chip applications to trap and manipulate proteins using an AC electric field. It causes the protein to experience a force, which depends on the proteins shape, size, and charge properties. However, in calculations this force is usually oversimplified due to the absence of a good model describing the interaction between the proteins and a spatially inhomogeneous electric field. Therefore, we aim to gain a deeper understanding of protein DEP. We combine interferometric scattering microscopy (iSCAT) with DEP actuation in a nanoelectrode trap to investigate the behaviour of proteins in high electric fields (up to 10^7 V/cm). By alternating the electric field strength, we want to actuate the trapped particle. We already showed this on 40 nm polystyrene beads. Here, we were able to see the actuation frequency and its multiples in the fourier-transform of the scattered light intensity, showing actuation, which validates the feasibility of our novel approach. For proteins this motion is dependent on it's environment and parameters of the AC field. By observing known proteins of different size, charge and dipole moment in different salt environments we want to understand this interaction. With the data from our new setup, we want to close the knowledge gap in protein DEP and develop a model of proteins in AC electric fields. Once we understand the interaction of proteins with the electric fields in our setup, we can start to use it as a new tool to study and characterize proteins.

P1.123

Generating, imaging, and characterising DNA plectonemes with combined transverse magneto-optical tweezers, fluorescence microscopy, and all-atom molecular dynamics simulation

Dr Jack W Shepherd[1,2], Sebastien Guilbaud[1], Zhaokun Zhou[3], Jamieson AL Howard[1], Matthew Burman[1], Agnes Noy[1], Mark C Leake[1,2]

[1]School of Physics, Engineering, and Technology, University Of York, , United Kingdom, [2]Department of Biology, University of York, , United Kingdom, [3]Guangdong Provincial Key Lab of Robotics and Intelligent System, Shenzhen Institute of Advanced Technology, , China

In recent years considerable evidence has emerged of DNA function beyond the central dogma. Far from a passive information store acted on by other agents, DNA topological response to perturbation is as important as chemical gene regulation markers, and topological change may indicate damage. To truly understand cellular biology, DNA's inherent mechanical properties and response to mechanical stimuli must be completely understood. However, imaging topological response to force and torque is challenging and requires a combination of techniques. Here we present our novel, bespoke apparatus which allows the decoupled application and measurement of pN forces and single-turn level supercoiling alongside simultaneous imaging with fluorescence microscopy. This technique, called Combined Optical and Magnetic BIomolecular TWEEZer (COMBI-Tweez), fuses optical tweezing using an infrared optical trap and magnetic tweezing with two pairs of Helmholtz coils around the sample. The trapped molecule is held transversely and imaged through its entire contour length, allowing us to generate an experimental model linking tension and supercoiling state with plectoneme size and dynamics through micrograph analysis with an adapted version of our single-molecule intensity and trajectory analysis suite PySTACHIO. We find that in the low force regime plectonemes formed by positive and negative supercoiling appear in different locations with different mobilities, which we link to predicted sequence-related bubble formation. Using all-atom molecular dynamics, we demonstrate that melting bubbles at the tips of plectonemes differ between under- and overtwisted DNA, which we hypothesise explains differing plectoneme mobility and has implications for genome structure and organisation.

P1.125

Visualising NDP52 shape DNA Confirmation

Daniel Rollins[1], Mingxue Du[1], Dr Ália dos Santos[2], Faeeza Lorgat[2], Dr Christopher Toseland[2], Dr Alice Pyne[1]
[1]*Department of Materials Science and Engineering, University of Sheffield, Sheffield, United Kingdom,*
[2]*Department of Oncology and Metabolism, University of Sheffield,, ,*

There is a growing appreciation that the structural and cellular context of DNA is vital to its function. DNA binding proteins are at the core of the structural and functional regulation of the genome. Here we study the interaction of DNA with NDP52, a protein with a role in cell adhesion and autophagy within the cytoplasm of eukaryotic cells but with a poorly understood role within the nucleus the mechanism of its interaction with DNA is also unknown.

We set out to understand the structure and interaction of NDP52 with DNA to discover how it may play a role in regulating or manipulating DNA structure. We use atomic force microscopy (AFM), a technique capable of achieving sub- molecular resolution on biomolecules in physiologically relevant conditions to directly image the structure of the protein. We show that we can visualise the globular C- and N-terminal domains, and the single coiled-coil between them. We observe significant heterogeneity in the protein's structure, one of the reasons the structure has not been solved by x-ray crystallography or cryo-EM. We confirm the presence of dimerisation, noting this occurs via the globular regions in addition to the coiled-coil as previously postulated. Finally, in the presence and absence of NDP52 we observed changes in the conformation of short linear strands of dsDNA mediated by NDP52 bridging and bending DNA strands. We combine these findings with extensive biochemical studies to further propose that the nuclear functions of NDP52 are related to transcription and DNA regulation through chromatin organisation.

P1.126

Unpicking DNA Translocation in Nanopores with Simultaneous Single-Molecule Fluorescence and Optical Single Channel Recording

Mark Wallace[1], Daisy Rogers-Simmonds, Steven Vanuytsel
[1]*King's College London, London, United Kingdom*

Nanopore-based DNA sequencing offers high throughput and long reads by measuring blockades in current as the strand is translocated, providing a single-molecule readout with high time resolution. However, electrical measurements alone offer no spatial information. To improve nanopore sequencing, a molecular picture of the steps of DNA binding, capture and translocation is required. While simulations have provided important insights, a direct step-by-step readout of this process has not been produced. Previously, optical single channel recording of calcium flux through the nanopore has enabled individual single-molecule capture events to be distinguishable in a droplet interface bilayer containing multiple nanopores. Here, we combine optical single channel recording with single molecule fluorescence readout of DNA unzipping to correlate the steps of individual translocations and image the location of the system components. By annealing short oligomers bearing fluorophore-quencher pairs to the translocating DNA strand, we make preliminary measurements that can map the links between capture, threading and unzipping.

P1.127

Tractor beams and single molecules: How to visualize and manipulate single biomolecules in real-time

Emma Verver[1], Artur Kaczmarczyk[1], Jack O'Sullivan[1], Aida Llauro[1], Andrea Candelli[1]
[1]*LUMICKS, Amsterdam, Netherlands*

Imagine you could directly see the location and dynamics of individual proteins binding to a piece of single DNA. What if you could assemble your biological complex step by step and see it in action in real-time? What if you could manipulate the structure of your biomolecule and quickly change buffer conditions to test your experimental hypotheses? By using “molecular tractor beams”, the C-trap makes that a reality.

Essential biological processes performed by proteins interacting with DNA or cytoskeletal protofilaments are key to cell metabolism and life. In order to understand the molecular basis of life, as well as the pathological conditions that develop when processes go wrong, it is critical to get detailed insights into these processes at the molecular level. Not only at the highest resolution, but also in real time.
Here, we will take you through an exciting journey of different research topics in which dynamic single molecule studies revealed remarkable insights. In each study, the C-Trap system was used; our easy-to-use platform that combines optical tweezers with fluorescence microscopy. We will explain how the ability to control, visualize and manipulate single molecules in real time, changes the way we answer tough scientific questions in the field of DNA processing, single-protein dynamics, liquid-liquid phase separation (LLPS) and beyond.

P1.128

Tracking single molecules on fluorous coated surfaces: New toolkit to study biomolecule interactions

Carlos J Bueno Alejo[1], Marina Santana Vega[3], Alexander Axe[5], Glenn A. Burley[5], Cyril Dominguez[2,4], Hesna Kara[2,4], Vasileios Paschalis[2,4], Sumera Tubasum[2,4], Ian C. Eperon[2,4], Alasdair W. Clark[3], Andrew J. Hudson[1,2]
[1]School of Chemistry, University of Leicester, Leicester, United Kingdom, [2]Leicester Institute of Structural & Chemical Biology, Henry Wellcome Building, University of Leicester, Leicester, United Kingdom, [3]School of Engineering, Advanced Research Centre, University of Glasgow, Glasgow, United Kingdom, [4]Department of Molecular and Cellular Biology, Henry Wellcome Building, University of Leicester, Leicester, United Kingdom, [5]WestCHEM & Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow, United Kingdom

Single molecule tracking is becoming a powerful tool to analyze how biomolecules interact on different situations. From chromatin formation in the nucleus to the movement of proteins over the cell membrane, to learn the way that biomolecules moves is of paramount importance. When single molecule techniques are used for biomolecular analysis adequate surfaces are needed, and it is of upmost importance that those surfaces have good antifouling properties. Very recently we proposed the use of fluorous coated surfaces in order to avoid non-specific binding of biomolecules1. We showed that these surfaces could have a great potential for its use in single molecule microscopy since they would only bind biomolecules with specific fluorous tags and reject any other molecules. In this work we show that combining those fluorous surfaces with different fluorous tags is possible to analyze how biomolecules move free on a surface opening a myriad of opportunities. We will show that when a biomolecule like an oligonucleotide or a protein is tagged with a certain fluorous tags it can freely diffuse over fluorous coated surfaces. We have analyzed and categorized the movement of the different combinations of biological entities and fluorous tags so it can be applied to analyze different biomolecular problems.

P1.129

Structural Conversion of alpha-Synuclein at the Mitochondria Induces Neuronal Toxicity

Dr Minee Choi[2], Mr Alexandre Chappard[1], Dr Bhanu P. Singh[1], Prof Andrey Y. Abramov[2], Prof Sonia Gandhi[2], Dr Mathew Horrocks[1]

[1]University Of Edinburgh, , United Kingdom, [2]Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, UK., , United Kingdom

The aggregation of alpha-synuclein, which normally exists as an intrinsically disordered monomer, is a major hallmark of synucleinopathies, including Parkinson's disease and Lewy body dementia. Rather than the larger aggregates being toxic, it is now thought that the earlier soluble oligomers are the damaging species. These, however, are challenging to study due to their high heterogeneity and low abundance. In the past, we have developed a range of single-molecule and super-resolution techniques [1–3] to visualise the earliest aggregation events in vitro and in biofluids. Here, we report a Förster Resonance Energy Transfer (FRET) based method that enables the kinetics of intracellular oligomerisation to be tracked in live cells [4]. We have shown that cells rapidly uptake monomeric alpha-synuclein, which then forms two distinct oligomeric states in neurons in a concentration-dependent and sequence-specific manner. Furthermore, 3D FRET-correlative light and electron microscopy revealed that intracellular seeding events occur preferentially on membrane surfaces, especially at mitochondrial membranes. Our study highlights a mechanism of de novo alpha-synuclein oligomerisation at mitochondrial membranes and subsequent neuronal toxicity.

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P1.130

Single molecule experiments and theory of the bending and looping dynamics of DNA at the scale of its persistence length.

Dr Bhavin Khatri[1,2], Dr George Pobegalov[1], Dr Maxim Molodtsov[1]
[1]*Imperial College London, Ascot, United Kingdom,* [2]*The Francis Crick Institute, London, United Kingdom*

The dynamics of DNA on short length scales is of fundamental importance in molecular biology as proteins interact with DNA on scales of order the persistence length. However, our understanding of the mechanics of DNA is encapsulated by the Worm-Like Chain Model, which is limited to equilibrium information. To probe the dynamics of double-stranded DNA, we developed a new more sensitive single-molecule force probe analysis to characterise the viscoelastic response of double-stranded DNA from its velocity fluctuations. Using optical tweezer apparatus, we measure a force-dependent frictional response, which we explain in terms of the Frictional Worm-like Chain model and a new fundamental constant for the mechanics of DNA — the bending friction ζB — which here gives rise to a characteristic persistence time for the shape of short loops. We measure ζB = 137± 22 μg nm^3/ms which corresponds to a persistence time of ~1ms, which is many orders of magnitude slower than predictions of solvent limited dynamics; this gives fundamental insight to the importance of local energy barriers within a DNA molecule and how it limits its dynamic flexibility. To exemplify the impact of this result we focus on two toy-models of important biological processes: the time to spontaneous initiation of DNA loops within SMC complexes and the spontaneous time to wrapping of DNA around nucleosomes — both highlight an interesting interplay between geometry, curvature and Brownian motion, and give rise to predicted timescales many orders of magnitude longer than those based on solvent limited dynamics.

P1.131

Single cohesin molecules generate force by two distinct mechanisms

Dr. Maxim Molodtsov[1]

[1]*University College London, , United Kingdom*

Spatial organization of DNA is facilitated by cohesin protein complexes that move on DNA and extrude DNA loops. However, how cohesin works mechanistically as a molecular machine is poorly understood. Here, we measured mechanical forces generated by conformational changes in single cohesin molecules using an optical trap. We show that bending of SMC coiled coils occurs via two mechanical steps of 17 and 15 nm and it is driven by random thermal fluctuations that do not allow overcoming forces above 1 pN. On the contrary, engagement and disengagement movements of the ATP binding SMC head domains occur in single steps of ~ 10 nm and engagement generates a power stroke capable of overcoming forces up to 15 pN. Our molecular dynamic simulations suggest that the energy of SMC head engagement can be stored in a mechanically strained conformation of the NIPBL subunit and released during disengagement. These findings reveal how single cohesin molecules generate force by two distinct mechanisms. We present a model, which proposes how this ability may power different aspects of cohesin-DNA interaction.

P1.132

Rapid and Reversible Conformational Switching of Single DNA Hairpins

Miss Sarah Graham[1], Francisca D'Rozario[1], Steven Johnson[1], Mark Leake[1,2,3], Steven Quinn[1,2]
[1]School of Physics, Engineering and Technology, University of York, York, United Kingdom, [2]York Biomedical Research Institute, University of York, , United Kingdom, [3]Department of Biology, University of York, , United Kingdom

DNA hairpins play an essential role in replication initiation, and are commonly used across a number of synthetic bionanotechnological applications. The thermodynamic details of hairpin formation have been extensively investigated, but the kinetic aspects remain underexplored.

Here I use Förster Resonance Energy Transfer (FRET), as a powerful approach to quantify the opening and closing dynamics of both freely diffusing and surface immobilised adenosine-rich hairpins, specifically conjugated with the fluorophores Cy3 and Cy5. By modelling FRET efficiency trajectories with Hidden Markov-based algorithms, relative populations and time-dependent kinetic switching rates can be extracted.

Two environment-sensitive states corresponding to "open" (low FRET) and "closed" (high FRET) forms of the hairpin are identified, with the folding-reaction strongly dependent on factors including pH, buffer composition, presence of crowding agents and ionic strength.

I also implement a label free sensing strategy based on quartz crystal microbalance with dissipation (QCM-D) monitoring to quantify the conformation of surface-immobilised hairpins via measurement of the acoustic frequency and dissipation. These results also distinguish between open and closed forms of the molecule confirming that transitions between states are reversible.

The acquisition of FRET trajectories from both freely-diffusing and immobilised hairpins, coupled with QCM-D analyses, will serve to enhance our fundamental mechanistic understanding of DNA hybridization, and facilitate the rational design of functional DNA nanoswitches, including devices for computation and memory storage.

P2.01

Investigating the evolution of developmental strategies using spatiotemporally patterned telencephalic organoids.

Miss Taniya Mandal[1,2], Afnan Azizi[1,2], Zena Hadjivasiliou[1,3], Corinne Houart[1,2]
[1]Francis Crick Institute, London, United Kingdom, [2]Centre for Developmental Neurobiology, King's College, London, United Kingdom, [3]University College London, London, United Kingdom

Vertebrate adult forebrains show a large variation in size and complexity across species, despite their morphology and gene expression profiles being similar in early embryonic stages. In the developing telencephalon, Shh (ventral) and Wnt/BMP (dorsal) signals induce the expression of region-specific transcription factors thereby dividing it into discrete zones of progenitor cells. The relative size and cell type composition of the progenitor zones influence the complexity of the adult brain. The mechanism of how diverse telencephalic structures emerge utilizing a handful of conserved morphogens remains poorly understood.

To tackle this gap in the field, we are establishing mouse and human organoid systems suitable for in vitro patterning. We will impose anti-parallel gradients of ventral (Shh) and dorsal (Wnt) morphogens to recapitulate dorsoventral organisation of the nascent telencephalon at 5-12 weeks post-conception in human and at the corresponding stages in mouse. We hypothesize that differential regulation of morphogen dynamics and its context-dependent interpretation by gene regulatory networks (GRNs) together initiate the evolutionary divergence between the two species. To test our hypothesis, we will quantify cellular responses to morphogen input and analyse how spatiotemporal patterns change in response to perturbations in morphogen dynamics and in downstream GRNs. We will distinguish between different hypotheses of early brain patterning that emerge from experimental quantifications using mathematical modelling. We aim to identify the species-specific properties of how cells and tissues respond to morphogen signals and subsequently determine the size and complexity of the developing telencephalon using standardized spheroids, controllable signal induction and mathematical modelling.

P2.02

Novel mathematical models for fate selection in neural crest stem cells

Karol Bacik[1], Saeed Farjami[3], Karen Camargo Sosa[2], Jonathan Dawes[1], Andrea Rocco[3,4], Vsevolod Makeev[5], Robert Kelsh[2]

[1]Department of Mathematical Sciences, University Of Bath, Bath, United Kingdom, [2]Department of Life Sciences, University of Bath, Bath, United Kingdom, [3]Department of Physics, University of Surrey, Guildford, United Kingdom, [4]Department of Microbial Sciences, University of Surrey, Guildford, United Kingdom, [5]Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia

The development of an embryonic cell has historically been conceptualized by considering the motion of a ball rolling through an abstract epigenetic landscape, with valleys representing alternative developmental pathways and final states. In more modern times there has been a vibrant debate centering around two alternative views of fate specification in neural crest stem cells: the Progressive Fate Restriction (PFR) and Direct Fate Restriction (DFR) hypotheses. In PFR, partially restricted intermediate cell states exist; in DFR they do not.

Here we introduce a third, unifying, possibility which we term Cyclical Fate Restriction (CFR). In CFR stem cells have an intrinsically dynamical character which cannot be captured by the epigenetic landscape idea. Mutually inhibitory interactions in an underlying genetic regulatory network (GRN) give rise to robust temporal oscillations in gene expression patterns and admit, in a mathematically generic way, a new mechanism for the transition from multipotency to fate-specified cell types. Mathematical modelling focuses in particular on the role of symmetry in the core GRN and reveals that these model dynamics are extremely robust to parameter choices. We relate our findings to recently published and ongoing in vivo experiments on the zebrafish neural crest.

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P2.03

Capillary forces functionally remodel membrane-bound organelles and condensates inside cells

Roland L. Knorr[1]

[1]*Humboldt-Universität zu Berlin, , Germany*

Membrane-bound organelles have long been considered solely responsible for intracellular compartmentalization, but more recent work has demonstrated that condensates also organise the interior of the cell. Forming from localised, liquid-like concentrations of biomolecules such as proteins and RNA, condensates are often described as 'membrane-less organelles' due to their lack of a bounding membrane. Our recent breakthrough research has shown that these compartments can in fact interact with membrane-bound compartments, resulting in remarkable membrane deformations, the physiological importance of which is only just becoming apparent: Evidence suggests that condensate clearance involves autophagy, a highly-conserved cellular recycling system in which membrane sheets expand and bend to isolate and degrade portions of the cell interior.

Here, we investigate the mechanisms of condensate sequestration by membrane sheets in both living and synthetic cells. A minimal theoretical model shows that the condensate surface tension determines whether membrane sheets isolate condensates in a whole or piecemeal fashion. We also find that wetting condensates induce local membrane spontaneous curvature changes, resulting in the reversal of the bending direction of membrane sheets and, thus, in cytosol sequestration [Nature 2020, 2021]. Further, we demonstrate that the morphogenesis of protein storage vacuoles in plants underlies similar physical principles [PNAS, JCB 2021]. I propose that condensate-mediated autophagy and vacuole remodelling represent a novel class of cellular processes controlled by capillary forces.

P2.04

Gene expression dynamics during cell fate decisions in the retina

Cerys Manning[1]

[1]*University Of Manchester, , United Kingdom*

Eye development is a dynamic process with cell state transitions between retinal progenitor cells and differentiated cell fates such as ganglion cells, yet many of the current techniques only provide a single timepoint snapshot. As a result, little is known about the dynamic gene expression changes that occur during the shift from progenitor to differentiated state. It is vital that this knowledge gap is filled because these cell state transitions are critical for tissue development and when mis-regulated lead to developmental disorders. Recent evidence shows that cell state transitions are not merely binary on-off switches in gene expression. Significantly, my own live imaging of neural development shows that changes in the expression dynamics of key cell fate determinants HES1 and HES5 (HES1/5) are concurrent with and important for cell fate transitions between neural progenitors and neurons. These can be from ultradian (short-period) oscillations to sustained expression or from noisy aperiodic to ultradian oscillations. Oscillatory expression is beneficial as it encodes more information than simply levels of expression, by modulation of frequency, amplitude, duration of oscillations and phase differences between oscillations. However, the critical information on expression dynamics of key cell fate determinants and how these oscillations are decoded is lacking in retinal development. Here we use live imaging of fluorescent reporters of the key transcription factors HES1, HES5 and NGN2 in retinal progenitor cells and observe complex dynamic behaviours, including in-phase and out-of-phase oscillations. We also show extrinsic regulation of HES5 levels and dynamics by YAP and substrate stiffness.

P2.05

A study on the spatiotemporal dynamics and fitness landscape of bacteriophages

Hassan Alam[1], Dr. Diana Fusco[1]
[1]*Department of Physics, University of Cambridge, Cambridge, United Kingdom*

Bacteriophages (also called phages) are viruses that infect bacteria. Microbial systems consisting of bacteriophage and their host bacteria are very useful to understand the population dynamics and ecology of different complex microbial systems. This study utilizes a Partial Differential Equations (PDE) model to predict the spatiotemporal dynamics and fitness landscape of bacteriophages under different host bacteria conditions.

Interactions between bacteriophages and their bacteria depend on different biological and physics factors, especially phage absorption rate, burst size, lysis time, phage decay rate, bacteria growth rate, and their diffusivity and motility. In addition, different spatial distributions of bacteria play important role in determining the fixation of resident and mutant phages. This research provides insight into how different spatiotemporal patterns spontaneously emerge in the population of phages and bacteria based on these parameters.

The work provides a mathematical framework to investigate the selective pressure of phage and bacteria as a function in complex dynamical environments.

P2.06

The interplay of size and pattern during evolution

Amy Bowen[1,2], Zena Hadjivasiliou[1,2,3]

[1]Francis Crick Institute, London, United Kingdom, [2]UCL, London, United Kingdom, [3]London Centre of Nanotechnology, London, United Kingdom

During development, tissues undergo growth and patterning concomitantly. The mechanisms that regulate this process are tightly controlled to ensure that tissues reach well-defined and reproducible patterns, shapes and sizes. Morphogens are molecules that have a significant role in both growth and patterning, and are highly conserved in evolution. Despite this, morphogens generate a wide range of size and patterning phenotypes in nature. The dual role of morphogens implies that genetic changes impacting morphogen dynamics may alter tissue size and pattern simultaneously. This indicates that the pleiotropy of morphogens may play a role in how evolution has generated the wide diversity of size and pattern in nature. We are aiming to understand how the interplay between growth and patterning interacts with natural selection during evolution.

We have developed a reaction-diffusion model of morphogen dynamics in growing tissues to investigate the effects of coupling growth and patterning. We have tested this model computationally and verified results with previously published data. These results will be compared to morphometric data obtained from quantifying the size-pattern co-variance in Drosophila wings, across various species and between different patterning mechanisms. This project will establish how the dual role of morphogens impacts the diversification of size and pattern, and investigate how that duality could have evolved under different selective pressures.

P2.07

Curve registration – an approach for comparing gene expression dynamics over different developmental timescales

Ruth Kristianingsih[1], Alexander Calderwood[1], Gurpinder Singh Sidhu[1], Shannon Woodhouse[1], Hugh Woolfenden[1], Rachel Wells[1], Richard J. Morris[1]
[1]*John Innes Centre, Norwich, United Kingdom*

How gene expression changes over time can provide valuable insights into developmental processes and responses to the environment. Differences in expression dynamics may be indicative of potential differences in regulation. Comparing gene expression may help identify correspondences between developmental stages within and between species, differences in the timing of key events during development, and transcriptional response to treatments or perturbations. A straightforward comparison between the dynamics is, however, hindered by different timepoints and timescales of datasets. To address this, we developed a Bayesian approach based on statistical model comparison, which finds the optimal alignment of gene expression dynamics by inferring temporal shifts and stretches. To make this this approach accessible, we developed an R package ('greatR') which is available from CRAN at https://CRAN.R-project.org/package=greatR and GitHub at https://github.com/ruthkr/greatR. We validated our approach using simulated data and applied to several transcriptomic datasets, including comparisons between different plant species, as well as between mouse and human. Our development facilitates knowledge transfer from model systems to less studied species, the identification of modules of co-regulated genes, and the discovery of condition-specific temporally differentially expressed genes.

P2.08

Exploring the design principles of Arabidopsis in response to temperature changes

Dr Paula Avello[1], Seth J Davis[2], James Ronald[3], Jonathan W Pitchford[2]
[1]University of Leeds, , United Kingdom, [2]University of York, , United Kingdom, [3]University of Glasgow, , United Kingdom

Biological experiments and mathematical models have provided useful for understanding what genes integrate into the plant circadian system, how they interact together and how light affects this complex system. This is captured by a series of published ordinary differential equation (ODE) models. Recently, we have incorporated temperature dependence by applying Arrhenius equations into these models to help understand the role of temperature in periodicity, and the relationship between temperature entrainment and compensation. Despite these enormous advances, the underlying network system of the plant clock in circadian mechanisms is still poorly understood. Here by mathematical simulations, we explore the link between plant clock functions and the clock network structure. For this, we use two modelling frameworks: 1) exploring the design principles of the Arabidopsis clock that may explain temperature compensation, and 2) elucidating the role of HSP90 in thermal entrainment by simulating the effect of geldanamycin. Results from our models 1) show that a highly repressive network structure of the plant circadian network together with autoregulation patterns and three-node feedback loops, favour temperature compensation, and 2) support the hypothesis, suggested from experiments, that HSP90 influences the morning loop during thermal entrainment. In addition, our numerical results support the repressive role found of CCA1 and LHY on PRR9; however, they also suggest that activation on PRR7 is needed.

P2.09

Blastoid-on-a-chip: development of a microfluidic platform for dynamic visualisation of pre-implantation embryogenesis

Dr Georgina Glover[1], Dr Ge Guo[1], Prof Austin Smith[1]
[1]*Living Systems Institute, University of Exeter, Exeter, United Kingdom*

Human embryogenesis is a topic of major interest in scientific research, but studies are limited by virtue of the in vivo environment being inaccessible, the limited supply of donated IVF embryos, and strict ethical considerations. To circumvent these limitations we recently described a human embryo model (blastoid) that develops via guided self-organisation from naïve pluripotent stem cells . The blastoid faithfully recapitulates a natural blastocyst with correct specification and segregation of trophectoderm, hypoblast and epiblast. Here, we engineer a microfluidic platform that allows for dynamic visualisation of lineage segregation in blastoids. We track how dissociated naïve pluripotent stem cells cluster into aggregates and then form a cavitated blastoid with trophoblast, hypoblast and epiblast lineages. The 'Blastoid-on-a-chip' microfluidic device relies on manipulation of device orientation to permit culture of blastoids on a suitable physical surface and precise imaging via an appropriate material. Single cells are introduced into the device and are able to settle into culture chambers via gravity whilst vertically oriented, then briefly laid horizontal for imaging through 0.17mm thick glass coverslip. The microfluidic device is designed to enable precise control over the extracellular environment, specifically the delivery and removal of nutrients, chemicals, growth factors and waste materials. This system will permit comprehensive evaluation of microenvironmental and biophysical parameters governing blastoid formation and morphology.

