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AQV Days 2024

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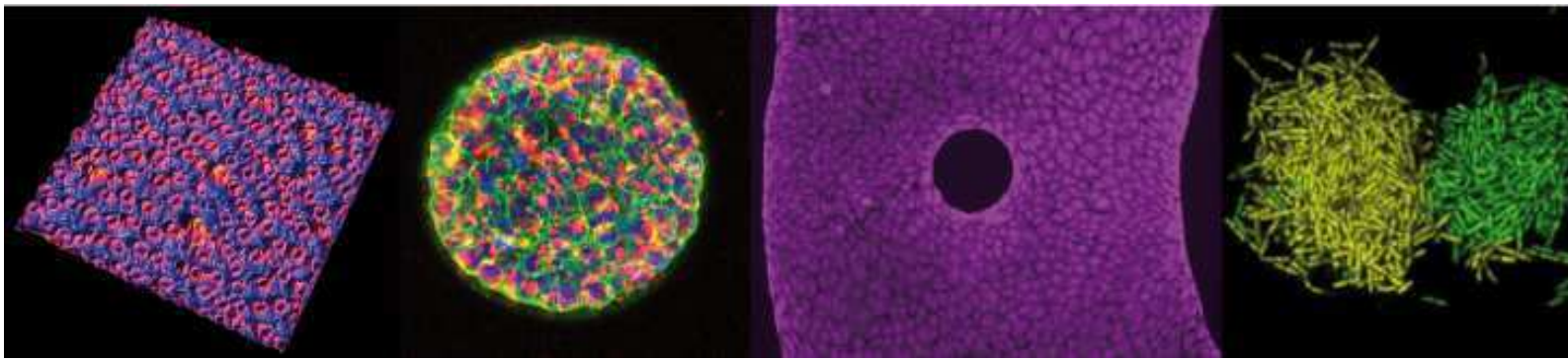

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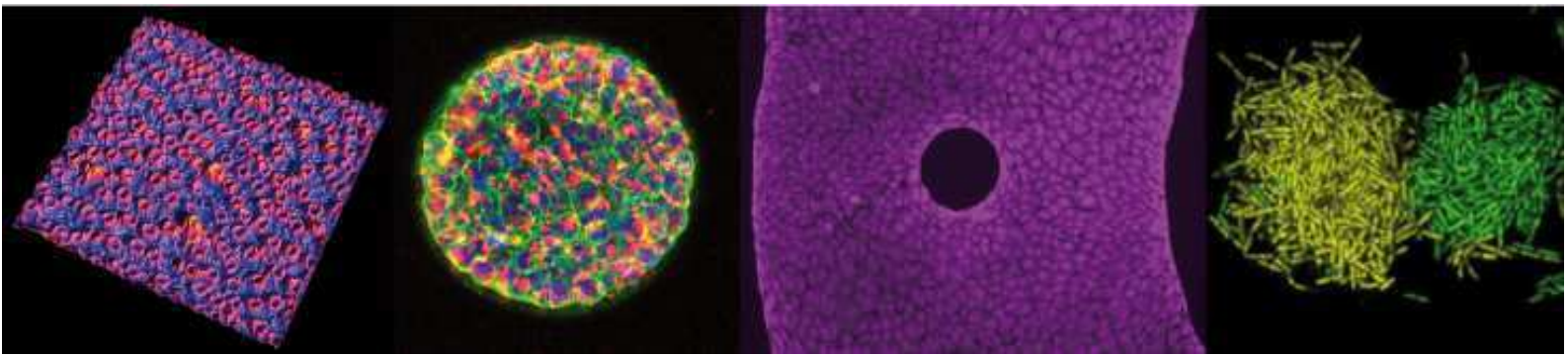
Oral Communications



Bacterial Superheroes: An Introduction to Mechano-Micro-Biology

Nicolas Biais
Laboratoire Jean Perrin UMR 8237, Sorbonne Université - CNRS

We all have, in all likelihood, more bacterial cells in us and on us than our own cells. While we have studied bacteria in the lab mostly in extremely rich environments where they swim freely, we now understand that most bacteria are part of highly dense, physically interconnected aggregates with limited resources at their disposal. Many bacteria bear appendages called Type IV pili. These long retractable polymers enable bacteria to exert forces on their surroundings and between each other. We will show how the characterization of the forces exerted by these ubiquitous bacterial nanonomachines and their dynamics, across evolution, provides an insightful framework to study the impact of mechanical cues on bacterial physiology.

Influence of mechanical constraints on tropomyosin-actin interaction

C. Bagès[1]*, R. Pagès[1], G. Romet-Lemonne[1], A. Jégou[1], H. Wioland[1]

[1] CNRS - Institut Jacques Monod, Paris, France
*camille.bages@ijm.fr

In cells, actin filaments assemble in multiple networks, including lamellipodia, filopodia and stress fibres, composed of various actin binding proteins (ABP) and which undergo different mechanical constraints. The dozens of tropomyosin isoforms are key components of these networks as they cover most actin filaments and regulate the activity of the other ABP in an isoform specific manner. For example, Tpm3.1 localizes in stress fibres and promotes myosin II activity.

One fundamental question is to understand how tropomyosin isoforms selectively localise into actin networks. The hypothesis of this project is that the mechanical context of each actin network regulates tropomyosin recruitment.

To carry out this study, I perform in vitro single filament experiments, using a novel tropomyosin design, with wild type N-terminal acetylation and fluorescence labelling. To apply controlled mechanical constraints, I use microfluidics: the drag force of the fluid flow generates a tension over tens of single actin filaments simultaneously. I also designed experiments combining microfluidics with either optical or magnetic tweezers to apply precise tension or torsion. I quantify several tropomyosin assembly parameters - nucleation rate, elongation rate and saturation rate - as well as the competition between tropomyosin isoforms. These experiments allow me to assess whether forces tune the affinity of specific tropomyosin isoforms. Overall, this study will shed light on the mechano-regulation of actin binding proteins activity.

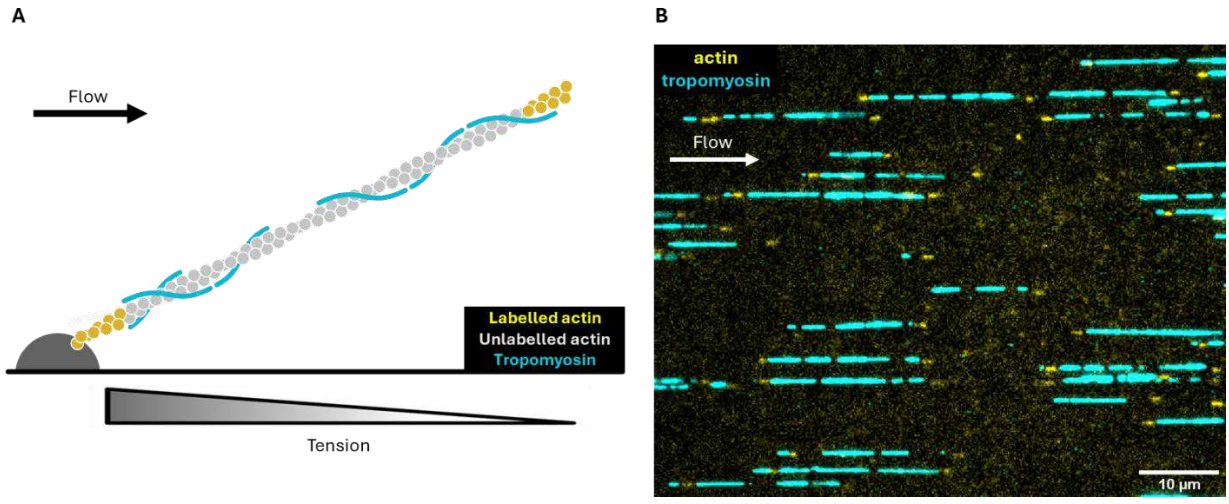


Figure 1: Typical microfluidic experiment where tropomyosin domains (cyan) bind to long segments of unlabelled actin (grey in the sketch) surrounded by short, labelled actin segments at the two extremities (yellow). The drag force of the fluid flow generates a tension gradient alongside filaments.

(A) Sketch of an actin filament inside a microfluidic chamber.

(B) TIRF microscopy image of a typical experiment.

DistNet2D: Leveraging long-range temporal information for efficient segmentation and tracking

Maxime Deforet[1]*

[1]Laboratoire Jean Perrin, Sorbonne Université, Paris, France
*maxime.deforet@sorbonne-universite.fr

Extracting long tracks and lineages from video-microscopy requires an extremely low error rate, which is challenging on complex datasets of dense or deforming cells. Leveraging temporal context is key to overcoming this challenge. We propose DistNet2D [1], a new deep neural network (DNN) architecture for 2D cell segmentation and tracking that leverages both mid- and long-term temporal information. DistNet2D considers seven frames at the input and uses a post-processing procedure that exploits information from the entire video to correct segmentation errors. DistNet2D outperforms two recent methods on two experimental datasets, one containing densely packed bacterial cells and the other containing eukaryotic cells. It is integrated into an ImageJ-based graphical user interface for 2D data visualization, curation, and training. Finally, we demonstrate the performance of DistNet2D on correlating the size and shape of cells with their transport properties over large statistics, for both bacterial and eukaryotic cells.

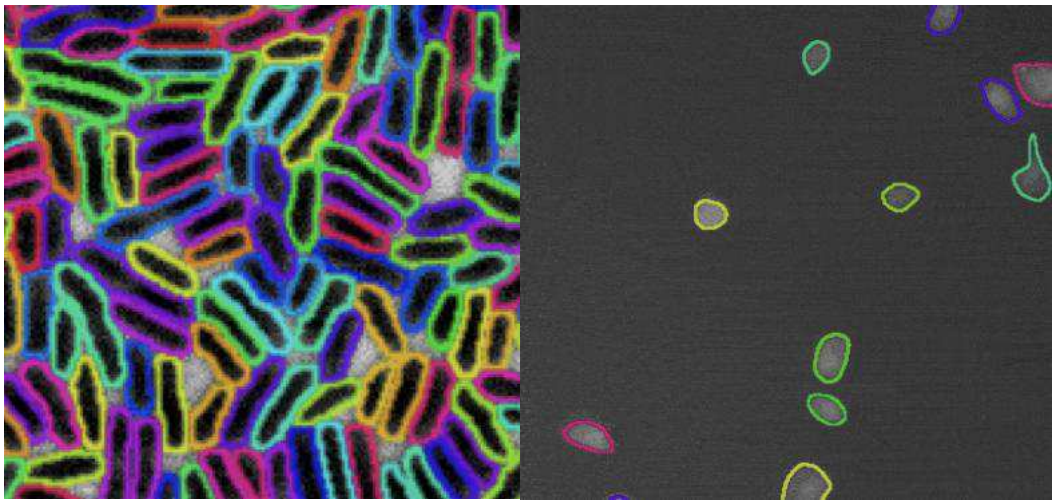


Figure 1: Left: Dense monolayer of rod-shape bacteria. Right: Migratory adherent eukaryotic cells. The colored outlines depict the results of segmentation and tracking from DistNet2D.

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Mechanical characterization of oocytes using AFM to evaluate their developmental potential

Rose Bulteau[1,2], Lucie Barbier[2], Guillaume Lamour[1] ,Tristan Piolot[2], Martin Lenz[3], Elsa Labrune[4], Marie-Emilie Terret[2], Clément Campillo[1]

[1]Université d'Evry Val d'Essonne, LAMBE, Boulevard F Mitterrand, Evry 91025, France, [2]CIRB, Collège de France, Paris F-75005, France, [3]Laboratoire de Physique Théorique et de Modèles Statistiques, 2ème étage, Bât100, Campus d'Orsay, [4]Hospices Civils de Lyon, Service de Médecine de la Reproduction, 59 Bd. Pinel, 69500 Bron, France
clement.campillo@univ-evry.fr

Oocyte production is essential for sexual reproduction. However, this process is error-prone and generates a basal rate of poor-quality oocytes, having deleterious consequences for fertility and offspring development. Aberrant oocyte stiffness, a common defect in mouse and human oocytes, has been shown to alter oocyte quality. Thus, mechanical properties could be used to predict oocyte developmental potential in assisted reproductive technologies. The mechanical properties of these large non-adherent cells have been measured using the micropipette aspiration (MP) technique. However, it only provides access to one mechanical parameter, cortical tension. Thus, we used AFM (Atomic Force Microscopy) as it gives access to various mechanical parameters. We designed a protocol and analysis pipeline, including an elasto-capillary description of oocyte mechanics, to measure the evolution of its mechanical properties during morphogenesis with AFM. We validated this approach with mouse oocytes engineered to be extra-soft and stiff. We show that cortical tension decreases concomitant to oocyte morphogenesis, confirming our previous measurements by MP. Our AFM approach allows measuring the evolution of other mechanical parameters: oocyte elasticity, dissipated viscous energy, and capillary indentation rate, quantifying whether the oocyte behaves predominantly as a droplet with surface tension or as an elastic material. We show how these parameters correlate to specific cortex organization. Finally, we transfer our approach to human oocytes and show that the mechanical parameters of human and mouse oocytes are in the same range. Still, in humans, only cortical tension decreases, and at a later stage of morphogenesis than in mice, suggesting a different cortex organization. Next, we will test whether mechanical measurement of human oocytes could provide information on their quality.

