Physics of Cells: From Biochemical to Mechanical (PhysCell 2018)

3–7 September 2018
The Majestic Hotel, Harrogate, UK

EMBO
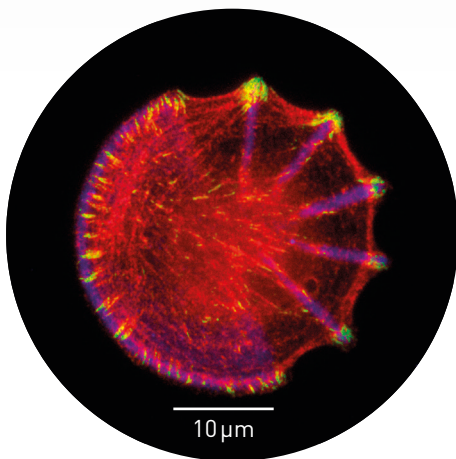*Workshop*
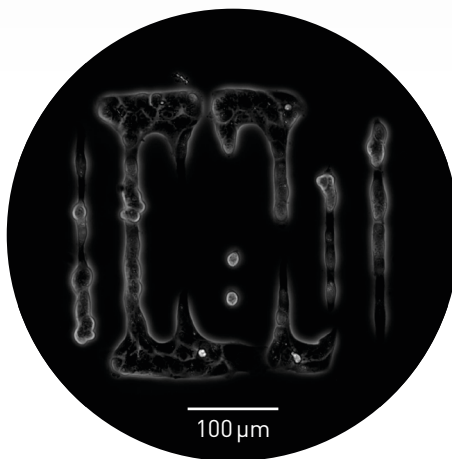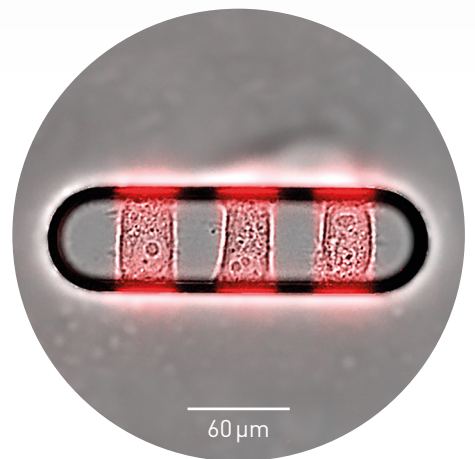
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IOP Institute of Physics

Physics of Cells: From Biochemical to Mechanical (PhysCell 2018)

Welcome

After much planning I am happy and excited to welcome you to PhysCell 2018. This is the 4th in the EMBO workshop series "Physics of Cells" with previous meetings held in Croatia, France and Germany. Welcome to the UK, this year's host country and to the historic Yorkshire spa town of Harrogate. Prepare yourself to be inspired this week with fantastic new science, new friends and a taste of the proud county of Yorkshire. The Yorkshire Dales (big green patch on google maps) means Yorkshire Valleys and is a national park known for its outstanding scenery, walking, cycling, castles, abbeys, idyllic villages, country pubs, local beers, sheep, dry stone walls, rolling green hills and wild heather moors.

The ethos of this workshop is discussion and sharing of ideas. Whether you are a new student or an emeritus professor we want you to both contribute and learn from this week. After each talk by our eminent invited speakers there will be 10 minutes of questions/discussion. This is for everyone to join in. A free drink will be offered to the first student comment/question after each talk so get your hand up fast! The contributed talks, flash presentations, poster sessions and exhibition stands will highlight some of the excellent talent and fantastic science in our field. There are poster prizes to be won and previous workshops have had very lively enthusiastic poster sessions so please make the most of the time and space to discuss during these sessions. The Physics of Cells is a very interdisciplinary field and there will be people here from a wide variety of scientific backgrounds. Please bear this in mind when you are talking about your own work - be prepared to explain the basics of your own sub field and don't be shy to ask basic questions. There are no 'stupid' questions - sometimes a seemingly simple question from someone from a different background can lead to the next ground breaking research project. You have 3 years to work on it to present it at the next PhysCell meeting! Please make the most of the social side of this workshop - your next scientific success may happen at the bar or during the excursion. Please treat everyone you meet with respect whatever their background, gender, ethnicity, age etc.

I hope this conference leads you to a new project idea, collaboration, friend, postdoc, breakthrough in your current work or just generally inspires you. Enjoy the science and the social side of this conference!

Rhoda Hawkins

Local Organising Committee Chair
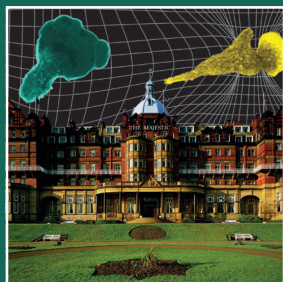
Programme

Monday 3 September

08:00–09:00	Breakfast *Reading and drawing room*	
09:00–13:00	BIOPOL satellite meeting *Carriage Suite*	
10:00–13:00	Registration *Reception Room*	
13:00	Lunch *Main Dining Room*	

Session: Cytoskeleton
Carriage Suite

14:00	**(invited) Disassembling actin filaments with proteins and mechanical stress** Guillaume Romet-Lemonne, Institut Jacques Monod, CNRS/ University Paris Diderot, France	
14:30	Questions and discussion	
14:40	**(invited) A systems view on starfish surface contraction waves: from cell cycle regulation through actomyosin contractility to cytoplasmic flows** Ulrich Schwarz, Heidelberg University, Germany	
15:10	Questions and discussion	
15:20	**Role of the Arp2/3 complex in the architecture and propagation of actin waves** Marion Jasnin, Max Planck Institute of Biochemistry, Germany	
15:40	**Controlling the mechanical and biochemical properties of cell culture substrates** Pierre-Olivier Strale, Alvéole, France	
15:45	Coffee break *Main Dining Room*	

Session: Membranes
Carriage Suite

Biopol

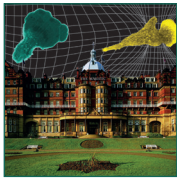|		
16:10	**(invited) Buckling of epithelium growing under spherical confinement** Aurelien Roux, University of Geneva, Switzerland	
16:40	Questions and discussion	
16:50	**(invited) The OrganoPlate: Human organ-on-a-chip tissue models for predictive drug testing in any throughput** Henriette Lanz, MIMETAS, The Netherlands	
17:20	Questions and discussion	

17:30	**Cargo composition and clathrin light chains determine the mode of membrane bending by the clathrin lattice** Hannes Maib, University of Sheffield, UK	
17:50	**(invited) Mechanical regulation of cell membranes revealed by model membrane systems** Margarita Staykova, Durham University, UK	
18:10	Break and discussion time	
19:00	Dinner *Billiard Room*	

Keynote lecture
Carriage Suite

20:30	**(invited) From skin to brain, cyclic strain is a potent cue for our cells** Rudolf Merkel, Forschungszentrum Jülich, Germany

Tuesday 4 September

08:00–09:00	Breakfast *Reading and drawing room*

Session: Development
Carriage Suite

Biopol

9:00	**(invited) Epithelial order and planar polarity: uncoupling the coupled** David Strutt, University of Sheffield, UK
09:30	Questions and discussion
09:40	**Physical model of non-polarized cell migration during the epiboly of zebrafish embryo** Rodrigo Soto, Universidad de Chile, Chile
10:00	**Control of zippering by transient cytoskeletal scar during dorsal closure** Amélie Godeau, Centre de Regulació Genòmica (CRG), Spain
10:20	**Plasma membrane and cell surface mechanics in embryonic stem cells** Henry De Belly, University College London, UK
10:40	Coffee break *Main Dining Room*

Session: Nucleus
Carriage Suite

11:10	**(invited) Matrix Mechanosensing: from scaling concepts in 'Omics data to mechanisms in the nucleus and tumor heterogeneity** Dennis Discher, University of Pennsylvania, US
11:40	Questions and discussion
11:50	**(invited) Polymer choreography in the nuclear pore complex** Bart Hoogenboom, University College London, UK
12:20	Questions and discussion
12:30	**Laminar density determines formation mechanism of nuclear blebs** Dan Deviri, Weizmann Institute of Science, Israel
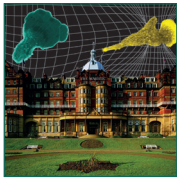
13:00	Lunch *Main Dining Room*

Session: Tissues
Carriage Suite

14:00	**(invited) Shaping cell contacts during tissue morphogenesis** Pierre-Francois Lenne, IBDM/Aix-Marseille University/CNRS, France
14:30	Questions and discussion
14:40	**(invited) Living tissues as active materials** Cristina Marchetti, Syracuse University, USA
15:10	Questions and discussion
15:20	**Polarity-induced gradients in surface tension drive the positioning of sensory hair cells to form a mirror symmetric organ** Anna Erzberger, The Rockefeller University, USA
15:40	Coffee break *Main Dining Room*

Session: Mechanical and biochemical signalling
Carriage Suite

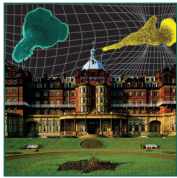|	
16:10	**(invited) Mechanical signaling and cell fate** Kevin Chalut, University of Cambridge, UK
16:40	Questions and discussion
16:50	**Mechanical communication in cardiac cell beating and in the sensory nervous system** Shelly Tzlil, Israel Institute of Technology, Israel
17:10	**mTORC1 controls phase separation and the biophysical properties of the cytoplasm by tuning crowding** Liam Holt, New York University, USA
17:30	**Cell context-dependent CD95 activation drives apoptosis or tumorigenesis by CD95L pre-confinement** Cornelia Monzel, Heinreich-Heine University, Germany
17:50	**Flash poster talks 1** *Carriage Suite* **F.P1: Flash poster**
18:10–19:00	Break and discussion time
18:15–19:00	Committee and advisory board meeting
19:00	Dinner *Terrace*
20:30	**Poster session 1** *Main Dining Room* **P1: Poster**

Physics of Cells: From Biochemical to Mechanical (PhysCell 2018)

EMBO *Workshop*

Wednesday 5 September

08:00–09:00	Breakfast *Reading and drawing room*

Session: Fibres, bundles and extracellular matrix
Carriage Suite

09:00	**(invited) How collections of regulatory proteins give rise to actin bundles** Jenny Gallop, The Gurdon Institute, UK
09:30	Questions and discussion
09:40	**(invited) Mechanics of biological soft matter across scales** Gijsje Koenderink, AMOLF, The Netherlands
10:10	Questions and discussion
10:20	**Mechanical interaction of cells with the fibrous non-linear elastic environment** Ayelet Lesman, Tel-Aviv University, Israel
10:40	Coffee break *Main Dining Room*

physical**biology**

Session: Cell division
Carriage Suite

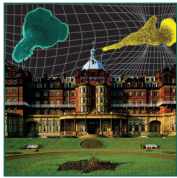|	
11:10	**(invited) Cell Division: mechanical integrity with dynamic parts** Sophie Dumont, University of California San Francisco, USA
11:40	Questions and discussion
11:50	**(invited) Active contraction or expansion of disordered cytoskeletal networks** Francois Nedelec, European Molecular Biology Laboratory, Germany
12:20	Questions and discussion
12:30	**Flash poster talks 2** *Carriage Suite* — **F.P2: Flash poster**
13:00	Lunch *Main Dining Room*
14:00	Excursions
18:00	Dinner *Billiard Room*
19:30	**Poster session 2** *Main Dining Room* — **P2: Poster**

Physics of Cells: From Biochemical to Mechanical (PhysCell 2018)

EMBO Workshop

Thursday 6 September

08:00–09:00	Breakfast *Reading and drawing room*

Session: Microbes and pathogens
Carriage Suite

09:00	**(invited) Physical virology: unveiling self-assembly principles and mechanics of viral particles** Wouter Roos, Rijksuniversiteit Groningen, The Netherlands
09:30	Questions and discussion
09:40	**(invited) Adaptive division control and cell shape regulation in bacteria** Shiladitya Banerjee, University College London, UK
10:00	**(invited) Bacterial collective behaviour** Knut Drescher, Max Planck Institute for Terrestrial Microbiology, Germany
10:30	Questions and discussion
10:40	Coffee break *Main Dining Room*

Session: DNA/chromatin/epigenetics
Carriage Suite

11:10	**(invited) Single molecule manipulation and imaging of complex DNA-protein transactions** Gijs Wuite, Vrije Universiteit, The Netherlands
11:40	Questions and discussion
11:50	**(Invited) Nuclear reprogramming: a leap forward through mechanobiology** G V Shivashankar, National University of Singapore, Singapore/ FIRC Institute of Molecular Oncology (IFOM), Italy
12:20	Questions and discussion
12:30	**Sperm chemotaxis in turbulent flows** Steffen Lange, TU Dresden, Germany
13:00	Lunch *Main Dining Room*

Session: Adhesion
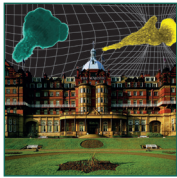*Carriage Suite*

14:00	**(invited) Integrin adhesions at the crossroad between microtubules and the actomyosin cytoskeleton** Alexander Bershadsky, National University of Singapore, Singapore
14:30	Questions and discussion
14:40	**(invited) Physical effects in cell adhesion** Kheya Sengupta, Centre Interdisciplinaire de Nanoscience de Marseille, France
15:10	Questions and discussion
15:20	**Control over the mechanical interface between fibronectin and silicone elastomers regulates fibroblast adhesion and polarization** Dimitris Missirlis, Max-Planck-Institute for Medical Research, Germany

15:40	Coffee Break *Main Dining Room*

Session: Migration and microswimmers
Carriage Suite

16:10	**(invited) Oscillations in single-cell motility: simple one-dimensional models** Nir Gov, Weizmann Institute, Israel
16:40	Questions and discussion
16:50	**(invited) Exploring protein-signaling stochasticity by FRET in single cells** Tom Shimizu, AMOLF, The Netherlands
17:20	Questions and discussion
17:30	**Spiral actin waves as modulators of dendritic cells random movement** Franziska Lautenschlager, Saarland University, Germany
17:50	**Curvotaxis directs cell migration through cell-scale curvature landscapes** Laurent Pieuchot, CNRS, France
18:10	Break and Discussion time
19:00	Drinks reception and conference dinner *Main Dining Room*

Friday 7 September

08:00–09:00	Breakfast *Reading and drawing room*

Session: Protein/membrane systems
Carriage Suite

| 09:00	**(invited) Physics of cell adhesion: The role of the membrane in the protein recognition process** Ana-Suncana Smith, FAU Erlangen-Nürnberg, Germany and Institute Ruđer Bošković, Zagreb, Croatia
09:30	Questions and discussion
09:40	**(invited) Evolutionary self-organisation: lessons from the polarisation machinery in budding yeast** Liedewij Laan, Delft University of Technology, The Netherlands
10:10	Questions and discussion
10:20	**Phospho-regulation of Tropomyosin Cdc8 during cytokinesis is crucial for actin cable turnover in fission yeast** Darius Koester, Warwick University, UK
10:50	Coffee break *Main Dining Room*

Session: Cell mechanics
Carriage Suite

11:10	**(invited) Actin flows in cell migration: from locomotion to trajectories** Raphael Voituriez, CNRS/Sorbonne Universite, France
11:40	Questions and discussion
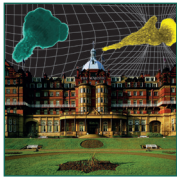
| 11:50 | **Active prestress leads to an apparent stiffening of cells through geometrical effects** |
| | Elisabeth Fischer-Friedrich, TU Dresden, Germany |

Keynote lecture
Carriage Suite

| 12:10 | **(Invited) Polarized Cell locomotion in soft tissues mediated by microtubule actin crosstalk** |
	Erich Sackmann, Technical University Munich, Germany
12:40	Questions and discussion
12:50	Closing remarks
13:00	Lunch and departure
	Main Dining Room

Flash Poster programme
Carriage Suite

FP.1 Tuesday 4 September		FP.2 Wednesday 5 September	
FP1.1 17:50	**Formin processivity on actin bundles** Emiko Suzuki, Institut Jacques Monod / CNRS, France	**FP2.1** 12:40	**Simultaneous cell tracking and visualization of flagellar dynamics of *Pseudomonas putida* in chemoattractant gradient** Zahra Alirezaeizanjani, Potsdam university, Germany
FP1.2 17:54	**Active torque generation by actomyosin cytoskeleton drives chiral cell-cell rearrangement** Lokesh Pimpale, TU Dresden, Germany	**FP2.2** 12:44	**Breast cancer cell migration in the bone microenvironment** Natasha Cowley, University of Sheffield, UK
FP1.3 17:58	**Cultured vs. mechanically isolated muscle cells: is there any biomechanical difference?** Karla Garcia-Pelagio, Universidad Nacional Autonoma de Mexico, Mexico	**FP2.3** 12:48	**Ligand-free EGFR activity enhances E-cadherin junction formation** Chaoyu Fu, National University of Singapore, Singapore
FP1.4 18:02	**Buckling of epithelium by apical-only actomyosin action** Jocelyn Etienne, CNRS — University Grenoble Alpes, France	**FP2.4** 12:52	**The role of tip pressure in fungal growth: mechanical and microfluidics study of *Aspergillus nidulans* and mutants** Blanca González Bermúdez, Universidad Politécnica de Madrid, Spain
FP1.5 18:06	**Traction forces mediate cell activity during cell polarization** Zeno Messi, Ecole Polytechnique Federale De Lausanne, Switzerland	**FP2.5** 12:56	**Structure and dynamics of the trypanosoma brucei plasma membrane** Marie Schwebs, University Würzburg, Germany
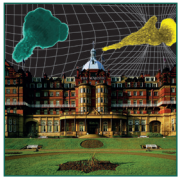
Poster programme

Main Dining Room

P.1 Tuesday 4 September

P1.1 Elastic properties of cytoskeletal networks in confinement and implications for mechanics of cells
Somiealo Azote, Stellenbosch University, South Africa

P1.2 Structural stability of a mitotic spindle: parametric finite element approach
Andrii Iakovliev, University of Southampton, UK

P1.3 Cell cortex structure and dynamics before, during, and after adhesion
Emmanuel Terriac, Leibniz Institute for New Materials, Germany

P1.4 Tension in the actomyosin cortex from 3D simulations
Jiri Pesek, KU Leuven, Belgium

P1.5 Actin organization in cells responding to a perforated surface, revealed by live imaging and cryo-electron tomography
Marion Jasnin, Max Planck Institute of Biochemistry, Germany

P1.6 Global turnover of contractile actin in frog egg extract
Jianguo Zhao, Georg-August-Universität Göttingen, Germany

P1.7 Investigating the collective behaviors of motor proteins pulling a cargo along cytoskeletal filaments
Naruemon Rueangkham, University of Sheffield, UK

P1.8 Modelling force generation in phagocytosis
James Bradford, University of Sheffield, UK

P1.9 Hydro-osmotic instabilities in active membrane tubes
Sami Al-Izzi, University of Warwick, UK

P1.10 The flexibility and dynamics of the tubules in the endoplasmic reticulum
Thomas Waigh, The University of Manchester, UK

P1.11 Glycosphingolipid- and lectin-dependent endocytosis studies using a chemical biology approach
Joanna Zell, Institut Curie, France

P1.12 Narrow escape: how long does it take for a camel to go through the eye of a needle?
Elisabeth Meiser, Universität Würzburg, Germany

P1.13 Membrane structural remodeling upon stress/compression
Celine Dinet, Durham University, UK

P1.14 Minimal molecular dynamics model provides insight into the connection between the structure and mechanics of ESCRT-III filaments
Lena Harker-Kirschneck, University College London, UK

P1.15 Simulation of the thermal fluctuations of red blood cells with the inclusion of hydrodynamic interactions
Thomas Hunt, University of Kent, UK

**P1.16 The actomyosin cytoskeleton drives spontaneous folding of hydra fragments**
Xinpeng Xu, Guangdong Technion - Israel Institute of Technology, China

P1.17 Modeling of mechanical forces during annual fish pre-epiboly and epiboly
Fernanda Pérez, Universidad de Chile, Chile

P1.18 Deformation experiments of MDCK II model tissue
Simone Gehrer, Friedrich Alexander Universität Erlangen-Nürnberg, Germany
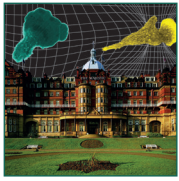
P1.19 Curvature-dependent control of oriented epithelial tissue growth by anisotropic cell-scale topography
Pablo Rougerie, Universidade Federal do Rio de Janeiro, Brazil

P1.20 Tissue fluidity promotes epithelial wound healing
Michael Staddon, University College London, UK

P1.21 A role for autophagy in YAP/TAZ dependent tumor plasticity and somatic cell reprogramming
Qiuyu Zhuang, University of Padova, Italy

P1.22 Investigating the dynamics of apico-medial Myosin-II foci
Nilankur Dutta, Universite Grenoble Alpes, France

P1.23 Geometry and resilience of biological transport networks: Local rules for robust global transport in liver networks
Jens Karschau, TU Dresden, Germany

P1.24 High-throughput platform for rapid TEER measurement of Organ-on-a-Chip endothelial and epithelial tubules
Arnaud Nicolas, Biopol - Mimetas B.V., The Netherlands

P1.25 Interplay between tissue organisation and planar cell polarity
Sara Tan, University of Sheffield, UK

P1.26 Constricted migration increases DNA damage and independently represses cell cycle
Charlotte Pfeifer, University of Pennsylvania, USA

P1.27 The dystroglycan LINC: the functions of dystroglycan in the nuclear envelope
Ben Stevenson, University of Sheffield, UK

P1.28 The reduced approach to the stochastic modelling of cooperative Ca^{2+} release through IP_3R channels yields the global characteristics of the cell regulation
Svitlana Braichenko, University of Southampton, UK

P1.29 Role of hydrodynamic forces in beating orientation of mammalian motile cilia
Nicola Pellicciotta, Cambridge University, UK

P1.30 Deformable active nematic shells
Luuk Metselaar, Rudolf Peierls Centre for Theoretical Physics, UK

P1.31 Emergent hunting behaviors of the unicellular predator Lacrymaria encoded in coordination of its active molecular systems
Scott Coyle, Stanford University, USA

P1.32 Mechanical environment influences macrophage morphology and inflammatory response
Joan-Carles Escolano, TU Dresden, Germany

P1.33 A role for Caveolin-1 as a potential integrator of mechanoadaptive and metabolic networks in the cell
Victor Jiménez, Centro Nacional de Investigaciones Cardiovasculares Carlos III, Spain

P1.34 Regulation of the Hippo pathway via the multi-PDZ domain protein MAGI-1 in epithelial cells
Claire Murzeau, The University of Sheffield, UK

P1.35 Study of the mechanical role of caveolae in 3D tumoral proliferation
Carlos Ureña Martín, Institut Curie, France

P1.36 On the thermodynamic principles of nonlinear acoustic propagation on lipid monolayers
Kevin Heeyong Kang, Technical University Dortmund, Germany

P1.37 Single-molecule biomechanics of HIV binding to broadly neutralising antibodies probed through novel integration of magnetic tweezers with digital holography
James Flewellen, The Francis Crick Institute, UK

P2.38 Nuclear mechanical issues during direct neuronal reprogramming
Marcelo Salierno, King's College London, UK
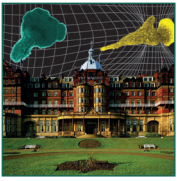
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Poster programme

Main Dining Room

P.2 Wednesday 5 September

P2.1 Epithelial cell proliferation under 3D constraint
Ilaria Di Meglio, University of Geneva, Switzerland

P2.2 Directly probing how kinetochore-fibers are locally anchored in the mammalian spindl
Pooja Suresh, University of California, USA

P2.3 Modulation of APC expression in mesenchymal stem cell during nomadic culture on heterogeneous field of elasticity
Satoru Kidoaki, Kyushu University, Japan

P2.4 Structuring of the epithelial tissue
Jakov Lovrić, Ruđer Bošković Institute, Croatia/Friedrich Alexander Universität Erlangen Nürnberg, Germany

P2.5 Reconstituting *in vitro* perineuronal nets, a specialised extracellular matrix structure
Luke Souter, University of Leeds, UK

P2.6 Change in ECM composition affects sensory organ mechanics and function
Abeer Hassan, Israel Institute of Technology, Israel

P2.7 A novel mechanotransduction gene library for RNAi screening of extracellular matrix remodelling-dependent tumor Invasion
Antonio Quílez-Álvarez, Centro Nacional de Investigaciones Cardiovasculares, Spain

P2.8 Migration model of crawling cells driven by persistent fluctuation of cell shape
Hiroyuki Ebata, Kyushu University, Japan

P2.9 Cellular dynamics and cellular preferences for adhesion site geometry on two-state micropatterns
Alexandra Fink, University of Munich, Germany

P2.10 Multi geometry calibration of a cellular potts model
Sophia Schaffer, Ludwig-Maximilians-Universität München, Germany

P2.11 Mechanics of cilia beating: a relationship between metachronal wavelength and fluid flow rate
Jon Hall, University of Sheffield, UK

P2.12 Motility and waves in a hydrodynamic model of confined cell fragments
Ido Lavi, University of Barcelona, Spain

P2.13 Seawater bacteria on technical surfaces: lateral and vertical adhesion forces and nanomechanical properties
Linda Hofherr, Technische Universität Kaiserslautern, Germany

P2.14 Supported lipid bilayer platforms to study cadherin-mediated cell-cell adhesion
Feyza Nur Arslan, IST Austria, Austria

P2.15 Near real time analysis of stress fibre formation in stem cells
Lara Hauke, Georg-August-Universität Göttingen, Germany

P2.16 Actin-spectrin cytoskeleton regulates mechanical responses of neurons
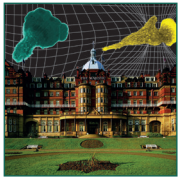Sushil Dubey, Raman Research Institute, India

P2.17 Characterization of the interactions between mesenchymal stem cells and microcarriers
Neda Davoudi, University of Kaiserslautern, Germany

P2.18 Physical model for durotaxis in non-polarized cells
Susana Márquez, Universidad de Chile, Chile

P2.19 Mechanical characterisation of the bone microenvironment by atomic force microscopy for studying breast cancer metastasis
Xinyue Chen, University of Sheffield, UK

P2.20 Cell volume modulation in response to deformations
Larisa Venkova, Institut Curie/Institut Pierre-Gilles de Gennes, France

P2.21 Levy walks in intracellular transport
Daniel Han, The University of Manchester, UK

P2.22 Elasticity threshold of the gel matrix to manipulate migration and differentiation vectors of mesenchymal stem cell
Kosuke Moriyama, Kyushu University, Japan

P2.23 Mimicking tubular environments to study epithelial sensing to curvature
Caterina Tomba, University of Geneva, Switzerland

P2.24 Biomechanics of living skin cells during wound healing and melanoma progression
Barbara Orzechowska, Institute of Nuclear Physics PAN, Poland

P2.25 Microfluidic cell deformation under inertial and shear flow conditions: probing cell structure and determining disease state
Fern Armistead, University of Leeds, UK

P2.26 Unraveling the relationship between nanoscale architecture and force generation in podosomes
Liisa Hirvonen, King's College London, UK

P2.27 Mechanical communication as a noise filter
Ido Nitsan, Israel Institute of Technology, Israel

P2.28 Advanced physical studies of cells by micropipette aspiration
Gustavo R Plaza, Universidad Politécnica de Madrid, Spain

P2.29 Actin crosslinkers and cortex tension during cell division
Neza Vadnjal, University College London, UK

P2.30 Computational study on the interplay between active tension and cortical elasticity in governing cell adhesion mechanics
Bart Smeets, KU Leuven, Belgium

P2.31 Desmoglein-3 acts as a mechanosensor in keratinocytes
Hong Wan, Queen Mary University of London, UK

P2.32 Entrainment and persistence time of beating cardiomyocytes
Ohad Cohen, Weizmann Institute of Science, Israel

P2.33 AFM-based microrheology to quantify viscoelastic properties of cells
Shada Abu Hattum, JPK instruments, Germany

**P2.34 Model based estimation of the mechanical micro-environment inside tissue spheroids**
Maxim Cuvelier, MeBioS, Belgium

P2.35 Exploring the mechanics of phagocytosis
Jaime Cañedo, University of Sheffield, UK

P2.36 The establishment of the patient customized in vitro platform to evaluate CAF-induced anticancer drug resistance
Jung-Yeon Yi, Ministry of Food and Drug Safety, Korea
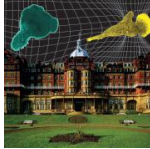
Physics of Cells: From Biochemical to Mechanical (PhysCell 2018)

Session: Cytoskeleton

(invited) Disassembling actin filaments with proteins and mechanical stress

G Romet-Lemonne

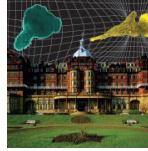Institut Jacques Monod/University Paris Diderot, France

The actin cytoskeleton comprises several networks of filaments which are perpetually assembled and destroyed, in a finely controlled manner. A well-regulated filament disassembly is vital, and proteins of the ADF/cofilin family are the central players in this process. Here, using microfluidics to monitor the action of ADF/cofilin on individual actin filaments, we characterize their ability to sever filaments and promote their disassembly from both ends. Strikingly, we show that the barbed ends of ADF/cofilin-decorated filaments can hardly stop depolymerizing, even when actin monomers and capping proteins are available (Wioland et al. Current Biology 2017). In addition, actin filaments in cells are exposed to various forms of mechanical stress, which are likely to affect the action of regulatory proteins such as ADF/cofilin. Here, we quantify the impact of mechanical tension, curvature and torque on the binding and severing activity of ADF/cofilin. We find that the mechanical context can dramatically increase the rate of filament severing by ADF/cofilin.

(invited) A systems view on starfish surface contraction waves: from cell cycle regulation through actomyosin contractility to cytoplasmic flows

U Schwarz[1], C Brand[1], N Klughammer[1], J Bischof[2] and P Lenart[2]

[1]Heidelberg University, Germany, [2]EMBL Heidelberg, Germany

The mechanics of animal cells is strongly determined by contractility of the actomyosin system, which in turn is regulated mainly through the small GTPase Rho. Through its two main effectors, the formin mDia and the Rho-associated kinase (ROCK), the master regulator Rho makes sure that actin polymerization and myosin activity are coordinated in time and space. Here we discuss the function of the RhoA-module as part of a cell-wide system that steers starfish oocytes through the first (meiotic) cell divisions. Using live cell imaging and a mechanical whole-cell model, we first show that surface contraction waves in starfish oocytes are generated by a traveling band of RhoA-activation and that a negative feedback exists in the Rho-module that is needed to end the contractile phase. We then turn to the regulation of the Rho-module and show that it is activated by removal of a Cdk1-inhibition. Cyclin dependent kinase I (Cdk1) forms a complex with CyclinB that is released into the cytoplasm after nuclear envelope breakdown and then degraded. Because of the asymmetric positioning of the nucleus, this leads to a gradient in Cdk1-CyclinB that guides the wave from the vegetal to the animal pole, as described by a simple reaction-diffusion model and verified by changing cell shape with microfabricated chambers. We finally discuss the downstream consequences of the surface contraction waves. Solving the Stokes equation for a deformable sphere with the observed surface deformations as boundary conditions, we demonstrate that the cytoplasmic flows as measured by particle image velocimetry are fully determined by the cortical contractions. We calculate the resulting pressure gradients and find that they are insufficient to explain polar body extrusion.