P2.10

YAP levels and dynamics control cell fate and proliferation

Kirstin Meyer

Transcription factors transmit extracellular information to gene expression programs to drive important developmental processes such as pluripotency, proliferation and differentiation. How individual transcription factors transmit signals to control different sets of genes and cellular behaviors remains largely unclear. Importantly, cells make use of time-varying signaling features such as the duration, amplitude and frequency to encode information. Here, we probe how concentrations and temporal dynamics of YAP, a key developmental regulator, direct pluripotency gene activation and developmental decision making. Using an optogenetic approach to control nuclear YAP dynamics with light, we find differential control of Oct4 and Nanog through YAP levels and dynamics. While offset repression thresholds provide differential control of both genes under steady-state YAP levels, dynamic YAP inputs preferentially activate Oct4 at frequencies mimicking the dynamics of the endogenous system. Live imaging of transcription and computational-theoretical analysis of transcriptional regulation demonstrates that Oct4 decodes dynamics by acting as an adaptive change sensor. Using cell fate and proliferation readouts, we identify a similar decoding logic directing cellular decision-making: germ layer fates respond to YAP steady-state levels in a dose dependent manner, while cell proliferation is preferentially activated by oscillatory YAP inputs. Together, we reveal how YAP concentrations and dynamics enable multiplexing of information transmission for the control of developmental gene activation and cellular decision making.

P2.11

Dynamic Sigma Factor Patterning in Bacillus Subtilis Biofilms

Alexander Mckinnon[1], Chris Schwall[1], James Locke[1]
[1]*University Of Cambridge, Cambridge, United Kingdom*

As nutrient resources diminish, B. subtilis colonies can adapt by forming a biofilm. Biofilms have a spatial structure, with cells performing different functions in different parts of the biofilm. However, the degree of specialisation of cell function during biofilm formation is unclear. Here, we investigate the role of B. subtilis sigma factors in generating spatial patterns of gene expression during biofilm formation. B. subtilis has 19 alternative sigma factors, with each sigma factor regulating functionally specific downstream genes. We are studying how sigma factor gene expression patterns emerge and develop over time, as the biofilm grows and matures. We are achieving this by growing fluorescent sigma factor reporter strains on biofilm inducing Msgg media and imaging the fluorescent patterns using a confocal microscope. Quantitative analysis of this 3-dimensional temporal-patterning can then be used to construct a simulation which aims to capture the underlying dynamics. In the future we intend to study how this colony-wide pattern is created on a single-cell level by studying regulatory dynamics of single cells within the biofilm.

P2.12

Fast fingerprint of insulin structure and stability assessment with A-TEEM (Absorbance-Transmission Excitation Emission Matrix) spectroscopy.

Dr Giorgia Marucci[1]

[1]*HORIBA UK, NORTHAMPTON, United Kingdom*

The different types of commercial insulin therapeutics divide into short-acting and long-acting insulin. In some cases, the difference between short-acting and long-acting insulin is only one to three residues in the protein sequence. We present A-TEEM™ (Absorbance-Transmission Excitation Emission Matrix) spectroscopy to characterize protein therapeutic formulations for aggregation behaviour in a matter of seconds, even when there are only small differences in the protein sequence. A-TEEM™ acquires simultaneously three-dimensional fluorescence spectroscopy data (EEM) and the absorbance spectroscopy data, to return a unique fingerprint of the sample, independently from its concentration, thanks to the correction of the so-called inner filter effect (IFE). The IFE is the absorption of the excitation photons and the reabsorption of the emitted photons by the sample itself that will change the shape and intensity of the emission spectrum. Because commercial insulin formulations are high in concentration, the IFE correction becomes critical to measure their signature. The acquisition of fluorescence and absorbance data with the same optical configuration is essential to have a meaningful correction, therefore they are performed with the same instrument, with the employment of a CCD detector to acquire the A-TEEM™ data in only a few seconds. Highly sensitive and specific compared to Raman and UV-VIS-NIR spectroscopy respectively, A-TEEM™ does not require complicated sample preparation, is faster and more sustainable than separation techniques, not requiring any consumable, and is cheaper than NMR. This new technique positions itself as a valid alternative for rapid protein characterization.

P2.13

Advances in Localization Atomic Force Microscopy

George Heath[1], Tabitha Storer[1]
[1]*School Of Physics & Astronomy, University of Leeds, Leeds, United Kingdom*

Understanding structural dynamics of biomolecules at the single-molecule level is vital to advancing our knowledge of molecular mechanisms. Currently, there are few techniques that can capture dynamics at the sub-nanometre scale and in physiologically relevant conditions. Atomic force microscopy (AFM) has the advantage of analysing unlabelled single molecules in physiological buffer and at ambient temperature and pressure, but its resolution can limit the assessment of conformational details of biomolecules. Here I will present recent advances in localization AFM (LAFM), a technique developed to overcome current resolution limitations. By applying localization image reconstruction algorithms to height fluctuations in high-speed AFM and conventional AFM data, we increase the resolution beyond the limits set by the tip radius, and resolve single amino acid residues on soft protein surfaces in native and dynamic conditions. LAFM enables the calculation of high-resolution maps from either images of many molecules or many images of a single molecule acquired over time, facilitating single-molecule structural analysis. LAFM is a post-acquisition image reconstruction method that can be applied to any biomolecular AFM dataset.

P2.14

Role of Sam68 in phase separation and fibre formation

Dr Cyril Dominguez[1]
[1]*University Of Leicester, Leicester, United Kingdom*

Sam68 is an RNA binding protein involved in alternative splicing regulation of many oncogenic genes, such as Bcl-x, CD44, Cyclin D1, and Sam68 overexpression is associated with poor prognosis in various cancers (1). While its predominant cellular localisation is diffused in the nucleus, Sam68 has also been shown to localise in condensates, such as Sam68 nuclear bodies (SNBs) or stress granules (1).

Previously, we have unraveled the structural basis of dimerization and RNA recognition by Sam68 and proposed that Sam68 could affect some alternative splicing events by looping out regions of the pre-mRNA (2). We further demonstrated that Sam68 is specifically phosphorylated by Cdk1 at residues T33 and T317 and these phosphorylation events reduce the binding of Sam68 to RNA, control its cellular localization, and reduce its alternative splicing activity, leading to a reduction in the induction of apoptosis and an increase in cell proliferation (3).

Recently, we used NMR spectroscopy to investigate Sam68 in physiological conditions. Strikingly, while addition of nuclear extract had little effects on Sam68 NMR spectra, we observed that in the presence of cytoplasmic extract, the N-terminal intrinsically disordered region (IDR) of Sam68 becomes structured and induces the formation of a macroscopic fibre enriched in cytoskeletal proteins. To our knowledge, this is the first observation that an IDR 1) can become structured in cell extract and 2) can induce phase separation and fibre formation. Mass Spectrometry analysis suggest that the fibre is enriched in cytoskeletal proteins, such as myosin and tubulin. We believe our data provide a unique and powerful in vitro tool to investigate Sam68's properties in phase separation and fibre formation, as well as the role of Sam68 in cytoskeletal organization.

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P2.15

The Cellular Electrome: The Extracellular Significance of Potassium

Ms Oreoluwa Griffiths[1], Dr Fatima Labeed[1], Dr Rebecca Lewis[2], Professor Michael Hughes[3], Mr Federico Bertagna[2], Dr Kamalan Jeevaratnam[2]

[1]University of Surrey, Centre for Biomedical Engineering, School of Mechanical Engineering Sciences, Guildford, United Kingdom, [2]School of Veterinary Medicine, Faculty of Health and Medical Sciences, Guildford, United Kingdom , [3]Khalifa University, Department for Biomedical Engineering, Abu Dhabi, United Arab Emirates

It is theorised that K+ from burst cells will align with an endogenous electric field (EF) produced through injury, creating an ionic gradient with higher [K+] at the cathode (wound site). This gradient on a nm scale may influence the directional migration of macrophages through redistribution of the negatively charged actin skeleton by direct current polarisation.

In literature it has been demonstrated that macrophages migrate anodally in the presence of an EF and neurites have been observed to grow towards a cathode. Bacterial biofilms have been shown to utilise K+ to call distant bacteria to their surface but neurites will not grow up a [K+] gradient, suggesting evolutionary conservation.

In the absence of the gradient inducing EF, cells may instead react to the ionic gradient itself, indicating this as a secondary signal. Based on evidence discussed above, it would be expected for the migration of macrophages in a [K+] gradient to be directed away from an area of increased [K+].

To investigate this phenomenon, the influence of K+ gradients on the migration of THP-1 derived macrophage like cells (MLCs) was studied. THP-1 monocytes were differentiated to MLCs on gradient chips. Once matured into MLCs, a [K+] gradient of 5 - 15 mM was introduced and the motile cell behaviour was observed for 14 hrs using time-lapse imagery. Preliminary results show biased directional migration of 60% of MLCs towards a lower [K+], indicating that in the absence of an EF cell migration may be directed by ion gradients.

P2.16

The Antagonistic Effect Of Oxysterols In ClyA Pore Formation Pathway

Mr. Samlesh Choudhury[1], Aditya Upasani[1], Diksha Parwana[1], K. G. Ayappa[1], Rahul Roy[1,2]
[1]Department of Chemical Engineering, Indian Institute of Science, Bangalore, India, [2]Center for BioSystems Science and Engineering, Indian Institute of Science, Bangalore, India

Pore-forming toxins are a class of proteins secreted by various bacteria that form nanopores on target cell membranes, causing leakage of cellular components and resulting in cell death. Previous experimental and all-atom MD simulations have emphasized the intricate and supportive role of cholesterols in increasing the pore-formation activity of Cytolysin A (ClyA). On the contrary, oxidized cholesterol derivatives such as 25-hydroxycholesterol produced naturally from cholesterol by the cell have shown antiviral effects and innate immunity against bacterial infections by altering the accessible cholesterol content in the cell. Dye leakage experiments carried out with oxysterols-treated small unilamellar vesicles demonstrate reduced pore-forming activity in a concentration-dependent manner. We observe that the replacement of about one-third of cholesterol by 25-hydroxycholesterol negates the enhancement in ClyA activity observed in the presence of cholesterol. Using all-atom MD simulations, we observe a distinct tendency for oxysterols to replace cholesterols which bind in the β-tongue pockets to stabilize the membrane-inserted oligomeric complex of ClyA. Oxysterols also induce large structural deviations in the membrane-inserted N-terminus protein domain compared to ClyA in purely cholesterol membranes. Unlike cholesterols, an uphill free energy profile is also observed in the presence of oxysterols between the initial monomer and final protomer states, indicating a non-spontaneous conversion from monomeric to protomeric states of ClyA. Our study reveals the mitigating influence of oxysterols in the prevention of pore formation by bacterial toxins and has implications for understanding bacterial infection pathways in aged and senescent cells where oxidative stress levels are elevated.

P2.17

Heterogeneous endosomal dynamics within eukaryotic cells

Nickolay Korabel[1]
[1]*Department of Mathematics, The University of Manchester, Manchester, United Kingdom*

The transport processes of many structures inside living cells, such as endosomes in eukaryotic cells, are described by anomalous diffusion. This type of transport is characterized by a non-linear relationship between the mean square displacement of particles and time and is often heterogeneous in space and time. The study of large ensembles of single particle trajectories can provide insights into the mathematical modeling of these transport processes and help to quantify the heterogeneities present.

Anomalous diffusion in endosomes is particularly intriguing as it is both ergodic and exhibits aging behavior. This paradoxical behavior is caused by ensemble heterogeneity, which is an inherent property of endosomal motion in addition to the space-time heterogeneity within a single trajectory.

In this research, we aim to investigate the anomalous dynamics of endosomes by analyzing large ensembles of single particle trajectories. Our goal is to quantify the heterogeneities present in endosomal motion and provide insights into the mathematical modeling of this transport process. We will also investigate the underlying mechanisms that lead to the paradoxical behavior of endosomal motion and explore the potential implications for cellular transport processes. By studying the anomalous diffusion of endosomes, we hope to gain a deeper understanding of the complex transport processes that occur within living cells.

P2.18

Self-quenching behaviour of fluorescent probes incorporated within lipid membranes explored using electrophoresis and fluorescence lifetime imaging microscopy

Dr Sophie Meredith[1], Yuka Kusunoki[2], Dr Simon Connell[1], Dr Kenichi Morigaki[2], Prof. Stephen Evans[1], Dr Peter Adams[1]

[1]University Of Leeds, Leeds , United Kingdom, [2]Kobe University, Kobe, Japan

Fluorescent probes are useful in biophysics research to assess the spatial distribution, mobility and interactions of biomolecules. However, fluorophores can undergo “self-quenching” of their fluorescence intensity at high concentrations. A greater understanding of concentration-quenching effects is important for avoiding artefacts in fluorescence images and is relevant to energy transfer processes in photosynthesis. Here, we show that an electrophoresis technique can be used to control the migration of charged fluorophores within supported lipid bilayers (SLBs) and that quenching effects can be quantified with Fluorescence Lifetime Imaging Microscopy (FLIM). Confined SLBs containing controlled quantities of Texas Red (TR) fluorophores were generated within 100 × 100 μm corral regions on glass substrates. Application of an electric field in-plane with the lipid bilayer induced the migration of negatively-charged TR molecules towards the positive electrode and created a lateral concentration gradient across each corral. The self-quenching of TR was directly observed in FLIM images as a correlation of high concentrations of fluorophores to reductions in their fluorescence lifetime. By varying the initial concentration of TR fluorophores incorporated into the SLBs from 0.3% to 0.8% (mol/mol), the maximum concentration of fluorophores reached during electrophoresis could be modulated from 2% up to 7% (mol/mol), leading to the reduction of fluorescence lifetime down to 30% and quenching of the fluorescence intensity down to 10% of their original levels. Overall, these findings prove that electrophoresis is effective at producing microscale concentration gradients of a molecule-of-interest and that FLIM is an excellent approach to interrogate dynamic changes to molecular interactions.

P2.19

Quantitative Microbiology with Microscopy: Effects of Projection and Diffraction

Mr Georgeos Hardo[1]

[1]*University Of Cambridge, Cambridge, United Kingdom*

Live cell imaging of microbial cells with microscopy has revolutionised quantitative microbiology. Micrographs are one of the most information-rich data captured about a microbe, allowing quantification of the size and morphology of individual cells and their gene-expression over time. However, an optical microscope is a diffraction-limited system, and the comparable size of a microbial cell with the system's point spread function can lead to imaging artefacts which corrupt and bias the data. Additionally, the comparable depth of a microbe to the microscope's depth of field means that the 2D image contains compressed, projected 3D information. This makes it difficult to extract the underlying 3D distribution of photon emitters. For unknown distributions, the problem can be as ill-posed as a deconvolution problem, usually not having a unique solution. Together the diffraction and projection effects affect our ability to accurately quantify the size and shape of microbial cells from their images and their contents from intensity measurements. We use simulation of image formation of microbial cells to illustrate the effects of diffraction and projection on cell segmentation and signal quantification. We also use the knowledge of these effects to design experiments which can help to reduce the artefacts and biases in our analysis of cell dimensions and intensity quantification. Awareness of these effects and the approaches towards alleviating them will help to accurately quantify microbiology from microscopy data.

P2.20

The importance of water in membrane receptor function

Professsor Anthony Watts[1]
[1]*Oxford University, Oxford, United Kingdom*

Resolving conformational changes in membrane receptors in response to a stimulus, and capturing their functionally relevant dynamics, is very challenging. Over the years we have addressed this challenge using spectroscopy [1,2] on functionally competent photoreceptors, in membranes [4] or Lipodisqs™ [5]. We have complemented this work with functional studies and mass spec [6], very high resolution (1.07Å) crystallography [7,8] and photo-induced XFELs, detergent-free and including natural lipids. High resolution structures reveal individual waters, and their importance in receptor activation-desensitization. QM(SCC-DFTB)/MM MD trajectories give insights into the activation process. The system studied is achearhodopsin-3 (AR3), a photoreceptor utilized in optogenetics. We suggest that the different arrangement of internal water networks in AR3 is responsible for the faster photocycle kinetics compared to homologs – AR3 is ~10x more efficient than bacteriorhodopsin at current generation. These insights may well have generic implications for other receptors.

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P2.21

Experimental investigation of non-classical excited-states energy transfer dynamics in green fluorescent protein tandem assemblies using time-resolved fluorescence anisotropy

Mr. Alejandro Sanchez-pedreno Jimenez[1], Dr. Youngchan Kim[1]
[1]*University Of Surrey, Guildford, United Kingdom*

It is implicitly assumed that any quantum mechanical effects (e.g., quantum superposition, tunneling, entanglement) created in a biological system are instantaneously dissipated due to random molecular interactions in the warm, wet, and disordered biological environment. However, owing to advances in sensing and molecular biology technologies, mounting evidence indicates that biological molecules and biomolecular systems can give rise to the quantum effects in such complex biological environments. Notably, a recent study suggests that the dimeric fluorescent proteins exhibit room-temperature exciton coupling when they dimerise (Kim et al., Biophysical Journal, 2019). Here, we use enhanced green fluorescent protein (eGFP) tandem dimers and oligomers to investigate the excited-states energy transfer dynamics in eGFP oligomers. We employ time-resolved fluorescence anisotropy that is used to measure the dynamics of energy transfer between fluorescent molecules. Our results indicate the presence of anomalous ultrafast dynamics in the energy transfer between multiple fluorescent proteins upon oligomerization.

P2.22

A neutron diffraction study finds that Trimethylamine-N-oxide drives urea out of a β-turn's solvation shell

Mr Mazin Nasralla[1], Dr Harrison Laurent[1], Dr Oliver Alderman[2], Dr Tom Headen[2], Professor Lorna Dougan[1]
[1]University Of Leeds, Leeds, United Kingdom, [2]ISIS neutron and muon source, Didcot, United Kingdom

TMAO (Trimethylamine-N-oxide) and urea have opposing effects on the structural stability of proteins. Urea is a denaturant, and TMAO is a protective osmolyte i.e., a small biological molecule expressed by marine life to protect proteins against denaturation by urea and the effects of high pressure. Urea is believed to exhibit a preferential interaction towards the protein's surface, vis a vis water, and once there it binds to the peptide's amide groups, inhibiting the formation of stabilising intra-peptide bonds. The mechanism by which TMAO counteracts urea is uncertain although molecular dynamics (MD) simulations suggest it breaks urea's affinity for the peptide surface. Previously, using NMR spectroscopy to study aqueous urea and TMAO, we found evidence for a urea-TMAO complex and in the presence of TMAO strengthened hydrogen bonding involving water. Motivated by previous studies of the β-turn GPG.NH_2 in aqueous urea, we present here a neutron diffraction study of GPG.NH_2 in TMAO, urea and water in the molecular ratio 1:3:6:58. We find that TMAO's interacts with the β-turn solely through its nitrogen groups. We also find that TMAO generates a differential effect on hydrogen bonding in the bulk solution where it hydrogen bonds to urea, tightens the urea--water network and weakens water-water hydrogen bonding. We suggest that by enhancing hydrogen bonding involving urea, and curtailing the extent of the water network, TMAO simultaneously drives urea out of, and water into the β-turn's solvation shell.

P2.23

Viral RNA Conformation Analysis via Nanotechnology at Single Molecule Resolution

Dr Chalmers Chau[1], Dr Andrew Tuplin[1], Dr Paolo Actis[1]
[1]*University Of Leeds, Leeds, United Kingdom*

Solid-state nanopore is a raising platform for single molecule analysis, molecule passing through the nanopore generates unique electrical translocation signal that is associated with the translocating molecule, this nanotechnology has been used for molecule shape analysis including proteins, DNA, and DNA nanostructures.

The conformation of RNA has a deep connection with its functionality and this depends heavily on the thermodynamic landscape, which is largely affected by its sequence and the electrolyte environment. RNA conformation is typically probed by biochemical and biophysical technique such as SHAPE analysis and cryo-EM. Here we present data on using solid-state nanopore to probe changes in the conformation of the chikungunya virus RNA genome by subjecting the in vitro synthesised RNA to thermodynamic folding process, this process caused the RNA to switch from its co-transcriptionally folded state to its native state. We envision further development in this platform can be used to probe RNA unfolding kinetics, different conformation analysis and aid the design of RNA nanostructures in the future. Furthermore, the conformation and structure of other biomolecules could also be studied with this nanotechnology.

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[3] Marcuccio, F. et al. Mechanistic Study of the Conductance and Enhanced Single-Molecule Detection in a Polymer–Electrolyte Nanopore. ACS Nanoscience Au (2023) doi:10.1021/acsnanoscienceau.2c00050.

P2.24

Uncovering conserved mechanisms in the assembly and activity of eukaryotic and archaeal minichromosome maintenance proteins

Oliver Noble[1], Clement Degut[1], Michael Hodgkinson[1], James Chong[1], Michael Plevin[1,2,3]
[1]Department of Biology, University of York, York, United Kingdom, [2]York Structural Biology Laboratory, University of York, York, , [3]York Biomedical Research Institute, University of York, York, United Kingdom

Replicative helicases are central components of the cell cycle, catalyzing separation of parental DNA ahead of DNA polymerases. In eukaryotes, the role of the replicative helicase is fulfilled by six closely related minichromosome maintenance proteins (MCM2-7), which assemble into a heterohexameric complex. By contrast, most archaea express a single MCM which functions as a homohexamer. Recent cryoEM studies have mapped out key steps in the assembly pathway of MCM2-7. However, while archaeal MCMs are commonly used as a model for the eukaryotic MCM2-7 complex, we know little about how they assemble into a functional homohexamer. We hypothesized that the longstanding practice of studying MCMs from thermophilic archaea may explain our lack of information about their assembly.

We identified an MCM from a mesophilic archaeon (MesoMCM) with strong activity under ambient conditions. A thorough analysis of its biochemistry and structural biology revealed closer similarity to eukaryotic MCM2-7 than to any previously studied archaeal MCM. Biochemical and biophysical assays showed eukaryotic-like assembly and oligomerisation properties. A 2.6 Å 3D structure of apo-MesoMCM revealed oligomerization interfaces with similar composition to MCM2-7. Lastly, a cryoEM structure of DNA-bound MesoMCM showed a cracked ring similar to that seen between subunits 2 and 5 in eukaryotic MCM2-7; the first time such a feature has been observed in an archaeal MCM. These data suggest important and previously unseen mechanistic similarities between eukaryotic and archaeal MCMs, presumably conserved from an ancient homoheteromeric archaeal ancestor, and provide a basis for better understanding the evolution of the heteromeric MCM2-7.

P2.25

Single-molecule imaging of Botulinum Neurotoxin translocation

Mrs Joanne Carniello[1], Dr. Jason Sengel[1], Miss Daisy Roger-Simmonds[2], Prof Mark Wallace[1]
[1]King's College London, London, United Kingdom, [2]Imperial College London, London, United Kingdom

Botulinum neurotoxin (BoNT) is infamous as the most potent toxin currently known. BoNT arrests synaptic exocytosis causing severe muscle paralysis and death. It's neurospecificity also means BoNT has found widespread use as a therapeutic agent; treating muscle spasms, chronic migraines, and excessive sweating.

Despite BoNT's importance, the molecular mechanism underlying synaptic vesicle escape remains poorly understood. Here we combine single-molecule tracking and optical single channel recording of BoNT/A to interrogate the molecular steps of BoNT translocation across the membrane. Contrary to the most accepted model of BoNT translocation, our experiments support a model where channel formation is not a prerequisite for synaptic vesicle escape.

P2.26

Probing the Redox Chemistry and Structure Function Relationship of LPMO's via Electrochemistry

Mr Connor Miles[1], Miss Ella Reid[1], Mr Alexander Ascham[1], Dr Nicholas Yates[1], Dr Glyn Hemsworth[2], Dr Alison Parkin[1]

[1]University Of York, York, United Kingdom, [2]University of Leeds, Leeds, United kingdom

Lytic polysaccharide monooxygenases (LPMO) are copper containing metalloenzymes that play a key role in the economical conversion of lignocellulosic waste into biofuels. The critical industrial function of these enzymes is that they can reduce the crystallinity of recalcitrant structural carbohydrates (e.g. cellulose, chitin) via oxidative cleavage of surface exposed endo-glycosidic bonds. The widespread application of LPMOs within industrial enzyme mixes, however, belies the underlying poor characterisation of LPMOs and their mechanism. While spectral and structural studies have clearly identified the novel architecture of the so-called “histidine-brace” active site, there is substantive debate around the nature of the co-substrate for the carbohydrate oxidation. Different evidence has suggested both molecular oxygen and hydrogen peroxide could be the essential oxidant, and the role of residues in the protein outside of the copper coordinating histidine brace has barely been explored. To explore these questions, detailed kinetic and thermodynamic data is required. Electrochemistry provides a solution; by utilising multi walled carbon nanotubes to immobilise LPMO’s we can “see” electrons exiting and entering the copper active site of enzymes from a variety of sub-classes and relate this reactivity to a catalytic H2O2-induced current. This can now be coupled to molecular biology studies to deconvolute the role of structure in controlling LPMO function.

P2.27

A new twist on drug design: AdhE spirosomes as cross species anti-virulence targets (withdrawn)

Ester Serrano[1], Andrew Roe[1], Olwyn Byron[1]
[1]*School of Infection and Immunity, University Of Glasgow, Glasgow, United Kingdom*

Enterohemorrhagic Escherichia coli (EHEC) is a major human pathogen that causes bloody diarrhoea, haemorrhagic colitis, and life-threatening haemolytic uremic syndrome. In our quest to develop new compounds to block a range of infections, we have studied the EHEC virulence factor AdhE. Deletion of adhE results in a huge attenuation of virulence and a reduction in expression of the major system used by EHEC to attach to host cells. AdhE is a bidirectional enzyme that catalyses the conversion of acetyl-CoA to ethanol. Unusually, AdhE oligomerises in vivo and in vitro to form long (15-120 nm) filaments heterogenous in length called spirosomes.

Here, we report progress on understanding the solution behaviour of AdhE in partially fractionated, but still functional, form. Analytical ultracentrifugation (AUC) sedimentation velocity and small angle X-ray scattering (SAXS) analysis of fractionated AdhE demonstrates that it is not possible, with conventional size exclusion chromatography, to generate homogenous AdhE samples. Instead, we were able to determine the constituents of fractions and building on this, we observed that the length of the spirosomes has no effect on the enzymatic activity of the protein in the forward reaction. However, in the reverse reaction there is a huge reduction in activity when the spirosome length decreases. Taken together these results suggest that the spirosome formation drives the direction of the enzymatic activity of the protein. These results are key because understanding how AdhE spirosomes work will help us to develop, in the longer-term, specific inhibitors that might function against a range of Gram-negative pathogens.