Stiffening cells with light

Eva Gonzalez[1], Julien Husson[1*]

[1] Laboratoire d'Hydrodynamique (LadHyX), CNRS, Ecole polytechnique, Institut Polytechnique de Paris, Palaiseau, France
[*]julien.husson@polytechnique.edu

Fluorescence imaging is at the core of cell biology. However, excessive illumination can result in the production of reactive oxy-gen species (ROS), leading to phototoxicity. ROS are known to destabilize cytoskeleton fibers in vitro, so that the generation of intracellular ROS could soften cells. We addressed this question using profile microindentation [1] and showed that the outcome was the exact opposite: fluorophore-loaded cells stiffen dramatically upon fluorophore excitation. This is consistent with an alternative mechanism, in which light-induced ROS induce crosslinking of their surrounding proteins and DNA. We used profile microindentation to quantify cell stiffness and demonstrated that upon loading with various fluorescent probes and 10-s exposure to light, several types of cells including T lymphocytes, endothelial cells, and neutrophil-like PLB cells became up to ten times stiffer (Fig. 1). To demonstrate a dose effect, we showed that consecutive shorter flashes stiffen the cells at a rate that increases with lamp intensity. In addition to cells becoming stiffer, their function was impaired: T lymphocytes loaded with the calcium probe Fluo-4 cease activation within seconds of probe excitation. Cell stiffening correlates with increased fluorescence of a ROS sensor, supporting the hypothesis that light-induced intracellular ROS mediate stiffening. Cells treated with cytoskeletal inhibitors still stiffened massively, consistent with ROS affecting more molecules than cytoskeletal structures. Our results show that cell stiffening is a new indicator of phototoxicity and opens avenues for exploring how cells cope with ROS, especially leukocytes such as neutrophils, which are accustomed to managing high ROS levels.

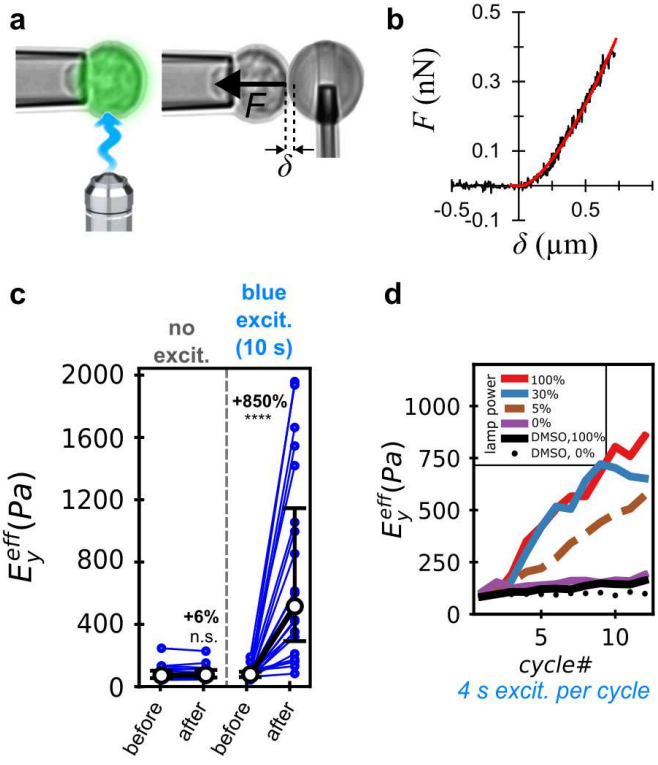

Figure 1: Stiffening of fluorophore-loaded cells upon excitation. **a**, setup. After exposing a fluorophore-loaded cell to light, the cell is compressed by a force F exerted by a spherical indenter, which leads to an indentation δ of the cell. **b**, force–indentation curve, whose slope is proportional to cell stiffness quantified by an effective Young's modulus E_y^{eff}. **c**, Fluorescent T lymphocytes stiffen massively after a 10-s exposure to blue light. **d**, T cells loaded with the Fluo-4 calcium probe exposed to successive 4-s flashes of blue light stiffen continuously. Cell stiffening increases with the power of the light source. In control experiments (DMSO), cells were not loaded with the fluorophore and exposed or not to light. Curves represent average values over three donors and at least 15 cells for each condition.

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Interplay between flux and affinity within biomimetic nuclear pores

S. Brugère[1]*, P. Guegan[2], C. Raillon[3], S. Mailfert[4], D. Marguet[4], F. Montel[1]

[1]Laboratoire de Physique ENS de Lyon-CNRS, Lyon, France
[2]Institut Parisien de Chimie Moléculaire Sorbonne Université-CNRS, Paris, France
[3]Laboratoire Physiologie Cellulaire & Végétale CEA Grenoble, Grenoble, France
[4]Centre d'Immunologie de Marseille Luminy Inserm-CNRS, Marseille, France
*saskia.brugere@ens-lyon.fr

The Nuclear Pore Complex (NPC) controls the exchanges between the cell nucleus and the cytoplasm. Its remarkable selectivity is due to the presence of a network of intrinsically unstructured proteins (FG-nups) inside its central channel [1, 2]. While thousands of molecules evolve in the cytoplasm, solely few proteins with specific features can cross the NPC. Recent theoretical studies predict a non-trivial relation between the affinity for the pore and the flux of molecule [3]. In cellulo approaches are limited to tackle these subjects because of the complexity of the natural system. Our project aims to study these phenomena thanks to a biomimetic system. To this end, we use synthetic nanoporous membranes grafted with hydrophobic tunable polymers mimicking the FG-nups networks [4]. Flux of molecules are determined by an optical detection which allows us to measure the effective diffusion constant of proteins through the pores. Affinities of the different proteins for grafted and ungrafted surfaces are determined by Biolayer Interferometry. We show that selectivity depends mainly on the affinity of the proteins for the pore surface and not on the size of the molecules. We develop a simple model to describe our results and highlight the facilitated diffusion observed when proteins interact with the nanopores.

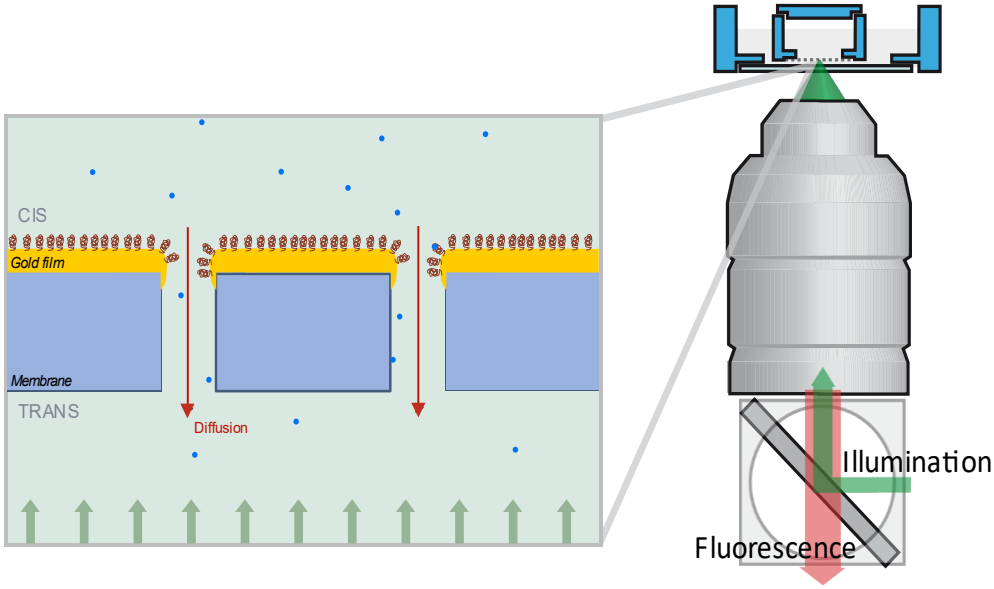

Figure 1: Experimental setup for translocation of fluorescent biomolecules through a synthetic nanoporous membrane

References

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Dynamics of tube feet adhesion and mass-driven adaptation during sea star locomotion

A. Deridoux[1,2*], P. Flammang[2] and S. Gabriele[1]

[1] Mechanobiology & Biomaterials Lab, University of Mons, CIRMAP, Research Institute for Biosciences, Mons, Belgium
[2] Biology of Marine Organisms and Biomimetics Unit, Research Institute for Biosciences, University of Mons, Mons, Belgium
[*]amandine.deridoux@umons.ac.be

Although they have no proper brain and are not very mobile, sea stars have the remarkable ability to move around actively, whether hunting or climbing rocks. These movements are enabled by numerous tubular adhesive organs, referred to as tube feet or podia, which cover their oral surface and are connected to a water vascular system. The intricate stepping motion of sea stars is achieved through the extension, flexion, and retraction of these tube feet. Despite extensive studies on the internal morphology and ultrastructure of sea star tube feet, their dynamics and the precise locomotion mechanism remain incompletely understood.

To address this challenge, we developed an optical method based on frustrated total internal reflection (TIRF) to dynamically visualize and quantify the adhesion of tube feet to the substrate in real-time. Our investigation, conducted on individuals from the species *Asterias rubens* spanning a range of sizes, revealed that crawling speed is not directly correlated with the sticking contact area — a metric linearly proportional to mass. Instead, our findings showed an inverse relationship between crawling speed and tube foot adhesion time, a factor influenced by mass. These results suggest a mechanical adaptation of crawling speed to mass through the modulation of tube foot adhesion time. We found that less than 10% of tube feet adhere to the surface during sea star locomotion. Surprisingly, high magnification experiments revealed that the same tube feet are reused, with a recovery stroke phase occurring approximately every 20 seconds during locomotion.

To validate these findings, some individuals were equipped with a 3D-printed harness loaded with a weight equivalent to 25% or 50% of their initial mass. The artificial increase in mass resulted in a significant increase in tube foot adhesion time, confirming the existence of an adaptation mechanism wherein crawling speed adjusts to mass through the modulation of tube foot adhesion time. Further insights were gained by studying how tube feet dynamics in *A. rubens* adapt to inverted locomotion, when sea stars walk upside down. Finally, we measured forces exerted by tube feet during locomotion by using a traction force microscopy (TFM) based set-up (Figure 1). In this set-up, when sea stars are allowed to crawl, the tube feet movements cause beads displacements within the soft elastomer surface which are then recorded with a camera placed under the aquarium.

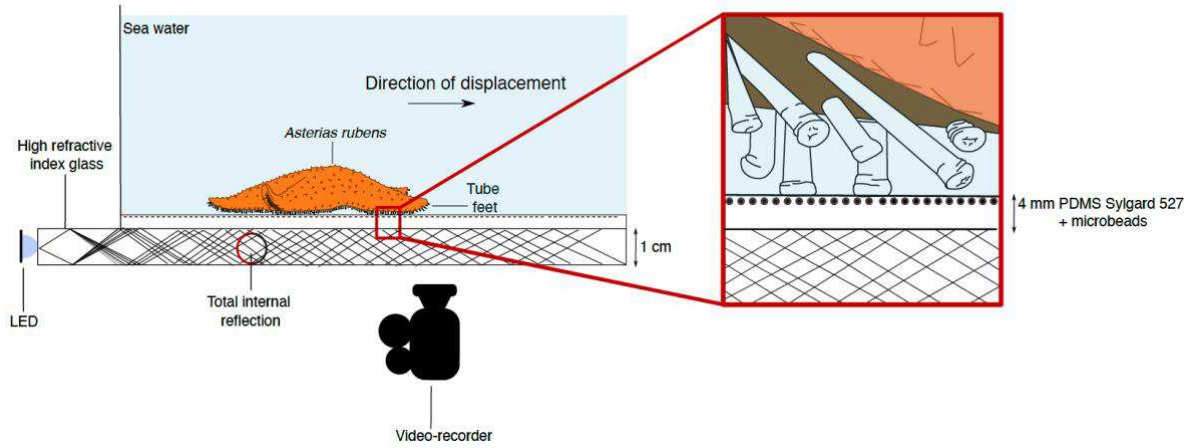

Figure 1: Experimental recording of the interaction between tube feet and the surface using a frustrated total internal reflection (TIRF) and traction force microscopy (TFM).

Combining strength and dynamics in supramolecular DNA hydrogels.

R. Merindol[1*], P. Le Bourdonnec[1], C. Ferkous[1], L. Cipelletti[1]

[1]Laboratoire Charles Coulomb, Université de Montpellier, CNRS, Montpellier, France.
*remi.merindol@umontpellier.fr

Biological tissue combine strength, reorganisation and self-healing properties. In synthetic hydrogels the implementation of reorganization comes at the cost of strength and structural stability. Vitrimers are a new class of plastic materials which overcome a similar antagonism between stable thermoset and processable thermoplastics.[1] The key mechanism at the origin of their exceptional mechanical properties lies in an associative exchange mechanism, where new bonds form before the former breaks. Yet existing chemistries driving this mechanism are water sensitive which impedes the development of hydrogel vitrimers. Here, we demonstrate the transfer of this concept into supramolecular hydrogels using DNA.

In solution DNA strands with complementary domains can form supramolecular hydrogels hold by duplexes. We use enzymatic synthesis to produce large amounts (mg) of sequence controlled DNA and prepare macroscale hydrogels.[2,3] We encoded into these hydrogels a strand displacement mechanism allowing duplexes to swap without melting, *i.e.* a supramolecular associative exchange mechanism. Such vitrimer inspired DNA hydrogels, can reorganize and meld far below their melting temperature (Figure 1). This presentation describes the mechanical behaviour of static and dynamic DNA hydrogels, discuss quantitatively the molecular mechanism controlling their reorganization and present key advantages of the proposed associative exchange mechanism to control reorganization independently from the strength and elastic modulus in supramolecular hydrogels.

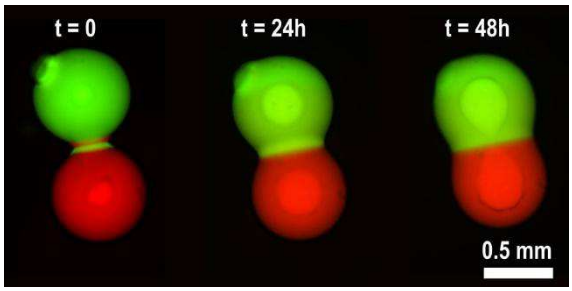

Figure 1: Melding of dynamic DNA hydrogels. Widefield fluorescence images of two DNA hydrogels covalently labelled with Atto488 (Green, top) and Atto565 (Red, bottom) fluorophores, after 0, 24 and 48h at 30 °C. The hydrogels are immersed in mineral oil to force contact and prevent evaporation.

