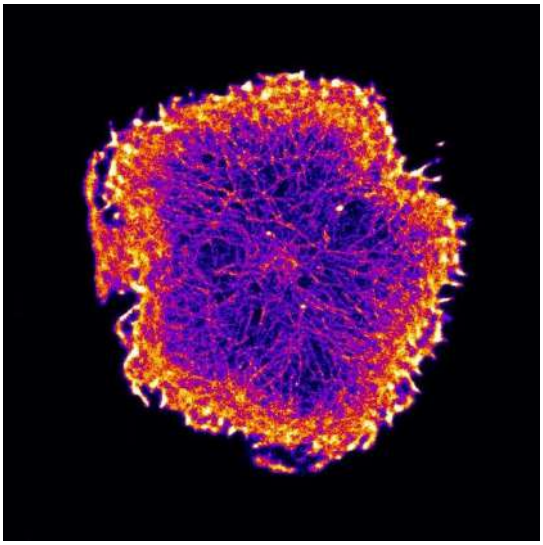

From cells to tissues: quantitative approaches to living systems!

CellTiss Days 2019

NOVEMBER 5-7, 2019,
AT BELAMBRA,
GIENS, FRANCE

Invited speakers:

Matthieu Coppey, Institut Curie, Paris, France.

Susanne Fenz, Wuerzburg University

Ray Goldstein, Cambridge University

Gijsje Konderink, TU-Delft

Cécile Leduc, Instit. Pasteur, Paris

Manos Mavrakis, Institut Fresnel, Marseille

Florence Niedergang Inst. Cochin, Paris

Jean-François Rupprecht , CPT, Marseille

Frank Schnorrer, IBDM, Marseille

Organizers :

Marc Lefranc, PhLAM, Lille

Loïc Le Goff, Institut Fresnel, Marseille

Marc Léonetti, CNRS/LRP/SFP, Grenoble

Kheya Sengupta, CINaM/CNRS, Marseille

Programme

Tuesday, November 5th 2019

14:45 – 14:50 Introduction: Kheya Sengupta & Loïc Le Goff

Session 1: Collective Phenomena in tissue modelling

14:50-15:50 Ray Goldstein: Upside-Down and Inside-Out: The Biomechanics of Cell Sheet Folding

15:50-16:50
Francis Corson: Self-organization and patterning dynamics in Drosophila
Antoine Fruleux: Analyzing tissue growth at different scales
Joseph d'Alessandro: Emergent modes of collective motion in confined epithelia
Herve Turlier: Physics of lumen coarsening and blastocoel formation

16:50 – 17:10: Coffee Break (put up posters)

Session 2: Active systems in biology

17:10 – 17:40 Jean-Francois Rupprecht: Modelling active stress fluctuations from the cell cortex to the tissue scale

17:40-18:40
Anis Senoussi: Tunable corrugated patterns in an active nematic sheet
Sham Tlili: Shaping the zebrafish myotome by inter-tissue friction and active stress.
Somanna Kollimada Aiyappa: Quantifying the relationship between the mechanical status of cells and the associated molecular organization of its components
Dario Dell'Arciprete: A growing bacterial colony in 2D as an active nematic

18:40 – 20:00 Aperitif and Poster session (odd numbers present)
20:00 Dinner. Poster viewing continued after dinner (all posters)

Wednesday, 6th November 2019

Session 3: Biopolymers: from molecules to mechanics and function

8:30-9:00 Gijsje Koenderink: The role of cytoskeletal crosstalk in cell form and function

9:00-10:15
Renaud Poincloux: Actin filaments store elastic energy to produce nanoscale forces
Mehdi Bouzid: Mechanical properties of branched actin networks
Valentin Laplaud: Cell cortex dynamics: the role of myosin II in cortex thickness fluctuations

Angughali Sumi: Adherens Junction Length during Tissue Contraction Is Controlled by the Mechanosensitive Activity of Actomyosin and Junctional Recycling
Matthias Merkel: An analytical approach unifies mechanical rigidity in cell-based tissue models and biopolymer networks

10:15 – 11:00: Coffee Break (with posters)

Session 4: Light for life and other tools for quantitative biology

11:00-11:30 Matthieu Coppey: Optogenetic dissection of cell polarity and migration

11:30-12:45
Aurelie Dupont: QuanTI-FRET: a framework for quantitative FRET measurements in living cells
AnnaMaria Kiss Segmentation of 3D images of plant tissues at multiple scales using the level set method
Aya Nassereddine: Surface patterning strategies to dissect T-cell adhesion and actin organisation
Elie Wandersman: A quantitative approach of cell-cell communication
Audrey Cochard: ArtiGranules, a novel tool to examine intracellular phase transitions and RNA-Protein membrane-less organelles

12:45 – 14:15 Lunch

Session 5 Cytoskeletal organization and new tools to probe it

14:15-14:45 Manos Mavrakis: Genetically-encoded fluorescence-based reporters for measuring actin filament organization in living cells and tissues
14:45-16:00
Anne-Cecile Reymann: Modulation of cortical actin assembly dynamics in the early embryo.
Clemence Vigouroux: In vitro reconstitution reveals that actomyosin force dissociates the talin-RIAM complex to promote talin-vinculin interaction
Vincent Mirouse: How to sculpt an organ: Drosophila follicle development as a model of morphogenesis involving extracellular matrix
Jing Xie: Visco-elastic properties of bulk cytoplasm maintain the mitotic spindle in the center of large cells
Raphael Clement: Spontaneous symmetry breaking in oocyte meiosis
16:00-16:30 Cécile Leduc: Molecular architecture and dynamics of intermediate filaments in glial cell migration

16:30 – 17:10: Coffee Break (with posters)

Session 6: Biophysics of microbes

17:10-17:40 Suzanne Fenz: Examination of the trypanosomes' VSG coat by biophysical methods

17:40-18:40
Maxime Ardre: Pathways and Biophysical Consequences of Cellulose Production by the wild type Pseudomonas fluorescens SBW25 at the Air-Liquid Interface.
Raphael Jeanneret: Sinking for sex in the ocean
Duc Quang Tran: Microfluidic study of individual and collective swimming of P. parasitica zoospores
Nelly Henry: Bacterial biofilm: The resort of microbes

18:40 – 20:00 Aperitif and Poster session (even numbers present)

20:00- Dinner

21:00 – open-ended: Florence Niedergang & Marc Lefranc: Round table on the GDR project "quantitative approaches to life"

Thursday, 7th November 2019

Session 7: Force generation, confinement and tools to measure them

8:30-9:00: Frank Schnorrer: Do titins rule sarcomeres of insect muscles?
9:00-10:30
Charlotte Riviere: Soft-cell-confiner development to decipher the impact of mechanical stimuli on cell
Alexandre Glentis: Tubular geometry induces cohesive whole tissue epithelial rotation
Anna Francesca Rigato: Growth-associated constraints and cell mechanics during epidermal morphogenesis in Drosophila
Nicolas Borghi: Force transmission at cell adhesion and the nucleus
Alice Nicolas: Quantification of cellular forces on rigidity patterned substrates evidences distinct length scales in rigidity sensing
Adrien Hallou: A new tool to characterize mechanical properties of soft biological tissues

10:30 – 11:00: Coffee Break (take-off posters)

Session 8: Biophysics of the immune system

11:00-11:30: Florence Niedergang: Integrin-mediated phagocytosis: mechanosensitivity and closure mechanisms
11:30-12:15
Laurent Limozin: From single nanobody-antigen bond to antibody dependant cell cytotoxicity
Nicolas Garcia Seyda: Microfluidic tools to image chemotaxis of swimming leukocytes
Julien Husson:Single-cell immuno-mechanics reveal large and fast cell-type-specific mechanical changes during white blood cell activation.

12:15 – 12:30 Closing of the Meeting

12:30 – 14:00 Lunch and Departure (Bus leaving at 13:30)

Poster List (alphabetical order)

1. **Beatrici Carine:** Tissue Mechanics: Stokes Flow in Confluent Cell Simulations
2. **Boccara Martine:** Interferometric microscopy and dynamic OCT: two optical tools to study nanoparticles inside and outside the cell
3. **Boudjema Amélie-Rose:** Developing tools to study self-organizing properties of centriole amplification in MCC
4. **Bouzakarne Sara:** Actin Dynamics and cellular forces in vivo and in vitro.
5. **Brezin Louis:** Spontaneous Flows and Defects in Active Cellular Nematics
6. **Bruot Nicolas:** Puncture and penetration in soft materials
7. **Chevalier Louis:** The Mechanobiology of cell wall growth in filamentous fungi
8. **Colombo Jessica:** A system to test the molecular requirements for the formation of a dynamic actin cortex
9. **Contreras Diego:** Geometry and mechanics of a model epithelium with irregular cells and a clonal inclusion
10. **Delgehyr Nathalie:** Actin and nuclear deformation contribute to differentiation of brain multiciliated cells
11. **Dhayer Nathalie:** Patch-Clamp Electrophysiology and BioMembrane Force Probe to assess mechanisms of Cell Penetrating Peptide uptake into cells.
12. **Escoubet Nicolas:** Swimming behavior of a mechanosensitive organism
13. **Gasecka Paulina:** Imaging metabolic dynamics by deuterium oxide-Stimulated Raman Scattering (DO-SRS)
14. **Gehan Pauline:** Translocation of the cell penetrating peptide penetratin through asymmetric model membranes: role of the lipids and microfluidic approach
15. **Gelin Matthieu:** Study of the regulation of the microtubule network architecture.
16. **Gergely Csilla:** Single cell nanomechanics and luminescence based monitoring of nanodiamonds internalization in MCF7 cells
17. **Glentis, Alexandre:** Tubular geometry induces cohesive whole tissue epithelial rotation
18. **Golovkova Iaroslava:** Biomimetic emulsions to probe the role of adhesion in tissue remodeling processes
19. **Jebane Cecile:** Cell nucleus mechanics and premature senescence
20. **Kukhaleishvili:** Determination of turgor pressure of filamentous yeast
21. **Kuony Alison:** Unravelling the mechanical and physical cues involved in lacrimal gland ductal compartment formation
22. **Lardier Nathan:** Mechanical properties of the cytoskeleton in living cells
23. **Lefranc Marc:** A mathematical model of the liver clock linking feeding and fasting cycles to clock function
24. **Le-Goff Thomas:** Molecular segregation in actin networks

25. **Lombard Alain:** Coupling magneto-active substrates with FRET biosensors to decode mechanotransduction
26. **Luciano marine:** Substrate Curvature Modulates The Nuclear Behavior Of Epithelial Tissues Grown On Wavy Hydrogels
27. **Luna Juan:** Proneural patterning in the *Drosophila* eye disc
28. **Manesco Clara:** Broadband coherent anti-Stokes Raman micro- spectroscopy for optical label-free readout of spinal cord injury; towards new therapeutic strategies in mice and non- human primates
29. **Matthias le-bec:** Spatio-temporal control of division of labor in yeast communities
30. **Mercey Olivier:** Centriole self-assembly is sufficient to organize centriole amplification in multiciliated cells
31. **Molcrette Bastien:** Directional transport through nanopores: an experimental approach of a nanoscale Brownian ratchet.
32. **Montel Lorraine:** Adhesive emulsions as a model system
33. **Moreau Alexis:** Microcirculation of Red Blood Cells in biomimetic splenic slits
34. **Noblin Xavier:** Microfluidic technique for rapid measurement of intracellular pH dynamics
35. **Pernier Julien:** Deciphering the molecular links between actomyosin force, integrin regulation and ECM properties through an *in vitro* reconstitution
36. **Proces Anthony:** Deciphering the role of activated glial cells on neuronal connectivity using in vitro models of traumatic brain injury
37. **Robert Bruno:** Peptides as chalcogenide-organic linkers for biosensing
38. **Rulquin Charlotte:** Heterogeneity and cell clustering in *Drosophila* central-nervous system cancers
39. **Saadaoui mehdi:** A tensile ring drives tissue flows to shape the gastrulating amniote embryo
40. **Seveau Valentine**: In vitro T lymphocyte adhesive haptotaxis towards lower Icam-1 density areas
41. **Shaozhen Lin:** Collective Cell Migration in Monolayer Systems
42. **Valencia-Gallardo Cesar:** Elasticity from entanglements in branched actin networks
43. **Van-Der-Hofstadt Marc:** Patterning cell cultures by self-organising biochemical landscapes
44. **Varga Bela:** Cardiomyocyte structural parameters of a Duchenne muscular dystrophy murine model revealed by automated image analysis on SHG images
45. **Vassaux Maxime:** A biophysical model for curvature-guided cell migration
46. **Vercruysse Eléonore:** How do cell-cell adhesions impact on the migration speed of epithelial cohorts?
47. **Versaevel Marie:** Collective migration during a gap closure in a two-dimensional haptotactic model
48. **Villars Alexis**: Orchestration of epithelial cell elimination by the effector caspases
49. **Wang Hong:** Regulation of actin dynamics by vinculin and talin
50. **Wioland Hugo:** Biochemical and mechanical regulation of actin filament disassembly

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Abstracts

Invited Talks

Upside-Down and Inside-Out: The Biomechanics of Cell Sheet Folding

Raymond Goldstein

Department of Applied Mathematics and Theoretical Physics, University of Cambridge, UK.

Folding of cell sheets is a ubiquitous phenomenon in embryonic development, from the formation of the gastric system to the development of the eye, and a common underlying driving force of these morphological transformations is changes in cell shape. This lecture will describe perhaps the simplest example of this process – embryonic inversion in green algae – where a combination of light-sheet microscopy and elasticity theory has led to a quantitative understanding.

Modelling active stress fluctuations from the cell cortex to the tissue scale

Jean-Francois Rupprecht

Centre Physique Théorique (CNRS/Université Aix-Marseille), Marseille, France

Cells and tissues are constantly out-of-equilibrium characterized by a local conversion of chemical energy into heat and mechanical work. In the cell cortex, part of this task is performed by molecular motors (myosins), which act as active cross-linkers putting under tension a meshwork of polymeric filaments (the actin cortex). Motivated by the case of the cell cortex -- whereby fluctuations in motor contractility typically occur on a larger time scale than the life-time of actin cross linkers - we developed a fluctuating active gel theory with stress fluctuations that are correlated on a long time scale compared to the characteristic viscoelastic time of the medium. We then investigated the effect of such fluctuations on the dynamics of an embedded tracer. We find that the tracer is attracted towards regions with lower diffusion coefficient, in stark contrast to expectations based on the assumption of an effective thermal-like temperature. We apply this generic result to the dynamics of deformations of the cell nucleus; we demonstrate the appearance of a fluctuation maximum at a critical level of activity, in agreement with experimental data. We then consider the effect of active fluctuations on the rheology of a network of active gel segments that models an epithelial tissue.

The role of cytoskeletal crosstalk in cell form and function

Gijsje Koenderink

Bionanoscience Department, Delft University of Technology, The Netherlands

Cell shape and mechanics are determined by the interplay of four distinct cytoskeletal networks, made of actin filaments, microtubules, intermediate filaments and septins. These four types of cytoskeletal polymers differ in their structural and physical properties, enabling specific cellular functions. However, there is growing evidence that the four cytoskeletal subsystems also exhibit strongly coupled functions necessary for cell polarization, cell migration, and mechano-responsiveness. In this talk, I will discuss our own recent work addressing the biophysical mechanisms of cytoskeletal crosstalk using in vitro reconstitution with purified cellular components. I will discuss the role of crosstalk in the establishment of cytoskeletal polarity and in cell mechanics.

Optogenetic dissection of cell polarity and migration

Matthieu Coppey

Physical-chemistry department, Curie Institute, Paris, France

The migration of eukaryotic cells requires a high coordination of molecular activities in space and time. When migrating, cells present a highly polarized state as revealed by the many subcellular biochemical gradients, the directed transport of materials, or the spatially arranged cytoskeletal structures. A complex signaling circuitry orchestrate this coordination, and we are currently trying to understand the logic of this circuitry from biophysical principles. As a main theme, we use light-gated dimerization (non-neuronal optogenetics) to manipulate in space and time the biochemical activities of the RhoGTPases, which are small molecular switches at the core of the signaling network controlling cell polarity and migration. I will present the principle of these acute perturbations and illustrate their application in the understanding of cell polarity during mesenchymal single cell migration.

Genetically-encoded fluorescence-based reporters for measuring actin filament organization in living cells and tissues

Manos Mavrakis

MOSAIC, Institute Fresnel, Marseille, France

Essential physiological functions, including cell division, cell adhesion and motility, and tissue morphogenesis, rely on the capacity of animal cells to change and adapt their shape. To accomplish these force-dependent tasks, animal cells make use of actin cytoskeletal filaments. The precise way in which actin filaments organize, i.e. how actin filaments are physically oriented in space, and how filament organization is remodeled in time, is determinant for force generation. Thus, being able to measure actin filament organization directly in living cells and tissues promises to advance our understanding of how proteins and signaling pathways individually and collectively control actin-driven cellular functions. In my talk I will present the development of novel genetically-encoded, fluorescent-protein-based reporters that allow non-invasive, quantitative measurements of actin filament organization in living cells and tissues by using polarization-resolved fluorescence microscopy.

Molecular architecture and dynamics of intermediate filaments in glial cell migration

Cecile Leduc

Institute Pasteur, Paris, France

Intermediate filaments (IFs) are key players in the control of cell morphology and structure as well as in active processes such as cell polarization, migration and mechano-responses. However the regulatory mechanisms controlling IF dynamics and organization in motile cells are still poorly understood. In a first part of the talk we will show how IF network reorganizes in a polarized manner during the cell polarization and migration and provide new insights into the mechanism of coordination between the three cytoskeleton components. In a second part we will show how the molecular architecture of single intermediate filaments can be deciphered using super-resolution microscopy, and discuss how their structural organization is compatible with their cellular function as stress absorber.

Examination of the trypanosomes' VSG coat by biophysical methods

Susanne Fenz

Biozentrum, Universität Würzburg

African trypanosomes are the causative agents of human sleeping sickness and Nagana in livestock. They are not only highly relevant subjects of research for their own sake, but they are also excellent model organisms to address fundamental questions in membrane biophysics, for example protein diffusion in crowded membranes and the narrow escape problem. In the bloodstream of their host, trypanosomes exhibit a remarkably clean and extremely dense layer of membrane-anchored variant surface glycoproteins (VSGs). Fluidity of this surface coat is fundamental for the evasion of the host's immune system and consequently for the survival of the parasite. The maintenance of the VSG coat is accomplished by shuffling the proteins through the tiny flagellar pocket, which is the sole site for endo- and exocytosis while it makes up for only 5% of the cell surface.