P2.28

Ultrasensitive fluorescence detection of conformational changes in single lipid vesicles

Lara Dresser[1], Sarah Graham[1], Dr Donato Conteduca[1], Dr Jack Shepherd[1], Dr Lisa Miller[1], Dr Charley Schaefer[1], Prof Steven Johnson[1], Prof Mark Leake[1], Dr Steven Quinn[1]
[1]*University Of York, Heslington, York, United Kingdom*

Encapsulation of physiochemical indicators and incorporation of FRET probes within lipid vesicles – valuable mesoscale molecular confinement vessels - has enabled valuable insights into membrane interactions, including pore formation, solution exchange, changes to membrane morphology and fusion dynamics. While vesicle architecture can often be modified by changing the lipid composition, the influence of extramembrane agents on vesicle morphology has remained an under-explored area owing to a lack of experimental tools capable of capturing morphological changes on the nanoscale. To characterize and quantify vesicle remodelling events induced by disruptive agents, including detergents, protein aggregates, and molecular crowders, we have implemented high-throughput and easily-adaptable fluorescence assays based on measuring the extent of ion flux into, and lipid mixing within, single lipid vesicles. Our approaches involve the surface-immobilisation of highly-curved, sub-micron sized vesicles containing lipophilic membrane stains and encapsulated calcium indicators, and quantifying changes in fluorescence and FRET as a result of membrane perturbation via single-vesicle imaging. Here, we show that widely-used non-ionic detergents can controllably induce pore formation and vesicle fusion, protein aggregates composed of the A-beta peptide trigger vesicle swelling, and molecular crowding agents, including polyethylene glycol and Ficoll lead to reversible vesicle compaction. The ability to precisely control vesicle morphology has vast implications for drug delivery and trafficking systems and our observations may serve to act as mechanosensitive readouts of the local extramembrane environment. We expect the presented tools to be applicable beyond the sub-micron sizes studied here, and be of value for unveiling otherwise hidden membrane interactions with ultrasensitive precision.

P2.29

Interaction between the chlamydia effector protein TarP and the SH2 domain of Vav2

Dr Tharin M. A. Blumenschein[1], Karen Chau[1], James Tolchard[2], Caragh Scull[1], Jairah R. Lubay[1]
[1]University Of East Anglia, Norwich, United Kingdom, [2]Université Claude Bernard Lyon 1, Lyon, France

TarP (Translocated actin-recruiting phosphoprotein) is an intrinsically disordered protein conserved across chlamydia species. It is injected into the host cell in the initial stages of infection, where it remodels the actin cytoskeleton. Throughout its sequence, TarP contains different motifs for interactions with a number of host proteins. Despite being conserved, there is variation in the number of times each motif appears, with some motifs entirely absent from some isoforms. We have previously characterized the interaction between the WH2-like motif and G-actin, and here we present initial studies on the Tyr-rich region.

The Tyr-rich region is formed by a variable number of Tyr-rich repeats, each 50 amino acid residues long, 4 of which tyrosines. TarP from the Chlamydia trachomatis serovar L2 contains 6 Tyr-rich repeats, which are phosphorylated by host kinases once transferred into the host cell by Type III secretion. We show that individual Tyr-rich repeats are intrinsically disordered, with uniform motions along their sequence. Src kinase phosphorylates the Tyr-rich repeat in vitro, with a clear order of preference for the different tyrosines in the repeat. The phosphorylated Tyr-rich repeat binds the SH2 domain of the signalling protein Vav2, undergoing clear changes in its [^{1}H, ^{15}N]-HSQC NMR spectrum.

P2.30

A novel RNA thermosensor element regulating teichoic acid biosynthesis in obligate human pathogen Streptococcus pneumoniae (SPN)

Mr Kuldeep Sharma, Prof Anirban Banerjee
[1]*Indian Institute Of Technology Bombay (IIT Bombay), India, MUMBAI, INDIA*

Opportunistic bacteria i.e., Streptococcus pneumoniae (SPN) share a complex life phase with its obligate human host. Despite being the normal commensal in the nasopharyngeal region, it is a major etiological agent of pneumonia, bacterial meningitis, sepsis, and otitis media globally. Additionally, viral-bacterial synergism during co-infection has been reported to surge the mortality rate significantly. The manifestation of febrile condition alongside the cytokine storm remains the primary characteristic feature of inflammation induced by preceding viral infection which can be further sensed by 'RNA thermosensor' like elements present in virulence-associated genes in bacteria. We uncovered presence of such an RNA thermosensor in the 5'-UTR segment of capD mRNA encoding the enzyme that catalyzes the formation of AAT-Gal, an essential sugar moiety of teichoic acid (TA) polymer in SPN. A 34 base pair long mRNA segment tends to form the secondary structure as stem-loop (SL) spontaneously, occluding the ribosome binding site at lower temperature which is prevalent in the nasopharyngeal area or outside of the host. However, the Stem-loop is melted upon sensing the elevated body temperature i.e., 38.5˚C hence liberating the RBS site from the occlusion in the stem-loop. As a result, efficient translation of capD gene is achieved which further ramps up the TA biosynthesis. The SPN strains grown at febrile temperature i.e., 38.5˚C were able to synthesize an enhanced amount of teichoic acid compared to those grown at nasopharyngeal temperature i.e., 33˚C. The increase in 'chemical shafts' i.e., teichoic acid polymer provides additional attachment sites available for phosphorylcholine (Pcho) moiety and choline-binding proteins (CBPs) subsequently. Such regulation at the mRNA level allows the SPN to attain improved attachment capability and thwart the host immune system in order to establish successful infection in the deeper sterile tissues.

P2.31

Transient structural dynamics during allosteric regulation of glycogen phosphorylase

Ms Monika Kish[1], Dr Jonathan Phillips[1]
[1]*University Of Exeter, , United Kingdom*

Whilst it is becoming routinely possible to determine accurate structural models of proteins with high resolution, it is still challenging to ascertain the specific structurally dynamic changes that underpin protein functional switching. The archetypal allosteric enzyme, glycogen phosphorylase (GlyP) is one of the most studied and has a substantial therapeutic potential in treating metabolic diseases and cancers. However, a lack of understanding of its complex regulation, mediated by dynamic structural changes, hinder its exploitation as a drug target. Here, we precisely locate dynamic structural changes upon allosteric activation of GlyP, by developing a time-resolved non-equilibrium millisecond hydrogen/deuterium-exchange mass spectrometry (HDX-MS) approach. We resolved obligate transient changes in localized structure that are absent when directly comparing active/inactive states of the enzyme, thus rationalizing the mechanism of action of an allosteric activator. This approach has broad application to determine the structural kinetic mechanisms by which proteins are regulated.

P2.32

Tackling topology with TopoStats

Max Gamill
[1]*University Of Sheffield, Sheffield, United Kingdom*

The heterogeneity of many biomolecular structures, such as DNA, is driven by its inherent flexibility. This allows self-interactions and with cellular proteins, intertwining its strands into complex topologies. However, this flexibility makes visualising DNA structure challenging. Atomic force microscopy (AFM) can image single molecules in liquid with sub-molecular resolution, without the need for labelling. Enabling visualisation of biomolecular structures in native-like states. However, a large conformational landscape, and lack of generalised automated analysis tools for AFM data make high-throughput structural characterisation difficult, and much is done by hand.

Here I present a new pipeline for our open-source AFM image analysis software TopoStats (https://github.com/AFM-SPM/TopoStats.git) which enables single molecule analyses of DNA topology. By focusing on DNA crossings, we can determine crossing direction at each point and therefore explicitly determine topology. This is achieved by adapting skeletonisation algorithms to take advantage of AFM height data. By biasing the segmentation towards higher regions, we obtain more accurate molecular traces while retaining the simplicity of single pixels. Local area-based convolutions identify crossing points and their emerging branches, used to enable quantitative per-node statistics such as branch numbers, crossing angles, and classify which branch lies atop another. This work provides an open source, high-throughput, automated methodology to identify and calculate the statistics of complex, intertwined molecules. By including this tool in our wider AFM analysis toolbox, TopoStats, we hope this software will help the wider community in classifying the topology of complex biomolecular species such as DNA catenanes and knots.

P2.33

A toolkit of customised protein sensors for interrogating mechanical forces in the cell

Dr Maria Zacharopoulou[1], Dr Marie Synakewicz, Dr Janet R Kumita, Dr Mohsin Mubarak Naqvi, Prof Laura S Itzhaki

[1]*University of Cambridge, Cambridge, United Kingdom*

Mechanical forces play a role in a wide variety of cellular processes, such as activation of ion channels upon mechanical stimulation, force-bearing proteins in the muscle and extracellular matrix, and spindle tension in chromosome segregation. Specific proteins must, therefore, be capable of sensing mechanical signals and giving biological outputs. The understanding of mechanical stimuli at the molecular level has been limited to date due to the lack of tools that can accurately measure low forces in vivo. Moreover, these force sensors must be customised for each specific use, to cover the appropriate force regime. Here we report a toolkit for measuring different ranges of forces in the cell, made possible by the striking spring-like properties of the tandem-repeat protein class and their exceptional amenability to rational design. We design a panel of Fluorescence Resonance Energy Transfer (FRET)-based tension sensors using consensus tetratricopeptide (CTPR) proteins as the mechanosensitive linkers. These are flanked by the fluorescent protein mCherry and contain an engineered tetracysteine motif in the protein sequence, which then binds tightly to the fluorescein arsenical hairpin binder dye (FlAsH), resulting in a FRET pair. Optical tweezers experiments on the CTPR linkers show that the proteins respond to physiological forces in a highly distinctive manner. We dissect the mechanics of the proteins by mutationally creating and then characterising a series of variants with systematically modified properties, to build create a toolbox of customisable, calibrated force sensors. Our toolkit is capable of probing the varied mechanotransduction processes within the cell beyond what is currently possible.

P2.34

A novel sliding interaction between the extracellular matrix polysaccharide hyaluronan and its lymphatic vessel endothelial receptor LYVE-1 that regulates immune cell trafficking

Dr Fouzia Bano[1], Dr Suneale Banerji[2], Dr Tao Ni[2], Dixy Green[3], Prof Paul DeAngelis[3], Dr Martin Lepsik[5], Dr Emanuele Paci[4], Prof Robert Gilbert[2], Dr Ralf Richter[1], Prof David Jackson[2]

[1]University of Leeds, Leeds, United Kingdom, [2]University of Oxford, Oxford, United Kingdom, [3]University of Oklahoma Health Sciences Center, Oklahoma, United States of America, [4]University of Bologna, Bologna, Italy, [5]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic, [6]CERMAV, Université Grenoble Alpes and CNRS, Grenoble, France

The exit of leucocytes from tissues through lymphatic vessels is essential for both immunity and inflammation. Critical to the process is engagement of the linear polysaccharide hyaluronan (HA), which is part of glycan coat enveloping the leucocyte, by the lymphatic endothelial receptor LYVE-1, which enables docking and subsequent transit in the low shear environment at the inner surface of lymphatic capillaries. However, the molecular details of how the interactions of LYVE-1 with HA support these events are currently unknown. Using a combination of dynamic force spectroscopy and structural analyses, we demonstrate the receptor engages in a novel mode of sugar binding that leads to threading of the long HA polysaccharide chains through adjacent LYVE-1 molecules via an unusual type of sliding interaction distinct from the conventional sticking interaction typified by the related leucocyte HA receptor CD44. These findings identify a specialised mode of carbohydrate binding that is exclusively adapted to the adhesion and transmigration of leucocytes in the low-shear environment of the lymphatic vasculature.

P2.35

Structural dynamics of membrane-associated proteins at microsecond timescales and sub-nanometre resolution with High-Speed AFM

Tabitha Storer[1,3], George Heath[1,3], Neil Thomson[1,2,3]

[1]*Molecular and Nanoscale Physics Group, School of Physics and Astronomy, University of Leeds, LS2 9JT. , , ,*

[2]*Division of Oral Biology, School of Dentistry, St. James Hospital Campus, University of Leeds, LS9 7TF. , , ,*

[3]*Bragg Centre for Materials Research, University of Leeds, LS2 9JT. , ,*

Understanding the structural dynamics of biomolecules is essential to develop a complete picture of molecular function and thereby advance our knowledge of diseased states and possible intervention mechanisms. However, few techniques follow the motions of individual biomolecules at high enough speeds or resolutions to capture the underlying behaviour. High-speed atomic force microscopy (HS-AFM) accesses the topography and dynamics of molecules under physiological conditions at a rate of 50 frames per second, with sub-nanometre resolution. However, biomolecular processes typically occur faster than this, including diffusive and intra-molecular motions of membrane-associated proteins and ion channels. The development of HS-AFM height spectroscopy (HS-AFM-HS), whereby the cantilever tip is held at a fixed position and directly detects the motions of unlabelled molecules beneath, achieved microsecond temporal resolution (Heath & Scheuring, Nat Comms 19, 4983, 2018). HS-AFM-HS can be applied to study both the diffusion of many biomolecules and the internal structural dynamics of single biomolecules at previously inaccessible time scales. Furthermore, to overcome the spatial resolution limit, localisation AFM (LAFM), a post-acquisition image reconstruction method, was recently developed achieving Angstrom spatial resolution (Heath et al, Nature 594, 385390, 2021). LAFM takes advantage of fluctuations in topographic features in the HS-AFM images, allowing super-resolution principles to be applied to the data, localising structural fluctuations with sub-pixel precision. Here we present new experimental data on annexin proteins along with optimisation of these HS-AFM methods to further push the spatial and temporal resolution and gain deeper fundamental understanding of protein structural dynamics.

P2.36

Deciphering the structure of integration host factor with supercoiled DNA minivectors

Ms Neha Ramteke[1], Professor Mark Leake[2], Dr. Agnes Noy[3]

[1]School of physics engineering and technology university of york, Heslington York, United Kingdom, [2]School of physics, engineering and technology and department of biology university of york, Heslington York, united kingdom, [3]School of physics engineering and technology University of york , Heslington York , United Kingdom

In this study, we propose the study of supercoiled DNA in conjugation with a DNA bending using the protein integration host factor (IHF) as an example. The research is considered to be done in various parts which includes the computational and experimental aspects. Molecular dynamics simulation plays a key role in the predetermined studies and proposing experimental setup of the protein and IHF. It is then accompanied by biochemical techniques like electrophoresis and atomic force microscopy to study the dynamics of the DNA along with the complex structure supported by the bending . Analytical methods like smFRET would also be a part of this study. The analysis of data is proposed to be done with the various tools that will be accompanying the results. This study will decrypt the relation between supercoiled DNA as minivectors and its importance in many areas such as gene therapy and medicine research.

P2.37

Single-molecule and super-resolved imaging deciphers membrane behavior of onco-immunogenic CCR5

Mr Patrick Hunter[1], Dr Alex Payne-Dwyer[1], Dr Michael Shaw[2], Dr Nathalie Signoret[1], Prof Mark Leake[1]
[1]University Of York, York, United Kingdom, [2]National Physical Laboratory, Teddington, United Kingdom

The ability of tumors to establish a pro-tumorigenic microenvironment is an important point of investigation in the search for new therapeutics. Tumors form microenvironments in part by the ‘‘education’’ of immune cells attracted via chemotactic axes such as that of CCR5-CCL5. Further, CCR5 upregulation by cancer cells, coupled with its association with pro-tumorigenic features such as drug resistance and metastasis, has suggested CCR5 as a therapeutic target. However, with several conformational ‘‘pools’’ being reported, phenotypic investigations must be capable of unveiling conformational heterogeneity. Addressing this challenge, we performed super-resolution structured illumination microscopy (SIM) alongside single-molecule partially TIRF-coupled HILO (PaTCH) microscopy of CCR5 in fixed cells. A newly developed technique, PaTCH microscopy utilises a novel intermediary angle of excitation beam delivery which benefits from the high signal-to-noise ratio of total internal reflection fluorescence (TIRF) microscopy and the increased penetration depth of highly inclined and laminated optical sheet (HILO) microscopy. SIM data revealed a non-random spatial distribution of CCR5 assemblies, while intensity-tracking of CCR5 assemblies using PaTCH indicated dimeric sub-units independent of CCL5 perturbation. Extended studies investigate the effect of CCR5 antagonists such as Maraviroc, a HIV therapeutic considered for clinical trials in cancer patients, on the behaviour of CCR5 on the cell surface. These biophysical methods can provide important insights into the structure and function of onco-immunogenic receptors and many other biomolecules, with the capability of these techniques being showcased in a recently published research article ‘https://www.sciencedirect.com/science/article/pii/S2589004222019472’.

P2.38

Passive microfluidics for the characterisation of neuronal signals in live nematodes

Dr Nino Läubli[1], Prof. Gabriele Kaminski Schierle[1]
[1]*University Of Cambridge, Cambridge, United Kingdom*

Neurodegenerative diseases, such as Alzheimer's (AD) or Parkinson's, are a global health issue and despite intensive efforts by the research community, only little is known about the root causes of these neurodegenerative diseases – a factor further highlighted by more than 200 failed clinical trials for AD. As an alternative to in-human studies, small model organisms such as C. elegans nematodes have proven valuable for the investigation of various diseases and the development of suitable treatment methods. However, due to their high motility, so far, optical characterisations have relied on the application of toxic paralysations which often lead to the specimen's death, thus, preventing the study of disease progression in individual samples, while also significantly affecting the relevance of the obtained results, especially with regard to neuroscience. Here, we explore the use of an accessible microfluidic design suitable for passive immobilisations of C. elegans nematodes. By minimising their mobility, the device permits fluorescence-based evaluations of neuronal activity affected by varying experimental and environmental conditions, such as different cholesterol concentrations. Furthermore, observed fluctuations in GCaMP-based neuronal signals are compared to data obtained from non-paralysed GFP-expressing mutants to determine if the signal variations are caused by residual internal movements or result from live action potentials and Ca2+ fluxes. Finally, potential negative effects induced by the microfluidic-based trapping are evaluated using a sensitive computational behaviour assay.

P2.39

Biofilm Water Channel Network Model for
Bacterial Communication

Yanahan Paramalingam[1], Dr Hamidreza Arjmandi[1], Dr Adam Noel[1]
[1]*University Of Warwick, Coventry, United Kingdom*

In nature, bacteria are often found in enclosed colonies called biofilms. Biofilm includes extracellular polymeric substance (EPS), which acts as a protective layer. Biofilms are an issue in healthcare since they contribute to antimicrobial resistance. Bacteria are able to chemically communicate cell-to-cell via extracellular signalling molecules called autoinducers. This process is called quorum sensing (QS) and relies on the production of and response to the autoinducers. Soluble signalling molecules are transported throughout the biofilm through both water channels and the EPS. One way proposed to disrupt biofilm is to interrupt the quorum sensing process.

Within a biofilm, communication takes place primarily via diffusion using water channel networks and the EPS. Thus, a biofilm can be considered as a porous material, resulting in anisotropic diffusion. A good analogy representing the network of water channels within a mature biofilm is the London Tube map, where the tube routes are the water channels, the passengers are the autoinducers, and the destination is the receiver. In the centre, there are multiple routes branching out into all directions. However, as you travel away from the centre, there are fewer options available. A greater understanding of molecule propagation, including that of autoinducers, could lead to strategies to improve the inhibition effectiveness of antimicrobial agents. Such improvements could increase our capability to disrupt and disperse the bacteria in biofilms. This presentation will introduce molecular communication theory and propose a mathematical model for signal propagation in a biofilm.

P2.40

Modeling the Growth of Kidney Organoids subject to optogenetically-induced BMP4 Morphogens

Jonas Pleyer[1]

[1]*Freiburg Center for Data Analysis and Modeling, Freiburg Im Breisgau, Germany*

BMP4 plays an important role in early embryogenic morphogenesis. In particular, it was shown to promote differentiation of epithelial tubules and unbranched, ureter-like 'trunks' in kidney organoids. Recent advancements now allow researchers to engineer mammalian cells such that expression of BMP4 can be light-modulated.

We propose an experiment comprised of two cell-types acting as sender and receiver. An external controller regulates light exposure at the sender cell and thus indirectly BMP4 concentration. This device resembles a Digital Micromirror Device (DMD) and is also able to spatially vary light intensity and track cells.

We present a fully integrated multi-scale agent-based model which closely resembles the experimental setup. Advancements and challenges of this particular computational model and its experimental implementation will be discussed.

P2.41

Decoherence and Energy Transfer Dynamics of Green Fluorescent Proteins

Anna Cusick[1], Marian Florescu, Adam Burgess
[1]*Surrey University, , United Kingdom*

The study of decoherence in biological systems has become an active area of research for quantum mechanics. Such systems involve highly complex quantum dynamics which are often difficult to decompose into exact interactions. Some of these biological systems have been thought to have structured themselves to preserve coherences in the system. One example is Fluorescent Proteins (FPs) maintaining coherent interactions under physiological conditions. Expanding our understanding of such systems would help us elucidate some of the mysteries between the transition from quantum to classical dynamics. As well as further understanding quantum decoherence at the nanoscale.

In this work, we use an open quantum systems approach to explore the dynamics and decoherence timescales of energetic processes in the excited quantum states of a Green Fluorescent Protein (GFP). We investigate the dynamical decoherence of a GFP in a finite-temperature solvent environment by using a modified version of the McKenzie-Gilmore model. We were able to study the non-Markovian and non-perturbative nature of the system's dynamics by utilising the Hierarchical Equations of Motion (HEOM), a numerically exact approach to open quantum systems. We first examine GFPs in their monomeric state and then extend this model to elucidate why the GFP homo-dimers experience extremely prolonged decoherence timescales for coherent energy transfer. This model utilises the associated dielectrics of the GFP structure and the surrounding solvent, which has been overlooked in previous models, to describe the system-environment interactions. We derive spectral densities using the Fluctuation-Dissipation theorem and a modified Debye dielectric model for the solvent environment and found several novel dynamical features. We discover that the timelines of decoherence are significantly extended compared to weak coupling Markovian theories.

P2.42

DNA origami with fluorescent proteins

Callum McKeaveney[1], Wooli Bae, Catxere Andrade Casacio, Alejandro Sanchez-Pedreno Jimenez
[1]*University Of Surrey, Guildford, United Kingdom*

Förster resonance energy transfer (FRET) is an energy transfer process between fluorophores mediated by Coulombic dipole-dipole coupling. The efficiency of FRET is mainly determined by three primary conditions: close proximity between the donor and acceptor molecules (i.e., 10 nm or less), the spectral overlap between the acceptor's absorption and the donor's fluorescence emission, and the permissible transition dipole moment orientations of the donor and acceptor. Because of its strong dependence on the separation distance between the donor and accepter, FRET has become the powerful research to study in the life sciences where to determine the distances, structures, and dynamics of biomolecular systems. Reliable interpretation of experimental results obtained from FRET measurements is predicated on the FRET theory in addition to experimental validation using a FRET standard. Here, to provide a better standard for the proximal distance for homo-FRET interactions (i.e., FRET between spectrally identical fluorophores), we propose and demonstrate conjugating enhanced green fluorescent proteins (eGFP) with DNA origami nanostructures using a C-terminal cysteine residue to allow site-selective coupling by a heterobispecific cross-linker. Due to their high degree of customisation, DNA origami nanostructures allow for the creation of complex and precise nanoscale structures. These structures have a high degree of versatility which has led to various applications of this nanotechnology, including light harvesting constructs and nucleic acid analysis. To compare the FRET theory with measurements of the FRET standard, time-resolved fluorescence anisotropy is used to evaluate the energy transfer kinetics between mEGFPs when using different sequences and shapes of DNA origami.

P2.43

How to tune the tempo of embryonic development across species: a mathematical toolkit

Charlotte Manser[1], Dr Ruben Perez-Carrasco[1]
[1]*Imperial College London, , United Kingdom*

Development is dynamic, with the timings and sequences of gene expression vital for proper growth. Many of the genetic programs that have evolved to coordinate these sequences are common amongst species; however, the timescale of these programs varies greatly between species [1]. Differences in developmental tempo has been observed in both somitogenesis [2] and motor neuron differentiation [3].

My work focuses on how we can explore possible timing mechanisms through a mathematical approach. I have developed and utilized a framework called 'orbital equivalence', which is a property of two dynamical systems. If the two systems have the same orbit, ignoring how quickly the orbits are traversed, then they are orbitally equivalent. This gives us a way to measure how similar two dynamical systems are in terms of their biological function, independently of their timescale.

I have used this notion successfully with the repressilator GRN. I have developed a toolset which can identify equivalent systems, and tune them to control tempo, without changing their function. This method can be applied to many other GRNs, and thus paves the way for a deeper understanding of the control of developmental tempo.

[1] Iwata and Ryohei, Tempus fugit: How time flies during development, Science, 369(6510), 2020.
[2] Matsuda et al., Species-specific segmentation clock periods are due to differential biochemical reaction speeds, Science, 369(6510), 2020.
[3] Rayon et al., Species-specific pace of development is associated with differences in protein stability, Science, 369(6510), 2020.

P2.44

Using Shape Fluctuations to Probe the Mechanics of Stress Granules

Dr Jack Law[1], Dr Carl Jones[1], Mr Tom Stevenson[1], Mr Thomas Williamson[2], Prof Matthew Turner[3], Prof Halim Kusumaatmaja[2], Prof Sushma Grellscheid[1]

[1]University Of Bergen, Bergen, Norway, [2]Durham University, Durham, UK, [3]Univeristy of Warwick, Coventry, UK

Many of the functions of biological condensates are mechanical in nature, such as wetting and deforming lipid membranes and biological filaments. Condensates also coalesce or form droplet-in-droplet structures. In each of these cases, surface tension plays a significant role. We present a high-throughput flicker spectroscopy technique, which analyses the thermal fluctuation of surfaces of tens of thousands of droplets, to extract the surface tension distribution. We demonstrate this approach on stress granules: a class of biological condensate which forms in response to stress and are thought to protect and stall the translation complexes. They are also involved in the development of neurodegenerative diseases such as ALS. We show that the measured fluctuation spectra of stress granules cannot be explained by surface tension alone. Instead, it is necessary to include an additional energy contribution, that we attribute to an elastic bending rigidity due to the presence of structure at the droplet-cytoplasm interface. Furthermore, we find that the stress granule surface tensions and bending rigidities span several orders of magnitude. Therefore, biomolecular condensates can only be differentiated through large-scale surveys.

P2.45

Multimodal quantum sensors for detecting nanoscale dynamics in C. elegans

Sophia Belser[1], David Jordan[2,3], Louise Shanahan[1], Jack Hart[1], Qiushi Gu[1], Eric Miska[2,3], Mete Atatüre[1], Helena Knowles[1]

[1]Cavendish Laboratory, University of Cambridge, JJ Thomson Avenue, Cambridge CB3 0HE, , United Kingdom, [2]Wellcome/CRUK Gurdon Institute, University of Cambridge, , United Kingdom, [3]Department of Biochemistry, University of Cambridge, , United Kingdom

Nanodiamonds (ND) can be used as powerful quantum sensors at the nanoscale. They are of particular interest for biosensing applications due to their low cytotoxicity, amenability to surface functionalisation, and optical properties. Because of the characteristic response of nitrogen vacancy centres (NVs), point defects in the diamond carbon lattice, NDs can be employed to obtain a sensitive readout of temperature in the vicinity of the ND probe. Sub-degree temperature sensitive NDs allow for real time in vitro and in vivo sensing with nanoscale spatial resolution that could drastically improve our current understanding of intracellular processes.