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Unraveling the dynamic evolution of tissue rheology during Gastruloid self-organization

D. El Arawi[1], A. Rostan[1], PF. Lenne[1], PH. Puech[2] & S. Tlili[1]

[1]IBDM: Aix Marseille Univ, CNRS, Turing Centre for Living systems, Marseille, France
[2]LAI: Aix Marseille Univ, INSERM, CNRS, Turing Centre for Living systems, Marseille, France
*dalia.el-arawi@univ-amu.fr

Mouse embryonic stem cells can self-organize into 3D embryonic organoids, known as Gastruloids, sharing similarities with their *in vivo* counterparts. Recent studies have shown that, within a few days, gastruloids undergo significant morphological changes, developing structures that closely resemble organs both genetically and morphologically, such as the development of neural tube-like structures. In our group, we have recently investigated gastruloid culture protocols designed to guide their differentiation into tissues containing both anterior mesoderm (cardiac and vascular mesoderm) and endoderm (gut structures). The critical early morphogenesis steps for gastruloids occur between 48 and 96 hours post-aggregation. Specifically, at 48 hours, they transition from spherical and rugous pluripotent stem cell aggregates to smooth, spherical cell aggregates composed of a mix of endoderm and emerging mesoderm by 72 hours. Afterwards, between 72 and 96 hours, gastruloids undergo a phase known as "symmetry breaking", gradually elongating along a primary axis, with a gene expression gradient colinear to the axis of elongation [1]. This differentiation process involves an epithelial-to-mesenchymal transition and collective cell movements, driven by surface tension gradients among cell populations [2]. However, the dynamic evolution of these processes over space and time, as well as their impact on tissue viscosity, remains unknown. To address this, we use micropipette aspiration on gastruloids to characterize their viscoelastic properties at different developmental stages. The analysis of resulting creep curves allows us to derive mechanical parameters, such as viscoelastic properties, revealing that the tissue becomes more liquid-like after 48 hours. Moreover, following symmetry breaking, we quantify spatial differences in tissue viscosity associated with the gene expression gradient. Finally, combining this method with a microfluidic device to observe cell rearrangements and movements, provides valuable insights into gastruloid behavior and responsiveness to mechanical cues.

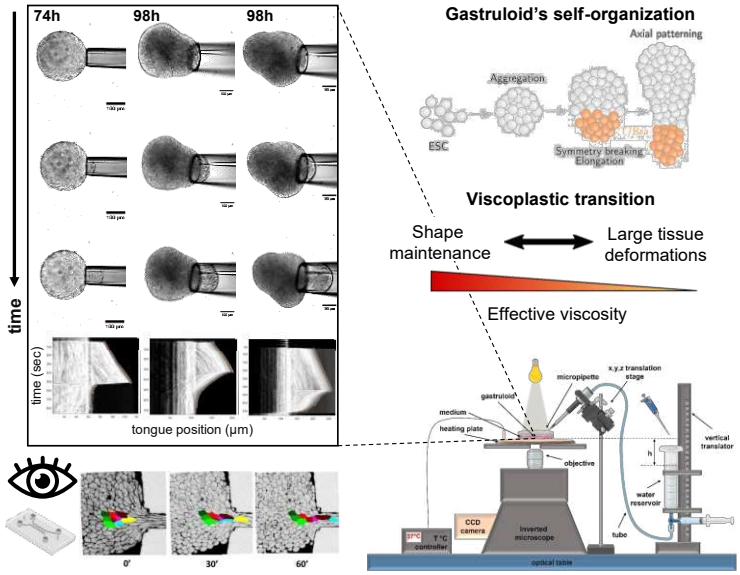

Figure 1: Different approaches to understand gastruloids local rheological properties

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How to create form and dynamics in a living embryo

E. W. Gehrels[1,2,*], **B. Chakrabortty**[3], **M. Merkel**[2,4], **T. Lecuit**[2,5,6]

[1]Aix Marseille Université & CNRS, CINaM - UMR7325, Marseille, France
[2]Turing Centre for Living Systems, Marseille, France
[3]IISER Thiruvananthapuram, Kerala, India
[4]Aix Marseille Université & CNRS, Centre de Physique Theorique - UMR7332, Marseille, France
[5]Aix Marseille Université & CNRS, IBDM - UMR7288, Marseille, France
[6]Collège de France, 11 Place Marcelin Berthelot, Paris, France
*email: emily.gehrels@univ-amu.fr

Biological organisms are able to develop from simple eggs to complex adult forms via a series of dynamic rearrangements, during which embryonic tissues generate stress, deform, and flow. What can we learn about the rules and possibilities of autonomous structure formation by studying their development? I will present one such study where we uncover how *Drosophila* embryos use symmetric forces to create a polarized flow of cells needed to achieve their final form.

Cell flows in the early *Drosophila* embryo are driven by an interplay between biological signaling and tissue mechanics. Using live imaging, we observe how changes in the presence of force-generating proteins and the geometry of the tissue relate to tissue dynamics at the onset of morphogenesis. We use theoretical and computational methods to model the behavior of the tissue and experimentally challenge our findings using select genetic perturbations of the embryos. With this combination of experimental and modeling approaches, we have uncovered how organized multicellular dynamics emerge from genetic, mechanical, and geometric "information" during early *Drosophila* development.

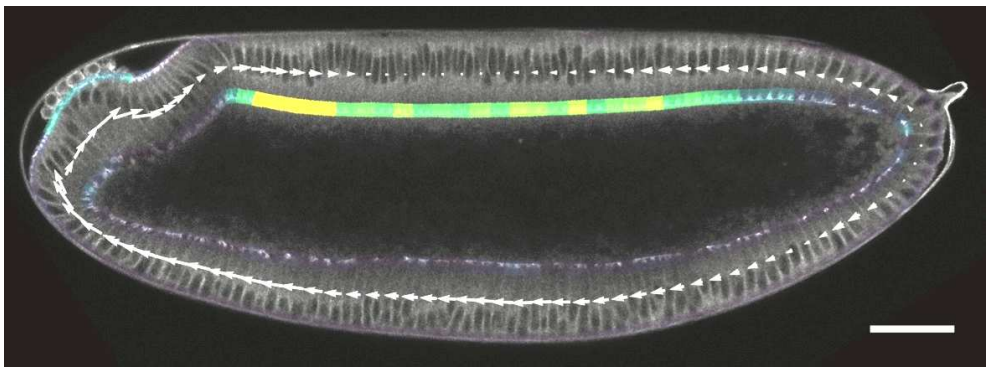

Figure 1: Two-photon micrograph of an early *Drosophila* embryo expressing fluorescently labeled myosin. Myosin levels on the apical (outer) and basal (inner) cell surfaces have been quantified and are denoted by colors ranging from purple for low levels to yellow for high levels. Polarized tissue flow, quantified using particle image velocimetry, is shown by white arrows. Scale bar is 50 μm.

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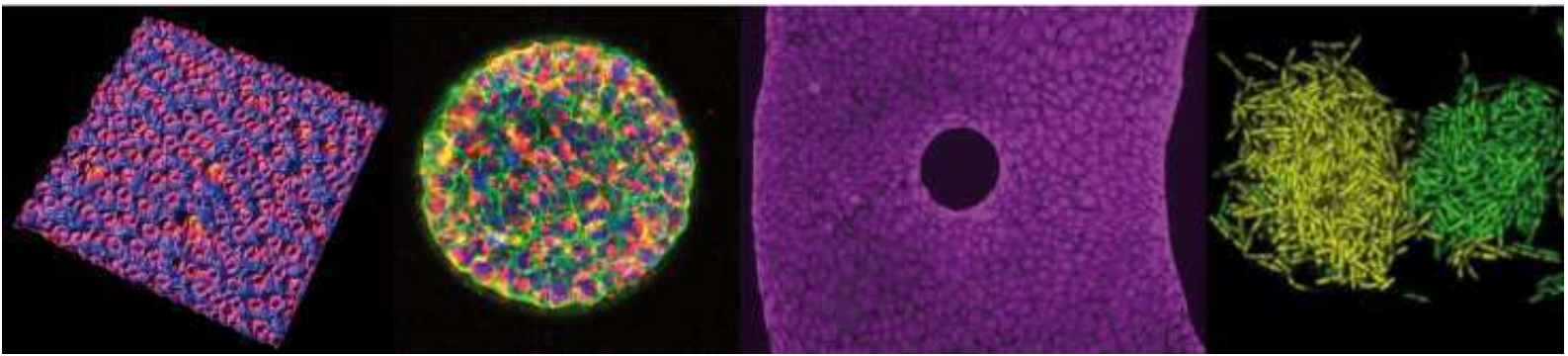
Poster Communications



Cooperative dynamics of PARP1 zinc-finger domains in the detection of DNA single-strand breaks

Parvathy.A.P.Sarma[1]*, Fabrizio Cleri[1], Corrine Abbadie[2]

[1]Institut d'Electronique Microelectronique et Nanotechnologie (IEMN CNRS UMR8520) and Department de Physique, Université de Lille, 59652 Villeneuve d'Ascq, France
[2]Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, UMR9020-U1277 - CANTHER - Cancer Heterogeneity, Plasticity and Resistance to Therapies, F59000 Lille, France
Email: *parvathy.ananthapadmanabhasarma.etu@univ-lille.fr

Abstract

The Single-Strand Break Repair (SSBR) pathway is initiated by the identification of single-strand break (SSB) by PARP1 (poly(ADP)ribose polymerase-1) protein, utilizing its zinc finger domains (Zn1 & Zn2) [1, 2]. Despite extensive research on Zn1-2 in the detection of SSBs, the dynamics of the entire process are still unclear. To investigate, we conducted all-atom molecular dynamics (MD) simulations involving SSB and Zn1-2 domains of PARP1 (Figure 1). We performed simulations on various single-strand break configurations with different 3' and 5' terminations, and explored PARP1's interaction with the break. Additionally, we studied the action of PARP1 as a dimer which highlighted the cooperative dynamics of Zn2 & Zn1. Our findings largely mirror the quick Zn2 interaction observed in experiments, followed by Zn1 attempting to interact with the break. From our MD simulations, we deduce that DNA can spontaneously open due to thermal fluctuations, exposing the break and allowing Zn2 to interact with the exposed nucleotides, which are extremely rare in free long DNA. The bending of DNA in specific configurations results from the phosphate backbone grip of Zn1-2, enables hydrogen bonds formations with the major/minor groove. Although Zn1's role is secondary, its orientation with terminal nucleotides and relative orientation with Zn2 are crucial. This study elucidates the dynamics of the PARP1-mediated single-strand break repair pathway, unveiling the cooperative actions of Zn1 and Zn2.

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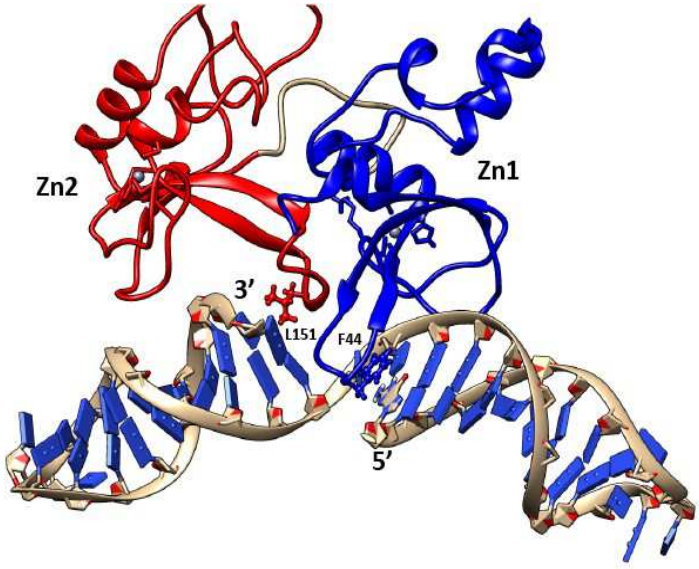

Figure 1: Zn1 & Zn2 of PARP1 interacting with the Single strand break – obtained after 200ns simulation

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Revisiting Ameoboid Cell Motility: Swimming mediated by membrane treadmilling

Dalia Arawi[1,3], Ahmad Awada[1,3*], Laurene Aoun[1], Nicolas Garcia-Seyda[1], Sham Tlili[3,4], Alexandre Farutin[2], Chaouqi Misbah[2], Marie-Pierre Valignat[1], Olivier Theodoly[1,3]

[1] Laboratoire Adhesion et Inflammation, Marseille, France
[2] LIPhy, Grenoble, France
[3]Aix Marseille Universté, Marseille, France
[4]IBDM, Marseille, France
[*]ahmad.awada@univ-amu.fr

Mammalian cells developed two main migration modes. The slow mesenchymatous mode, like crawling of fibroblasts, relies on maturation of adhesion complexes and actin fiber traction, whereas the fast amoeboid mode, observed exclusively for leukocytes and cancer cells, is characterized by weak adhesion, highly dynamic cell shapes, and ubiquitous motility on two-dimensional and in three-dimensional solid matrix. In both cases, interactions with the substrate by adhesion or friction are widely accepted as a prerequisite for mammalian cell motility [1], which precludes swimming. We show here experimental and computational evidence that leukocytes do swim, and that efficient propulsion is not fueled by waves of cell deformation but by a rearward and inhomogeneous treadmilling of the constituents of the cell's membrane. Our model consists of a molecular paddling by transmembrane proteins linked to and advected by the actin cortex [2], whereas freely diffusing transmembrane proteins hinder swimming. Furthermore, continuous paddling is enabled by a combination of external treadmilling and selective recycling by internal vesicular transport of cortex-bound transmembrane proteins. This mechanism explains observations that swimming is five times slower than the retrograde flow of cortex and also that lymphocytes are motile in nonadherent confined environments. Resultantly, the ubiquitous ability of mammalian amoeboid cells to migrate in two dimensions or three dimensions and with or without adhesion can be explained for lymphocytes by a single machinery of heterogeneous membrane treadmilling.

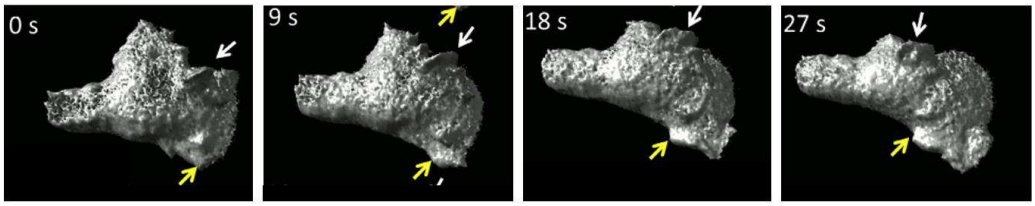

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Figure 1: Protrusion paddling alone seems not efficient enough to propel swimming. (A) Image sequence of swimming cell with micron-scale protrusions traveling along cell body. soSPIM images of a cell transfected with RFP-Lifeact reveals the shape and motion of waves of actin protrusion in three dimensions. White and yellow arrows point to particular protrusions. Protrusions (white and yellow arrows) travel backward in the frame of the cell and of the lab. Scale bars, 10 mm.