In our work, we aim to elucidate the governing principles that ensure coat fluidity at high densities and efficient recycling in the presence of a bottleneck. We perform both in vitro measurements of reconstituted coats in self-organized model membranes as well as live-cell super-resolution imaging. In vitro, we systematically vary properties of the VSG coat, like density, ectodomain length, glycosylation state, overall structure, and probe their influence on the protein dynamics on the single-molecule level [1-3]. Live-cell super-resolution imaging of intrinsically fast-moving flagellates is a highly challenging task. We recently introduced a solution based on cyto-compatible hydrogel embedding [4]. Building on this work, we are now able to track VSG dynamics in living trypanosomes at high spatial (localization precision ~25 nm) and temporal resolution (f = 100 Hz).

[1] Hartel et al., Sci. Rep., 5, 10394, (2015).
[2] Hartel, Glogger et al., Nat. Comm., 7:12870, 2016.
[3] Bartossek et al., Nat. Microbiol., 2:1523-32, 2017.
[4] Glogger et al., JPD: Applied Physics, 50:074004, 2017.

Do titins rule sarcomeres of insect muscles?

Vincent Loreau[1], Wouter Koolhaas[2] and Frank Schnorrer[1,2]

[1] Aix Marseille University, CNRS, IBDM, Marseille, France
[2] Max Planck Institute of Biochemistry, Martinsried, Germany

Sarcomeres are the force producing molecular machines of muscles in all higher animals. Each sarcomere consists of a quasi-crystalline assembly of cross-linked parallel actin filaments with bipolar myosin filaments, both of which are linked by gigantic titin molecules. Members of the titin family are essential for sarcomere formation across evolution and were shown to determine sarcomere length by spanning from the sarcomeric Z-disc to the M-band in mammals. While this titin ruler model is well supported for mammalian sarcomeres, titin isoforms in insects are shorter and thus may not simply determine sarcomere length. Here, we are investigating the two Drosophila titin homologs Sallimus and Projectin, both of which are essential for the formation of sarcomeres in Drosophila. Using CRISPR-based genome engineering we modified the various parts of both proteins and quantified the consequences for myofibrillogenesis, sarcomere length and muscle function.

Integrin-mediated phagocytosis : mechanosensitivity and closure mechanisms

Anna Mularski[1], C. Le Clainche[2], M. Balland[3] and Florence Niedergang[1]

[1]Biology of Phagocytes, Institut Cochin (Inserm, CNRS, Université Paris Descartes)
[2]Department of Biochemistry, Biophysics and Structural Biology , Institute for Integrative Biology of the Cell, Gif-sur-Yvette, France
[3]Laboratoire interdisciplinaire de Physique, Université Joseph Fourier (Grenoble 1)

Phagocytosis is a mechanism of internalization and degradation of micro-organisms or cellular debris. Phagocytosis is important for remodelling of tissues, disposal of dead cells and bacterial clearance. Actin polymerisation provides the force that drives the membrane deformation required to engulf particulate matter during phagocytosis. Key to dissecting the mechanism by which this occurs, is understanding how the complex mechanosensitive machinery of actin binding proteins sense force and stabilize actin anchoring during phagocytosis, from initial receptor binding, through to phagosome formation and closure.

A novel experimental approach utilizing traction force microscopy was used to observe phagocytosing macrophages on substrates of biologically relevant stiffness. We show that the capacity of macrophages to perform efficient phagocytosis varies with the substrate stiffness. In addition, phagocytosing macrophages display different contractile energy and polarization degree as compared with non-phagocytosing cells. The mechanotransduction pathways implicated are under study.

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Abstracts

Contributed Talks

Self-organization and patterning dynamics in Drosophila

Francis Corson

Laboratoire de Physique Statistique - ENS

The emergence of spatial patterns in developing multicellular organisms relies on positional cues and cell-cell communication. Drosophila sensory organs have informed a paradigm where these operate in two distinct steps: prepattern factors drive localized expression of proneural genes, then Notch-mediated lateral inhibition singles out neural precursors. Revisiting this classic model system, we have found that in the fly thorax, Notch signaling also organizes a series of proneural stripes that resolve into rows of sensory bristles. Patterning, initiated by a gradient of Delta ligand expression, progresses through inhibitory signaling between and within stripes. Thus, self-organized Notch activity establishes an ordered, tissue-wide pattern. We are now investigating whether similar dynamics are at play in other tissues, such as the Drosophila eye.

F. Corson, L. Couturier, H. Rouault, K. Mazouni, F. Schweisguth, Self-organized Notch dynamics generate stereotyped sensory organ patterns in Drosophila, *Science* (2017)
L. Couturier, K. Mazouni, F. Corson, F. Schweisguth, Regulation of Notch output dynamics via specific E(spl)-HLH factors during bristle patterning in Drosophila, *Nat. Comm.* (2019)

Analyzing tissue growth at different scales

Ecole Normale Supérieure de Lyon
RDP, *Biophysics and Development* team
Antoine FRULEUX & Arezki BOUDAOUD

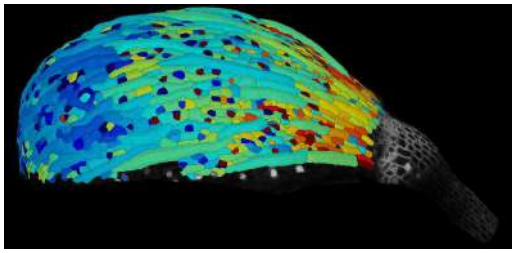

Figure 1: Cell growth is heterogeneous in space and time. Example of a sepal (green organ that protects a flower before it opens) from the model plant Arabidopsis thaliana. The colour scale corresponds to growth rates (high in red, low in blue).

The two hands of most humans almost superimpose. Similarly, flowers of an individual plant have similar shapes and sizes. This is in striking contrast with growth and deformation of cells during organ morphogenesis, which feature considerable variations in space and in time, raising the question of how organs and organisms reach well-defined size and shape. In order to link cell and organ scales, we built a theoretical model of growing tissue with fibre-like structural elements that may account for the plant cell wall or animal cytoskeleton or extracellular matrix [1]. It predicts that fluctuations occurring at cellular scale exhibit long-range correlations and relate it to the mechanical response of tissues.
Investigating this relation is experimentally challenging because of many constraints such as the curvature or the cellular nature of tissues. To tackle these difficulties I am setting up an analysis inspired by recent developments in signal analysis on graphs [2]. During this presentation, I will introduce the analysis and its first results when applied to live imaging data of the development of plant floral organs (Figure 1).

References

[1] Antoine Fruleux and Arezki Boudaoud. Modulation of tissue growth heterogeneity by responses to mechanical stress. *Proceedings of the National Academy of Sciences*, 116(6):1940–1945, 2019.

[2] David I Shuman, Sunil K Narang, Pascal Frossard, Antonio Ortega, and Pierre Vandergheynst. The emerging field of signal processing on graphs: Extending high-dimensional data analysis to networks and other irregular domains. *IEEE Signal Processing Magazine*, 30(3):83–98, 2013.

Emergent modes of collective motion in confined epithelia

Joseph d'Alessandro[1], Grégoire Peyret[1], Philippe Marcq[2], Romain Mueller[3], Simon Bégnaud[1], Amin Doosthmohammadi[3], Julia Yeomans[3], Benoît Ladoux[1]

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[2] Laboratoire Physique et Mécanique des Milieux Hétérogènes, CNRS UMR 7636, Sorbonne Université, ESPCI, 75005 Paris
[3] Peierls Center for Theoretical Physics, University of Oxford, United Kingdom

In many processes *in vivo*, and particularly in embryonic development, the collective migration of epithelial tissues plays a pre-eminent role. Although those movements take place in confined environments, it is still unclear to which extent mechanical signals arising from the interplay between confinement and collective motion could contribute to the emergence of spatial structures in the tissues. The aim of this study was to systematically analyse the effect of geometrical confinement on epithelial movements.

Methods

Epithelial cells from various cell lines were cultured on confining patterns of diverse shapes and sizes, prepared by micro-contact printing of fibronectin on stiff or deformable PDMS substrates. Then, combining video-microscopy and image analysis, several physical and biological observables were quantified: velocities, traction forces, internal tissue stress, cell density, protein localisation and cell polarity. We studied the spatio-temporal relationships of those quantities, as well as the effect of drug and genetic perturbations on the patterns of motion, to gain insight in the main mechanisms at stake. We used this knowledge to build a phase-field model accounting for cell mechanics, cell motility, and their coupling through cell-cell interactions.

Results

Keratinocytes confined on square patterns exhibit oscillatory movements that are coordinated over the whole tissue, up to millimetre-sized confinement. Those coordinated movements rely on the coupling of the motility of individual cells to the mechanical signals arising from their neighbours. At a multicellular level, the oscillations induce regular patterns of cell deformations, which transduce into the activation of the transcription factor YAP1. Thus, this phenomenon, originating from the interplay of confinement and cell-cell coordination, provides the tissue with a potential mechanism to robustly define spatio-temporal patterns of mechanical signals. As such, it could define a new route to structure formation driven by cell motion.

The measurement of tissue stress also allowed us to estimate some rheological properties of the tissues. Our findings point to correlations with the different dynamical behaviours exhibited by the various cell types. This suggests that fine tuning of cell and cell adhesion mechanics directs the selection of the mode of motion arising at the tissue scale.

Physics of lumen coarsening and blastocoel formation

Hervé Turlier
Center for Interdisciplinary Research in Biology, Collège de France

Early embryos are shaped via a series of cell divisions, shape changes and rearrangements leading generally to a stereotypical blastula structure before gastrulation. I will summarize our recent analysis and modeling efforts to decipher the self-organizing principles of the mouse embryo morphogenesis, combining 3D numerical simulations with simple concepts of soft-matter physics (1-3). Pre-implantation mammalian embryogenesis leads to the blastocyst, a structure composed of an epithelial layer surrounding an inner mass of cells and a fluid-filled cavity. In a collaborative efforts with biologists, we demonstrated in the past years that differential changes in cell surface tensions are sufficient to drive the process of compaction at 8-cell stage (1) and the formation of the inner-cell mass at the 8-to-16 cells transition (2). Here I will focus on the formation and positioning of the blastocoel cavity after the 32-cell stage. I will present our recent results demonstrating that the blastocoel forms by hydraulic fracturing of cell-cell contacts into hundreds of microlumens, which then coarsen into a single cavity though hydro-osmotic luminal fluid exchange (3).

(1) Maître, J. L., Niwayama, R., Turlier, H., Nédélec, F., & Hiiragi, T. Pulsatile cell- autonomous contractility drives compaction in the mouse embryo. *Nat. Cell Biol.* 17, 849-855 (2015).

(2) Maître, J. L., Turlier, H., Illukkumbura, R., Eismann, B., Niwayama, R., Nédélec, F., & Hiiragi, T. Asymmetric division of contractile domains couples cell positioning and fate specification. *Nature*, 536, 344-348 (2016).

(3) Dumortier, J., Le Verge-Serandour, M., Tortorelli, A. F., Mielke, A., de Plater, L., Turlier*, H., & Maitre*, J.L. Hydraulic fracturing and active coarsening position the lumen of the mouse blastocyst. *Science*, 365, 465-468 (2019).

Tunable corrugated patterns in an active nematic sheet

A. Senoussi[1,*], S. Kashida[1], R.Voituriez[1,2], J.-C. Galas[1], A. Maitra[1], and A. Estévez-Torres[1]

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[2]Laboratoire de Physique Théorique de la Matière Condensée, Sorbonne Universite and CNRS, F-75005, Paris, France
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To what extent can we engineer matter that shapes itself? To investigate this question we study a 3D solution of multimeric kinesin motors and microtubule filaments. In addition to previously described patterns [1] [2], we report that such a solution can spontaneously form a 2D free-standing nematic active sheet that actively buckles out of plane into a centimeter-sized periodic corrugated sheet [3]. This pattern is stable at low activity and is transient at higher activities and will ultimately break into chaotic flows. Using the hydrodynamic theory of active fluids, we demonstrate that the wavelength and dynamics of the corrugations are controlled by the motor concentration. Our results underline the importance of both passive and active forces in shaping active matter and provide some insights how active fluids can be sculpted into a static material through an active mechanism.

[1] F. J. Nédélec *et al.* Nature 389(6648), 305 (1997)
[2] T. Sanchez *et al.* Nature 491(7424), 431 (2012)
[3] A. Senoussi *et al.* Proc. Natl. Acad. Sci. USA, In press (2019) [*arXiv:1904.09249*]

Shaping the zebrafish myotome by inter-tissue friction and active stress.

S.Tlili[1,2,*], J.Yin[1,*], J-F Rupprecht[1], M. A. Mendieta-Serrano[1], G. Weissbart[1], N. Verma[1], X. Teng[1], Y. Toyama[1], J. Prost[1,3], T. E. Saunders[1,2,4].

1 Mechanobiology Institute, National University of Singapore.

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During zebrafish embryogenesis, large muscle segments, called myotomes, acquire a characteristic chevron morphology, which is believed to aid swimming. Myotome shape can be altered by perturbing muscle cell differentiation or the interaction between myotomes and surrounding tissues during morphogenesis. To disentangle the mechanisms contributing to shape formation of the myotome, we combine single-cell resolution live imaging with quantitative image analysis and theoretical modeling. We find that, soon after segmentation from the presomitic mesoderm, the future myotome spreads across the underlying tissues. The mechanical coupling between the future myotome and the surrounding tissues appears to spatially vary effectively resulting in spatially heterogeneous friction. Using a vertex model combined with experimental validation, we show that the interplay of tissue spreading and friction is sufficient to drive the initial phase of chevron shape formation. However, local anisotropic stresses, generated during muscle cell differentiation, are necessary to reach the acute angle of the chevron in wildtype embryos. Finally, tissue plasticity is necessary for formation and maintenance of the chevron shape, which is mediated by orientated cellular rearrangements. Our work sheds light on how a spatio-temporal sequence of local cellular events can have a non-local and irreversible mechanical impact at the tissue scale, leading to robust organ shaping.

Quantifying the relationship between the mechanical status of cells and the associated molecular organization of its components

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Contractile forces are involved in many cellular processes including cell division, migration, or differentiation. The propagation of forces throughout the cell depends mainly on the assembly of actomyosin bundles called stress fibers (SF), which are connected to the surrounding substrate through adhesion complexes. Although structural and molecular composition of both SF and their associated adhesions have been well characterized, the co-assembly mechanism of these structures and the key parameters setting the magnitude of the traction forces exerted at the cellular level still remain unclear[2].

In order to identify key parameters involved in force generation process, we designed dumbbell-shaped micropatterns on which epithelial cells were shown to display essentially two peripheral SF, thereby mimicking the minimal system required for contractility establishment. While measuring associated traction forces, we surprisingly observed large variations in contractile energies among the cell population, although the apparent actin architecture remained quite conserved. This intriguing finding indicates that a key feature of the production and transmission of forces is missing in our description of contractile networks and is the focus of our study.

We first studied the possibility that some internal/subcellular contractile efforts could be dissipated instead of being transmitted to cell anchorages by looking at the interplay between actin dynamics and traction forces in these cells. We next decided to quantify both the molecules acting on the network and its mechanical state. In particular, we focused our study on myosin, alpha-actinin and focal adhesions associated with the stress fibers in these cells. As standard TFM requires detachment of the cell to get the undeformed configuration of the soft substrates, it however eliminates the ability to fix and label cells, a necessary step to explore further the molecular composition of the SF and the surrounding network. To overcome this technical issue, we established a new protocol that combines micropatterning and TFM with fluorescent labelling. An alignment grid with numbering patterned along with the dumbbells allow for finding cells of interest and aligning the images. This unique procedure allowed us to access simultaneously to the forces exerted by cells and without any genetic perturbation, the associated molecular state of its network.

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A growing bacterial colony in 2D as an active nematic

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Abstract

Although our knowledge of molecular mechanisms and genetics of bacterial cells is quite advanced, there is still much work to do about the underlying physical mechanisms ruling the way these cells interact with each other and with the surrounding environment.

I will talk about the experiment on the growth of single-layer micro-colonies of *E. coli* - that is, colonies which develop from a single mother cell to about thousands of daughter cells in 2D - confined to just under the surface of soft agarose by a glass slide.

The idea is to provide a physical interpretation of such a biological system in terms of liquid crystals, more specifically as an active nematic. I will discuss: (*i*) the formation of microdomains of a well-defined size induced by growth, (*ii*) the tangential alignment to which the cells at boundary are subject, and (*iii*) the creation and dynamics of topological defects with charges ±½.

For CellTiss days 2019 : Quantitative Approaches of Living Systems

Actin filaments store elastic energy to produce nanoscale forces

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The elongation of single actin filaments produces forces that do not exceed a few piconewtons. Yet, the forces generated by branched actin networks, essential for many fundamental biological processes, can reach up to tens of nanonewtons. How the 3D organization of branched actin networks allows the production of nanoscale forces remains unknown. We employed *in situ* cryo-electron tomography to reveal the architecture of podosomes, mechanosensitive actin structures formed at the ventral surface of human macrophages that generate nN-scale protrusion forces (1-3). We found three populations of actin filaments: a core of upright filaments surrounded by radial filaments and a layer of cortical filaments. Quantitative analysis of podosome architecture showed that core filaments are denser, more oblique, and shorter than radial and cortical filaments. Core filaments are bent and store high elastic energy, supporting that the podosome core consists of a set of highly compressed actin filaments. Additionally, subtomogram averaging revealed the spatial distribution and orientation of Arp2/3 complex-mediated branches within the core. Altogether, these findings provide a mechanical basis to understand how branched actin networks are able to transmit nanoscale forces.