Role of the Arp2/3 complex in the architecture and propagation of actin waves

M Jasnin[1], F Beck[1], Y Fukuda[1], M Ecke[1], A M Sanchez[1], J Prassler[1], J Faix[2], G Gerisch[1] and W Baumeister[1]

[1]Max Planck Institute of Biochemistry, Germany, [2]Hannover Medical School, Germany

Assembly and interconversion of specialized actin structures modulate cell shape and movement. Arp2/3 complex-branched organization has been visualized in the quasi-two-dimensional lamellipodial networks at the leading edge of the cell. Here we address actin branch nucleation relative to a polymerizing membrane in a three-dimensional cellular actin structure. Cryo-electron tomography of *Dictyostelium* cells revealed the architecture of actin waves propagating at the ventral membrane. Characteristic structural elements of the waves are clusters of actin filaments arising in a tent-like array at high angle from the membrane, intertwined with a dense meshwork of crosslinked actin filaments along the membrane. Subtomogram averaging provided the native structure of Arp2/3-nucleated branch junctions at 3.1 nm resolution. Quantitative analysis of branch orientation showed that actin polymerization is independent of wave propagation direction. Branch nucleation is clustered in the tent-like arrays, originating from filaments along the membrane and directed toward the ventral membrane, including at high angles from the membrane. Location of the Diaphanous-related formin B (ForB) in the actin waves suggests a potential regulation of Arp2/3-mediated actin polymerization by ForB. We propose a model of actin wave propagation, in which formin-nucleated filaments along the membrane may serve as mother filaments for Arp2/3-nucleated and formin-elongated tent-like arrays.

Controlling the mechanical and biochemical properties of cell culture substrates

P O Strale

Alvéole, France

In vivo, the cellular microenvironment has a crucial impact on the regulation of cell behavior and functions such as cellular differentiation, proliferation and migration. One of the challenges confronting cell biologists is to mimic this microenvironment *in vitro* in order to more efficiently study living cells and model diseases. Here, we present PRIMO : a contactless and maskless UV projection system, which allows to control both the mechanical and biochemical properties of *in vitro* microenvironments.
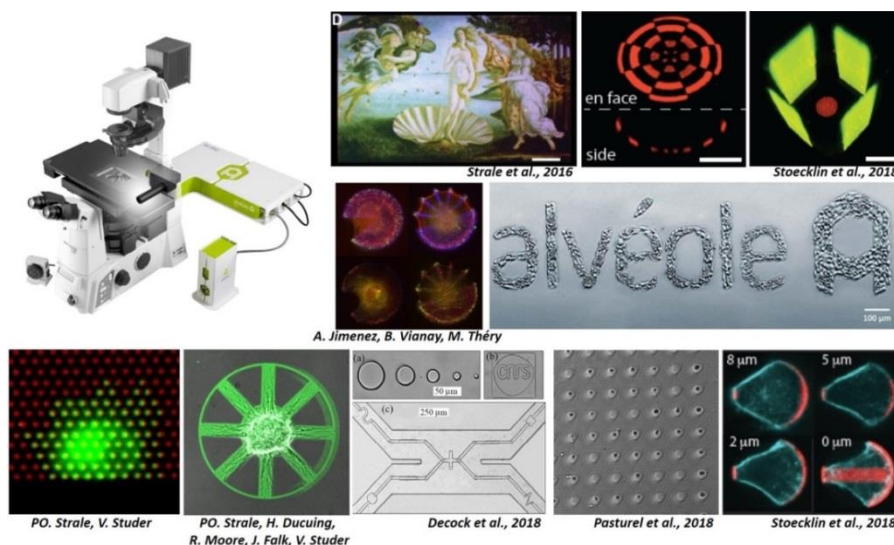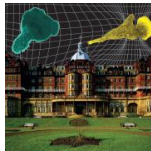
Fig. 1 : A glimpse of PRIMO and its applications - 2D and 3D micropatterning for single/pluri cells experiments, photopolymerization of UV-sensitive materials, structuration/functionnalization of hydrogels.

Session: Membranes

(invited) Buckling of epithelium growing under spherical confinement

A Trushko[1], I di Meglio[1], A Merzouki[1], C Blanch-Mercader[1], S Abuhattum[2], J Guck[2], K Alessandri[3], P Nassoy[3], K Kruse[1], B Chopard[1] and A Roux[1]

[1]University of Geneva, Switzerland, [2]Technische Universität Dresden, Germany, [3]Université de Bordeaux/CNRS and Institut d'Optique, France

Many organs, such as the gut or the spine are formed through folding of an epithelium. While genetic regulations of cell fates leading to epithelium folding have been investigated, mechanisms by which forces sufficient to deform the epithelium are generated are less studied. Here we show that cells forming an epithelium onto the inner surface of spherical elastic shells protrude inward while growing. By measuring the pressure and local forces applied onto the elastic shell, we show that this folding is induced by compressive stresses arising within the epithelial layer: while growing under spherical confinement, epithelial cells are subjected to lateral compression, which induces epithelium buckling. While several fold initiations can be observed within one capsule, final shapes often show one or two folds. While analytical theory of epithelium buckling predicted a single fold at equilibrium, multicellular simulations showed several folds occurred from a competition between epithelium bending, growth and adhesion to the shell. By quantitatively comparing the shapes of buckled epithelium predicted by theory and simulations, with experimental shapes, we determined how epithelium bending rigidity, adhesion and proliferation control buckling, and extracted their values in our experimental conditions. As proposed for gastrulation or neurulation, our study shows that forces arising from epithelium proliferation are sufficient to drive epithelium folding.
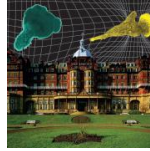
(invited) The OrganoPlate: Human organ-on-a-chip tissue models for predictive drug testing in any throughput

H Lanz

MIMETAS, The Netherlands

Organ-on-a-chip has recently emerged as the new paradigm in enhanced, 3D tissue culture. The field builds on almost 26 years of developments in microfluidic and associated microfabrication techniques on the one hand and an urge towards ever more physiologically relevant cell and tissue culture approaches on the other hand. Application of microengineering techniques in cell culture enables structured co-culture, 3D culture, the use of flow and associated shear stress and application of controlled gradients. MIMETAS develops a commercially available platform based on a microtiter plate format that harbors up to 96 chips and enables perfused 3D co-culture in a membrane-free manner[1]. The OrganoPlate® facilitates growth of tubules and blood vessels under continuous flow of medium, it allows engineering of organ complexity without usage of artificial membranes. The OrganoPlate® is fully compatible with liquid handling equipment and high-content readers and is easily adopted by end-users. Current flagship models in OrganoPlates® comprise the human kidney proximal tubule, central nervous system[2], colon[3], liver and blood vessels[4].

[1]   S. J. Trietsch, G. D. Israëls, J. Joore, T. Hankemeier, and P. Vulto, "Microfluidic titer plate for stratified 3D cell culture.," Lab Chip, vol. 13, no. 18, pp. 3548–54, Sep. 2013.
[2]   N.R. Wevers, R. van Vught, K.J. Wilschut, A. Nicolas, C. Chiang, H.L.Lanz, S.J.Trietsch, J.Joore and P.Vulto, "High-throughput compound evaluation on 3D networks of neurons and glia in a microfluidic platform.," Scientific Reports, vol. 6, pp. 38856, Dec. 2016.
[3]   S.J. Trietsch, E. Naumovska, D. Kurek, M.C. Setyawati, M.K. Vormann, K.J. Wilschut, H.L. Lanz, A. Nicolas, C.P. Ng, J. Joore, S. Kustermann, A. Roth, T. Hankemeier, A. Moisan, and P. Vulto.

"Membrane-free culture and real-time barrier integrity assessment of perfused intestinal epithelium tubes.," Nat Commun, vol 8, no. 1, pp. 262, Aug 2017.

[4]   V. van Duinen, A. van den Heuvel, S. J. Trietsch, H.L. Lanz, J.M. van Gils, A.J. van zonneveld, P. Vulto and T. Hankemeier. "96 perfusable blood vessels to study vascular permeability in vitro." Scientific Reports, vol. 7, pp. 18071, Dec. 2017.

Cargo composition and clathrin light chains determine the mode of membrane bending by the clathrin lattice
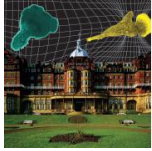
H Maib[1], F Ferreira[1], S Vassilopoulos[2] and E Smythe[1]

[1]University of Sheffield, UK, [2]Institute of Mycology, UK

Clathrin coated pits (CCPs) form when clathrin triskelia, composed of three molecules of clathrin heavy chains and three of clathrin light chains (CLCs) assemble onto the cytoplasmic surface of the plasma membrane. Clathrin-mediated endocytosis (CME) requires a flat membrane patch to be deformed into a spherical vesicle to capture cargo proteins for intracellular transport. The generation of curvature during invagination requires physical forces to overcome the tension and rigidity of the plasma membrane. Currently two distinct models are proposed to explain CCP invagination. In one model of "constant curvature", the membrane is directly deformed by the polymerization of clathrin as coated pits assemble, while the alternative model of "constant area" proposes that flat lattices are initially formed and are subsequently rearranged to gain curvature.

Here we show that the cargo composition of coated pits can bias which mode of curvature generation is followed in a clathrin light chain (CLC) dependent manner. Using Total Internal Reflection Fluorescent- as well as electron-, microscopy, we demonstrate that the CLCs affect the stability of the clathrin lattice and are required for the transition of flat lattices into invaginated buds. This flat-to-curved transition requires extensive rearrangement of the clathrin lattice and Fluorescent Recovery after Photobleaching experiments demonstrate that this rearrangement depends on the CLCs ability to be phosphorylated. On a molecular level, this rearrangement is mediated by the chaperone protein Auxilin and depletion of either the CLCs or Auxilin inhibits the uptake of the G protein-coupled receptor (GPCR), $P2Y_{12}$, but not transferrin receptor. Cargoes such as GPCRs form higher order oligomers and cluster tightly inside CCPs which is predicted to increase the local membrane rigidity whereas the large ectodomain of the Transferrin receptor is likely to prevent this high packing density.

Taken together, these data support a model in which the local concentration of cargo inside a CCP dictates the way it can be deformed. For energetically 'difficult' cargo, such as the $P2Y_{12}$ receptor, this deformation would follow the constant area pathway where CCPs start as flat lattices that require clathrin rearrangement and CLC phosphorylation. However, for energetically less difficult cargo, such as transferrin receptor, CCPs could gain curvature directly through clathrin polymerization along the constant curvature mode, independently from lattice rearrangement and the CLCs or, more likely, as recently shown, by 'variable curvature' which would be defined, at least in part, by the nature of the cargo incorporated by CCPs.

(invited) Mechanical regulation of cell membranes revealed by model membrane systems

M Staykova

Durham University, UK

Cells are well adapted to sustain mechanical perturbations. For example, upon stretch cells can actively increase their surface area by adding more lipids to it and preventing their membranes from rupture; upon compression membrane lipids get removed. However these mechanisms may be too slow to ensure that cells remains intact and functional upon quick mechanical perturbations.

In our work we use model lipid membrane systems to understand the mechanisms behind the quick area and shape regulation in cells. We use microfluidic channels to subject the membrane systems to area dilation or osmotic shock. Our results suggest that the cellular responses can be of simple mechanical nature and that they are critically dependent on the adhesion of the lipid membrane to its neighbouring structures, such as solid substrates, polymeric networks or other lipid membranes. The strength and spatial distribution of the adhesion contacts determine whether a membrane would respond by forming invaginations and protrusions, whether it would slide relative to the neighbouring surface or form pores [1,2]. We compare observations from model systems and living cells to get insights on the mechanics of the cell interface.

[1]   Staykova, M., Arroyo, M., Rahimi, M. & Stone, H. A. Confined bilayers passively regulate shape and stress. *Phys. Rev. Lett.* 110, 1–5 (2013).
[2]   Stubbington, L., Arroyo, M. & Staykova, M. Sticking and sliding of lipid bilayers on deformable substrates. *Soft Matter* 13, 181–186 (2017).

Keynote lecture

(invited) From skin to brain, cyclic strain is a potent cue for our cells

R Merkel

Forschungszentrum Jülich, Germany

Throughout the body each tissue cell is constantly experiencing mechanical signals. A prominent example for which is mechanical strain. To investigate the effect of this signal on cells we developed a device where cells are cultivated on a soft elastomeric substrate that is undergoing predominantly uniaxial strain with carefully controlled amplitude and repeat frequency. All cells studied up to now reoriented and remodeled their cytoskeleton upon cyclic straining. These effects were imaged by immunofluorescence against actin and subsequent digital image processing to quantify reorientation. Due to large sample sizes (> 100 cells) even small effects of e.g. mutations can be detected.

In this talk results on skin cells (keratinocytes) will be presented. Upon addition of Ca^{2+} these cells form strong cell-cell adhesions with at the same time reduced numbers of cell-matrix adhesions. We found that this maturation process is accompanied by a switching of the mechanosensory mechanism from focal adhesions to cell-cell adhesions.

While it is obvious that mechanical strain is a physiologically relevant cue for cells of the skin, the same holds surprisingly also for neuronal cells. First results on cortical neurons exposed to cyclic strain will be presented. The most important observation is that these cells survived cyclic strain of substantial amplitude (up to 30%) for several days. As a result of this signal they reoriented their projections.
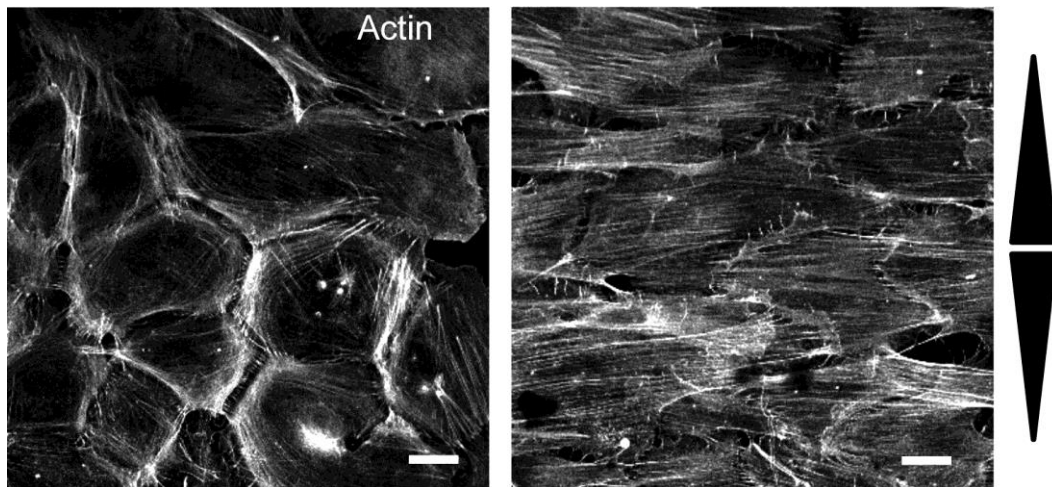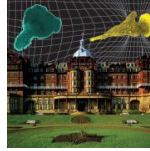
Fig 1: Keratinocytes reaction to cyclic strain (left control, right 14%, 300 mHz, 4 h). Shown are immunofluorescence micrographs against actin, scale bars 20 μm, double arrow indicates strain direction.

Session: Development

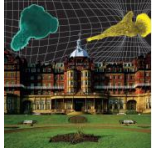### (invited) Epithelial order and planar polarity: uncoupling the coupled

S E Tan, K H Fisher, and D Strutt

University of Sheffield, UK

Epithelial sheets provide a simple 2D context in which to study tissue patterning and organisation. By definition the cells exhibit apicobasal polarity with distinct upper and lower surfaces, and cells are linked to their neighbours via apicolateral adhesions. Within the plane of epithelial sheets two further levels of organisation are evident. One is the orderliness of cell packing, with a regular hexagonal array of evenly-sized cells being an example of a well-ordered state. The other is planar polarisation: the coordinated orientation of cells in the plane of the epithelium, often manifest (for instance) via the polarised production of cytoskeletal elements such as hairs/trichomes.

The developing *Drosophila* pupal wing is a well-established system for studying epithelial organisation. Pioneering quantitative work, in particular from the Eaton lab (Dresden), has established that in the developmental period prior to production of polarised wing hairs, orderliness of both cell packing and cellular planar polarisation increase in concert. Furthermore, disruption of molecular pathways that control planar polarisation can also result in reduction in orderliness of cell packing. Conversely, it is less clear whether disruption of cell packing can reduce the ability of the tissue to planar polarise.

We are taking a number of approaches to attempt to understand the relationship between cell packing and planar polarisation in the epithelium of the *Drosophila* pupal wing. We wish to know how the planar polarisation machinery is affected by different cell shapes, sizes and packing geometries, as a route to understanding molecular mechanisms of tissue planar polarisation. Ultimately, we would also like to understand how planar polarisation affects cell packing and the feedback between these processes. Our starting point has been to develop and validate tools for quantitating cell packing and planar polarisation. We have gone on to use these to confirm the monotonic relationship between orderliness of cell packing and of planar polarisation during normal development. Moreover, we find that this relationship holds in a variety of genetic conditions that lead to altered cell packing and/or cell shape. We therefore conclude that over normal developmental timescales in the developing pupal wing, measures of orderliness of cell packing and planar polarisation are tightly correlated.

To probe the relationship further, we have done experiments where we cause rapid changes in planar polarisation (by turning on planar polarity pathway activity at a particular developmental stage) or rapid changes in cell packing, by using laser cutting to release tissue tensions. Interestingly, these break the relationship between cell packing and planar polarisation. Notably, *de novo* cellular planar polarisation occurs at the same rate in regions of the wing with different degrees of orderliness of cell packing. Furthermore, whereas orderliness of pre-existing planar polarisation is slow to recover in tissue where cell packing has been rapidly disrupted via laser cutting, *de novo* planar polarisation of such disrupted tissue regions occurs rapidly. These results suggest that poor cell packing is not in itself inhibitory to the ability of a tissue to planar polarise, but that remodelling of planar polarity in poorly packed tissues is slow.

Physical model of non-polarized cell migration during the epiboly of zebrafish embryo

R Soto[1], S Marquez[1], E Pulgar[2] and M Concha[1]

[1]Universidad de Chile, Chile, [2]Universidad Austral, Chile

During the epiboly of zebrafish, the laterality organ progenitor (LOP) cells arise by delamination from the extraembryonic surface epithelium by apical constriction. As LOPs delaminate they retain apical contacts with the epithelium, which expands in the direction of the vegetal pole by mechanical traction (Fig. a). Apical contacts serve as mechanical links that transmit pulling forces from the epithelium to guide the LOP movement. In the process, the apical contacts decline and are lost, resulting in an increasing fraction of LOPs move without attachment to the epithelium. Short- and long-range intra LOP forces cooperate with pulling to protect the integrity of the progenitor cell cluster and allow its collective movement, thus avoiding the loss of organ progenitors. To describe the motion of LOPs, an agent-based model is developed, where LOP cells are described as particles that move on the surface of a sphere. They are subject to random motion, with diffusion coefficients measured in experiments. The traction force due to the attachment to the epithelium is modeled by elastic springs with one end fixed to the pulling zone, which moves at constant speed toward the vegetal pole (Fig. b). For the intra LOP forces, a history-dependent Lennard-Jones potential is used, with potential wells that take different values depending if cells are in the attraction or in the adhesion regime. The rates of cell division and of attachment loss are taken from the experiments. Finally, the values of the potential wells are fitted to reproduce the experimental clustering of LOPs, center of mass displacement, cell fluctuation during the motion, and average number or attached-nonattached pairs. Simulations of the model reproduce the global motion of LOPs, their spatial distribution, and the fraction of lost cells (Fig. c). Our findings reveal a tissue-guided developmental mechanism that integrates the origin, morphogenesis and fate specification of progenitor cells at early stages of organogenesis in a vertebrate.
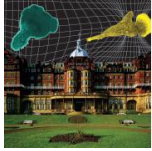
Control of zippering by transient cytoskeletal scar during dorsal closure

A Godeau[1], M M Riera[2], T Pujol[1], A d'Angelo[1], P Hayes[1], J Colombelli[1], J Sharpe[2] and J Solon[1]

[1]Centre de Regulació Genòmica (CRG), Spain, [2]European Molecular Biology Laboratory, Spain

Epithelial sealing is a fundamental process occurring during animal development and wound healing. Dorsal Closure (DC), in Drosophila melanogaster development, is a classical model to investigate the mechanisms underlying epithelial sealing. The completion of DC involves a tissue zippering that relies on microtubule dependent filopodia (Jankovics et al., 2006) leading eventually to the controlled sealing of epidermal segments (Jacinto et al, 2000). For epithelial sealing to progress efficiently, the angle at the zipping point (or canthus) has to be controlled and reduced to allow the maximum contact between the two epidermal leaflets. How the zipping point angle and consequently zipping progression is controlled still remains elusive.

Here we show that, during DC, while the tension within an actin cable at the leading edge cells increases, the angle at the canthus diminishes, correlating with an increase in zipping velocity. We find that the zipping angle is set by a force balance that is actively controlled by the epidermal tissue. During the zipping process the actin cable surrounding the epidermal gap remains transiently stable within the epithelium after zipping and forms a "cytoskeletal scar".

This scar is connecting the zipping point to the closest segment boundaries (SB) and generating the mechanical tension necessary for the reduction of the zipping angle. Only once an epidermal segment is entirely sealed, scar disassembly will occur, leading to a sequential assembly and release of segments during zipping. Reducing the tension within segment boundaries via Hedgehog overexpression directly affects the zipping angle and slows down zipping progression. This indicates that the SBs are generating forces together with the scar to reduce zipping angle, as in a tug of war.

In addition, we find that the scar is reinforced by the attachment of the Amnioserosa tissue (AS), underneath the epidermis, to segment boundaries. These AS-SB attachments are sequentially removed, together with scar disassembly, after whole segment zipping. Probing AS tension with laser dissection or disrupting AS-SB adhesion by genetic interference, reveals a force contribution of the attached AS tissue to the tension

generated by cytoskeletal scar. Interestingly, this contribution is dependent on the microtubule cytoskeleton and the molecular motor dynein rather than actin and myosin.

A simple physical description, based on vertex model, shows that scar maintenance allows a reduction in the angle at the canthus due to force balance. Furthermore, we model zipping process in absence and with SBs confirming experimental observations.

We propose that the interplay of the epidermal scar and the AS enables the embryo to counteract the increasing force of the actin cable during DC, controlling the zippering angle and thus allowing epithelial fusion progression.

[1]   Jankovics, F. & Brunner, D. Transiently Reorganized Microtubules Are Essential for Zippering during Dorsal Closure in Drosophila melanogaster. *Dev. Cell* 11, 375–385 (2006).
[2]   Jacinto, A. *et al.* Dynamic actin-based epithelial adhesion and cell matching during Drosophila dorsal closure. *Curr. Biol.* 10, 1420–1426 (2000).

Plasma membrane and cell surface mechanics in embryonic stem cells

H De Belly[1], K Chalut[2] and E Paluch[1]

[1]University College London, UK, [2]Cambridge University, UK

Embryonic Stem Cells (ESCs) are able to generate any tissue in a given organism; this ability is called naïve pluripotency. When ESCs differentiate and exit naive pluripotency they undergo an important shape change, going from round to spread. Membrane mechanical properties, such as tension, have been shown to influence cell shape and differentiation in many systems. However, very little is known about plasma membrane dynamics and mechanics in ESCs. Here, we investigate the link between the mechanical properties of the plasma membrane and the molecular factors that lead to exit from pluripotency. Using optical tweezers, we show that naïve mouse ESCs have a higher effective membrane tension compared to cells exiting pluripotency. We show that naïve cells have a higher expression and level of activation of protein regulating membrane-to-cortex attachment and that affecting these proteins results in defects in shape change and exit from pluripotency. We also observe that physically preventing spreading using micropatterns also results in defects in exiting pluripotency. Together, our data show that membrane organization and tension regulate shape changes during exit from naïve pluripotency in mouse ESCs. Preventing shape changes, physically or genetically, results in defects from exiting pluripotency. We are currently investigating how shape changes regulate the pluripotency signaling network.
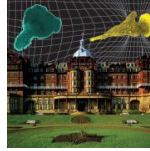
Session: Nucleus

(invited) Matrix mechanosensing: from scaling concepts in 'Omics data to mechanisms in the nucleus and tumor heterogeneity

D Discher

University of Pennsylvania, USA

Scaling concepts have been successfully applied for many years to synthetic polymers, but application to biology seems infrequent even though cells and tissues are built from polymers. Tissues such as brain and fat are very soft while tissues such as muscle and bone are stiff or even rigid – even when probed at the nanoscale, but the effects on cells are just now being discovered. Having shown that matrix stiffness helps specify tissue lineages *in vitro* [1], we developed mass spectrometry algorithms to quantify protein levels in embryonic, mature, and cancerous tissues, and then studied some mechanosensitive factors in cells on

substrates of tuned stiffness [2, 3]. Tissue stiffness is directly determined by extracellular collagen polymers with near-classical scaling, and for embryonic heart, contractile beating of the organ and of isolated cells on gels is maximal when the stiffness is that of the normal tissue. The results are consistent with a 'use it or lose it' mechanism of tension-inhibited degradation. Acto-myosin assembly likewise increases with stiffness and stresses the nucleus, which upregulates a nuclear structure protein called lamin-A (related to keratin in fingernails) that again scales with stiffness via a 'use it or lose it' gene circuit that we solve mathematically. Lamin-A assembly has evolved to control nuclear stiffness and strength, and it varies widely between tissues and diseases including cancer. Differentiation is generally modulated by lamin-A levels downstream of matrix stiffness [2], with various pathways co-regulated by lamin-A. Complementary insights are obtained for DNA damage and repair with stem cells and with cancer cells [4], and evidence of invasion through rigid pores provides insight into scaling of cancer mutation with tissue stiffness [5].
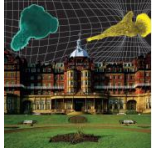
[1] A. Engler, S. Sen, H.L. Sweeney, and D.E. Discher. Matrix elasticity directs stem cell lineage specification. *Cell* 126: 677-689 (2006).

[2] J. Swift, I.L. Ivanovska, A. Buxboim, T. Harada, P.C. D.P. Dingal, J. Pinter, J.D. Pajerowski, K. Spinler, J-W. Shin, M. Tewari, F. Rehfeldt, D.W. Speicher, and D.E. Discher. Nuclear Lamin-A Scales with Tissue Stiffness and Enhances Matrix-directed Differentiation. *Science* 341: 1240104-1 to 15 (2013).

[3] S. Majkut, T. Idema, J. Swift, C. Krieger, A. Liu, and D.E. Discher. Heart-specific stiffening in early embryos parallels matrix and myosin levels to optimize beating. *Current Biology* 23: 2434-2439 (2013).

[4] J. Irianto, Y. Xia, C.R. Pfeifer, A. Athirasala, J. Ji, C. Alvey, M. Tewari, R.R. Bennett, S.M. Harding, A.J. Liu, R.A. Greenberg, and D.E. Discher. DNA damage follows repair factor depletion and portends genome variation in cancer cells after pore migration. *Current Biology* 27, 210–223 (2017).

[5] J. Irianto, C. Pfeifer, Y. Xia, D.E. Discher. Mechanosensing matrix. *Cell* 165: 1820 (2016).

(invited) Polymer choreography in the nuclear pore complex

B Hoogenboom

University College London, UK

The nuclear pore complex (NPC) is a large molecular machine that allows selective macromolecular transport into and out of the cell nucleus. In spite of a wealth of knowledge on the NPC, the mechanism of this transport selectivity remains to be fully elucidated. Much of the difficulty lies in the unstructured, intrinsically disordered nature of the proteins (named FG-nups) in the NPC transport barrier: This makes them poorly amenable to conventional biological approaches. Here I will discuss physics-based approaches to determine the mechanism of NPC transport selectivity. Our approaches include nanomechanical assays, in-vitro reconstituted mimics of the NPC, and computational implementations of polymer physics. In combining these, we show that there can be differently condensed states (or phases) of polymers in the NPC, allowing us to speculate about the transport of large cargoes and about how it may be facilitated by transitions between different collective polymer arrangements.

Laminar density determines formation mechanism of nuclear blebs

D Deviri[1], C Pfeifer[2], L Dooling[2], D Discher[2] and S Safran[1]

[1]Weizmann Institute of Science, Israel, [2]University of Pennsylvania, USA

The cell nucleus is surrounded by a laminar layer, which among other functions, maintains the structural integrity of the nucleus via the viscoelastic response of its lamin protein constituents. Mechanically perturbed laminae are correlated with formation of nuclear blebs (nuclear envelope protrusions) whose rupture can cause DNA damage by exposing chromatin to the cytoplasmic environment. Here, we present measurements that correlate bleb formation with controlled constrictions through which migrating cells must pass. This motivates our theoretical model for hole nucleation which leads us to suggest two molecular mechanisms for laminar hole nucleation: (i) Holes that are initiated by disassembly of proteins that bind lamins, such as the nuclear pore complex. (ii) Holes that result from localized depletion of the lamin filament concentration in homogeneous regions of the lamina. We predict the nucleation rate of the laminar holes for each of these scenarios as a function of the nuclear deformation and compare the theoretical scaling relations to our measurements as well as to previous [1,2] ones. Based on this, we suggest that laminar density determines the mechanism of laminar hole nucleation: lamin-rich nuclei nucleate holes preferentially via nuclear pore disassembly, while lamin-poor nuclei do so by displacing lamin filaments.

[1]   Denais, Celine M., et al. "Nuclear envelope rupture and repair during cancer cell migration." Science (2016): aad7297.
[2]   Raab, Matthew, et al. "ESCRT III repairs nuclear envelope ruptures during cell migration to limit DNA damage and cell death." Science (2016): aad7611.
[3]   Irianto, Jerome, et al. "DNA damage follows repair factor depletion and portends genome variation in cancer cells after pore migration." Current Biology 27.2 (2017): 210-223.
[4]   Swift, Joe, et al. "Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation." Science 341.6149 (2013): 1240104.
[5]   Deviri, Dan, Dennis E. Discher, and Sam A. Safran. "Rupture dynamics and chromatin herniation in deformed nuclei." Biophysical journal 113.5 (2017): 1060-1071.
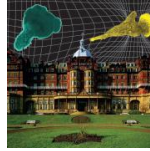
Session: Tissues

(invited) Shaping cell contacts during tissue morphogenesis

P-F Lenne[1,2,3]

[1]IBDM, France, [2]Aix-Marseille University, France, [3]CNRS, France

During animal morphogenesis cell contacts are constantly remodeled. This stems from active contractile forces that work against adhesive forces at cell contacts. Using quantitative approaches, we study the mechanics of cell contacts during early embryogenesis of Drosophila. By analyzing shrinkage and extension of cell junctions and using optical manipulation, we found that junctional deformations are stabilized by mechanical dissipation. To understand how contractile forces transmit to adhesion complexes, we combine mechanical inference, laser ablation and a ratiometric readout based on Vinculin and E-Cadherin fluorescence intensities. We show that tensile and shear stresses have distinct mechanical effects on adhesion complexes.

(invited) Living tissues as active materials

M C Marchetti

Syracuse University, USA

The mechanical properties of dense tissues control many biological processes, from wound healing to embryonic development to cancer progression. Theoretical work combining established developmental models with active matter physics has shown that confluent tissue can exhibit a jamming-unjamming transition tuned by cell shape and cell motility. Cell division and death, as well as mechanical feedback that coordinates cell migration, can modify the transition resulting in novel tissue ``materials'' properties. In this talk I will discuss the implications of these findings for cell sorting and patterning in wound healing and development.

Polarity-induced gradients in surface tension drive the positioning of sensory hair cells to form a mirror symmetric organ

A Erzberger, A Jacobo and A J Hudspeth

The Rockefeller University, USA

Mechanosensory epithelia are highly sophisticated force sensors in the auditory, vestibular and lateral line systems of vertebrates, all of which rely on the mechanoreceptive properties of sensory hair cells. Hair cells display a characteristic polarity in the plane of the epithelium, which governs the orientation of their mechanosensitive apparatus. Although the mechanosensory tissues of many non-mammalian vertebrates possess regenerative capacities, the hair cells of mammals are not replaced after loss or damage, leading to hearing and balance disorders including permanent deafness.