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Metastability of Uniform-Density Flocks

M. BESSE[1]*, H. CHATE[2,3], A. SOLON[3]

[1]Laboratoire Jean Perrin, CNRS-Sorbonne, Paris, France
[2]Service de Physique de l'Etat Condensé, CEA, Saclay, France
[3]Laboratoire de Physique Théorique de la Matière Condensée, CNRS-Sorbonne, Paris, France
*marc.besse@sorbonne-universite.fr

We numerically study two-dimensional uniform density flocks. More precisely we investigate the Toner-Tu field theory where the density field is maintained constant, a limit case of "Malthusian" flocks.

Surprisingly we show that such uniform-density flocks are metastable to the nucleation of a specific defect configuration, as shown in Figure 1. The ordered phase is thus replaced by a globally disordered phase consisting of a foam of asters, namely asters surrounded by shock lines that constantly evolve and remodel themselves.

We further demonstrate that this foam is endowed with a slow-fast dynamics: a fast dynamics for the fluctuations of the shock lines and a slow dynamics for the centers of the asters (in fact for the associated topological charges). This renders this nonequilibrium foam fundamentally different from the corresponding equilibrium system.

Interestingly, a simple nonreciprocal spin model is shown [1] to display the same nonequilibrium foam state that is found for uniform-density flocks, as it was suggested in [2]. These results could be of relevance in some real systems in spite of the simplicity of the framework we considered. Prime candidates are found in cytoskeletal active matter, i.e. in vitro mixtures of (mostly) biofilaments and molecular motors, for which the formation of asters have been reported [4,5].

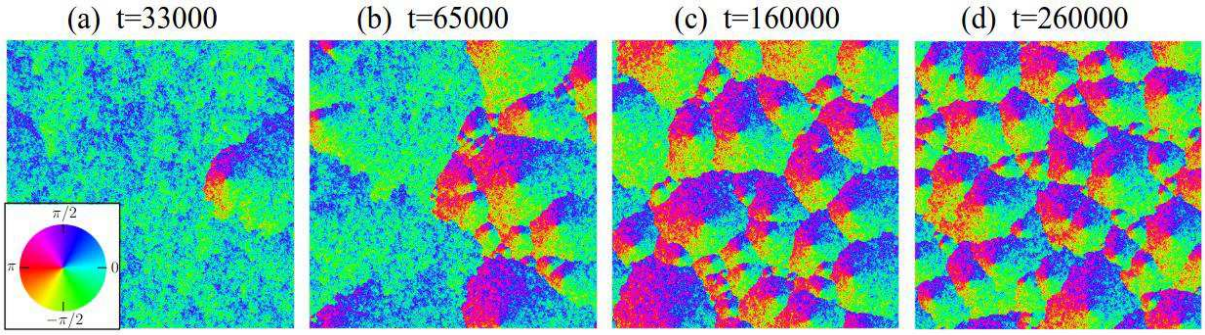

Figure 1: Nucleation of a first defect in the (metastable) ordered phase of 2D uniform-density flocks, yielding to an everevolving foam of asters.

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Roles of cell forces and ECM remodeling on fibrous tissue self-assembly.

T. Bram[1]*, A. Méry[2], V. Misiak[1], M. Balland[1], G. Cappello[1], T. Boudou[1]

[1]Univ. Grenoble Alpes, CNRS, LIPhy, Grenoble, F-38000, France
[2]I. Mech. Eng., Ecole Polytechnique Fédérale de Lausanne, Lausanne CH-1015, Switzerland
*thibault.bram-dit-saint-amand@etu.univ-grenoble-alpes.fr

Mechanical signals play a key role in the regulation of biological processes such as morphogenesis, homeostasis, or wound healing. These signals come from deformations induced by large-scale phenomena (blood flow, body movements, etc.) but also, on a smaller scale, by contracting cells. These deformations propagate over long distances and, together with the remodeling of the extracellular matrix (ECM), lead to large-scale reorganization events such as tissue formation or repair [1]. The understanding of the interactions between tissue contractility and its surrounding ECM can provide detrimental information on physiological mechanisms, such as tissue assembly, but also pathologic mechanisms such as fibrosis onset and expansion [2]. To investigate this feedback between cell contractility and ECM remodeling, one would need to simultaneously modulate and measure cell forces, while evaluating cytoskeletal and extracellular architecture.

To this end, we use NIH 3T3 cells stably expressing a Cry2-CIBN optogenetic probe to control with blue light the activity of RhoA, a major regulator of cellular contractility [1]. When placing a mixture of these cells and collagen in PDMS microwells containing flexible micropillars, we succeed in engineering microtissues and trigger their contraction by illuminating them (Fig. 1). Measuring the deflection of the pillars allows quantifying the forces generated by the microtissues in real-time. We observe that varying the frequency of stimulation modifies the dynamic of cells contractility.

We now plan to study the impact of contraction frequency on the speed and efficacy of tissue formation, as well as on tissue contractility, architecture, and mechanical properties. Altogether, our results will provide unique opportunities to elucidate how mechanical cues dynamically regulate tissue formation and function.

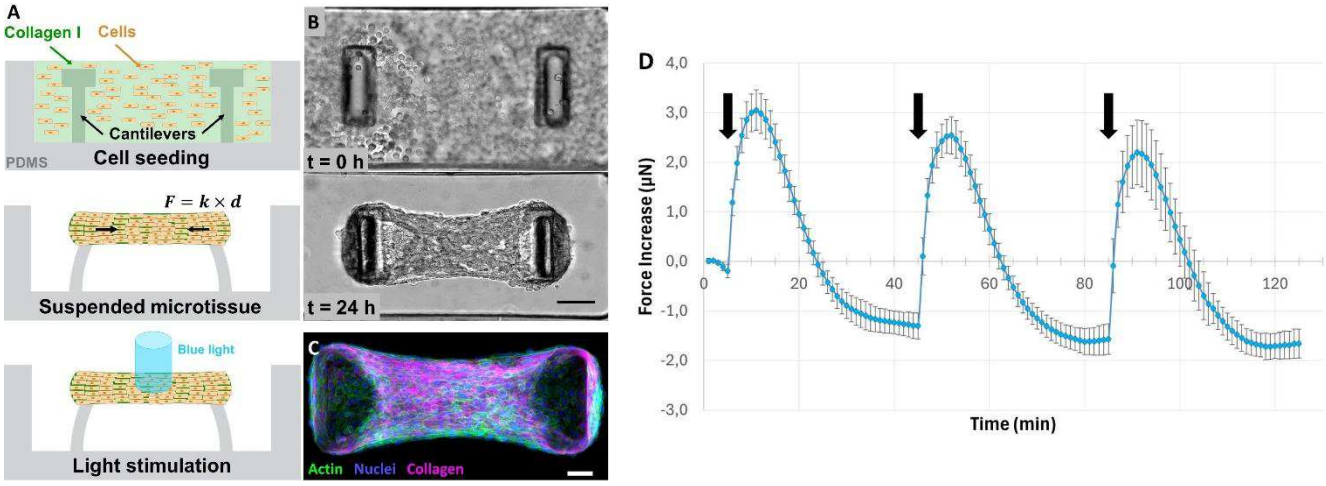

Figure 1: Engineering of light-controllable microtissues A. Schematic side-view of microtissue formation and stimulation in PDMS microwells. Tissue-generated force *F* is obtained thanks to the deflection *d* and the spring constant *k* of the pillars **B.** Top-view brightfield images illustrating microtissue formation, scale bar = 100µm **C.** Representative top-view confocal projection of a microtissue stained for actin (green), nuclei (blue) and collagen (magenta), scale bar = 50 µm. **D.** Mean force increase in tissues stimulated every 20 minutes (n = 12). Arrows indicate time of light stimulation; error bars represent standard deviation (A., B. & C. adapted from [1]).

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Probing Red Blood Cell stiffness with fluorescent probes : toward new markers of pathology

Alice Briole[1*], **Bérengère Abou**[1]

[1]Laboratoire MSC, Paris, France
[*]alice.briole@gmail.fr

We propose a new method for intracellular rheology of red blood cells (RBCs) based on molecular rotors, fluorescent viscosity probes [1]. We identify DASPI, a molecular rotor adapted to hemoglobin, which spontaneously penetrates into RBCs. Its sensitivity to viscosity in simple fluids makes it attractive for the study of erythrocyte pathologies characterized by the rigidification of RBCs. DASPI is capable of distinguishing between healthy RBCs whose stiffness varies with temperature or between donors, demonstrating the potential of our nanorheology technology [2]. The relevance of our new technique is studied in comparison with ektacytometry, the reference technique for deformability measurements in erythrocyte pathologies. In the case of sickle cell disease, recognized as a public health priority, we propose new indicators that could complement current clinical monitoring.

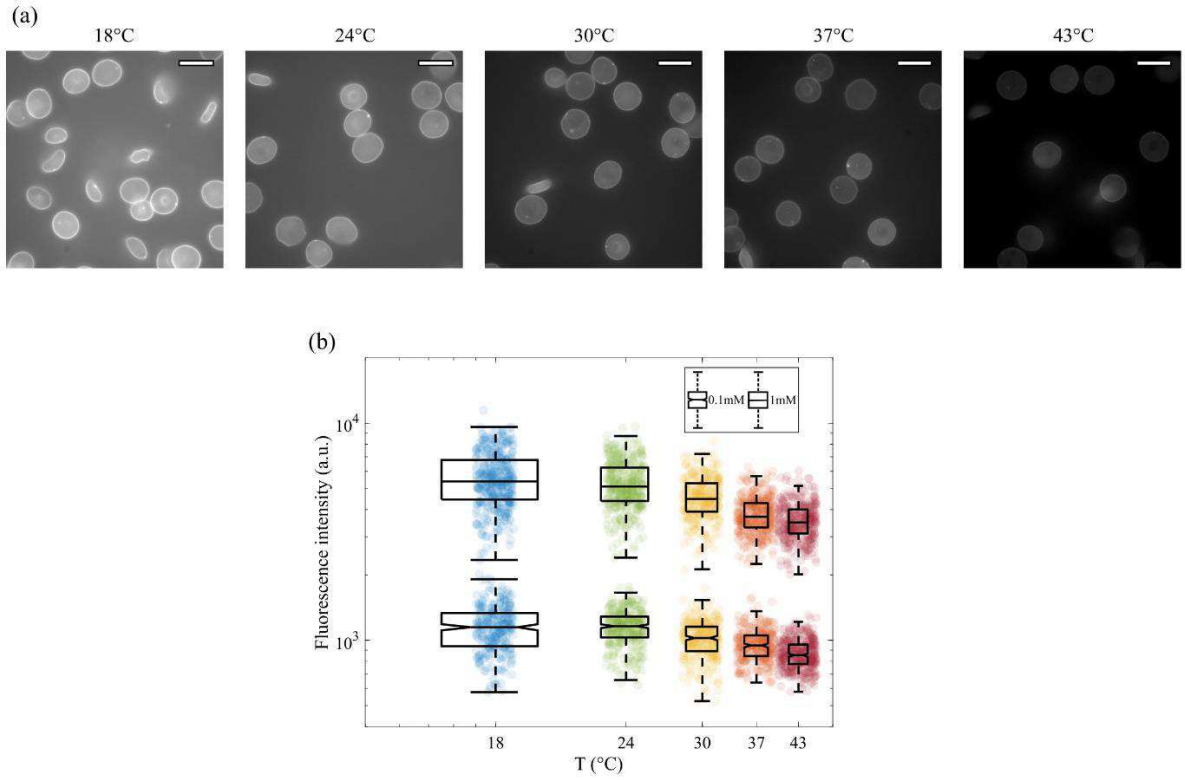

Figure 1: Fluorescence microscopy of RBCs at different temperatures (excitation 480 ± 20 nm, emission 595 ± 30 nm). (a) Images of RBCs with increasing temperature in a 1 mM DASPI solution (scale bar: 10 µm); (b) fluorescence intensity of RBCs as a function of temperature in 0.1 mM and 1 mM DASPI solutions; RBCs come from five donors, approximately four hundred cells were imaged at each temperature and their average intensity was measured. The fluorescence intensity increases with decreasing temperature i.e with the stiffness of the red blood cells.

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Lymph node mechanics and its impact on immune cells

L. Bruno[1]*, E. Donnadieu[1], J.F. Rupprecht[3], J. Fattaccioli[2] and P. Pierobon[1]
[1] Institut Cochin, CNRS UMR8104, INSERM U1016, Université Paris Cité, Paris, France
[2]Ecole Normale Supérieure, Institut Pierre Gilles de Gennes, CNRS UMR 8640, Paris, France
[3]Centre de Physique Théorique , UMR7332, Université Aix-Marseille, Marseille, France
*luisa.bruno@inserm.fr

B cells are key components of the adaptive immune response. [1] They are mostly known for producing antibodies; in order to do that, naïve B cells have to recognize and internalize the antigen through their B cell receptor (BCR). This process takes place in the lymph node and is known to be a mechanosensitive process. This suggests that the microenvironment mechanics plays an important role in B cells immune function.

To establish the role of lymph node mechanics in B cell function we use a system of lymph node slices kept alive to characterize the stiffness of the tissue and its rheological properties. We characterized the stiffness of the tissue by atomic force microscopy (AFM). We assessed micro rheology information by analyzing lipid droplets movements and deformation to determine the flow inside the tissue and to define the stress experienced by cells.[2] We will extend our measurements to inflamed tissues [3] subjected to external perturbations.