We employ ND quantum sensors to probe temperature and viscoelastic properties in live Caenorhabditis elegans (C. elegans). Temperature is known to influence processes in living organisms and is one of the most fundamental parameters to affect biological activity. Usually, thermoregulation through homeostasis is responsible for stabilising the core body temperature of mammals. In contrast, nematodes like C. elegans belong to the group of cold-blooded organisms that cannot actively regulate their body temperature. Despite temperature being such an important contributor to the health of biological organisms, little is known about the systemic response and adaptation to thermogenesis. Thus, we aim to induce a temperature change through chemical uncouplers that disrupt the electron transport chain and are expected to lead to a localised heat release in mitochondria [1]. We employ ND sensing for in vivo thermometry to understand more about the ectothermic nature of C. elegans.

[1] Nakano, M. et al. PLoS One12, e0172344 (2017).

P2.46

Modelling DNA in Complex Topologies: The Role of Gyrase

Katy Hollands[1], Mark Leake[1,2], Anthony Maxwell[3], Agnes Noy[1]

[1]School of Physics, Engineering and Technology, University Of York, York, United Kingdom, [2]Department of Biology, University of York, York, United Kingdom, [3]Department of Biological Chemistry, John Innes Centre, Norwich, United Kingdom

DNA gyrase are type IIA topoisomerases, which are enzymes that can relax and induce supercoils in DNA through the strand passage of the double helix. They only occur in bacteria and are key in the DNA replication and transcription processes, as they relax superhelical tension caused by DNA polymerase. They are also capable of inducing negative supercoils through the hydrolysis of adenosine triphosphate (ATP). Due to their importance in the survival of the cell, they are a common target for antibiotics.
It is assumed that the DNA gate opens to allow the transport segment to pass through the other DNA strand; however, experiments haven't shown how this occurs. The exact mechanism of the enzyme also hasn't been observed with experiments and so these will be looked at using Molecular Dynamics (MD) simulations using implicit solvent, to be able to see the system in a dynamic way with the hope of finding new potential targets for antibiotics.

P2.47

Correlative light electron microscopy using small gold nanoparticles as single probes

Professor Paola Borri[1], Dr Iestyn Pope[1], Hugh Tunner[2], Dr Francesco Masia[1], Dr Lukas Payne[1], Kenton Paul Arkill[2], Judith Mantell[2], Professor Wolfgang Langbein[1], Professor Paul Verkade[2]
[1]Cardiff University, Cardiff, United Kingdom, [2]University of Bristol, Bristol, United Kingdom

Correlative light electron microscopy (CLEM) combines the strengths of light microscopy (LM) and electron microscopy (EM) and is receiving growing attention in the life sciences. CLEM aims to combine the live cell imaging capability, large field of views, and molecular specificity of LM with the spatial resolution and ultrastructural information of EM. To highlight biomolecules of interest and determine their position with high accuracy in CLEM, they need to be labelled with probes that are visible both in LM and in EM.

In this work, we demonstrate a novel CLEM approach using small gold nanoparticles as a single probe [1]. Individual gold nanoparticles bound to the epidermal growth factor protein were located with nanometric precision background-free in human cancer cells by light microscopy using an in-house developed technique based on transient resonant four-wave mixing (FWM), and were correlatively mapped with high accuracy to the corresponding transmission electron microscopy images. We used nanoparticles of 10nm and 5nm radius and show a correlation accuracy below 60nm over an area larger than 10µm size, without the need for additional fiducial markers. Correlation accuracy was improved to below 40nm by reducing systematic errors, while the localisation precision is below 10nm. Notably, we found that polarisation-resolved FWM correlates with nanoparticle shapes, promising for multiplexing by shape recognition in future applications. Owing to the photostability of gold nanoparticles and the applicability of FWM microscopy to living cells, FWM-CLEM brings a powerful alternative to fluorescence-based methods.

[1] PREPRINT: https://doi.org/10.48550/arXiv.2209.07771

P2.48

Broadband Cavity Enhanced UV-VIS Absorption Spectroscopy for Picolitre Liquid Samples

Ms Imogen Fermor-Worth[1], Dr Catalin Chimerel[1,2]

[1]University of Exeter, Exeter, United Kingdom, [2]Transilvania University of Brașov, Brașov, Romania

Absorption spectroscopy is a widely used analytical technique due to its label-free nature. However, its application to small liquid samples is hampered by the associated short absorption pathlengths, limiting sensitivity. A concept for the development of an ultrasensitive broadband absorption spectrometer optimised for thin liquid films is presented. We implemented an optical cavity within a fibre-based absorption spectrometer, to enhance sensitivity of the absorbance measurements. In the setup, light propagates multiple times through the sample of interest resulting in greatly increased sensitivity. The bandwidth of the instrument is determined by the choice of two dielectric mirrors forming the optical cavity and, in this implementation, has been set to be optimised for UV detection (250-450 nm). The sensing volume of the spectroscope is prescribed by the choice of optical fibres employed to deliver light to the sample, here we employed 400 µm diameter fibres, giving a sensing volume of 630 picolitres for a thin film of 5 µm in thickness. As a proof-of-concept, we have used our platform for the ultrasensitive detection of the antifungal drug Amphotericin B. Cavity enhancement factors, the equivalent pathlength increase over classical absorption spectroscopy, in the range of 200X have been achieved across a broad wavelength range. Taking advantage of the extended path length the limit of detection for Amphotericin B in a 5 µm thick aqueous film has been dropped from ~125 µg/ml to ~20 µg/ml. We envision multiple applications of our technology ranging from low concentration nucleic acid quantification to label-free cellular drug uptake.

P2.49

A view to a kill: using 3D holographic microscopy to study the motility behaviour of predatory bacteria

Dr Emma Brock[1,2], Dr Laurence Wilson[2], Dr Christoph Baumann[2]
[1]University Of Cambridge, Cambridge, United Kingdom, [2]University of York, York, United Kingdom

Bdellovibrio bacteriovorus (B. bacteriovorus) are a promising tool in the fight against antimicrobial resistance. With a predatory lifestyle and a wide range of prey, including antibiotic drug resistant pathogens, they are novel solution to the growing issues presented by antimicrobial resistant bacteria. Despite the proven importance of motility to B. bacteriovorus predation, their swimming behaviour remained little understood including the key characteristics of their motility such as re-orientation mechanisms. This study has utilised digital inline holographic microscopy (DIHM), a novel three-dimensional and high speed imaging technique, to shed new light on B. bacteriovorus motility. The cells were tracked in a range of conditions including in bulk fluid, near to surfaces and in the presence of live prey cells. The resulting trajectories were analysed to quantify the key motility characteristics, patterns, and differences in behaviour dependent on condition changes. I have shown, for the first time, that B. bacteriovorus have a complex bi-phasic swimming style with run-reverse-flick re-orientations. Their motility behaviour including swimming speeds, run lengths and re-orientation angles remains consistent over a co-culturing window of 19-24 hours. However, it changes radically near to surfaces showing a significant drop in swimming speed and no longer performing a run reverse flick style re-orientation. In contrast, in the presence of live prey cells B. bacteriovorus retain the run reverse flick behaviour but increase their swimming speed. This is likely a mechanism to increase predation efficiency in areas of high prey density. These results represent the first in-depth three-dimensional study of B. bacteriovorus motility.

P2.50

A fluorescence, microfluidic microscope built for microgravity and extreme Earth environments

Miss Katrina Crompton[1], Miss Koren Murphy[2], Mr Thomas Wareing[2], Mr Alexander Stokes[2], Mr Jack Dawson[2], Mr Yusuf Ugurluoglu[2], Mr Connor Richardson[2], Dr Adam Wollman[1]
[1]Newcastle University Biosciences Institute, Newcastle Upon Tyne, United Kingdom, [2]Newcastle University School of Engineering , Newcastle Upon Tyne, United Kingdom

With planned missions to send humans to the Moon and Mars, researching the impact of altered gravity on human health and the microbes needed to supply astronauts with food and medicines through synthetic biology has never been more important. Microgravity platforms can provide scientists with intervals of microgravity (10^-2g) and hypergravity (1.8g and above) to test the impact of altered gravity. A parabolic flight offers 20 seconds of microgravity and 40 seconds of hypergravity. A microscope is the essential tool for cell biology but there have been few microscopes built specifically for use on microgravity platforms offering brightfield and fluorescence capabilities. None of these have incorporated microfluidics into their design. Combining microfluidics with fluorescence microscopy allows imaging of live cell-signalling processes dynamically as they occur. We built a fluorescence microscope and microfluidics platform to withstand the vibrations and effective gravity changes experienced on a parabolic flight. We tested this aboard a European Space Agency parabolic flight imaging live yeast, Saccharomyces cerevisiae, cells. We were able to capture images successfully and operate our microfluidic system to inject fluorescent dye. Our technology will be useful for future live cell microgravity research and for performing microscopy in extreme environments on Earth.

P2.51

Using light to control cellular energetics in Escherichia coli

Tommy Schmidlechner[1], Jochen Arlt[1], Luke McNally[1], Teuta Pilizota[1]
[1]*University Of Edinburgh, , United Kingdom*

Optogenetics is a powerful research tool due to the unrivalled spatiotemporal precision and non-invasiveness of light. Proteorhodopsin, a light-driven proton pump, has previously been used to power the swimming of bacteria. This shows that the major electrochemical ion gradient of the cell, the proton motive force (PMF), can be controlled optogenetically. But what is the extent of the control we have? My objective is to investigate the optogenetic control of cell physiology and energetics using proteorhodopsin and other types of microbial rhodopsins.

I introduced a set of microbial rhodopsins to physiologically relevant Escherichia coli strains and measured the average swimming speed of nutrient-limited cell populations under the light control of the best expressing microbial rhodopsins, proteorhodopsin and PoXeR, using differential dynamic microscopy. The swimming powered by the bacterial flagellar motor has been shown to be proportional to the PMF, and the dynamics of PMF in relation to time and rhodopsin activity can tell us how nutrient depletion and respiration rate respond to PMF manipulation.

The lower proteorhodopsin expression of the physiologically healthy strain was sufficient to reproduce the light-powered swimming of bacteria, and the depletion of PMF using PoXeR causes the cell to counter this perturbation by increasing its respiration rate and thus consuming nutrients faster. The light-driven proton pumps likely have little effect on respiring cells with excess nutrients, but it will be interesting to see how we can control the growth of nutrient limited cells and how different ion gradients affect the cell and its other ion gradients.

P2.52

Selective manipulation of mitochondria function and cell viability in cancer cells through blue light and photosensitizer agent

Emily Skates[1], Munehiro Asally[1], Orkun Soyer[1]
[1]*University of Warwick, Coventry, United Kingdom*

Traditional cancer treatments such as chemotherapy, surgery and radiotherapy do not always target cancer cells specifically and this can lead to serious side-effects that greatly affect patient quality of life. Consequently, alternative therapies that can target cancer specifically to reduce side effects and improve success rates are continually being developed. One emerging treatment modality is photodynamic therapy (PDT). Major advantages include improved cancer specificity, reduced toxicity and is non-invasive. PDT involves a photosensitier (PS) agent that together with oxygen, when exposed to light, causes cell death. A novel mitochondrial membrane potential dye, Thioflavin T (ThT), together with blue light, dramatically reduces cell viability in a model cancer cell line (HeLa), and ThT dark toxicity is not significant. Cell viability can be altered by changing both light exposure levels and ThT concentration and that the loss of mitochondrial membrane potential is linked to cell viability. There is evidence that the mechanism behind the loss of mitochondrial membrane potential and subsequent cell death is via reactive oxygen species production. ThT is therefore a good photosensitiser candidate for photodynamic therapy for the killing of cells or inhibiting single mitochondria. The former can be developed into a photodynamic therapy against cancer, while the latter can develop into an existing research tool to study cell physiology at sub-cellular level.

P2.53

Investigating the processes of life in the cold: high resolution imaging of Antarctic fish cells

Anne-pia Marty[1,2], Professor Clemens Kaminski[1], Professor Melody Clark[2]
[1]University Of Cambridge, Cambridge, United Kingdom, [2]British Antarctic Survey, Cambridge, United Kingdom

Cold-adapted species perform their biological functions in very constrained environments which combine high oxygen diffusivity with an increased risk of oxidative damage, low available energy, high fluidity of membranes and low stability of proteins.[1] Despite these environmental pressures, entire ecosystems thrive in the -2° to 2°C range. Little is known about how cold-adapted species perform basic biological functions such as folding and maintaining unstable proteins. In fact, high levels of ubiquitination and high basal expression of Heat Shock Proteins (HSPs) seem to indicate these cells struggle to maintain proteostasis.[2] This, combined with a highly viscous and energy deprived environment containing high concentrations of osmolytes and undegraded RNA, adds to questions surrounding how these living systems manage resources. To investigate different processes including protein folding, aggregation and degradation as well as cell division and organelle dynamics, we propose to use microscopy. This in itself is challenging because of the lack of appropriate instrumentation. Super-resolution microscopes do not allow for the study of cold species in their physiological conditions. This work also presents progress to develop sub-0°C super-resolution microscopy. We anticipate this work to shed some light on fundamental living processes and the general behavior of proteins and aggregates in extreme systems. We also hope the methods developed to tackle these challenges will benefit other areas in microscopy.

[1] Hawkins,S. J., et al. Oceanography and Marine Biology: An Annual Review 58 (2020): 395-440.
[2] Fraser. K. P. et al. Royal Society open science 9 (2022) 211-272

P2.54

Analysis of Common Motifs in Metabolic Systems with emphasis on the role of conserved moieties

Dr Robert West[1]

[1]*University Of Warwick, Coventry, United Kingdom*

Cycling of co-substrates, whereby a metabolite is converted among various forms via different reactions, is ubiquitous in cellular metabolism. Cycled co-substrates include the ATP/ATP and NAD/NADH pairs, but there are also other metabolites that act as co-substrates in various parts of central carbon pathways and biosynthetic pathways. Besides introducing a "cycling motif" within cellular metabolism, co-substrates also cause many connections among otherwise independent metabolic pathways. Despite this central role, system dynamics relating to co-substrates has not been explored in detail.

Here, I will present a mathematical analysis on the effects of "co-substrate cycling" on pathway flux, using several reaction motifs that are common in cellular metabolism. The analytical and numerical results presented show that co-substrate cycling: (1) introduces a limit on reaction flux in linear systems, (2) allows regulation of branch point fluxes for pathways sharing a common upstream metabolite, and (3) allows for correlated outfluxes despite input noise in pathways coupled by a common co-substrate.

P2.55

Intracellular multimodal temperature and viscoelasticity sensing using nitrogen-vacancy defects in carbon nanocrystals

Mr Jack Hart[1], Mr Qiushu Gu[1], Miss Louise Shanahan[1], Miss Sophia Belser[1], Dr Helena Knowles[1], Prof. Mete Atature[1]
[1]*University Of Cambridge, Cambridge, United Kingdom*

Recent experimental evidence suggests that mitochondria operate at 50°C, well above ambient body temperature and theoretical predictions [1]. Furthermore, it has been shown that some cells can actively respond to external temperature changes by altering their internal viscoelastic properties [2]. To investigate this further, we have developed an intracellular multimodal temperature and viscoelasticity sensor that utilises the nitrogen-vacancy defect in the carbon lattice of diamond nanocrystals. Nanodiamonds (NDs) are biocompatible and readily ingested by eukaryotic cells. Non-invasive temperature readout of the zeptolitre volume surrounding the ND is performed by microwave spectroscopy, achieving sensitivities of ~2 K/√Hz. Simultaneously, the viscoelastic properties of the local environment can be extracted by real-time tracking and passive nanorheology.
In this work, we demonstrate the efficacy of our sensor by performing concurrent readout of viscoelasticity and temperature in abiotic environments. We next show uptake and active trafficking of NDs in HeLa cells and probe the intracellular temperature response to external thermal modulations.

[1] - Lane, N., 2018. Hot mitochondria?. PLoS biology, 16(1), p.e2005113.
[2] - Persson, L.B., Ambati, V.S. and Brandman, O., 2020. Cellular control of viscosity counters changes in temperature and energy availability. Cell, 183(6), pp.1572-1585.

P2.56

Effects of molecular noise on cell size control

Motasem ElGamel, Andrew Mugler
[1]*University of Pittsburgh, Department of Physics and Astronomy, Pittsburgh, United States*

Cells employ control strategies to maintain a stable size. Dividing at a target size (the 'sizer' strategy) is thought to produce the tightest size distribution. However, this result follows from phenomenological models that ignore the molecular mechanisms required to implement the strategy. Here we investigate a simple mechanistic model for exponentially growing cells whose division is triggered at a molecular abundance threshold. We find that size noise inherits the molecular noise and is consequently minimized not by the sizer but by the 'adder' strategy, where a cell divides after adding a target amount to its birth size. We derive a lower bound on size noise that agrees with publicly available data from six microfluidic studies on Escherichia coli bacteria.

P2.57

Unobtrusive wearable sensing to estimate human circadian process

Nemanja Cabrilo[1], prof.dr.ir. Jean-Paul Linnartz[1,2], dr. Charikleia Papatsimpa[2]
[1]TU/e, Eindhoven, Netherlands, [2]Signify, , Netherlands

The central biological clock in the brain has a near-24h rhythmicity that is a main determinant of individuals' sleep/wake cycles, hormonal secretion and influences in alertness and performance[1]. Having a solution to determine and track the state of an individual's internal circadian state can allow us to make major steps to optimize human daily rhythms. Some estimates[2] of the circadian state demonstrated that, even if not up to clinical standards, can greatly enhance human-centric lighting to improve wellbeing of, for example, office, industry or warehouse workers. In a quest to investigate the use of wearable sensing for tracking the underlying circadian process, we developed a model that combines a physiology-based model of the human biological clock with non-invasive but possibly inaccurate ambulatory data (in particular actigraphy data) in a statistical framework[3]. A dataset is collected from a field study to validate our model in a real-life setting and to test to what extent this leads to meaningful estimates. We compare model-based predictions based on wearable data against the "gold standard" circadian state estimation, namely, individual subject's bathyphase, or timing of the daily Core Body Temperature (CBT) nadir (minimum point) which is a widely accepted circadian phase marker[4]. We aim to assess further physiological signals and identify the best possible predictors, with reasonable user comfortability, as non-invasive circadian biomarkers, for instance: Skin temperature, Heart-Rate Variability, Electro- Dermal Activity. Accurate and unobtrusive estimation of the circadian phase can unlock the potential of numerous applications, including the development of innovative personalized Human-Centric Lighting.

P2.58

Tracking the life history of chromosomes (kinetochores) in human cell division

Dr Abdullahi Daniyan[1], Alessio Inchingolo[1], Catriona Conway[1], Professor Nigel J Burroughs[1], Professor Andrew McAinsh[1]

[1]*University of Warwick, Coventry, United Kingdom*

During human cell division duplicated chromosomes are released into the cytoplasm at nuclear envelope breakdown then captured and congressed as the spindle assembles and matures, forming the metaphase plate prior to initiating anaphase when all chromosomes satisfy the spindle assembly checkpoint. By imaging the kinetochore, a macromolecular complex that forms attachments between microtubules and the chromosomes, we are able to track throughout cell division (lattice light sheet microscopy, 2s/frame for 15-20 mins), obtaining a life-history through mitosis. We have built a pipeline from imaging through to tracking of the kinetochores in human (RPE1) cells. Here we examine capture and congression dynamics at the cell level, examining typical and atypical behaviours.

P2.59

Optical control of a synthetic oscillatory circuit

Maria Cristina Cannarsa[1], Giacomo Frangipane[1], Filippo Liguori[1], Nicola Pellicciotta[1], Roberto Di Leonardo[1]
[1]*Sapienza University Of Rome, Rome, Italy*

Synthetic biology has enabled the creation of new genetic circuits by combining modular parts. One of the earliest examples is the repressilator, a genetic oscillator that turns individual bacteria into independent clocks. However, like all clocks, individual oscillators lose synchronization over time. So far, the system has been controlled with chemical inputs added to the external cellular environment, with obvious limitations to the range of spatial and temporal modulations. We combined a light-controlled gene expression module with the repressilator to control the system's oscillations, achieving high temporal resolution. Interfacing synthetic circuits with optical switches requires careful calibration of the components' dynamic range. Mathematical models were used to guide and optimize the connection between circuit components. The resulting system allowed us to robustly synchronize a population of oscillators using light inputs. Our optimization method can also be applied to other circuits using light to regulate their performance.

P2.60

Dynamics of membrane proteins using high-speed atomic force microscopy

Abeer Alshammari[1,2], Robin Bon[3], Edmund Kunji[4], George Heath[1]
[1]School of Physics and Astronomy, University of Leeds, Leeds, United Kingdom, [2]Department of Physics, College of Science, University of Hafr Al Batin, Hafr Al Batin, Saudi Arabia, [3]School of Medicine, University of Leeds, Leeds, United Kingdom, [4]Medical Research Council Mitochondrial Biology Unit, University of Cambridge, Cambridge, United Kingdom

Membrane proteins such as ion channels and transporters are involved in a variety of biological processes such as sensing, signal transmission, and molecule distribution regulation. The dysfunctions of these membrane proteins have been implicated in many diseases, and they might be a great therapeutic target for a variety of diseases. In order to gain deeper insights into the mechanism of action of these proteins, it is important to require knowledge of protein structures and dynamics. High-Speed Atomic Force Microscopy (HS-AFM) allows us to monitor individual proteins in action under physiological environments with high spatiotemporal resolution of around 1 nm and 100 ms [1]. Here, we start to use HS-AFM to study the structural dynamics of Transient receptor potential canonical 5 (TRPC5) ion channels, to understand how these ion channels open and respond to small molecules. TRPC structures reported so far represent static pictures of closed channel states in non-native environments [2]. Also, we investigated the effect of curvature on membrane proteins conformation. In another project we aim to quantify the interaction strength between mitochondrial ATP/ADP carriers to determine whether they exist and operate as monomers or dimers, because of conflicting reports in the literature [3].

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P2.61

Developing a system for probing phase behaviour in synthetic proteo-liposomes/polymersomes.

Mr Thomas Gregson[1], Dr Peter Adams[1], Dr Simon Connell[1]

[1]*School of Physics and Astronomy and the Astbury Centre for Structural Molecular Biology, University of Leeds, UK, Leeds, United Kingdom*

Lipid bilayers are integral to life, and their phase behaviour is important in controlling many cell functions. However, their use in bioinspired applications has been limited due to the short lifetime of the lipids and proteins remote from the living cell. Hybrid lipid polymer bilayers have been shown to sustain protein activity over months, but little is known about the fundamental mechanisms that govern activity levels and long-term stability. In this work, we are probing the detailed structure and phase behaviour of both lipid and polymeric vesicles containing the plant light-harvesting complex LHCII. We have shown that the protein can easily be inserted into a fully synthetic polymersome with a high incorporation yield, and that the protein then remains stable and active within the polymer bilayer. The behaviour of multiple fluorescent dyes sensitive to lipid phase has been characterised in binary and ternary model lipid systems, using fluorescence microscopy, fluorescence lifetime imaging microscopy (FLIM), and bulk fluorescence spectroscopy. We show that the fluorescence lifetime of environment-sensitive lipid dyes in mixed liquid-ordered (Lo) and liquid-disordered (Ld) phases correlates well with a shift in emission wavelength, and that the signals from an environment sensitive dye are sensitive not to lipid order as understood, but to cholesterol content. Having built a toolbox of techniques using model lipid membranes we are now using these techniques with the polymer systems to understand phase behaviour and the relationship with LHCII activity and stability.

P2.62

"Each Drop of Blood Measures its Time": Electrophysiological Rhythms in Blood Cells

Dr Fatima Labeed[1], Dr Emily Kruchek[1], Dr Andrew Beale[1,2], Ms Krista Clarke[1], Mr Matthew Johnson[1], Mr Stephen Kitcatt[1], Ms Oreoluwa Griffiths[1], Mr Rashedul Hoque[1], Dr Petra Schneider[3], Prof Malcolm von Schantz[1], Dr Rebecca Lewis[1], Prof Sarah Reece[3], Dr Rula Abdallat[4], Dr Rita Jabr[1], Prof Debra Skene[1], Prof Jonathan Gibbins[5], Dr John O'Neill[2]

[1]University Of Surrey, Guildford, United Kingdom, [2]MRC Laboratory for Molecular Biology, Cambridge, United Kingdom, [3]University of Edinburgh, Edinburgh, United Kingdom, [4]The Hashemite University, Zarqa, Jordan, [5]University of Reading, Reading, United Kingdom

Whilst the cellular electrical properties of membrane potential (Vm), cytoplasm conductivity (σcyto), membrane conductance (Geff) and capacitance (Ceff) have been well-characterised for excitable tissues such as muscle and nerve, they are less commonly investigated in non-excitable cells, such as those found in blood. However, such cells are unique in the body in that their interaction with their environment is affected to a much greater degree by electrostatic interactions with their surrounding media. We have taken a multifactorial approach to the study of the electrical properties of blood cells, particularly red blood cells and platelets to ascertain their role in cellular function. We identified endogenous circadian rhythms in several electrical properties including Geff, σcyto, Vm and zeta potential (ζ). These rhythms persist when cells are removed from the body, resuspended in plasma-free medium and maintained at a constant temperature. Rhythms were found to be temperature-compensated. Similar rhythms were observed in platelets. Investigation of the electrical rhythm suggests that the movement of potassium across the cell membrane is a key component. Further, ζ is of potential significance due to its role in governing interactions between the cell and its environment, including other cells. Rather than being solely a function of surface chemistry, we have identified a mechanism by which the cell ζ is modulated by Vm. Experimental evidence suggests that this phenomenon may play a significant role in blood disease, from myocardial infarctions and strokes to malarial infection.

P2.63

Effect of Integrin αIIb/β3 proteins on lipid properties

Mr. Ubeiden Cifuentes Samboni[1], Natalia Wilke[1], Agustina Godino[1], José Luis Barra[1], Guillermo Montich[1]
[1]*Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC, UNC-CONICET), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, , Argentina*

Membrane proteins may stretch or disorder the chains of surrounding lipids, promote transbilayer movement of lipids, influence their lateral organization, induce the formation of different macroscopic phases, or promote processes such as membrane fusion or fission. Here we are interesting to study how the segments of integrin αIIb and β3 affect the membrane properties. Each peptide corresponding to the transmembrane-cytoplasmic domain αIIb/β3 were expressed by recombinant protein expression in BL21 (DE3)-transformed cells, purified by metal affinity chromatography (IMAC) and re-purified by reversed-phase HPLC. Langmuir monolayers were carried out to evaluate the lateral interfacial stability of the hydrophobic peptides by generating an insoluble peptide film at the air-phosphate interface and then laterally compressing the monolayer. The compression isotherms of pure αII and β3 monolayers at the air-water interface indicated that both peptides formed stable films up to a surface pressure of ~32 and 35 mN/m, respectively, with molecular areas of ~140 Å² at close packing. The higher collapse pressure suggests that the monolayer is stable, which was demonstrated by the compression-expansion cycles.

The surface pressure-area (π vs A) isotherms recorded for POPC monolayers with αIIb or β3 peptide were carried out in a Teflon through, the compression isotherms for lipid/peptide co-spread (with amounts of peptides in a range of 2.0 and 7.0 mol%) films revealed that αIIb induced lipid molecules to a further disordered phase than the β3 mixter, generating a more favourable environment and preventing the lipid molecules from forming stiff films.