Here we study the cellular processes underlying the development and regeneration of mechanosensory epithelia, focusing on the formation of planar polarity patterns in the neuromasts of the zebrafish lateral line. Neuromast hair cells are arranged in a mirror-symmetric configuration, with oriented cell divisions along the tissue polarity axis giving rise to cell pairs of opposite polarity. Using fluorescent live imaging, we quantify a stereotyped sequence of shape changes and rotational movements that nascent hair cell pairs undergo after division. We find that polarized motile and protrusive activities robustly position the cells within the appropriate tissue polarity regions, giving rise to bilateral organ symmetry. Using a continuum mechanics framework, we investigate the cellular forces arising from opposite cell-intrinsic gradients in effective surface tension, and calculate the resulting cell shape changes and rotational movements, recovering closely the cellular trajectories and contact angle dynamics measured *in vivo*. Genetic perturbations to planar cell polarity signalling or polarity reversal give rise to phenotypes consistent with the predictions of our theory. Taken together, we propose that cells exploit the mechanical effects arising from polarity-regulated spatiotemporal changes in their surface properties to position themselves in a mirror-symmetric polarity pattern which underlies optimal mechanosensory function.

Session: Mechanical & biochemical signalling

(invited) Mechanical signaling and cell fate

K Chalut

University of Cambridge, UK

The role of mechanical signaling in cell fate choice has been largely overlooked; however, it plays a significant role in tuning cellular response to signals. My lab is investigating the interplay between biochemical signaling and mechanical signaling in cell fate decisions. In this talk, I will present a new substrate technology we are employing to show that mechanics tunes the response of stem cells to biochemical signaling to steer fate choice. This hypothetical feedback loop between mechanics and biochemical signaling likely has significant impact on cellular plasticity both in development and stem cells. I will also present an example demonstrating the functional impact of mechanics on stem cell function. In this example, we have shown that we can reverse the loss of plasticity associated with ageing by controlling the mechanical microenvironment. Ultimately, I will advance the hypothesis that mechanical sensing acts as a switch to modulate growth factor signaling to modulate cell fate choice.

Mechanical communication in cardiac cell beating and in the sensory nervous system
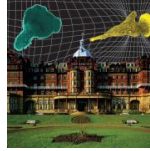
S Tzlil

Israel Institute of Technology, UK

Cell-cell communication enables cells to coordinate their activity and is essential for growth, development and function. Intercellular communication is discussed almost exclusively as having a chemical or an electrical origin, however; recent experiments demonstrate that cells can communicate mechanically by responding to mechanical deformations generated by their neighbors. The characteristics of mechanical communication, its role and its ability to regulate biochemical processes within the cell are still largely unknown.

In this talk, I will describe the progress made in our lab in understanding the role of mechanical communication in cardiac cell beating and in the sensory organ of the fly.

We have recently shown that an isolated cardiac cell can be trained to beat at a given frequency by mechanically stimulating the underlying substrate using a 'mechanical cell'. The 'mechanical cell' consists of an oscillatory probe that mimics the mechanical aspect of a cell by generating substrate deformations identical to the ones induced by a neighboring beating cell. Cardiac cell beating is stochastic and beating cells can go in and out of phase even if beating at the same average frequency. Therefore, continuing on this work, we study the regulation of beating noise by mechanical coupling and the influence of beating stochasticity on cardiac cell pacing. We measure the mechanical interaction between neighboring cells and show that beat-to-beat variability decays exponentially with mechanical coupling and that noise reduction by mechanical coupling is sensitive to the stochastic nature of the 'master' cell. Both pacing and noise reduction persist long after mechanical stimulation stops, implying that mechanical communication induces changes in the biochemical network kinetics that governs spontaneous beating in cardiac cells. By quantitatively measuring the reduction of noise with mechanical coupling strength, we could identify microtubule integrity, NOX2 and CaMKII as key mediators of mechano-transduction.

We also explore the role of the mechanical properties of the connective tissue on muscle-neuron mechanical coupling using the sensory organ of the fly as a model system. Changes in ECM composition alter the viscoelastic properties of the sensory organ and consequently interfere with the propagation of mechanical deformations and proper sensing.

mTORC1 controls phase separation and the biophysical properties of the cytoplasm by tuning crowding

L Holt, M Delarue and G Brittingham

New York University, USA

Macromolecular crowding has a profound impact on reaction rates and the physical properties of the cell interior, but the mechanisms that regulate crowding are poorly understood. We developed Genetically Encoded Multimeric nanoparticles (GEMs) to dissect these mechanisms. GEMs are homomultimeric scaffolds fused to a fluorescent protein. GEMs self-assemble into bright, stable fluorescent particles of defined size and shape. By combining tracking of GEMs with genetic and pharmacological approaches, we discovered that the mTORC1 pathway can tune the effective diffusion coefficient of macromolecules ≥15 nm in diameter more than 2-fold without any discernable effect on the motion of molecules ≤5 nm. These mTORC1-dependent changes in crowding and rheology affect phase separation both *in vitro* and *in vivo*. Together, these results establish a role for mTORC1 in controlling both the biophysical properties of the cytoplasm and the phase separation of biopolymers.

Cell context-dependent CD95 activation drives apoptosis or tumorigenesis by CD95L pre-confinement

C Monzel[1], G S G Balta[2], S Kleber[2], J Beaudouin[3], T Kaindl[4], M Thieman[5], M Tanaka[4] and A Martin-Villalba[2]

[1]Heinreich-Heine University, Germany, [2]German Cancer Research Center, Germany, [3]Université Grenoble Alpes, France, [4]Heidelberg University, Germany, [5]Apogenix AG, Germany

The behaviour of a cell, following an external challenge, is dictated by its cellular context. Cancer cells react to CD95 activation with either apoptotic or tumorigenic responses. Yet, the determinants of these two antithetic reactions are fundamentally not understood. Here, we show that CD95L molecules, pre-confined to a supported lipid membrane and tuned in their nanometric spacing, activate apoptosis in cancer cells *in-vitro.* For particular CD95L pre-confinement at intermediate spacing, signal activation is most efficient. Surprisingly, in tumor models, the same pre-confinement yields enhanced proliferation of cancer cells. This shift is rooted in cell-cell interactions, as proliferation was also observed in tumorspheres *in-vitro*. Indeed, proliferation required death domain tyrosine phosphorylation of CD95 that was facilitated by cell-cell contacts, whereas decreasing the levels of global tyrosine kinase activity favoured apoptosis. Hence, response to CD95 activation is cell context dependent and tunable by CD95L pre-confinement, thus opening therapeutic opportunities in cancer.
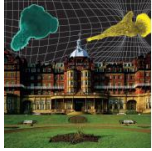
Session: Fibres, bundles & extracellular matrix

(invited) How collections of regulatory proteins give rise to actin bundles

U Dobramysl, I K Jarsch, H Shimo, B Richier, Y Inoue, J Mason, J R Gadsby, A Walrant, R Butler, E Hannezo, B D Simons and J L Gallop

The Gurdon Institute, UK

When filopodia form at the plasma membrane in cells, actin regulatory proteins assemble which incorporate new actin monomers into characteristic bundled architectures. To understand the how these protein assemblies make the actin bundle we have used live imaging in fruit flies and high throughput quantitative imaging of a cell-free model of filopodia-like structures and placed the experimental data within a theoretical framework. We were surprised to discover that the regulatory protein composition is very heterogeneous for otherwise indistinguishable actin structures, while at the same time the actin dynamics is constrained. Growth and shrinkage velocities fall on the bi-exponential Laplace distribution. Our work links redundant and

dynamic subcomplexes of actin regulatory proteins to constrained patterns of actin behaviour. Such a mechanism may allow the maintenance of actin properties when it is co-opted in response to different upstream cellular signals and requirements.

(invited) Mechanics of biological soft matter across scales

G Koenderink

AMOLF, The Netherlands

This talk will focus on the polymeric load-bearing structures that support our cells and tissues. Cells have a fibrous *cytoskeleton,* whilst tissues are supported by the *extracellular matrix*. An extensive body of experimental and theoretical work has shown that fibrous networks have many advantageous mechanical properties. Fibers can form space-filling elastic networks at low volume fractions and at subisostatic connectivities and they reversibly stress-stiffen, which provides protection from damage. Two features of biopolymer networks whose ramifications are less well understood is that they are multiscale and viscoelastic materials because they are built from supramolecular protein assemblies held together by short-lived bonds. I will showcase recent findings on the elastic, viscoelastic and plastic properties of biopolymer networks based on quantitative measurements on reconstituted biopolymer networks, ranging from macroscopic rheological measurements on whole networks to microscopic mechanical measurements on single fibers using optical tweezers.
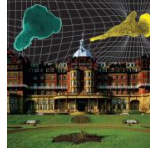
Mechanical interaction of cells with the fibrous non-linear elastic environment

A Lesman, R Sopher, S Goren, Y Koren, S Natan and O Tchaicheeyan

Tel-Aviv University, Israel

Biological cells apply contractile forces against fibrous extracellular matrices (ECM) that exhibit complex mechanics. One of their mechanical properties is the highly non-linear response to applied stress, both in tension and compression. This study is aimed at exploring the effects of elastic nonlinearity of the fibers contained in the ECM, on the transmission of mechanical loads in the ECM and particularly between contracting cells.

I will describe our experimental and computational efforts to understand how cell-induced deformations propagate, decay and distribute throughout the ECM. Our experiments with fibroblast cells embedded in biologically-relevant fibrin gels show that cells align and densify the gel in a highly directed manner toward neighboring cells. This observation is linked with cell shape changes and migration capabilities. We have developed finite-element modeling of one and two contracting cells within a structural fibrous network. The individual fibers were modelled as showing linear elasticity, compression-microbuckling and/or tension-stiffening. Strain-induced alignment of the ECM, fiber compression-buckling and tension-stiffening all induces slower decay of displacements that propagate to a longer-distance. When two cells contract in a non-linear elastic matrix, the distributions of deformations are more concentrated in the inter-cellular region of the matrix and more directed toward the neighboring cell. These results highlight the contribution of ECM non-linear mechanics in supporting long-range transmission of forces, and to the directionality of mechanical-signal transfer between contractile cells. Both can ultimately allow distant cells to mechanically communicate over long distances.

Session: Cell division

(invited) Cell division: mechanical integrity with dynamic parts

S Dumont, C Hueschen, P Suresh and A Long

University of California San Francisco, USA

The spindle is the machine that segregates chromosomes at cell division. To perform its job, the spindle must be flexible and dynamic over short timescales, and yet maintain its mechanical integrity and function over long timescales. How it does so is not understood. How do the spindle's nanometer-scale parts self-organize to form its micron-scale architecture, and how do robust mechanics and function emerge? I will present our recent efforts to understand how the mammalian spindle's steady-state geometry emerges, far from equilibrium, and the consequences of not achieving a steady-state. Then, I will discuss our work aiming to connect molecular-scale and cellular-scale forces in the spindle. Inspired by classic Nicklas experiments, we can now pull on the spindle inside mammalian cells with microneedles: from the spindle's deformation map in different molecular backgrounds, we infer mechanical connectivity between different spindle components and its molecular basis. Looking forward, this work will inform on simple design rules that allow the spindle to be dynamic yet robust – two properties central to the spindle's function.

(invited) Active contraction or expansion of disordered cytoskeletal networks

F Nedelec, J Belmonte and M Leptin

European Molecular Biology Organisation, Germany

The cytoskeleton drives many essential processes in vivo, but for this, the system of filaments will arrange itself into different overall spatial organizations, e.g., random, branched networks, parallel bundles, antiparallel arrays, etc. A general objective of our research is to understand what makes these architectures adapted to their tasks. In this talk, I will first focus on 2D disorganized actin networks in which the filaments are oriented randomly and connected both by active molecular motors and passive crosslinkers. Systems with these properties have been reconstituted in vitro, and serve as a model of the cortical actomyosin networks that drive morphogenesis in animal tissues, or cytokinesis during cell division. Although the network components and their properties are known, the requirements for contractility are still poorly understood. I will describe a theory that predicts whether an isotropic network would contract, expand, or conserve its dimensions, depending on the properties of the filaments and the elements that connect them. This simple theory encompasses mechanisms of contractions previously proposed and predicts how the contraction rate depends for example on the ratio of motors to crosslinkers.
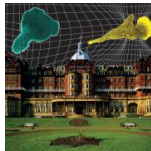
Session: Microbes and pathogens

(invited) Physical virology: unveiling self-assembly principles and mechanics of viral particles

W H Roos

Rijksuniversiteit Groningen, The Netherlands

Cellular life harbours a fascinating variety of complex processes and we are still at the beginning of our understanding of how the cell manages all these processes. Using Atomic Force Microscopy imaging and force spectroscopy we are now making big steps towards the elucidation of the mechanisms behind (supra)-molecular cellular processes [1]. Here we show how we are studying the physics of this fascinating sub-cellular dynamics. This will be illustrated by discussing the mechanics and material properties of viruses and

cellular protein nanocages; In particular by revealing the existence of pre-stress in nanoshells, by scrutinizing the interactions between viral RNA and its capsid and by showing how viral infectivity is in essence a mechanical process [2-4]. Furthermore, recent studies on viral assembly and disassembly will be discussed, as well as dynamic properties of artificial viruses [5].
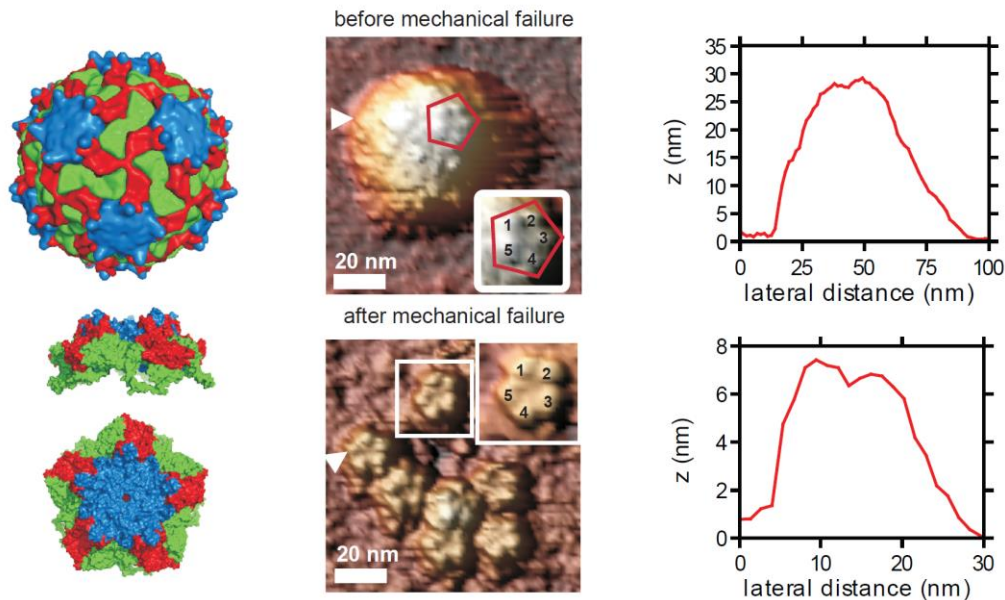
[1]   M. Krieg *et al.* (2018) Nature Physics, *accepted*
[2]   M. Baclayon *et al.* (2011) Nano Letters, 11, 4865
[3]   J. Snijder *et al.* (2013) Nature Chemistry, 5, 502
[4]   J. Snijder *et al.* (2017) J Virol, 91, e00123-17
[5]   Van Rosmalen *et al.* (2018) Biophys J, *accepted*

(invited) Adaptive division control and cell shape regulation in bacteria

S Banerjee

University College London, UK

Control of cell shape is a fundamental adaptive trait that underlies the coupling between cell growth and division. Bacterial cells possess the unique ability to adapt their morphologies in response to environmental cues, thereby translating extracellular information into decisions to grow or divide. However, the quantitative principles and mechanisms relating cell shape and division timing remain poorly understood. In this talk, I will discuss our recent work on adaptive cell size and shape control in bacteria, where the decision to divide is tightly regulated by the spatiotemporal patterning of cell wall growth modes [1-3]. Using a combination of theoretical modelling and single-cell experiments, I will elucidate the implications of the size control model for cellular fitness adaptation under antibiotic stress. In particular, our results show that morphological transformations provide robust fitness and survival advantages to bacteria under sustained and periodic exposure to antibiotics.

[1]   Banerjee S., et al, *Nat. Microbiol* 2, 17116 (2017).
[2]   Banerjee S., et al, *Soft Matter* 12, 3442 (2016).
[3]   Wright C.S., Banerjee S., et al., *Sci Rep* 5, 9155 (2015).

(invited) Bacterial collective behaviour

K Drescher

Max Planck Institute for Terrestrial Microbiology, Germany

In nature, bacteria often engage in a range of collective behaviours. In this presentation, I will demonstrate how two bacterial behaviours, swarming and biofilm formation, are related by physical interactions, chemical signalling, and dynamical transitions. I will show how these collective behaviours arise from cell-cell interactions, and the physiological state of individual cells. Furthermore, I will introduce new experimental methods for investigating bacterial collective behaviours.

Session: DNA/chromatin/epigenetics

(invited) Single molecule manipulation and imaging of complex dna-protein transactions

G Wuite

Vrije Universiteit,The Netherlands

The genetic information of an organism is encoded in the base pair sequence of its DNA. Many specialized proteins are involved in organizing, preserving and processing the vast amounts of information on the DNA. In order to do this swiftly and correctly these proteins have to move quickly and accurately along and/or around the DNA constantly rearranging it. In order to elucidate these kind of processes we perform single-molecule experiments on model systems such as restriction enzymes, DNA polymerases and repair proteins. The data we use to extract forces, energies and mechanochemistry driving these dynamic transactions. The results obtained from these model systems are then generalized and thought to be applicable to many DNA-protein interactions.

In this presentation I will show (Super-resolution) Quadruple Trap Correlative Tweezers-Fluorescence Microscopy (CTFM), a single-molecule approach capable of visualizing individual DNA-binding proteins on densely covered DNA and in presence of high protein concentrations. Moreover, proteins on DNA can be visualized on multiple DNA strand. Using this instrument we have investigated human non-homologous end joining (NHEJ).

NHEJ is the primary pathway for repairing DNA double-strand breaks (DSBs) in mammalian cells. Such breaks are formed, for example, during gene-segment rearrangements in the adaptive immune system or by cancer therapeutic agents. Although the core components of the NHEJ machinery are known, it has remained difficult to assess the specific roles of these components and the dynamics of bringing and holding the fragments of broken DNA together.

I will present data using dual and quadruple-trap optical tweezers combined with fluorescence microscopy, on how human XRCC4, XLF and XRCC4–XLF complexes interact with DNA in real time. We find that XRCC4–XLF complexes robustly bridge two independent DNA molecules and that these bridges are able to slide along the DNA. These observations suggest that XRCC4–XLF complexes form mobile sleeve-like structures around DNA that can reconnect the broken ends very rapidly and hold them together. (Brouwer et al., Nature, doi:10.1038/nature18643 , 2016).

[1]   I. Heller, G. Sitters, O.D. Broekmans, G. Farge, C. Menges, W. Wende, S.W. Hell, E.J.G. Peterman & G.J.L. Wuite, *Nature Methods*, 2013
[2]   Brouwer et al., *Nature*, 2016

(invited) Nuclear reprogramming: a leap forward through mechanobiology

G V Shivashankar[1,2]

[1]National University of Singapore, Singapore, [2]FIRC Institute of Molecular Oncology, Milan, Italy

In landmark experiments, exogenous biochemical factors were shown to enable the reprogramming of somatic cells into induced Pluripotent Stem Cells (iPSC) in vitro. However, in vivo, cells undergo de-differentiation and trans-differentiation in the absence of exogenous factors, thereby suggesting that the local tissue micro-environmental signals are sufficient to induce such transitions. In this talk, I will first describe our efforts in engineering gene expression programs by tuning cell geometry. Then I will show that nuclear reprogramming can be induced solely by cell-geometric constraints (without the need for any exogenous factors) with very high efficiency. Furthermore, I will describe the physiological implications of such reprogramming processes in regulating cell-fate decisions and oncogenic transformations. Importantly, the ability to mechanically reprogram somatic cells has major implications for understanding the onset of various diseases and in regenerative medicine.

[1]   Laterally confined growth of cells induces nuclear reprogramming without any exogenous factors, Roy et al, Proceedings of the National Academy of Sciences-USA (2018-in press)
[2]   Cell geometry dictates TNFα-induced genome response, Mitra et al, Proceedings of the National Academy of Sciences-USA (2017) 114(20) E3882-E3891
[3]   Nuclear deformability and telomere dynamics are regulated by cell mechanical constraints, Makhija et al, Proceedings of the National Academy of Sciences-USA (2016) 113(1):E32-40
[4]   Cell geometric constraints induce modular gene-expression patterns via redistribution of HDAC3 regulated by actomyosin contractility, Jain et al, Proceedings of the National Academy of Sciences-USA (2013) 110(28):11349-11354
[5]   Regulation of genome organization and gene expression by nuclear mechanotransduction, Caroline Uhler & G.V.Shivashankar, Nature Reviews Molecular Cell Biology (2017) 18(12):717-727

Sperm chemotaxis in turbulent flows

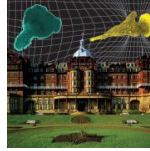S Lange and F Benjamin

TU Dresden, Germany

Chemotaxis - the navigation of biological cells guided by chemical gradients - is crucial for bacterial foraging, immune responses, and guidance of sperm cells to the egg during fertilization.

Previous work on chemotaxis focused predominantly on idealized conditions of perfect chemical gradients. However, natural gradients are affected by distortions, e.g. by turbulent flows in the ocean.
Recent experiments with bacteria and sperm cells from marine invertebrates have surprisingly revealed the existence of an optimal turbulence strength at which the chemotaxis is more effective than for still water conditions with perfect gradients.

Using sperm chemotaxis in steady and unsteady shear flows as a prototypical example, we reproduce an optimal turbulence strength in numerical simulations. We can understand the origin of this optimum and quantify it using a minimal description. Specifically, we use a simplified characterization of a turbulent vortex and resultant filamentous concentrations fields, and apply a linear response theory of chemotactic navigation. We explain how external flows distort sperm swimming paths and concentration gradients of signaling molecules released by the egg, but at the same time extend the spatial range of these gradients. The combination of these two competing effects accounts for the optimal turbulence strength.

We compare our theoretical results to previous experiments. Our results represent a first step towards a theory of cell navigation in dynamic and disordered environments shaped by turbulent flows.

Session: Adhesion

(invited) Integrin adhesions at the crossroad between microtubules and the actomyosin cytoskeleton

A D Bershadsky[1,2]

[1]National University of Singapore, Singapore, [2]Weizmann Institute of Science, Israel

Actomyosin cytoskeleton and cell-matrix adhesions are the key elements determining cell morphogenesis. Peripheral domains of the actin cytoskeleton associated with the clusters of integrin transmembrane receptors, comprise several types of mechanosensing cell-matrix adhesions, such as focal adhesions and podosomes. Myosin-IIA filaments assemble into superstructures ("stacks") organizing and remodeling actin filament networks, including cell-matrix adhesions. Multiple signaling pathways control the integrin adhesions through regulation of myosin-IIA filament assembly. Myosin-IIA filaments affect the adhesion structures in a differential manner, promoting the integrity and growth of focal adhesions but disrupting the podosomes. A feedback response from the integrin adhesions to the myosin IIA filaments is in part mediated by another essential cytoskeletal system, microtubules. Focal adhesions and podosomes capture microtubules through KANK family proteins connecting the integrin-binding protein talin with the cortical microtubule-stabilizing complex (CMSC). Myosin-IIA filaments function as effectors in the microtubule-driven regulation of integrin adhesions. Capturing of microtubules by integrin adhesions suppresses, while detachment - promotes the myosin-IIA filament formation. The mechanism underlying these effects depends on Rho activation by guanine nucleotide exchange factor GEF-H1, which is trapped by microtubules when they are coupled with integrin adhesions via KANK proteins. Microtubule uncoupling from the integrin adhesions triggers a release of GEF-H1 from microtubules, activation of Rho and Rho-associated kinase (ROCK), and consequently the assembly of myosin-IIA filaments. Thus, microtubule capturing by integrin-mediated adhesions modulates the effect of microtubules on the actomyosin cytoskeleton. The myosin-IIA filaments then remodel the focal adhesions and podosomes, closing the regulatory loop.
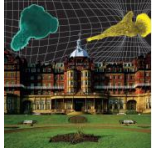
[1]   S.Hu et al (2017). Long-range self-organization of cytoskeletal myosin II filament stacks. Nature Cell Biol. 19, 133-141.
[2]   Y.Nishimura, N.Mohd Rafiq et al. (2017). Microtubule regulation of integrin-based adhesions is mediated by myosin-IIA. BioRxiv doi: https://doi.org/10.1101/195495.

(invited) Physical effects in cell adhesion

K Sengupta

Centre Interdisciplinaire de Nanoscience de Marseille, France

One of the most important triggers of cell activity is adhesion, a process by which cells and their organelles interact and attach to substrates, internal scaffolds, external interfaces, or other cells. The physiological and pathological significance of cell adhesion is immense and adhesion is therefore ubiquitous in the living world. Adhesive contacts need to be able to function in widely varying circumstances and must be established in an extremely noisy environment. For these reasons, the control mechanisms of adhesion have had to develop to be able to permanently monitor and correct cellular performance. While a lot of effort has been invested into understanding the biochemical aspect of these processes, the underlying physical principles of adhesion regulation have been much less appreciated. Only in recent years have these two approaches begun to converge in a unified view. I will present insights into how physics of the membrane modulates cell adhesion, in particular focusing on membrane fluctuations that mediate lateral interaction between bonds. I will additionally discuss how friction effects at an adhesive cellular interface may modulate cell adhesion and mechanosensing, with special focus on T lymphocytes.

Control over the mechanical interface between fibronectin and silicone elastomers regulates fibroblast adhesion and polarization

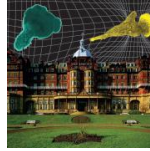D Missirlis[1], T Haraszti[2] and J Spatz[1]

[1]Max-Planck-Institute for Medical Research, Germany, [2]DWI Leibniz Institute for Interactive Materials, Germany

A mechanically deregulated extracellular matrix, often characterized by abnormal fibronectin (FN) deposition, contributes to the progression of pathological conditions such as fibrosis and cancer. Our current work provides experimental evidence that the physical coupling between FN and the underlying substrate is critical in determining cell adhesion and migration behavior in vitro. While the notion that the mechanical properties of the substrate direct cell decisions and fate is widely accepted, it is based predominantly on studies performed on hydrogels, where functional ligands are covalently coupled to the substrate. Interestingly, studies on silicon elastomers appear to challenge this dogma, and we have considered two potential reasons for this discrepancy: the viscoelastic character of elastomers and the difference in tethering mechanics.

We thoroughly characterized by AFM and oscillatory rheology fibronectin (FN)-functionalized silicon elastomers prepared using the CY52-276 formulation. Bulk viscoelasticity was controlled by the mixing ratio of base to curing agent, achieving materials with stiffness from a few kPa down to ultrasoft substrates with Young's moduli of 300 Pa. Despite having a sol fraction greater than 0.6, a viscous modulus larger than the elastic and exhibiting significant creep, these ultrasoft elastomers did not exhibit bulk viscoplasticity. AFM nanoindentation studies revealed the formation of a stiff protein layer that was firmly attached to the substrate. Pulling FN using an AFM cantilever resulted in FN unfolding, rather than FN adsorption. This was in stark contrast to the situation on polyacrylamide hydrogels, where pulling on FN caused gel deformation.

Fibroblasts readily adhered, spread and formed robust FAs on FN-coated elastomers, independent of bulk substrate properties. In contrast, polarization and directional persistence in migration were dependent on mechanical properties and were enhanced on stiffer substrates. These results suggest that polarization emerges from signal integration at the cell - and not the focal adhesion - level. Taking a closer look on the underlying reasons for this behavior revealed that cells accumulate FN under their surface on soft substrates, due to the deformable nature of the elastomer. Changing substrate hydrophilicity to decrease FN adhesion strength, or cross-linking FN altered the ability of cells to remodel the FN coating with opposite effects. Cross-linking essentially abolished FN fibril formation, but had minimal effects on cell adhesion. Reducing FN adhesion, on the other hand, enhanced FN remodeling and enabled fibroblast polarization at reduced stiffness values.

Overall, our data demonstrate that both substrate elasticity and tethering mechanics control fibroblast mechanosensing, polarization and consequently migratory behavior. In other words, cells feel the surrounding mechanical environment depending on how their ligands are anchored to it and therefore, how efficient is force transmission to the substrate.

Session: Migration & microswimmers

(invited) Oscillations in single-cell motility: simple one-dimensional models

N Gov

Weizmann Institute, Israel

We present an extension of our one-dimensional cell motility model, which was based on the coupling between actin treadmilling and an internal gradient of a polarity marker that in turn drives the actin flow. We extend this model to allow for time-varying cell length, due to stick-slip adhesion at the cell back. The resulting modes of motility are shown to include stable polarized motion, as well as oscillations where the cells detach their trailing edge periodically. Oscillations are shown to depend on the time-scales of relaxations of the different internal processes. We compare these results to experiments.

(invited) Exploring protein-signaling stochasticity by FRET in single cells

T Shimizu

AMOLF, The Netherlands

The importance of gene-expression noise in cellular processes is widely appreciated, but stochasticity in the interaction between gene products has been more challenging to address experimentally, especially within signaling networks of live cells. I will present recent data from *in vivo* single-cell FRET measurements that have allowed us to quantify protein signaling noise in the bacterial chemotaxis system. In the absence of changes in gene expression, we find that single cells demonstrate strong temporal fluctuations in signaling activity. We provide evidence that such signaling noise can arise from at least two sources: (i) driven stochastic kinetics of adaptation enzymes, and (ii) fluctuations in the allosteric chemoreceptor arrays. Surprisingly, we find that under certain conditions (ii) can generate giant fluctuations that drives the signaling activity of the entire pathway into a stochastic two-state switching regime. I will discuss both the functional significance of such protein-signaling noise, as well as mechanistic insights gained by analysis of these fluctuations.

Spiral actin waves as modulators of dendritic cells random movement

F Lautenschläger[1], K Kaub[1], D Florman[2] and E Terriac[2]

[1]Saarland University, Germany, [2]Leibniz Institute for New Materials, Germany

The cellular cortex is crucial for a variety of cellular functions such as shaping the cell or transmitting forces during migration. We characterized and compared the structure and dynamics of the cellular cortex of suspended versus adherent cells by Fluorescent Recovery After Photobleaching and Electron microscopy.

Curvotaxis directs cell migration through cell-scale curvature landscapes

L Pieuchot

CNRS, France

Cells have evolved multiple sensing mechanisms to apprehend and adapt finely to their micro-environment and fulfil their biological functions. Here we report a new cellular sense which we term "curvotaxis" that enables the cells to respond to cell-scale curvature variations, a ubiquitous trait of cellular biotopes. We develop ultra-smooth sinusoidal 3D surfaces presenting modulations of curvature in all directions, and monitor cell behavior on these simplified topographic landscapes. We show that cells avoid convex regions during their migration and position in concave valleys. Computational modeling combined with functional analysis and live imaging show that curvotaxis relies on a dynamic interplay between the nucleus and the cytoskeleton - the nucleus acting as a curvature sensor that guides the migrating cell towards more concave curvatures. Further analysis show that substratum curvature affects nuclear shape, intracellular tensions, focal adhesions organization and dynamics, and gene expression. Altogether, this work identifies curvotaxis as a new cellular guiding mechanism and promotes cell-scale curvature as an essential physical cue fully integrated by the cells.

Session: Protein/membrane systems

(invited) Evolutionary self-organisation: lessons from the polarisation machinery in budding yeast

L Laan

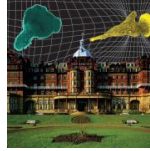Delft University of Technology, The Netherlands

In this talk I will present our work on how the biochemical networks underlying cellular functions evolve. The biochemical network we focus on is the polarisation machinery in budding yeast, which establishes a polarized protein pattern on the cell membrane and is essential for proliferation. We combine bioinformatics, experimental evolution, quantitative cell biology and reaction-diffusion based modelling to understand how proteins that are essential for polarity establishment in one species can be absent in a closely related species or strain, while both species have the same functional requirement. So, how can these biochemical networks reorganize during evolution without significantly compromising fitness along the way?