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Mechanical properties of branched actin networks

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Gels of fibrous biopolymers are ubiquitous within cells and their rigidity is crucial for their function [1]. Our current understanding of their elastic response is usually understood as an interplay between the bending and stretching of their filaments [2]. This point of view however fails when applied to the weakly coordinated branched actin networks found throughout the cell [3, 4]. Through experiments and theory, we show that their elasticity crucially involves reversible entanglements between their filaments. Additional entanglements may get locked in during network growth, setting the final properties of the network [5]. These properties could be key to understanding how moving cells dynamically adapt their cytoskeleton to their environment.

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Cell cortex dynamics: the role of myosin II in cortex thickness fluctuations

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A cell's survival and ability to execute its function rely on many processes. Amongst those, cell migration, division and endocytosis are central ones that rely on the actin cortex. But we know surprisingly few things about this structure and its dynamic. One of the problem encountered to study the cortex is its small size, typically a few hundreds of nanometers in thickness, making it too small to be properly observed with conventional microscopy techniques.

We developed a new tool to study the behavior of the cortex at this scale. We use super-paramagnetic beads under a controlled magnetic field: in this situation, the beads develop their own dipolar moment and are attracted to each other with a known force. For this study we first used dendritic cells, which easily ingest micron-size objects, and that are known to migrate in complex 3D physico-chemical environments. With this system we measured the thickness of the cortex, by having one bead inside the cell and one outside and applying just enough force to get the beads in contact with the cortical layer.

Once a cortex is pinched between beads, we track these beads with image analysis, then we can compute the distance between the two beads which is equivalent to the thickness of the cortex. With this technique we measured the thickness of the cortex to be around 270nm on average with a precision of 30nm. This level of precision is possible because beads are extremely monodisperse in size, and because we compute their position using image with 16-bits gray level dynamics. By doing observation over a few minutes, we also realized that said thickness is highly fluctuating in time, on a scale of a few hundred nanometers.

By using different drugs we showed that this fluctuation activity is entirely dependent on actin. Furthermore, inhibiting polymerization reduces it but does not completely stop it. Surprisingly inhibiting myosin II motors with Blebbistatin drastically reduced cortex thickness fluctuations. Both this results and the scale of the fluctuations led us to conclude that they were not actin-related protrusions growing from the cell but rather a dynamical process happening within the cortex. In agreement with our experimental results, a 3D active gel model of the cortex describes contractility-driven mechanical instability that creates actin density fluctuations in time and space in the cortex. One of the predictions of this model is that the spatial amplitude of fluctuations is correlated to the thickness of the cortex, a result we also see in our experiments.

Adherens Junction Length during Tissue Contraction Is Controlled by the Mechanosensitive Activity of Actomyosin and Junctional Recycling

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During epithelial contraction, cells generate forces to constrict their surface and, concurrently, fine-tune the length of their adherens junctions to ensure force transmission. While many studies have focused on understanding force generation, little is known on how junctional length is controlled. Here, we show that, during amnioserosa contraction in Drosophila dorsal closure, adherens junctions reduce their length in coordination with the shrinkage of apical cell area, maintaining a nearly constant junctional straightness. We reveal that junctional straightness and integrity depend on the endocytic machinery and on the mechanosensitive activity of the actomyosin cytoskeleton. On one hand, upon junctional stretch and decrease in E-cadherin density, actomyosin relocalizes from the medial area to the junctions, thus maintaining junctional integrity. On the other hand, when junctions have excess material and ruffles, junction removal is enhanced, and high junctional straightness and tension are restored. These two mechanisms control junctional length and integrity during morphogenesis.

An analytical approach unifies mechanical rigidity in cell-based tissue models and biopolymer networks

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Understanding how mechanical properties of biological tissues arise from collective cellular behavior is vital for understanding the mechanisms that guide embryonic development, cancer growth, and wound healing. Recently, a new type of rigidity transition was discovered in a family of cell-based models for 2D and 3D tissues. Here I discuss these transitions and show that they are an instance of a much more general class of transitions, which appear when introducing geometric incompatibility into under-constrained systems. This kind of transition also provides an important limiting case to understand stiffening in fiber network models, which are used to describe biopolymer networks like collagen. We show that all of these models exhibit generic elastic behavior close to the transition, which is independent of the microscopic structure and the disorder in the system. In particular, we obtain analytic expressions for the elastic stresses and moduli, and we numerically verify our findings by simulations of under-constrained spring networks as well as 2D and 3D vertex models for dense biological tissues. Several of our predictions are parameter-free, and we thus expect them to be general hallmarks for stiffening in under-constrained materials induced by geometric incompatibility. Hence, these predictions provide direct experimental tests for whether stiffening behavior observed in a material is due to geometric incompatibility or not.

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QuanTI-FRET: a framework for quantitative FRET measurements in living cells

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Fluorescence Resonance Energy Transfer (FRET) allows for the visualization of nanometer-scale changes. This performance is achieved in single-molecule experiments but still challenging in biological materials. Despite many efforts, quantitative FRET in living samples is still either restricted to specific instruments or limited by the complexity of the required analysis. With the recent development and expanding utilization of FRET-based biosensors, it becomes essential to allow biologists to produce quantitative results that can directly be compared. Here, we present a new calibration and analysis method allowing for quantitative FRET imaging in living cells with a simple fluorescence microscope. Aside from the spectral crosstalk corrections, two additional correction factors were defined from photophysical equations, describing the relative differences in excitation and detection efficiencies. The calibration is achieved in a single step, which renders the Quantitative Three-Image FRET (QuanTI-FRET) method extremely robust. The only requirement is a sample of known stoichiometry donor:acceptor, which is naturally the case for intramolecular FRET constructs. We show that QuanTI-FRET gives absolute FRET values, independent of the instrument or the expression level. Based on the calculated stoichiometry and FRET probability, we present a framework for assessing the quality of the data and of the analysis to make QuanTI-FRET usable confidently by non-specialists.

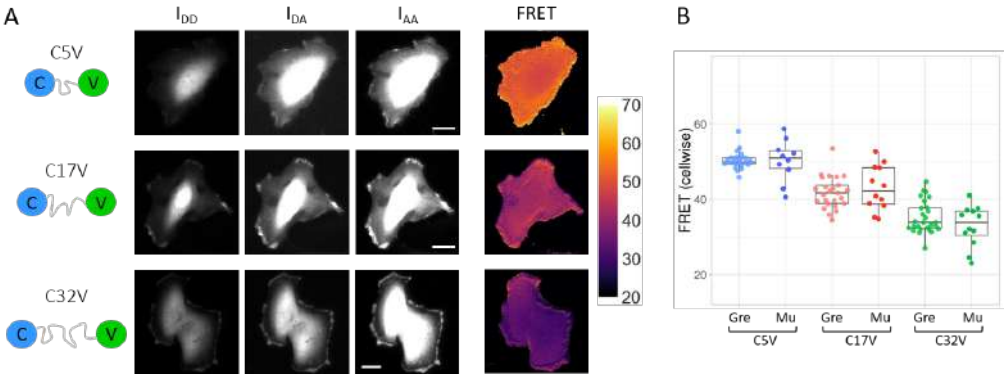

Figure 1: FRET measurements on FRET standards. **A** Triplet fluorescence images of cells expressing the three FRET standards: C5V (short linker), C17V (medium) and C32V (long linker). FRET maps showing the highest FRET for the shortest linker construct C5V and decreasing to the lowest FRET construct C32V. Scale bar 20μm. **C** Boxplot gathering cellwise FRET values of C5V, C17V and C32V measured independently in two different labs (in Grenoble and Munich). After calibration, same FRET median values were obtained.

SEGMENTATION OF 3D IMAGES OF PLANT TISSUES AT MULTIPLE SCALES USING THE LEVEL SET METHOD

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Background: Developmental biology has made great strides in recent years towards the quantification of cellular properties during development. This requires tissues to be imaged and segmented to generate computerised versions that can be easily analysed. In this context, one of the principal technical challenges remains the faithful detection of cellular contours, principally due to variations in image intensity throughout the tissue. Watershed segmentation methods are especially vulnerable to these variations, generating multiple errors due notably to the incorrect detection of the outer surface of the tissue.

Results: We use the level set method (LSM) to improve the accuracy of the watershed segmentation in different ways. First, we detect the outer surface of the tissue, reducing the impact of low and variable contrast at the surface during imaging. Second, we demonstrate a new edge function for a level set, based on second order derivatives of the image, to segment individual cells. Finally, we also show that the LSM can be used to segment nuclei within the tissue.

Conclusion: The watershed segmentation of the outer cell layer is demonstrably improved when coupled with the LSM-based surface detection step. The tool can also be used to improve watershed segmentation at cell-scale, as well as to segment nuclei within a tissue. The improved segmentation increases the quality of analysis, and the surface detected by our algorithm may be used to calculate local curvature or adapted for other uses, such as mathematical simulations.

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Surface patterning strategies to dissect T-Cell adhesion and actin organisation

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For an efficient immune response, an optimal interaction between T-cells and antigen presenting cells (APC) is required; it takes the form of a cell-cell contact involving different scales ranging from the molecular (1-10 nm) to the cellular (1-10 micrometre). The ligation of the special T cell receptors (TCR) to its ligands on an APC, leads to larger scale molecular reorganisation leading first to formation of TCR micro-clusters, and later to cell-scale restructuring of both the membrane and the cytoskeleton. Patterning an artificial substrate with ligand-clusters that in turn induce TCR-clustering is an important tool to understand the link between the organisation of TCR and its ligand, the organisation of the actin cytoskeleton and the impact of both on overall cell behaviour including adhesion and signalling. We developed a new nanotechnology based substrate (ligand-dot size down to 250 nm) and also used an alternative strategy based on colloidal self-assembly (700 or 400 nm) to show that TCR is clearly clustered on 700 nm dots but not on smaller 400 nm dots. Actin is homogeneously distributed in the form of a network in most cells but in a few of them, it appears as dots that co-localize with the ligand clusters. However, even for cells where the actin network appears homogeneous, a novel image analysis based on deep learning strategy, developed specifically for cells on the nanotech based substrates, indicates that the actin organisation on the dots and outside the dots may be different at least for some of the cells. While the nature of the difference is not clear from this analysis, finer observation using stochastic optical reconstruction microscopy hints that the dots may in fact be sites where actin bundles cross each other forming nodes that are not visible at lower resolution. This work confirms a close link between T cell receptor organisation and actin structure.

A quantitative approach of cell-cell communication

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We have developed an experimental model biomimetic of cell-cell communication. It consists in networks of aqueous droplets bathing in oil and connected by lipid bilayers decorated with ion channels. To produce these 1D/2D droplet networks, we first develop an original printing method based on the periodic extraction through an oil/air interface of a capillary in which the aqueous phase is injected [1]. When the bilayers are decorated with the ion channel hemolysin, we then study the diffusion of a fluorescent probe in 1D nanoporous networks, using epifluorescence microscopy [2]. We establish that the characteristic diffusion time depends non-linearly on the nanopores concentration. We show that our results are well captured within a first passage time theoretical description, in which nanopores are clustered rather than being independent.

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ArtiGranules, a novel tool to examine intracellular phase transitions and RNA-Protein membrane-less organelles

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Membrane-less organelles are ubiquitous functional sub-units of cells that are involved in many vital functions such as RNA regulation (storage, translation and degradation), thus shaping the general gene expression output. Importantly, their dysfunction is linked to viral infection, cancer, and neurodegenerative diseases. Beyond their molecular composition, these organelles are very complex regarding their biochemical and biophysical properties, which implies the development of novel tools for their study. By combining synthetic biology and biophysics, we have developed a novel methodology allowing the controlled formation in cells of artificial membrane-less granules that are made of synthetic RNA-protein scaffolds (ArtiGranule)[1]. Our artificial RNA-protein granules recapitulate the hallmarks of phase-separated liquid membrane-less organelles and can be used to dissect RNP granule biogenesis and functions. We used ArtiGranule approach for testing predictions and examined the contribution of RNA elements to the formation of a condensed phase within a living system.

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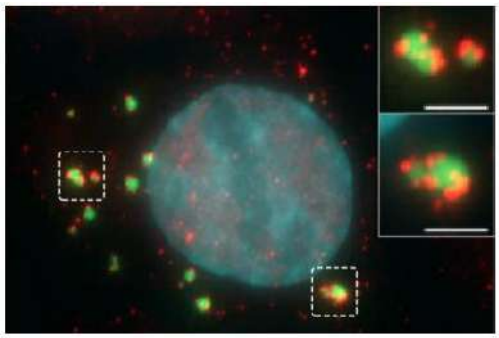
Phase transitions in cells



Modulation of cortical actin assembly dynamics in the early embryo

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The cortex is a thin layer of actin filaments attached to the cellular membrane, controlling cell's architecture through a constant remodeling of its networks. From the cortex emerge transient structures such as filopodia, endocytosis patches or cytokinesis ring for example. All these architectures are modulated by signaling pathways, numerous regulatory proteins, but are also modified through mechanical feedbacks. As a result, actin architectures are tightly spatially and temporally regulated to perform different functions.

One of the goals of my lab is to understand how the dynamic control of actin nucleation is regulated in the *C. elegans* early embryo. By the use of *in vivo* endogenous labeling of proteins and state of the art microscopes, we are now able to resolve the dynamics of individual molecules and molecular components of the cytoskeleton. We are characterizing the dynamics of an essential *C. elegans* formin in the zygote (CYK-1) and notably its cooperativity with Arp2/3 and Capping proteins to nucleate filopodia.

In vitro reconstitution reveals that actomyosin force dissociates the talin-RIAM complex to promote talin-vinculin interaction

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Mechanical cues govern a variety of biological processes. For example, cell-matrix adhesion complexes sense changes in intra- and extracellular mechanical properties to adjust adhesion strength and cytoskeleton anchoring. The force-dependent conformational change of the actin-binding protein talin might be the initial switch that triggers the maturation of short-lived nascent adhesions into stable focal adhesions. In this process, the mechanical stretching of talin exposes cryptic binding sites for vinculin to reinforce actin anchoring. Several studies further suggested that distinct force-dependent conformations of talin select specific binding partners, including vinculin and RIAM, to trigger the appropriate mechanical response. However, the nature, sequence and interdependence of these molecular events are largely unknown. To address these issues, we designed a quantitative in vitro microscopy assay with purified proteins, in which actomyosin force controls RIAM and vinculin binding to a talin-micropatterned surface. This in vitro reconstitution revealed for the first time that actomyosin force is sufficient to provoke the dissociation of RIAM from talin, establishing the mechanosensitivity of the talin-RIAM complex. This work demonstrates how actomyosin force controls a key cellular decision-making process.

How to sculpt an organ: Drosophila follicle development as a model of morphogenesis involving extracellular matrix

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Epithelial tissue morphogenesis is studied in many frameworks, especially in invertebrate organisms that allow an efficient combination of genetics and imaging. However, in many well-described examples, there is no role for the basal extracellular matrix (basement membrane), which probably contrast with morphogenesis processes in vertebrates. In our group we are studying how Drosophila follicle elongates, which is highly dependent on the interplay between epithelial cells and their basement membrane. This process simple in appearance - change a sphere in an ovoid – involves several mechanisms that can be temporally or genetically unconnected. More precisely, we are looking at the role of the actomyosin and of several actin cytoskeleton regulators to understand their impact on elongation at the molecular, cellular and tissular levels. I will give an overview on our last advances on the generation of a basal planar cell polarity by the Dystrophin/Dystroglycan complex that links extracellular matrix with the actin cytoskeleton.

Visco-elastic properties of bulk cytoplasm maintain the mitotic spindle in the center of large cells

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Mitotic spindles specify the position and orientation of cell divisions. Spindles orientation and position is canonically determined by forces exerted by astral microtubules (MTs) which radiate from centrosomes and contact the cell cortex to apply pushing and/or pulling forces that move and rotate spindles. In large cells, such as eggs and early blastomeres of echinoderms and vertebrates embryos, however, spindles do not contact the cortex and are pre-positioned by large interphase MT asters. In spite of their lack of interaction with the cortex, mitotic spindles appear stable and still for few tens of minutes. We used *in vivo* magnetic tweezers to directly apply calibrated forces and torques to mitotic spindles of dividing sea urchin zygotes. By applying forces parallel or perpendicular to the spindle axis, we reveal the existence of visco-elastic forces which hold the spindle in the middle of the cells. Moving spindles are associated with large and anisotropic drags, and are submitted to elastic forces which partially restore their initial centered positon. The absence of MT contact with the cell cortex suggests that those visco-elastic forces may directly emerge from the interaction of the spindle and its associated endomembranes with bulk cytoplasm. Thus, those data document a MT contact-independent mechanism that stabilizes the mitotic spindle in the middle of an animal cell.

Spontaneous symmetry breaking in oocyte meiosis

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In mammals, oocyte meiosis consists in two rounds of asymmetric divisions that generate a large haploid egg and two small polar bodies. Unlike symmetric cell division, in which the spindle is usually located at the cell center, the spindle is apposed and parallel to the oocyte periphery. Indeed, chromatids signal to the periphery and polarize the oocyte cortex, which generates an attractive flow. Upon fertilization, sister chromatids are separated in two clusters located at each end of the spindle, and cleavage furrow ingression starts at the spindle center. This is quickly followed by the spindle rotation. One chromatid cluster is extruded in the nascent polar body, while the other remains in the oocyte. Here we show that as furrow ingression progresses, the symmetric configuration (spindle aligned with the cell boundary) becomes unstable. This instability relies on two antagonistic forces exerted on the spindle: the progression of the cleavage furrow pushing the spindle inwards, and the attraction of the chromatid clusters to the periphery. This results in a spontaneous symmetry breaking event and to the rotation of the spindle in either direction. We devise a numerical model of this instability and make predictions that we verify experimentally. Our observations show that neither the rotation direction nor the identity of the extruded chromatid cluster need to be biologically predetermined during meiosis.