P2.64

Interfacial residues in protein-protein complexes are in the eyes of the beholder

Ms Jayadevan Parvathy[1], Dr Aragasamy Yazhini[3], Prof Narayanaswamy Srinivasan[1], Prof Ramanathan Sowdhamini[2]

[1]Indian Institute of Science , Bangalore, India, [2]National Center for Biological Sciences (NCBS), Bangalore, India, [3]Max Planck institute for multidisciplinary sciences, , Germany

Interactions between proteins are vital in almost all biological processes. The characterization of protein-protein interactions is hence inevitable for understanding the mechanistic basis of biological processes, enabling the manipulation of proteins for biotechnological and clinical purposes. The interface residues of a protein-protein complex are assumed to have the following properties: a) They always interact with a residue in its partner protein, which forms the basis for distance-based interface residue identification methods; b) They are solvent-exposed in the isolated form and become buried in the complex form, which forms the basis for Accessible Surface Area (ASA)-based methods. The study interrogates this popular assumption by recognizing interface residues in protein-protein complexes through these two complementary methods. To our knowledge, this is the first study to focus on the interface residues unique to these two methods. The study shows that a few residues could be identified uniquely by each method and the extent of conservation, propensities and contribution to the protein-protein interaction stability varies substantially between these residues. The case study analyses showed that interface residues unique to distance form interactions that hold the proteins together, whereas the interface residues unique to ASA have a potential role in the recognition, dynamics and specificity of the complex and can be a hotspot. Overall, the study suggests the consideration of residues identified by both distance and ASA methods as an interface so that the residues (other than the directly interacting residues) which have a crucial role in specificity, recognition, dynamics and function are also captured.

P2.65

Evaluating the Structural Dynamics of Photosynthetic Proteins using High-Speed Atomic Force Microscopy and Advanced Fluorescence Methods

Maya Tekchandani[1], Prof Stephen Evans[1], Dr George Heath[1], Dr Peter Adams[1]
[1]*University of Leeds, Leeds, United Kingdom*

During photosynthesis, photons of sunlight are absorbed by thylakoid membranes within chloroplasts, where large numbers of Light-Harvesting Complex (LHC) proteins act as antennas for channelling energy to Photosystem (PS) proteins. This project aims to use High-Speed Atomic Force Microscopy (HS-AFM) and Fluorescence Microscopy (FM) to record the assembly and disassembly of supercomplexes of PS-LHC proteins in real time, and to assess the effect of membrane composition and temperature. This will reveal the interaction strength and remodelling capabilities of these critically important PS/LHC clusters. Here, we will present an introduction to the project and some initial data. Preliminary measurements were made to assess the quality of supported lipid bilayers (SLBs) comprised of dioleoylphosphatidylcholine (DOPC) and a fluorescent lipid, by using FM and Fluorescence Recovery After Photobleaching (FRAP). Images revealed a relatively homogeneous fluorescence and a high lateral lipid mobility, indicative of a high-quality SLB which resembles a natural membrane. These SLBs will act as artificial membranes for future work. Isolated LHC trimers incorporated into SLBs will provide a test sample for assessing the dynamics and optical properties of photosynthetic proteins and preliminary measurements using AFM and FM measurements are ongoing. The results provide a baseline standard for the protein height/width, aggregate size, and fluorescence intensity. Our future plans will be to use FM and HS-AFM to assess the dynamics of these individual subunits and the whole PS-LHC supercomplex, including evaluating the flexibility and rearrangement of single LH proteins with a newly developed ultra-fast height spectroscopy mode.

P2.66

Kinetics of surface-immobilized, pH-sensitive DNA triplex switches

Francisca D'Rozario[1], Steven Quinn[1,2], Steven Johnson[1,2]

[1]School of Physics, Engineering and Technology, University of York, York, United Kingdom, [2]York Biomedical Research Institute, University of York, York, United Kingdom

Dynamic DNA machines exploit the specificity and predictability of DNA base pairing and/or regulation of local environment to fuel the reversible switching of DNA between conformational states. One such example is pH-sensitive DNA triplex switches that are driven by proton-mediated parallel Hoogsteen interactions. While these switches have been studied widely for a range of medical and biotechnological applications, the vast majority of these studies have focussed on DNA machines that are freely diffusing in solution phase. However, for many applications, the DNA switches must be integrated with solid-state devices which requires immobilisation on surfaces. Here, we have explored the kinetics of a DNA triplex switch immobilized as a dense, 2-dimensional DNA monolayer on gold surfaces via gold-thiol chemistry. Using the quartz crystal microbalance with dissipation monitoring (QCM-D) method, we prove that despite the high density of DNA within the monolayer (ca. 10^12 molecules/cm^2), pH-switching between open and closed states remains highly reversible and repeatable, and occurs within timescales on the order of 10's of seconds. Moreover, closing of the switch in acidic pH environments follows a bi-exponential process attributed to a triplex "nucleation" process followed by "zipping" of the strands and reshaping of the loop region to form an equilibrium-favoured structure. In contrast, switch opening which occurs at pH > 8, follows a single-exponential process assigned to the dissociation of the triplex structure into a duplex by deprotonation of cytosines. This study not only provides insight into the kinetics of surface-immobilised DNA machines but also supports the development of new technologies such as switchable diagnostics and hybrid electronic-DNA technologies for information processing and storage.

P2.67

Structural analysis of the influenza genome by high-throughput single virion DNA-PAINT

Christof Hepp[2], Qing Zhao[2], Ervin Fodor[3], Achillefs Kapanidis[2]
[1]Oxford University, Biological Physics, Oxford, United Kingdom, [2]Kavli Institute for Nanoscience Discovery, Oxford, United Kingdom, [3]Sir William Dunn School of Pathology, Oxford, United Kingdom

Influenza A, a negative-sense RNA virus, has a genome that consists of eight single-stranded RNA segments. During influenza co-infections, re-assortant virus strains containing gene segments from either strain can occur, occasionally leading to pandemic outbreaks with severe, worldwide consequences for human health.

To better understand the formation of these potentially pandemic re-assortants, we analysed the selective packaging of all eight RNA segments into single virions. We designed a multiplexed DNA-PAINT approach that is capable of a) detecting the presence or absence of all eight gene segments inside of more than 10,000 individual virus particles in one experiment and b) spatially resolving the individual segments inside complete virus particles with a precision of ~ 10 nm. With its high throughput and the capability of unambiguously identifying specific gene segments, this experiment complements data from previous electron microscopy studies.

Our preliminary results show that 1) there is a cooperative effect in influenza genome assembly, with a tendency towards virions with higher segment counts, 2) all segments interact to some extent, while certain segment pairs co-appear preferentially, indicating segment-specific interactions, and 3) inter-segment distances and the spatial distribution of segments inside virions suggest a flexible spatial arrangement.

Overall, our data points to a flexible network of inter-segment interactions that form a robust genome assembly mechanism for influenza A, in agreement with data from previous studies. In the long term, we will develop our experimental approach for the structural and functional study of viral nucleoprotein complexes.

P2.68

Investigating the binding pocket of the glycine receptor through atomic simulation and metadynamics

Jacob Clark[1], Guangpeng Xue[1], Carla Molteni[1]
[1]*Kings' College London, London, United Kingdom*

Pentameric ligand gated ion channels (pLGIC) play an important role in the nervous system and are involved in many neurological disorders. However, due to their complexity, how pLGICs function at the molecular level is still far from being fully understood. In our work, we use molecular dynamics and the enhanced sampling method metadynamics to investigate the activation mechanism of a prototypical pLGIC, the glycine receptor, which is initiated by the binding of glycine to the receptor extracellular domain. We focus specifically on computational strategies to describe the kinetics of ligand binding/unbinding.

P2.69

High-resolution mid-infrared imaging of cervical lymph node metastasis in oral squamous cell carcinoma

Safaa Al Jedani[1], Cassio Lima[2], Asterios Triantafyllou[3], Caroline Smith[1], Janet Risk[4], Steve Barrett[1], Roy Goodacre[2], Peter Weightman[1]

[1]Department of Physics, University of Liverpool, Liverpool, United Kingdom, [2]Centre for Metabolomics Research, Department of Biochemistry and Systems Biology, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, United Kingdom, [3]Department of Pathology, Liverpool Clinical Laboratories, University of Liverpool, Liverpool, United Kingdom, [4]Department of Molecular and Clinical Cancer Medicine, Institute of Systems, Molecular and Integrative Biology, Liverpool, United Kingdom

Oral squamous cell carcinoma (OSCC) is a deadly disease that predominantly metastasises to the lymph nodes. The overall 5-year survival rate for OSCC ranges from 50% to 65%. Currently, Haemoxylin and Eosin (H&E) staining of biopsies is the gold standard diagnostic tool in pathology practice. An alternative imaging method is label-free, Optical-Photothermal infrared imaging (O-PTIR), an innovative technology that overcomes the diffraction limitations of traditional benchtop infrared (IR) microscopy. Here we present the translation of knowledge from IR microscopy into O-PTIR to classify OSCC nodal metastasis tissue using five specific wavenumbers and the ratio 1252:1285. This Ratio results shows that the improved spatial resolution of O-PTIR allows the generation of a contrast similar to that of H&E images. The O-PTIR intensity ratio images result in a distinct difference between the two tissue types. This is primarily due to the nucleic acid band at 1252 cm^{-1} and the collagen and protein band at 1285 cm^{-1}.

Additionally, we present pixel-wise and spatial-spectral models that cross-validate for 46-patients samples with nodal metastasis. Pixel-level models performed poorly in reproducing the discrimination between tissue types. This failure arises from the loss of information arising from neighbouring pixels. Improvement in AUC and accuracy scores is enabled by a spatial-spectral model using hybrid convolutional-neural-network-Random-forest (CNN-RF) that is optimised for the O-PTIR dataset to capture the spatial and spectral information. The CNN-RF model achieves a reliable classification with an AUC of 0.93. As a result of combining spatial-spectral models with O-PTIR, images of OSCC biopsies were classified precisely.

P2.70

Progress in interferometric microscopy: from nanoparticles detection to dynamic cell imaging

Martine Boccara[1,2], Samer Alhaddad[2], Nathalie Jolie[3], Richard Dorrell[3], Ignacio Izeddin[2], Olivier Thouvenin[2], Claude Boccara[2]

[1]Museum of Natural History Paris, Paris, France, [2]Institut Langevin ESPCI, Paris, France, [3]IBENS , Paris, France

We previously described a set up which is able to count phages and determine their diameter from 30-35nm in diameter to 100nm and more by following their Brownian motion (diffusion). In addition we showed that we could differentiate viruses from membrane vesicles based on their different refractive index (a).

Progresses have been directed towards a noticeable gain in sensitivity and we are now able to differentiate full and empty capsids (T5 phage) as well as to detect virus of smaller sizes (25 nm MS2 phage).
Moreover we have developed a new tomographic approach that has led to explore new fields:
We were interested to quantify metabolic activity within algae in different environmental conditions (b). We extended our method to study diatoms facing the polar night and haptophytes a cosmopolitan group growing at different temperatures. We also coupled interferometry with polarisation measurements to visualize structured organelles within algae.
All these results will be presented and discussed for more applications.

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(b) Houda Bey, Florent Charton, Helena Cruz de Carvalho, Shun Liu, Richard G. Dorrell, Chris Bowler, Claude Boccara & Martine Boccara (2022): Dynamic Cell Imaging: application to the diatom Phaeodactylum tricornutum under environmental stresses, European Journal of Phycology, DOI: 10.1080/09670262.2022.2081732

P2.71

High-Speed and Sensitive Flow Cytometry using Fluorescence Oblique Plane Microscopy

Amir Rahmani[1], Aleks Ponjavic[1]
[1]*School of Physics and Astronomy, University of Leeds, Leeds, United Kingdom*

Flow cytometry is routinely used in clinical applications to screen patient cells in order to identify suitable drug treatments. A notable example is in cancer immunotherapy, where patient T cells are redirected to attack tumours. Although flow cytometry enables the classification of cells in a rapid and high-throughput manner, it lacks subcellular resolution and is limited in terms of sensitivity. Imaging flow cytometry (IFC) techniques have been developed to provide characterisation and quantitative image data of single cells in large heterogeneous populations. However, the performance and utility of IFC are still constrained by the fundamental trade-off between throughput, sensitivity, and spatial resolution.

Here, we combined high numerical aperture oblique plane microscopy (OPM) with flow cytometry, which provides a versatile platform to image live samples at a high spatial-temporal resolution. The enhanced light collection efficiency associated with the numerical aperture of our system enables the imaging of fluorescent biological samples at the single-molecule level. We evaluated the sensitivity of our OPM system by characterising the transfer efficiency and detection limit of fluorescent beads at various excitation powers and exposure times. Then, we incorporated microfluidic chips into the system to enable flow cytometry experiments. Hence, we performed imaging through a microfluidic platform by first observing fluorescent beads passing through the channel in an aqueous solution. Finally, we studied membrane labelled cells flowing in the channel and created 3D images of the cells with single-molecule sensitivity.

The ability to enhance the sensitivity of flow cytometry has exciting opportunities for low expression biology and cell sorting and we aim to apply our new device to explore new regimes in flow cytometry as well as to improved screening of patient cells in immunotherapy applications.

P2.72

Molecular Mechanisms of Lipid-Induced Amyloid Fibril Formation from Global Fitting of Kinetic Models.

Mr Alisdair Stevenson[1], Dr Thomas Michaels[1]
[1]*Institute of Biochemistry, ETH Zürich, Department of Biology, Zürich, Switzerland*

Elucidating the molecular mechanisms of amyloid fibril aggregation from soluble peptides is central to building an understanding and the potential control of protein aggregation disorders, such as Alzheimer's and Parkinson's diseases. A fundamental challenge in achieving this is the complexity of the aggregation reaction network itself, which makes it difficult to analyse kinetic data of protein aggregation in terms of the underlying mechanisms. Here we present a theoretical framework, based on chemical reaction kinetics and global fitting, that allows the interpretation of quantitative experimental measurements of α-synuclein aggregation on lipid membranes, a process linked to Parkinson's disease. These results provide a framework for modelling amyloid forming systems with lipid-dependent interactions and has the potential to guide the rational design of small-molecule inhibitors of α-synuclein aggregation.

P2.73

High Throughput Single Cell Bacterial Imaging

Mr. Morten Kals[1], Dr. Jurij Kotar[1], Dr. Allen Donald[2], Professor Pietro Cicuta[1]
[1]University of Cambridge, Cambridge, United Kingdom, [2]Synoptics Ltd., Cambridge, United Kingdom

Antibiotic resistance is a growing issue in healthcare worldwide and is currently responsible for over one million deaths annually. We seek to develop a new method for antibiotic susceptibility testing (AST) using single-cell microscopy. The assay is based on a multi-pad agarose plate (MPAP), where one or more bacteria samples are placed on 96 small agarose pads. Each pad provides a growth environment where media, nutrients, and toxins can be added independently. The bacteria are confined to a single imaging plane using a glass coverslip and imaged for two hours with single-cell microscopy. We then analyse the image sets with a fully automated segmentation pipeline that outputs statistics for colony growth and single-cell characteristics. We demonstrate how, by adding concentration gradients of antibiotics to the pads, the minimum inhibitory concentration (MIC) can be consistently determined. Additional experiments were also conducted to demonstrate that the pads on the MPAP are not affected by their neighbours and that the method is not sensitive to agarose concentration and seeding density.

P2.74

Fusogenic liposome interactions with bacterial envelopes

Anna Scheeder[1], Marius Brockhoff[1], Edward Ward[1], Ioanna Mela[1], Clemens F. Kaminski
[1]*Univeristy of Cambridge, Cambridge, United Kingdom*

The diffusion-limited outer membrane of gram-negative bacteria poses a significant challenge in antibiotic delivery. The reduced drug sensitivity of bacteria and the emergence of antibiotic resistance pose a major threat to global health and have significant socio-economic implications. To overcome this challenge, researchers have developed antibiotic-loaded lipid nanocarriers fusing with the outer membrane of gram-negative bacteria and releasing the antimicrobial payload directly into the periplasmic space. However, the interactions of single nanocarriers with both gram-positive and gram-negative bacteria, as well as the impact of artificial lipid integration into the bacterial envelope, are still not fully understood. In this study, we investigate the interactions of positively charged fusogenic liposomes (FL) composed of the lipid DOPE and the cationic co-lipid DOTAP against bacterial extract-supported lipid bilayers (SLB), Gram-positive (Bacillus subtilis) and Gram-negative (Escherichia coli) bacteria. Total internal reflection microscopy (TIRF) demonstrates the rapid fusion of individual liposomes with SBLs and whole gram-negative bacteria cells. Using structured illumination microscopy (SIM), we observed FL targeting of both bacterial strains. While the liposomes fused with the envelopes of gram-negative bacteria, lipids were internalised by gram-positive bacteria. Furthermore, our findings indicate that liposome fusion with gram-negative envelopes results in membrane bulging and release making bacteria more susceptible to their environment. Additionally, we found that antimicrobial-loaded FLs demonstrated enhanced antibiotic activity. Overall, our research provides a comprehensive examination of the interactions between fusogenic liposomes and both gram-positive and gram-negative bacteria cells and will aid in the design of next-generation liposomes for more effective antimicrobial delivery.

P2.75

Seeing is believing: Imaging across scales to investigate the Actin nucleation activity of Adenomatous Polyposis Coli (APC)

Dr Mari Angeles Juanes[1,2,3], Lautaro Baro[1,2], Dr Asifa Islam[1,2]

[1]Teesside University , Middlesbrough, United Kingdom, [2]National Horizons Center, Darlington, United Kingdom, [3]Centro de Investigacion Principe Felipe, Valencia, Spain

The cytoskeleton plays fundamental cellular roles, including regulation of cell dynamics and motility. We have used imaging techniques across scales to investigate the actin nucleation activity of the tumour suppressor Adenomatous Polyposis Coli (APC). At the molecular level, we showed that APC binds to G-actin and dimerize to form filaments in vitro. Disruption of APC dimers abolishes APC's actin nucleation activity. Imaging individual cells showed that this APC activity is critical for levels, organization and dynamics of F-actin at focal adhesions. To assess the relevance of our findings to collective cell migration, we imaged epithelial cell monolayers and found that the APC-dependent actin pool contributes to sustaining levels of F-actin and adhesive components at cell-cell junctions, thus preserving cell junction dynamics and integrity, facilitating collective cell remodelling and consequent cell motility. Studies in 3D spheroids showed that loss of this actin activity alters spheroid remodelling events along with the ability to form invasive protrusions. Our research offers a new perspective to probe the importance of APC's cytoskeletal functions in tumorigenesis. Further, this is one example of the power of imaging across scales to fully understand complex cellular processes in order to advance their translation into developing more effective cancer therapies.

P2.76

Mapping nanostructural changes in E.coli Peptidoglycan

Dr Abimbola Feyisara Olulana[1,4,5], Dr Jacob Biboy[2], Dr Oliver Meacock[1], Dr Laia Pasquina-Lemonche[1,4,5], Dr William M. Durham[1], Prof Simon J. Foster[3,4,5], Prof Waldemar Vollmer[2], Prof Jamie K. Hobbs[1,4,5]
[1]Department of Physics and Astronomy, University of Sheffield, Sheffield, United Kingdom, [2]The Centre for Bacterial Cell Biology, Newcastle University, Newcastle, United Kingdom, [3]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom, [4]Krebs Institute, University of Sheffield, Sheffield, United Kingdom, [5]The Florey Institute, University of Sheffield, Sheffield, United Kingdom

E.coli is a rod-shaped Gram-negative bacterium whose shape is maintained by a biopolymer known as Peptidoglycan (PG). The chemical composition of PG is well understood but the following questions remain unanswered; 1) what is the detailed molecular organization of the PG; 2) In the case of antibiotic-induced shape change and death, what happens to the PG organization? To this end, we utilized high-resolution atomic force microscopy (AFM) to map the changes in the PG organization from the pole to the cylindrical section of the rod. We extend this location-dependent imaging to interrogate different areas of the PG under different antibiotic treatment times.

To quantify the associated nanoscale directionality and orientation, we developed a MATLAB script that uses ridge detection to automatically select strands in the AFM image, quantify its orientation, and output an orientation color map and angular histogram. In addition, we developed an image segmentation pipeline—a combination of Otsu thresholding and particle analysis to measure holes and breaks in the PG network. Using these approaches, our results reveal unprecedented detail of nanometric molecular organizations with location-dependent orientation across the PG and the distribution of holes and breaks as a function of antibiotic treatment times. The increase in the frequency and size of breaks suggest the progression of death due to antibiotic-induced enzyme inactivation.
Our findings reveal remarkable application of high resolution AFM in deciphering the bacterial cell wall; bringing us closer to the understanding of how antibiotics affect PG molecular organization and in turn, regulates its function & stability.

P2.77

Integrating analog and digital modes of gene expression at Arabidopsis FLC

Svenja Reeck[1,4], Rea Antoniou-Kourounioti[1,2], Anis Meschichi[3], Stefanie Rosa[3], Caroline Dean[4], Martin Howard[1]

[1]Department of Computational and Systems Biology, John Innes Centre, Norwich, United Kingdom, [2]School of Molecular Biosciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom, [3]Plant Biology Department, Swedish University of Agricultural Sciences, Uppsala, Sweden, [4]Department of Cell and Developmental Biology, John Innes Centre, Norwich, United Kingdom

Quantitative gene regulation at the cell population-level can be achieved by two fundamentally different modes of regulation at individual gene copies. A “digital” mode involves binary ON/OFF expression states, with population-level variation arising from the proportion of gene copies in each state, while an “analog” mode involves graded expression levels at each gene copy. At the Arabidopsis floral repressor FLOWERING LOCUS C (FLC), “digital” Polycomb silencing is known to facilitate quantitative epigenetic memory in response to cold. However, whether FLC regulation before cold involves analog or digital modes is unknown. Using quantitative fluorescent imaging of FLC mRNA and protein, together with mathematical modelling, we find that FLC expression before cold is regulated by both analog and digital modes. We observe a temporal separation between the two modes, with analog preceding digital. The analog mode can maintain intermediate expression levels at individual FLC gene copies, before subsequent digital silencing, consistent with the copies switching OFF stochastically and heritably without cold. This switch leads to a slow reduction in FLC expression at the cell population-level. These data present a new paradigm for gradual repression, elucidating how analog transcriptional and digital epigenetic memory pathways can be integrated.

P2.78

How worms explore 3D space

Mr Thomas Ilett[1], Omer Yuval[1], Felix Salfelder[1], Robert Holbrook[1], Thomas Ranner[1], David C. Hogg[1], Netta Cohen[1]

[1]*University Of Leeds, Leeds, United Kingdom*

From microorganisms to animals, navigation and exploration of the natural environment requires a variety of locomotion gaits that are combined and modulated across a wide range of time scales. Caenorhabditis elegans lives in granular and complex fluid habitats which it must explore and forage for survival. However, the nature and mechanisms of its explorations are largely unknown in volumetric environments. In studies of planar motion of C. elegans, local area search is well described in terms of tumble and run dynamics consisting of undulations (runs) separated by random turning events. In 3D neither the locomotion primitives nor the exploration strategies are known. Here we present a high resolution triaxial recording pipeline for capturing both microscopic postures and macroscopic trajectories across a range of homogeneous non-Newtonian environments. Using our new corpus of reconstructed postures and trajectories, we identify non-planar undulatory behaviours, as well as non-planar manoeuvres, including new reversals and new turning behaviours. We find that C. elegans explores its local volume using a nested hierarchy of these locomotion gaits and manoeuvres. Finally, we show that this volumetric exploration can be explained with a simple three-state model that uses rates which exhibit a strong separation of timescales. These results demonstrate that hierarchies of timescales and non-planarity are essential components of foraging and survival in the worm's natural environment.

P2.79

Deciphering oligomeric states in nuclear condensates using single-molecule step calibrated confocal microscopy

Dr Alex Payne-Dwyer[1], Prof Mark Leake[1]
[1]*University of York, York, United Kingdom*

Liquid condensates are ubiquitous and vital drivers of biological function, yet extracting information on the underlying molecular scale presents a challenge for commercial confocal microscopes. Standard 'number and brightness' methods [1] can extract oligomeric distributions of labelled proteins, but for low-mobility conditions inside condensates, this requires traces of several seconds.

Fluorescent proteins offer exceptionally stable brightness in most physiological media. This property delivers a consistent photobleaching intensity step under controlled imaging conditions, and can be detected directly by single-molecule techniques.

Our approach lends this molecular precision to live AiryScan imaging of Arabidopsis root epithelia. This enables rapid oligomeric counting within condensates of Flowering Control locus A (FCA), a regulatory protein that associates with chromatin to facilitate flowering [2].

We establish a fluorescent protein standard in live yeast cells containing the same EGFP reporter as fused to FCA in plant nuclei, but on a consistently expressed metabolic regulator [4]. Single-molecule microscopy generates precise statistics of the molecular tracks in each nucleus. We then complete a simple calibration dataset, using commercial AiryScan microscopy

We then apply the calibrated AiryScan to count FCA oligomers in 60 ms exposures [3]. Our approach can be applied to determine protein expression and stoichiometries of individual assemblies, even in challenging samples such as liquid condensates in plant tissues.

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P2.80

CRISPR-trapping bacteriophages to shine the light on infection events

Dr Temur Yunusov[1], Dr Racha Majed[3], Dr Michael Hunter[2], Mr Charlie Wedd[1], Mr Georgeos Hardo[1], Dr. Somenath Bakshi[1], Mr Gil Kirkler[1], Mr Hassan Alam[1], Dr Diana Fusco[1]
[1]University Of Cambridge, Cambridge, United Kingdom, [2]University of British Columbia, Vancouver, Canada, [3]Refeyn, Oxford, United Kingdom

Despite the long history and wide-spread use of bacteriophage-derived molecular biology tools in molecular biology and microbiology, the early infection events of bacteriophage T7 infecting an E. coli cell are not well elucidated. We are using the Crispr approach to genetically edit the phages and introduce fluorescent markers. To this end we have developed a series of plasmids which contain both the guide RNA targeting specific sites in the T7 genome, and Homology-directed Repair Template (HRT)s with desired T7 genetic edits. We transform E. coli cells with these plasmids and upon infection by T7, the incoming bacteriophage genome gets Crispr-edited in the infected cell. We then use these Crispr-edited phages to infect E.coli cells set up in a mother machine, which enables us to document the early infection events in individual cells with high spacial and temporal resolution. This allows us to go beyond averaged bulk phage infection parameters and estimate cell-to-cell variability in infection dynamics. We hope to use the combination of precise editing of T7 genome and the follow-up detailed survey of its behaviour in E. coli infections to better understand the full sequence of events upon infection.

P2.81

Significant Electrophysiological Changes in White Blood Cells due to Hyperosmotic Stress in ME/CFS

Ms Krista Clarke[1], Mrs Caroline C Kingdon[2], Dr Eliana M Lacerda[2], Dr Emily J Kruchek[1], Ms Oreoluwa V Griffiths[1], Mr Rashed Hoque[1], Professor Michael P Hughes[3], Dr Fatima H Labeed[1]

[1]University of Surrey, Centre for Biomedical Engineering, Guildford, United Kingdom, [2]London School of Hygiene and Tropical Medicine, Department of Clinical Research, , United Kingdom, [3]Khalifa University, Department for Biomedical Engineering, , United Arab Emirates

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a debilitating condition characterised by profound disabling fatigue, not relieved by rest, and exacerbated by mental or physical exercise. No reliable and validated laboratory tests currently exist to quantifiably diagnose ME/CFS, with reliance instead on diagnostic criteria which specify mandatory symptoms, and blood tests to eliminate differential diagnoses. However, heterogeneity of clinical symptoms in ME/CFS patients and variability in different diagnostic criterion mean this is subjective and can result in years before a diagnosis is reached; a quantifiable biomarker is greatly needed.