(invited) Physics of cell adhesion: The role of the membrane in the protein recognition process

A-S Smith[1,2]

[1]FAU Erlangen-Nurnberg, Germany, [2]Institute Ruđer Bošković, Zagreb, Croatia

In embryogenesis, vertebrate cells assemble into organized tissues. In metastatic cancer, cells spreading in the circulatory system build cell-cell contacts with the surrounding tissue to establish new tumors. At the root of these life-forming or life-threatening biological phenomena is cell adhesion, the binding of a biological cell to other cells or to extracellular matrix. The most obvious fundamental question to ask is then as follows: What factors control or govern cell adhesion? For a long time, the paradigmatic answer to this question was that specific protein molecules embedded in the cell wall (or membrane) were responsible for cell adhesion, in either a key-lock fashion (in cell-cell adhesion) or a suction-cup fashion (in cell-matrix adhesion). But, a new realization has emerged during the past two decades that physical mechanisms, promoted by the cell membrane, play an unavoidable, yet not fully understood role. Although these physical elements, namely membrane fluctuations and ability to change shape, do not at all depend on any specific proteins, they can have a major impact on the protein-mediated adhesion, and can be viewed as mechanism that controls the

binding affinity to the cell-adhesion molecules. In my talk I will show how these mechanisms can be studied in mimetic models both experimentally and theoretically, the result of which will be discussed in the cellular context.

Phospho-regulation of Tropomyosin Cdc8 during cytokinesis is crucial for actin cable turnover in fission yeast

S Palani[1], D Koester[1], A Kamnev[1], T Hatano[1], T Kanamaru[2], H Brooker[3], J Fernandez[1], A M Jones[1], J Miller[1], D Mulvihill[3] and M K Balasubramanian[1]

[1]Warwick University, UK, [2]University of Heidelberg, Germany, [3]Kent University, UK

Actin filaments, which constitute one of the major cytoskeletal networks in eukaryotes, are known to exhibit a high rate of turnover. How cells regulate actin filament turnover is not well understood, despite detailed studies of contributors to actin turnover such as actin severing proteins and actin nucleators. Here, we describe a phosphorylation-controlled mechanism for actin cable turnover in *Schizosaccharomyces pombe* that is involved in cytokinesis. We show that Cdc8-tropomyosin phosphorylation on serine-125 does not affect its structure and stability, but leads to a weaker interaction with actin filaments *in vitro* in pelleting and total internal reflection microscopy based assays. Furthermore, phosphorylation-mediated release of Cdc8 from actin filaments facilitates occupancy of filaments by the actin severing protein Adf1 and subsequent filament disassembly. In *in vivo* experiments, phospho-mimetic mutant of Cdc8 showed decreased actin cable-stability and conversely a non-phosphorylatable Cdc8 mutant shows increased actin cable stability. Our study provides a novel relay mechanism for actin filament turnover in which actin filament bound tropomyosin is phosphorylated, causing its dissociation from actin filaments, followed by Adf1 loading and actin filament severing.
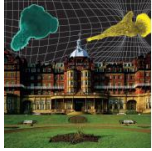
Session: Cell mechanics

(invited) Actin flows in cell migration: from locomotion to trajectories

R Voituriez[1,2]

[1]CNRS, France, [2]Sorbonne Université, France

Eukaryotic cell movement is characterized by very diverse migration modes. Recent studies show that cells can adapt to environmental cues, such as adhesion and geometric confinement, thereby readily switching their mode of migration. Among this diversity of motile behaviors, actin flows have emerged as a highly conserved feature. Based on active gel models of cytoskeleton dynamics, I will argue that the various observed migration modes are continuous variations of elementary locomotion mechanisms, which rely on a very robust physical property of the actin/myosin system: its ability to sustain flows at the cell scale. This central role of actin/myosin flows will be shown to affect the large scale properties of cell trajectories.

Active prestress leads to an apparent stiffening of cells through geometrical effects

E Fischer-Friedrich

TU Dresden, Germany

Tuning of active prestress e.g. through activity of molecular motors constitutes a powerful cellular tool to adjust cellular stiffness through nonlinear material properties. Understanding this tool is an important prerequisite for our comprehension of cellular force response, cell shape dynamics and tissue organisation. Experimental data obtained from cell-mechanical measurements often show a simple linear dependence between mechanical prestress and measured differential elastic moduli. While these experimental findings could point to stress-induced structural changes in the material, we propose here a surprisingly simple alternative explanation in a theoretical study. We show how geometrical effects can give rise to increased cellular force response of cells in the presence of active prestress. The associated effective stress-stiffening is disconnected from actual stress-induced changes of the elastic modulus and should therefore be regarded as an apparent stiffening of the material. We argue that new approaches in experimental design are necessary to separate this apparent stress-stiffening due to geometrical effects from actual nonlinearities of the elastic modulus in prestressed cellular material.

Keynote lecture

(invited) Polarized cell locomotion in soft tissues mediated by microtubule actin crosstalk

E Sackmann

Technical University Munich, Germany

Fundamental differences between the physical principles of the globally coordinated directed migration of cells on resilient tissue surfaces and in soft tissue (such as the brain) are described. The mechanisms of the protrusion force generation differ fundamentally. Migration on resilient surfaces is driven by thrust generating solitary actin gelation pulses (SAGP) followed by the uropod retraction by stress fibers activation. In the soft tissues (such as of the brain) the forward directed thrust of the cell front is generated by spreading of cell lobes on the cell surfaces. The forward directed thrust is mediated by microtubule-dynein machines while actin myosin assemblies serve the saltatoric retraction of the nucleus to the centrosome. Finally the role of the antagonistic pair of GTPases, RhoA and Rac-1, for the pulsative migration of cells is described.
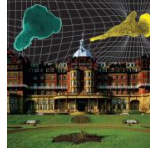
Flash poster session 1

FP1.1 Formin processivity on actin bundles

E Suzuki, A Jégou and G Romet-Lemonne

Institut Jacques Monod/CNRS, France

Many essential cellular processes including cell division, migration and morphogenesis are carried out largely thanks to the actin cytoskeleton, which consists of actin filaments and its associated actin binding proteins that organize filaments into different architectures. Filopodia, which are dynamic finger-like structures consisting of actin filaments bundled in parallel, emerge at the cell front and are important for various functions such as cell sensing and cell motility. Actin filaments within filopodia are elongated at their barbed ends by elongation-accelerating actin binding proteins such as formins linked to the cell membrane. A key characteristic of formins that is important for filopodia dynamics is its processivity, which refers to the duration of time that the formin remains attached to actin barbed ends and hence accelerates

elongation of filaments. These formin-elongated actin filaments are cross-linked in parallel by the bundling actin binding protein fascin, stiffening structures and allowing filopodia to emerge from the cell surface. These formin/fascin machineries are thought to collaborate to design a unique type of actin network that governs filopodium dynamics, yet the exact mechanism by which these two key proteins synergize to create an actin structure with specific capabilities/features is not well understood. We are interested in understanding how the fascin-imposed bundle architecture of actin filaments affect formin-mediated elongation and formin processivity, and in particular the processivity of formins at the cell membrane.

To tackle these questions, we use an *in vitro* microfluidics-based approach to reconstitute a minimal system of fascin-induced actin bundles. We have probed the activity of mDia1 formins and fascins in a large range of biochemical conditions : first on single filaments, then scaling up to bundles of multiple filaments. We have experimented with different formin anchoring methods, ranging from setups that limit formin movement, to anchoring to lipid bilayers to allow free diffusion and rotation of formins, mimicking formins that are anchored to cell membranes. We observe that formin elongation rate is slowed down and its processivity is strongly diminished by fascin bundling if formin rotation is hindered. On the other hand, when formins are allowed to freely rotate, elongation rates and processivity are comparable to those of single filaments. Our results offer insight into the conditions formins require in order to successfully elongate and retract actin bundles. We will also present recent results on the mechanosensitivity of formin-induced bundles.

FP1.2 Active torque generation by actomyosin cytoskeleton drives chiral cell-cell rearrangement

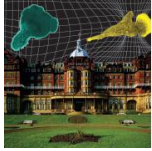L Pimpale, T Middelkoop and S Grill

TU Dresden, Germany

Most animals are left-right (LR) asymmetric, however the molecular and physical mechanisms that drive LR symmetry breaking are largely unexplored. In *C. elegans* LR symmetry breaking is thought to be linked to the cytoskeleton. Recently, Naganathan et al 2014, showed that the actomyosin cortex generates active chiral torques in the single cell embryo. Similar chiral flows lead to chiral cell movements that are subsequently involved in establishing the LR axis at the 6-cell stage. We speculate that chiral movements are much more prevalent in development, driving cellular rearrangements and axis repositioning. I explore these chiral morphogenetic features in a quantitative manner in early stages of worm development and determine the extent of chiral cortical flows and chiral morphogenesis in the first few divisions of the nematode. Using RNAi mediated perturbations for actomyosin regulators, I modulate chiral flows and monitor their effect on axis establishment and cellular rearrangement until the 6-cell stage. Finally, by combining thin film active chiral fluid theory with experimental data, I plan to deliver a physical description of how torques are generated in the actomyosin cortex and how these events lead to morphogenetic changes in development.

FP1.3 Cultured vs. mechanically isolated muscle cells: is there any biomechanical difference?

K Garcia-Pelagio[1], E Hernandez-Ochoa[2], S Pratt[2] and R Lovering[2]

[1]Universidad Nacional Autonoma de Mexico, Mexico, [2]University of Maryland School of Medicine, USA

Duchenne muscular dystrophy (DMD), the most common and severe dystrophy, is caused by the absence of dystrophin. Muscle weakness, fragility, and increased susceptibility to damage are presumably due to structural weakness of the myofiber cytoskeleton, but recent studies suggest that malformed/split myofibers in dystrophic muscle may also play a role. We have previously studied the biomechanical properties of the sarcolemma in single myofibers isolated mechanically from extensor digitorum longus (EDL) muscles in wild-

type (WT) and dystrophic (*mdx,* mouse model for DMD) mice. We now introduce similar biomechanical methods on enzymatically-dissociated myofibers (both normal and malformed) from the flexor digitorum brevis muscle (FDB) of WT and *mdx* mice. FDB muscles were enzymatically dissociated and plated on specialized coverslips, cultured. Suction pressures (P) applied through a pipette to the membrane generated a bleb, which increased in height with increasing P. Larger increases in P ruptured the connections between the membrane and myofibrils, at costameric region and eventually caused the membrane to burst. The results from cultured FDB myofibers match findings from dissected EDL myofibers, but separation P was up to 14-fold higher in the FDB than EDL. P at which the membrane separated from the underlying myofibrils was 27% lower in cultured *mdx* myofibers and 50% less in branches of split fibers compared to the trunk. We also asked whether the abnormal biomechanical phenotype of the *mdx* myofibers is associated with further deficits on excitable properties. To this end we use high-speed confocal microscopy and the voltage-sensitive indicator di-8-butyl-amino-naphthyl-ethylene-pyridinium-propyl-sulfonate. We found that the action potential amplitude is not altered in *mdx* 'normal' or *mdx* 'split' FDB muscle fibers when compared to WT. Data indicate a reduction in muscle stiffness, increased membrane deformability and instability in *mdx* muscle from cultured fibers compared to mechanically isolated ones. Findings also suggest mechanical differences due to altered morphology.
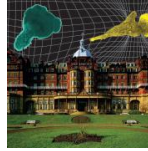
FP1.4 Buckling of epithelium by apical-only actomyosin action

J Etienne[1], J Fierling[1], A Torzinsky[1], G Blanchard[2], C Lye[2], C Quilliet[2] and P Marmottant[2]

[1]CNRS — University Grenoble Alpes, France, [2]University of Cambridge, UK

Embryos acquire their shape through large movements of primitive epithelia, of which gastrulation is an archetypical and fundamental example. In Drosophila, it consists in the formation of the ventral furrow, oriented along the long axis of the embryo in the anteroposterior direction.

Numerous models have aimed at understanding how the causal mesodermal actomyosin activity governs the corresponding local change of curvature of the epithelium. However, models so far resort to some apicobasal mechanism of force production or length control, for which there is no evidence of cytoskeletal cause. Here, we address whether a model relying on apical force balance only can predict ventral furrow formation. We show that an isotropic actomyosin prestress in the mesoderm region leads to a mediolateral pattern of apical area change that is comparable with experimental observations. We show that these area changes are not cell-intrinsic and the pattern obtained is due to mechanical interactions over the whole embryo. Simultaneously, the ventral region flattens and then abruptly folds as observed experimentally. Our modelling gives insight on the role of the mesoderm and three-dimensional embryo geometries in this process, which can be described as the buckling of the epithelium.
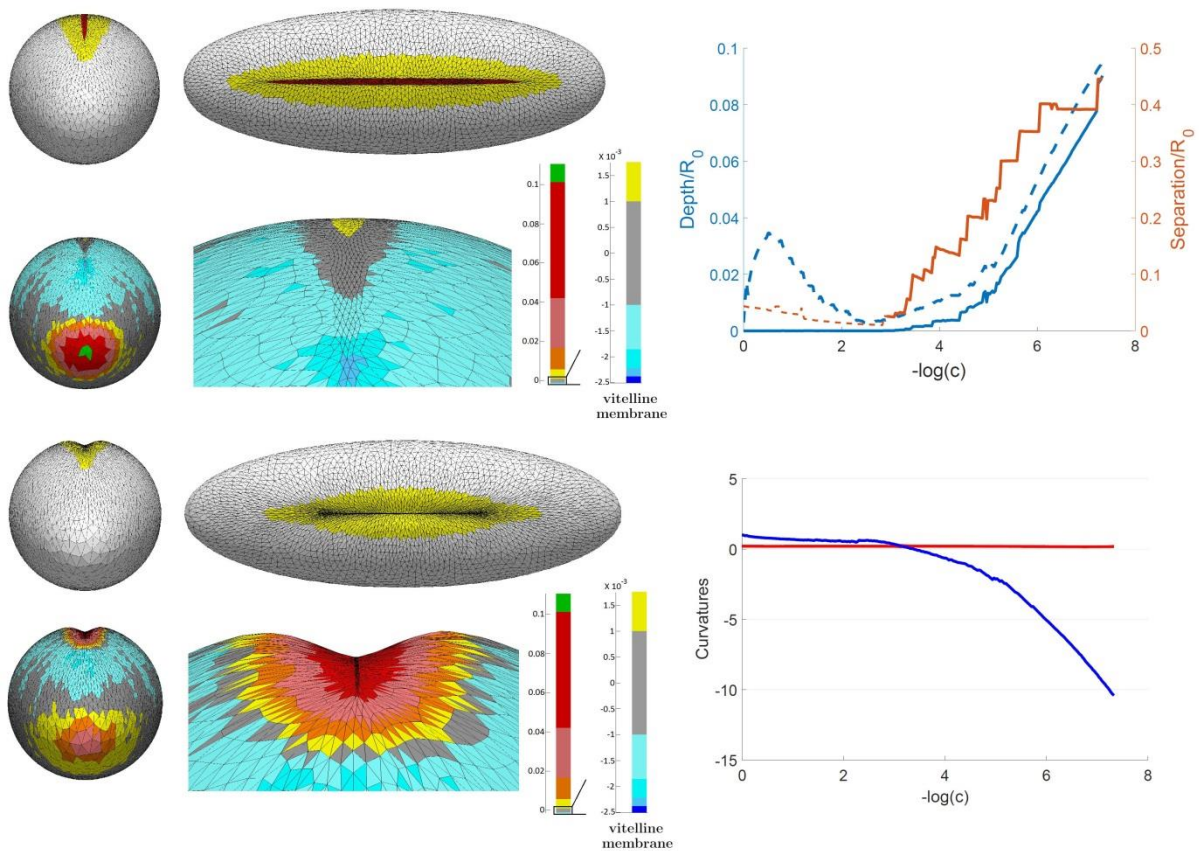
Fig 1: Formation of the ventral furrow. (*Top left four images*) Simulated mesoderm geometry after flattening and colour-coded distance between epithelial surface and vitelline membrane. The flattening has caused an increase of distance along midline ventrally, posteriorly there is a decrease of the distance towards the ventral side and a strong increase in a circular region more dorsal. (*Bottom left four images*) Same after VFF, there is now a well marked furrow at the ventral midline. (*Bottom right*) Evolution of the curvature at ventral midline as the actomyosin prestress increases. *Red* is mediolateral curvature, it is initially positive (since the transverse section is circular), decreases as soon as actomyosin prestress appears (flattening phase) and then plateaus. After this plateau, it starts decreasing more sharply and becomes strongly negative (folding phase). *Blue* is anteroposterior curvature, it is initially positive also (due to the ventral convexity of embryo) and starts decreasing marginally once the folding has occurred, corresponding to a straightening of the midline in the furrow in the sagittal plane. (*Top right*), Evolution of the furrow geometry as the actomyosin prestress increases (proxy for time). Dashed blue line, distance from midline to vitelline membrane: flattening initially increases this distance sharply, then as mesoderm area reduces the distance reduces, and increases again during the folding phase. Solid blue line, depth of the furrow itself (difference between the position of the furrow borders and the furrow bottom). Red, separation between the furrow borders in the mediolateral direction (dashed before actual furrow formation, solid after). It increases during the initial stage of furrow formation and then decreases.
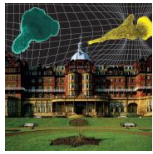
FP1.5 Traction forces mediate cell activity during cell polarization

Z Messi[1], A Bornert[2], F Raynaud[3] and A Verkhovsky[1]

[1]École Polytechnique Fédérale De Lausanne, Switzerland, [2]Etablissement Francais du Sang, France,[3]University of Lausanne, Switzerland

How cells break symmetry and organize their activity to move directionally is a fundamental question in cell biophysics. Models of cell polarization commonly rely on front-to-back gradients of functional components or regulatory factors, but it is not clear how the front-back axis is defined in the first place. We have recently proposed a novel and simple principle of self-organizing cell activity, in which local cell-edge dynamics depends on the distance from the cell center, but not on the orientation with respect to the front–back axis (Raynaud et al., Nat. Physics 12, 367-373, 2016). Stochastic modeling demonstrated that distance-sensitivity is sufficient for the symmetry breaking and the emergence of persistent cell motion and stable shape. To understand physical mechanisms of distance-sensitivity, we investigate the distribution and dynamics of traction forces exerted by polarizing cells on the substrate, and show that these forces increase with the distance from the cell center, and correlate in space and time with protrusion retraction switches. However, no correlation is observed between traction forces and local edge curvature. Traction forces increase gradually during cell protrusion, and maximal forces are observed shortly after the onset of retraction, coinciding with maximal velocity of edge retraction and actin retrograde flow. However, increase of traction forces before the onset of retraction is not accompanied by a significant increase of actin flow rate. These results suggest that the modes of force transmission are different before and after the onset of retraction but adhesions persist and transmit frictional stress even during retraction. Inhibition of cell contractility leads to diminished traction forces, reduced edge activity, and abnormal cell polarization. Collectively, our results suggest that traction forces mediate distance-sensitivity and organize cell activity during symmetry breaking.
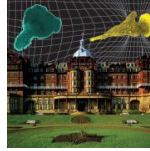
Flash Poster session 2

FP2.1 Simultaneous cell tracking and visualization of flagellar dynamics of *Pseudomonas putida* in chemoattractant gradient

Z Alirezaeizanjani, V Waljor, M Hintsche and C Beta

Potsdam University, Germany

Biological membranes form hydrated, quasi-2D elastic interfaces, and it has been proposed that mechanical signals along the membrane plays a fundamental role in biological communication. Such interfaces have also been shown to be held at physiological conditions in which they are susceptible to undergo phase transition. Here we investigate whether thermodynamic principles can be applied on interfaces to study mechanical signaling along membranes and whether the signaling characteristics can be derived from the state diagram of the system. Using fluorescent probes embedded on an lipid monolayer assembled at the air-water interface, the state (pressure, density, etc.) of the interface is observed using FRET. Optical measurements show that a localized, mechanical impulse imposed on the interface propagates through the monolayer as acoustic waves. Notably, an above-threshold stimulation near the phase transition region of the state diagram (liquid-expanded/liquid condensed) can drive the waves into the nonlinear regime. This generates all-or-none type solitary pulse, and the threshold behavior and the pulse shape show striking similarity with the nervous impulse. When such nonlinear pulses collide head on, they can interact and annihilate each other, a behavior observed in collision of action potential in excitable cells. Altering the macroscopic state (pH, ionic concentration ($Na+$, $Ca2+$), temperature, etc.) changes the material properties

of the membrane (e.g. lateral compressibility) and consequently affects pulse velocity, amplitude, and relaxation. These results altogether show that the thermodynamic state and its changes determine the existence and propagating properties of interfacial waves, and they support a physical basis of communication in living systems.

FP2.2 Breast cancer cell migration in the bone microenvironment

N Cowley and R Hawkins

University of Sheffield, UK

We are investigating how material confining a cell affects cell motility and behaviour. In particular we look at how rigidity and geometry play a role by investigating the mechanical response of materials with different rigidities to cell generated forces. We model a cell as a viscous droplet with an active contractile boundary analogous to the actin cortex. We use the immersed boundary method to simulate a cell interacting with deformable elastic walls of various geometries. We look at the specific case of breast cancer metastasis to the bone.

The majority of patients who die from cancer do so not from the primary tumour, but from the metastasis of cancer to other sites in the body. Breast cancer most commonly metastasises to the bone. We are examining how mechanical forces are involved in this process. Bone tissue is very varied in composition and vastly different from breast tissue. In order to model this varied niche we combine analytical calculations and our simulations, with AFM data from our collaborators. This will further our understanding breast cancer metastasis to the bone and it's motility in the bone microenvironment.

FP2.3 Ligand-free EGFR activity enhances E-cadherin junction formation

C Fu, V Viasnoff, W Engl and M Sheetz

National University of Singapore, Singapore

E-cad homophilic interactions transiently activate epidermal growth factor receptor (EGFR) in a ligand-independent manner during cell-cell contact formation. The functional consequences of this activation process are not fully characterized and understood. In this work, we focus the role it plays in regulating cytoskeletal elements distal from the cell-cell contact and in absence of any integrin signaling. To this end, we use a suspended cell doublet system to achieve the best control over junction formation dynamics. First, we observe that changes of cell-cell contact size (ρ) in newly-formed cell doublets is positively correlated with changes of cortical tension in cell singlets. Inhibiting ligand-free EGFR activity decreases the contact size (ρ) of newly-formed cell doublets but did not affect contacts in matured ones. Our results suggest that the ligand-free activity of EGFR transiently regulates the strength of E-cad junction during junction formation. Secondly, we find that junction formation time (t) is determined by myosin II contractility and actin dynamics of the cortex. Low myosin contractility and low actin dynamics will lead to fast junction formation. Strikingly, actin reorganization during E-cad junction formation reduces the actin dynamics in the free cortex of doublets, and this reduction of actin dynamics speeds up further junction formation. In addition, inhibiting ligand-free EGFR activity prevents the changes of dynamics of the actin free cortex of doublets, and abolishes the enhancement of second junction formation.
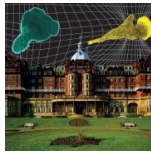
FP2.4 The role of tip pressure in fungal growth: mechanical and microfluidics study of *Aspergillus nidulans* and mutants

B G Bermúdez[1], Q Li[1], G V Guinea[1], M A Penalva[2] and G R Plaza[1]

[1]Universidad Politécnica de Madrid, Spain, [2]Biological Research Center, Spain

Mechanical studies of fungal cells have facilitated the understanding of a range of cellular processes that are characteristic of these model organisms, including fermentation, pathogenesis or tissue invasion. These studies have also revealed that the differential pressure between the hyphal cytoplasm and the surrounding medium is essential for fungal growth, being correlated with the growth rate [1]. Importantly, during the invasion of tissues the external pressure at the tip of the hypha may differ from the pressure in the surrounding medium. Aiming to develop an experimental procedure to study the mechanics of hyphal growth and tissue invasion, we report a novel microaspiration-based technique which permits probing the external pressure at the hyphal tip [2].

The proposed procedure allows one to both control the external pressure at the tip of the hyphae and to simultaneously measure the internal length of the cell (see Fig. 1). In addition, this technique enables the characterization of hyphal growth mechanics in the case of very thin hyphae, which are not accessible to other techniques (e.g. turgor pressure probes). We have also developed a simple analytical model that incorporates the external pressure at the tip during the experiments.
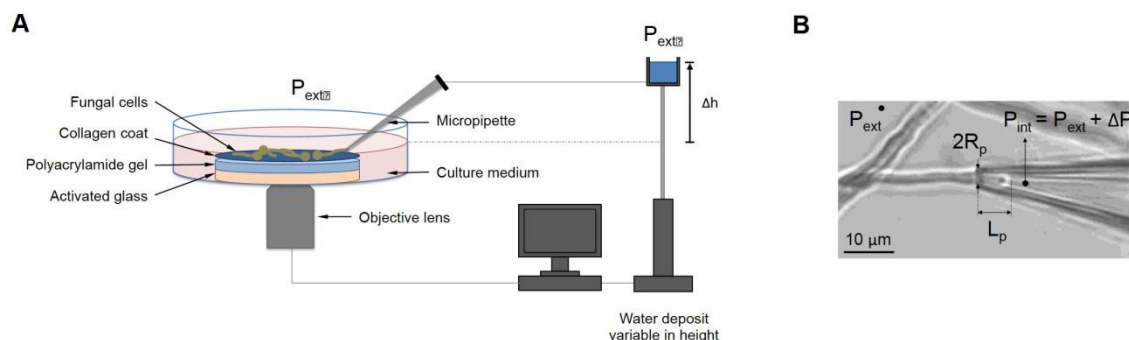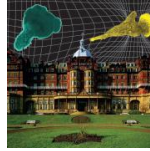

Fig 1: (A) Sketch of the experimental procedure to perform tip-pressure experiments on *Aspergillus nidulans*. (B) Representative experiment.

In the tip-pressure experiments conducted in this work, we found a correlation between the local pressure at the tip and the growth rate for the model species *Aspergillus nidulans*, as predicted by the model. Consistently, this method allows measuring the pressure at the tip required to arrest the hypha growth, what may have implications in the field of medical treatments for fungal infections.

Uncovering the impact of the local conditions at the tip is relevant in the frame of fungal invasiveness. Using this procedure, we are also investigating the relation between growth rate and external pressure in thermosensitive mutants of *Aspergillus nidulans*, which may provide new insights on the mechanics of hyphal growth and the effect of external pressure and temperature. Thus we consider that these experiments can ultimately offer new clues for treatments against fungal invasion of tissues, where the local pressure at the tip is significantly different from the pressure in the surrounding tissues, as it is the case in blood vessels.

[1]   Taheri-Talesh, N., T. Horio, L. Araujo-Bazan, X. Dou, E.A. Espeso, M.A. Penalva, S.A. Osmani & B.R. Oakley. The tip growth apparatus of aspergillus nidulans. Mol. Biol. Cell. 19, 1439–1449 (2008).

[2]   González-Bermúdez, B., Li, Q., Guinea, G. V., Peñalva, M. A., & Plaza, G. R. Probing the effect of tip pressure on fungal growth: Application to Aspergillus nidulans. Physical Review E, 96(2), 022402. (2017).

FP2.5 Structure and dynamics of the trypanosoma brucei plasma membrane

M Schwebs, M Glogger, M Engstler and S Fenz

University Würzburg, Germany

African trypanosomes are the causative agents of sleeping sickness in human and Nagana in livestock. In the bloodstream of their host, they exhibit a dense coat of variant surface glycoproteins (VSG). Fluidity of the VSG coat is fundamental for parasite survival. However, the diffusion behavior of the VSGs is also limited by the physical properties of their lipid matrix. We have recently introduced super-resolution imaging of intrinsically fast moving flagellates based on cyto-compatible hydrogel embedding [Glogger et al. JPD: Appl Phys 17]. Building on this work we employ leaflet-specific membrane probes and single-molecule fluorescence microscopy to elucidate the structure and dynamics of the plasma membrane and VSG coat in living trypanosomes. Using expressed lipid-anchored eYFP as a probe for the inner membrane leaflet, we found specific domains where the probe accumulates or appears diluted rather than being homogenously distributed. We hypothesize that this structuring of the membrane is associated with the underlying microtubule cytoskeleton. The next steps include employment of a more stable fluorescent label to resolve dynamic interaction of single probes with the observed domains. Moreover, we aim to track fluorescently labeled lipids in the outer leaflet to gain insight in inter-leaflet coupling in vivo, and we plan a two-color experiment to simultaneously investigate membrane and VSG dynamics.

Poster session 1

P1.1 Elastic properties of cytoskeletal networks in confinement and implications for mechanics of cells

S Azote and K Müller-Nedebock

[1]Stellenbosch University, South Africa

The spatial organisation and orientation of cell cytoskeletal networks, especially branching actin networks due to the Arp2/3 protein complex, are strongly influenced by the constraints that the cell-sized confinement introduces.
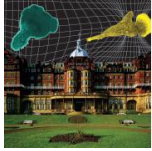
We develop a grand canonical formalism that allows the computation of densities, order profiles and some of the elastic properties of cytoskeletal structures under cellular confinement. We compare various geometries and topologies. These tools and insights allow us to confirm the stability of cells and, we make predictions of how more than one cell might interact with another, based on the structure and properties of cytoskeletal networks predicted.

P1.2 Structural stability of a mitotic spindle: parametric finite element approach

A Iakovliev, S Dasmahapatra and A Bhaskar

University of Southampton, UK

Mitotic spindles are mechanical structures that play a critical role in cell division by generating forces to separate chromosomes. They are ordered assemblages of proteins that make up microtubules (MT) and microtubule connectors whose mechanical properties are responsible for their structural integrity under mitotic forces. We use a continuum mechanics approach to study the stability of equilibrium of a mitotic spindle as a whole. We create and apply a finite element (FE) parameterised model of interpolar MTs, astral MTs and MT connectors varying the number of MT filaments and the arrangement of their interconnections. The model is based on the experimental data on Fission Yeast spindles in late anaphase B and mitotic HeLa

cells [1]–[3]. We account for the complex interactions between interpolar MTs, astral MTs, connectors and centrosomes through mechanical coupling. Comparing the results with experiments and Molecular dynamics-based simulations [1], we demonstrate the great potential of Structural mechanics methods to address the stability of spindles. Here we report how buckling states of the spindle get localised towards either of centrosomes due to the irregular placement of microtubules and irregularities in MT coupling. In certain cases, such behaviour may result in nuclear misplacement, asymmetric division or other abnormalities.

[1]   J. J. Ward, H. Roque, C. Antony, and F. Nédélec, "Mechanical design principles of a mitotic spindle," *eLife*, vol. 3, p. e03398, 2014.
[2]   F. Pampaloni, G. Lattanzi, A. Jonas, T. Surrey, E. Frey, and E.-L. Florin, "Thermal fluctuations of grafted microtubules provide evidence of a length-dependent persistence length," *Proc. Natl. Acad. Sci. U.S.A.*, vol. 103, no. 27, pp. 10248–10253, 2006.
[3]   F. M. Nixon, C. Gutiérrez-Caballero, F. E. Hood, D. G. Booth, I. A. Prior, and S. J. Royle, "The mesh is a network of microtubule connectors that stabilizes individual kinetochore fibers of the mitotic spindle," *eLife*, vol. 4, pp. 1–21, 2015.

P1.3 Cell cortex structure and dynamics before, during, and after adhesion

E Terriac, D Flormann, K Kaub and F Lautenschläger

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The cellular cortex is crucial for a variety of cellular functions such as shaping the cell or transmitting forces during migration. We characterized and compared the structure and dynamics of the cellular cortex of suspended versus adherent cells by Fluorescent Recovery After Photobleaching and Electron microscopy.

P1.4 Tension in the actomyosin cortex from 3D simulations
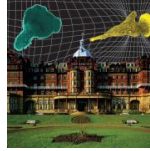
J Pesek and H Ramon

KU Leuven, Belgium

The acto-myosin cortex is in the center of research interest since early 80's, e.g. [1,2], due to its prominent role in biology of eukaryotic cells. Namely, it is the part of the muscle cells essential for their contraction, it forms cell protrusions known as filopodia and lamellipodia, creates a contractile ring in eukaryotic cells during mitosis and plays a major roll in cell adhesion and greatly influences cell's effective stiffness.