Pathways and Biophysical Consequences of Cellulose Production by the wild type Pseudomonas fluorescens SBW25 at the Air-Liquid Interface

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Cellulose-overproducing wrinkly spreader mutants of *Pseudomonas fluorescens* SBW25 have been the focus of much investigation, but conditions promoting the production of cellulose in ancestral strain SBW25 and its effects and consequences have escaped in-depth investigation through lack of an in vitro phenotype. Here, using a custom-built device, we reveal that in static broth microcosms, ancestral SBW25 encounters environmental signals at the air-liquid interface that activate, *via* three diguanylate cyclase-encoding pathways (Wsp, Aws, and Mws), production of cellulose. Secretion of the polymer at the meniscus leads to modification of the environment and growth of numerous microcolonies that extend from the surface. Accumulation of cellulose and associated microbial growth leads to Rayleigh-Taylor instability resulting in bioconvection and rapid transport of water-soluble products over tens of millimeters. Drawing upon data, we built a mathematical model that recapitulates experimental results and captures the interactions between biological, chemical and physical processes

Sinking for sex in the ocean

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[3]Jupiter Intelligence

Diatom species constitute a major group of non-motile phytoplankton found everywhere on the globe. These cells have the peculiarity of being encased in a fairly rigid silica-based shell that is thought to be part of their success. Oddly though, this shell seems to impose a constraint during cytokinesis and repeated cell division in this group is accompanied by a slow reduction in cell size. As a matter of survival therefore, cells must reproduce sexually once in a while (i.e. every $\sim$ couple of years) to restore a sufficiently large body size and resume a life cycle based on mitosis and cytokinesis. However, pairing for sexual reproduction in an inherently turbulent and three-dimensional system like the ocean seems a difficult task for non-motile organisms having poor control over their position and orientation.

In this presentation we will see how, from field observations in the sea and laboratory experiments, the sinking (in calm water) of suspensions of elongated diatoms (called "pennate" diatoms) favors cell encounter via a hydrodynamically-mediated density instability. The characteristic time-scale (i.e. the linear growth rate) of this instability depends on cell concentration, sinking speed and length of the diatoms. This naturally promotes encounters between healthy kins that sink synchronously. Therefore we conjecture that this selective pairing mechanism is ideal to robustly go through obligatory sexual reproduction when needed.

Microfluidic study of individual and collective swimming of *P. parasitica* zoospores

Quang D. Tran*[a], Eric Galiana[b], Philippe Thomen[a], Céline Cohen[a], François Orange[c], Fernando Peruani[d], Xavier Noblin[a]

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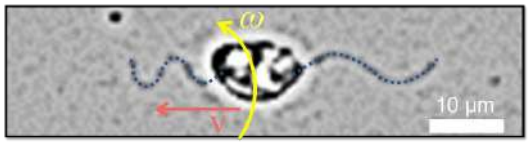
Abstract

Phythophthora diseases are dangerous to agriculture and eco-systems due to their pervasive spreading through water. From an infected host, multiple biflagellate fungal microswimmers, called as 'zoospores', are released to the environment with high-speed swimming up to 250 µm/s, and attack nearby plants. However, there is still lack of understanding about the swimming mechanism of zoospores and their interactions against chemical gradients and physical obstacles in their surrounding environments. In this study, we first conduct microscopy observations to achieve characteristics of their individual swimming and develop a theoretical model to explain its hydrodynamics resulted from the two diametric flagella. Then, we study their response to ionic gradients and physical obstacles using various microfluidic systems. Our experimental results show that the zoospores react significantly against potassium gradients. High concentration of potassium makes them reduce speed, change swimming patterns and even stop moving. We also find out that the density and arrangement of physical obstacles play a big impact on the swimming and spreading speed of the zoospores. These findings contribute to better understand *Phythophthora* diseases and the development of disease control.

a)



b)

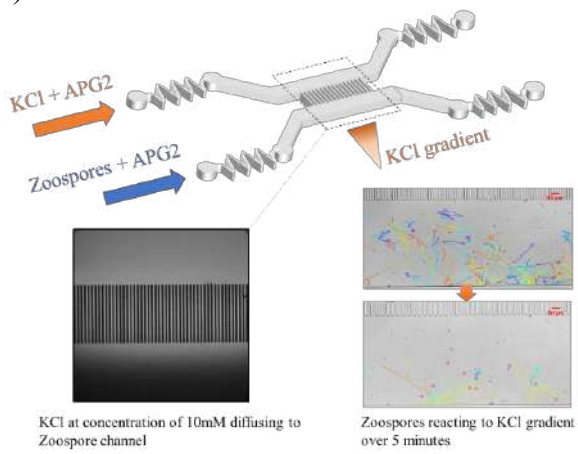


Figure: a) A single zoospore captured swimming in water. b) The microfluidic system to investigate the collective motion of zoospores under potassium gradient.

Bacterial biofilm: The resort of microbes

P. Thomen, A. F. Bitbol, A.Monmeyran, W.Ben-Youssef, N.Henry

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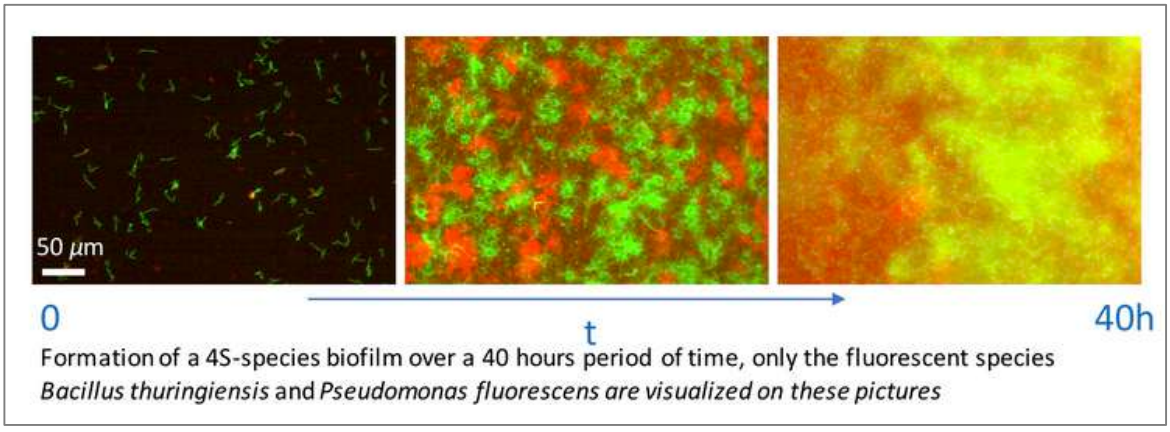
Adherent microbial communities, so called biofilms, are widely spread on earth in living hosts such as animals or plants but also on inert materials such as soil grains or ship hulls. Exhibiting very diverse traits and multiple impacts, they all have in common a three-dimensional architecture in which the confined cells, hold together by an extracellular polymer matrix, acquire specific properties and display significant advantages in comparison with their planktonic counterparts. To unravel the outperforming mechanisms operating in these communities, we investigate the role of their physical properties and of the physico-chemical parameters of the environment on their development. I propose here to give a short overview of the experimental approaches we run to address questions such as which general principles govern biofilm physics, response to flow or community assembling rules. I will show results obtained on a simple model system such as *E. coli* biofilm but also more complex multi-species adherent communities.



Formation of a 4S-species biofilm over a 40 hours period of time, only the fluorescent species *Bacillus thuringiensis* and *Pseudomonas fluorescens are visualized on these pictures*

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Soft-cell-confiner development to decipher the impact of mechanical stimuli on cell

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There are emerging evidences showing the importance of mechanical stimuli in cancer progression [1]. While the effect of matrix stiffness is extensively studied in the context of tumor progression [2], limited studies have focused in the role of solid stresses [3]. The field is lacking standard *in-vitro* test reproducing this long-term compression, without affecting cells by other means.

We have developed an integrated agarose-based microsystem with rigidity close to physiological conditions and enabling passive medium renewal. The set-up is compatible with time-lapse microscopy, in-situ immunostaining, as well as classical molecular analysis.

The confining agarose stiffness can be adjusted in a large range (100 Pa-5kPa). The soft-cell confiner can hence be used to decipher the influence not only of confinement, but also of matrix stiffness (in combination or separately). It appears thus as a powerful tool that could be of major interest to answer key biological questions in the growing field of mechanobiology. The ultimate design is now user friendly and has been transferred successfully to different users from both engineer and biological background.

The versatility of the set-up was analyzed by confining both adherent and non-adherent cells lines (TF1 Leukemic cells, MCF10A breast cancer cells and HS27A stromal cells). Cell proliferation was followed up to 3 days using video-microscopy and further documented by western-blot and EdU immunostaining. Live (calcein) and dead staining (Propidium Iodide) was also performed to assess cell viability. Nuclei and cell deformation were further characterized using in-situ immunostaining for actin and nucleus.

All tested cells were properly confined, with no major impact on cell proliferation for controls, while there was a decrease in cell proliferation for confined cells (5 µm-height). The cell viability was maintained for up to 8 days in the soft-confiner.

Noteworthy; the nuclear projected area is much larger upon confinement, with many nuclei highly deformed, exhibiting a non-circular shape. On-going experiments are performed to decipher the interplay between such high nuclei deformation, the expression of key mechanosensitive pathways, and cell phenotype.

We anticipate that the soft-cell-confiner could be a valuable tool for the fundamental understanding of the effect of cell confinement on various hallmarks of cancer progression and resistance, and in particular to decipher the role of nuclear mechanosensitivity.

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Tubular geometry induces cohesive whole tissue epithelial rotation

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Collective cell migration occurs in various physiological and pathological processes, including development, wound healing and cancer metastasis. Numerous studies have established that collective cell migration is regulated by 2D external mechanical constraints, like substrate geometry and confinement. Numerous studies have characterized epithelial collective movements, focusing mainly on flat surfaces. However, epithelia in vivo usually migrate on surfaces that are not flat but rather curved or grooved. Here we report the effect of curvature on epithelia migration, using micro-fabricated tubes of various curvature degrees (ranging from 25 to 250μm in diameter) in order to mimic in vivo curved surfaces. Using MDCK cells and real-time confocal microscopy, we show that epithelia migrating in hollow microtubes adopt a cohesive helicoidal movement and upon confluency, cells adopt a persistent collective rotational motion. Our findings suggest that collective rotation depends on the extent of tubular curvature, displays a distinct acto-myosin organization in rotating cells and requires lamellipodia-driven cell polarity. Altogether, our results indicate that surface curvature regulates collective cell migration, underlining the effect of geometry on tissue dynamics and could induce further insights into in vivo multicellular motions

Growth-associated constraints and cell mechanics during epidermal morphogenesis in Drosophila

Annafrancesca Rigato, Loïc LeGoff, Institut Fresnel, Marseille.

The morphology of a cell in a tissue is determined by the interplay of biochemical signaling and physical cues imposed by neighboring cells and the extracellular matrix. A particular case is that of a growing tissue, which increases in size and volume. In this context, each cell both exerts and is subjected to a passive viscoelastic resistance and an active force. Growth is thus a source of stress. Among others, cell divisions are reckoned as a fundamental stress-releasing mechanism that allow to maintain the mechanical homeostasis of the tissue. Therefore, growth and cell cycle are considered as tightly coupled phenomena. We thus wondered how the morphology and mechanics of a tissue are influenced when growth and cell divisions are uncoupled. A perfect model to investigate such case is that of the larval epidermis of *Drosophila*, formed by larval epithelial cells (LECs), and adult epidermis precursors (histoblasts). Histoblasts are organized in nests forming a continuous layer with the surrounding LECs. The epidermis is thus a simple binary system where two cell populations compete for space. While growth and cell divisions are usually coupled in epithelia, histoblasts grow without dividing throughout the whole larval life. Constraints due to the surrounding LECs and growth of the histoblasts in the absence of cell divisions lead to a dramatic change of histoblasts' morphological and mechanical state. In fact, in third instar larvae, they lose the typical aspect of a tensed cell layer and present folded junctions. Such mechanical transition is reminiscent of buckling instability, where growing LECs seem to exert a compressive force on histoblasts. By altering cell cycle or growth of either cell type, we show that uncoupling these two processes leads to unexplored mechanical regimes.

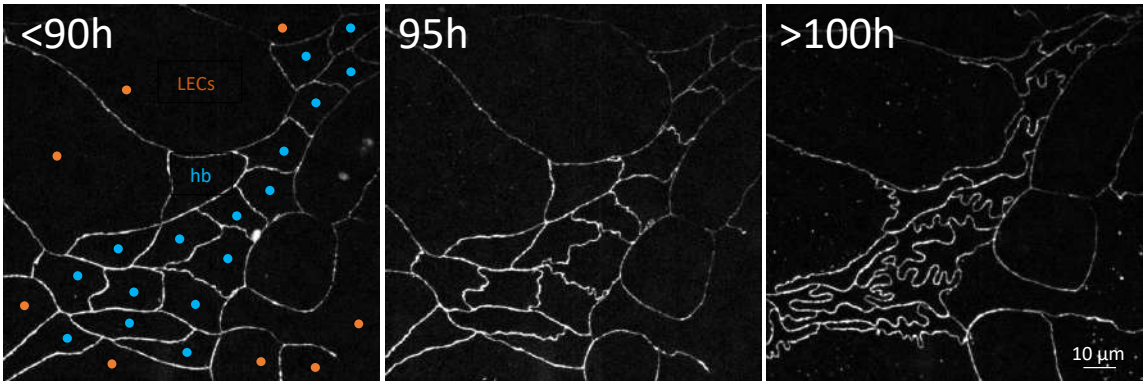

Figure 1. Cell outlines (e-cadherin:mKate fusion) **of histoblasts (hb, blue) and larval epithelial cells (LECs, red) through the mechanical transition**.

Force transmission at cell adhesion and the nucleus

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Institut Jacques Monod (IJM), Paris, France

In multicellular organisms, cells generate and undergo mechanical forces that may shape tissues and regulate genetic programs, but the underlying mechanisms remain largely unknown. Using genetically encoded biosensors of molecular tension, it is now possible to monitor in space and time the forces exerted on specific cell adhesion proteins in situ. Doing so, we have assessed in cell culture models how adhesion proteins respond to intra- and extracellular mechanical cues, and how their tension relates to the activation of cell signaling pathways and cell-scale forces upon induction of generic morphogenetic processes. Our results reveal varied mechanisms for force transmission across scales and their efficiencies, and how cell mechanosensation may arise from these processes.

Quantification of cellular forces on rigidity patterned substrates evidences distinct length scales in rigidity sensing

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ABSTRACT

Cell rigidity sensing comes from the ability of the cells to pull on their adhesions and adapt to the resistance cellular forces are opposed. Both micron and cell-scaled assemblies of myosin motors and actin or acto-myosin filaments have been shown to pull on cell adhesions, thus contributing to rigidity probing. But how these different scales interact to drive rigidity sensing is still unknown. Here we propose a mechanical approach to this issue: we designed a synthetic extracellular matrix with subcellular modulations of the rigidity and implemented a methodology to assess intra and extracellular stresses as well as cell Young's modulus with submicron resolution. By quantifying the cellular forces that pull on the rigidity-textured matrix as well as the intracellular contractility, we show that intra and extracellular stresses contribute to a partly independent probing of the rigidity: the pulling forces that the adhesions transmit to the extracellular matrix adapt to the local, adhesion-scaled rigidity while the intracellular stresses predominantly adapt to the cell-averaged rigidity. These findings evidence distinct rigidity-sensing machinery in cell adhesion and question signaling pathways that may control this parallel adaptation.

A new tool to characterize mechanical properties of soft biological tissues

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The mechanical properties of soft biological tissues play a crucial role in their normal physiology, contributing not only to their formation, maintenance and repair, but also crucially to diseases such as cancer, asthma or fibrosis. Mechanics has been proposed to exert its effect by impacting cells fate decisions and cell behaviours including proliferation, differentiation and motility, amongst others. However, despite its critical relevance, a comprehensive analysis of the biomechanics of soft biological tissues is still lacking due to the limitations of the existing characterization tools. In this work, we address this issue and describe the development of a novel device for uniaxial tensile testing of small samples (in the millimetre range) of epithelial and connective tissues, based on the real-time computer control of the interaction between a magnetic actuator and an electromagnet subject to feedback-controlled current.

After validating the device on synthetic materials of known mechanical properties and comparing its performance with conventional mechanical testing methods, we use a newly developed mounting chamber with controlled environmental conditions to characterize, as a case study, the mechanical properties of the oesophagus and of each of its constitutive tissue layers (epithelium, stroma, muscle). Through an analysis of strain-stress curves, we demonstrate that the whole oesophagus behaves as a trilayered composite material, whose overall mechanical response depends of the properties of each of its tissue layer. At high strains, the mechanical behaviour of the oesophagus is dominated by the stromal layer which exhibits the highest stiffness, whereas the muscle provides the major contribution to the oesophagus mechanical response at low and intermediate strains. At physiological strains, the mechanical behaviour of the oesopahgus is dominated by the rigidity of the muscle layer, the epithelium being the softest of all three layers and the stroma having an intermediate rigidity between muscle and epithelium. We discuss the potential relevance of these results on understanding the mechanisms which regulate oesphagus epithelial morphogenesis during post-natal development and oesophgus self-renewal dynamics at homeostasis.

Overall, we demonstrate that our set-up is able to measure the mechanical properties of biological soft tissues with an unprecedented reliability and precision, and discuss its tremendous potential as a diagnostics tool using patients sample biopsies in pathologies such as cancer.

From single nanobody-antigen bond to antibody dependant cell cytotoxicity

Cristina Gonzalez [1], Adrien Aimard [2], Martine Biarnes [1], Brigitte Kerfelec [2], Daniel Baty [2], Philippe Robert [1], Patrick Chames [2], Laurent Limozin [1]

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Mechanical forces are taking an emerging role in immunological processes, from molecular to cellular scales. We focus here on the potential role of mechanics to regulate antibodies behaviour, when they serve as bridges between immune effector cells and target cells, including cancerous cells. Some anti-cancerous antibodies have proved their efficiency by promoting antibody dependent cell cytotoxicity (ADCC). How their collective mechanical response may be involved and harnessed in this process remain unexplored. We have characterized the force dependence of the interaction between nanobodies (or single domain antibodies) and immune receptors and shown how it can evidence the production of force by spreading Natural Killer (NK) cells. Furthermore, we combined two panels of nanobodies to produce original bispecific molecules, mimicking the bridging role of conventional antibodies in the NK immunological synapse formed during ADCC. We discuss the consequences to produce potentially more efficient molecules for immunotherapy.