Esfandyarpour et al. (Proc. Nat. Acad. Sci., 116, 10250, 2019) recorded a differential response in the overall impedance of white blood cells in platelet rich plasma under hyperosmotic stress in ME/CFS versus healthy controls when incubated for 1.5 hours. To understand in more detail the differential responses to hyperosmotic stress seen in ME/CFS to healthy controls, this preliminary study used dielectrophoresis (DEP), to characterise the electrophysiological properties of cells. We incubated peripheral blood mononuclear cells from a total of 12 donors from four donor cohorts: severe ME/CFS, mild/moderate ME/CFS, multiple sclerosis and healthy controls in physiological media and hyperosmotic media supplemented to 200 mM NaCl. After 1.5 hours, the electrophysiological properties of white blood cells were characterised using the 3DEP. A normalised arbitrary value of hyperosmotic – physiological incubation significantly differentiated severe ME/CFS donors and healthy control donors ($p<0.05$). This suggests that DEP may potentially pave the way towards a biomarker for ME/CFS.

P2.82

Emergence of chiral muscle fibers and its role in tissue deformation during mouse heart morphogenesis

Dr Naofumi Kawahira[1], Dr Haruko Nakano[1], Dr Atsushi Nakano[1]
[1]*UCLA, Los Angeles, United States*

Organ morphogenesis is a multi-scale process in which numerous cells behave in a coordinated manner to construct three-dimensional macroscopic forms. In adult heart, intricate organ shape is composed of well-ordered muscle fibers that support the organ function. Anatomical studies over the past few hundred years have clarified that, in addition to the asymmetric organ shape, there is also a specific distribution in fiber angles varying from about 60° (from the circumferential direction) at the inner surface of the muscle wall to about -60° on the outer surface. However, little is known about how proper heart shape is created from cardiomyocytes during development along with the twisted fiber structures. Here we show that local tissue undergoes anisotropic deformation along the muscle fiber orientation. Three-dimensional imaging of the mouse embryonic heart from different developmental stages revealed that the specific fiber orientations emerge between gestational day 12.5 and day 15.5. There is a rapid increase in twisting angle concomitant with the increase in the wall thickness, suggesting a narrow time window where the global fiber pattern is established in the embryonic heart. We further investigated the relationship between fiber orientations and local tissue deformation and found that local tissue tends to stretch along the fiber orientation. These results suggest that fiber orientation guides the local tissue deformation, thereby contributing to the organ morphogenesis. For future studies, we plan to apply these analyses to elucidate how genetic and metabolic defects deviate the course of organ growth in congenital heart diseases.

P2.83

scPrisma infers, filters, and enhances topological signals in single-cell data using spectral template matching

Jonathan Karin[1], Yonathan Bornfeld[1], Mor Nitzan[1,2,3]

[1]School of Computer Science and Engineering, The Hebrew University of Jerusalem, Jerusalem, Israel, [2]Racah Institute of Physics, The Hebrew University of Jerusalem, Jerusalem, Israel, [3]Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

Single-cell RNA-sequencing has been instrumental in uncovering cellular spatiotemporal context. This task is challenging as cells simultaneously encode multiple, potentially cross-interfering, biological signals. I will present scPrisma, a computational method that uses topological priors to decouple, enhance, and filter different classes of biological processes in single-cell data, such as periodic and linear signals. We apply scPrisma to analysis of the cell cycle in HeLa cells, circadian rhythm and spatial zonation in liver lobules, diurnal cycle in Chlamydomonas, and circadian rhythm in the suprachiasmatic nucleus in the brain. scPrisma can be used to distinguish mixed cellular populations by specific characteristics such as cell type and uncover regulatory networks and cell-cell interactions specific to predefined biological signals, such as the circadian rhythm. We show scPrisma's flexibility in incorporating prior knowledge, inference of topologically informative genes, and generalization to additional diverse templates and systems. scPrisma can be used as a stand-alone workflow for signal analysis, and as a prior-step for downstream single-cell analysis.

P2.84

Spatial and temporal heterogeneity in human cell division dynamics

Prof Nigel Burroughs[1], Prof Andrew McAinsh[1], Dr Joanthan Harrison, Dr Peter Embacher, Dr Constandina Koki, Dr Lina Germanova

[1]*University Of Warwick, Coventry, United Kingdom*

Cell division is a self-organised and highly regulated process. By tracking the protein complex that attaches chromosomes to the spindle in human cells (the kinetochore), and fitting biophysical mechanical models to the dynamics (metaphase through to anaphase), we demonstrate a diverse heterogeneity both in time and space across the spindle (orthogonal to the spindle axis). The latter in fact has higher variation than that between cells, i.e. spatially matched kinetochores are dynamically more alike between cells than with kinetochores in the same cell. This suggests that chromosome dynamics is extremely robust to variation in forces throughout the spindle. We also have evidence that sisters (kinetochores attached to duplicated chromatids and held together by the chromatic spring until anaphase) have asymmetric dynamics in 20% of kinetochore pairs. This raises the question of why cell division dynamics is so robust to this heterogeneity. To grapple with this question, we have examined dynamics within the kinetochore structure itself using fluorescent imaging, showing that there are two ensemble level conformations, that associated with microtubule attachment and that associated with tension.

P2.85

Unnatural Light Stimulation of a Natural System in the Cyanobacterium Synechococcus elongatus

Mr Kieran Randall[1], Dr Bruno Martins[1]
[1]*University Of Warwick, , United Kingdom*

Eukaryotes and prokaryotes alike utilise circadian rhythms to regulate gene expression. In the cyanobacterium Synechococcus elongatus, the circadian clock contains only three core protein components: KaiA, KaiB, and KaiC. The clock is free running and maintains a period of approximately 24 hours in constant light but can be reset with long dark pulses.

In this study, we aim to investigate the limits of operation of this circadian system under unnatural fast-frequency light-dark (LD) perturbations. Does the clock maintain phase robustness and the ability to respond to light cues under this regime?

Cells carrying a fluorescent clock reporter were imaged with perturbations of one hour LD cycles commencing at (i) subjective dawn and (ii) subjective dusk under a microscope to examine the effect on the stability of clock period and phase compared to a free running clock under constant light.

We found a perturbation induced clock phase delay and evidence of post-perturbation maintenance of clock phase and period synchrony across the cell population compared to a constant light control. Applying both minimalistic and mechanistic mathematical models, we reproduced these trends by setting up a light entrainment factor that depends on recent environmental history.

A further aim is to determine the effect of unnatural 1 h LD pulses on cellular growth. Surprisingly, there is detectable, clock-dependent, growth under dark pulses, with growth rate in the dark phase-shifted from growth rate in light, which we aim to model within a cellular resource allocation framework.

P2.86

Rapid detection and classification of motile cell tracks in 3D

Samuel Matthews[1], Dr Laurence Wilson[1], Dr James Walker[1], Dr Victoria Hodge[1]
[1]*University Of York, , United Kingdom*

Tracks of motile microbes can be used to identify species, such as pathogens, with different swimming behaviours. They provide detailed information on responses to external stimuli such as chemical gradients and physical objects. Digital holographic microscopy (DHM) is a well-established, but computationally intensive method for obtaining three-dimensional cell tracks from video microscopy data. We use DHM data as ground truth libraries for a deep learning object detection network. The trained network allows a 100-fold increase in processing speed, and is suitable for implementation in real-time applications on modest computing hardware.

P2.87

From Live Cells to Single Molecules: Unravelling the Interplay Between 3D Genomic Structure and Gene Expression

Miss Faeeza Lorgat[1]

[1]*University Of Sheffield, Sheffield, United Kingdom*

The nuclear dot protein 52 (NDP52) is a known cytoplasmic protein despite being initially localised in the nucleus. The protein's main cellular functions were identified in the cytoplasm, particularly its role in selective autophagy. NDP52 plays an active and key role as an autophagy receptor in selective autophagy of ubiquitin-coated bacteria. Additionally, the protein has also been shown to be involved in viral infection and immune response. NDP52 interacts with the NF-κB and IFN production pathways against viral pathogens. More recently, the nuclear role of NDP52 has been revisited. It has been suggested to act as transcriptional factor and may be involved in the DNA damage response pathway with links to the cGAS-STING pathway. Therefore, the structure of NDP52 was investigated to gain more insight on the cellular interactions and functions of NDP52 and its interactions with DNA. This work aims to (i) clearly define how the protein is linked to the cGAS-STING pathway and (ii) explore how oligomerization of the protein impacts DNA binding and protein function. The former investigates the cellular response of NDP52 to DNA damage agents and transcription inhibitors in different cell lines. The latter uses more biochemical approaches such as DNA binding assays, FRET, Microscale thermophoresis, and Atomic Force Microscopy to determine oligomerisation and protein interactions. The biochemical approach has shown NDP52 dimerises via its termini, and the protein binds directly to double-stranded DNA. Initial indications suggest NDP52 can bend or bridge DNA strands which may support changes in genome organisation to modulate transcription activity.

P2.88

New chemical and microscopy tools targeting the complement system in neuroinflammation

Mr Dominic Alderson[1], Dr Ayaz Ahmad[2], Dr Agnieszka Bronowska[2], Dr Katrina Madden[2], Dr Adam Wollman[1]
[1]Newcastle University Biosciences Institute, Newcastle upon Tyne, UK, [2] Chemistry — School of Natural and Environmental Sciences, Newcastle upon Tyne, UK

Alzheimer's disease is one of the leading causes of late-life disability and death. The amyloid beta neuronal plaques are a well-known hallmark of the disease however growing evidence suggests that the simultaneous neuroinflammation is a driving factor in disease pathogenesis thus it is reasonable to assume that by reducing neuroinflammation disease progression can be slowed. Neuroinflammation is driven by the complement system and its interactions with these plaques so complement targeted therapeutics may provide an avenue to slowing disease progression. To fully understand complement driven inflammation requires new imaging techniques specific to the complex protein interactions that take place in the complement cascade specifically the cleavage of C5 to its daughter products C5a and C5b. A single molecule TIRF microscopy assay is under development that will be capable of imaging C5 cleavage in real-time using fluorescent reporters thus allowing for characterisation of the molecular dynamics of the process. We will design and produce small molecule cleavage inhibitors which will then be tested in the TIRF assay to visualise cleavage inhibition. Finally, the original hypothesis that C5 cleavage inhibition reduces neuroinflammation will be tested in human cell lines. Overall, the study aims to enhance our understanding of how neuroinflammation drives neurodegenerative disease pathogenesis through novel imaging assays and analysis methods.

P2.89

Optimal Control Theory in Cancer Chronotherapy

Mr Byron Tzamarias[1], Dr Annabelle Ballesta[2,3], Professor Nigel Burroughs[4]

[1]Mathematics Of Real-World Systems CDT, Mathematics Institute, University of Warwick, Coventry, United Kingdom, [2]INSERM ATIP-Avenir and INSERM UMR-S 900, Institut Curie, MINES ParisTech CBIO, PSL Research University, Saint-Cloud, France, [3]Chronotherapy, Cancer and Transplantation, Faculty of Medecine, CNRS Campus, University of Paris-Saclay, Villejuif, France, [4]SBIDER, Mathematics Institute, University of Warwick, Coventry, United Kingdom

The circadian clock is a biochemical oscillator in every cell that is synchronized to the day night cycle and regulates processes in the human body including cell division. Cancer cells typically lose synchronisation of cell division with the diurnal rhythm, with full decoupling from the circadian clock in mature tumours. Cancer chronotherapy exploits this loss of synchronization, utilising drugs whos activity is dependent on the circadian clock and timing drug infusion in order to maximize anti-tumor efficacy while limiting toxicity to healthy cells. We use optimal control theory to optimise chronotherapy for cancer cells that have totally lost synchronization with the circadian clock, minimizing a cost function with a periodically driven running cost. We analyse drugs that increase cell death rates (cytotoxic drugs) and drugs that target cell division. By applying the Pontryagin Minimum Principle we show that in both case studies optimal solutions are bang-bang whilst aperiodic optimal drug infusion schedules are typical, with the duration of the daily drug administration interval increasing as the treatment progresses.

P2.90

Using Nanomagnetic Heterostructures for the Improved Detection and Treatment of Kidney Cancer

Rachel Matthews[1], Professor Russell Cowburn[1], Professor Grant Stewart[1,2]
[1]University Of Cambridge, Cambridge, United Kingdom, [2]Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom

Renal Cell Carcinoma (RCC) affects 13,000 people per year in the UK. Upon identification of renal masses, accurate diagnosis with core biopsy remains challenging due to single-point sampling, which often does not reflect the tumour heterogeneity, leading ultimately to overtreatment (around 30% of post-surgical biopsies reveal a benign tumour).

This project seeks to investigate whether the less invasive technique of Fine Needle Aspirate (FNA) could be used to improve the diagnosis of kidney cancer. FNA uses a needle to extract a small number of cells from multiple tumour sites; this sampling method provides a better capture of the cell populations that constitute the tumour.

This project combines FNA with a new, high-sensitivity assay technique based on nanomagnetic heterostructures. It is predicted that the development of an accurate FNA diagnostic approach would save ~800 patients per year from having unnecessary surgery for benign tumours.

By adhering kidney cancer cells onto the nanomagnetic heterostructures, they can be tested for gene expression using fluorescence antibody staining. As genes such as carbonic anhydrase 9 (CA9) have altered expression levels in cancerous cells compared to healthy cells, the staining intensity can be interpreted as a diagnostic indicator.

This poster presents fluorescent antibody staining data from A704 cells (RCC cell line) and A549 cells (lung cancer cell line used as a control). It shows initial studies of how cell lines loaded onto the nanomagnetic heterostructures behave. This work is the first step towards using nanomagnetic heterostructures for the diagnosis of kidney tumours using FNA.

P2.91

Lithographically defined magnetic heterostructures for the targeted screening of kidney cancer.

Dr Selma Leulmi Pichot[1], Tarun Vemulkar[2], Jeroen Verheyen[2], Grant Stewart[3], Russell Cowburn[1]
[1]The Cavendish Laboratory, Department of Physics, University of Cambridge, , United Kingdom, [2]Semarion Ltd, , United Kingdom, [3]Department of Surgery, Addenbrookes Hospital, University of Cambridge, , United Kingdom

Renal cell cancer (RCC), is the 7th commonest cancer in the UK and the most lethal urological malignancy; 50% of all RCC patients will die from the condition. However, if identified early enough, small RCCs are usually cured by surgery or percutaneous procedures, with 95% 10-year survival.
In this context, our group is currently developing a non-invasive, urine-based, diagnostic technology for the detection of RCC. Our approach uses barcoded nanomagnetic heterostructures that retrieve specific RCC biomarkers (such as aquaporin-1 and perilipin-2) from the urine.
Inspired by the well established capture/detection sandwich immunoassays, the nanomagnetic heterostructures are functionalised with specific capture antibodies that pull the biomarker of interest out of the urine. A complementary specific detection antibody highlights the captured target of interest, through the emission of fluorescence.

The nanomagnetic heterostructures developed for this study have a magnetic configuration designed for a remote control of their orientation by external magnetic fields. This property ensures that the functionalized side with captured biomarkers is always aligned with the detector during imaging. Furthermore, each set of heterostructures have a unique barcode allowing to assign the fluorescence to a specific biomarker. This feature provides a multiplexed analyte capture platform, as different sets of heterostructures can be mixed together in a patient sample.

Our initial results confirm the feasibility of detecting urinary biomarkers in RCC patients with the barcoded nanomagnetic heterostructures. Comprehensive optimisation cycles are in progress to validate the robustness of this technology as a new screening method for RCC.

P2.92

Dielectrophoresis and Zeta Potential as Potential Bladder Cancer Diagnostics

Mr Rashedul Hoque[1], Dr Fatima Labeed[1], Professor Michael Hughes[2]

[1]Centre for Biomedical Engineering, University of Surrey, Guildford , United Kingdom, [2]Department of Biomedical Engineering, Khalifa University, Abu Dhabi, United Arab Emirates

The task of accurately distinguishing and isolating cancer cells from complex samples, such as blood and urine, pose a continuing challenge in the clinical setting. Dielectrophoresis (DEP) is a technique that exploits the unique electrophysiological properties of cells, enabling separation and characterisation of cells from heterogenous samples in a label-free manner. The 3DEP system, is an instrument capable of rapidly analysing thousands of cells, using a cost-effective disposable chip; the 3DEP has already demonstrated promising application in the clinical setting, both with oral and bladder cancer. We present the preliminary results from an ongoing study, based on the published findings from a prior bladder cancer pilot study, where heterogeneous cellular components of voided urine samples from healthy participants were analysed via the 3DEP system (n = 19).

The mean difference value (MDV) was used as the main analysis parameter, which suggested females possess higher MDVs than males. A positive correlation between MDV and participant age was observed (P = 0.0384). Furthermore, zeta potential analysis identified a negative correlation between mean zeta potential and age (P = 0.0204), suggesting older participants produce more stable and repulsive samples. An increase in participant age was also associated with increased sample cellularity. These early findings highlight notable electrophysiological variability within urine samples of a healthy population. We hope to establish the “electrophysiological blueprint” of voided urine samples from bladder cancer patients, clinically distinct from healthy controls and potential intragroup variability due to cancer stage and grade, when large-scale patient recruitment commences this year.

P2.93

Optimisation of patient futures in cancer therapy

Prof Nigel Burroughs[1], Mr Byron Tzamarias, Dr Annabelle Ballesta
[1]*University Of Warwick, Coventry, United Kingdom*

We propose a new payoff function for cancer treatment based on the expected lifetime of the patient, averaging over all outcomes. Multiple risk factors can be included, such as the risk of metastasis and risk of generating resistant mutants. The futures pay-off is proposed in a deterministic control theory framework, but is motivated and based on branching processes. We demonstrate the futures payoff on simple ordinary differential equation tumour growth models, specifically logistic and Gompertz growth models. Using Pontryagin's maximum principle we prove the optimal solutions are bang-bang and, for these growth models, have either no switch or only a single switching time giving solutions that are continuous treatment at maximum tolerated dose, no treatment or treat-and-stop solutions. Optimising over the time horizon, the optimal treatments are either no treatment or MTD for a specified time. The futures payoff is in principle parametrisable, requiring parametrisation of the rate of these risks and the associated expected lifetimes under each risk event. It thus directly incorporates tumour characteristics into therapy optimisation.

P2.94

Vinculin recruitment to α-catenin halts the differentiation and maturation of enterocyte progenitors to maintain homeostasis of the Drosophila intestine

Dr Jerome Bohere[1], Miss Buffy Eldridge-Thomas[1], Dr Golnar Kolahgar[1]
[1]*University Of Cambridge, Cambridge, United Kingdom*

Mechanisms communicating changes in tissue stiffness and size are particularly relevant in the intestine because it is subject to constant mechanical stresses caused by peristalsis of its variable content. Using the Drosophila intestinal epithelium, we investigate the role of vinculin, one of the best characterised mechanoeffectors, which functions in both cadherin and integrin adhesion complexes. We discovered that vinculin regulates cell fate decisions, by preventing precocious activation and differentiation of intestinal progenitors into absorptive cells. It achieves this in concert with α-catenin at sites of cadherin adhesion, rather than as part of integrin function. Following asymmetric division of the stem cell into a stem cell and an enteroblast (EB), the two cells initially remain connected by adherens junctions, where vinculin is required, only on the EB side, to maintain the EB in a quiescent state and inhibit further divisions of the stem cell. By manipulating cell tension, we show that vinculin recruitment to adherens junction regulates EB activation and numbers. Consequently, removing vinculin results in an enlarged gut with improved resistance to starvation. Thus, mechanical regulation at the contact between stem cells and their progeny is used to control tissue cell number.

P2.95

Mechanisms underlying symmetry breaking in embryonic development

Shayan Shamipour[1], Lucas Pelkmans[1]
[1]*University of Zurich, Zürich, Switzerland*

During development, initially unpatterned cells obtain distinct cellular identities to ultimately give rise to a mature organism. To achieve this milestone, cells are required to integrate multimodal signals from their environment with that of their internal cellular state and exhibit an orchestrated signaling response. Yet, how signaling and cellular state landscapes function together to ensure robust and coordinated cell fate specification during embryonic development remains largely unknown. Here, we set out to investigate the interplay between these landscapes and cellular decisions occurring at the onset of zebrafish gastrulation as germ layers become specified. To this end, we are currently employing iterative immunofluorescence imaging and multiplexed fluorescence in situ hybridization technologies to simultaneously capture the physiochemical, signaling and fate properties of cells across the whole embryo prior to and at the onset of pattern formation. We hypothesize that each signaling pathway likely provides non-redundant but limited information for cellular decisions and that only by considering the full signaling landscape we will be able to faithfully predict the formation of cell types as observed within the embryo. In addition, we expect that heterogeneities in cellular state properties, such as cell cycle state or cytoskeletal abundance, will also contribute to this process by providing additional information for obtaining robust cellular decisions. Collectively, such multiplexing approaches, in combination with 4D live-imaging and perturbation experiments, will generate novel insights into the information provided by each of the intra- and extracellular inputs and ultimately into our understanding of the mechanisms underlying symmetry breaking during development.

P2.96

Mechanical drivers of early mammalian development

Marta Urbanska[1], Aurelien Roux[2], Kevin Chalut[3], Ewa Paluch[1]
[1]University of Cambridge, Cambridge, United Kingdom, [2]University of Geneva, Geneva, Switzerland, [3]Altos Labs, Cambridge, United Kingdom

During early embryonic development, pluripotent cells differentiate, change shapes, and rearrange spatially to form the blueprint of the adult body. Classically, the orchestration of cell fate commitment in the developing embryo has been attributed to the activity of biochemical signalling pathways. More recently, biophysical properties of cells and their environment have emerged as important players in this process.

The aim of my research project is to explore how cell fate is influenced by cell surface mechanics in mouse gastruloids, a 3D multicellular model system mimicking a gastrulating embryo. We are in the process of establishing a pipeline for tracing changes in cell shape and cell mechanics concomitant with the differentiation towards mesendoderm in the multicellular context of the gastruloid. Our approach combines advanced microscopy techniques, cell segmentation, morphometric analysis, and in situ mechanical characterisation of cells. In the final stages of the project, we aim to perturb cell mechanics during gastruloid growth, and monitor the impact of these perturbations on cell fate and spatial patterning.

This work will shed light on the interplay between cell surface mechanics, cell shape, and cell fate in developing gastruloids, and thus contribute to answering the long-standing question in developmental biology of how physical determinants coalesce with biochemical signalling to drive embryogenesis.

P2.97

Intraoperative gamma imaging technologies: Could it be a game changer?

Mohammed Alqahtani[1,2], Ali Alqahtani[3]

[1]Radiological Sciences Department, King Khalid University, Abha, Saudi Arabia, [2]University of Leicester, Leicester, United Kingdom, [3]Applied College, King Khalid University , Abha, Saudi Arabia

Detecting sentinel lymph nodes (SLNs) during cancer surgeries to minimize the chance of spreading is a challenging task. For SLNs detection in breast cancer, the sensitivity of 85 % and a false-negative rate of 5 % have been recommended by the American Society of Breast Surgeons as tolerable. However, the need for further improvement exists, hence, optimum methods need to be developed to improve the sensitivity and reduce the false-negative rates of sentinel lymph node detection. The requisite improvement in SLN detection might be delivered by intraoperative gamma camera imaging. In a case report, the existence of an additional metastatic sentinel lymph node (with low radiotracer uptake), that was not detected by a gamma probe or with preoperative imaging, was actually observed by intraoperative gamma camera imaging, and it has been suggested that the false-negative detection rate can be reduced by using an intraoperative gamma camera.

The conventional gamma cameras used in the hospitals are large and bulky, which will make their use in the surgical theatres infeasible. Well-established, easy to handle, intraoperative gamma imaging systems that could enhance the current surgical services for cancerous patients, will make these systems a potential surgical imaging tool that can be commercialised nationally and internationally.

The aim of this study is to evaluate the existing technologies and artificial intelligence solutions utilised to develop and assess small field of view (SFOV) gamma imaging systems that would be used during cancer surgical procedures to detect the possible route of cancer spreading in the lymphatic system.

P2.98

Condensate's ageing modelled through molecular dynamics: protein structural transitions critically transform the network connectivity and viscoelasticity of RNA-binding protein condensates

Ignacio Sanchez Burgos[1], Andres R. Tejedor[2], Maria Espinosa[3], Jorge Ramirez[2], Rosana Collepardo Guevara[2], Jorge R. Espinosa[1,4]

[1]University of Cambridge, Cambridge, United Kingdom, [2]Universidad Politécnica de Madrid, Madrid, Spain, [3]University College London, London, United Kingdom, [4]Universidad Complutense de Madrid, Madrid, Spain

Biomolecular condensates, some of which are liquid-like during health, can age over time becoming gel-like pathological systems. Ageing of RNA-binding protein condensates can emerge from the progressive accumulation of inter-protein β-sheets. To bridge microscopic understanding of such time-dependent transformation with the modulation of FUS and hnRNPA1 condensate viscoelasticity, we develop a multiscale simulation approach. Our method integrates atomistic simulations with sequence-dependent coarse-grained modelling of condensates that age over time due to accumulation of inter-protein β-sheets. We reveal that ageing notably increases condensate viscosity but does not transform the phase diagrams. Strikingly, the network of molecular connections within condensates is drastically altered during ageing and culminates in gelation when the network of strong inter-protein β-sheets fully percolates. High concentrations of RNA decelerate the accumulation of inter-protein β-sheets, abrogating the effects of ageing.

P2.99

Microskøpe: a 3D-printed flat-pack microscope for mapping phase diagrams

Clement Degut[1]
[1]*University Of York, York, United Kingdom*

There are many open source designs for 3D printable microscopes, including models with various levels of automation in image acquisition. However, there are no designs that include automated sample stage movement for imaging microtitre plates over time. Here, we describe simple and very low cost microscope design in which a 3D printed structure houses a Raspberry Pi HQ camera, a standard microscope lens, and off-the-shelf or 3D printed components for automation. The design is straightforward to assemble and programme, and is constructed using easy to print or easy to source components. Importantly, the microscope can be programmed to automatically image across different positions in an XY grid (e.g. well positions in a microtitre plate), and to monitor these coordinates through repeated imaging over time. We will present data to show that the microscope can be used to monitor liquid-liquid phase separation of biomolecular mixtures, and that the images recorded can be used to map phase diagrams. Our microscope offers a low-cost tool for broad-scale evaluation of phase diagrams at the bench ahead of advanced analysis of a more focussed set of conditions using more expensive technologies. More broadly, the design can benefit any application in which it is desirable to cheaply image 96 well microtitre plates using a bench top device.