The acto-myosin cortex is not only interested from a biological point of view. A full characterization of its properties from a physical point of view is a challenging problem on its own. This is due to its nature, as it is a complex polymer network formed by actin driven out of equilibrium by the activity of molecular motors (myosin) and by persistent polymerization and depolymerization of actin. Moreover, the network itself can be crosslinked via proteins like fascin, or branched via Arp2/3. Such variety naturally leads to a very rich behavior of the network, see e.g. [3,4].

Recent advances in numerical computations are slowly making simulations of such complex systems on relevant time and space scales possible. Here we present a preliminary results of crosslinked actin cortex simulations without any branching proteins, where we study the effect of the polymerization rate on the tension buildup inside the network.

[1]   Nagashima, H. Asakura, S. - *J. Mol. Bio.* 136(2), 169-182 (1980).
[2]   Pollard T. - *J. Cell Biol* 95, 816-825 (1982)

[3]   Alvarado, J., Sheinman, M., Sharma, A., MacKintosh, F. C., Koenderink, G. H. - *Nature Physics* **9**(9), 591-597, 2013

[4]   Backouche, F., Haviv, L., Grosswasser, D., Bernheim-Grosswasser, A. - *Phys. Biol* **3**(4), 264-273, 2006

P1.5 Actin organization in cells responding to a perforated surface, revealed by live imaging and cryo-electron tomography

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Max Planck Institute of Biochemistry, Germany

In a 3D environment, motile cells accommodate their protruding and retracting activities to geometrical cues. Dictyostelium cells migrating on a perforated film explored its holes by forming actin rings around their border and extending protrusions through the free space. The response was initiated when an actin wave passed a hole, and the rings persisted only in the PIP3-rich territories surrounded by a wave. To reconstruct actin structures from cryo-electron tomograms, actin rings were identified by cryo-correlative light and electron microscopy, and thin wedges of relevant regions were obtained by cryo-focused ion-beam milling. Retracting stages were distinguished from protruding ones by the accumulation of myosin-II. Early actin rings consisted of filaments pointing upright from the membrane, entangled with a meshwork of filaments close to the membrane. Branches identified at later stages suggested that formin-based nucleation of filaments was followed by Arp2/3-mediated network stabilization, which prevented buckling of the force-generating filaments.
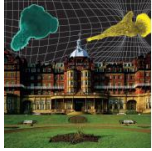
P1.6 Global turnover of contractile actin in frog egg extract

J Zhao and C Schmidt

Georg-August-Universität Göttingen, Germany

The turnover dynamics of actin cytoskeleton in eukaryotic cells is of crucial importance for various biological functions [1]. While components are constantly exchanged, the spatio-temporal distribution and concentration of components, including monomeric and polymeric actin often maintain a steady-state. On the other hand, cytoskeletal structures are also quite adjustable in the presence of stimuli, and different steady states can be accessed in response to regulatory signals [2]. However, the unifying principle of interplay between molecular-scale protein interactions and actin assembles organization, the local actin dynamics and global cell-scale behavior remains elusive. Given the complexity of cells, we study such dynamic organizations in reconstituted water-in-oil droplet composed of *Xenopus laevis* egg cytoplasmic extract. In our preliminary experiments, we observed actin networks were continuously generated near the droplet edge, and contracted towards the center. They were eventually dissembled when reaching critical concentration, supplementing the pool of actin monomers. The actin assemblies were quite active and such cycling dynamics could last for hours in a balanced state.

[1]   Pollard T.D. and Cooper J.A. Science, 2009, 326:1208–1212.

[2]   Suarez, C. and Kovar, D. R. Nat. Rev. Mol. Cell Biol., 2016, 17, 799–810.

P1.7 Investigating the collective behaviors of motor proteins pulling a cargo along cytoskeletal filaments

N Rueangkham and R Hawkins

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Molecular motors stepping along cytoskeletal filaments facilitate intracellular transport and organelle positioning. Molecular motors can work either alone or cooperatively. They are often classified according to their processivity. Processive motors never unbind from the filaments during transporting whereas non-processive motors unbind from the filaments frequently. In this work, we both study the collective behaviors of processive and non-processive motors using Monte Carlo based simulations. The motor dynamics follow biased random walks subjected to a simple exclusion process.

We compare our simulation to both analytical and experimental results for the velocity as a function of applied force and number of motors. *In vivo*, we assume that the applied force is given by a calculation of the drag of the cargo. We present the comparison of analytical, simulation and experimental results for both processive and non-processive motors.

P1.8 Modelling force generation in phagocytosis

J Bradford, R Hawkins and S Johnston

University of Sheffield, UK

Phagocytosis is the mechanism whereby large particles are internalised by specialised cells known as phagocytes. This process forms a crucial component of the mammalian immune response to infection, as phagocytes form the first line of defence against invading pathogens.

Actin is known to be necessary to generate forces on the cell membrane, deforming it to engulf the target. However, it is still unclear what type and geometry of actin network is necessary. Various regulatory proteins are known to be involved but the relative importance of their roles is not yet understood.
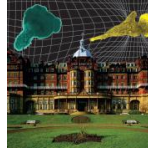
To address this we have developed a 2D stochastic simulation of actin dynamics, which uses both Monte Carlo and Brownian dynamics methods. We model processes governed by regulatory proteins including nucleation, capping, branching and severing. This model is now being coupled to a model for the membrane to investigate how the actin deforms the membrane to wrap a target particle. With this combined model we will be able to test different conditions of the underlying actin (kinetic parameters, protein concentrations, initial conditions, geometries) to see which scenarios lead to successful phagocytosis.

P1.9 Hydro-osmotic instabilities in active membrane tubes

S Al-Izzi[1], G Rowlands[1], P Sens[2] and M Turner[1]

[1]University of Warwick, UK, [2]Institut Curie, France

We study a membrane tube with unidirectional ion pumps driving an osmotic pressure difference. A pressure-driven peristaltic instability is identified, quantitatively distinct from similar tension-driven Rayleigh-type instabilities on membrane tubes. We discuss how this instability could be related to the function and biogenesis of membrane bound organelles, in particular, the contractile vacuole complex. The unusually long natural wavelength of this instability is in agreement with that observed in cells.

P1.10 The flexibility and dynamics of the tubules in the endoplasmic reticulum

D Han, S Fedotov, N Korabel, T Waigh and V Allan

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The *endoplasmic reticulum* (ER) is a single organelle in eukaryotic cells that extends throughout the cell and is involved in a large number of cellular functions. Recent super-resolution fluorescence microscopy measurements have played a key role in defining the ER's architecture in eukaryotic cells; triply coordinated matrices of rapidly fluctuating tubules of ~90 nm in diameter [1]. Using a combination of fixed and live cells (human MRC5 lung cells) in diffraction limited and super-resolved fluorescence microscopy (STORM) experiments, we determined that the average persistence length of the ER tubules was 3.03 ± 0.24 μm [2]. Removing the branched network junctions from the analysis caused a slight increase in the average persistence length to 4.71 ± 0.14 μm, and provides the tubule's persistence length with a moderate length scale dependence. The average radius of the tubules was 44.1 ± 3.2 nm. The bending rigidity of the ER tubule membranes was found to be 10.9 ± 1.2 *kT* (17.0 ± 1.3 *kT* without branch points). We investigated the dynamic behaviour of ER tubules in live cells, and found that the ER tubules behaved like semi-flexible fibres under tension. The majority of the ER tubules experienced equilibrium transverse fluctuations under tension, whereas a minority number of them had active super-diffusive motions driven by motor proteins. Cells thus actively modulate the dynamics of the ER in a well-defined manner, which is expected in turn to impact on its many functions.
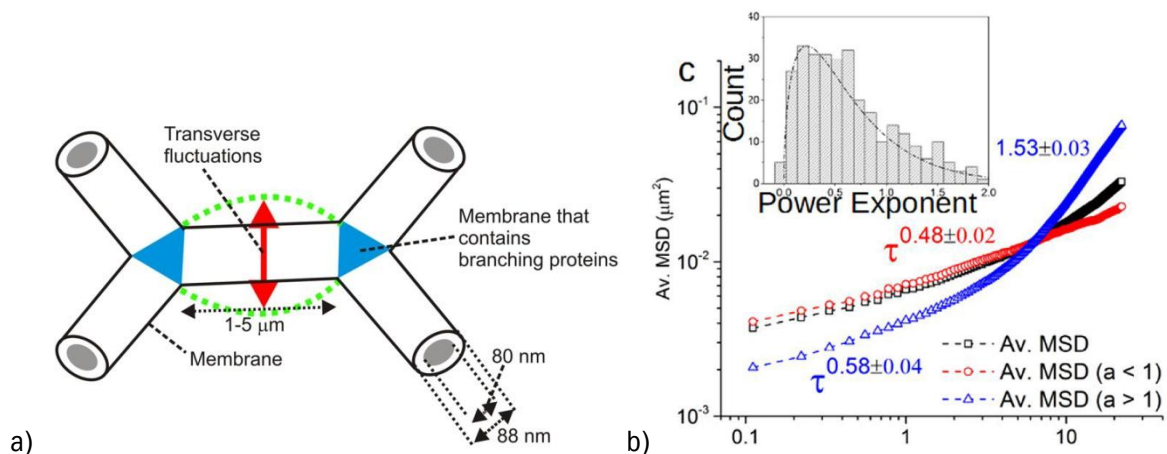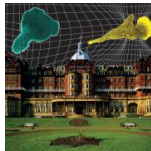

Fig. 1: a) Schematic diagram of the transverse fluctuations of the ER tubules. b) Average transverse mean square displacement of the ER tubules in live eukaryotic cells [2].

[1]   J.Nixon-Abell et al, 'Increased spatiotemporal resolution reveals highly dynamic dense tubular matrices in the peripheral ER', *Science*, 2016, 354, 6311, aaf3928.

[2]   P.Georgiadis et al, 'The flexibility and dynamics of the tubules in the endoplasmic reticulum', *Scientific Reports*, 2017, 7, 16474.

P1.11 Glycosphingolipid- and lectin-dependent endocytosis studies using a chemical biology approach

J Zell[1], J Sampaio[1], H Gao[1], R Guignard[2], J-C Florent[1], F Schmidt[1] and L Johannes[1]

[1]Institut Curie, France, [2]Institut de Recherche Servier, France

The bacterial Shiga toxin gains entry to cells on binding to its glycosphingolipid (GSL) cell receptor, Gb3, through a recently described mechanism of endocytosis, independent of the canonical intracellular clathrin machinery. We hypothesise that endocytic pits can be built in an endogenous process of cargo uptake using GSLs and lectins, which can spontaneously bend a lipid bilayer when bound. Currently we are unable to reconstitute the functionality of long-chain GSLs, such as Gb3, in cells. We aim to develop molecular tools permitting cellular reconstitution of functional GSLs in a controlled manner, employing copper-free click chemistry, and use these tools to gain mechanistic insight on GL-Lect-mediated endocytosis.

P1.12 Narrow escape: how long does it take for a camel to go through the eye of a needle?

E Meiser, M Glogger, M Engstler and S Fenz

Universität Würzburg, Germany

The narrow escape (NE) problem is a common problem in biology and biophysics. It deals with Brownian particles confined to a given domain with reflecting borders and only a small opening where the particles are absorbed. This is reminiscent of variant surface glycoproteins (VSGs) diffusing on the plasma membrane of *Trypanosoma brucei*. *T. brucei* are unicellular parasites and the causative agents of sleeping sickness in human and Nagana in cattle. In contrast to other eukaryotic cells, *T. brucei* have a sole site for endo- and exocytosis, the flagellar pocket. This small membrane invagination covers only approx. 5% of the surface. The time a particle needs to reach the target by pure Brownian motion, the mean first passage time, has been analytically calculated for several geometries in two and three dimensions. Comparison of the theoretical predicted time with measurements of VSG coat exchange in trypanosomes yields a clear discrepancy in timescales.

We will address the problem in two ways. First, by measuring site-specific VSG diffusion *in vivo* with single-molecule fluorescence microscopy. For this purpose, we recently introduced super-resolution imaging of intrinsically fast moving flagellates based on cyto-compatible hydrogel embedding [Glogger et al. JPD: Appl Phys 2017, Glogger et al. Exp Parasitol 2017]. Second, by challenging the theory of NE experimentally in micro-patterned model membranes. We will vary geometric parameters systematically and test the validity of the theoretical model in a wide phase space. We will present our first results on membrane patterning and VSG diffusion in solid supported lipid bilayers on the single-molecule level.
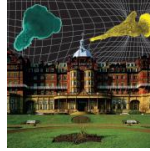
P1.13 Membrane structural remodeling upon stress/compression

C Dinet and M Staykova

Durham University, UK

Lipid bilayers supported on solid substrates have been extensively used as model systems to get insights into the physical properties of cell membranes. However, in nature cell membranes are supported on highly dynamic assemblies such as the cytoskeleton and the extra cellular matrix rather than a solid support.

To address this, we have deployed an experimental system in which a membrane is coupled to a flexible polymeric support. Our results reveal important mechanistic insights into how supported membranes can adapt to changes in the substrate area by out of plane remodeling and, by sliding [1,2]. The membrane response strongly depends on the nature of the membrane-substrate coupling.

In this work we extend the experimental model by incorporating specific membrane substrate linkages, such as biotin-neutravidin links. Our long-term plans are to study the friction force that arise between cells or cell and substrates linked by integrin and cadherin adhesion receptors.

[1]   M. Staykova et al., PNAS, 108, 9084-9088 (2011)
[2]   L. Stubbington et al., Soft Matter, 13, 181-186 (2017)

P1.14 Minimal molecular dynamics model provides insight into the connection between the structure and mechanics of ESCRT-III filaments

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University College London, UK

The endosomal sorting complex required for transport (ESCRT) machinery is a protein complex that can bend and bud membranes away from the cytoplasm. ESCRT-III is the sub-complex that is involved in a vast range of membrane deformation events, ranging from viral budding to multi-vesicular body formation and even cell division itself. Because it is so important, it has been conserved throughout evolution and across different species.

Our goal is to strip the complexity away from this machinery and identify the core functions that allow it to deform membranes. To do this, we developed a biophysical, minimal model of polymerized ESCRT-III filaments and tested their membrane deformation abilities in mesoscopic molecular dynamics simulations.
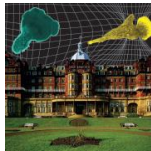
In vitro experiments have revealed the chiral nature of these filaments, as well as their strong internal bonds that allow them to be very flexible while withstanding large tensions. As the filaments polymerize on membranes, their intrinsic curvature makes them form spirals, which induce buckles to form away from the cytoplasm. With our model, we can identify the physical mechanisms that give these spirals the ability to deform membranes in such a rich variety of ways and on such different scales.

Our model consists of 3 filaments that are strongly connected to each other laterally and that have slightly different minimal energy configurations. This causes them to relax in the shape of a helix when they are not attached to a membrane. We found that these inter-filament bonds and the three-dimensional structure of the filament are essential to its ability to maintain a chiral curvature. Without them, the filament would release its inner tension (due to its being under- or over-bent) by shifting its internal structure rather than by deforming the membrane.

Using our model, we observe buckles and vesicles that develop away from the cytoplasm in molecular dynamics experiments. The spirals' inner filaments are over-bent and the outer ones under-bent compared to their preferred in-plane curvature. Releasing this tension fuels the buckle formation. However, the membrane spiral attraction itself also seems to play a vital role in pushing the membrane down, rather than letting the spiral relax upwards.

Furthermore, we discovered that a second angle acts away from the membrane surface and it is this angle that takes over once the buckles are forming.

The buckle may deepen into a tube, the dimensions of which are influenced by the filament's length, curvature and stiffness. We also found a simple way to adapt the model to work on spherical topologies, as required in modelling cell division or membrane neck scission.

P1.15 Simulation of the thermal fluctuations of red blood cells with the inclusion of hydrodynamic interactions

T Hunt[1], B McGill[2], G Marcelli[1], C Lorenz[4], P Winlov[2] and P Petrov[2]

[1]University of Kent, UK, [2]University of Exeter, UK, [3]King's College, UK

The mechanical properties of the red blood cell (RBC) membrane are of considerable significance to blood flow. This is especially so in the case of microcirculation where the cell must deform to pass through the narrow capillaries. One experimental technique for determining the mechanical properties of the membrane involves observing the fluctuations of the equatorial contour of the cell via phase-contrast microscopy[1,2]. We further extend a previous analysis, which used molecular dynamics simulation and extending them with dissipative particle dynamics simulations[3] (DPD) we analyse the fluctuations of the RBC in the equatorial contour and infer the mechanical properties of the cell. The advantage of using DPD is that we are able to calculate both the time correlation functions of fluctuations of the RBC as well as the equilibrium average of the amplitudes of the modes. The time correlation function allows us to determine the cytoplasmic viscosity, which can be affected in diseased cells. Comparison between experimental and computational spectra of the fluctuations allow the determination of the bending and shear moduli of the membrane, which are in the range of values reported in the literature.

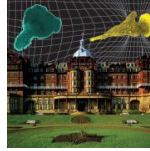## P1.16 The actomyosin cytoskeleton drives spontaneous folding of hydra fragments

X Xu[1] and Samuel Safran[2]

[1]Guangdong Technion-Israel Institute of Technology, China, [2]Weizmann Institute of Science, Israel

Hydra is a multicellular fresh-water polyp that exhibits remarkable regeneration capabilities, making it an excellent model system for studying animal morphogenesis [1]. Hydra consists of a single-axis hollow-cylindrical tube (~5-10 mm long) with a tentacle-ringed head and an adherent foot at two ends. The hydra body-tube is formed by two (endoderm and ectoderm) epithelial cell layers [2]. Hydra body shape is maintained by a contractile actomyosin cytoskeleton that spans many cells [3]. The contractile cytoskeleton fibers are longitudinally oriented (along the body axis) in the ectoderm and circularly oriented in the endoderm. Regenerating hydras use their cytoskeleton to regulate their cells and direct the regeneration process [3]. When pieces are excised from hydras, the cytoskeletal pattern survives and becomes part of the new animal. The existing cytoskeleton pattern generates a small but potent amount of mechanical force that aligns the cells during the hydra regeneration. This mechanical force can serve as a form of "memory" that stores information about the body plan [3].

At an early stage of the regeneration of hydra fragments excised from adult hydra body [3,4], the hydra fragments spontaneously and quickly fold; their cytoskeleton then remodels to find a balance between maintaining its preexisting organization and adapting to the new curved conditions. After about one hour of the folding-remodeling loop, the excised pieces fold into small hollow spheroids [3].

We focus on the theory behind the observed spontaneous folding of freshly excised hydra fragments. We propose that the spontaneous folding is driven by the contractility of the supracellular cytoskeleton fibers. This contractility effectively generates a "spontaneous curvature" c0 to the fragment, which can be calculated using a simple elastic multilayer model. We find that c0 is proportional to the cellular contractility and elastic asymmetry of the ectoderm and endoderm layers. In addition, we have also explored the curling kinetics of hydra strips and their dependence on the fragment length. We expect that short hydra strips fold quickly into a circular arc, while for long strips, the folding starts quickly at the edges and then slows down as the two curls at the edges approach each other. Similar models can be applied to square hydra fragments where the two perpendicular cytoskeleton fibers cooperate and drive the squares to fold into spherical caps. Interestingly, in this case, we predict potential "active auxetic" responses of hydra squares to external

gentle bending. We compare our theoretical predictions to experiments on the shape and folding of hydra segments. Finally, we point out that the concept of spontaneous curvature and active auxetic generated by the contractility of the aligned, supracellular cytoskeleton structure can also be useful to better understand the spontaneous folding of other tissues and organs in other animal species.
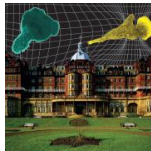
[1]   Galliot, B. Regeneration in Hydra. eLS (2013).
[2]   Fujisawa, T., Developmental dynamics, 226, 182 (2003).
[3]   Livshits, A. et al, Cell Reports, 18, 1410 (2017).
[4]   Krahe, M. et al New J. Phys. 15, 035004 (2013).

P1.17 Modelling of mechanical forces during annual fish pre-epiboly and epiboly

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Universidad de Chile, Chile

Both pre-epiboly and epiboly processes in annual fishes (Austrolebias sp) are analyzed modelling the epithelial tissue as an elastoplastic medium. To describe its dynamic we use a Vertex Model, that is, the epithelium is taken as a network of well-defined vertices forming polygonal cells. Within a cell, we account for forces driven by pressure, and cortical and adhesion tensions. Mechanical forces are applied to the vertices, which result in the tendency of the cells to attain target areas and perimeters. We consider plasticity for the target area as well as for the target perimeter. During the pre-epiboly stage (approximately ten hours) we allow for cellular rearrangements while keeping their volume constant as they get thinner. Cells accommodate over a sphere-shape surface of radius 594 μm , which models the yolk cell. It is found that a linear function in time models the evolution of the target area due to the cells rearrangements in the tissue. A Monte Carlo optimization is performed to find the best parameters that fit the experimental data obtained by tracking the cell edges and vertices. In this stage some cells experience active contractive pulses, which are related to frustrated cellular divisions. Each pulse lasts for about two hours and via the elastic network moves the surrounding cells. We account for these pulses by reducing, for a given time, the target area of the contracting cell. During the epiboly stage (approximately twenty hours), on the other hand, we consider an active force acting on the edge of the tissue, forcing those vertices to move at a constant velocity, 20 μm/hr, in the polar direction (theta direction in spherical coordinates). For this stage, we performed another Monte Carlo optimization, finding the best parameters that fit experimental data through this embryonic process. For the pre-epiboly stage, we obtain plastic relaxation times of 0.2 and 7.2 hours, for the area and the perimeter, respectively. The area elastic constant is 5.3 $hr^{-1}\mu m^{-2}$, and the perimeter elastic constant is 2.3 10^{-4} hr^{-1}. The constant related to the adhesion-driven tension is found to be -40 μm/hr. Finally, the target areas reductions are of the order of 10^3-10^4 μm^2. For the epiboly stage, we obtain plastic relaxation times of 0.5 and 3.7 hours, for the area and the perimeter, respectively. The area elastic constant is 18.2 $hr^{-1}\mu m^{-2}$, and the perimeter elastic constant is 1.8 10^{-4} hr^{-1}. The constant related to the adhesion-driven tension is found to be -130.6 μm/hr.

P1.18 Deformation Experiments of MDCK II model tissue

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Epithelia forms active two-dimensional cell sheets that act as barriers between different tissue types and form boundaries of the majority of organs. These are oft exposed to mechanical stress, i.e. in the peristaltic tract or in the expansion of the lung. The tissue quickly responds to this mechanical stimulation by changing shape and internal organization of the tissue on short time scales. On long time scales tissue can release stress by proliferation and growth.

To study the response of MDCK II model tissue, we grow cell colonies on PDMS substrate and expose them to a uniaxial stress (10-30%) using a stretching device [1]. The resulting changes are monitored from minutes over days by phase-contrast or confocal microscopy. The reversible changes in the overall shape of the cell confirms that on short time scales the tissue responds as an elastic material. This is furthermore confirmed by analysing the morphology and connectivity of individual cells before and after stress. On longer time scales, we find that the growth rate of the whole colony is affected by the continuous deformation, while after 24-48h the tissue adapts and resumes its initial morphology, depending on the stretching amplitude.
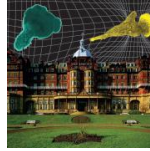
[1]   Faust et al. "Cyclic stress at mHz frequencies aligns fibroblasts in direction of zero strain." *PloS one* 6.12 (2011): e28963.

P1.19 Curvature-dependent control of oriented epithelial tissue growth by anisotropic cell-scale topography

P Rougerie[1], L Pieuchot[2], R S dos Santos[1], T Canuto[1], J Marteau[3], M Bigerelle[4], K Anselme[2] and M F de Souza[1]

[1]Universidade Federal do Rio de Janeiro, Brazil, [2]Université de Haute-Alsace, France, [3]Université de Technologie de Compiègne, France, [4]Université de Valenciennes et du Hainaut Cambresis, France

Symmetry breakage and transition between isotropic to anisotropic tissue growth is an essential and widespread phenomenon during embryo morphogenesis or tissue regeneration. The parameters of the microenvironment controlling this transition and the repercussions on tissue homeostasis are poorly understood. Here, we investigate the role of the environment topography by developing an *in vitro* model based on a series of microstructured substrata to study anisotropization of epithelial monolayers. We first show that anisotropic cell-scale topographies can instruct the epithelial colonies to grow in a preferred direction. Importantly, we show that it is possible to control the extent of anisotropy reached by the growing epithelium by modulating the transverse positive (convex) curvature of the surface. We establish that increasing convexity leads to local heterogeneity of F-actin density and the progressive exclusion of cell nuclei. This results in cell reorientation along the substrate's major axis. Interestingly, we found that substrate anisotropy also reorients mitotic spindles and cell division axis, which further enhances the overall oriented tissue growth. We hypothesize that these curvature-dependent "topographical barriers" induce a symmetry breaking of the overall mechanical tension in the epithelia due to an alignment of the actin stress fibers. Altogether, this work demonstrates that it is possible to use the anisotropy of cell-scale topographies as a guidance direction and local curvature as a tuning parameter to orientate the mechanical tension of epithelial tissues and control their morphogenesis.

P1.20 Tissue fluidity promotes epithelial wound healing

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Many developmental processes, such as morphogenesis, wound repair, and apoptosis, involve the closure of epithelial tissue gaps to maintain mechanical integrity. While the biochemical pathways for wound healing are well characterised, it remains poorly understood how the tissues mechanical properties regulate wound healing. Using a combination of computational modelling and laser ablation experiments on the Drosophila wing imaginal disc, we find two stages of closure. First, an actomyosin purse-string assembles around the wound leading to rapid reduction in wound area. In the second phase, the wound closure rate slows down dramatically, with cell intercalations prevalent at the wound edge that progressively decreases the number of cells contacting the wound. Using a computational model, we find that these intercalations necessary for efficient closure, with wounds jamming when intercalations are disabled. The model further predicts that tissue fluidization by intercalations, and the rate of wound closure can be tuned by modulating tissue tension. Finally, we demonstrate that by tuning the activity of myosin II, and therefore contractility, we can systematically vary the rate of intercalations and healing. This new mode of wound closure via tissue fluidisation potentially opens new therapeutic avenues, through which wound healing can be accelerated.

P1.21 A role for autophagy in YAP/TAZ dependent tumor plasticity and somatic cell reprogramming

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University of Padova, Italy

YAP and TAZ are potent inducers of cell proliferation and important drivers of tumorigenesis. Yet, the mechanisms of how they execute their functions remains under-investigated. Here, through a siRNA-based loss-of function screen among YAP/TAZ direct transcriptional targets, we found that autophagy can be regulated by YAP/TAZ and this regulation can modulate YAP/TAZ-dependent cancer cell proliferation and plasticity. Of note, autophagy can also participate in YAP/TAZ-induced conversion from differentiated cells into somatic stem-like cells. Therefore, our work not only suggests autophagy as one of the underlying mechanism of YAP/TAZ biological functions in tumorigenesis, but also reveals a novel role of autophagy in somatic cell reprogramming.

P1.22 Investigating the dynamics of apico-medial Myosin-II foci

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[1]Universite Grenoble Alpes, France, [2]University of Cambridge, UK, [3]Universidad Europea de Madrid, Spain

During development, tissue morphogenesis is often driven by pulsatile cell shape changes. These changes are driven by Myosin-II which localizes at the apical cortex to form foci. While it is well known that there is a strong correlation between myosin pulses and cell shape changes, the apparent motion of these foci has been less studied. We investigate whether the dynamics of actomyosin foci can be explained by biochemical or mechanical factors within the tissue, since these may have implications on the function of foci themselves.

Using automated image analysis of amnioserosa cells with tagged Myosin-II during Drosophila dorsal closure, we isolate the foci and track the movements of thousands of them in multiple cells. We observe that it is common that several foci are present simultaneously within the same cell apex, and that events of merging and splitting are equally frequent. Asking whether the motion of foci behaves as a random walk, we
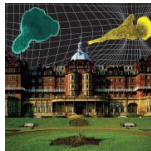
found that the distance they travel grows nearly linearly in time, showing that the motion is not random. It is therefore possible to define a direction of movement for each focus. We find that this direction is correlated with the orientation of the cell when it has an elongated shape. We hypothesize and test three possible contributions that may explain this deviation from random walk behaviour: the role of the underlying tissue motion, a self-avoiding behaviour due to cortex properties, and the directed motion of foci induced by a biochemical or mechanical cause.

P1.23 Geometry and resilience of biological transport networks: Local rules for robust global transport in liver networks

J Karschau[1], A Scholich[2], J Wise[1] and B Friedrich[1]

[1]TU Dresden, Germany, [2]Max Planck Institute, Germany

The liver resembles a "chemical factory" that is characterized by intertwined transport networks for the transport of toxins and metabolites. Each hepatocyte cell interacts with two space-filling networks of bile canaliculi and sinusoids, which transport bile or blood, respectively. How these networks grow and establish their distinct geometry to supply all cells – besides adapting to a variable load and being robust against local perturbations – remains elusive.

Here, we analyse experimental high-resolution imaging data from mice, and report design principles of these microcirculatory networks. We find the networks to represent true sub-graphs of the Delaunay graph of their nodes, highlighting a strong preference for nearest neighbour edges. Similarly, the minimum spanning tree of the experimental networks approximately equals the true minimum for the given set of nodes, suggesting that experimental network growths follows a cost optimisation algorithm.

We provide a simple network generation algorithm that faithfully reproduces local network statistics, starting from a set of simulated node positions, using multi-objective optimization for node degree and edge length distribution. We compare transport properties of experimental and simulated networks, including their resilience to random perturbations using a novel "transport-at-risk" measure. By connecting statistical properties of network geometry and transport capacity, we address the link between form and function in a complex tissue.
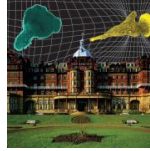
P1.24 High-throughput platform for rapid TEER measurement of Organ-on-a-Chip endothelial and epithelial tubules

A Nicolas[1], B Trietsch[1], D Kurek[1], T Hankemeier[2], J Joore[1] and P Vulto[1]

[1]Biopol - Mimetas B.V., The Netherlands, [2]Leiden University, The Netherlands

Organ-on-a-chip technology has rapidly grown in the past decade, driven by the need for better predictive in vitro models for drug efficacy and toxicity assessment. These systems enable the formation of endothelial and epithelial tubules that are used to mimic in vivo cues such as flow exposure, mixed co-culture and overall micro-environment. In conventional barrier transport studies, Trans Epithelial/Endothelial Electrical resistance (TEER) is used to determine the integrity of barrier tissues. However, current approaches to TEER measurement involve the use of chopstick electrodes, incompatible with high-throughput Organ-On-a-Chip platforms.

Using the OrganoPlate®, a microfluidic platform for perfused 3D cell culture developed by MIMETAS (The Netherlands), one can replicate ECM-supported tubular structures and study the transport of drugs across a cellular barrier [1]. The format of the OrganoPlate makes it very suitable for high-content imaging [2], but the need for TEER-based measurements of cell models in the OrganoPlate still needs to be addressed.

To this end, we developed a fully automated TEER measurement platform capable of addressing up to 96 tubules in an OrganoPlate. The developed system makes use of an electrode interface compatible with the OrganoPlate microfluidics layouts. The system is lightweight, fits in an incubator and can be used in combination with a rocker platform to provide perfusion in parallel to long-term TEER experiments. The device can read out an entire OrganoPlate with 96 perfused tubules within 60 seconds and allows programmable measurements over the entire duration of an epithelial/endothelial study. We quantified TEER values in multiple epithelial/endothelial tubules, including widely used primary and immortalized cell lines such as Caco2, Huvec and HepG2, and developed an automated signal analysis solution, suited for high-throughput assays in the OrganoPlate. To validate the system for compound exposure studies, experiments were conducted on an OrganoPlate Caco2 gut model. Collagen I at 4mg/ml was layered in the gel compartment of the OrganoPlate. A suspension of Caco2 cells at 10e6 cells/ml was added to the perfusion compartment and incubated at 37 degrees on a perfusion rocker. TEER was monitored for 11 days until a plateau was reached at 490 Ohm.cm² +- 87 Ohm. Additionally, inflammatory cytokines were added on a subset of the tubules at day4. Subsequent decrease in TEER at 72h was quantified showing compound dependent decrease in TEER.