Microfluidic tools to image chemotaxis of swimming leukocytes

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Naïve T lymphocytes circulate between blood and lymph nodes in their search for antigen. The contribution of different chemokines to this process is inferred from loss-of-function experiments where the entry, homing within or exit from lymph nodes are impaired. However, their actual migration along defined chemokine gradients has not been imaged so far. This gap arises due to the lack of a proper *in vitro* substrate, since the migration of these cells is independent of integrins. Therefore, current knowledge about lymphocyte chemotaxis is derived from Transwell experiments, which circumvent the necessity of cell adhesion. Nevertheless, this technique presents several caveats as the gradient profile is unknown, the chemokines may bind to the porous membrane and function as adsorbed rather than soluble gradients, and because random migration (chemokinesis) can be mistaken with directed one (chemotaxis). Finally, real information on migration parameters such as speed, directionality and persistence, is missing. To circumvent these issues, we are developing a set of microfluidic tools to create stable gradients on cells migrating without adhesion (swimming). The optimization of a collagen matrix will also allow us to explore the contribution of confinement to lymphocyte migration in 3D.

Single-cell immuno-mechanics reveal large and fast cell-type-specific mechanical changes during white blood cell activation.

Alexandra Zak[1,2], Sara Violeta Merino Cortés[3], Anais Sadoun[4,5,6], Avin Babataheri[1], Stephanie Dogniaux[7], Elodie Hudik[2], Sophie Dupré[2], Yolanda R Carrasco[3], Yannick Hamon[8], Pierre-Henri Puech[4,5,6], Claire Hivroz[7], Oliver Nüsse[2], and Julien Husson[1*].

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White blood cells (leukocytes) perform diverse functions, including removing dead, infected cells and pathogens. They also interact with other leukocytes to exchange information about possible threats present in the organism. To perform these diverse functions, leukocytes activate by forming specialized interfaces called synapses with other cells. Different cell types form different types of synapses, which still share many common molecular features. Similarities are also mechanical. For instance, we have shown that when forming a synapse, a T lymphocyte generates forces and stiffens [1-4], recapitulating pioneering observations that during phagocytosis, the cortical tension of a phagocytic leukocyte increases dramatically while the cell engulfs its prey. The mechanics of other leukocytes such as B lymphocytes was still unknown, and some common mechanical aspects were also unexplored, such as the evolution of viscous properties of leukocytes during activation. Cellular matter is indeed peculiar in that elastic and viscous properties of resting cells are usually not independent: resting leukocytes conserve a ratio of cortical tension to cell viscosity of about 0.2-0.3 µm/s in neutrophils and macrophages, even though the latter can be ten times more tensed. Many studies exhibited this peculiar aspect of biological matter in other cell types. What remained unknown was whether this link between elastic and viscous properties would be conserved over time even during mechanical changes.

In this study, we quantified the evolution of both elastic and viscous properties during the activation of three types of leukocytes, and asked if elastic and viscous showed parallel time evolution. To address this question, we used a micropipette rheometer and activated leukocytes with standardized activating antibody-covered microbeads. We show that mechanical properties vary within seconds following the onset of leukocyte activation, and lead to up to a dramatic increase of both elastic and viscous properties of cells, while maintaining a relationship between elastic and viscous properties. This relationship constitutes a mechanical signature of leukocytes activation and it is cell-type-specific. This work opens new perspectives for a better understanding of cell mechanical properties and their evolution over time, and it suggests new ways to characterize cells during active physiological and pathological processes.

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CellTiss2019

Poster Abstracts

Alphabetical Order

Tissue Mechanics: Stokes Flow in Confluent Cell Simulations

Carine P. Beatrici, Leonardo G. Brunnet, François Graner

Cell migration plays an important role in embryogenesis, wound healing and cancer metastasis. Cell monolayer migration experiments provide data to understand what determines the movement of cells. However the simple monolayer set up in a linear channel cannot discriminate the differences in migration created by each cell ingredient. In our team, cell monolayers dynamics are observed in a discriminant benchmark: the Stokes flow, i.e. a cell flow around a circular obstacle. In the present work (in progress), we simulate cell monolayer migration using different computation models of active agents. The aim is to list the minimum cellular ingredients necessary to correctly simulate real cell migration. We take the same coarse grained measurements in each one: velocity field, density field, deformation field and others. We compare several computation cell models, not only with with *in vitro* and *in vivo* experiments, but also among themselves: *i*) Vicsek like particles; *ii*) Vicsek like particles with Voronoi neighborhood; *iii*) Cellular Potts i*v*) Multiparticle. We will determine which model represents best the cell flow in each measurement and for each cell ingredient. We also begin to simulate active vertex model. Each model has its own specific advantages. The phase diagrams for the interesting parameters in these models is being build. Preliminary results show that the cells form a viscous-elasto-plastic material like foams but with activity as an extra ingredient, and help understand the emerging collective behavior.

Interferometric microscopy and dynamic OCT: two optical tools to study nanoparticles inside and outside the cell

Martine Boccara[1,2], Samer Alhaddad[1], Ignacio Izedin[1], Kassandra Groux[1], Jules Scholler[1], Jean Marie Chassot[1], Olivier Thouvenin[1] and Albert Claude Boccara[1]
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We recently developed an interferometric microscope devoted to the study of nanoparticles in culture media or natural environment (aquatic, soil or microbiota). This new microscope uses the incoherent flux of a LED as a light source and a single microscope objective whose focal plane is imaged on a CMOS camera chip. The wave from the source interferes with the light scattered by the particles. The signal of the detected diffraction spot is amplified by interferences, which can be constructive or destructive. With the interferometric microscope we determine the total number of particles in a fluid and use two physical measurements, the light scattering level and the Brownian motion, to measure the size of the particles and to identify the type of particles (viruses vs membrane vesicles, according to their different refractive index for example).

The laboratory has also extended the use of Full field Optical Coherence tomography (FFOCT). Here the interferometer enables imaging through the depth of tissues. To get inside the dynamical properties of subcellular structures parallel acquisition of time series (<3ms/frame)) within the selected layer are performed. Here we display, after a mathematical treatment, the backscattering signals of intracellular nanoparticles and its time characteristics. With this method we were able to record metabolic activity of cancer cells in tumors, or plant cells from leaves under stress.

Results and applications of these new tools will be presented

Title : **Developing tools to study self-organizing properties of centriole amplification in MCC.**

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Ependymal cells are multiciliated epithelial cells (MCC) lining the brain ventricular cavities. They play a crucial role in the development of the central nervous system. They display at their apical surface hundreds of motile cilia to ensure essential physiological functions like propelling the cerebro-spinal fluid (CSF). Defects in their formation lead to serious neuro-developmental diseases. To nucleate these motile cilia, the MCC progenitors undergo a massive amplification of the centrosomal centrioles through a phenomenon called « centriole amplification » where newly formed procentrioles are assembled on MCC specific platforms known as « deuterosomes » (Al Jord et al. 2014). However, our recent data suggest that procentrioles are able to self-assemble without the help of deuterosomes (Mercey et al. 2019), and that amplification is driven by a combination of centriole self-organizing properties and by spatiotemporal variations of favorable material properties of the cytoplasm during MCC differentiation.

To test this hypothesis I am developing tools to (i) characterize the spatiotemporal changes in the material properties of MCC cytoplasm and MCC specific deuterosome organelles during centriole amplification progression (passive micro-rheology, FRAP, SUMO-SIM assay), (ii) assess how modulation of these material properties affect the dynamics of centriole amplification, and (iii) identify how the molecular pathways involved in centriole amplification, cytoplasm viscosity and phase separation affect each process (CDKs, mTORC1, DYRK3). Beyond the principal aim of understanding the regulation of centriole number and multiciliation, our well characterized and easy to handle MCC culture assay offers the opportunity to tackle how self-organization provides a scaffold to coordinate spatio-temporally, a complex cellular process.

ACTIN DYNAMICS AND CELLULAR FORCES IN VIVO AND IN VITRO

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October 7, 2019

Morphogenesis is a developmental process by which shape is acquired. During morphogenesis, tissues change their shape, a process often mediated by myosin-driven cell contractility. The C.elegans embryo has been established as a model to investigate the relationship between cell contractility and shape changes.
One important morphogenetic step in C.elegans is elongation, that converts the embryo from a bean-shaped embryo to the characteristic elongated worm shape. Elongation occurs via changes in cells' shapes that depend on the forces created by the embryonic cells and the resistance of the biological tissue.
Actin organization was shown to play an essential role during this elongation [1]. Its transition from disordered, thin filaments to parallel thick bundles is suspected to be at the origin of an anisotropic tension that correlates with a planar polarization of the cortex and the elongation of the embryo [2].
Here we address the role of actin organization on embryonic tensions. First, we use laser ablations in the actin cortex to probe actin tension during the elongation of the embryos. The ablation is performed in a rectangular-shaped torus, to mechanically separate a small patch of actin from the rest of the cortex and allow the analysis of the tissue relaxation with well-defined boundary conditions.
Second, we develop an in vitro model that mimics actin organization during elongation in order to correlate it with cellular forces. Using A431 as an epithelial cellular model, we correlate actin organization with cellular forces. Actin organization will further be tuned either via chemical or mechanical patterning on soft hydrogels, so to explore the mechanical effects of the actin patterns that are observed in vivo.
Exploitation of both techniques is expected to provide a better characterization of actin dynamics during the course of C.elegans embryonic elongation.

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Spontaneous Flows and Defects in Active Cellular Nematics

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Nematic cells are cells that have a preferred direction, for example due to an elongated shape. When those cells are plated on a substrate, at confluence they align over a typical size of ~500µm and form an active nematic layer. We are interested in the collective behavior of these cells, typically at the tissue scale ~1mm. At such a scale, it is relevant to neglect fluctuations and to look at a hydrodynamic description. Cells consume energy locally and are therefore considered as active materials.

The hydrodynamic theory of active matter predicts that by confining active nematics in particular geometries one can create spontaneous flows, with a transition analogous to the Frederiks transition in liquid crystals. This has been recently verified experimentally, in particular in the group of P. Silberzan with different types of nematic cells. In collaboration with this group, we look at what happens to this active Frederiks transition with the combined effect of activity and patterning of the substrate to impose a preferential direction to the cells, as a magnetic field would do for a liquid crystal.

In this confluent layer of nematic cells, there are topological defects where the orientation is no longer well defined. Because the cells are active, defects create local flows and can be self-propeled. We are particulary interested in the influence of cell division on these flows.

Puncture and penetration in soft materials

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The strength of soft materials against puncture by sharp objects is a property of condensed matter relevant to various situations spanning the design of strong gloves, to understanding animals bite mechanics or modelling the invasion of filament-shaped yeasts breaking through human skin. The overall processes of puncture and penetration are described by an interplay between several mechanisms involving the material elastic response, fracture, crack propagation, friction and adhesion. This variety of mechanisms raises the question of which are mostly relevant to any particular situation.

We present here puncture and penetration experiments at the micron-scale in crosslinked polydimethylsiloxane (PDMS) in two different systems. First, in nanoindentation experiments with micropipettes of known shape, we measure critical forces and indentations at puncture depending on crosslinker concentration and speed of indentation. We find in particular that the strength against puncture is the highest for an optimal crosslinker concentration. Second, we measure deformations of PDMS as an invasive yeast, *Candida albicans*, grows in this substrate in the shape of a filament with a tip continuously moving forward by cell wall creation. Here, the forces at the tip are driven by the internal pressure of the cell and are strong enough to induce indentation and penetration in materials such as human tissues or PDMS. Using a dedicated "compression force microscopy" technique, we monitor in 3d the displacement of fluorescent particles in the PDMS. We show it sustains deformations that are surprisingly smaller compared to the nanoindentation tests and we also highlight more detailed features that the deformation maps reveal.

The Mechanobiology of cell wall growth in filamentous fungi

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The growth of individual cells has crucial implications for cell size determination, tissue homeostasis or cancer progression. To date the mechanisms which control cellular growth remain poorly understood. This is because growth is an integrated output of multiple intertwined biochemical and biomechanical elements which dynamically probe and alter the cell surface to accommodate surface expansion. The growth of walled cells, like bacteria, fungi and plants is limited by the expansion of the cell wall (CW), a thin and stiff sugar-made layer encasing the plasma membrane. Growth speeds can vary over 3-4 orders of magnitude among walled cells, with fungal hyphal cells being some of the fastest growing cells. How CW mechanics adapts to such rapid expansion is not known, in part because of the lack of imaging methods to monitor CW dynamics in live growing cells. Using the genetically tractable model *Aspergillus nidulans*, we implemented a sub-resolution imaging method to map cell wall thickness with nanometric resolution around live growing hyphae. This combined with laser-based wall relaxation also allows to compute bulk elastic moduli of the cell wall in a local manner. We found interesting modulations in patterns of thickness and elasticity. For instance, growing tips were systematically thinner and softer than cell sides, likely accounting for de novo deposition of cell wall material there. The CW then becomes gradually stiffer away from cell tips, highlighting a gradual maturation and stiffening. Those data bring quantitative numbers and understanding in the regulation of cell wall synthesis during extremely rapid growth.

A system to test the molecular requirements for the formation of a dynamic actin cortex

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The actin cytoskeleton is a force-generating machinery implicated in a variety of cellular processes. One common property of these actin networks is that they are not static, but undergo a continuous recycling cycle called actin turnover.
Despite the importance of actin turnover in cells, its molecular mechanism is still not well understood.
One of the reasons is that most reconstituted systems, such as bead motility assays, are performed in open systems and are not powerful to study how components of actin networks are recycled as they are in cells. For this reason, we reconstitute in this study the formation of actin cortices inside water-in-oil emulsion droplets, where the importance of the proteins involved in actin recycling can be tested easily.

Geometry and mechanics of a model epithelium with irregular cells and a clonal inclusion

Diego A.C. Contreras, Vincent Hakim, Francis Corson

October 7, 2019

Abstract

An important role in the modeling of epithelial tissue mechanics has been played by vertex models, in which cells are idealized as polygons, with tricellular junctions represented as vertices joined by straight interfaces. Numerical simulations of these models in the presence of cell divisions display geometrically irregular cells, similar to those of epithelia, even when cell properties are homogeneous. Yet existing theoretical analyses are mostly confined to the mechanics of regular hexagonal lattices. Here, we develop an analytical description of geometrically disordered vertex models. We first quantify, in numerical simulations, geometrical properties such as the distribution of cell areas and perimeters, and mechanical properties such as the tissue bulk and shear moduli, with different sources of disorder, e.g different division rules or simply relaxation in the presence of noise. We then develop a simple mean-field description that accounts for these properties. Finally, we apply our analytical description to a simple case that is of interest in different biological contexts: a clonal group of cells with material properties that differ from the surrounding tissue and that may also grow at a different rate.

Actin and nuclear deformation contribute to differentiation of brain multiciliated cells

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In the brain, radial glial cells differentiate in various cell types one of them being the ependymal cells. These cells line all the brain ventricles, form a protective barrier for the parenchyma and contribute to the neural stem cell niche. Ependymal cilia beating participates in the cerebrospinal fluid flow regulating delivery of signalling molecules, toxin washout, oriented migration of new-born neurons and responses to injury. Impairment of the flow is correlated with age-related dementia and hydrocephalus.

We noted that differentiation into ependymal cells is concomitant with actin reorganisation, itself depending on primary cilia elongation. These phenomena are also accompanied by nucleus changes in volume, shape and position. Mechanical stresses on the nucleus, propagated through the cytoskeleton, modify chromatin organisation and gene expression. Thus, we hypothesize that cytoskeleton rearrangements may be instructive to nuclear reshaping and cell fate during ependymal differentiation. By targeting the actin pathway with drugs or shRNA expressed directly in the developing brain, we show that actin reorganisation contribute to nuclear deformation and to differentiation. Similarly, disrupting the complex linking the nuclear envelope to the actin cytoskeleton impaired the migration and deformation of the nucleus and blocked ependymal differentiation. Altogether, our results show that the relationship between the cytoskeleton and the nucleus is crucial to induce differentiation into multiciliated cells of the brain.

Patch-Clamp Electrophysiology and BioMembrane Force Probe to assess mechanisms of Cell Penetrating Peptide uptake into cells.

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Cell membrane is required for integrity and survival of living cells but can be a barrier for intracellular delivery of therapeutic agents. To counter this, Cell Penetrating Peptides (CPP) can be used: these peptides are able to cross cell membrane even linked to a cargo. By this way, it is known that the cargo - like small molecules, RNAs, fluorophores or drugs – can be delivered into the cytosol. Nevertheless, the cargo can be destroyed when it is addressed to lysosomes. That is why a perfect understanding of the CPP uptake mechanisms is required to improve its efficacy.

For this purpose, patch-clamp electrophysiology technique, highlighted **Figure 1**, is used to measure transitory current into cell membrane. When certain peptides are added in extracellular medium, a brutal current drop is reported which results from pore formation in the cell membrane. We will thus determine whether transient pore formation is part of the CPP uptake mechanism.

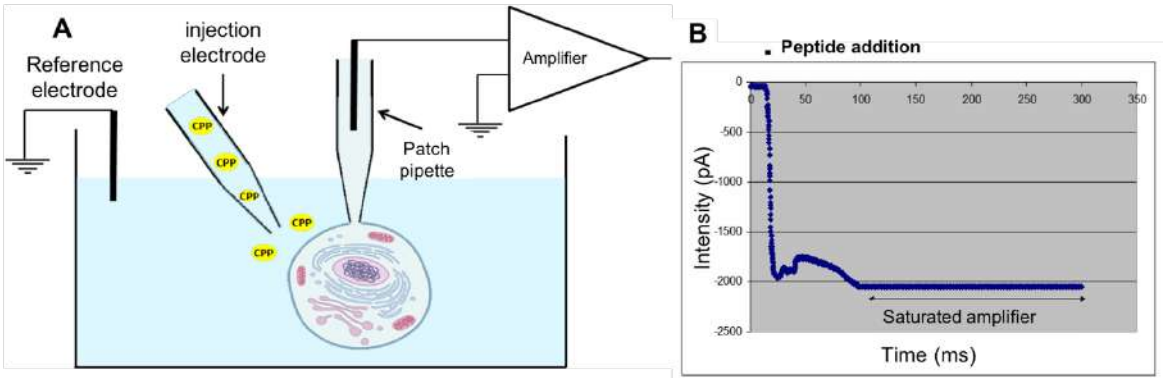

Figure 1 : A. Patch-clamp electrophysiology setup. The membrane cell is attached to the pipette containing an electrode, potential difference is applied between this electrode and a reference electrode so that current through membrane can be measured with CPPs in bath. B. Current through membrane of CHO vs time after R10W6 peptide addition.