P2.100

A Molecular Backbone Reduces the Minimal Kit Required for Structure Assembly

Jeremy Guntoro[1], Jordan Juritz[1], Thomas Ouldridge[1]
[1]*Imperial College London, London, United Kingdom*

Many structures fundamental to life are assembled with guidance from a molecular backbone. A key example is the folding of proteins from their amino acid sequences into their secondary and tertiary structures. Backbone-directed assembly processes have a key advantage over generic self-assembly processes in that they are able to assemble a large number of structures with high accuracy from only a small handful of fundamental building blocks. For example, the enormous space of possible proteins is encoded using a set of only 20 amino acids. While intuitively understood, a fundamental theory of exactly how much a backbone benefits assembly does not currently exist in literature. To explore this question, we study models of backbone-assisted and backbone-free self-assembly using the polyomino tile assembly model as a starting point. We first show that backbone-assisted polyominoes can use a finite set of building blocks to deterministically assemble any structure through which the backbone can be routed, a property absent from backbone-free polyominoes. Then we explore the question of how much of this advantage is due to the sequence of monomers being specified, and how much is due to the geometric restriction imposed by the backbone. In contrast to backbone-assisted polyominoes, polyomino assembly with a defined sequence of added polyomino tiles but without the geometric constraints of the backbone cannot assemble all possible structures. The latter is non-intuitive in light of the exponential scaling of both the structure space of polyominoes and the number of sequences for a fixed set of building blocks.

P2.101

Optimisation of FLIM parameters for quantifying NAD(P)+/ NAD(P)H dynamics in cellular metabolism.

Dr Adil Mustafa[1], Dr Zoe Schofield, Professor Orkun Soyer

[1]University of Warwick, Coventry, United Kingdom, [2]University of Warwick, Coventry, United Kingdom, [3]University of Warwick, Coventry, United Kingdom

Redox couples NAD(P)+/ NAD(P)H enable many reactions in cellular metabolism. For example, NAD+ reduction is coupled to oxidation of carbon compounds through glycolysis, while NADH oxidation fuels mitochondrial respiration. Similarly, NADP+/NADPH pair enables flux in the pentose phosphate pathway and in maintaining other redox pairs involved in scavenging of reactive oxygen species (ROS). Both mitochondrial respiration and ROS scavenging are central processes linked to gene expression and aging. Therefore, the steady state ratios of NAD(P)+/NAD(P)H pairs, and their temporal dynamics are crucial factors that can influence cell physiology, yet they are not well understood – especially at single cell level. Here, we present optimisation of conditions for multiphoton fluorescence lifetime imaging (FLIM) to investigate NAD(P)+/ NAD(P)H dynamics at single cell level. FLIM has been reported to successfully detect NAD(P)H auto-fluorescence at the single cell level but has not been used widely for temporal studies. Our aim is to establish appropriate protocols for temporal FLIM imaging of NAD(P)H of HeLA and yeast cells. We show that, for HeLA cells, time-lapse FLIM imaging increases the rate of generation ROS within a few hours leading to loss of cell viability and concurrently, a diminishing of NAD(P)H signal. We found that the ROS effects are variable over cells and that the introduction of antioxidant solutions in cell culture media reduces it. We further investigated the effect of media replenishing on the rate of ROS generation by using a flow through microfluidic device.

P2.102

Microfluidic DNA self-assembly methods for digital data storage

Max Earle[1], Dr. Casey Platnich[1], Prof. Ulrich Keyser[1]
[1]*KeyserLab, University Of Cambridge, , United Kingdom*

In the modern world data is a key commodity, but the rate of data production is growing faster than our capacity to store it. New data storage technologies are needed in order to sustain this pace. Deoxyribonucleic acid (DNA) offers a potential solution. Promising areal data densities hundreds of thousands of times greater than current data storage technologies such as magnetic and electronic storage, DNA has been a focus of molecular data storage research for the past 50 years. However, DNA-based approaches that focus on encoding data in a base pair sequence are hampered by cost and rate limitations in DNA sequencing and synthesis techniques [1]. In order to circumvent these issues, I propose herein a DNA data storage platform based on DNA origami, high-throughput microfluidics and nanopore sensing. DNA origami allows the construction of two- and three-dimensional nanostructures using a bottom-up self-assembly approach, replacing bespoke DNA synthesis with mixing. High-throughput mixing can be achieved with droplet microfluidics and picoinjection, wherein droplets act as micro-reactors. In these micro-reactors, DNA components could be mixed to produce self-assembled user-defined data strings at a high rate with minimal waste. Using this approach, sequencing could be replaced by nanopore sensing [2], a single-molecule technique using cheap and miniaturisable nanopore chips to acquire structural information about nanoscale objects. A developed technology based on this proof-of-concept could enable long-lived, economical and compact molecular data storage and reduce the footprint and cost of archived data in the future.

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P2.103

Data-driven mathematical modeling of the Wheat circadian clock

Dr. Abhishek Upadhyay[1], Ms. Jamila Rowland-Chandler[1], Dr. Gabriela Pingarron-Cardenas[2], Prof. Alex Webb[2], Prof. James Locke[1]

[1]Sainsbury Laboratory, University of Cambridge, , , [2]Department of Plant Sciences, University of Cambridge , ,

Circadian rhythms, daily oscillations with ~24 hr periodicity due to the Earth's rotation on its own axis, have evolved in species across the kingdoms of life, They allow the timing of a large range of physiological, metabolic, and behavioral processes to anticipate environmental cycles. Circadian timing in plants is governed by a clock gene network that exists in each cell. This clock network consists of interlocked feedback loops of transcriptional regulators Wheat is a key crop and understanding the clock in wheat will have broader agriculture and economical significance. However, the underlying clock mechanisms in wheat are not well understood.

Utilising experimental datasets from wheat (Wittern et al. 2022), we built models of the wheat clock to determine how it differs from the clock of the well-studied plant Arabidopsis (Fogelmark et al. 2014, Caluwe et al. 2016). Our optimised models suggest a mechanism for the dawn-expression of the key clock gene ELF3 in wheat, which peaks at dusk in Arabidopsis. Our unpublished finding involves using parameter optimization methods to develop the first Ordinary Differential equation model of the Wheat circadian clock.

Going forward, I will analyse new wheat RNA-seq time course data and use machine learning techniques to develop a further optimised model of the wheat clock network. In addition, we will investigate the effects of temperature and noise in gene regulation on the clock and incorporate yield related modules to explore chronoculture (Steed et al. 2021) strategies.

P2.104

Understanding the Photophysics of Carotenoids in Bacterial Light Harvesting Protein Complexes

Dr Sagar Satpathi[1], Dr Matthew S. Proctor[2], Dr Andrew Hitchcock[3], Dr Peter G. Adams[4]
[1]University Of Leeds, Leeds, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom, [3]University of Sheffield, Sheffield, United Kingdom, [4]University Of Leeds, Leeds, United Kingdom

Light harvesting (LH) proteins are an integral component of photosynthesis, where they absorb the light energy and then efficiently transfer it to the reaction centre (RC) protein complexes for the conversion of light energy to chemical energy. Along with chlorophylls (Chls), carotenoids (Cars) are widely distributed pigments among light-harvesting protein complexes and play a critical role in regulating energy transfer and photoprotection. This project will explore how different Cars will lead to different energetic pathways within LH2. Here, we present an introduction to the project and some initial data comparing LH2 proteins that were stabilized either in detergent or nanodiscs. Preliminary experiments were conducted to optimize the protocol of forming lipid nanodiscs that incorporate LH2 protein complexes. The lipid type, detergent type, lipid-to-detergent ratio, protein-to-lipid ratio and the absolute concentrations of all components appear to be essential for the successful stabilization of LH2. Solution spectroscopy has been used to characterize the absorption and fluorescence of the LH2 and show that it LH2-nanodiscs can be formed successfully. We are also exploring how nanodiscs can be attached to glass coverslips to allow their characterization by fluorescence microscopy and single-particle spectroscopy. In ongoing experiments, we are analysing a series of LH2 proteins each containing one specific type of Car pigment that have a sequence of energy levels. Spectroscopy data will reveal how excited states are transferred between Chl and Car, or how the energy is quenched. These findings will provide a new understanding of the function of these crucial light-absorbing proteins.

P2.105

Building and breaking epithelial tubes

Xuan Liang[1], Helena Crellin[1], Millie Race[1], Clare Buckley[1]
[1]*University Of Cambridge, , United Kingdom*

My group strives to understand the early morphogenesis and disease of epithelial organs. Broadly, we want to know how epithelial tubes/cavities first polarise, what controls whether they open vs. close and how these developmental processes might be linked to congenital diseases. We are currently particularly interested in understanding the links between apical-basal cell polarity, cell-cell adhesion and cellular mechanics during the process of vertebrate secondary neurulation and mammalian early epiblast formation. To investigate these processes, we use high resolution imaging and optogenetic approaches in vivo, with the developing zebrafish neural tube and in vitro, within multicellular mouse embryonic stem cell (mESC) cultures. This allows us to quantitatively image the behaviour of cells before, during and after a precise manipulation in, for example, actomyosin contractility at a subcellular scale. We have recently found a new Cadherin-mediated mechanism of apical membrane initiation site (AMIS) localisation within developing epithelial tubes and cavities. This suggests that cell-cell adhesion acts as the first symmetry-breaking event in de novo polarising epithelial tubes. We are now investigating whether differential actomyosin contractility is involved in stabilising early Cadherin adhesions (and therefore AMIS localisation) and in mediating the switch between hollowing or folding modalities of lumenogenesis.

P2.106

Mechanical mapping of fate maps in the early Xenopus embryo

Ross McGinn[1], Prof. Kristian Franze[2]

[1]University Of Cambridge, Cambridge, United Kingdom, [2]Institute of Medical Physics, Friedrich-Alexander-Universität Erlangen-Nürnberg, , Germany

There has been lots of in vitro work over recent decades that has shown substrate stiffness to be an important regulator of cell fate. However, we currently know very little about the contribution of viscosity, and in vivo investigations are still missing. Here we used in vivo atomic force microscopy to map time-dependent viscoelastic properties of developing Xenopus laevis embryos. We found distinct, reproducible patterns of viscoelastic properties across embryonic regions that contribute to distinct lineages in the adult organism. Dextran-based lineage tracing and comparison to known Xenopus laevis fate maps allow us to determine correlation between fate choice/lineage restriction and local mechanical properties during embryonic development.

P2.107

Universal codes for the phase behaviour of prion-like low-complexity domains

Maria Julia Maristany[1], Anne Aguirre[1], Dr Jerelle Joseph[2], Dr Rosana Collepardo[1]
[1]University of Cambridge, Cambridge, United Kingdom, [2]Princeton University, Princeton, United States

Biomolecular phase separation is a critical mechanism that contributes to intracellular spatiotemporal organization via the formation of biomolecular condensates. In addition to their widespread implications in aggregation-related disorders, prion-like low complexity domains (PLCDs) have been shown to exhibit demixing behavior and their aberrant condensation has been linked to disease. Here, we investigate the relationship between amino acid sequence and phase behaviour of the PLCDs exploring an unprecedented set of 140 different protein sequences. To this end, we perform direct coexistence molecular dynamics simulations of our residue-resolution coarse-grained protein model, which we have shown accurately predicts quantitative phase diagrams. For variants where experimental phase behaviors have been characterized, we show that our predictions are in excellent agreement with the experimental results. Analysis of the full simulation set of 140 variants reveals that the identity and patterning of sticker residues (e.g., Y, F) are crucial to define the phase behaviour of PLCDs. Notably, aromatic and charged residues have context-dependent effects on the critical temperature of PLCD condensation. Regarding spacer residues (e.g., S, G, N, T), PLCDs condensates are remarkably stable in front of mutations of glycine and serine but are more sensitive to mutations of asparagine and glutamine. Remarkably, these results extend to the entire family of PLCDs tested and reveal universal codes for PLCDs phase behavior, In this work, we defined such a code, through the definition of scaling laws that can predict phase behaviour changes based on sequence composition mutations. Furthermore, the findings here help shed light on the functions of PLCDs and aid in the design of modifications to counter their aberrant phase separation.

P2.108

The hyperactivity of estrogen receptor fusion proteins in breast cancer

Ana-Maria Gherghelas[1], Dr Chris Toseland[1]
[1]*University Of Sheffield, Sheffield, United Kingdom*

Estrogen Receptor-positive (ER+) cancers represent 70% of all breast cancer cases. Despite their initial response to anti-hormonal treatments, acquired resistance eventually arises, allowing the progression of disease into a more aggressive, metastatic form. An emerging mechanism of therapeutic resistance is through chromosomal translocations that result in a diverse set of genes to fuse with the ER-alpha (ESR1) gene in such a way that it eliminates its ligand-binding domain. The resulting chimeric proteins possess constitutive, ligand-independent activity which has been associated with increased cell growth, proliferation and initiation of the metastatic cascade.

Preliminary research in the Toseland lab revealed that ESR1 fusion proteins have the ability to form nuclear condensates, which we hypothesised to confer transcriptional hyperactivity. To test this, the ER+ epithelial cell line MCF7 was transiently transfected with the patient derived fusions ESR1-DAB2 and ESR1-SOX9 which were Halo-tagged. With the use of SIM we demonstrated that ESR1 fusion proteins strongly colocalised with RNA polymerase II (RNAPII), in particular the pSer2 active elongation state, in areas of active transcription. This was accompanied by altered levels of various histone marks of activated and silenced transcription. We postulate that ESR1 fusion proteins interact with the transcriptional machinery in order to drive chromatin re-organisation and aberrant gene expression programmes associated with breast cancer. Gaining a deeper understanding of these aberrant nuclear structures, their dynamics, and functional relevance should provide new insight on oncofusion-driven cancers and may open new therapeutic avenues for the treatment of advanced, therapy-resistant breast cancer disease.

P2.109

Understanding Dynamin Polymerization by Single-molecule Counting and Particle-tracking Using Mass Photometry

Manish Singh Kushwah, Dr. Roi Asor, Eric. D. B. Foley, Prof. Philipp Kukura
[1]*University Of Oxford, Oxford, United Kingdom*

Protein polymerisation is important for a range of cellular functions including locomotion, division, adhesion, communication, and nutrient uptake. Studying protein polymerization is challenging as it is difficult to detect all intermediate species with existing technologies. In this study, we use mass photometry (MP) to study dynamin polymerization, an aspect central to dynamin's function as a membrane fission catalyst during clathrin-mediated endocytosis.

Using MP, we quantify the oligomeric distribution of wild-type dynamin (full-length (FL) and ΔPRD) and polymerizing centronuclear myopathies (CNM) mutants in non-polymerizing and polymerizing conditions, and on supported-lipid bilayers via single-particle-tracking with MP. Free energy estimation for the oligomers for every condition enables us to derive mechanistic and regulatory details of dynamin polymerization.
We find that even under non-polymerizing conditions, both WT and ΔPRD contain a heterogeneous mix of species, dominated by dimers and tetramers, in an equilibrium controlled by bulk protein concentration. For polymerization, we find that both tetramer and dimer are required for the rate limiting step of hexamer formation, and polymer elongation occurs by spontaneous dimer addition. We show that the presence of the PRD domain in Dynamin-FL suppresses its polymerisation in solution. Furthermore, we find that membrane binding significantly decreases the free energy for hexamer formation thus promoting polymerisation. This membrane binding-polymerisation coupling is weakened in CNM mutants, leading to abundant hexamer formation and dynamin polymerisation in solution. Taken together, we provide a quantitative model for dynamin polymerisation that not only describes the mechanism of polymerization but also illustrates how membrane binding and the PRD domain form a two-tiered regulatory module preventing dynamin polymerization in solution. Furthermore, our model also allows us to understand how conditions affecting dimer and tetramer abundance could affect dynamin polymerization.

P2.110

Viscosity of intrinsically disordered proteins in phase separated condensates. Three different approaches for simulations

Andres R. Tejedor[1,2], Prof. Rosana Collepardo-Guevara[2,3], Dr. Jorge Ramirez[1], Dr. Jorge R. Espinosa[4]
[1]Universidad Politécnica de Madrid, Dept. of Chemical Engineering, Calle José Gutiérrez Abascal 2, 28006, Madrid, Spain, [2]University of Cambridge, Yusuf Hamied Dept. of Chemistry, Lensfied Road, CB3 0HE, Cambridge, United Kingdom, [3]University of Cambridge, Department of Genetics, Downing Site, Cambridge CB2 3EH, Cambridge, United Kingdom, [4]University Complutense of Madrid, Faculty of Chemistry, Plaza de las Ciencias 2, 28040, Madrid, Spain

Membraneless assemblies, known as biomolecular condensates, are fundamental for the organization of the cell components. Liquid-like behaviour represents a key feature for the correct function of biomolecular condensates, so investigation of their viscoelastic response is crucial. In this work, we apply three distinct computational methods to characterize the viscoelasticity of biomolecular condensates upon their formation. These methods the shear stress relaxation modulus integration (SSRMI), the oscillatory shear (OS) technique, and the bead tracking (BT) method. First, we test the performance and computational efficiency of the three methods using a simple CG model for studying intrinsically disordered proteins. We find that SSRMI and OS techniques provide better estimations of the viscosity. Then, we apply these two methods for a set of 12 different protein/RNA systems using a sequence-dependent high-resolution coarse-grained model identifying a strong correlation between condensate viscosity and protein/RNA length as well as with the number of stickers vs spacers along the sequence. Finally, we study the progressive accumulation of β-sheet fibrils that yields an effective rigidification of the condensate and is related to the onset of neurodegenerative illness. More precisely, we study hnRNPA1, FUS, and TDP-43, finding that both SSRMI and OS techniques successfully predict the transition from the functional liquid-like behaviour to kinetically trapped pathological states. In conclusion, our work provides a compilation of different modelling rheological techniques to evaluate the viscoelastic properties of biomolecular condensates, a critical magnitude that delimits function from misregulation.

P2.111

Single-molecule trajectories in chemically active condensates

Stefano Bo[1], Frank Jülicher[2]

[1]King's College London, London, United Kingdom, [2]Max Planck Institute for the Physics of Complex Systems (MPI-PKS), Dresden, Germany

Biomolecular condensates provide distinct chemical environments, which can organise various cellular processes. The fluorescent labeling of molecules enables molecular tracking and provides an invaluable tool to probe key processes in cell biology. We discuss how biomolecular condensates govern the kinetics of chemical reactions and how this is reflected in the dynamics of individual labeled molecules. Our theoretical approach provides insights into how the dynamics of labeled molecules can be used to measure key physical properties of the condensates, such as diffusion coefficients, the partition coefficient, and the chemical reaction rates inside and outside bimolecular condensates.

P2.112

Targeted disruption of transcription bodies causes widespread activation of transcription

Martino Ugolini[1,2], Ksenia Kuznetsova[2], Haruka Oda[3], Hiroshi Kimura[3], Nadine L. Vastenhouw[1,2]
[1]UNIL, Lausanne, Switzerland, [2]MPI-CBG, Dresden, Germany, [3]Cell Biology Center, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan

Transcription is highly organized in sub-nuclear structures called transcription bodies1. While intensely studied, it is still unclear whether such bodies have a function in transcription regulation.

In conventional models, hundreds of such transcription bodies form. These are difficult to observe and impossible to perturb for functional study2. During zebrafish early development, on the other side, two prominent transcription bodies are nucleated from the mir430 locus3,4. By deleting this locus, we can specifically disrupt their formation. This provides us with an excellent system to study the function of transcription bodies in vivo5-6.
To assess the role of these bodies in gene regulation, we measured the nascent transcriptome using enriched SLAM-Sequencing (eSLAM-Seq), a combination of metabolic labeling and enrichment of nascent transcripts7. We observed, as expected, many down-regulated genes in the mir430 deletion mutant, compared to the wild type, suggesting that transcription bodies do indeed increase transcription efficiency.

Remarkably, we also observed a set of up-regulated genes. These genes are expressed multiple cell cycles earlier in the mir430 mutant than in wild type embryos and seem to be expressed in supplementary transcription bodies that can be observed in the mir430 deletion mutant.
Our observations suggest that the transcriptional machinery involved in the transition from initiation to elongation is sequestered by the two large transcription bodies in wild type nuclei and becomes available for the transcription of other genes in nuclei that do not form these bodies.

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7. 10.1038/nmeth.4435

P2.113

Molecular and emergent dynamics of recombinant algal pyrenoids by single-molecule tracking and optical tweezers

Catherine Read[1], Daria Gusew[2], Eleanor Goffee[1], Dr Alex Payne-Dwyer[1]
[1]University of York, York, United Kingdom, [2]Freie Universität Berlin, Berlin, Germany

Most of the oxygen we breathe originates from marine algal photosynthesis. These algae organise their Rubisco, the enzyme responsible for capturing CO2 and releasing oxygen, into a liquid droplet called the pyrenoid [1]. CO2 concentrated into the pyrenoid is fixed up to 60% more efficiently. However, Rubisco requires a 'linker' featuring multivalent, transient binding to form this liquid matrix [2]. What then are the minimal properties of the linker and the droplet that promote internal dynamics and this incredible boost in photosynthetic output?

Our model system uses recombinant Rubisco and linkers to nucleate pyrenoid-like droplets. Connecting their molecular and emergent behaviour demands techniques with high specificity and spatiotemporal resolution, and the application of perturbative forces. We correlate fluorescence and optical phase tomography [3] to generate maps of protein concentrations within the droplets. Combining single-molecule tracking [4] and fluorescence recovery reveals the diffusive dynamics of individual components, while stretching droplets in optical tweezers [5] probes the emergent rheology of the matrix as a whole. We find the pyrenoid's mechanical stiffness is linked to slow fluorescence recovery, with an unexpected differential between the two components. Since Rubisco is chiefly reactivated near the pyrenoid surface, inhibited mixing has implications for enzymatic carbon fixation.

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P2.114

Microfluidic technologies for dynamic compositional control of artificial cell systems

Dr Marcus Fletcher[1], Dr Yuval Elani
[1]*Imperial College London, London, United Kingdom*

Understanding the interactions between biomolecules, such as membrane proteins, and lipid bilayers is crucial for the controlled engineering of synthetic cells with advanced membrane functionalities. Moreover, quantifying physicochemical parameters like binding state diagrams between specific protein and membrane systems is essential for unravelling the mechanisms which underpin their assembly. However, in typical membrane binding phenomena, many variables may influence the association stability such as lipid composition, ionic strength and pH etc. Binding state diagrams thus require combinatorial numbers of measurements that thus far have been slow to acquire and consume large samples of potentially expensive protein analytes. To remedy this, we present the development of microfluidic tools to produce biomimetic Giant Unilamellar lipid Vesicles (GUVs) with dynamic and rapid control of the aqueous and lipid compositions. Our tools allow us to quickly and efficiently produce and study lipid vesicles with 100s of precisely defined compositions. Exploiting this, we characterise our platform's potential for elucidating binding interactions between peptides and membranes of different charge. In addition, we demonstrate the use of our compositional control to create membrane bound microgel particles as novel cell-mimetic models.

P2.115

Minimal polymer physics modelling of biological assemblies

Dr. Luke Davis[1,2], Dr. Philip Pearce[1,2]

[1]Department of Mathematics, University College London, London, United Kingdom, [2]Institute for the Physics of Living Systems, University College London, London, United Kingdom

Proteins, nucleic acids, and filamentous phages form biomolecular condensates as a consequence of liquid-liquid phase separation (LLPS). These condensates are essentially concentrated droplets that are known to form a dynamic environment for important cellular biochemical and physical processes. Despite the importance of biomolecular condensates to the functioning of the cell, and through their association with diseases and even bacterial antibiotic resistance, the key molecular mechanisms underpinning their formation, coalescence, ageing, and composition remain rather incomplete. Here, we present coarse-grained simulations and scaling theories, based on rudimentary polymer physics, which allows us to predict how microscopic properties determine the relative importance of bulk and surface energies. Taken together, our modelling provides mechanistic insights into how the patterning, shape, and strength of inter-molecular interactions between polymeric units determine the size, geometry, and coalescence behaviour of modelled biomolecular assemblies.

P2.116

Protein-RNA condensates: complementary or competing interactions in ALS progression?

Dr Mark Driver[1], Mr Jasper Postema[1], Prof. Dr. Patrick Onck[1]
[1]*University Of Groningen, , Netherlands*

Membraneless organelles within cells provide organization of the intracellular environment through the process of liquid liquid phase separation (LLPS). The Fused in Sarcoma (FUS) protein is an RNA binding protein that undergoes LLPS with RNA as part of normal activity in healthy cells. PolyPR and polyGR, dipeptide repeat proteins (DPRs) caused by C9orf72 repeat expansion disorders, have been shown to promote protein aggregation of RNA binding proteins, including FUS. This aggregation contributes to the progression of the neurodegenerative diseases ALS (amyotrophic lateral sclerosis) and FTD (frontotemporal dementia). The pathway from stable condensates towards protein aggregation is poorly understood, but has been linked to changes in condensate behavior induced by the addition of DPRs. Here we investigate the effect of DPR and RNA on the stability of FUS condensates through molecular dynamics simulations.

Condensate formation is driven by electrostatic, cation-pi and hydrophobic/aromatic interactions between molecules. Our previously developed coarse-grained molecular dynamics (CGMD) models are used to study the competition between these molecular interactions and how this depends on relative concentrations. Results of simulations on FUS-DPR-RNA mixtures using our 1 bead-per-amino-acid (1BPA) and 3 bead-per-nucleotide (3BPN) models are presented in a ternary phase diagram. Molecular contact maps and radial density profiles were used to categorize topological changes inside the condensates across the phase diagram. A DPR length dependence was observed to influence transition between distinct topological regimes. We anticipate these insights to shed light on the toxic effect of arginine-containing DPRs and potentially contribute to the disclosure of new therapeutic avenues.

P2.117

The potential role of liquid-liquid phase separation in long-distance RNA transport via the phloem

Dr Ruth Veevers[1], Dr Melissa Tomkins[1], Franziska Hoerbst[1], Dr Richard Morris[1]
[1]*John Innes Centre, Norwich, United Kingdom*

Molecules ranging in size from small molecules to large structures including proteins and RNA are transported long distances within plants. While there is evidence, such as the results of grafting experiments, that these biomolecules are being trafficked long distances between plant organs via the phloem, the mechanism and function behind this movement is often not completely clear.

Some phloem proteins are known to undergo liquid-liquid phase separation; abundant in the phloem, the recently characterised PARCL protein binds to and chaperones many RNAs and has also been shown to phase separate.

We have trained a convolutional neural network using the Chaos Game Representation of the sequences of mRNA observed to be mobile and, by extracting features and weights learned by the model, have begun to identify common characteristics in their sequence length and composition. We consider the potential links between the formation and motion of condensates in the phloem and the transport of biomolecules between plant organs. We will present our latest results on the properties of proteins found in the phloem, including their propensities towards phase separation and disorder, working towards a new understanding of phase separation in the phloem and how condensates may play a role in long distance RNA transport.