Complementing the OrganoPlate scope of application, we developed a novel technique for on-a-Chip epithelial/endothelial tissue TEER investigation with very fast measurement times, automated signal extraction and compatible with tissue culture incubator environments. This provides a valuable tool for drug toxicity and transport studies in Organ-on-a-Chip.

[1]   Trietsch, S.J., Israels, G.D., Joore, J., Hankemeier, T., Vulto, P. , Microfluidic titer plate for stratified 3D cell culture, 2013, Lab chip, vol. 13, no. 18, pp. 3548-54.
[2]   Trietsch, S.J. et al., Membrane-free culture and real-time barrier integrity assessment of perfused intestinal epithelium tubes, Nature Communications, vol.8, no. 262 (2017)
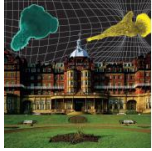
P1.25 Interplay between tissue organisation and planar cell polarity

S Tan and D Strutt

University of Sheffield, UK

Planar cell polarity (PCP) is a primary feature of epithelia cells, which controls epithelial cell morphogenesis and cellular pattern formation. The mechanisms underlying planar polarisation have been most thoroughly studied in the *Drosophila melanogaster* wing, where PCP pathways orient wing hair outgrowth on the distal edge of the cell. During wing morphogenesis, cells undergo dynamic division, elongation and rearrangement into a hexagonally organised tissue from 15 hAPF to 30 hAPF. Interestingly, the magnitude of PCP protein polarisation intensifies when cells achieve a regular and well organised tissue geometry. The precise temporal correlation of these events indicates the possibility of cross regulation. Could the increase in polarisation magnitude be related to tissue organisation and vice versa? In this project, we would like to investigate the interplay between PCP proteins polarisation and epithelial tissue organisation in the wing epithelium. In order to comprehend the complex spatiotemporal relationship of planar polarity and cell packing geometry, we have developed unique computational tools capable of harnessing the rich content of images in terms of cell morphological parameters, epithelial tissue organisation and cell polarisation. We altered epithelial cell packing in vivo and assay for PCP polarisation using genetic manipulations and laser ablation assay. our preliminary results suggested that the epithelial organisation of pupal wing might influence the competence of core PCP proteins to polarise.

[1]   Strutt, Helen and David Strutt (2009) S*eminars in Cell & Developmental Biology 20.8*: 957-963
[2]   Classen, Anne-Kathrin et al (2006) *Developmental Cell 9.6*: 805-817

P1.26 Constricted migration increases DNA damage and independently represses cell cycle

C Pfeifer[1], Y Xia[1], K Zhu[1], D Liu[1], J Irianto[1], V M Garcia[1], L S Millan[1], B Niese[1], D Deviri[2], R Greenberg[1] and D Discher[1]

[1]University of Pennsylvania, USA and [2]Weizmann Institute of Science, Israel

Cell migration through dense tissues or small capillaries can elongate and even damage the nucleus, with any effect on cell cycle potentially impacting processes such as carcinogenesis. Here, nuclear rupture and DNA damage increase with constricted migration in different phases of cell cycle—which we show is partially repressed. We study several cancer lines that are contact inhibited or not and that exhibit diverse frequencies of nuclear lamina rupture after migration through small pores. DNA repair factors mis-localize after migration, and we consistently measure an excess of DNA damage based on pan-nucleoplasmic foci of phospho-activated ATM and γH2AX. Foci counts are suppressed in late cell cycle in agreement with expected checkpoints, and migration of contact-inhibited cells through large pores into sparse microenvironments leads to cell cycle re-entry and no effect on a basal level of damage foci. Constricting pores delay such re-entry while excess foci occur independent of cell cycle phase. Knockdown of repair factors increases DNA damage independent of cell cycle, consistent with effects of constricted migration. Because such migration causes DNA damage and impedes proliferation, constricted migration illustrates "go or grow".

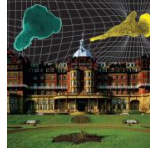### P1.27 The dystroglycan LINC: the functions of dystroglycan in the nuclear envelope

B Stevenson, S Winder and J Hobbs

University of Sheffield, UK

Dystroglycan is a ubiquitously expressed protein which goes through proteolytic cleavage to produce a- and b-subunits which are transported to the plasma membrane (PM) where they link the extracellular matrix with the cytoskeleton. The bsubunit is a transmembrane protein important for the aforementioned connection, however, there is also a growing body of evidence for b-DG being present in the nuclear envelope (NE). The translocation of b-DG from the PM to the NE has been well characterised but despite this the role b-DG plays within the NE remains to be elucidated.

We have preliminary evidence suggesting an interaction between b-DG and the LINC complex in the NE. The LINC complex connects the cytoskeleton to the nuclear lamina, thus nuclear b-DG may play an analogous role to b-DG in the PM. Based on this the mechanical properties of the nuclei from both wild type and DAG1 knockout cell lines will be assessed. This will be evaluated primarily using atomic force microscopy to determine the Young's modulus of isolated nuclei. Additionally, the mechanical properties will also be assessed using microchannels to understand the effects b-DG deficiency has on the migration of cells through extremely narrow channels (<5mm) and ultimately the deformability of the nucleus.

Our current data suggests morphological differences between wild type cell lines and the DAG1 knockouts. In addition, there appears to be deficiency in proper emerin localisation to the NE in the knockout cells suggesting a potential role for nuclear b-DG in the stability of the nuclear lamina.

P1.28 The reduced approach to the stochastic modelling of cooperative Ca^{2+} release through IP_3R channels yields the global characteristics of the cell regulation

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University of Southampton, UK

Clusters of IP_3 receptor channels in the endoplasmic reticulum (ER) membranes of many non-excitable cells release calcium ions in a cooperative manner giving rise to dynamical patterns such as Ca^{2+} puffs, spikes, waves and oscillations that occur on various spatial and temporal scales. We introduce a stochastic reaction-diffusion model of randomly distributed IP_3 receptors using a principled reduction [1] of a detailed Markov chain description [2] of individual channels. Our model reveals how the crucial characteristics of cell regulation, such as inter-spike intervals (ISI), depend on the IP_3 loads in agreement to various theoretical [3] and experimental [4] studies. We employ the reduced approach to obtain the ISI to standard deviation relation which is found to be critical in the cell regulation [5]. From the correlation analysis of the $[Ca^{2+}]$ at the neighbouring clusters, we obtain the threshold value of $[IP_3]$ for initiation and propagation of Ca^{2+} wave. Thus, we aim to link the local properties of IP_3R channels and clusters, such as channel type or coupling between channels, with the global patterns which may emerge from these channels, e. g. spikes, waves or oscillations.
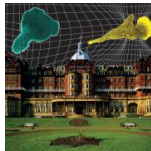
[1]   S. Rüdiger, "Excitability in a stochastic differential equation model for calcium puffs", Phys Rev E, vol. 89, no. 6, pp. 062717–1–062717–6, 2014.

[2]   G. W. De Young and J. Keizer, "A single-pool inositol 1,4,5-trisphosphate-receptor-based model for agonist-stimulated oscillations in Ca^{2+}concentration", Proc Natl Acad Sci USA, vol. 89, no. 20, pp. 9895–9899, 1992.

[3]   M. Rückl, I. Parker, J. S. Marchant, C. Nagaiah, F. W. Johenning, and S. Rüdiger, "Modulation of Elementary Calcium Release Mediates a Transition from Puffs to Waves in an IP_3R Cluster Model", PLOS Comput Biol, vol. 11, no. 1, p. e1003965, 2015.

[4]   A. Skupin, H. Kettenmann, U. Winkler, M. Wartenberg, H. Sauer, S. C. Tovey, C. W. Taylor, and M. Falcke, "How does intracellular Ca^{2+}oscillate: by chance or by the clock?", Biophys J, vol. 94, no. 6, pp. 2404–2411, 2008.

[5]   K. Thurley and M. Falcke, "Derivation of Ca^{2+}signals from puff properties reveals that pathway function is robust against cell variability but sensitive for control.", Proc Natl Acad Sci USA, vol. 108, no. 1, pp. 427– 432, 2011.

P1.29 Role of hydrodynamic forces in beating orientation of mammalian motile cilia

N Pellicciotta

Cambridge University, UK

Fluid flow generated by a ciliated epithelium is a fascinating example of collective behaviour in nature: thousands of microscale whip-like structures called motile cilia beat at the same frequency and in a coordinated fashion. This dynamics has been reported to have fundamental physiological roles in microorganisms and in many organs of vertebrates. In the airways, the coordinated beating of motile cilia generates a fluid flow that is able to push the mucus towards the pharynx and so protect the lungs from any inhaled contaminants. Airway cilia must be aligned along the longitudinal tissue axis to generate proper mucociliary clearance. The establishment of such directional motility happens during ciliogenesis, a process that spans from late embryonic to early postnatal days. The biological or mechanical cue that drives such orientation is still unknown.

Our hypothesis is that hydrodynamic forces could be involved in the breaking of symmetry for cilia orientation during ciliogenesis. We developed a novel organ on-chip where airway cells can be grown in vitro and exposed to shear stresses during ciliogenesis. Specifically we apply fluid flow prior to ciliogenesis to mimic mechanical stresses from prenatal fetal breathing and air flow to study postnatal stresses caused by breathing. We have preliminary data suggesting that these shear stresses during development are indeed important for cilia alignment and global coordination.

P1.30 Deformable active nematic shells

L Metselaar[1], A Doostmohammadi[2] and J M Yeomans[2]

[1]Rudolf Peierls Centre for Theoretical Physics, UK, [2]University of Oxford, UK

When an active nematic is confined to the surface of a vesicle four +1/2 motile defects lead to complex spatiotemporal patterns [1]. Using a hybrid finite difference-lattice Boltzmann model we investigate how such active nematic shells can deform under the influence of activity. In particular we study the relationship between the shape of the vesicle and the positions of the topological defects on its surface.
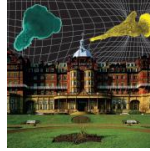
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P1.31 Emergent hunting behaviors of the unicellular predator Lacrymaria encoded in coordination of its active molecular systems

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Stanford University, USA

Conserved active systems operate inside all living cells, but it is often unclear how specific cellular behaviors are built up using these shared components. To gain insight into the origin of complex cellular behaviors, we analyzed the hunting strategy of *Lacrymaria olor*, a unicellular predatory ciliate that can extend and whip its "neck" over many body lengths to locate and strike prey. By tracking the morphology of single cells in real time over hours we found that the fast-timescale motions of the cell underlie an efficient hunting behavior apparent at longer timescales. This emergent behavior is governed by a tug-of-war between subcellular anatomical structures that use cilia and contractility to extend, deform, and retract the cell's neck, which remarkably is flexible enough to permit access to almost any nearby location. Using principle component analysis, we show that this flexibility can be described statistically in terms of a small number of normal harmonic modes and identify behavioral subroutines that the cell uses to provide reach and facilitate search during hunts. A choice between low-amplitude steering and buckling behaviors and high-amplitude whipping behaviors is controlled by the length of the neck relative to its mean extension, and perturbations to the cell's signaling controller demonstrate that executing these behaviors depends on coordination between ciliary and contractile programs; neither active system on its own permits access to the shape space necessary for effective search. Our results highlight the importance of how modular active subsystems are organized and coordinated to build up and encode cell behaviors, suggesting alternative paths to engineering complex behaviors at the molecular scale.

P1.32 Mechanical environment influences macrophage morphology and inflammatory response

J-C Escolano[1], G Ng[1], S Abuhattum[2], W Shen[3], C Lamaze[3], U Freudenberg[4], C Werner[4], C Bryant[5] and J Guck[1]

[1]TU Dresden, Germany, [2]JPK Instruments, Germany/ TU Dresden, Germany, [3]Institut Curie, France, [4]Leibniz Institute of Polymer Research, Germany, [5]University of Cambridge, UK

Mechanical mismatch between compliant tissue and stiff materials hinders biointegration and limits the long-term success of implanted prostheses. This limitation is primarily due to the foreign body reaction, a proinflammatory response that damages surrounding tissue and engulfs the region in fibrotic tissue. We have previously shown that microglia, macrophage-like immunosensory cells in neural tissue, are mechanosensitive to inert polyacrylamide gels both *in vitro* and *in vivo*. These cells, like macrophages, direct inflammatory reactions and the foreign body response. However, the mechanism of their inflammatory mechanosensitivity has remained elusive. Here, we utilize an engineered novel polymer with independently tunable mechanical properties, star-polyethylene-glycol (star-PEG) crosslinked to heparin, to investigate how bone marrow derived macrophages (BMDM) sense the stiffness of their substrate and trigger inflammatory reactions. Using this system, we also examine the potential role played by different membrane reservoir components and the activity of certain ion channels in macrophage mechanosensing. Our findings could contribute to better explain how engineered bioinert *in vitro* polymers can trigger robust inflammatory reactions *in vivo*. Furthermore, information of the underlying molecular and biophysical mechanism could aid in the rational design of biomaterials, enable pharmacological control against mechanosensitive pathways, and have further implications in amyloid-driven and chronic inflammatory disorders.

P1.33 A role for Caveolin-1 as a potential integrator of mechanoadaptive and metabolic networks in the cell
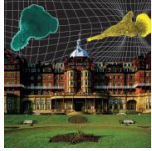
V Jiménez[1], M Sánchez-Álvarez[1], J Guck[2], F Sánchez-Cabo[1] and M Á del Pozo[1]

[1]Centro Nacional de Investigaciones Cardiovasculares Carlos III, Spain, [2]TU Dresden, Germany

Caveolin-1 (CAV1) participates of multiple processes in the cell, including plasma membrane organization (through structures termed caveolae), lipid metabolism and trafficking, signaling integration and regulation, and mechanotransduction. However, we are far from fully understanding its contribution to cell behavior and disease. CAV1 can directly interact with many regulators of fundamental cellular processes such as cell cycle or mechanotransduction- but it can also dramatically impact cell physiology due to its scaffolding activity on specific membrane nanodomains, which are in turn responsible for the indirect modulation of the activity of other protein receptors and effectors, by controlling their spatiotemporal distribution along the different cellular membranes.
We hypothesize that CAV1 expression levels specifically couple mechanical phenotypes with other cell functions such as cell proliferation control and metabolism. This coupling would thus underpin key functional emerging properties across tissues, as well as their propensity to develop pathological alterations such as neoplastic disease.
To explore these systems-level aspects of CAV1 biology, we are combining complementary approaches that integrate bioinformatics and comparative biophysics with molecular biology and genetic engineering. Here, we discuss the expected challenges and advantages of each approach, and the insights we envisage our studies will potentially contribute to our understanding of the principles ensuring cellular homeostasis, and how their disruption might favour the onset and progression of diseases such as cancer or neurodegeneration.

P1.34 Regulation of the Hippo pathway via the multi-PDZ domain protein MAGI-1 in epithelial cells

C Murzeau and K S Erdmann

The University of Sheffield, UK

YAP/TAZ are transcriptional coactivators that control essential cellular processes such as proliferation. Their level of expression and subcellular localization are regulated by an intricate network of signalling pathways among which is the Hippo signalling cascade. The core Hippo pathway is composed of a kinase cascade which phosphorylate and thereby inhibit the activity of YAP/TAZ. Tight and accurate control of YAP/TAZ activity is paramount to ensure tissue homeostasis as hyper-activation of YAP/TAZ leads to tissue overgrowth and cancer formation. However, the mechanisms at play in the signal transduction from upstream mediators, such as tight junctions, to the core Hippo cascade remain largely elusive.

MAGI-1 is a tight junction, multi-PDZ domain scaffolding protein and therefore plays a crucial role in ensuring epithelial integrity. This essential feature of epithelial tissues is often disrupted in cancer and gives way to metastasis. MAGI-1 was recently reported to regulate YAP subcellular localization in a cell density-dependent manner. However, the mechanism remains unclear. Here, we provide novel evidence further establishing MAGI-1 as a regulator of the Hippo pathway. We used CRISPR/Cas9 technology to generate a MAGI-1 knockout cell line in colorectal cancer epithelial cells, DLD-1. Moreover, we have further confirmed that MAGI-1 physically interacts with key regulators of the Hippo pathway such as the LATS1/2 kinases, the protein tyrosine phosphatase PTPN14 and YAP itself. We therefore suggest that MAGI-1 acts as a scaffolding protein, assembling part of the Hippo signal transduction machinery at the membrane and provides a link between tight junction integrity and YAP regulation.

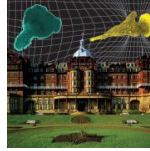## P1.35 Study of the mechanical role of caveolae in 3D tumoral proliferation

C U Martín and C Lamaze

Institut Curie, France

Caveolae are plasma membrane invaginations that require caveolin (Cav) and cavin proteins for their biogenesis. Recently, our laboratory discovered a key function of caveolae in membrane tension homeostasis. When membrane tension is increasing through mechanical stress, caveolae immediately flatten out into the PM such as to "buffer" the membrane tension increase and protect the plasma membrane. Cancer cells are facing a wide range of mechanical stresses and grow in a complex three-dimensional (3D) environment. In order to reproduce a more relevant to physiology environment I took advantage of two different but complementary technologies to exert a 3D long term confinement and mimic carcinoma in situ environment. I first used a unique microfluidic co-extrusion device to form alginate-encapsulated multicellular spheroids. My first results showed a loss of the caveolar ATPase EHD2 expression and increase of Cav2. It is interesting as changes in expression of caveolins and EHD2 have been related to tumor progression in vivo. I recently confirmed my results with a complementary technology based on hyperosmotic induced compression in multicellular spheroids. We believe that these data may represent a caveolae-mediated adaptation of the cells to compressive stress. For the remaining time of my thesis project, I will determine by which mechanism(s) EHD2 is lost and the consequence(s) of this loss on breast cancer cells using the two unique compressive systems.

I aim to:

Explore the caveolae response to mechanical stress in a 3D environmentInvestigate the possible roles of caveolae in the regulation of different signaling pathways upon mechanical confinement and its interrelation with cancer growth and invasion.

P1.36 On the thermodynamic principles of nonlinear acoustic propagation on lipid monolayers

K H Kang and M F Schneider

Technical University Dortmund, Germany

Biological membranes form hydrated, quasi-2D elastic interfaces, and it has been proposed that mechanical signals along the membrane plays a fundamental role in biological communication. Such interfaces have also been shown to be held at physiological conditions in which they are susceptible to undergo phase transition. Here we investigate whether thermodynamic principles can be applied on interfaces to study mechanical signaling along membranes and whether the signaling characteristics can be derived from the state diagram of the system. Using fluorescent probes embedded on an lipid monolayer assembled at the air-water interface, the state (pressure, density, etc.) of the interface is observed using FRET. Optical measurements show that a localized, mechanical impulse imposed on the interface propagates through the monolayer as acoustic waves. Notably, an above-threshold stimulation near the phase transition region of the state diagram (liquid-expanded/liquid condensed) can drive the waves into the nonlinear regime. This generates all-or-none type solitary pulse, and the threshold behavior and the pulse shape show striking similarity with the nervous impulse. When such nonlinear pulses collide head on, they can interact and annihilate each other, a behavior observed in collision of action potential in excitable cells. Altering the macroscopic state (pH, ionic concentration (Na+, Ca2+), temperature, etc.) changes the material properties of the membrane (e.g. lateral compressibility) and consequently affects pulse velocity, amplitude, and relaxation. These results altogether show that the thermodynamic state and its changes determine the existence and propagating properties of interfacial waves, and they support a physical basis of communication in living systems.

P1.37 Single-molecule biomechanics of HIV binding to broadly neutralising antibodies probed through novel integration of magnetic tweezers with digital holography
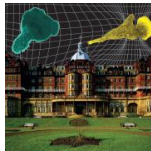
J Flewellen[1], I L Garcia[2] and P Tolar[1]

[1]The Francis Crick Institute, UK,[2]University College London, UK

Magnetic tweezers offer a straightforward, non-invasive technique for high-throughput force spectroscopy experiments on the microscale. We present a bespoke magnetic tweezer system and molecular biology assay capable of probing the biomechanical interactions between single molecules. In our system, DNA tethers, bound to a glass surface at one end, are conjugated to antigen molecules of interest at the other. The antigens bind to complementary antibodies, which in turn are fixed to superparamagnetic microspheres. These microspheres respond to an external magnetic field gradient, which supplies an exquisitely controllable force clamp.

Our system works on the premise of being able to track in three dimensions the position of the microspheres, which yields the biomechanical interaction between the antigens and antibodies. By incorporating digital holographic microscopy into our tweezer apparatus – thus encoding the 3D electromagnetic field of the sample – and by extending 2D image processing techniques to work on three-dimensional data, we have developed a novel approach to acquire and process force spectroscopy data. This approach simplifies magnetic tweezer force spectroscopy experiments by removing the need to pre-record a look-up table of microsphere images at different z-positions in order to obtain bead height by comparison. Video microscopy facilitates high-throughput recording of many single-molecule interactions simultaneously; our holographic imaging technique enables a greater number of these to be recorded per field of view than current techniques.

We use this system to probe the biomechanics of interactions between human immunodeficiency virus (HIV) gp120 envelope proteins and antibodies derived from co-evolved B lymphocytes. The high mutability of HIV

means it evades the typical antibody response. However, a class of antibodies, known as 'broadly neutralising', capable of counteracting HIV does develop naturally in a minority of chronically infected patients. Such broadly neutralising antibodies are typically selected against by normal cellular differentiation processes throughout the immune response, a process that is thought to be influenced by the strength and nature of physical binding between the B cell receptor and antigen proteins. Our apparatus is capable of probing biomechanical interactions between HIV gp120 envelope proteins and both specific and broadly neutralising antibodies. This will lead to a greater understanding of the physical forces involved in immune cell-pathogen interaction – necessary for the development of next-generation vaccines.

P1.38 Nuclear mechanical issues during direct neuronal reprogramming

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[1]King's College London, UK, [2]Johannes Gutenberg University, Germany

In the course of cell reprogramming, the genetic expression is modified at the same time as the cell body shape is rebuild. In particular, astrocytes undergo dramatic morphological changes to become induced-neurons (iNs) among which the cell cytoplasmic volume must be radically reduced. At the same time, we found a systematic gradual shrinkage of the cell nuclear volume at the beginning of reprogramming. Here, we demonstrate that nuclear shrinkage must escort the reprogramming process for a successful occurrence. Moreover, the modification of the nuclear shape by pharmacological and mechanical stimuli can induce per se alterations of gene expression and protein production suggesting cell fate can be manipulated by readjusting nuclear morphology. Our results, pave the way to engineer cell μenvironments in order to mechanically prepare and promote glial reprogramming to iNs for applications in regenerative therapies.
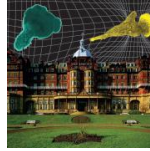
Poster session 2

P2.1 Epithelial cell proliferation under 3D constraint

I D Meglio, A Trushko and A Roux

University of Geneva, Switzerland

The formation of organs and tissues during early embryogenesis is characterized by profound changes in the shape and form of these tissues. Many of these developmental processes involve the invagination of a continuous sheet of epithelial cells. Morphogenesis is thus a physical process where tissues must deform, fold and push to give rise to predefined shapes and sizes. Importantly, these changes in form are dictated by processes that take place at the cellular level, chiefly cell proliferation, cell shape changes, cell rearrangement and cell death. These cellular processes will (I) eventually define the shape of the tissue and (II) give rise to forces that are propagated within and between tissues. Once the tissue has fully formed, cells also encounter a range of different geometrical conditions, such as curvature or substrate stiffness. Forces that arise during development and that are present during tissue homeostasis must be tightly regulated, yet our understanding of the mechanisms underlying this regulation remains elusive. With the goal of better understanding the coupling between mechanical forces and cellular processes like proliferation, we are using an *in vitro* model consisting of encapsulated epithelial cells inside alginate hollow microspheres, produced using a microfluidic co-extrusion technique (Alessandri et al., 2016). The shell constrains the growth of the monolayer and thus allows us to investigate the effect of mechanical forces, such as compression, while the spherical shape allows us to investigate the effect of curvature, on cell growth. We find that cells continue to divide past monolayer confluence through (i) buckling and (ii) inflation of the capsule, both mechanism probably allowing release of the pressure that arises through cell division and the resulting later confinement.

P2.2 Directly probing how kinetochore-fibers are locally anchored in the mammalian spindle

P Suresh, A F Long and S Dumont

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The spindle is a dynamic and self-organizing machine. Key to its function, it must both generate and resist mechanical force, with nanometer-scale molecules driving cellular-scale events. A defining structure of the mammalian spindle is the kinetochore-fiber (k-fiber), the microtubule bundle holding on to chromosomes. Our recent work suggests that k-fibers need not be anchored at poles to move chromosomes, and that they are instead locally anchored to the spindle, near kinetochores. To what structure and how they are anchored there remains poorly understood. To address this question, we developed a microneedle-based approach to exert spatially and temporally controlled, local forces on the spindle in live cells. We pull on k-fibers in different places and over different timescales, and monitor the real-time displacement map of spindle markers. Pulling the k-fiber rapidly sideways, outside the spindle, reveals that it is easier to drastically bend the k-fiber than detach it from its pole or kinetochore, or to stretch the chromosome, and that neighboring k-fibers are only weakly coupled. Strikingly, the k-fiber does not adopt its lowest energy bending mode ('U'-shape) when pulled, and instead remains unbent near kinetochores, parallel to the sister k-fiber ('S'-shape). These observations suggest strong lateral crosslinking along the k-fiber's length, in particular near kinetochores, and we are now probing its physical and molecular bases. Looking forward, we aim to map the key mechanisms that robustly connect the k-fiber to the rest of the spindle.

P2.3 Modulation of APC expression in mesenchymal stem cell during nomadic culture on heterogeneous field of elasticity
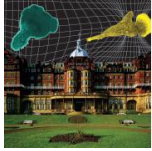
S Kidoaki[1], K Moriyama[1], T Kuboki[1], R Sawada[2], Y Tsuji[1], H Ebata[1], S Sasaki[1], A Yamamoto[1], K Tanaka[1]and K Kouno[1]

[1]Kyushu University, Japan, [2]National Institute of Health Sciences, Japan

Mesenchymal stem cell (MSC) is one of the most potent stem cells, and is a strong cell-resource closest to the clinical application for regenerative medicine. However, standardization and quality assurance of the MSC are essential issues because the quality of MSC is easiliy varied depending on collection method, individual differences, and changes in stemness during culture. Therefore, to establish the evaluation criteria and culture techniques for assuring the quality of MSC has been stongly required.

As an important factor to cause quality change of MSC during culture, involvement of memory of MSC on mechanical conditions of culture environment has recently been reported [1]. Precise definition of mechanics of the culture substrate that can preserve quality of MSC is a problem. For this problem, we try to establish the design of micro-mechanical field of cell culture hydogel that can maintain undifferentiated state of MSC. The main strategy is to regulate the mechanical signals input to the cells from local mechanical field during spontaneously migration of MSCs, i.e., when MSCs are cultured in a periodically-designed elastic field such as alternating patterns of stiff and soft regions, a kind of vibrating mechanical signal input to MSCs from each of stiff and soft region are generated especially in the nomadic mode of movement between those two regions. We have observed before that the MSCs after the nomadic movement keep the undifferentiated state due to inhibition of fixation of mechanical memory (we call this phenomenon as "frustrated differentiation"[2]).

In this project, we aim to fully prove the phenomenon and concept of the frustrated differentiation, and to apply the culture methodology for finding standardized MSCs because the MSC culture in nomadic mode of movement on heterogeneous mechanical field always initilize mechanical memory from culture substrate in principle.Recently, we have established complete design of the microelastically-patterned gelatinous gels to realize the nomadic movement of MSCs and quasi-oscillatory input of mechanosignals to the MSCs, which

employs the triangular elasticity patterns to induce both forward and reverse durotaxis [3]. After culture of MSCs on the gels for 4days, the highest up-regulation of APC gene was first found from comprehensive analysis for gene expression with DNA microarray, which is deeply related to Wnt signaling and cell motility. We will discuss the effect of forced nomadic movement of MSCs across the elasticity boundaries on the maintenance of stemness through modulating APC expression and resulted regulation of Wnt signaling.

[1]   C. Yang, M. W. Tibbitt, L. Basta, K.S. Anseth, Nat. Mater. 13, 645-652 (2014)
[2]   S. Kidoaki, S. Jinnouch, Biophys. J., 102, Suppl.1 (2012), p716a.
[3]   A. Ueki, S. Kidoaki, Biomaterials, 41, 45-52 (2015).

P2.4 Structuring of the epithelial tissue

J Lovrić[1], S Kaliman[2], M A Klatt[3], G E Schröder-Turk[4] and A-S Smith[1]

[1]Ruđer Bošković Institute, Croatia/FAU Erlangen-Nürnberg, Germany, [2]FAU Erlangen-Nürnberg, Erlangen, Germany, [3]Karlsruhe Institute of Technology, Germany, [4]Murdoch University, Australia

Structural properties of space tessellations are important to understand various problems in many fields of science and industry. One of the existing questions is how to tessellate space with the maximized centrality of the cells, usually known as Quantizer problem. Here we study stable solutions of Quantizer problem by applying Lloyd's algorithm on various disordered random point processes. We find that Lloyd's algorithm converges to a universal amorphous structure with a long-range order. Furthermore, we investigate the role of cell centrality in the epithelium tissue. First we find that the tissue can be represented by the tessellation based on the nuclear shape of constituting cells. In the following we explore the interplay between finite size effects and the Lloyd minimisation and find that during the epithelial tissue development, centrality as a concept may play a role and is tightly controlled by the activity of the cell.

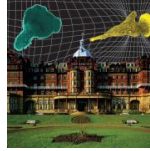## P2.5 Reconstituting *in vitro* perineuronal nets, a specialised extracellular matrix structure

L Souter, R Hall, R Richter and J Kwok

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A perineuronal net (PNN) is a layer of condensed pericellular matrix molecules that forms around the soma and dendrites of sub-populations of neurons in the central nervous system (CNS). PNNs have been implicated in a variety of biological functions including: reducing synaptic plasticity, increasing synaptic stability and as a neuroprotective layer.

PNNs are primarily made up of hyaluronan, link proteins, chondroitin sulfate proteoglycans (CSPGs) and tenascin-R. Enzymatic removal of PNNs reactivates plasticity, which improves recovery from spinal cord injury, and improves memory retention in the mouse model of Alzheimer's disease and in ageing. This makes them an interesting therapeutic target for enhancing neuronal repair and regeneration. Currently little is known about how this extracellular matrix structure is assembled, or the biomechanical properties it confers to the surface of the neurons. Equally the mechanisms by which PNNs are exerting their function remain unclear.

PNNs, as a layer of aggregated matrix, are highly likely to change the biomechanical properties of the neuronal surfaces they surround. Cells in the CNS are sensitive to mechanical changes and can regulate their behaviour depending on the properties of surface they are in contact with. The aim of this project is to understand how the formation of PNNs changes the microenvironment and local mechanics on the neuronal surface, therefore affecting cell behaviour.

Using an acoustic surface measurement technique called quartz crystal microbalance (QCM-D) we aim to assemble these structures to produce a biomimetic surface that simulates the specialised ECM structure of the PNN with tuneable properties. By altering the order in which molecules are presented to the surface we can gain insights relating to their molecular interactions and the self-organisation processes driving PNN formation, the compliance of the surface and the surface thickness. Altering the order the molecules are presented has already demonstrated that link protein is important for the stable binding of CSPGs to hyaluronan. The results have also demonstrated that the size of hyaluronan is important. If the hyaluronan chains are too short then CSPGs cannot interact with them.