To go further, Biomembrane Force Probe (BFP) is used to measure the force exerted by the CPP on the cell membrane. This technique was successfully coupled to the electrophysiology one, as shown on **Figure 2**, so we can measure at the same time current through membrane and CPP action on it. This will give details to the level of insertion of the CPPs and the concomitant membrane destabilization.

Both techniques should lead us to a better understanding of the CPP uptake mechanisms.

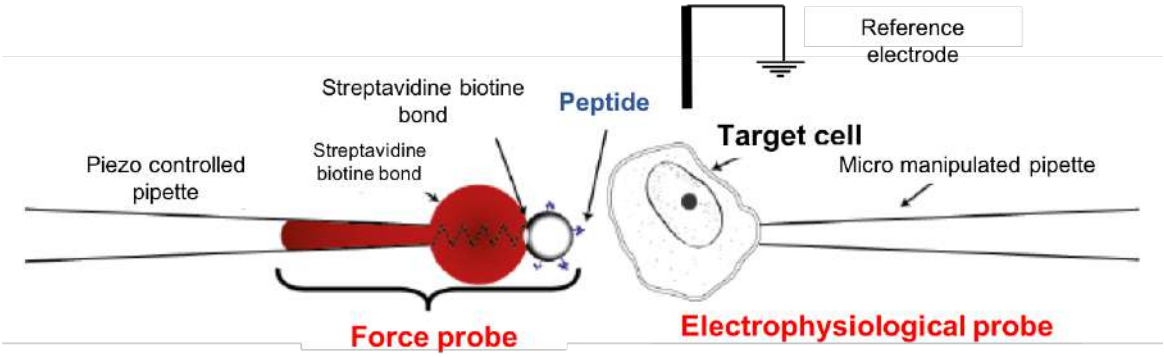

Figure 2 : Patch-Clamp electrophysiology coupled to BFP setup.

Swimming behavior of a mechanosensitive organism

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Paramecium is a large unicellular ciliate (100-300 μm long) that lives in still fresh water and that typically swims in straight lines segmented by abrupt changes in direction [1]. These reorientations can either be spontaneous or induced by a mechanosensitive response of the Paramecium upon its collision with obstacles. We have studied its swimming behavior in free and crowded environments by using tracking methods.

In a free environment, we found that Paramecium presents a ballistic motion at low timescales followed by a diffusive-like one at large timescales. We also measured the distribution of run lengths between reorientation events and found an exponential distribution, reminiscent of what has already been observed with Escherichia coli [2].

We have also studied how this behavior is affected by the presence of obstacles in the path of the Paramecium. To this end, we have micro-engineered 2D elastomer pools decorated with cylindrical pillars that are distributed in hexagonal or square lattices. We find that the diffusion constant of Paramecium decreases with the density of obstacles, independently of the topology. These results will ultimately allow us to decipher the role of mechanosensitivity on the swimming behavior of micro-organisms.

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Imaging metabolic dynamics by deuterium oxide-Stimulated Raman Scattering (DO-SRS)

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How growth is regulated at the scale of tissues or entire individuals is a central question of Developmental biology[1]. A downstream effector of this regulation is biosynthetic metabolic activity. Visualization and quantification of biosynthetic metabolic activities in a living organisms is thus a key objective to achieve. Yet, commonly available techniques able to provide metabolic information lack of sufficient spatial resolution.

We report on our attempt to image protein and lipidic biosynthetic rates using stimulated Raman scattering imaging. DO-SRS microscopy is a noninvasive approach able to probe metabolic dynamic in situ[1]. SRS imaging is based on a vibrational contrast that images C-H molecular vibrations to reports on the concentration of pre-existing molecules (proteins, lipids). Targetting of carbon–deuterium (C–D) bonds, can be used to visualize de novo lipid and protein synthesis upon exposure of cells to Deuterium. Here, we use this strategy to probe the metabolic incorporation of deuterium into yeast cells that can be used later on as a food source for developing *Drosophila* larvae with C-D molecular vibrations as a signature of metabolic activity.

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TRANSLOCATION OF THE CELL PENETRATING PEPTIDE PENETRATIN THROUGH ASYMMETRIC MODEL MEMBRANES: ROLE OF THE LIPIDS AND MICROFLUIDIC APPROACH

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Cell penetrating peptides (CPPs) are small polycationic peptides able to cross the cell membrane. They are interesting candidates as efficient vectors to deliver drugs directly inside the cell. It has been shown that this crossing can occur through endocytosis pathway or direct translocation, however the molecular mechanism of the latter remains unknown[1].

We focused on the molecular description of translocation and the determination of the peptide's partners for this mechanism. We demonstrated the role of certain lipids in the interaction and internalization of the CPP Penetratin. For this purpose, we used a model lipid bilayer formed at the interface of two adhering aqueous droplets in oil[2]. By introducing the fluorescently labelled CPP into one of the droplet, we can monitor its translocation through the bilayer. Moreover, this system allows us to form asymmetric bilayers and thus provides a versatile model for studying the effects of lipid composition on CPP translocation and obtaining insights on the mechanisms involved. We have showed that anionic lipids are required on both leaflets and that the specific nature of the head group on the proximal leaflet has a crucial importance to induce translocation of the CPP.

To pursue this study, we are developing a microfluidic device to form and trap the two-droplet populations. We will thus screen a lot of parameters and evaluate the role of lipids in CPP internalization mechanisms. We are also studying the possible modifications of the bilayer in the presence of the CPP and the effect of the transmembrane potential by manipulating droplets with micropipettes containing electrodes.

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Study of the regulation of the microtubule network architecture

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The centrosome is a key organel of cells. It is the main organizing center of the microtubule network and has a critical role during cilia formation. Thus its location within cells must be tighly controlled and different mechanisms trying to explain this phenomenon have been proposed throughout the years ([1]L. Laan et al., Cell, 2012. [2]H. Tanimoto et al, Nature Physics, mai 2018.
). In our study we use a combination of micropatterning and In Vitro approaches to try to better understand the possible role of another actor of the cytoskeleton, the actin network, in this complex behaviour. By interacting with the microtubules a branched actin network could alter the behaviour of those microtubules and possibly change the position of a microtubule nucleating center.

Single cell nanomechanics and luminescence based monitoring of nanodiamonds internalization in MCF7 cells

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Luminescent nanodiamonds (ND) are attractive tools for nanoscale biologic cellular imaging allowing both photoluminescence (PL) and magnetic resonance imaging [1]. Recent technological developments enable to fabricate bright NDs with high content of nitrogen-vacancy centres [2] that are anticipated to serve as a cell probes. In this work we present novel method of NDs detection in cellular environment. We demonstrate simultaneous visualization of NDs and of the non-labeled nucleus of living cells based on Raman and PL detection as a new tool for the localization of internalized nanoparticles.

To this end, NDs of size ranging from ultra-small particles ~ 5 nm to 60 nm were used, prepared from Ib synthetic diamond. Cells used for this experiment were from mammalian breast cancer (MCF7). We report on accomplishing to successfully internalize ND particles in MCF7 cells. We show that ND internalization can be monitored by Raman imaging method using K-mean cluster analysis. Whilst standard Raman imaging methods of NDs make use of the sp^3 diamond Raman signal, which limits their use to 100 nm size particles or bigger [3], here we employ Raman imaging in a novel way to detect small near-IR cellular probe. Changes of cells stiffness were detected by force measurement in atomic force microscopy after incubating cells with NDs, suggesting cell membrane hardening upon ND uptake.

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Tubular geometry induces cohesive whole tissue epithelial rotation

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Collective cell migration refers to the coordinated motion of a group of cells and is fundamental in the regulation of various processes, such as morphogenesis, tissue organization, wound healing and cancer metastasis. Numerous studies have characterized epithelial collective movements, focusing mainly on flat surfaces. However, epithelia in vivo usually migrate on surfaces that are not flat but rather curved or grooved. Here we report the effect of curvature on epithelia migration, using micro-fabricated tubes of various curvature degrees (ranging from 25 to 250μm in diameter) in order to mimic in vivo curved surfaces. Using MDCK cells and real-time confocal microscopy, we show that epithelia migrating in hollow microtubes adopt a cohesive helicoidal movement and upon confluency, cells adopt a persistent collective rotational motion. Our findings suggest that collective rotation depends on the extent of tubular curvature, displays a distinct acto-myosin organization in rotating cells and requires lamellipodia-driven cell polarity. Altogether, our results indicate that surface curvature regulates collective cell migration, underlining the effect of geometry on tissue dynamics and could induce further insights into in vivo multicellular motions

Biomimetic emulsions to probe the role of adhesion in tissue remodeling processes

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We use biomimetic systems to understand the physical basis of tissue remodeling in a simplified framework. In particular, we study how cell-cell adhesion controls the behavior of tissues under mechanical constraint. We use biomimetic emulsions that were shown to reproduce the minimal mechanical and adhesive properties of cells in biological tissues. We flow the emulsions through 2D microfluidic constrictions and use image analysis to characterize their elasto-plastic response to the applied perturbation. To validate our system, we firstly introduced non-specific depletion attraction forces between the droplet surfaces by tuning the concentration of SDS micelles in the continuous phase. We find that, for similar surface tensions, higher depletion attraction forces induce larger deformations of the droplets and delays plastic rearrangements in the channel. This shows that the elastic response of adhesive assemblies is favored when the plastic response is impaired by adhesion. We then induce specific adhesion between the droplets by grafting their surface with binding molecules. Preliminary results on the effect of specific adhesion also indicate that adhesion modifies the flow of compact biomimetic tissues through the constrictions. In the future, this work should allow us to predict tissue behavior as a function of cell-cell adhesion.

Cell nucleus mechanics and premature senescence

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Abstract

This project aims to propose an integrated model to better understand **a pathway to premature senescence**. Cellular senescence is a normal process defined as the progressive decline of all cellular functions ending with cell cycle arrest. When senescence is accelerated, it induces premature ageing and pathologies such as metabolic syndrome or cardiovascular diseases. It can affect parts of the organism or the whole body. In the most severe case of Progeria, where the patient ages prematurely, a pathway to senescence is related to a defect in the lamina. Lamina is a component of the nuclear envelope (NE) which ensures the mechanical stability and rigidity of the nucleus and chromatin integrity. NE and associated proteins play a major role in the cell response to various external environmental mechanical stimuli. Two hypotheses are evoked in laminopathies, genetic diseases induced by lamina mutations. The first one, the **mechanical hypothesis**, proposes that alterations of the NE protein network lead to increased nucleus susceptibility to mechanical stress (mechanotransmission). The second, the **gene expression hypothesis** implies that NE structural changes impact directly on gene transcription (mechanotransduction). In this project, we investigate the relationship between lamina mutations, alteration of cell mechanotransduction and severity of the disease, from nuclear to multi-cellular scale. We propose an **interdisciplinary approach** combining cell biology, biochemistry, and physics. Firstly, we will determine the mechanical and rheological properties of single cells. Secondly, we will explore how shear flow affects cell organisation and mechanotransduction process in a 2D-layer of lamina-deficient cells. Thirdly, we will investigate the effect of isotropic pressure on the geometry and gene expression of 3D-aggregates. From our results, we aim to develop a physical model of the senescence pathway.

Determination of turgor pressure of filamentous yeast

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The fungus *Candida albicans* is a commensal yeast that is found on mucosal surfaces of the gastrointestinal and urogenital tract in most healthy individuals. However, this organism can cause superficial as well as life-threatening systemic infections in response to alterations of its environment, and is particularly aggressive in immuno-compromised individuals. A switch from budding to filamentous growth is critical to invade host tissues [1], as well as to burst out of host macrophages.

The aim of this interdisciplinary project is to examine the biophysics of filamentous growth of this human fungal pathogen. The project uses a combination of micro-fabrication, live cell microscopy, physical measurements and modeling in order to understand how C. *albicans* adapts itself while invading.

One part of this project focuses on determining quantitative relationships between physical forces (due to turgor pressure) and cell growth. To this end, we grow *C. albicans* in microchambers composed of polydimethylsiloxane (PDMS), of different stiffnesses [2]. We visualize the invasion of *C. albicans* to obtain relations between growth speed, substrate resistance and tip shape. To better understand the material puncture threshold and the details of the rupture process (crack, friction, adhesion), we also mimic *C. albicans* invasion by indentation of a macroscopic probe into PDMS substrates [3]. By combining the live observations with the macroscopic puncture tests, we expect to quantify the internal pressure for wild type and several cell wall mutants.

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Unravelling the mechanical and physical cues involved in lacrimal gland ductal compartment formation

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While the impact of mechanical forces on single cell fate decision is well documented, their role on collective cell behavior remains largely unknown. However, cellular recognition of physical changes (mechanosensing) and the associated intracellular transmission of these signals (mechanotransduction) are crucial for epithelial tissue morphogenesis, maintenance and renewal.
As epithelial organs with secretory functions, like intestinal crypt and lacrimal gland are composed of a mosaic of different cells types interacting to produce and pour protective components in the digestive tract and the lacrimal ducts, respectively. Although their secretion's composition is relatively well described, the physical cues driving their morphogenesis and determining the cell distribution is largely under studied.
During my post-doctoral project in the Ladoux-Mège laboratory, I will investigate how mechanical constraints and mechanotransduction influence epithelial tubes and ducts formation and homeostasis. I will take advantage of 3D microfabrication techniques developed in the Ladoux-Mège lab to create environments depicting cylindrical domes, cavities and tubes. In addition, Together with my own expertise on lacrimal gland biology (Kuony and Michon, 2017), these experimental procedures will be used to investigate lacrimal gland ductal formation in a near-physiological context. The research will contribute more largely to understand how mechanical constraints and mechanotransduction influence organ development, self-renewal and maintenance.

Mechanical properties of the cytoskeleton in living cells

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The cytoskeleton allows eukaryotic cells to resist deformation, move, change shape, transport intracellular cargoes, and exchange materials with their environment. Although extensively studied in vitro (1), the mechanics of the cytoskeleton is still largely unexplored in living cells. We are using an intracellular optical tweezers-based micromanipulation (2) technique to apply forces directly on cytoskeletal filaments. We are focusing on microtubules and intermediate filaments and on how they interact mechanically.
By measuring the force-deflection curves of the filaments, *i.e.* the deformation of the filaments as a function of the applied force, we have first measured the rigidity of vimentin intermediate filaments and shown that the intermediate filament composition impacts the mechanical behavior of vimentin filaments.
We are currently investigating the effects of microtubule dynamics, microtubule stability and tubulin post-translational modifications on the mechanical properties of the microtubules network. Preliminary results suggest that increased acetylation of the microtubules tends to soften them, making them less subject to breakage.
Finally, we are characterizing the physical links between microtubules and intermediate filaments by measuring intermediate filaments mechanics in cells treated with microtubule destabilizing drugs.

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A mathematical model of the liver clock linking feeding and fasting cycles to clock function

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October 10, 2019

To maintain energy homeostasis along the diurnal cycle, the liver relies on a circadian clock synchronized to food timing. Perturbed feeding and fasting cycles have been associated with clock disruption and metabolic diseases; however, the mechanisms are unclear. To address this question, we have constructed a mathematical model of the mammalian circadian clock, incorporating the intracellular metabolic sensors SIRT1 and AMPK [1]. The clock response to various temporal patterns of AMPK activation was simulated numerically, mimicking the effects of a normal diet, fasting, and a high-fat diet. The model reproduces the dampened clock gene expression and NAD+ rhythms reported for mice on a high-fat diet [2] and predicts that this effect may be pharmacologically rescued by timed REV-ERB agonist administration. However, it is known that besides intracellular factors such as AMP or NAD+, systemic factors such as insulin, glucagon, or free fatty acids (FFA) also can reset the clock. A natural question then is the relative importance of these different driving signals in synchronizing the clock hepatic clock. To address this question, we have constructed a simple 5-gene mathematical model driven by insulin, glucagon and FFA to study how well it can reproduce the clock phase shift observed when the feeding schedule is suddenly shifted by 12 hours [4]. We intend to eventually integrate all these stimuli in the same model to obtain a comprehensive mathematical description of how feeding/fasting cycles entrain the hepatic and other peripheral clocks.

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Molecular segregation in actin networks

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Abstract :

In the cytoplasm of cells, several actin networks coexist with distinct sizes, geometries, and protein compositions. These specific properties are essential for actin networks to achieve their functions (e.g. endocytosis, migration, division, etc.). An important question which we address in this work is: How do actin binding proteins (ABPs) localize specifically to a given actin network?

To address this question, we identified three possible mechanisms that we study independently : 1/ the cooperative binding of individual ABPs to actin filaments; 2/ the competitive binding between ABPs and 3/ the role of the architecture of the actin network. Both theoretical and experimental approaches are used, with computational models and minimal reconstituted systems. During this presentation, I will mainly focus on the impact of cooperativity for the distribution of ABPs on actin filaments.

CellTiss days 2019 : quantitative approaches of living systems - November 2019

Coupling magneto-active substrates with FRET biosensors to decode mechanotransduction

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Abstract

Living cells process mechanical signals into intracellular biochemical ones that regulate essential cellular functions ; this mechanism is called mechanotransduction. We think of cells as signal processing machines that convert mechanical inputs into biochemical outputs. Our goal is to experimentally study the coupling between the two types of signals to infer the mechanotransduction "transfer function" in space and time. To apply local and dynamic mechanical constraints at the single cell scale through a continuous surface, we have developed and modelled magneto-active substrates made of magnetic micro-pillars embedded in an elastomer. Constrained and unconstrained substrates are analysed to map surface stress resulting from the magnetic actuation of the micro-pillars and the adherent cells. These substrates have a rigidity in the range of cell matrices, and the magnetic micro-pillars generate local forces in the range of cellular forces, both in traction and compression. As an application, we followed the protrusive activity of cells subjected to dynamic stimulations. The biochemical response to the spatio-temporally controlled forces exerted by our substrates is read with FRET-based biosensors. They report in live the biochemical activity of Rho-GTPases in fibroblasts given a quantitative computation of the FRET efficiency. Our goal is to compute the spatial maps of FRET efficiency over time and correlate them with the spatio-temporal maps of the mechanical stimulation or the membrane activity. Our magneto-active substrates combined with FRET-based biosensors thus represent a new tool to study mechanotransduction in single cells.