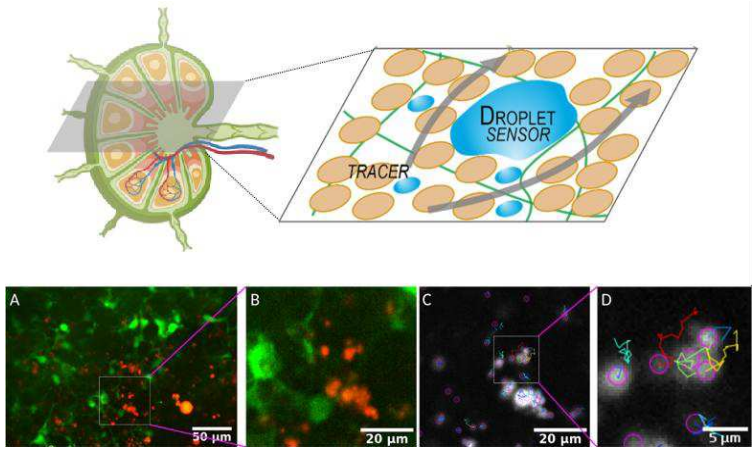

Figure 1: Lymph nodes slices can be used as a system to assess micro rheology information

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Nanofluidics for the transport of viral particles

Léa Chazot-Franguiadakis[1]*, Fabien Montel[1]
[1]Laboratoire de Physique, UMR CNRS 5672, ENS de Lyon, Université de Lyon, Lyon, France
*lea.chazot-franguiadakis@ens-lyon.fr

The human cell is organized into compartments. The transport of biomolecules between them is an essential step in order to maintain cell function. Among the cell's communication pathways, the nuclear pore complex (NPC), which regulates transport between the cell nucleus and the cytoplasm [1], is certainly the most complex. This pore has exceptional adaptability and selectivity properties, due to the presence of a network of dynamic polymers inside its central channel. Many viruses (adeno-associated virus, hepatitis B virus, HIV, ...) must transport their genetic material across the nuclear membrane, via the NPC to replicate inside the cell nucleus.

Our project addresses the issue of virus transport through the NPC in a biomimetic environment, i.e. simplified and controlled, in order to facilitate the study. To this end, we mimic the nuclear pore by grafting nanoporous membranes with hydrophobic polymers [2]. We rely on a highly sensitive optical system, developed within our team, which allows us to detect in real time and at the level of the nanopore the transport of a single viral particle [3,4]. We then measure the translocation frequency of viruses (labeled with a fluorophore) through the pores as a function of a control parameter (pressure, concentration).

Using this device, we have shown that it is possible to accurately quantify the concentration of viral particles directly in biological samples with an error of 4% and a low detection limit (LOD<105 particles/mL) [4]. In a different regime, we have also revealed a jamming phenomenon caused by the confinement of the viruses under flow [5]. We study the determinants (physical, chemical) of this effect and propose a physical model of the phenomenon seen as a phase transition under flow. Extracted parameters are related to the interaction of the viruses with the pore. They can be used to study subtle structural and geometrical modifications of the viruses induced by topological defect modulators.

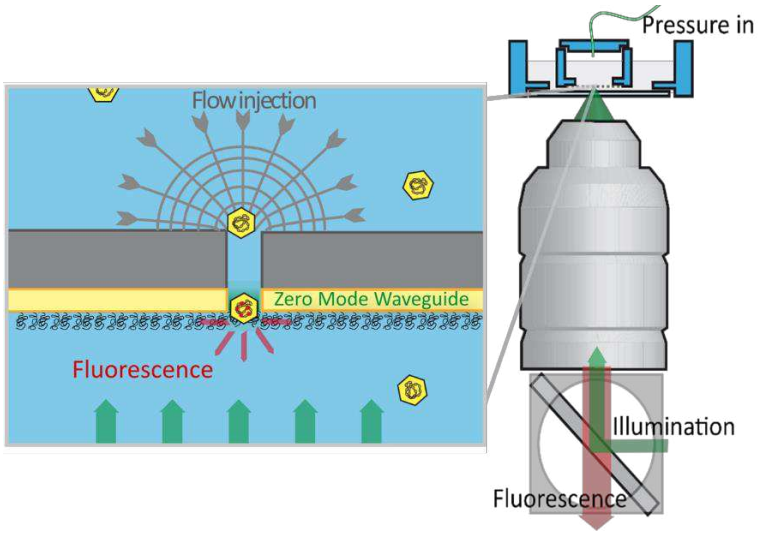

Figure 1: Experimental setup for translocation of (fluorescently labeled) viral particles through a synthetic nanoporous membrane grafted with hydrophobic polymers.

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Impact of actin variants on molecular dynamics and cortical mechanical properties in living embryos

M. Dufour Nicodex[1]*, T. Hecquet[1], A-C. Reymann[1]

[1]Institut de génétique et de biologie moléculaire et cellulaire, Strasbourg, France
*dufournm@igbmc.fr

Actin, a self-assembling biopolymer, plays a crucial role via the formation of dynamic networks that determine cellular shape and mechanical properties. *De novo* mutations in cytoskeletal actin (ACTB and ACTG1 genes) lead to the rare diseases termed non-muscle actinopathies (NMA) and associated with a wide range of symptoms with diverse severity levels. On one side, gene deletion can lead to reduced availability of actin, whereas single point substitution, due to the numerous essential interaction with a myriad of Actin Binding Proteins (ABP), can alter significantly actin dynamics. As the mechanism leading precisely actin disfunction to pathologies remains unknown, the Reymann team is investigating the molecular to functional consequences of some cytoplasmic actin variants using the model organism *C. elegans*.

Nine human substitutions, chosen to span a large range of severity in patients, were successfully recapitulated in *C. elegans* actin-coding gene using CRISPR/Cas9 editing. A phenotypic characterisation of these mutants shows notably embryonic lethality and signs of different mechanical perturbation at various scales. Our collaborators performing *in vitro* experiments on purified human mutant actin, showed some impaired polymerization kinetics or impaired interaction with some ABP for specific mutations. My PhD project is thus 1. to assess how actin kinetics is impaired in the actin cellular cortex of our living embryos, 2. to assess if the mechanical properties of the cortex are perturbed, 3. To reconcile the two scales to propose some mechanisms at the origin of phenotypes we observe.

I will present first quantifications of actin assembly kinetics by performing microscopy of the actin nucleator formin in the early *C. elegans* mutant embryonic cortex. The tracking of single formin particles (Fig 1-A) that are actively elongating actin filaments enables to extract actin polymerization velocities (Fig 1-B) as well as formin turnover rate [1]. Second, I will present quantifications of the actin cortical mechanical properties. To do so I performed laser ablation within the actin cortex as well as deduced cortical properties from measurement of myosin induced cortical flow rates.

Considering the obtained results for actin assembly kinetics, we suspect that some act-2 mutations present differences in actin polymerization velocities compared to the control, either by a slowed polymerization or by a sped up one. A new direct act-2 labelling strain recently obtained in the lab, will allow us to verify if actin turnover is also affected in vivo. At the macroscopic scale, the cortical properties quantifications are still running but we hope to obtain conclusive results soon. Altogether these results will allow to study the consequences at larger scales of polymerisation defects.

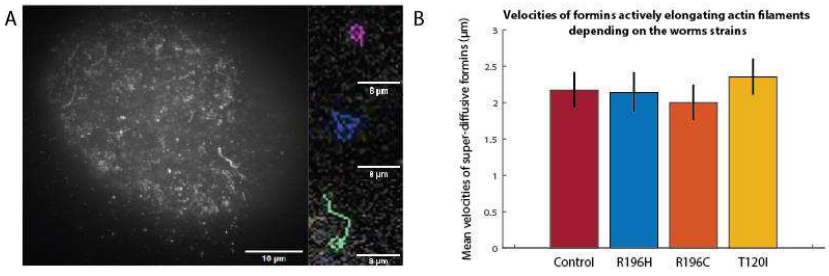

Figure 1: Single particle tracking in early embryonic cortical plane – (A) TIRF microscopy of CYK-1::Halo formins. Intensity projection allows to distinguish trajectories of the particles with 3 main dynamics, sub-diffusive (pink), diffusive (blue), super-diffusive (green) – (B) Mean velocity of super-diffusive formins (actively elongating actin filaments) for different *act-2* mutant strains

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Mechanical coupling of aECM to the epidermis

J. Eichelbrenner[1,2*], T. Sonntag[1], M. Labouesse[2], N. Pujol[1]

[1]CIML, Aix Marseille University, INSERM, CNRS, Turing Centre for Living Systems, Marseille, France
[2]IBPS, Sorbonne University, Paris, France
[*]eichelbrenner@ciml.univ-mrs.fr

All multicellular organisms must protect themselves from injury and pathogens. Lacking an adaptive immune system or motile immune cells, *C. elegans* relies on its epithelial barrier to defend against environmental threats. This makes it a powerful model to address the question of how epithelial cells detect damage. In *C. elegans*, the skin is characterized by a rigid but flexible apical extracellular matrix (aECM), the cuticle, which surrounds and protects the epidermis. Like the cell wall of plants or yeast, the nematode aECM also provides mechanical support to the underlying epithelium. Several features led us to postulate that the epidermis mechanically monitors the integrity of the aECM. We found that any change in cuticle mechanics, such as during molt when the aECM is remodeled, or in mutants of certain collagens with a change in matrix stiffness, induces a prophylactic immune response in the epidermis, similar to that induced during infection or damage. During molting, mechanical support is thought to be transiently transferred to the cytoskeleton, thanks to actin and microtubules alignment in periodic circumferential bands along the whole worm in the epidermal cell [1].

How does the epidermis read the changes in the aECM mechanics and induce coordinated cytoskeletal rearrangements and an immune response? To answer this question, we are currently exploring the mechanosensitivity of the different structures at the interface of the aECM and the epithelium. Using molting as a proxy for mechanical changes, we have observed specific transitory alignment of several structures, each appearing and disappearing at a precise timing during the formation of the new cuticle. We found attachment structures like hemidesmosomes and meisosomes, but also cytoskeleton regulators like spectrins and non-muscle myosins that are concomitant to actin and microtubule alignment. While doing a precise time series of appearance of these aligned structures, we are running epistasis analysis to explore their intimate dependency. In parallel, we have run genetic screens that led so far to the isolation of several candidates altering the immune response, including cadherin and microtubule regulators and are exploring their role in the mechanical response of the epidermis during molting. We expect to discover potentially novel signaling pathways involved in the mechanical coupling of the aECM to the epidermis.

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Nuclear volume reduction under mechanical stress regulates nucleolus.

K. Elias[1,2], S. Badawi[1], Y. Shetty[1], M. Dolega[1]

[1]Institute for Advanced Biosciences, Allée des Alpes, 38700 La Tronche (France)
[2]kenny.elias@etu.univ-grenoble-alpes.fr

Biochemical signals and mechanical cues regulate cell fate and function by triggering specific intracellular signaling pathways to allow the cell to respond, adapt, and maintain its homeostasis.
A previous project within the team unveiled the impact of static mechanical compression on an epithelial monolayer. Mechanical compression triggered a disassembly of the nucleoli by perturbing its organization and resulted in a reduced ribosomal DNA transcription, demonstrating a strong impact of mechanical stress on the ribosome biogenesis process.
Since nucleoli are bio-condensates stabilized by numerous physico-chemical parameters (such as temperature, protein concentration, etc.), we investigate how strain rate and associated changes in nuclear volume regulate the stability of nucleoli and their function.

Fibroblast persistent migration on soft hydrogel: how substrate stiffness affects cell direction of motion

S. Faour[1,2], C. Vezy[1], B. Langlois[2], S. Salesse[2], S. Dedieu[2] and R. Jaffiol[1]

[1]L2n, UMR CNRS 7076, UTT, Troyes, France
[2] MEDyC, UMR CNRS 7369, URCA, Reims, France
Email: sara.faour@utt.fr

As matrix stiffness has become a key factor in the differentiation of fibroblasts into cancer-associated fibroblasts (CAF), soft polymer-based substrates have been now widely employed to study such kind of mechanotransduction process [1,2]. Previous works have revealed the effect of substrate stiffness on 2D and 3D cell adhesion and migration (spreading area, speed, focal adhesion size, directionality) with different gels and elastomers [3,4,5,6]. However, in most of these studies, it was not possible to control separately substrate stiffness and adhesion molecules density (ECM proteins, RGD peptides). Moreover, the cell tracking data processing implemented does not allow to unbiasedly assess the persistence phenomena in cell migration [7]. Thus, we proposed a parametric study based on the use of fibronectin coated PEG-based hydrogels of well controllable stiffness to discern the impact of its mechanical properties on 2D fibroblast migration. These hydrogels are prepared via covalent cross-links between poly(L-lysine) dendrigrafts (DGL) and PEG-NHS molecules. The mechanical properties of our hydrogel were characterized using optical tweezers with micro-beads through (i) active bulk microrheology and (ii) surface micro-indentation. Two different subsets – normal fibroblasts (WPMY-1) and activated fibroblasts (exp-CAF2) –were studied on different elastic hydrogels of varying DGL concentrations to adjust the stiffness. The behavior of these cells was analyzed through single cell tracking with combined epi-fluorescence and phase contrast imaging. Direction and speed autocorrelations were computed and analyzed with the relevant "stick-slip" model proposed by H. Flyvbjerg [8], well suited to interpret the double-exponential decay of the velocity autocorrelation function. Finally, we clearly demonstrated that fibroblasts adopt a directional motion when the stiffness increases, characterized by an increase of the time associated to the direction changes and a decrease of the corresponding angles.

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The extracellular matrix stiffness promotes the invasiveness of breast cancer epithelial cells

Lucie Ergot[1]*, Sylvain Gabriele[1]

[1]Laboratory for Complex Fluids and Interfaces, Mechanobiology and Biomaterials Group, Research Institute for Biosciences, University of Mons, Mons, Belgium
*Lucie.ERGOT@umons.ac.be

Tumor progression alters the composition and physical properties of the extracellular matrix (ECM). Particularly, increased matrix stiffness has profound effects on tumor growth and metastasis in breast tissues. While one of the major contributing factors is increased density of collagen fibers in the ECM, the influence of the ECM stiffness on the epithelial-mesenchymal transition (EMT) and dissemination of breast cancer epithelial cells remain unclear. Here we used Gelatin hydrogels (GelMA) derived from native type I collagen through partial hydrolysis and functionalized with methacrylate groups to reproduce in vitro the main physico-chemical properties of breast tissues. We used the Irgacure 2959 photoinitiator to control the polymerization of GelMA hydrogels through UV illumination. Our findings show that the rigidity of the hydrogels increases from ~2 kPa (soft) to ~15 kPa (stiff) by doubling the gelatin concentration, which allows to mimic the rigidity of healthy and tumoral breast tissues. Normal (MCF-10A) and tumoral (MDA-MB-231) epithelial cells were cultured on soft and stiff GelMA to investigate the influence of the ECM stiffness on the epithelial-mesenchymal transition (EMT) and dissemination of breast cancer epithelial cells.