QCM-D measurements also demonstrate that aggrecan softens hyaluronan surfaces, but the addition of link protein causes a decrease in compliance of the surface. The more link protein present, the less compliant the surface. It is unknown what the physiological amount of link protein in the PNN is, so it is yet unknown to what extent the reduction in compliance may be caused by the addition of link protein.
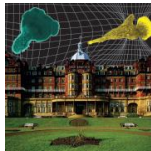
To date, work is ongoing on producing the biomimetic surface. The final aim of this project is to use the tuneable biomimetic surfaces to interrogate how neuronal behaviour is reacting to the change of biophysical properties of surfaces. This could be achieved by culturing neuronal cells on these biomimetic surfaces. The artificial net could also be used as a method for screening potential drug candidates that interact with PNNs, which may help produce translational therapies for spinal cord injury and Alzheimer's disease.

P2.6 Change in ECM composition affects sensory organ mechanics and function

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The ability to sense the posture and movement of body parts, based on signals within the body is termed proprioception. In mammals, a prominent proprioceptive input is provided by muscle spindles, whereas in invertebrates, proprioception is largely mediated through specialized stretch-responsive sensory organs termed chordotonal organs (ChO). Proprioceptive mechanosensing requires the transduction of mechanical strain generated by muscle contractions into sensory neuron impulses, via stretch-sensitive ion channels. However, the ChO sensory neuron does not attach directly to the muscle. Rather, the sensing unit (the neuron and a specialized glia) connects to the exoskeleton (cuticle) via four specialized accessory cells and the associated ECM. Thus, the neuron senses muscle contractions, indirectly, by sensing the deformations exerted on the cuticle by muscle contractions. The role of the mechanical properties of the connective tissue, (the accessory cells and ECM) has never been investigated. Collagen-IV-like protein Pericardin (Prc) is a filamentous protein which is incorporated only to the ECM enwrapping the cap and the sensory unit and is the major ECM component thereof. Here, we show in an *in-vivo* setup that by knocking down Pericardin, we alter the mechanical properties of the sensory organ. We further show that the modified mechanical properties of the prc-mutant result in short-wavelength buckling of the ChO sensory organ upon compression and low compressive strain within the organ, which interfere with proper sensing.

P2.7 A novel mechanotransduction gene library for RNAi screening of extracellular matrix remodelling-dependent tumor invasion

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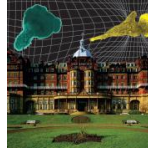
[1]Centro Nacional de Investigaciones Cardiovasculares, Spain, [2]Technical University of Madrid, Spain

Mechanotransduction (MT) refers to the mechanisms by which mechanical forces are converted to biochemical stimuli. The Extracellular Matrix serves as a substrate where cells can develop their function while maintaining the tissue homeostasis in response to disease or injury. However, under prolonged and abnormal loading conditions, the remodeling processes can become maladaptive, leading to altered physiological function and the development of pathologies, such as cardiomyopathies, fibrosis or tumor progression.

A MT gene library has been generated with the aim of identifying novel drug target genes involved in disease mechanisms related to mechanical signalling by using siRNA screening. With the purpose of providing a robust gene list in terms of response to mechanical forces and allow discovery of targets with potential therapeutic interest, a list of mechano-relevant genes was assembled from public databases and bibliographic sources. In addition, integrated analysis of transcriptomic and proteomic datasets was performed. These datasets are the resultant of experimental assays analyzing the genetic response of cells to mechanical stimuli such as substrate stiffness, flow, stretching, compression or indentation. Gene selection to produce the final library was performed based on risk levels: 1) Lower risk: includes well-characterized mechano-relevant genes from published databases and bibliography; 2) Intermediate risk: Broad list of genes featuring, with low/medium frequency, in the experimental datasets for mechanical response and filtered by Gene Ontology terms related to mechano-relevant pathways; and 3) Higher Risk: genes with higest appearance rate in the bioinformatic analysis of the experimental datasets.

To test the MT gene library, we have developed High-Content Screening assays which will enable us to decipher the molecular mechanisms by which mechanical forces regulate cellular responses relevant for cancer disease. Cancer-associated Fibroblasts (CAFs) play a fundamental role in the remodelling of the ECM. Our aim is to understand how the MT can modulate ECM fibre alignment and thus, tumour invasion. A primary 2D assay for siRNA screening to study ECM organization, fibre alignment and stroma mediated remodelling in fibroblast-driven cancer progression has been optimized. The use of FITC-labelled Fibronectin (FN) allows us to image ECM remodelling by cancer-associated Fibroblasts. Matrix organization or chaotic structures can be quantified by High-Content image analysis methods developed in MATLAB by the Cellomics Unit. Controls for chaotic and organized phenotypes will be used.

A secondary 3D assay mimicking the tumour microenvironment of breast carcinoma to measure tumour cell invasion will be assesed. CAFs embedded in Collagen-I gels are allowed to remodel the matrix. A monolayer of breast carcinoma cells will be seeded and allowed to invade into the collagen matrix before fixation and staining. An automatic image analysis tool will provide results in a multiparametric way to measure proliferation, cell morphology (polarized phenotype), cell migration and ECM remodelling.

P2.8 Migration model of crawling cells driven by persistent fluctuation of cell shape
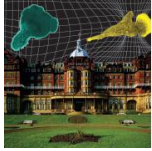
H Ebata, A Yamamoto, Y Tsuji, SSasaki, K Moriyama, T Kuboki and S Kidoaki

Kyushu University, Japan

In general, adherent cells such as fibroblast and mesenchymal stem cells spontaneously move on a substrate through repeated extension and contraction of the cell body. In a time-scale of hours, such cell movement often consists of an alternating series of directed motion and random turning, which is called persistent random motion [1]. With regard to the persistent random motion, the persistent random walk (PRW) model has been proposed to reproduce the migration patterns [2]. However, the PRW model does not adequately explain ordered patterns of migration, such as rotation, oscillation, and zig-zag trajectories, because the model cannot treat the cell trajectories that have strong spatiotemporal correlations. Recently, the pseudopodia have been reported to guide the cell movement [3]. Thus, the ordered motions derive from the spatiotemporal dynamics of pseudopodia, i.e., cell-shape dynamics. Thus, to explain spatiotemporally correlated motion, we should consider the effect of the shaping dynamics. However, previous approaches to formulate cell-crawling have not adequately quantified the relationship between cell movement and shape fluctuations based on experimental data regarding actual shaping dynamics.

In this study, we aimed to elucidate and formulate the relationship between movement and shape fluctuations through the quantitative analysis of cell-shaping dynamics. First, to clarify the quantitative relationship between velocity and shape, we experimentally characterized the crawling of fibroblast cells in terms of shape fluctuations, especially extension and contraction, by using an elasticity-tunable gel substrate to modulate cell shape. Through a Fourier-mode analysis of the shape, the cell velocity was found to obey the amoeboid swimmer-like velocity equation [4]. The equation formulates the movement caused by dynamics of pseudopodia, such as retraction of a trailing edge and extension of a lamellipodia. Next, to explain such shape fluctuation-based cell movement, we proposed a persistent random deformation (PRD) model by incorporating the amoeboid swimmer-like velocity equation into model equations for a deformable self-propelled particle [5]. In the PRD model, we assumed that the cell internal force are generated randomly, which activates the persistent random fluctuation of the cell shape. Then, velocity is induced by the shape fluctuation through the amoeboid swimmer-like velocity-shape relationship. The PRD model fully explains the statistics and dynamics of not only movement but also cell shape, including the characteristic back-and-forth motion of fibroblasts ([6], see Figure). This reciprocating motion is due to the time-reverse symmetry of the amoeboid swimmer-like velocity equation, which is essentially different from previous migration models. We will discuss the possible application of this PRD model to classify the different kinds of cells based on the phenotype of shaping dynamics.

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[4]   A. Farutin et.al, Phys. Rev. Lett. 111, 228102 (2013).
[5]   T. Ohta, M. Tarama, and M. Sano, Physica D 318-319, 3-11 (2016).
[6]   H. Ebata et.al, Sci. Rep. 8, 5153 (2018).
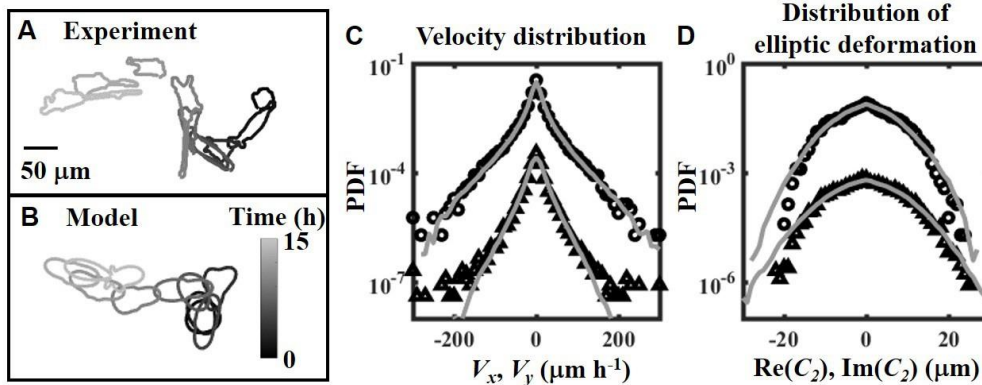
A, B Examples of the cell-shape dynamics. **A** Experimental result. **B** Result from numerical calculation **C** Probability distribution of velocity. **D** Probability distribution of elliptic deformation C_2. C_2 is 2nd Fourier mode of the cell shape. **C, D** Circle: 35 kPa gel. Triangle: 410 kPa gel. Gray line: Fitted curve calculated from PRD model. For clarity, symbols for the 410 kPa gel are shifted 1/100.

P2.9 Cellular dynamics and cellular preferences for adhesion site geometry on two-state micropatterns

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In response to a variety of complex environments encountered *in vivo*, migrating cells continuously change their shape. To understand the development of disease and to utilise cell migration in biomedical applications, it is crucial to understand both the dynamics of cells migrating in confined environments and the geometric determinants governing cell migration.

Micropatterning provides standardised, customisable microenvironments for the study of cells. Here, we report on fast, repeated transitions of MDA-MB-231 human breast carcinoma cells on two-state micropatterns. These micropatterns consist of two fibronectin-coated adhesive islands which are connected by a thin stripe. Cell nuclei are fluorescently labelled and semi-automatically tracked. Thus, we access a large number of nuclear trajectories which are the basis for all further analysis.

First, we vary the length of the connecting bridge to study the dynamics cell migration in confining environments. We find decreasing transition rates between adhesion sites for longer bridge lengths. A two-component stochastic equation of motion captures the observed cellular dynamics well. Through the frequent transitions between the adhesion sites, cells probe both microenvironments relative to each other. By varying the adhesion site area, shape and orientation we investigate preferential cell localisation. The steady-state occupation probabilities reveal that the occupation probability scales linearly with adhesion site area but is insensitive to square or circular adhesion site shape. In contrast, we find that rectangular adhesion sites of equal area but different orientation introduce a bias in occupancies.

Here, we have created an artificial two-state system which can be used to probe static and dynamic quantities of cell migration in a well-defined and confining environment. The two-state micropattern setup can potentially be used as a lab-on-a-chip device to distinguish cell lines and to probe cellular affinities for a variety of substrates.

P2.10 Multi geometry calibration of a cellular potts model

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Ludwig-Maximilians-Universität München, Germany

Cellular Potts Models have been successfully used to reproduce cell migration patterns in two dimensions, in particular on micropatterns as well as collective behavior. The model developed by the group of Erwin Frey aims at bridging from single cell to tissue mechanics by choosing a hybrid approach between a description on the molecular level inside cells and macroscopic modeling. It includes intracellular phenomena like cell polarization and substrate properties, making it a versatile tool for the description of a variety of migratory behaviors.

A particular challenge is optimization of model assumptions and parameters such that various aspects of different experimental data sets are fitted simultaneously. So far, no systematic approach for multi-experiment parameter optimization was made for this kind of models. Here, we combine a Bayesian Optimization approach and a set of basic micropatterns to determine parameter values in a systematic manner.

P2.11 Mechanics of cilia beating: a relationship between metachronal wavelength and fluid flow rate

J Hall and N Clarke
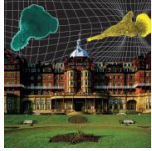
University of Sheffield, UK

Cilia driven fluid flow is a complex fluid mechanical system that is dependent on several physical parameters, including mucus rheology, cilia density, and beat coordination. We have developed a computational model of an array of beating cilia using a hybrid immersed boundary lattice Boltzmann algorithm in order to study the how the flow behaviour is affected by these key parameters. Our focus was to quantify the relationship between metachronal wavelength and the rate of fluid flow. We attempted to rationalise the observed relationship by considering how the fluid flow caused by each cilium is impeded by the presence of the adjacent cilia. We have also observed that this relationship changes significantly when the cilium spacing is reduced, implying that the height of adjacent cilia becomes more relevant than their proximity when the cilium spacing is small.

P2.12 Motility and waves in a hydrodynamic model of confined cell fragments

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[1]University of Barcelona, Spain, [2]Sorbonne Universites, France

We propose a minimal hydrodynamic model of polarization, migration, and deformation by biological cell fragments confined between two parallel plates (Hele-Shaw cell). The fragment is described as a viscous fluid drop that contains an active force-transducing solute. An increase in solute activity destabilizes a global translation-polarization mode, thereby prompting spontaneous symmetry breaking and fragment motility. Higher activity begets Hopf bifurcations of morphological deformation modes - characterized by normal shape oscillations evolving in concert with alternating solute distributions of the Bessel type (both standing and traveling waves). Due to its simplicity, this model presents an instructive paradigm of an over-damped hydrodynamic system that manifests slow inertial-like behaviors over diffusion timescales, akin to those exhibited by biological cells.

P2.13 Seawater bacteria on technical surfaces: lateral and vertical adhesion forces and nanomechanical properties

L Hofherr, N Davoudi, K Huttenlochner, J Chodorski, M Bohley, C Müller-Renno, J C Aurich, R Ulber and C Ziegler

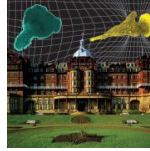Technische Universität Kaiserslautern, Germany

Biofilms are of growing interest for technical systems such as biofilm reactors. A key feature in the biofilm formation is the attachment of single bacteria to a solid surface, which results from different interactions of the cells with the substrate and is further affected by environmental influences. In our attempts to improve the bacterial attachment and therefore the overall biofilm formation process, we focus on adhesion and lateral forces as well as on forces acting on the cell wall due to the turgor pressure. Whereas the adhesion forces perpendicular to the surface dominate the first contact between the bacteria and the substrate, lateral shear forces – which are likely to occur for example in a flow reactor - are of major interest for a possible detachment. The forces caused by the turgor pressure can influence the viability of the cells.

We studied the gram-negative seawater bacterium *Paracoccus seriniphilus* on different materials like glass, titanium and stainless steel and under different environmental conditions like acidic and alkaline solutions or water with varying amounts of NaCl. Scanning Force Spectroscopy and Scanning Force Microscopy were the major tools in our investigations, supplemented by contact angle and zeta potential measurements. The vertical adhesion forces of *Paracoccus seriniphilus* were determined by Single Cell Force Spectroscopy as a function of pH, salinity and substrate. *Paracoccus seriniphilus* showed the strongest adhesion forces for acidic pH values and an increased ionic strength compared to the growth medium. The results of the Single Cell Force Spectroscopy experiments can be correlated to the electrostatic forces between cell and surface [1].

By applying Lateral Force Microscopy we could find a correlation between the applied force and the number of moved bacteria, accompanied by a correlation of the number of detached bacteria and the surface energy of the used substrate. Further, any structuring of the substrate hinders the detachment substantially [2]. In agreement with the results from the Single Cell Force Spectroscopy measurements, the bacteria are harder to detach at a pH of 4 than at a pH of 7 [3].

We further determined the turgor pressure of the bacteria as a function of pH and salinity and on different surfaces. *Paracoccus seriniphilus* can easily adapt to saline conditions. Its turgor pressure and thus its elasticity and size depend on the salinity. *Paracoccus seriniphilus* has its optimum pH at 7, but the results at pH 4 point to an active adaptation mechanism to acidic conditions which they didn't show at pH 11 [4].

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[4]   Davoudi, Neda, et al. "Nanomechanical properties of the sea-water bacterium Paracoccus seriniphilus—A scanning force microscopy approach." *Biointerphases* 10.1 (2015)

P2.14 Supported lipid bilayer platforms to study cadherin-mediated cell-cell adhesion

F N Arslan, C-P Heisenberg and M Loose

IST Austria, Austria

The cadherin-catenin complex is a key component regulating cell-cell adhesion. The spatial arrangement, dynamics and composition of the cadherin-catenin complex are thought to be influenced by tension of the actomyosin cortex to which this complex is anchored. Yet, how actomyosin tension influences the formation and function of the cadherin-catenin adhesion complex is not yet fully understood. To address this question, we have built a biomimetic system where we can visualize and manipulate cell adhesion at high spatiotemporal resolution. To this end, we have used supported lipid bilayers (SLBs) functionalized with zebrafish E-cadherin ectodomains as a cell-surface model where E-cadherin density and mobility can be precisely analyzed and controlled. Zebrafish progenitor cells expressing fluorescently tagged components of the cadherin-catenin adhesion complex or cytoskeleton were used to visualize the contact area. The preliminary images collected by total internal reflection fluorescence microscopy revealed pronounced accumulation of the cadherin-catenin adhesion complex at the margin of the cell-bilayer contact, mimicking our observations of contacts in cell-cell doublets. This marginal accumulation could also be promoted by increasing cortical tension of progenitor cells. We expect that our biomimetic analysis of cadherin-catenin adhesion complex dynamics and organization will reveal novel mechanisms underlying contact formation and mechanosensitivity.

P2.15 Near real time analysis of stress fibre formation in stem cells

L Hauke[1], B Eltzner[2], CWollnik[1], S Huckemann[2] and F Rehfeldt[1]

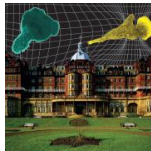[1]Georg-August-Universität Göttingen, Germany, [2] University of Münster, Germany

Human mesenchymal stem cells (hMSC) can be directed to differentiate into various cell types by growing them on matrices of different elasticities E. In contrast to changes in lineage specific protein expression, which occur over a period of days to weeks, significantly different structures of stress fibres are observable within the first 24 hours of planting [1]. Further analysis of stress fibre structure, quantified by an order parameter S, can be used as an early morphological marker. It also dictates the morphology of the nucleus and therefore could directly affect gene transcription.

We use a massively parallel live-cell imaging set-up to record cells under physiological conditions (37 °C, 5 % CO_2) over a period of 24-48 hours. This way we obtain a large, statistically sufficient data set. To minimize the impact of the fluorescent marker, we use an optimized lifeact-TagRFP transfection of hMSCs to visualize the structure and formation of actin-myosin stress fibres. We aim for a full representation of filament processes over time and space, allowing for statistical analysis. The current understanding and classification of stress fibres (dorsal, ventral, arcs) is based on their location in the cell and function during migration. In contrast, we concentrate on an unbiased classification due to their temporal and spatial persistence that should also correlate with function. This might for example been represented by significantly different persistence in space and time and crosstalk with other cytoskeletal components. For this task we developed the 'Filament Sensor' [2, 3], a freely available tool for near real-time analysis of stress fibres. We present experimental data where we can distinguish the cytoskeletal structures of hMSCs on 1 kPa, 10 kPa and 30 kPa elastic substrates with 99 % confidence. We are working on single filament tracking, a sophisticated analysis of the structure in terms of orientation fields, and 3D filament tracing and tracking.

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[2]   www.filament-sensor.de
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P2.16 Actin-spectrin cytoskeleton regulates mechanical responses of neurons

S Dubey[1], P Pullarkat[1], A Ghose[2], A Callan-Jones[3] and N Bhembre[1]

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The mechanical properties of the axonal cytoskeleton play an important role in development (growth and retraction). We are studying the mechanical responses of neurons using a home-built Force Apparatus which is an optical fiber based instrument. Mechanical response of neuronal cells subjected to tension using the force apparatus shows a non-linear viscoelastic behavior. We are also studying stress relaxation and creep behavior of the axon. Axons also show a transition from a viscoelastic elongation to active contraction and we aim to investigate the mechanism responsible for this effect. In short we plan to study (a) the roles played by the different cytoskeletal components, including motor proteins and MAPs in regulating the mechanical properties of axons, and (b) in exploring the feedback mechanism that regulate tension induced growth of axons. Our results show that f-actin plays important role in maintaining axonal tension which could be due to spectrin-actin skeleton arranged in periodic fashion.

P2.17 Characterization of the interactions between mesenchymal stem cells and microcarriers

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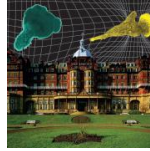
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Stem cells are undifferentiated cells with the ability to differentiate into various specialized cells if appropriate growth conditions are provided. The use of mesenchymal stem cells (MSCs) in cell therapy (1) and regenerative medicine (2) is still limited by cell source quantity. Human Mesenchymal Stem cells (hMSCs) are indeed only available in small numbers in the human body and their isolation and expansion are very limited. Therefore, an effective expansion process is crucial for achieving a therapeutic dose which is 100,000 to 200,000 times of the initial patient sample.

Microcarriers have been employed successfully in the expansion of hMSCs (3) as they provide a surface for the cells to attach and grow. Therefore, cell adhesion, interaction with microcarriers and detachment are important factors to study. In this work, cult Cytodex-1, plastic and plastic plus, plastic plus with fibronectin and hMSCs isolated from the Wharton's jelly of umbilical cords are investigated. The main goal of this Interreg project, Improve STEM, is the enhancement of the cell number of hMSCs by using microcarrier cultivation and by a correlation of the microcarrier properties (zeta potential, wettability, functionalization) to the expansion of the hMSCs.

In previous studies, it was shown that, due to bead-to-bead transfer, the addition of Cytodex-1 microcarriers during the culture of porcine MSCs could promote an extended growth of the cells while maintaining their stemness (3). However the use of 'recycled' microcarriers did not provide the same culture performance. Thus, to assess the possibility of recycling microcarriers or transferring cells from one microcarrier to another, a better understanding of the interactions between MSCs and microcarriers and, more specifically, of the possible changes in chemical structure of the microcarriers, seem necessary.

XPS studies of equilibrated microcarriers (prepared for cell culture), and microcarriers after cell detachment reveal the changes of the surface chemistry. While plastic and plastic plus do not show any significant difference in the chemical composition by XPS, Cytodex-1 clearly shows a consumption of oxygen probably caused by the cells. A reason could be that the cells are able to consume the dextran which is embedded in the Cytodex-1 microcarriers. Further measurements will help to understand the details.

In addition scanning force spectroscopy measurements reveal the nanomechanical properties of the cells beside the adhesion forces between the cells and the different microcarrier types (fresh respectively used ones). First results point at different adhesion forces as a function of the microcarrier type and between fresh and used microcarriers (depending on the microcarrier type).
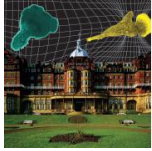
[1]   L. de Girolamo et al., Current pharmaceutical design 19, 2459-73 (2013).
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P2.18 Physical model for durotaxis in non-polarized cells

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During annual fish (Austrolebias sp) epiboly, between the egg yolk and the enveloping layer cells (EVL), there is a group of deep cells that move in contact with EVL layer. It is experimentally observed [Reig et al., Nat. Comm. 2017] that these cells migrate toward the EVL borders and move freely on them (figure 1). Apparently, the phenomenon is due to the edges of the EVLs being more rigid or under tension; therefore, the observed migration corresponds to durotaxis. This is one of the taxis or mechanism that cells use to migrate less understood so far. In this work, a model is developed to elucidate how this kind of migration works and what cells are sensing. It considers a cell modeled as a circle with four contacts on a thin elastic plate, representing the EVL layer. The process is studied when the substrate has or does not have a stiffer strip which simulates the EVL border, which could be responsible for cell attraction. Each of the four contact simulates a protrusion, which elongates and contracts their end out of the center of the cell randomly. We study the elastic response of the substrate and its implication for cell migration in three different cases. The first one considers protrusions that attach rigidly to the substrate and do not glide. That implies that after contractions the substrate remains wrinkled and its relaxation promotes the cell displacement. The second one considers both the cell gliding and the wrinkling of the substrate. In this case, the relaxation of the stresses is again the responsible for the cell movement. When the substrate is completely homogeneous and the EVL border is not being considered, all the cell's simulations show a random walk behavior. On the other hand, with the strip considered, in the first and the second cases, the results do not show the expected durotaxis effect. In fact, the cell shows a random walk movement added to a slight repulsion effect generated by the border, so that a stiffer strip on a thin plate is not enough to reproduce the phenomenon. Finally, a non-linear elasticity model is considered, where the substrate is under externally applied tensions. In this case, the results show that the cell movement depends on the stretch of the substrate and their Young modulus. Additionally, diffusion increases in the direction that the substrate is stressed, which could give more clues about the conditions in which experimentally durotaxis is observed.
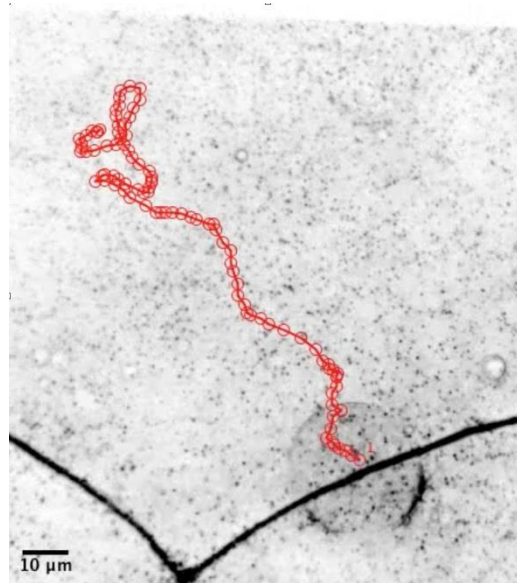
Fig 1: Deep cell tracking on the EVL substrate. At the beginning, the cell move randomly and later, the movement is oriented to the EVL border.

P2.19 Mechanical characterisation of the bone microenvironment by atomic force microscopy for studying breast cancer metastasis
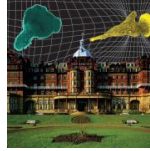
X Chen, R Hughes, N Mullin, I Holen, N Brown and J Hobbs

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The majority of patients with advanced breast cancer have incurable bone metastasis. However, the mechanisms used by breast cancer cells to colonise the bone, survive and resist cancer therapy are largely unknown. There is compelling evidence that bone homeostatic processes are closely correlated with metastatic breast cancer cell fate. Therefore an enhanced understanding of these processes may improve therapeutic options. Physical properties, in particular mechanical properties, have been identified as highly relevant in previous in vitro [1] or ex vivo biopsy [2] tumour investigations. Such mechanical studies have not been performed with respect to the bone microenvironment,. Therefore, the aim of the current study is to establish an atomic force microscopy (AFM) based technique for the mechanical characterisation of the bone microenvironment.

AFM was employed to map the mechanical response of the bone under indentation. Femurs from young (6-8 weeks) and mature (11-13 weeks) mice were split longitudinally using a custom designed tool with a razor blade, then placed in physiological buffer. No additional processing such as decalcification was required, which ensured the bone microenvironment remained intact. The force generated by indentation of the tissue surface and the subsequent relaxation process using a 25 micrometre spherical probe was measured on either individual points or an array (maximum size of 100 µm x 100 µm) within the regions of interest (potential metastatic region, pure marrow, cortical bone and epiphyseal plate). The Hertz model and standard linear solid model were adopted to quantify the elastic and viscoelastic properties respectively, from the obtained force responses.

Our preliminary results reveal the mechanical properties of different regions within the bone microenvironment are significantly different, with no differences identified between bones from young and mature mice. This study also indicates the feasibility of obtaining high-resolution maps reflecting both morphology in 3D and mechanical properties simultaneously on complex tissue like the bone

microenvironment by AFM. This is essential for further characterisation of bones containing tumour foci and their response to anti-cancer therapy. We aim to combine the mechanical maps with post-AFM optical/histological images to correlate the structure/mechanical properties, with the bone composition, as well as in vitro mechanical characterisation to better elucidate the mechanisms by which the bone microenvironment impacts on breast cancer metastasis.

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P2.20 Cell volume modulation in response to deformations

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Cells in tissues experience deformations of different timescales due to various normal and pathological conditions. Cells grow in crowded tissues (typical timescale - hours), increase membrane surface area while spreading (tens of minutes), and change shape during migration and cancer invasion. Cells can also be affected by fast external mechanical stress and osmotic perturbations (less than 1 minute). The mechanisms governing adaptive changes of surface area and volume in these contexts remain largely unexplored. I will show how cell volume and surface area are modulated in response to deformations of different timescales, such as cell spreading and fast mechanical compression. I will demonstrate the roles of the actomyosin cytoskeleton in cell volume regulation. I will also present the mechanisms ensuring cell survival in response to strong mechanical compression. Finally, I will discuss potential roles of surface tension homeostasis in modulating and coupling cell volume and surface area in response to deformations.

P2.21 Levy walks in intracellular transport

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The process of vesicle transport on microtubules remains partially characterized with recent active transport studies showing evidence for non-diffusive, anomalous statistics. Power-law distributions of vesicle run-length suggest that the movement of vesicles on microtubules is a Levy walk. We show that the turning time distribution of vesicle trajectories also follows a heavy tail power-law distribution (for times between 0.1s and 1s) consistent with Levy walk theory. Furthermore, the non-Markovian nature of vesicle transport is characterised by a decreasing rate function.

P2.22 Elasticity threshold of the gel matrix to manipulate migration and differentiation vectors of mesenchymal stem cell

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Mesenchymal stem cells (MSCs) have attracted attention in the field of regenerative medicine and tissue engineering because of their ability of self-renewal, differentiation into multiple lineages and generation of useful cytokines. Fabrication of functionalized substrates for cultivation of MSCs to control proliferation, migration and differentiation leads to the development of novel cell manipulation technologies. It has been reported that physical and mechanical factors from extracellular environment play an important role for differentiation and migration. For example, elasticity of extracellular environment determines direction of
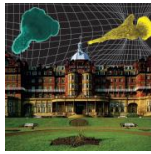
lineage specification, soft matrix biases neurogenesis and adipogenesis (soft tissue lineage) whereas stiff matrix biases osteogenesis (hard tissue lineage) [1]. Furthermore, mechanical factors of extracellular environment also affect the cellular migration. Cells on the substrate with heterogenous elasticity move from softer to stiffer regions, which termed durotaxis [2]. These results indicate that the matrix elasticity is important factor for cell manipulation. Although many studies reported conditions of matrix elasticity to modulate differentiation and migration of MSCs, precise threshold of elasticity to switch the mechanobiological responses of MSCs has not been established.

To address this issue, threshold of matrix elasticity to induce durotaxis and shuttling of mechano-transducing transcriptional factors of MSCs was characterized using elasticity-tunable photo-curable styrenated gelatin (StG) in this study. We firstly evaluated the elasticity condition to induce durotaxis of MSCs on StG gel. We fabricated microelastically-patterned gel that elastic modulus of soft region was fixed at ca. 2.5 kPa. The elasticity value of hard region was varied from 5 to 20 kPa and threshold elasticity to induce durotaxis was examined. After being seeded on the gel, MSCs around elasticity boundaries were monitored for 12 h. MSCs showed random migration on the gel with 5 kPa of elasticity at the hard region (Figure 1(A)). On the other hand, biased migration toward hard region was observed with 10 kPa of elasticity (Figure 1(B)), i.e. threshold elasticity of hard region for durotaxis was within range from 5 kPa to 10 kPa. Under the condition with >10 kPa, durotaxis was strongly induced compared with 10 kPa of hard region (Figure 1(C, D)). Additionally, we evaluated the threshold to induce durotaxis under the different condition of elasticity of soft region. As a result, threshold of hard region was strongly dependent on the elasticity of soft region. These insights can be applied to the fabrication of novel cultivation substrate to control MSCs migration. We will also report about the threshold elasticity value of StG gel that biased differentiation is switched and discuss the designing substrate for MSCs manipulation.
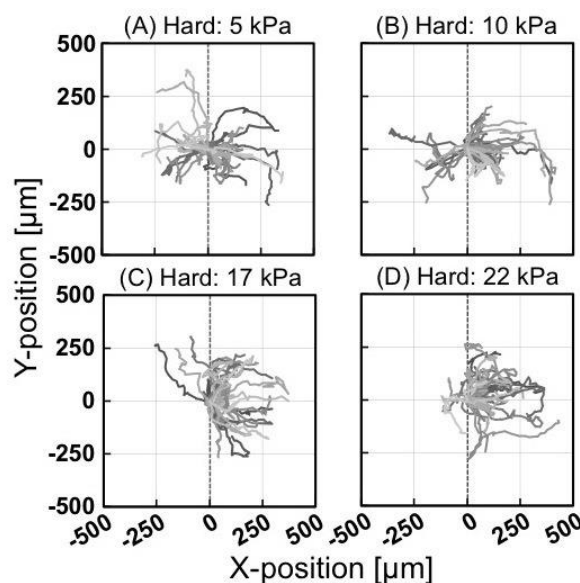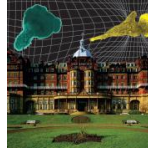

Figure 1. Migration trajectories of each cell close to the elasticity boundaries with the starting positions as the origin of the graphs. Dash lines shows elasticity boundary. X>0 and X<0 show hard and soft regions, respectively.