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SUBSTRATE CURVATURE MODULATES THE NUCLEAR BEHAVIOR OF EPITHELIAL TISSUES GROWN ON WAVY HYDROGELS

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In a large number of epithelial tissues, cells migrate within bidimensional folded monolayers, which topological and mechanical properties are specific of various organs. Despite accumulative evidence, the role of the topological variations in curved epithelial tissues remains misunderstood [1]. The current challenge is therefore to form *in vitro* folded epithelial tissues for understanding how assembly of epithelial cells detect and integrate topographical cues in terms of positive and negative curvatures.

To address this question, we developed a photopolymerization method for shaping hydroxy-polyacrylamide (hydroxy-PAAm) hydrogels [2, 3] with wavy topographical features ranging from 2 to 20 µm in wavelength. Using peak force AFM in liquid mode, we show that this method produces wavy hydrogels at the micrometer-scale resolution with a robust control of stiffness, wavelength and amplitude.

By growing MDCK cells on wavy hydrogels, we formed *in vitro* folded epithelial tissues with different wavelengths. We show that morphology, orientation and functional activity of the nucleus are modulated by positive or negative substrate curvatures. Interestingly, our findings show that the nuclear export of the transcriptional co-activator Yes-associated protein (YAP) is dependent on the local curvature only for dense epithelial tissues, suggesting an interplay between cell density and curvature sensing for mechanotransduction signaling. Altogether, our findings provide insights into the emerging architectures of epithelial tissues grown on folded surroundings, which are reminiscent of the *in vivo* scenario.

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Key Words: *Hydrogels, Epitelium, Curvature.*

Proneural patterning in the *Drosophila* eye disc

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The Drosophila eye, composed of regularly ordered units, or ommaditia, is an appealing model system to study tissue patterning. The cells that make up each ommatidium are recruited by a founder cell (R8 photoreceptor), which itself emerges from a cluster of cells expressing the proneural factor Atonal. The pattern develops through the periodic addition of R8 cells along a traveling differentiation front, with each column of R8s providing a template for the next. Although much is known about the underlying cellular and molecular events, a dynamic picture of proneural activity and R8 selection has been lacking. To better characterize patterning dynamics in the eye, we combine live-imaging of GFP-tagged Atonal in *ex-vivo* culture, 3D-segmentation and single cell tracking. Our observations of GFP-tagged Atonal in *ex-vivo* culture now reveal that the addition of R8 columns is accompanied by traveling waves of proneural gene expression, with individual cells undergoing oscillations in Atonal level before their fate is specified. Through a combination of modeling and perturbation experiments, we are now interrogating the mechanism by which these waves arise, and their possible role in the establishment of a regular arrangement of ommatidia.

Broadband coherent anti-Stokes Raman micro- spectroscopy for optical label-free readout of spinal cord injury; towards new therapeutic strategies in mice and non-human primates

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Spinal cord injuries (SCI) affect between 2.5 and 4 million patients worldwide (40 000 in France). Handicaps induced by SCI range from minimal sensory motor deficits to complete tetraplegia. Currently, there is no curative treatment on any symptoms associated with SCI.
A scar, called glial and composed mainly of astrocytes and microglia, inhibits axonal regeneration. Amongst other, SCI consists in a neuroinflammation and demyelination processes. These mechanisms are poorly understood. Better monitoring of animal models of SCI in particular through the development of bioimaging translational tools are therefore mandatory.
Our main objectives are to pursue and extend a multimodal imaging approach combining Broadband coherent anti-Stokes Raman Spectroscopy (BCARS), a novel technique [1] based on Raman spectroscopy with faster image aquisition[2], and multiphoton microscopy (MPM) to better understand mechanisms that underlie absence of spontaneous axonal regeneration following SCI, as well as to evaluate outcomes of therapeutic strategies that favor axonal regeneration in mice and non-human primates.
BCARS and MPM are conceived to reveal valuable information on structural changes in damaged tissue and monitor the effect of therapeutic strategies on regeneration, by co-localizing drugs within healthy/diseased tissues [3] and by imaging collagen fibers and axons with second harmonic generation, respectively.

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Broadband coherent anti-Stokes Raman micro- spectroscopy for optical label-free readout of spinal cord injury; towards new therapeutic strategies in mice and non-human primates

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Spatio-temporal control of division of labor in yeast communities

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The division of labor is the separation of a system into different parts specialized in one or multiple tasks. This concept is observed in social economics, in multicellular organism or ecosystems, and especially in microbial communities where the complementarity of different species can result in a more productive and robust consortium. Even among clonal colonies, nutrient uptake, inhibitory chemicals excretion or chemical communication considerably affect the individual microenvironment leading to cell-to-cell phenotypic differentiation.

The aim of my PhD is to get new insights in the role of space in community interactions. In particular, I am interested in the potential benefits of metabolism specialization resulting in a spatialized division of labor.

I am studying the interaction induced by Suc2 invertase secretion in the budding yeast Saccharomyces cerevisiae. This enzyme embedded in the cell wall allow yeast to grow on sucrose by breaking it into usable glucose and fructose. Thus Suc2 expressing yeast produce a public good that could be consumed by other cells.

We aim to control and study a yeast population subdivided in two parts: one slow-growing subpopulation specialized in Suc2 expression, and the other specialized in growth.

We built an optogenetic strain using CRISPR/Cas9 and MoClo technics, which produce Suc2 enzyme upon blue illumination. The yeast will be cultured in microfluidic chambers allowing the formation of monolayered colonies and gradients of shareable hexoses, and we will control quantitatively in space and time the production of the Suc2 enzyme using a Digital Micromirror Device to activate cells selectively.

All together this project will provide new insights in the spatial organization of microbial communities, toward a better understanding of microecology and more control in bioproduction processes.

Centriole self-assembly is sufficient to organize centriole amplification in multiciliated cells

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Centrosome is a highly conserved organelle that is present in almost all animal cells. It is composed of two centrioles surrounded by a protein matrix called the Pericentriolar Material (PCM). Centrosome operates different cellular functions such as cilium nucleation from its older centriole but also acts as the major microtubule organizing center (MTOC) of the cell. In order to keep the integrity of the centrosome throughout cell divisions, centrioles duplicate once per cell cycle, thanks to a tightly regulated process where one new procentriole arises on the proximal wall of each parent centriole.

Our lab focuses on an intriguing cell model, multiciliated cells (MCC), which form up to hundreds of motile cilia able to create fluid flows. MCC hijack the centriole duplication process to produce a high number of centrioles that will represent the foundation for these cilia. This process, called centriole amplification, has been described to rely on two different pathways: i) the centriolar pathway where around 10% of the procentrioles are formed on the proximal wall of the existing parent centrioles of the centrosome and ii) the deuterosomal pathway that involves MCC specific organelles, the deuterosomes, where the majority of procentrioles are thought to be formed.

The present study aims at assessing the role of the two centriole-nucleating platforms (centrosome and deuterosomes) in procentriole formation. Using a set of high resolution imaging techniques, we revealed that none of these platforms are actually needed for either correct centriole amplification (number, structure and timing) or cilia formation and function in MCC. Surprisingly, we also showed that in centrosome and deuterosome-depleted cells, procentrioles formed in a region characterized by a focused microtubule network and enriched PCM. We are now trying to understand how procentrioles self-organize their assembly without these platforms and what is the microenvironment sufficient to allow their biogenesis.

Directional transport of biomolecules through nanopores: an experimental approach of a nanoscale Brownian ratchet

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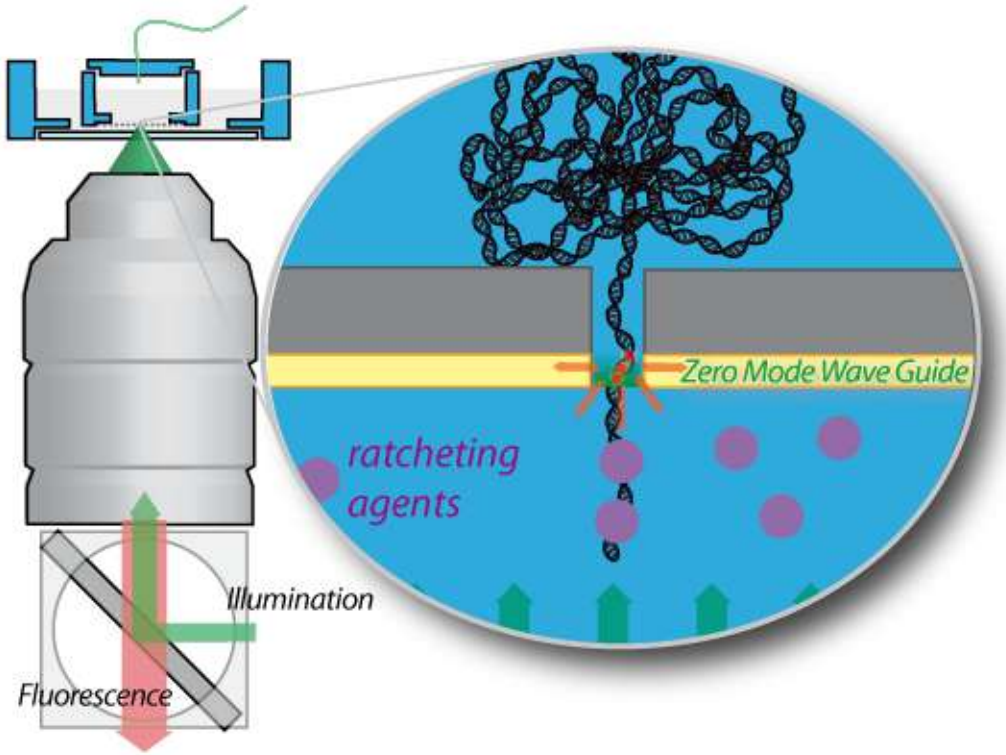
Some natural systems known as Brownian ratchets use thermal fluctuations to extract mechanical power. In these systems, diffusion is biased in order to achieve unidirectional biological processes. Good examples are the molecular motors as kinesins or myosins that walk on the cytoskeleton and use ATP to bias their movement. Another case is the transport of mRNA between the nucleus and the cytoplasm through Nuclear Pore Complexes (NPC). The NPC is a large molecular complex which selects molecules that enter or exit the nucleus. It has been shown to be highly selective and directional.

To study the directional transport of biomolecules through the NPC, a simplified mimetic device based on nanoporous membranes was designed. Our approach used near-field microscopy technique, Zero Mode Waveguide for Nanopores. With this method single DNA molecules translocation through nanopores was observed in real time. Ratchet agents which are polycations that bind strongly to the DNA and cannot diffuse through the pore were added on the exit side of the membrane. We quantified the effect of the ratchet agent on the translocation frequency of DNA molecules. An experimental measurement of the free energy gain associated with the Brownian ratchet was extracted and compared with coarse grained modeling.



Adhesive emulsions as a model system

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We develop biomimetic systems of adhesive soft tissues in order to study their elasto-plastic response under an applied mechanical perturbation. We thus use adhesive emulsions as a model system to unravel the role of adhesion in tissue mechanics in a simpler context, without active phenomena and complex protein-protein interactions.
In particular, we develop oil-in-water emulsions that can be readily functionalized with fluorescent specific binders and pushed through 2D microfluidic constrictions. Unlike solid particles, droplets can deform to flow through the constriction with an elastic response, and unlike repulsive particles, adhesive droplets resist plastic rearrangements. These emulsions are imaged through confocal microscopy, and images are systematically analyzed to track the droplets position, measure their shape, adhesion patches and identify their neighbors.
Thus, we can explore the microscopic details of the flow, and relate these different parameters through space and time, to compare the behavior of adhesive emulsions to theoretical predictions. The functionalization method will also allow us to explore a wide range of nature and binding energies, from classical biotin-streptavidin links, to tunable DNA complementary strands and cadherins.

Title: Microcirculation of Red Blood Cells in biomimetic splenic slits

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Red blood cells (RBCs), the most abundant blood cells, are responsible for distributing oxygen throughout the body. To fulfill this task, they must pass through the smallest blood capillaries that are smaller than RBC size. RBCs must therefore be highly deformable. Their deformability is probed in the spleen, where RBCs must pass a physical fitness test. They must squeeze through submicron inter-endothelial slits (IESs) to avoid being eliminated by surrounding macrophages[1]. The mechanisms of RBCs passage through IESs are still poorly understood because of the lack of experimental tools.

We have developed a microfluidic device containing slits with submicron width replicating the physiological dimensions of the IESs[2] where the RBC dynamics can be observed by video microscopy. We report the RBC transit time (Tt) as a function of relevant external parameters (slit dimensions, pressure drop), and shows an increase in Tt when decreasing slit size and/or in-slit pressure drop. We can also change RBC biological/mechanical parameters and observe for example that Tt decreases with hemoglobin viscosity or RBC volume. Conjointly, we developed numerical simulations that reproduce the observed RBC dynamics. By taking more interest in cell morphologies during this biomimetic filtration process, we show original modes of deformation with the formation of tips at the cell front. The computational approach and dimensional analysis allow us to fully understand the rheological behavior of the RBC transit through the slits.

Finally, we investigate the scenario of RBC active volume regulation under external mechanical stress by the mechanosensitive Piezo1 and the Ca^{2+} sensitive Gardos ion channels[3,4]. Using combinations of activators and inhibitors of the channels, we decipher the action mechanism of the channels on the RBC volume variation and their passage through the biomimetic slits.

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Microfluidic technique for rapid measurement of intracellular pH dynamics

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Abstract

The pH regulation of eukaryotic cells is of crucial importance and influences different mechanisms and pathologies including chemical kinetics, buffer effects, metabolic activity, membrane transport and cell shape parameters. In this study, we develop a microfluidic system to rapidly and precisely control a continuous flow of ionic chemical species to regulate the intracellular pH of NHE1-expressed fibroblast cells PS120 growing inside the device. Moreover, the microfluidic system is integrated with fluorescence microscopy to achieve the real-time measurement of intracellular pH values. We studied the effect of flow rate, cell density, rinse time on the cells pH evolution with time. Thanks to the fast and continuous flow, our system helps avoiding dead volumes and solution mixing, which results in overshooting behaviors of the intracellular pH, a phenomenon that is hardly produced in the previous classical perfusion systems. This overshooting phenomenon can be recovered in the mathematical model written by Bouret et al. [1]. Our future study aims to extend our understanding of intracellular pH in cancer cells and tumors as the pH of cancer cells are strongly acidic because of their different anaerobic metabolism from that of the normal cells.

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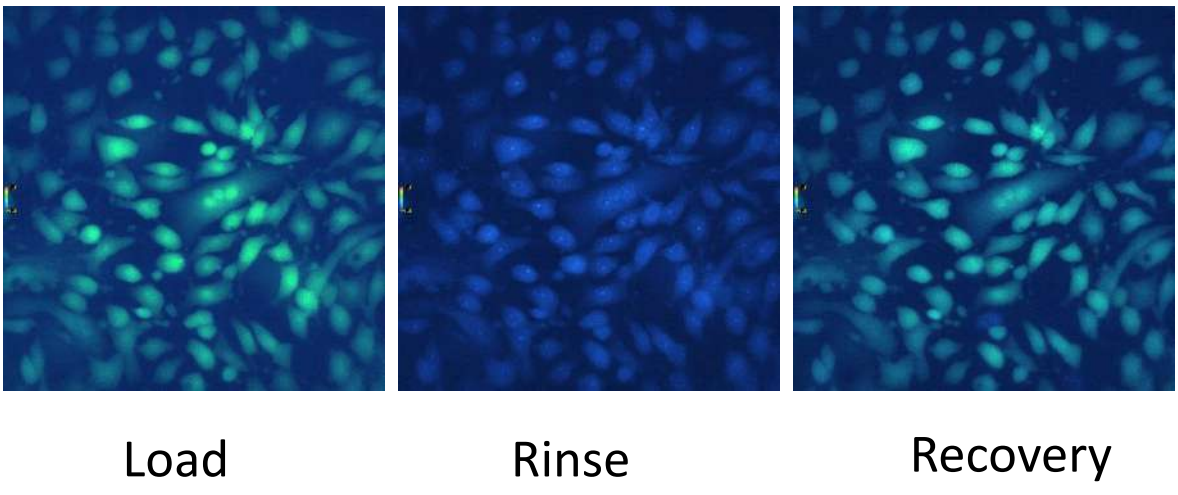

Deciphering the molecular links between actomyosin force, integrin regulation and ECM properties through an *in vitro* reconstitution

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Cells adapt to the biochemical and mechanical properties of their environment. Focal adhesions (FAs) are important mechanosensors that adapt their integrin-mediated adhesion to the internal force of the actomyosin cytoskeleton and to the physical properties of the extracellular matrix (ECM). The objective of our project is to decipher the elementary links between actomyosin force, integrin regulation and ECM properties, underlying FA mechanosensitivity. To this end, we will reconstitute a minimal adhesion complex made of an integrin-containing lipid bilayer that couples actomyosin cables, via regulatory proteins on one side, and a micropatterned ECM on the other side. Imaging the dynamics of FA components in our assay, where the concentrations, actomyosin force and the physical properties of the ECM are modulatable, will unambiguously identify mechanosensitive elementary reactions. We will present the first results of this project.

Deciphering the role of activated glial cells on neuronal connectivity using *in vitro* models of traumatic brain injury

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Traumatic brain injury (TBI) is currently one of the leading causes of global cognitive disorder and is mainly caused by falls, traffic accidents and sport contacts. During TBI, inertial forces cause shear stress in neurons and glial cells due to the deformation of brain tissues. Accumulative evidence suggests that neuroinflammation caused by mechanically activated glial cells plays an important role in the physio-pathological processes of TBI but this remains poorly understood.

To address this question, we first developed an inertia-driven model using organotypic hippocampal slice cultures (OHSC) providing near-*in vivo* cell interactions. Results showed a time-dependent diminution of synaptic connections due to the mechanical activation of microglia. Furthermore, our findings show that dendritic arborisation rapidly collapsed but progressively recovered over time alongside the microglial activation.