Friction when changing neighbours: adhesion-regulated junction slippage controls cell intercalation dynamics in living tissue

Alexander Nestor-Bergmann[#], Guy B. Blanchard[#] , Nathan Hervieux[#] , Alexander G. Fletcher[⊙], Jocelyn Étienne[⊗*] and Bénédicte Sanson[#]

[#] PDN, Univ. Cambridge, UK
[⊙] Maths, Univ. Sheffield, UK
[⊗] LIPHY, CNRS – Univ. Grenoble Alpes
* jocelyn.etienne@univ-grenoble-alpes.fr

During development tissues undergo dramatic shape changes to build and reshape organs. In many instances, these tissue-level deformations are driven by the active reorganisation of the constituent cells. This intercalation process involves multiple cell neighbour exchanges, where an interface shared between two cells is removed and a new interface is grown. The key molecular players involved in neighbour exchanges, such as contractile motors proteins and adhesion complexes, are now well-known. However, how their physical properties facilitate the process remains poorly understood. For example, how do cells maintain sufficient adhesive contact while actively uncoupling from one another? Then, how does a new interface grow in a contractile environment? Many existing biophysical models cannot answer such questions, due to representing shared cell interfaces as discrete elements that cannot uncouple.

Here, we develop a model where the junctional actomyosin cortex of every cell is modelled as a continuous viscoelastic rope-loop, explicitly representing cortices facing each other at bicellular junctions and the adhesion molecules that couple them. The model parameters relate directly to the properties of the key subcellular players that drive dynamics, providing a multi-scale understanding of cell behaviours. The code is distributed as an open-source free software.

We show that active cell neighbour exchanges can be driven by purely junctional mechanisms. Active contractility and cortical turnover in a single bicellular junction are sufficient to shrink and remove a junction. Next, a new, orthogonal junction extends passively. Our Apposed-Cortex Adhesion Model (ACAM) [1] reveals how the turnover of adhesion molecules regulates tension transmission and junction deformation rates by controlling slippage between apposed cell cortices. The model additionally predicts that rosettes, which form when a vertex becomes common to many cells, are more likely to occur in actively intercalating tissues with strong friction from adhesion molecules.

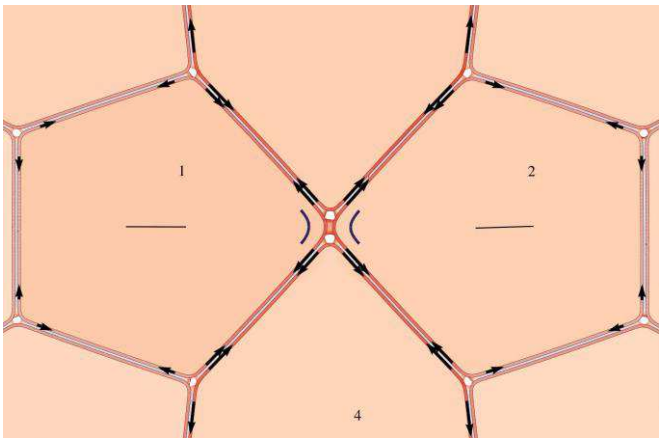

Figure 1: Mechanical balance during neighbour exchange in large cell--cell friction conditions. The junction outlined in dark blue is actively contracting. Black arrows, tension in the cortices of each cell. Cortices interact via spring-like adhesions. Observe that tension propagates across tissue, due to adhesion resistance to shear.

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Cell sensitivity to stiffness in 3D environment with controlled geometry

Aída G. Fernández Contreras [1,*], Jean-Hervé Tortai [1] and Alice Nicolas [1]

[1] Université Grenoble Alpes, CNRS, LTM, 38054, Grenoble, France

*aida-gabriela.fernandezcontreras@cea.fr

Cell culture is an important tool that enables the study of physiological and pathological cell activity in vitro with applications in fields such as cell biology, drug discovery, cancer research, tissue engineering, and stem cell studies [1]. In traditional cell culture, cells adhere to rigid two-dimensional (2D) surfaces, typically made of polystyrene or glass. However, within the body, many cells are supported by a complex three-dimensional (3D) extracellular matrix (ECM) [2].

There is a complex interplay between cells and this matrix, as cells exert mechanical forces to remodel the extracellular matrix according to their requirements. In turn, physical cues from the extracellular matrix, like stiffness or geometry, serve as stimuli that cells sense and transduce to regulate cellular behavior including adhesion, growth, migration, proliferation and differentiation [3]. Since this dynamic environment cannot be accurately represented by the static nature of traditional 2D cell culture surfaces, data obtained from in vitro studies with these models can be non-predictive of in vivo behavior, often resulting in disparities with animal and clinical tests [4].

To address this limitation, our aim is to develop 3D scaffolds with defined mechanical and geometrical properties at the micrometer scale, leveraging the high-resolution capabilities of two-photon polymerization. We first selected a commercial photoresist called OrmoComp that is compatible with cell culture and has been highly reported in literature [5]. Employing this material, we designed a process to achieve the fabrication of mesh structures with fiber thickness from 2 to 10 µm, and cavity sizes from 20 to 40 µm. Preliminary experiments with the adenocarcinoma cell line (A549) were conducted to assess the effect of pore size on cell behavior. Fluorescence microscopy images revealed differences in cell morphology and distribution in function of the pore size. Specifically, cells exhibited a propensity to adhere to the edges and encircle the cavities in models featuring larger cavity sizes. Conversely, in models with smaller cavity sizes, cells demonstrated a tendency to stretch out across the cavities.

We then advanced our approach to develop a model with tunable stiffness in addition to tunable pore size. Initial experiments using an acrylamide-based photoresist formulation were achieved. This hydrogel has high-swelling capabilities that allow tuning the scaffold stiffness in the range of biological tissues.

The results from this study collectively show that two-photon polymerization combined with material technology provides a promising path for the creation of artificial 3D scaffold models that better emulate in vitro the intricate natural environment of cells within the body, towards the advancement in basic cell behavior understanding as well as application in tissue engineering and drug development.

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Effects of microbial glycolipids on phospholipid membranes using Atomic Force Microscopy (AFM)

Y. Fok[1*], A.-L. Makanga-Boutoto[1], G. Lu[1], N. Baccile[2], C. Valotteau[1], L. Redondo-Morata[1]

[1]Aix-Marseille Université, INSERM, DyNaMo, Turing Centre for Living systems, Marseille, France
[2] Sorbonne Université, Centre National de la Recherche Scientifique, Laboratoire de Chimie de la Matière Condensée de Paris, LCMCP, Paris, France
[*]email of presenting author: yulia.fok@inserm.fr

Microbial glycolipids are biosurfactants comprising a hydrophilic saccharide moiety coupled to a hydrophobic fatty acid. Their antimicrobial properties are raising an increasing interest as they might be alternatives to antibiotics, offering biodegradability and low ecotoxicity, and their production is based on renewable-resource substrates. Antimicrobial activity involves changes and rupture of the cellular membrane inducing lysis. However, their mechanism of action is still not well-known. It might rely on the interaction of the saccharide moiety with the bacterial membrane and posterior penetration thanks to the lipophilic moiety [1]. The current project aims to study the effect of glycolipids on supported lipid bilayers using atomic force microscopy (AFM), to better understand the underlying molecular mechanisms at the nanoscale.

We observed the dynamic evolution of model phospholipid membranes after injection of two glycolipids. We also characterized the effect of glycolipids on the (nano)mechanical properties of membranes (Figure 1). Using a similar approach, supported lamellar structures can be formed by mixing phospholipids and glycolipids to form vesicle in suspension. We are exploring a wide range of phospholipid:glycolipid ratios to better understand their interactions and properties. These insights into the effect of microbial glycolipids on model membranes at the nanometre scale can contribute to a better understanding of their biological activity, as well as their potential use as nanocarriers.

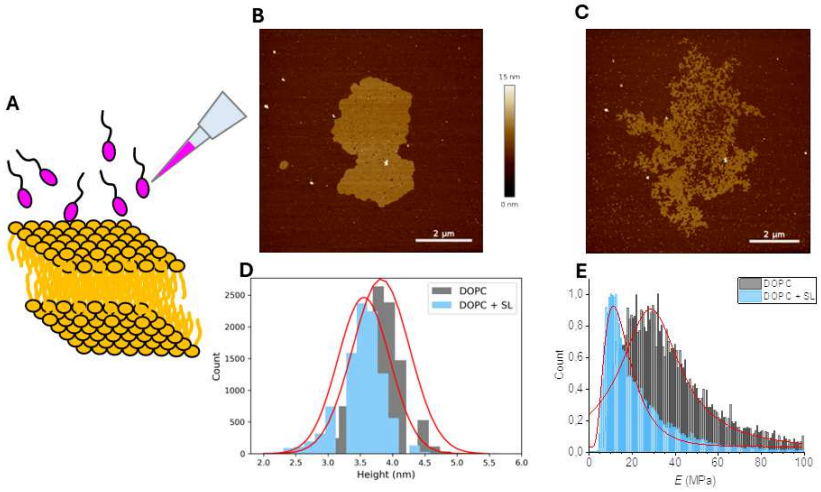

Figure 1: A) Glycolipid injection on a phospholipid membrane. B) AFM topography image of a DOPC lipid membrane before and C) after adding sophorolipid (SL) solution. D) Histograms of the height of the membrane and E) of the Young Modulus.

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Hypoxia triggers collective aerotactic spreading of eukaryotic cells

Nasser Ghazi[1], Mete Demircigil [2], Satomi Hirose[3,4], Amandine Chauviat[5], Vincent Calvez [2], Kenichi Funamoto [3,4,6], Christophe Anjard [1], Jean-Paul Rieu[1]

1 Institut Lumière Matière, UMR5306, Université Lyon 1-CNRS, Université de Lyon, 69622 Villeurbanne, France
2 Institut Camille Jordan, UMR5208, Université Lyon 1-CNRS, Université de Lyon, 69622 Villeurbanne, France
3 Graduate School of Biomedical Engineering, Tohoku University, Aoba-ku, Sendai, Miyagi 980-8579, Japan
4 Institute of Fluid Science, Tohoku University, Aoba-ku, Sendai, Miyagi 980-8577, Japan
5 Écologie microbienne, UMR 5557, Université Lyon 1-CNRS, Université de Lyon, 69622 Villeurbanne, France
6 Graduate School of Engineering, Tohoku University, Aoba-ku, Sendai, Miyagi 980-8579, Japan
*nasser.ghazi@univ-lyon1.fr

Oxygen (O_2) undoubtedly plays an essential role in the life of many organisms. Cells however need to adapt to surrounding, or internal, low oxygen regions which are either self-generated or occur due to the complex surrounding environment. We previously showed that a confined colony of *Dictyostelium* amoebae leads to self-generated gradients due to O_2 consumption – and in turn, the cells react with positive aerotaxis away from hypoxia and upwards the gradient in a collective manner [1]. We modelled this phenomenon with an original mean-field "Go-or-Grow" model with coupled reaction-diffusion equations between oxygen and cell density fields. We also previously showed, that the self-generated O_2 gradients arising are solely responsible for this collective phenomenon, and not other intercellular communication (secreted factors, ROS…) [2].

The main ingredient for this model is the cells' aerotactic response potential that we probe using a two-layered microfluidic device which allows the repeated control of externally subjecting cells to O_2 gradients [3]. We examine and compare the behavior under such O_2 gradients (both self-generated and externally applied) of two different amoebae: the social amoeba *Dictyostelium discoideum* (*Dd*) and the asocial amoeba *Acanthamoeba castellanii* (*Ac*) which explores more territory than *Dd*. While the former can be modelled well with a single cellular diffusion coefficient and aerotactic driving force, the latter seems to exhibit a unique dependence, both for cellular diffusion coefficient and for its aerotactic driving force, on cell density. It suggests that collisions play a strong role in spreading behavior and that perhaps microscopic or mesoscopic models should be pursued.

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ADHESION DIFFERENTIAL AND MECHANICAL FORCES APPLIED TO EMULSION BASED PROTO-TISSUES

Quentin Guigue[1]*, Marc Bess[1], Raphaël Voituriez[1], Alexis Prevost[1], Elie Wandersman[1], Matthias Merkel[2], Lea-Laetitia Pontani[1]

[1]Sorbonne Université, CNRS, Institut de Biologie Paris-Seine (IBPS), Laboratoire Jean Perrin (LJP), Paris, France
[2]Aix Marseille Université, Université de Toulon, CNRS, CPT (UMR 7332), Turing Centre for Living Systems (CENTURI), Marseille, France
*quentin.guigue@sorbonne-universite.fr

During morphogenesis, bio-chemical signaling and mechanical cues are at play in order to give rise to shape and function to future formed tissues, influencing each other through a dynamic feedback loop. Still, the role of such mechanical cues on the emergence of structure is not well understood.

In particular, in the context of adhesive tissues or cell aggregates, it is known that the hierarchy of cell-cell adhesion, together with cellular motility, can lead to the self-organization of identified structures, a phenomenon described within the framework of the differential adhesion hypothesis. Nevertheless, these kinds of processes have been mainly studied in biological systems in vivo or in vitro where deciphering the exact influence of individual factors like cell-cell adhesion or forces remain challenging.

In order to tackle this problem, we choose to work in a simplified framework. We use adhesive emulsions as a biomimetic system of epithelial tissues, in which each oil droplet mimics a cell in the tissue and we introduce differentials of adhesion using palindromic DNA sequences as the binders between the droplets. The obtained “proto-tissues” are then flowed in micro-channels of various geometry that directly define the stress field.