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P2.23 Mimicking tubular environments to study epithelial sensing to curvature

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During embryogenesis, epithelial tissues fold to establish the final shape of the different organs. For example, in gastrulation and neurulation processes, epithelium deformation into tubules is respectively at the origin of the gut and the central nervous system. These shape changes at the tissue level involve processes at the cellular scale, like cell shape changes and proliferation. Today the coupling between mechanical forces and cellular processes is more and more investigated in tissue dynamics [1-2]. However, the understanding of the mechanisms underlying epithelial tubes formation remains elusive due to the complexity to access to both the tissue and the cellular scale observation in 3D environments.
In order to mimic these tubular environments, we developed two complementary approaches where epithelial response to curved confinement is quantitatively studied. Microfabrication techniques are employed to control the 3D cell organisation. The main difference between the two methods is to be a passive or an active constraint. In the first case, based on a microfluidic device [3], we created tubular shells where we observed that curvature remodels epithelial growth. In the second case, based on the principle of pre-stressed bilayers [4], we reproduced the tissue folding occurring during embryogenesis and we studied the response at a single scale level.

In conclusion, these two approaches provide simple tools to control the mechanical properties of the cell environment and to decouple their effects on cell growth. We expect our results and these technologies will contribute to provide useful insights for new quantitative studies of cell adaptation to curved environments.

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P2.24 Biomechanics of living skin cells during wound healing and melanoma progression

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Skin, the largest human organ, mainly participates in protection against external mechanical, chemical and biological factors. Its structure is composed of distinct cells forming three main layers, i.e. epidermis, dermis and hypodermis. Epidermis and dermis are separated by basal membrane, organization of which (in adult skin) results partially from the cooperative interaction between fibroblasts and keratinocytes through production of extracellular matrix proteins. On molecular and cellular levels, wound healing and melanoma progression are strongly distinct processes. However, one common feature is the disruption of skin integrity leading to migration and/or various cooperation between skin cells. In our studies, mechanical properties of key cell types, namely fibroblasts and keratinocytes, were evaluated aiming at the quantification of elasticity changes induced by the presence of neighboring cells of the same and different types. For wound healing, co-cultures composed of human skin fibroblasts (FBs) and keratinocytes (HaCaT) were applied while for melanoma progression both FB and HaCaT cells were mixed with melanoma cells (WM35, from radial growth phase). Results showed that FBs appeared to be more sensitive to the presence of neighboring cells as compared to HaCaT cells. The latter cells alter their elasticity only under the influence of melanoma cells.
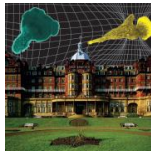
Melanoma cells were characterized by constant level of their deformability regardless of the presence of neighboring cells.

P2.25 Microfluidic cell deformation under inertial and shear flow conditions: probing cell structure and determining disease state
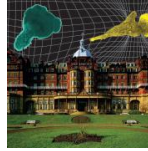
F Armistead[1], J G De Pablo[1], H Gadêlha[2], S Peyman[1] and S Evans[1]

[1]University of Leeds, UK, [2]University of York, UK

We report new insight into cell mechanics by using microfluidic deformation in both inertia- and shear-dominant flow regimes. We show that the deformability of HL60 cells changes with flow regime, which can be used to probe different aspects of a cell's internal structure. Additionally, we show the importance of flow regime when using deformability to distinguish between three colorectal cancer (CRC) cell lines, representing different disease stages.

Cell deformability is inherently linked to its biological constituents. Disease can alter the structure of these components, making deformability a biophysical marker for disease. Gossett et al (2012) introduced Deformability Cytometry (DC) which hydrodynamically stretches cells in extensional flow [1]. DC is dominated by inertial forces, deforming cells at high flow rates and high strains. Cells treated with an actin depolymerizing drug showed negligible deformability changes, suggesting that DC was more sensitive to cytoplasmic rather than cytoskeletal changes. Alternatively, Otto et al (2015) passed cells through a channel slightly bigger than the cell, and deformation occurred due the strong velocity gradient in the channel [2]. This system is dominated by shear forces, deforming cells at low flow rates and low strains, and treatment with an actin depolymerizing drug increased cell deformability. The sensitivity of these methods is therefore highly dependent on the flow-regime. Using the same device but in different flow regimes, controlled by changing the cell suspension buffer viscosity μ, we were able to probe different mechano-responses relating to different aspects of a cell's internal structure.

Figure 1a shows the microfluidic device used to deform cells, which was coupled with high speed imaging to capture thousands of events. We first showed that the mechano-response of HL60 cells are sensitive to whether inertial or shear forces dominate (Figure 1b). Next we studied three CRC cell lines: SW480, HT29 and SW620, which represent a model for metastatic cancer progression. Non-metastatic and metastatic CRCs were found to be distinguishable only in specific flow regimes. Additionally, by tracking deformation, multiple characteristic deformation parameters were extracted (Table 1). The Kelvin-Voigt model was also used to extract an elastic modulus, giving us an intrinsic mechanical parameter comparable to previous works using AFM. Results verified that the CRC cells become softer with disease progression due to changes in the cytoskeleton, emphasising the benefit of multiple parameter determination for furthering understanding of cell mechanics.
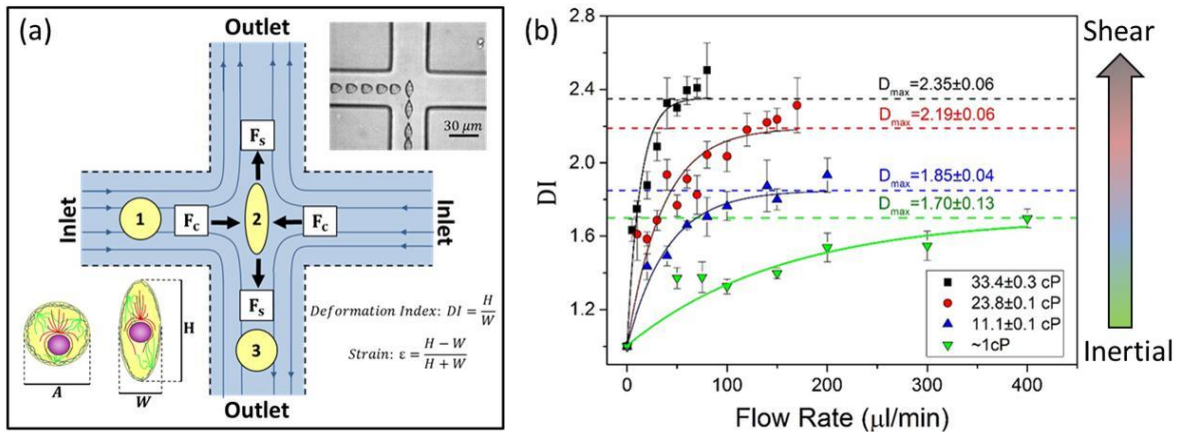
Fig 1: (a) Schematic of cell deformation microfluidic device and various deformation parameters. (b) The average deformation index DI±SE of HL60 cells over a range of flow rates (μl/min). The four data sets represent different flow regimes, adjusted by changing μ.

	HL60	SW480	HT29	SW620
Initial Diameter (μm)	12.3±0.3	15.1±0.2	14.6±0.2	11.5±0.2
ε_{max}	0.18±0.01	0.08±0.01	0.12±0.01	0.11±0.01
τ_r (ms)	3.52±0.14	1.36±0.06	0.89±0.05	1.04±0.05
τ_d (ms)	1.04±0.05	1.19±0.20	0.76±0.10	1.87±0.5
E (Pa)	301±29	542±66	309±50	372±98

Fig. 2: (a) Characteristic parameters of different cell lines, each found by deforming (N>50) cells at the stagnation point of an extensional flow at 5 μl/min in the shear-regime (μ=33 cP). Where ε_{max} is the maximum cell strain, τ_d is the deformation time, τ_r is the relaxation time and E is the elastic modulus.

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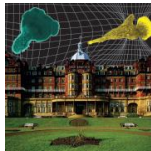[2]   Otto, et al., Nat. Methods, 12, 3, 2015.

P2.26 Unraveling the relationship between nanoscale architecture and force generation in podosomes

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Podosomes are mechanosensitive adhesion/invasion structures that form on the outer surface of macrophages and protrude into the extracellular matrix to probe, remodel and degrade. They consist of a rigid f-actin core surrounded by a ring ~0.5 μm in diameter containing adhesion proteins such as talin, vinculin and paxillin. Podosomes are highly dynamic, forming and dissociating in a timescale of a few minutes, and their mechanical properties (e.g. stiffness) fluctuate during their life cycle in the timescale of a few seconds.

Despite playing a central role in macrophage migration and invasion, the molecular structure of podosomes is still only partially understood. It is also unclear if the fluctuations in the mechanical properties are related to a change in molecular composition, and how this relates to the force generation capability of podosomes. To measure the molecular architecture and mechanical properties of podosomes simultaneously, we have combined localisation-based fluorescence super-resolution microscopy (PALM/STORM) and atomic force microscopy (AFM) in one setup. Localisation microscopy can determine the position of fluorescently labelled

proteins with accuracy of few tens of nanometers, yielding structural information of podosome composition. AFM, on the other hand, can measure topography as well as map mechanical properties, such as stiffness and adhesion. Simultaneous AFM and localisation microscopy measurements can help determine whether the changes in mechanical properties of podosomes are associated with changes in molecular composition.

In addition to fixed cell imaging with photoswitchable dyes (STORM), we have created cDNA constructs to label different podosome ring and core components with photoswitchable fluorescent proteins which enable localisation microscopy in living cells (PALM). We are investigating the relative localisation of the podosome ring components talin, vinculin and paxillin, and their spatiotemporal distribution relative to podosome stiffness, see Fig 1. This will enable us to form a dynamic molecular model of podosomes, which can then guide research into the diseases aided by uncontrolled macrophage movements, such as cancer metastasis and many chronic inflammations.
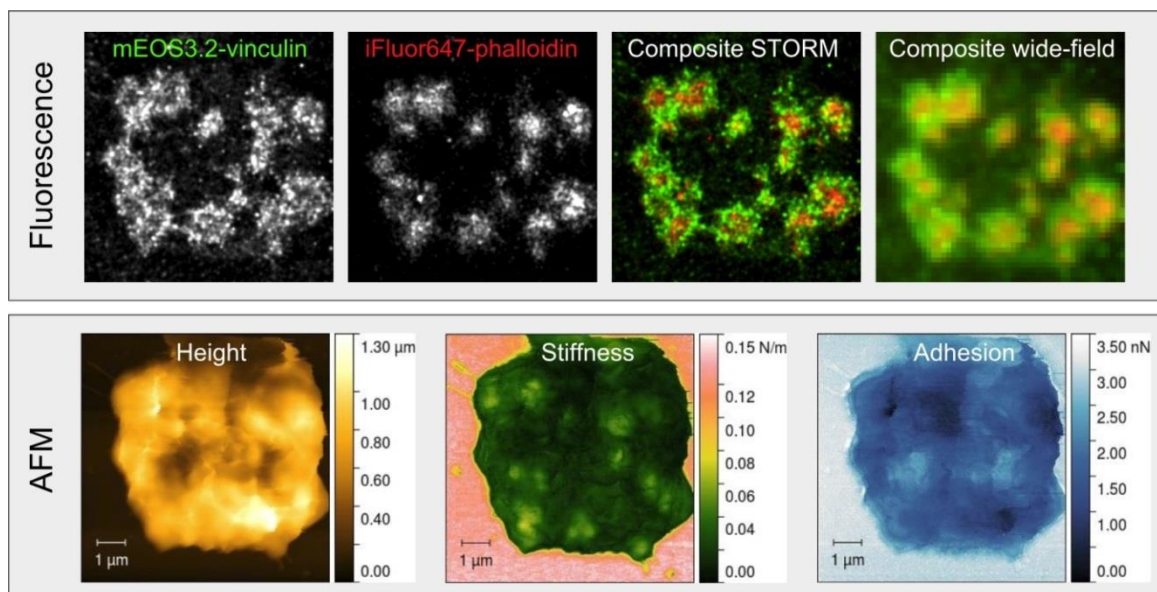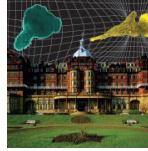

Fig 1: Podosomes in a fixed THP1 cell imaged with correlative AFM and 2-colour STORM. STORM (top row) gives the locations of the fluorescently labelled proteins with accuracy of few tens of nanometers, whereas AFM (bottom row) yields mechanical property maps; in the AFM stiffness image (bottom row, middle) the actin-rich podosome cores with higher stiffness stand out from the surrounding areas.

P2.27 Mechanical communication as a noise filter

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Cells can communicate mechanically by responding to mechanical deformations generated by their neighbors. Here we describe a new role for mechanical communication by demonstrating that mechanical coupling acts as a noise filter and reduces beat-to-beat variability in beating cardiac cells. We measure mechanical interaction between beating cells cultured on a patterned flexible substrate and find that the beat-to-beat variability of a cell decays exponentially with coupling strength. To demonstrate that such noise reduction is indeed a direct consequence of mechanical coupling, we reproduce the exponential decay in an assay where a beating cell interacts mechanically with an artificial stochastic 'mechanical cell'. The 'mechanical cell' consists of a probe that mimics the deformations generated by a stochastically beating neighboring cardiac cell. We show that noise reduction through mechanical coupling persists long after

stimulation stops, indicating that mechanical communication regulates noise by inducing long-lasting alterations in the interacting cells. We further identify NOX2 and CaMKII as key mediators of mechano-transduction in this case.

P2.28 Advanced physical studies of cells by micropipette aspiration

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The micropipette aspiration technique is widely used to measure elastic properties of cells. In this contribution (i) we analyze the important limitations of the commonly used models, which may lead to misinterpretations of the results, and the possibilities of simplified methods to estimate the viscous properties of the cytoskeleton[1] and elastic parameters for small deformation.[2,3] (ii) We provide a scheme for the different types of physical studies performed by this technique[4] and (iii) discuss the future avenues based on single-cell studies. In this regard, we describe our single-cell experiments allowing measuring a variety of biophysical parameters.
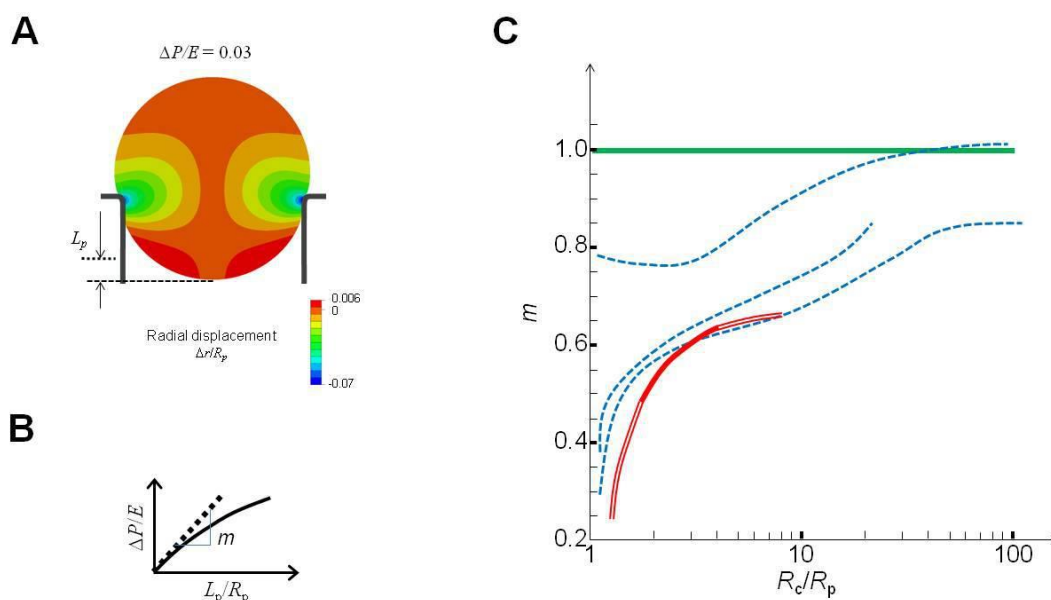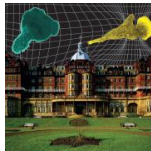

Fig 1: (A) Displacements in a spherical cell during the aspiration process. The aspirated legth, L_p, is shown for an aspiration pressure $\Delta P = 0.03E$, being E the elastic modulus. The radial displacement Δr, normalized by the cell radius R_c, is shown in color scale. (B) Scheme of an aspiration curve, showing the initial slope m. R_p is the radius of the capillary. (C) Slope of the curves pressure vs. aspirated length for various models (discussed in this contribution); the shaded area represents the range of values of the slope estimated by various models.

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Micropipette Aspiration and Its Application to Lymphocytes. *Annals of Biomedical Engineering*, doi:10.1007/s10439-017-1795-7 (2017).

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P2.29 Actin crosslinkers and cortex tension during cell division

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[1]University College London, UK, [2]University of Cambridge, UK

Animal cell shape changes during processes such as division, migration, and epithelial morphogenesis, are mostly controlled by the cellular cortex. The cortex is a thin network (less than 200 nm) of actin, myosin, and other actin binding molecules. Myosin motors pull on actin filaments in the cortex, generating contractile tension in the cortical layer. Changes in cortex tension result in cell shape changes. Most studies of tension regulation have focused on the role of myosin activity, while overlooking the impact of the organisation of the actin network itself. In particular, actin filament length and some crosslinkers have been shown to be involved in tension regulation.

To investigate the role of actin crosslinkers for cortical tension generation we firstly want to determine which actin crosslinkers are localized at the actomyosin cortex. To this aim we developed a method for the isolation of cortex fractions for proteomic analysis. We enrich for actomyosin cortex by isolating cellular blebs. Blebs are spherical protrusions involved in cell migration and division, that can also be induced by treating cells with Latrunculin B, which depolymerises actin. We showed that different actin binding proteins, including various crosslinkers are present in cortices reassembled in isolated blebs.

We have started investigating the localization of key cortical crosslinkers, and plan to explore how crosslinker size influences their interactions with the cortex and role in tension control.

P2.30 Computational study on the interplay between active tension and cortical elasticity in governing cell adhesion mechanics
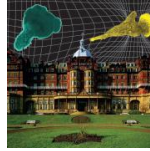
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The acto-myosin cortex regulates the cell's mechanical response. At short timescales it behaves as an elastic solid that provides mechanical rigidity, while at long timescales, its behavior more resembles a liquid with an active surface tension, governed by the interplay between cytoskeletal remodeling and myosin contractility [1]. By locally modulating active tension, cells control the extent of cell-cell contacts. At the tissue-level, this differential contractility has been suggested to drive cell sorting [2].

We propose a mechanical model of the cell that represents the cortex as a thin elastic shell that includes an active contractile tension. Cell-cell and cell-substrate adhesion are modeled using a Dugdale approximation of adhesive traction [3]. Using a particle-based simulation framework built around this model, we revise a variety of mechanical tests that have been performed on murine sarcoma s180 cells, such as micropipette aspiration (MA) [4], controlled cell spreading [5] and dual pipette aspiration (DPA) [6]. Doing so allows us to infer the mechanical properties of the cortex, and lets us explain the scaling laws of pull-off force, contact radius and cell deformation. By comparing between different experiments, we are able to examine the validity of our model assumptions in a broader range of conditions.

We present estimates of the cortical Young's modulus (15 kPa), effective mechanical thickness of the cortex (0.4 μm) and surface tension (0.4 nN/ μm) of s180 cells, and compare these to other estimates given in

literature. We show how the inclusion of an active surface tension can lead to JKR-like behavior [6] in controlled cell-cell pull-off experiments, and we quantify the role of 'differential interfacial tension' in controlling the extent and strength of cell-cell adhesions.

[1]   Kim et al. (2014) Biophys J, 106:526-534.
[2]   Maitre et al. (2012) Science Reports, 338:253-256.
[3]   Odenthal et al. (2013) Plos Comp Biol, 9(10): e1003267.
[4]   Engl et al. (2014) Nat Cell Biol, 16(6):587-94.
[5]   Cuvelier et al. (2007) Curr Biol, 17:694-699.
[6]   Chu et al. (2005) PRL, 94, 028102.

P2.31 Desmoglein-3 acts as a mechanosensor in keratinocytes

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Desmoglein 3 (Dsg3), plays a crucial role in cell-cell cohesion and tissue integrity. Increasing evidence suggests that Dsg3 acts as a regulator of cellular mechanotransduction, but little is known about its direct role in mechanical force transmission. The present study investigated the impact of cyclic strain and substrate stiffness on Dsg3 expression and its role in mechanotransduction. Exposure of oral and skin keratinocytes to equiaxial cyclic strain promoted cell-cell junction assembly, including recruitment of Dsg3 and E-cadherin, alongside acto-myosin cytoskeletal remodeling. Knockdown of Dsg3 by siRNAi blocked strain-induced junctional remodeling. Importantly, Dsg3 regulated the expression and localization of YAP, a mechanosensor and an effecter of the Hippo pathway. Dsg3 physically interacted with phospho-YAP and sequestered it at the plasma membrane, while Dsg3 depletion increased the sensitivity of keratinocytes to both strain- or rigidity-induced nuclear relocalization of pYAP. Moreover, Dsg3 depletion influenced the expression of YAP target genes, including MYC and FOXM1. Together, our findings provide evidence of a novel role for Dsg3 in keratinocyte mechanotransduction and a mediator for the cross-talk between Hippo signaling and the biomechanical regulation of YAP.

P2.32 Entrainment and persistence time of beating cardiomyocytes

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Isolated neonatal cardiomyocytes seeded on an elastic substrate tend to beat spontaneously with average frequency ω_c and amplitude A_p. It was recently shown [1] that the beating frequency of these cells can be controlled (paced) mechanically by an adjacent, inert probe that periodically deforms the underlying elastic substrate with a frequency ω_p and amplitude A_p. Here we theoretically predict the dynamics of synchronization using an effective, one-dimensional, non-linear dynamical equation [2] that can be derived analytically from the coupling of intrinsic calcium and acto-myosin contractility [3]. This model reproduces the three dynamical regimes observed in experiments (spontaneous, bursting and entrained beating) as well as a reduction in the effective beating noise (e.g. - "skipping" or "adding" a beat) by the external pacing force. By analyzing the fluctuations (beat-to-beat variability) in beating of isolated cells, we characterize the dynamical persistence time - the time over which the beating phase of these cells become decorrelated. New experiments by the Tzlil group [1] suggest that this decorrelation is not a simple exponential, and has a characteristic time-scale of $\sim$ 1 hr. We analyze this non-exponential time dependence theoretically in terms of a combination of white and colored cellular noise [4], which relates to the tendency of cells to beat with a coherent frequency over long times, a property with possible medical importance.
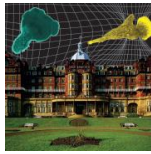
[1]   Ido Nitsan, Stavit Drori, Yair E Lewis, Shlomi Cohen, and Shelly Tzlil. *Mechanical communication in cardiac cell synchronized beating*. Nature Physics, 2016.

[2]   Ohad Cohen and Samuel A Safran. *Theory of frequency response of mechanically driven cardiomyocytes*. Scientic reports, 8(1):2237, 2018.

[3]   Ohad Cohen and Samuel A. Safran. *Mechanical and electrical entrainment of cardiomyocytes*. submitted, 2018.

[4]   Peter Haunggi and Peter Jung. *Colored noise in dynamical systems*. Advances in chemical physics, pages 239-326, 1995.

P2.33 AFM-based microrheology to quantify viscoelastic properties of cells

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Atomic force microscopy (AFM) has rapidly become one of the most used techniques to quantify the mechanical properties of biological samples ranging from single molecules to cells and tissues. The most conventional approach used in AFM-based techniques extracts the elastic modulus of the sample from force-indentation curves. However, the elastic modulus alone is not sufficient to characterize the mechanical properties of cells and tissues. Cells and tissues behave neither solid-like (elastic) nor fluid-like (viscous), but rather as a combination of both. This viscoelastic behavior plays a crucial role in various biological responses and, thus, it is imperative to advance current measurement and analysis methods. In this study, we extend AFM data acquisition and quantication to a wider range of probe-based mechanical interrogation modes. In particular, we developed and implemented AFM-based indentation methods to extract microrheological properties of cells from stress relaxation measurements without the need to t any preconceived models to the data. These measurements enabled us to quantify both the elastic and viscous responses of various cell types and cell morphologies over a wide range of frequencies.

## P2.34 Model based estimation of the mechanical micro-environment inside tissue spheroids

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The fate of cells in three-dimensional tissue cultures is strongly influenced by the cells' biophysical micro-environment [1]. Hence, by optimising the spatio-temporal properties of this micro-environment, the quality of engineered tissues can be enhanced. However, an adequate characterisation and quantification of the mechanical micro-environment is needed before this can be achieved. In this work we propose an individual cell-based modelling approach that predicts how the mechanical micro-environment is established as a result of individual cell mechanical properties in spheroid culture systems.

In the proposed deformable cell model [2], cells are considered as visco-elastic cortical shells, represented by a triangulated surface mesh. Over-damped equations of motion combined with constitutive equations, related to cell-specific mechanical properties, are solved for each node in the cortical shell. This allows the cells to deform and migrate.

We simulate self-assembly of cells into tissue spheroids [3] of varying size, mimicking different culture system set-ups. In a parameter study, we quantify the effect of key cell-specific properties on the mechanical micro-environment and its heterogeneity. Finally, a comparison is shown between the fast elastic response, as probed by mechanical tests, and the slow active and viscous behaviour which governs structure and mechanical environment at long time-scales.
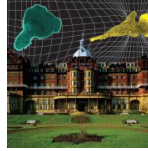
Cortex tension, Young's modulus and thickness as well as cell-cell adhesion energy are varied over a wide range. This allows us to capture the behaviour of a large variety of spheroid morphologies. These different morphologies are categorised based on how mechanical energy is stored and how mechanical forces are transmitted.

[1]   Janet, M. Tse, et al. "Mechanical compression drives cancer cells toward invasive phenotype." Proceedings of the National Academy of Sciences 109.3 (2012): 911-916.

[2]   Odenthal, Tim, et al. "Analysis of initial cell spreading using mechanistic contact formulations for a deformable cell model." PLoS computational biology 9.10 (2013): e1003267.

[3]   Moreira Teixeira, L. S., et al. "High throughput generated micro-aggregates of chondrocytes stimulate cartilage formation in vitro and in vivo." European cells & materials 23 (2012): 387-399.

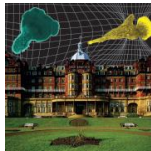## P2.35 Exploring the mechanics of phagocytosis

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Phagocytosis is a hallmark of adaptive immune cells such as macrophages, dendritic cells and neutrophils. This allows them to remove pathogenic microbes, effete cells and debris in the organism's body, precluding infection and supporting an environment for normal tissue development. Events of phagocytosis are highly complex and sequential that involve target identification, contact and internalisation, ultimately leading to intracellular transport and degradation. The underlying molecular mechanisms had been widely examined[1,2], however, information pertaining to the crucial mechanical properties are still scarce. Here, we explored the use of advanced imaging techniques and mathematical analysis to describe the mechanics of phagocytosis. Phagocytosis assays were carried out using J774 macrophage line and polystyrene beads and *Cryptococcus* as phagocytic targets. Slides were prepared accordingly by antibody cell staining; imaging was done using an inverted fluorescence microscope and cells were scored manually for phagocytosis. Cortex and membrane ruffles of a macrophage, which are essential for phagocytosis[3], were labelled by antibody staining. Prepared slides were imaged by Zeiss airyscan microscope, LSM 880. Fiji software was used to process the images and measure the dimensions of cortex and ruffles. Scanning electron microscopy (SEM) was used in parallel to measure the width and length of membrane ruffles.

Our results showed that internalisation of a phagocytic target is dependent on its size. For spherical beads, internalisation events decrease with increasing target size, and almost no beads of 3 um radius and larger were internalised. Notably, a sharp drift of internalised beads was found between 0.5 and 1.0 um radii, suggesting a bottleneck of phagocytosis in this size range. We are keen in further investigating this drift by examining the events of particle internalisation in relation to changes in cell's mechanical properties. On the other hand, we found a similar trend of phagocytosis using real biological target, *Cryptococcus.* Acapsular *Cryptococcus* which do not possess capsule, hence usually smaller, are internalised more than the capsular strain. Interestingly, internalisation of target is increased when more phagocytic targets are bound to the surface of the macrophage.

Our data in measuring the thickness of cell cortex and membrane ruffles showed a very remarkable consistency. Using airyscan imaging, both were found to be at 350 nm in every cell examined. This implies a fundamental mechanism of organising actin molecules in these cells to form such structures necessary for force generation and particle internalisation during phagocytosis. We are keen in further investigating the biological importance of maintaining such thickness by looking at the dynamics of actin molecules at

different stages of phagocytosis. Lengths of the ruffles were also measured and found to be highly variable, but can reach up to 5 um.

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[2]   P.P. Ostrowski, S. Grinstein, and S.A. Freeman, Dev. Cell 38, 135 (2016).
[3]   R.S. Flannagan, R.E. Harrison, C.M. Yip, K. Jaqaman, and S. Grinstein, J. Cell Biol. 191, 1205 (2010).

P2.36 The establishment of the patient customized *in vitro* platform to evaluate CAF-induced anticancer drug resistance

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Recent studies have showed that tumorigenesis and drug resistance are markedly influenced by the tumor microenvironment including cancer associated fibroblast(CAF). Especially in colorectal cancer(CRC) with abundant CAFs in the stroma, the mechanism study of CAF-induced drug resistance could be a crucial key in the treatment of CRC. Thus, we tried to set up *in vitro* platform to evaluate CAF-induced resistance against anticancer drugs using the CRC organoids and CAF cells derived from CRC-patients. We obtained individual 10 clonal organoids and 11 immortalized CAFs derived from colorectal cancer patients. Based on the 5-fluorouracil(5-FU) sensitivity using cell viability test, some organoids were selected among 10 and then we examined their responses on 5-FU after treating each 11 CAF-conditioned medium(CM). As well, we explored the biomarkers of CAF chemoresistance using the analysis of RNA sequence and protein expression such as Western blotting and cytokine array. The response on 5-FU in all of the selected organoids showed no remarkable change before and after of treating 11 different CAF's CM were prepared from the culture of each CAF. Thus, we modified the protocol several times to figure out how to obtain CAF-CM and evaluate the response on 5-FU in organoids, and distinctions of the response induced by each CAF soup have been observed. For the consequence we divided 11 CAFs into two groups, chemoresistance inducing group and non-inducing group. In addition, we found the differentially expressed genes between two groups using this platform. And interestingly, we figured out that cancer organoid's response on 5-FU was changed compared with the results of early stage experiment. It is expected that our study provides the CRC patient customized *in vitro* platform to be able to evaluate the CAF-induced anticancer drug resistance. And we suggested that it need to use small extent of passage of it with regard to evaluating drug response using the cancer organoid. In further studies, it will be required to confirm the correlation between target genes and the resistance on 5-FU induced by the CAFs using our platform.

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