P2.118

Processive and distributive non-equilibrium networks discriminate in alternate limits

David Jordan[1], Gaurav Venkataraman[2], Eric Miska[1]
[1]*University Of Cambridge, , United Kingdom,* [2]*Trisk Bio, , United Kingdom*

We study biochemical reaction networks capable of product discrimination inspired by biological proofreading mechanisms. At equilibrium, product discrimination, the selective formation of a 'correct' product with respect to an 'incorrect product', is fundamentally limited by the free energy difference between the two products. However, biological systems often far exceed this limit, by using discriminatory networks that expend free energy to maintain non-equilibrium steady states. Non-equilibrium systems are notoriously difficult to analyze and no systematic methods exist for determining parameter regimes which maximize discrimination. Here we introduce a measure that can be computed directly from the biochemical rate constants which provides a condition for proofreading in a broad class of models, making it a useful objective function for optimizing discrimination schemes. Our results suggest that this measure is related to whether a network is processive or distributive. Processive networks are those that have a single dominant pathway for reaction progression, such as a protein complex that must be assembled sequentially. While distributive networks are those that have many effective pathways from the reactant to the product state; e.g. a protein complex in which the subunits can associate in any order. Non-equilibrium systems can discriminate using either binding energy (energetic) differences or activation energy (kinetic) differences. In both cases, proofreading is optimal when dissipation is maximized. In this work, we show that for a general class of proofreading networks, energetic discrimination requires processivity and kinetic discrimination requiring distributivity. Optimal discrimination thus requires both maximizing dissipation and being in the correct processive/distributive limit. Sometimes, adjusting a single rate may put these requirements in opposition and in these cases, the error may be a non-monotonic function of that rate. This provides an explanation for the observation that the error is a non-monotonic function of the irreversible drive in the original proofreading scheme of Hopfield and Ninio. Finally, we introduce mixed networks, in which one product is favored energetically and the other kinetically. In such networks, sensitive product switching can be achieved simply by spending free energy to drive the network toward either the processive limit or the distributive limit. Biologically, this corresponds to the ability to select between products by driving a single reaction without network fine tuning. This may be used to explore alternate product spaces in challenging environments.

P2.119

Intracellular Wetting of Biomolecular Condensates

Prof Halim Kusumaatmaja[1]
[1]*Durham University, Durham, United Kingdom*

Biomolecular condensates composed of RNAs and proteins are formed through phase separation inside cells. There is now increasing evidence that these condensates interact with other cellular components [1,2]. Here, I will discuss two important examples of such interactions, and how they are pertinent to biological functions. First, I will focus on condensate-lipid membrane interactions during the formation of protein storage vacuole in the plant Arabidopsis thaliana [3]. During seed development, micrometer-sized condensates form within the vacuolar lumen and wet the tonoplast. Distinct tonoplast shapes arise in response to membrane wetting by condensates. Conditions of low membrane spontaneous curvature and moderate wettability favor droplet-induced membrane budding, whereas high membrane spontaneous curvature and strong wettability promote a membrane nanotube network that sits at the condensate interface. Second, I will consider condensate-virus capsid interactions for the translocation of HIV capsid through a nuclear pore complex. In particular, we hypothesise that two key asymmetries in the capsid shape and in the wetting conditions at the cytoplasm-pore condensate and pore condensate-nucleus interfaces allow the capsid to translocate through the nuclear pore complex with little to no energy barrier.

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P2.120

Enhanced stability and kinetics of bacterial toxin with cholesterol

Avijeet Kulshrestha[1], Sudeep N Punnathanam[1], K Ganapathy Ayappa[1]
[1]*Indian Institute Of Science, Bangalore, India*

There exist numerous infections caused by the bacterial released toxins. The bacteria utilize the fundamental differences between the bacterial and animal cell membrane, the first line of defense against pathogenic attacks, to code the specificity in the toxin activity. One such difference is the presence of cholesterol in the animal cell, unlike the bacterial cell membrane. Cytolysin A (ClyA), an α-pore-forming toxin, is expressed by E. coli bacteria as water-soluble monomers that convert to membrane-embedded protomers upon interaction with the plasma membrane. Subsequently, protomers oligomerize in the membrane to form transmembrane pores. Unregulated pore formation causes ion imbalance and passage to other pathogens, leading to cell death and various bacterial infections. In this work, we studied the role of membrane cholesterol in the conformational change of ClyA. We performed all-atom molecular dynamics simulations of the ClyA β-tongue motif embedded in the membrane with and without cholesterol, and utilized the string method approach combined with path collective variables to estimate the free energy landscape along the transition path. Our analysis suggests that the cholesterol induces unfolding, stabilizes the protomer state with low free energy, and increases the kinetics with a lower free energy barrier – hence, a higher activity. Thus, our free energy computations reveal that cholesterol plays a wider and more complex role in the pore-forming pathway of ClyA.

P2.121

Biophysics and immunology of PEGylated virus-like particle vaccines

Milad Radiom[1,2], Ahmed Ali[1], Suwannee Ganguillet[1], Erika Causa[2], Tim Keys[1], Yagmur Turgay[1], Pietro Cicuta[2], Raffaele Mezzenga[1], Emma Slack[1]

[1]ETH-Zürich, Zürich, Switzerland, [2]University of Cambridge, Cambridge, United Kingdom

Virus-like particle (VLP) vaccines offer certain immunological advantages over attenuated bacteria among which are their non-infectious nature and versatility of biosynthetic conjugation of antigens. We hypothesized that among the design parameters for an optimal VLP vaccine, its biophysical properties including mechanical and enzymatic stability are essential, but these properties are generally not accounted for leading to high dosage and repeated vaccination schedules. This is because, regardless of the route of vaccination, the microenvironment of vaccination sites up to the secondary lymphoid organs is biochemically and mechanically challenging for the resilient VLP capsids. In a recent publication, we showed that biosynthetic or chemical conjugation of external molecules (e.g., antigens) influences the biophysical properties and mainly the nanomechanics of VLPs assembled from Acinetobacter phage coat protein AP205 (Radiom, et al. Journal of Colloids and Interface Science, vol. 634, 2023). Here we extend the previous work to systematically vary these properties using polyethylene glycol (PEG) conjugation and examine simultaneously the effects on (a) antibody induction in in vivo mouse vaccination models, (b) mucus penetration in ex vivo human nasal tissue, and (c) enzymatic stability in vitro model of gastric digestion. It is expected that by understanding and overcoming the biophysical limits of efficacy, VLP vaccine technology will present highly effective vaccines with reduced administration dosage and number of schedules.

P2.122

Liquid droplets as a nonequilibrium control mechanism

Matthew Cotton[1]
[1]*University of Oxford, , United Kingdom*

Liquid-liquid phase separation is an exciting feature of subcellular organisation believed to be vital to the formation of membraneless organelles. It is generally believed that the main drivers of phase separation in such systems are attractive equilibrium interactions which are needed to overcome the entropic costs associated with phase separation. However, we developed a thermodynamically consistent model of a multicomponent mixture that leads to phase separation due to catalytic activity alone, without any equilibrium interactions between components [https://journals.aps.org/prl/abstract/10.1103/PhysRevLett.129.158101]. When these biomolecular condensates form, the overall catalytic rate in the system is reduced and as such this automatically regulates enzymatic activity. This is an exciting new mechanism for nonequilibrium phase separation, and a new way for catalytic activity to control the cellular environment. I will explain this mechanism and discuss how it fits into the puzzle of metabolic control.

P2.123

Collagen fibril self-assembly: a phase field crystal model

Dr Christopher Revell[1], Dr Jeremy Herrera[2], Dr Joan Chang, Dr Craig Lawless[2], Yinhui Lu[2], Prof Karl Kadler[2], Prof Oliver Jensen[1]

[1]Department of Mathematics, University Of Manchester, Manchester, United Kingdom, [2]Wellcome Centre for Cell-Matrix Research, University of Manchester, Manchester, United Kingdom

Collagen is one of the key components that give the body structure, and is arranged in a highly organised manner, indicating a strong cellular control in the deposition of collagen fibrils. However in structural tissues such as tendons, where the collagen bundles exist between cells and appear within 24 hours during development, the current model whereby cells coordinate deposition of collagen contradicts the speed of fibril appearance. Here, we hypothesize a phase-transition model for the rapid appearance of fibrils in tendon tissues, using a mathematical model to simulate collagen fibrillogenesis akin to that obtained in time-series electron microscopy of embryonic mouse tendon. Further, laser-capture microdissection coupled with mass spectrometry provides evidence for a rapid reduction of free collagen in the extracellular space that coincides with the appearance of less soluble collagen fibrils. Thus, we provide evidence for extracellular self-assembly of collagen fibrils in embryonic mouse tendon, thereby proposing a new model for collagen fibrillogenesis during embryonic development where rapid collagen fibril formation is required.

P2.124

Biology Exploits Geometry: Impact of Aspect Ratio on Protein Networks

Dr Matt Hughes[1], Ms Sophie Cussons[2,3], Dr Benjamin Hanson[1], Ms Kalila Cook[1], Dr Tímea Feller[4], Dr Najet Mahmoudi[5], Prof Robert Ariëns[4], Dr David Head[6], Prof David Brockwell[2,3], Prof Lorna Dougan[1,2]

[1]School of Physics and Astronomy, University of Leeds, Leeds, United Kingdom, [2]Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, United Kingdom, [3]School of Molecular and Cellular Biology, University of Leeds, Leeds, United Kingdom, [4]School of Medicine, University of Leeds, Leeds, United Kingdom, [5]ISIS Neutron and Muon Spallation Source, STFC Rutherford Appleton Laboratory, United Kingdom, [6]School of Computing, University of Leeds, Leeds, United Kingdom

Interconnected networks of high aspect ratio (AR) bio-polymers provide crucial structural and mechanical support to living systems and are ubiquitous in nature. Despite this ubiquity the functional advantage of high AR network building blocks is not understood. To address this, we engineer proteinaceous building blocks with varying numbers of protein L (pL) domains, creating building blocks with ARs from one to seven. Using, shear rheology and small angle neutron scattering (SANS) to characterise the mechanical and structural properties of photochemically crosslinked pL networks, we show that AR is a crucial property that defines network architecture and mechanics. Networks constructed from higher AR building blocks exhibit more homogeneous structures and higher storage moduli due to a shift from translational diffusion limited (TDL) to rotationally diffusion limited (RDL) network formation. High AR building blocks lack the space to rotate freely. For comparison, we study a fibrin network and observe the same transition from TDL to RDL formation, confirming that living systems exploit AR for their network assembly.

High AR bio-polymer building blocks confer significant functional advantages, which work to minimise the number of building blocks required to form an effective network. These include; increased mechanical strength at equivalent protein concentrations; and the rapid assembly of homogenous networks, above a critical concentration, crucial for in vivo biological processes e.g. blood clotting. In addition to uncovering the functional advantages of filamentous proteins for hydrogel formation in vivo, manipulating AR also provides a novel parameter in the design of new biomaterials.

P2.125

Optimal regimes of regulatory sequence evolution

Reka Borbely[1], Gasper Tkacik[1]
[1]*Institute of Science and Technology Austria, , Austria*

Cis-regulatory elements (CREs), such as enhancers and promoters, control gene expression by binding regulatory proteins called transcription factors (TFs). In contrast to their bacterial counterparts, metazoan CREs typically contain multiple functional TF binding sites of weak specificity. Despite extensive study, the origin and role of such sites remains unclear. Here we use simulations and scaling arguments to study adaptive evolution of CREs under selection for regulatory function. In a novel toy model that recapitulates the essential nonlinearities of metazoan regulation, function requires a gene to be activated by binding of cognate TFs, while remaining inactive in presence of non-cognate TFs that would otherwise cause regulatory crosstalk. Evolutionary outcomes in this model are controlled by two key parameters: selection strength and a newly-identified, dimensionless biophysical parameter. When this parameter is small, multiple strong sites must emerge in a CRE during exceedingly slow adaptation; evolutionary process is lost on the flat plateaus of the model's fitness landscape. When this parameter is large, nearly-random CRE sequences can activate the gene, but adaptation grinds to a halt, unable to select against non-cognate TF binding; evolutionary process is stuck in a frustrated fitness landscape. In the intermediate regime, adaptive evolution of CREs is orders-of-magnitude faster, naturally leading to a diversity of strong and weak binding sites, as reported in empirical studies. Our detailed characterization of this relevant regime suggests that evolution, over a very long timescale, might have optimized metazoan regulatory apparatus to enable rapid adaptation of regulatory sequences.

P2.126

Fluorescent Lifetime imaging of individual lipid domains

Miss Yiran Li[1], Mr Thomas Gregson[1], Dr Simon Connell[1], Dr Peter Adams[1]
[1]*University of leeds, Leeds, United Kingdom*

Despite the huge amount of research into membrane domains, commonly referred to as lipid rafts, there is still much to learn about the fundamental physical rules that govern lipid bilayer phase structure and dynamics. To understand even the simplest behaviour, well controlled model systems are required, and this has succeeded in revealing a great deal of complexity, but the correspondence with the reality of live cells is not straightforward. We are interested in the physics of domain formation and the mechanisms which control domain size. In live cells there is much evidence that the domains are < 200 nm, below the limit of standard optical resolution. To develop model systems that mimic these structures and to probe them in live cells, we must understand the origin and subtleties of the signals measured. Fluorescent molecular probes are one such tool that report on the properties of each phase, available in two types; the first are lipophilic fluorescent probes that partition specifically into liquid disordered or liquid ordered (raft) phases, the second are based on environment-sensitive dyes, which partition into both phases, but stain them by different fluorescence colour, intensity, or lifetime. In this work we report on recent results using a confocal laser Fluorescence Lifetime Imaging Microscope (FLIM) equipped with a spectrometer and AFM to study the behaviour of various lipid dyes in ternary separated model systems, discriminating the various signals down to the level of individual lipid domains.

P2.127

Modelling population bottlenecks in respiratory virus transmission

Patrick Sinclair[1], Dr Chris Illingworth
[1]University Of Glasgow, , United Kingdom, [2]University of Glasgow, , United Kingdom

The transmission bottleneck describes the number of viral particles that found an infection in a new host, and is of key importance for describing the evolutionary behaviour of a virus. Previous studies have used genome sequence data to suggest that transmission bottlenecks for influenza and SARS-CoV-2 usually involve few viral particles, but the general principles underlying bottlenecks remain unexplored.

Here we show that a physics-based model of airborne viral transmission predicts tight transmission bottlenecks across a broad set of environmental circumstances. Our model describes levels of exposure to viral particles arising from proximity to an infected individual. Levels of exposure are determined by the emission of infectious particles from that individual, and by the subsequent transmission of particles as they are affected by diffusion, sedimentation, evacuation by ventilation, and the degradation of viral particles. The extent to which exposure translates into infection is then determined by the contagiousness of the virus, described in our model by scaling the basic parameter of infectivity R0.

We model transmission in a variety of scenarios, including within an office, in a nightclub, and on a bus, and consider a model based upon the Skagit Chorale super spreading event, in which up to 51 out of 62 individuals present were infected. Our model suggests that across this broad range of circumstances, the great majority of transmission bottlenecks involve few viral particles, with a high proportion of infections being caused by a single viral particle. Infectious diseases spread by respiratory transmission have tight transmission bottlenecks.

P2.128

Evolving Tissue Pattern Scaling and Robustness Through Spatially Heterogeneous Feedback

Lewis Mosby[1,2,3], Zena Hadjivasiliou[1,2,3]

[1]The Francis Crick Institute, London, United Kingdom, [2]University College London: Department of Physics and Astronomy, London, United Kingdom, [3]London Centre for Nanotechnology, London, United Kingdom

The size, shape and patterning of animals varies greatly between species, despite these properties being governed by the distributions of highly conserved molecules such as morphogens. Following secretion, morphogen molecules form spatial concentration gradients in developing tissues through diffusion and degradation, and control patterning by activating specific genes within target cells in a concentration-dependent manner. Ideally, patterning should scale with organism size during growth while also being robust to fluctuations in morphogen production, but it is currently unclear how a single mechanism can generate morphogen gradients that are simultaneously adaptable while able to mitigate errors.

A popular model suggested that morphogen gradients are regulated via bidirectional feedback with a population of expander molecules, which act to increase the morphogen range as a function of tissue size, thereby mediating morphogen gradient scaling. This result gained support experimentally by studying the feedback between Dpp (morphogen) and Pentagone (expander) in the Drosophila Melanogaster wing imaginal disc. Using both analytical and computational approaches, we have studied how different types of morphogen-interactor feedback mechanisms can give rise to scaling and/or robust morphogen gradients. Further to this, we have developed an evolutionary algorithm to investigate how scaling and robustness can be jointly optimised by biological systems, with the aim of understanding whether these properties co-operate or compete with each other.

P2.129

Single-Molecule Structure and Topology of Kinetoplast DNA Networks

Pinyao He[2], Allard Katan[2], Luca Tubiana[3], Cees Dekker[2], Dr Davide Michieletto[1]
[1]School of Physics and Astronomy, Edinburgh, United Kingdom, [2]TU Delft, Delft, Netherlands, [3]University of Trento, Trento, Italy

The Kinetoplast DNA (kDNA) is a 2D network of mutually inter-linked DNA minicircles found in Trypanosomes that heavily resembles an "Olympic gel". Understanding the self-assembly and replication of this structure are not only major open questions in biology but can also inform the design of synthetic topological materials such as polycatenanes, dubbed as "the Holy Grail of Polymer Chemistry" by Sir Fraser Stoddart (Nobel Laureate in Chemistry, 2016).

In this talk I will present the first high-resolution, single-molecule study of kDNA network topology using AFM and steered molecular dynamics simulations. We accurately measure the spatial distribution of DNA in the network and quantify the distribution of valence of the minicircles. Additionally, we use sub-isostatic network theory to characterise the elastic Young modulus and bending stiffness of the network, and discover that they are 10^6 and 10^3 times smaller than the ones in typical 2D materials such as lipid membranes, thus rendering the kDNA the first example of a "ultra-soft" topological gel.

Our findings explain outstanding questions in the biology of kDNA and offer single-molecule insights into the properties of a unique topological material.

P2.130

Does phenotypic heterogeneity govern bacterial biofilm resilience?

Dr. Abhirup Mookherjee[1], Dr. Diana Fusco[1]
[1]*University Of Cambridge, Cambridge, United Kingdom*

Biofilms are the most common form of communal bacterial growth, which contains clusters of bacterial cells embedded within a matrix of extracellular polymeric substances (EPS). The complex interaction among cells, matrix and surrounding environment produces differential mechanical forces which create fascinating biofilm morphologies (e.g., wrinkled/rough, smooth, etc.). Heterogenous gene expression, even in clonal biofilm populations, is a frequently observed phenomenon, generating subpopulations with various phenotypic traits, e.g., morphology, motility, and adhesion. Furthermore, differential biofilm morphologies can also govern phenotypic differentiation. Previous studies have shown that phenotypic diversity confers fitness advantages through bet-hedging or division of labor in fluctuating environments. Despite the indispensable role of mechanics in biofilm morphogenesis, its effects on gene expression and its possible role in phenotypic heterogeneity are unknown. We hypothesized that phenotypic plasticity and biofilm morphology are interrelated and might have coevolved, which leads to biofilm resilience. We are using several fluorescent reporter Bacillus subtilis strains to report variance of gene expressions inside their colony biofilm in different physico-chemical conditions. Differential motility and EPS gene expressions were observed inside the colony biofilm in absence of critical nutrients. Gene expression at the single-cell level will also be measured in various physico-chemical conditions. Since direct measurements of local mechanical forces are difficult, we will use data generated from earlier experiments to a model based on computational continuum mechanics. The outcome of our results can shed light on the interplay between single-cell phenotypic expression, biofilm morphologies, and distribution of phenotypic heterogeneity.

P2.131

Are physics-informed neural networks (PINNs) the right tools for inverse reaction-diffusion problems? Success and Challenges

Dr Roozbeh Pazuki[1]

[1]*Roozbeh Pazuki, London, United Kingdom*

In 1952, Alan Turing proposed a novel mathematical model to explain pattern formation in chemical and biological phenomena. The model is a set of Partial Differential Equations (PDEs) that describes chemical species' spatiotemporal reactions and diffusion. For some regions in parameter space, diffusion-driven instability leads to stable steady-state patterns such as spots or stripes.

Meanwhile, Physics-Informed Neural Nets (PINNs) have recently been introduced as a new technique for solving PDEs, requiring only small training datasets. Like other numerical PDE solvers, PINNs are also used for inverse problems in PDEs, specifically in estimating parameters in high-dimensional space.

In this talk, we will explain some of the challenges of using PINNs for general parameter estimation in reaction-diffusion PDEs, especially in the case of equations that describe gene expression at the cellular level, and how one might be able to solve these.

P2.132

Phase2vec: Dynamical systems embedding with a physics-informed convolutional network

Dr Matthew Ricci[1], Noa Moriel[1], Zoe Piran[1], Professor Mor Nitzan[1]
[1]*The Hebrew University Of Jerusalem, Jerusalem, Israel*

Dynamical systems are found in innumerable forms throughout nature, yet all these systems intuitively fall into universal equivalence classes. Inferring these classes from data remains an important open challenge in computational physics, especially in life science applications, where the relationship between system parameters and dynamics is often obscure. Here, we propose phase2vec, a dynamical systems embedding method that learns high-quality, physically-meaningful representations of dynamical systems without supervision. Our embeddings are produced by a convolutional backbone that extracts geometric features from flow data and minimizes a physically-informed vector field reconstruction loss. In a training period, embeddings are optimized so that they robustly encode a data set of random ordinary differential equations over and above the performance of a per-equation fitting method. We then validate our architecture by using it to predict the governing equations of dynamical systems modeling phenomena from across the physical and life sciences, from population dynamics to gene regulation. We further demonstrate that inferred embeddings encode the dynamical properties of input data (stability of fixed points, conservation of energy, and the incompressibility of flow) much more accurately than standard blackbox classifiers and state-of-the-art time series classification techniques. Finally, we extend our method to the partial differential equation case, using phase2vec to classify spatial processes from climate data and outlining extensions to the setting of reaction-diffusion models. Collectively, our results demonstrate the viability of embedding approaches for unsupervised feature discovery in dynamical systems.

P2.133

CellPhe: a toolkit for cell phenotyping using time-lapse imaging and pattern recognition

Laura Wiggins[1]

[1]*University Of York, York, United Kingdom*

With phenotypic heterogeneity in whole cell populations widely recognised, the demand for quantitative and temporal analysis approaches to characterise single cell morphology and dynamics has increased. We present CellPhe, a pattern recognition toolkit for the characterisation of cellular phenotypes within time-lapse videos. To maximise data quality for downstream analysis, our toolkit includes automated recognition and removal of erroneous cell boundaries induced by inaccurate tracking and segmentation. We provide an extensive list of features extracted from individual cell time series, with custom feature selection to identify variables that provide greatest discrimination for the analysis in question. We demonstrate the use of ensemble classification for accurate prediction of cellular phenotype and clustering algorithms for the characterisation of heterogeneous subsets, validating and proving adaptability using different cell types and experimental conditions. Furthermore, we provide an example application for CellPhe to characterise response to chemotherapy, quantifying a population's response to varying concentrations of drug and identifying a subset of "non-conforming" treated cells that resist treatment. Our methods extend to other imaging modalities, such as fluorescence, and would be suitable for all time-lapse studies including clinical applications and drug screening.

P2.134

Combinatorial approaches for understanding morphogenesis in 3D embryos

Dr Salvish Goomanee[1]

[1]*Collège De France & CNRS, Paris, France*

Cells in tissues are inherently linked to their neighbours along their common interfaces which gives rise to mechanical forces being exerted onto each other and on their environment. These are complex interactions that allow for morphogenetic events to occur. Such interactions can be studied via vertex models that have been initially developed to study foams. For example, epithelial tissue can be viewed as a 2D network where one describes the epithelia by a set of vertices and interactions among them as edges which capture the mechanical constraints externally imposed. This is well documented in the literature. We are particularly interested in cell fate analysis as a result of such interactions in 3D embryos. We leverage techniques from discrete and computational geometry to construct 3D meshes from which we extract we equivalent graph with structured data. We employ state of the art machine learning techniques to study the resulting graphs in order to predict pertinent physical quantities such as line tensions between embryos and pressures within each embryo.

P2.135

Investigation of In Vitro and In Vivo of nano silver incorporate into bioglass/ceramics

Prof. El Sayed Yousef, Dr Mohamed S. Alqahtani, Prof. Dr Manuela Reben

The silver oxide was added to bioglasses and ceramics with unique compositions, as follows; 30P2O5-20CaO-15Ca(OH)2-20ZnO-8.0KF-5B2O3-2.0TiO2 and 25P2O5-20CaO-15Ca(OH)2- 20ZnO-8.0KF-10B2O3-2.0TiO2. The fabrication materials were synthesized by using X-ray, Transmission electron microscope (TEM), and Raman spectra. Thesis bioglass/ceramics were evaluated for their antimicrobial activity, anti-proliferative/cytotoxic effects on normal and activated splenic cells in vitro, anticancer activity, in vivo immunologic and hypersensitivity responses, in vivo and in vitro effects on cytotoxicity, and in vitro effects of activated splenic cells on produced samples. According to these studies, Gram-positive and Gram-negative bacteria as well as fungi were resistant to the antibacterial properties of the manufactured bioglass/ceramics. While the generated bioglass/ceramics demonstrated anti-proliferative/cytotoxicity against normal splenic cells, they exhibited no cytotoxic or proliferative effects on activated spleen cells. HepG2 cells were cytotoxic by it. In vivo testing showed that manufactured bioglass/ceramics is an inert substance that did not elicit local responses or the immune system. The products of activated splenic cells did not result in any modifications to the composition of bioglass or ceramics, nor did they induce RBCs to exhibit acute cytotoxicity or lysis. An inert substance that did not stimulate the immune system or produce liver cytotoxicity is the silver ions nanoparticle. Because the production of nanosilver bioglass and ceramics was unaffected by immune cells' lytic byproducts, they may be regarded as a safe and suitable material for implantation within the body of living organisms.

P2.136

Application of Infrared techniques to define molecular signatures in uveal melanoma samples

Mx Thiazzi Anakrazia[1], Prof Peter Weightman[1], Prof. Sarah Coupland[2], Dr. Steve Barrett[1], Dr Caroline Smith[1], Dr. Helen Kalirai[2], Dr. Karen Aughton[2], Dr Gianfelice Cinque[3]

[1]Department of Physics, University Of Liverpool, Liverpool, United Kingdom, [2]Liverpool Ocular Oncology Research Group's (LOORG's) Molecular and Clinical Cancer Medicine Institute of Systems, Molecular and Integrative Biology University of Liverpool, , United Kingdom , [3]B22: Multimode InfraRed Imaging And Microspectroscopy (MIRIAM), Diamond Light Source Ltd, , United Kingdom

There are major problems in identifying and characterising the molecular processes that initiate and drive cancers. The aim of this project is to utilise advances in infrared spectroscopy and microscopy to improve the understanding of Uveal Melanoma.It is hoped that this approach will lead to the development of improved methodologies for managing the disease in the form of a non-invasive probe for early diagnosis and prognosis. To this end five Uveal Melanoma cell lines with known genetic profiles have been chosen on the basis of their metastatic risk, specifically the status of Chromosome 3 loss which has a strong correlation with metastasis. Which are then imaged with an FTIR and analysed using a novel ensemble machine learning algorithm (MLA), known as Metrics Analysis, that uses wavenumber ratios as votes for discrimination. Unlike other machine learning techniques that discriminate on the basis of statistical variations within the data, our approach has the benefit of an intrinsic biological basis as any wavenumber identified as being important for discrimination is itself the product of the biology of the sample. Initial results on FPA data analysed by this MLA is extremely encouraging, with an above 90% confidence of discrimination for all five cell lines.