We seek to experimentally and theoretically elucidate the influence of local droplet deformations and T1 events on the global behavior of the emulsion flow. Next, we try to correlate those observations to the evolving structure of the emulsion along the channel as a function of the interdroplet adhesion hierarchy. By comparing our findings with structural features described in biological tissues we will thus be able to infer a potential role of adhesion regulations to facilitate the emergence of structure in developing tissues.

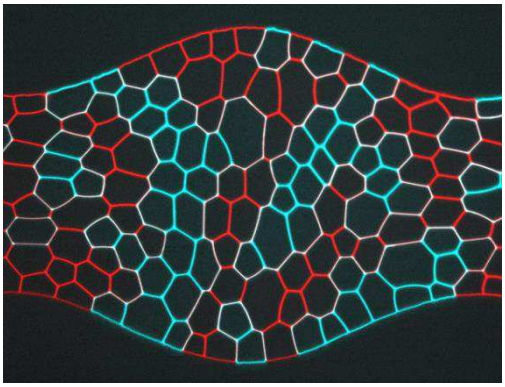

Figure 1: Fluorescence image of an adhesive emulsion with two populations of droplets exhibiting a differential of adhesion in a sinusoidal channel (higher binding energy between red droplets than between blue droplets).

Biofunctionalization and micropatterning of quantum dots and nanoplatelets for cell nanoimaging

L. Herry*, R. Jaffiol and C. Vézy

Light, nanomaterials, nanotechnologies (L2n), UMR CNRS 7076, Troyes, France
*lucie.herry@utt.fr

By mimicking the in vivo biochemical and mechanical cell microenvironment, microstructured surfaces offer a powerful framework for single-cell adhesion and migration assays [1]. We thus propose a FRET-based imaging technique that will integrate in its core these microstructuration and biochemical necessities: Non-radiative Excitation Fluorescence (NEF) nanoscopy (FRET: Förster Resonance Energy Transfer). Using a monolayer of emitters (quantum dots and nanoplatelets) as donors for FRET, NEF imaging allow selective observations of cell membrane components in a quantitative way, to measure for example cell-substrate distances with a nanometric resolution [2]. In order to combine NEF imaging and the microstructuration necessity to control cytoskeleton architecture, we have developed an innovative method to create micropatterns of highly concentrated quantum dots and nanoplatelets monolayer at large scale [3]. The obtained micropatterns will be used as a scaffold for surface structuration of the adhesion ligands (RGD peptide) specifically recognized by integrins on cell membrane. Therefore, we demonstrate that it is possible to bind any kind of his-tagged proteins on the nano emitters, thus allowing to observe cell membrane in real time.

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The role of mechano-chemical cues in vertebrate somite generation

Georgia Kefala[1]*, Benoit Sorre[1], Karine Guevorkian[1]

[1]Physique des cellules et cancer, Institut Curie, Paris, France
*georgia-maria.kefala@curie.fr

Somitogenesis is a crucial tissue patterning event in vertebrate embryonic development, in which epithelial structures, called somites, emerge from the pre-somitic mesoderm (PSM) in a periodic manner, as the PSM undergoes a mesenchymal to epithelial transition along the antero-posterior axis. Later in development, these structures differentiate into skeletal muscle, vertebrae and dermis. Previous research has shown that morphogen gradients such as fibroblast growth factor (FGF) and retinoic acid (RA), along with the activation of oscillatory genes, define the location and timing of segmentation. While the Clock and Wavefront model describes the biochemical signaling in the PSM segmentation, the role of geometrical confinement, tissue mechanics, as well as their crosstalk with chemical cues during this process is still poorly understood. Here, our aim is to study the physical mechanisms underlying somitogenesis in chicken embryos and their interplay with the chemical signals, originating from the morphogens, in *ex vivo* conditions. We are developing a novel microfluidic system to impose mechanical confinement on PSM explants and achieve precise control over both the geometry and the morphogen gradients within the PSM's environment. Studying the dynamics of somite formation in various morphogen conditions, and characterizing the mechanical properties of the mesoderm, will allow us to propose a quantitative mechanism of somite formation combining physical and chemical cues. Our findings will not only advance our understanding of this fundamental process but will also provide valuable insights into the principles of tissue self-organisation and morphogenesis.

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Self-limiting self-assembly of particles with complex interactions

Vincent Ouazan-Reboul[1]*, Martin Lenz[1,2]
[1]Université Paris-Saclay, CNRS, LPTMS, 91405, Orsay, France
[2]PMMH, CNRS, ESPCI Paris, PSL University,
Sorbonne Université, Université Paris-Cité, F-75005, Paris, France
*vincent.ouazan-reboul@universite-paris-saclay.fr

Many proteins self-assemble into higher-order structures in a controlled manner, and can in particular form into aggregates with a large but finite size at equilibrium. Understanding how this self-limiting property generically emerges from the characteristics of individual components is an open problem, relevant to both the understanding of biological self-assembly processes and the design of artificial particles. To uncover the broad design principles of objects which aggregate into finite-sized structures, we numerically study the self-assembly of three-dimensional lattice particles with simple geometries but complex interactions. We find that choosing particle interactions in a manner that introduces topological defects in the resulting aggregate is a viable strategy for size control.

Vimentin promotes actin assembly at the barbed end

L. Paty[1]*, Q. D. Tran[1], A. Jégou[1], G. Romet-Lemonne[1], C. Leduc[1]

[1]Institut Jacques Monod, CNRS, Université Paris Cité, Paris, France
*lilian.paty@ijm.fr

Vimentin is a cytoplasmic intermediate filament protein expressed in mesenchymal cells and a key regulator of cell migration [1], which relies on the assembly and disassembly of actin filaments. While there is evidence that the actin and vimentin cytoskeletons interact in cells [2-6], the underlying mechanisms of this interaction are only partially understood. Actin and vimentin can interact through biochemical signaling pathways [3] or via cross-linkers [2,6], but whether these two cytoskeletal filaments are involved in a direct protein-protein interaction remains controversial [7-8]. If such an interaction exists between actin and vimentin, it is probably weak and transient, making it almost impossible to observe and characterize in cells. By allowing direct observation of single filaments in well-controlled biochemical conditions, in vitro experiments from purified proteins represent a powerful tool to address this question. Here, using TIRF microscopy to monitor the elongation of single actin filaments, we show that vimentin promotes actin assembly at the barbed end in the absence of actin-vimentin cross-linkers. The acceleration of actin assembly by vimentin is dose-dependent, and does not require vimentin to be assembled into long filaments, as vimentin unit-length filaments increase actin assembly rate similarly to longer vimentin filaments. These findings reveal an unexpected new actor in the regulation of actin dynamics at the barbed end, and provide new insights to further understand the upregulation of cell migration by vimentin.

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How to electrically persuade cells to dance to your tune

N. Pishkari[1], V. Misiak[1], G. Shim[2] ,I. Breinyn[2], I. Wang[1],F. Fagotto[3], D. Cohen[2], G. Cappello[1] , T. Boudou[1] and M. Balland[1]

(1) Interdisciplinary Laboratory of Physics (LIPhy), Université Grenoble Alpes (UGA), 140 Rue de la Physique, 38402 Saint-Martin-d'Hères, France.
(2) Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ 08544, USA.
(3) CRBM, University of Montpellier and CNRS, Montpellier, France.
*niloofar.pishkari@univ-grenoble-alpes.fr

Studying large-scale directed collective cell motions offers insights into the mechanisms underlying multicellular biological processes like morphogenesis, wound healing, and cancer progression. Galvanotaxis, a specialized tool, facilitates the guided migration of cells, unveiling the intricacies of their behavior. Employing the unique 'SCHEEPDOG' [1] platform enables the precise herding of cell migration in desired directions, ideal for conducting long-term experiments. It is noteworthy that various regions within the tissue exhibit distinct behaviors in response to the applied stimulation. The 'SCHEEPDOG' use the principle of Galvanotaxis stimulation to initiate cell migration.

Distinct cell types exhibit varied responses to such stimulations [2], with the electric field intensity playing a crucial role. Migratory responses encompass not only the speed and velocity of cell motion but also changes in cell orientation as a feedback response to stimulation [3]. While perpendicular alignment of cells has been observed in several instances, this orientation does not necessarily coincide with accelerated directed migration.

We observed that, not only position of the cells Confluency of the cells in the monolayer is also another parameter affecting the magnitude of response to the stimulation in similar time scales.

Our ultimate goal is being able to relate the role of the electric field on the different molecular and physical parameters involved in the cell motile machinery.

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Mechanobiology of phagocytosis : force mapping by image analysis

H. Uhl[1,2*], S. Michelis[3], C. Pompili[4], B. Dumat[3], J-M. Mallet[3], F. Niedergang[4], J.Fattaccioli[1,2]

[1] PASTEUR, Département de Chimie, Ecole Normale Supérieure, PSL Université, Sorbonne Université, CNRS, 75005 Paris, France
[2] Institut Pierre-Gilles de Gennes pour la Microfluidique, 75005 Paris, France
[3] Laboratoire des BioMolécules, Département de Chimie, Ecole Normale Supérieure, PSL Université, Sorbonne Université, CNRS, 75005 Paris, France
[4] Université Paris Cité, Institut Cochin, Inserm, CNRS, 75014 Paris, France
[*]heloise.uhl@ens.psl.eu

Phagocytosis is a process involved both in immunity and tissues homeostasis, through which immune cells internalize targets as diverse as pathogens and worn-out cells, leading to their elimination from the organism. The uptake of targets requires rapid cytoskeleton reorganization and spatially controlled force generation. Progress has been made recently on the understanding of the cellular mechanisms and signaling pathways governing the mechanical behavior of cells during engulfment of model targets such as gel beads [1]. However, these materials have some intrinsic limitations that foster the development of alternative substrates to achieve a full description of the mechanobiology of phagocytosis.
Here, we propose to use deformable oil-in-water droplets with a controlled size as force sensors during uptake. These particles can be functionalized with ligands designed to target specific cellular receptors. Compared to other types of deformable particles, they allow these ligands attached to the interface to diffuse laterally and respond to the receptor clustering during recognition [2,3]. Their tunable deformability and known surface tension make them a relevant tool to study mechanical interactions as deformations provide direct measurement of the exerted stresses through Laplace's law.
By statistically analyzing the droplet deformation during engulfment by a large number of RAW 264.7 macrophages, we show that force generation is associated to the engagement of the Fcγ receptors. To precisely map the force generation at the single-cell level, we adapted an imaging and computational method [4] which allow for the reconstruction of the tridimensional shape of the droplet during internalization. Through the obtained deformation field, we are able to measure the range value of normal stresses exerted at each point of the surface.

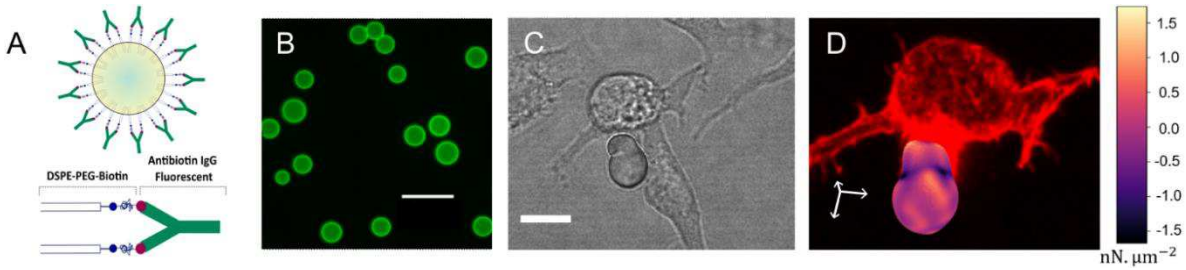

Figure 1 : (A) Schematic view of the immunoglobulin-functionalized droplet. (B) Epifluorescence image of IgGs-coated droplets. (Scale bar : 30 µm) (C) Confocal image of a macrophage internalizing IgG-coated droplets. (Scale bar : 10 µm). (D) 3D reconstruction of the droplet and the stress distribution on the surface.

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Spatially controlled basal stimulation reveals rules controlling the patterning of human pluripotent stem cells colonies

Tom Wyatt[1], Mingfeng Qiu[3], Vincent Hakim[3], Benoit Sorre[1,2*]

[1]Laboratoire Matière et Système Complexes, U. Paris Cité, Paris, France
[2]Laboratoire Physique des Cellules et Cancer, Institut Curie, Paris, France
[3]LPENS, ENS, Paris, France
[*]benoit.sorre@curie.fr

One of the most striking features of embryonic development is that differentiation is happening in a spatially ordered fashion: tissue self-organize to form well-defined patterns that pre-figure the body plan. During gastrulation, the cells of the embryo are allocated into three germ layers: ectoderm, mesoderm and endoderm. Molecular embryology has established that the FGF, WNT BMP and NODAL signaling pathways are necessary for the initiation of gastrulation in mammalian embryos and this knowledge has been used to guide stem cell differentiation *in vitro* for tissue engineering applications. However, studying the causality link between the spatio-temporal dynamics signaling and the resulting pattern formation is difficult in live embryos, because of their inherent lack of observability but also because it is not possible in an embryo to control in a quantitative manner the cells' physical and chemical environment.

Here, we used microfluidics to apply well defined gradients of morphogens on human embryonic stem cells colonies and study how the spatial organization of differentiating tissues depends on the signaling landscape. We showed that in a static parabolic gradient of BMP4, three cell types (amnion-like cells, mesoderm, epiblast) are generated at a well-defined position in the gradient, in perfect agreement with the classical "French flag model" [1]. However, in this static gradient configuration, the diversity in cell identities generated was much lower than in the embryo at similar stages as endoderm cells for instance were not observed. We were however able to generate them by changing the spatiotemporal properties of the gradient. Combining modeling and tracking of the cells signaling history of the WNT BMP and NODAL pathways, we show that the key element to explain our observations is a bistability in WNT and NODAL signaling. Our study paves the way toward predictive control of tissue organization thanks to precise control of the tissue environment.