Then, we used cortical neuronal networks of controlled architectures [1] to decipher the role of mechanically-activated astrocytes on neuronal network connectivity. We show that the culture medium of activated astrocytes modulates significantly the neuronal expression of TNF-α receptors. Indeed, we observed a decrease of TNFR1 expression, while TNFR2 expression was increased after 5 days. Moreover, ELISA experiments showed a large increase in the concentration of TNF-α in harvested stretched astrocyte culture medium compared to control astrocyte culture. Altogether, our results decipher positive or negative modulation of neuronal connectivity by mechanically activated glial cells.

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Peptides as chalcogenide-organic linkers for biosensing

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We report on functionalization of chalcogenide thin film with biotinylated 12-mer peptides peptides SVSVGMKPSPRP and LLADTTHHRPWT exhibiting high binding affinity towards our inorganic surface [1]. The specific biotin moieties were used to bind streptavidin proteins and demonstrate efficacy of the biofunctionalizated chalcogenide substrate to capture biomolecules. Atomic Force Microscopy (AFM) and Fourier transformed infrared spectroscopy in attenuated reflectance mode (FTIR-ATR) were used to monitor the effectiveness of the functionalization process. These methods enable to gather valuable information on the undergoing adsorption/binding mechanism of biomolecules. AFM provides high resolution images of the interface, whereas FTIR-ATR provides information on the structure of the bound proteins. Following absorption of the biotinilated peptides an bovine serum albumin (used to saturate the non-specific adsorption sites), a mostly homogenous coverage is obtained [2][3] The particle analysis of AFM images reveal an increase in the diameter and decrease in the height of the features formed on the chalcogenide film, whereas FTIR-ATR indicates a loss in the alpha-helix secondary structure according when de-convoluting the amide I band region (1700-1600 cm^{-1}). The subsequent incubation of streptavidin leads to an active protein layer as suggested by the native secondary structures obtained by IR spectroscopy, suggesting the effectiveness of the biotin-streptavidin bonding. Our study demonstrates that adhesion peptides are appropriate linkers to build a favorable interface on chalcogenide materials opening hereby promising biosensing applications.

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Heterogeneity and cell clustering in *Drosophila* central-nervous system cancers

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Central-nervous system cancers, that compose one quarter of human pediatric cancers, can be studied in the *Drosophila melanogaster*. These tumors composed of two types of cancer-neural-stem cells grow exponentially and converge at long times to an heterogeneous stationary state where the two populations are present in stable proportions and segregated, with clusters of cancer-stem cells surrounded by post-transition cells [1].
The aim of this work is to understand how the cancer-stem cell clusters form, and how they contribute to the tumor progression. For this we design a simple numerical model in which we can investigate how physical (adhesion) and biochemical cues (growth and transition factors) contribute to segregate clusters. The model mimics the growth of a three-dimensional tumor where cells are represented by finite-size particles that can divide, transit state, enter quiescence and perform small displacements.
As a first step, cell behaviors are extracted from a mean-field-like model [1] where cell growth and transition factors are homogeneous in space. Already the sole spatial-neighborhood constraint on daughter cells is enough for the formation of cancer-stem cell clusters, but their sizes and shapes are very different from the experimental ones. Supplementary ingredients are thus required to the formation of clusters with experimental features, and these should take the form of spatial cues affecting cell behaviors. Among them, we consider differential adhesion, which is a well-known mechanism leading to cell clusterization, and transitions rules that depend on the neighboring-cell identities. Already, these mechanisms seem to be able to recapitulate the formation of clusters that qualitatively mimics experimental observations.

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A tensile ring drives tissue flows to shape the gastrulating amniote embryo

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Abstract:

Tissue morphogenesis is driven by local cellular deformations, themselves powered by contractile actomyosin networks. Yet how localized forces are transmitted across tissues to shape them at a mesoscopic scale is still unclear. Analyzing gastrulation in entire avian embryos, we show that it is driven by the graded contraction of a large-scale supracellular actomyosin ring at the margin between the embryonic and extraembryonic territories. The propagation of these forces is enabled by a fluid-like response of the epithelial embryonic disk, which depends on cell division. A simple model of fluid motion entrained by a tensile ring quantitatively captures the vortex-like 'polonaise' movements that accompany the formation of the primitive streak. The geometry of the early embryo thus arises from a tug of war along its boundary.

In vitro T lymphocyte adhesive haptotaxis towards lower Icam-1 density areas

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During the immune response, lymphocytes are recruited and need to travel rapidly towards the inflammation site. For this, they migrate on the blood vessel walls and transmigrate through endothelial tissues. These mechanisms involve different biochemical and physical cues in which the role of adhesion is poorly understood.

Here, we studied crawling of human T lymphocytes on surfaces on which we spatially modulated adhesion for LFA-1 ligand. Using different technics of micro-patterning and micro-fluidics, we highlight a robust adhesive haptotaxis for a surface concentration found on high endothelial venules. Counterintuitively, cells favored migration toward low concentration areas of ICAM-1, the LFA-1 ligand. This suggest that adhesive haptotaxis could promote leukocyte recruitment. We hypothesized that this phenomenon would implicate lamellipodium dynamics rather than a mechanism of mechano-transduction or tug of war as it has been shown with mesenchymal cells.

Keywords : Micro-patterning, Haptotaxis, Lymphocytes.

Collective Cell Migration in Monolayer Systems

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Collective cell migration occurs and plays critical roles in various physiological and pathological processes such as wound healing, cancer metastasis, and embryo development. Many factors, both intrinsic (e.g. cellular properties) and extrinsic (e.g. geometric constraints), have been experimentally revealed to affect collective cell migration. However, the interplay between intrinsic and extrinsic factors in the regulation of collective cell migration remains to be explored theoretically. In this talk, we present theoretical studies on migratory modes, characteristic scales, and density fluctuations during collective migration of cells in a monolayer system, using an active vertex model. It is found that the competition between two kinds of intercellular social interactions — local alignment (LA) and contact inhibition of locomotion (CIL) — drives cells to self-organize into various dynamic coherent structures with a spatial correlation scale. The interplay between this intrinsic length scale and the external geometric constraint dictates the migratory modes of collective cells in a confined space. The LA–CIL coordination can induce giant density fluctuations in a confluent cell monolayer without gaps. Further, we investigate the collective migratory modes and density fluctuations in confluent cell monolayers adhering to curved substrates, focusing on the regulating roles of curvature and topology.

Elasticity from entanglements in branched actin networks

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The biologically crucial elasticity of actin networks is usually understood as an interplay between the bending and stretching of their filaments. This point of view however fails when applied to the weakly coordinated branched actin networks found throughout the cell. Through experiments and theory, we show that their elasticity crucially involves reversible entanglements between their filaments. Additional entanglements may get locked in during network growth, setting the final properties of the network. These properties could be key to understanding how moving cells dynamically adapt their cytoskeleton to their environment.

Patterning cell cultures by self-organising biochemical landscapes.

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Throughout the different stages of morphogenesis, different biomolecular concentration patterns emerge to guide cellular fate. While the initial biomolecular cue may be inherited from an external stimulus, the subsequent patterns are self-organised, forming complex landscapes that change and evolve continuously. For this reason, devising a methodology to create highly-controllable biomolecular patterns would help to understand and control morphogenesis.

Orthogonal to 3D functionalized-printed scaffolds and synthetic biology approaches, here we present an extracellular self-organizing system capable of interacting with living cell cultures. To achieve this, we use DNA nanotechnology due to its precise control of molecular interactions. In particular, we use the PEN DNA toolbox, that uses 3 enzymes (a Polymerase, an Exonuclease and a Nicking enzyme) and single stranded DNAs (ssDNA), to engineer chemical reaction networks that are able to tightly control the concentration of specific ssDNA over space and time. Using such system, we have recently created static two-band and three-band patterns that emulate those found during early Drosophila embryogenesis.

We demonstrate that these self-patterning synthetic solutions are compatible with cells *in vitro* and can spatiotemporally control cellular fate. Hence, this new methodology allows the co-existence of identical cells with independent phenotypes without suppressing cell signalling. In the long run, this technique, that achieves pattern formation of synthetic molecules in the presence of living cells, could be advantageously used to study tissue behaviour regarding long range signalling, in addition to exploitation in biomedicine research and tissue engineering.

Cardiomyocyte structural parameters of a Duchenne muscular dystrophy murine model revealed by automated image analysis on SHG images

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Duchenne muscular dystrophy (DMD) is a severe and lethal disease characterized by progressive muscle degeneration and weakness. It is linked to mutations in the gene that codes dystrophin, a large cytoplasmic protein having a crucial role to keep muscle cells intact. Previously we have shown that the development of dilated cardiomyopathy with aging in a DMD mouse model (mdx) is associated with a dysfunction of the contractile properties of the ventricular cardiomyocytes [1]. However, the link between the dystrophin deficiency, the sarcomeric structure and the contractile dysfunction of the cardiomyocytes is still not understood.

In this work, second harmonic generation microscopy (SHG) was used to acquire images on mdx and control alive cardiomyocytes at different ages and in different experimental conditions. SHG microscopy is based on second harmonic response of organized non-centrosymmetric biomolecules making functional imaging possible on muscle cells in absence of fluorescent staining [2,3]. This technique allowed us to study the cellular cytoskeleton and contractile apparatus organization in absence of artefacts generated by histologic fixation and use of antibody. Then recorded SHG images were the subject of an automated image analysis that was capable to identify the individual myofibrils. This allowed us to extract fibril-level data about the internal structure of cardiomyocytes gathering information about the orientation, the sinuosity or the organization of its constituent individual myofibrils.

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A biophysical model for curvature-guided cell migration

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The latest experiments have shown that adherent cells can migrate according to cell-scale curvature variations via a process called curvotaxis. Despite identification of key cellular factors, a clear understanding of the mechanism is lacking. At the moment multiple theories coexist, relying on pressure gradients inside the cell cytoplasm. We employ a mechanical model featuring a detailed description of the cytoskeleton filament networks, the viscous cytosol, the cell adhesion dynamics, and the nucleus. We simulate cell adhesion and migration on sinusoidal substrates. We show that cell adhesion on three-dimensional curvatures induces a gradient of pressure inside the cell that triggers the internal motion of the nucleus. We propose that the resulting out-of-equilibrium position of the nucleus alters cell migration directionality, leading to cell motility toward concave regions of the substrate, resulting in lower potential energy states. Altogether, we propose a simple mechanism explaining how intracellular mechanics enable the cells to react to substratum curvature, induce a deterministic cell polarization, and break down cells basic persistent random walk, which correlates with latest experimental evidences.

How do cell-cell adhesions impact on the migration speed of epithelial cohorts?

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In a large number of epithelial tissues, cells migrate collectively within a compact cohesive monolayer. This characteristic is necessary to ensure the barrier role of epithelia and relies on the formation of cadherin-based intercellular adhesions. Deregulation of cadherin adhesions is largely associated with tumor progression and metastasis in a large number of cancers. However, the exact role of cadherin-based adhesions in collective migration remains unclear. By combining fish epithelial explants with 2d adhesive microstripes, we develop an original experimental platform to study the role of cadherin interactions in standardized cell cohorts. We studied the migration velocity of isolated keratocytes with no cadherin adhesions and models of cellular aggregates with cadherin interactions. Our results indicate that head-to-tail interactions do not affect cell migration velocity, whereas the establishment of lateral adhesions slows down the migration speed of epithelial cell cohorts. In addition to the role of the spatial confinement, our results highlight the importance of the spatial localization of cell-cell adhesive interactions in the modulation of the migration speed of epithelium sheets.

Key Words: *collective cell migration, confinement, adhesion*

Collective migration during a gap closure in a two-dimensional haptotactic model

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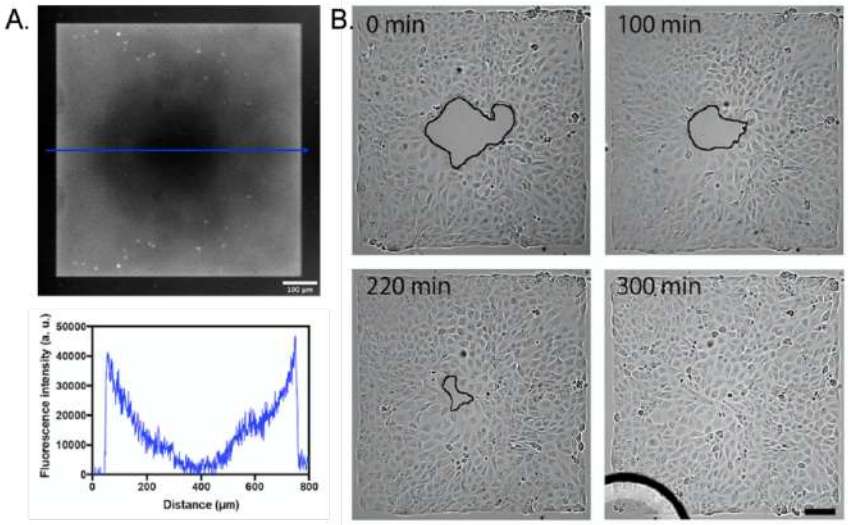
The ability of cells to respond to substrate-bound protein gradients is crucial for many physiological processes such as immune response, neurogenesis[1] and cancer cell motility[2]. However, the difficulty to produce well-controlled protein gradients limits our understanding of collective cell migration in response to haptotaxis. To address this issue, we benefit from the recent development of the PRIMO photopatterning technique[3]. We microprinted circular or linear gradients of fibronectin on two-dimensional (2D) cell culture substrates. We observed that epithelial cells spread preferentially on zones of higher fibronectin densities, creating a gap within the epithelium over circular or linear zones of lower fibronectin densities. Using time-lapse experiments, we demonstrate that gap closures in a 2D haptotaxis model require a significant increase of leader cell[4] area over the zone of lower protein density. Increasing cell spreading during closure enables to close the gap with similar dynamics than on homogeneous FN coatings but leads to regions of lower cell densities over the gaps. Interestingly, our findings show that proliferation increases rapidly after closure to restore the tissue density and strengthen cadherin junctions in matured tissues.



Epithelial tissue closure over a gradient of adhesiveness: A: Epifluorescent image of a gradient of TRITC-FN and plot profile of the fluorescence intensity according to the blue arrow. B: Time-lapse images of a gap closure in an epithelium grown on a circular gradient of FN. The edge of the closing gap was highlighted with a black line. All scale bars are 100 µm

We acknowledge the Alveole company, and particularly Pierre-Olivier Strale, Grégoire Peyret and Hélène Delobel for their help in this work.

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Orchestration of epithelial cell elimination by the effector caspases

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Cell extrusion is a sequence of remodelling steps leading to the elimination of a cell from an epithelium without impairing its sealing. The lab showed that caspases activation precedes and is required for extrusion. Thus, it suggests that extrusion in this tissue is driven by uncharacterized regulators including caspases substrates.

Therefore, we would like, to better characterize cell extrusion in the pupal notum, to study the remodelling steps affected by caspases and to identify new caspases substrates required for extrusion.

Our quantitative description of the distribution in time and space of cell shape modulators (E-cadherin and Myosin II, actin and microtubules) during cell extrusion shows that it differs from what was described during extrusion in other tissues. Interestingly, initiation of constriction was not associated with a drastic change in MyoII level. Moreover, upon caspase inhibition in clones, which block extrusion, we observed a significant increase in MyoII levels. This suggests that MyoII accumulation is not limiting extrusion. In addition, mechanically isolating cells by laser ablation show no difference in tension between these 2 cell populations.

These results suggest that caspases modulate the contractility of the cell without going through a change in MyoII level. This could be done either by changing the architecture / dynamics of the actomyosin network and facilitating the contraction or by "reducing" a factor that resists apical constriction. Supporting this second hypothesis we observed apical microtubules depletion with several markers, correlating with the initiation of constriction. Finally, modification of microtubules polymerisation was able to modulate cell size/shape arguing that microtubules depletion could be the main event triggering initiation of constriction by removing resisting forces.

Regulation of actin dynamics by vinculin and talin

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Focal adhesions (FAs) play a major role in many physiological functions. Focal adhesions mechanically couple the extracellular matrix (ECM) to the dynamic and contractile actomyosin cytoskeleton, via transmembrane receptors of the integrin family and actin-binding proteins (ABPs). Among these ABPs, talin plays major roles, including integrin activation and cytoskeleton anchoring to FAs. In response to force, talin recruits the ABP vinculin. The ability of isolated talin and vinculin to regulate actin assembly is known. However, because force is required to trigger vinculin association to talin, the activity of the talin-vinculin complex has never been investigated. Our project is to design talin and vinculin mutants that associate constitutively into a stable complex and determine its effect on actin dynamics by combining kinetic studies in fluorescence spectroscopy, binding assays and TIRF microscopy.

Biochemical and mechanical regulation of actin filament disassembly

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The actin cytoskeleton assembles into very dynamic structures that generate various forces. In this active processes, filament disassembly must be tightly regulated, either to maintain active units, or to discard excess filaments and replenish the pool of monomeric (G) actin. This regulation takes many forms: numerous binding proteins, the chemical environment, post-translational modifications, mechanical constraints, etc.

At the centre of all actin filaments disassembly machineries is the family of proteins ADF/cofilin. ADF/cofilin binds along filaments into domains, induces severing and regulate depolymerisation from filament ends.

To understand the action and regulation of ADF/cofilin, we performed *in vitro* experiments, on single actin filaments, inside a microfluidic chambers. This method allows us to perfectly control biochemical conditions, and to apply physiological mechanical constraints to filaments: tension, curvature and torsion.

Paradoxically, on its own, ADF/cofilin is not very efficient at disassembling filaments: it binds slowly, severs rarely and does not accelerate depolymerization significantly. We identified several biochemical and mechanical conditions which could explain ADF/cofilin-mediated rapid disassembly in physiological conditions. For example:

- ADF/cofilin and Cyclase Associated Protein (CAP) cooperation accelerates 100-fold depolymerization from the pointed-end.
- Actin filaments oxidized by the enzyme MICAL are extremely susceptible to ADF/cofilin.
- ADF/cofilin generates a torque on twist-constrained filaments to boost severing.

These findings highlights the many facets of actin regulation.