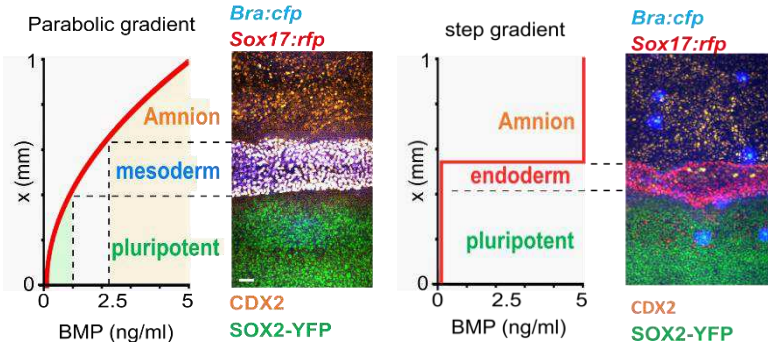

Figure 1: Different cell types are generated in human embryonic Stem Cells colonies depending on the steepness of the applied BMP gradient.

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Using engineered oil droplets to decipher how the extracellular matrix (ECM) transmits mechanical forces

Vasiljević Olga[1, 2]*, Breau Marie Anne[2], Fouchard Jonathan[2], Pontani Lea-Laetitia[1]

[1]Laboratoire Jean Perrin, Sorbonne Université, Paris, France
[2]Laboratoire de Biologie du Développement, Sorbonne Université, Paris, France
*olga.vasiljevic@sorbonne-universite.fr

Extracellular matrix (ECM) is a porous fiber network which lies in the interstices of tissues and organs. Thanks to its ability to transmit forces and adapt its mechanical properties in response to them, it plays a key role during morphogenetic events and tissue homeostasis [1]. The aim of this study is to decipher how the ECM is transmitting forces not only in between isolated cells, but also between two interacting tissues. For that, we develop deformable oil droplets, which are embedded within the ECM of developing zebrafish embryos or *in vitro* micro-tissues.

A recent study, using zebrafish embryos as a model system, showed that the developing eye exerts lateral traction forces on the olfactory placode *via* ECM, impacting morphogenetic movements and axon extension within the placode [2]. To tackle this question further and understand how the ECM transmits forces between the two tissues, we are developing self-functionalizing droplets that can display binders on their surface upon injection/formation of the droplet in the embryo. The droplets are designed to adhere to various components of ECM. By tuning the surface tension, they can be deformable enough to reveal transmission of forces through ECM. Furthermore, from droplet shape analysis and modeling, information about the forces can be extracted.

In parallel, cell-size oil droplets are introduced into *in vitro* micro-tissues composed of fibroblast cells cultured in a 3D network of type I collagen (the main component of the ECM). Here, fibroblasts rearrange the matrix through the traction forces they apply onto the ECM and can thus tune its mechanical properties [3]. We expect to quantify these forces and how they propagate into the ECM network by measuring the mechanical stresses applied on engineered oil droplets.

Altogether, this approach will bring new mechanistic knowledge on the role of ECM and its mechanical properties in tissue morphogenesis *in vivo*, with potential implications for ECM-related diseases, tissue engineering and regenerative approaches.

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Mechanoresponse of curved epithelial monolayers lining bowl-shaped 3D microwells

M. Versaevel[1#*], M. Luciano[1#], Y. Kalukula[1] and S. Gabriele[1]

[1] Mechanobiology & Biomaterials Group, Research Institute for Biosciences
University of Mons, 20 Place du Parc, Mons B-7000, Belgium
[#] Contributed equally to this work
[*] marie.versaevel@umons.ac.be

The optimal functioning of many organs relies on the curved architecture of their epithelial tissues [1]. However, the mechanoresponse of epithelia to changes in curvature remains misunderstood. Here, bowl-shaped microwells in hydrogels are designed via photopolymerization to faithfully replicate the shape and dimensions of lobular structures. Leveraging these hydrogel-based microwells, curved epithelial monolayers are engineered, and how in-plane and Gaussian curvatures at the microwell entrance influence epithelial behavior is investigated [2] (Fig 1). Cells and nuclei around the microwell edge display a more pronounced centripetal orientation as the in-plane curvature decreases, and enhanced cell straightness and speed. Moreover, cells reorganize their actin cytoskeleton by forming a supracellular actin cable at the microwell edge, with its size becoming more pronounced as the in-plane curvature decreases. The Gaussian curvature at the microwell entrance enhances the maturation of the supracellular actin cable architecture and leads to a vertical orientation of nuclei toward the bottom of the microwell. Increasing Gaussian curvature results in flattened and elongated nuclear morphologies characterized by highly compacted chromatin states. This approach provides better understanding of the mechanoresponse of curved epithelial monolayers curvatures lining lobular structures. In addition, bowl-shaped microwells offer a powerful platform to study curvature-dependent mechanotransduction pathways in anatomically relevant 3D structures.

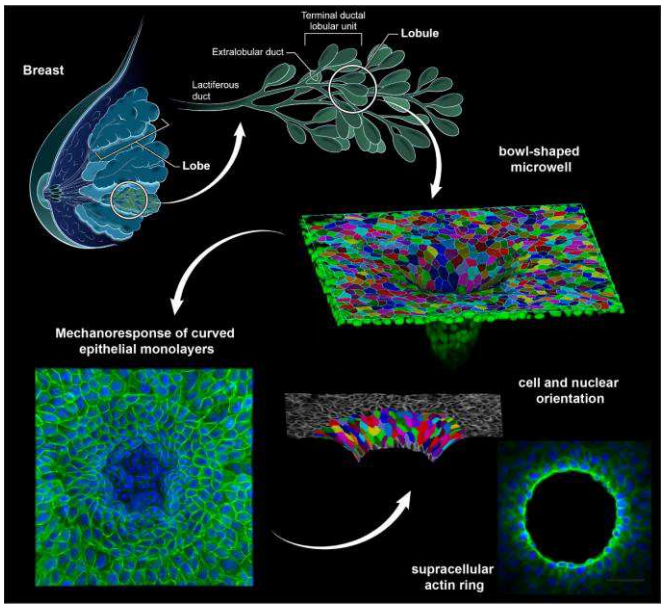

Figure 1: Bowl-shaped microwells that mimic the lobular structures present in many epithelial tissues and organs are used in this work to study the organization and mechanoresponse of curved epithelial monolayers.

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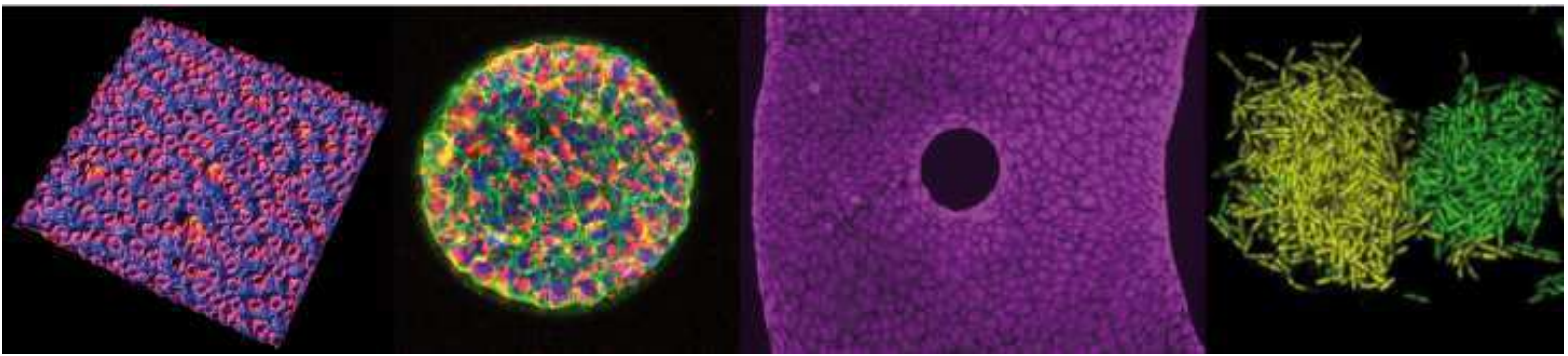

Optimization and historical contingency in protein sequences

Anne-Florence Bitbol[1,2]*

[1]Institute of Bioengineering, School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[2]SIB Swiss Institute of Bioinformatics, CH-1015 Lausanne, Switzerland
*anne-florence.bitbol@epfl.ch

Protein sequences are shaped by functional optimization on the one hand and by evolutionary history, i.e. phylogeny, on the other hand. A multiple sequence alignment (MSA) of homologous proteins contains sequences that evolved from the same ancestral sequence and have similar structure and function. In such an alignment, correlations in amino-acid usage at different sites can arise from structural and functional constraints via coevolution, but also from historical contingency (see Fig. 1).

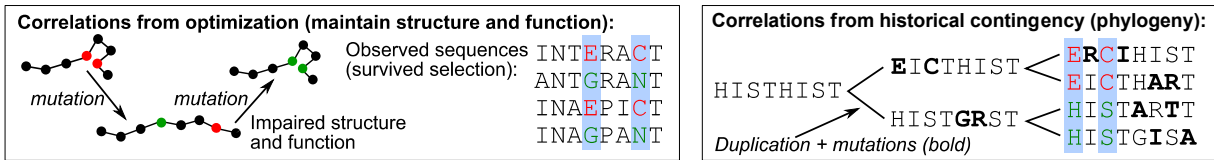

Figure 1: Different sources of correlations in MSAs of homologous proteins. Left: example of a functional constraint, here coming from the need to maintain a given three-dimensional structure. Right: example of correlations arising from common ancestry – two mutations appearing early give rise to correlations, even if these mutations were neutral. Correlated columns in the MSAs are shaded in blue in both panels.

Correlations arising from phylogeny often confound coevolution signal from functional or structural optimization, impairing the inference of structural contacts from sequences. However, inferred Potts models are more robust than local statistics to these effects, which may explain their success [1]. Moreover, phylogenetic correlations can in fact provide useful information for some inference tasks, especially to infer interaction partners from sequences among the paralogs of two protein families. In this case, signal from phylogeny and signal from constraints combine constructively [2], and explicitly exploiting both further improves inference performance [3].

Protein language models have recently been applied to sequence data, greatly advancing structure, function and mutational effect prediction. Language models trained on multiple sequence alignments capture coevolution and structural contacts, but also phylogenetic relationships [4]. These models can be used to predict which proteins interact among the paralogs of two protein families, which improves the prediction of the structure of some protein complexes [5]. In addition, they have promising generative properties [6].

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Mechanical characterization of regenerating Hydra tissue spheres

Thomas Perros[1]*, Anaïs Biquet-Bisquert[1,2], Zacchari Ben Meriem[3], Morgan Delarue[3], Pierre Joseph[3], Philippe Marcq[4], Olivier Cochet-Escartin[1]

[1]Institut Lumière Matière, Villeurbanne, France
[2]Centre de Biologie Structurale, Montpellier, France
[3]Laboratoire d'analyse et d'architecture des systèmes, Toulouse, France
[4]Laboratoire Physique et mécanique des milieux Hétérogènes, Paris, France
*thomas.perros@univ-lyon1.fr

Hydra vulgaris, long known for its remarkable regenerative capabilities, is also a longstanding source of inspiration for models of spontaneous patterning. Indeed, one can cut this animal into several pieces which will reform a full *Hydra* in a few days. In this process, the pieces must define a new organizing axis via biochemical means. Recently, it became clear that this early patterning during *Hydra* regeneration is actually an integrated mechano-chemical process where morphogen dynamics is influenced by tissue mechanics [1]. One roadblock to understand *Hydra* self-organization is our lack of knowledge about the mechanical properties of these organisms. I will present how we combined microfluidic tools for parallelized microaspiration rheological experiments and numerical simulations to characterize these mechanical properties [2]. Experimentally, we observed three different behaviors with increasing applied stress: an elastic response, a visco-elastic one and rupture of the epithelial tissues. Using models of deformable shells, we quantified their Young's modulus, shear viscosity as well as the critical stresses required to switch between these different behaviors. Based on these experimental results, we finally developed a description of the internal tissue mechanics during normal regeneration. Our results provide a first step towards the development of original mechano-chemical models of patterning grounded in quantitative, experimental data.

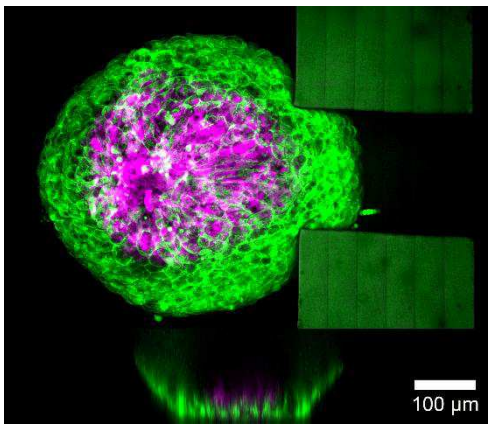

Figure 1: Fluorescent imaging of a Hydra pieces aspirated in a microfluidic tunnel.

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Perturbing and modeling the collective behavior of fish schools

R. Larrieu[1], B. Ventéjou[1], E. Bertin[1], C. Graff[2], P. Peyla[1], A. Dupont[1*]

[1]Université Grenoble Alpes, CNRS, LIPhy, Grenoble, France
[2]Université Grenoble Alpes, CNRS, LPNC, Grenoble, France
[*]Aurelie.dupont@univ-grenoble-alpes.fr

Crowd movements are observed among different species and on different scales, from insects to mammals, as well as in non-cognitive systems, such as motile cells. When forced to escape through a narrow opening, most terrestrial animals behave like granular materials and clogging events decrease the efficiency of the evacuation. Here, we explore the evacuation behavior of macroscopic, aquatic agents, neon fish, and challenge their gregarious behavior by forcing the school through a constricted passage. Using a statistical analysis method developed for granular matter, our results clearly show that, unlike crowds of people or herds of sheep, no clogging occurs at the bottleneck. The fish do not collide and wait for a minimum waiting time between two successive exits, while respecting a social distance. When the constriction becomes similar to or smaller than their social distance, the individual domains defined by this cognitive distance are deformed and fish density increases. We show that the current of escaping fish behaves like a set of deformable 2D-bubbles, their 2D domain, passing through a constriction. Schools of fish show that, by respecting social rules, a crowd of individuals can evacuate without clogging, even in an emergency situation.

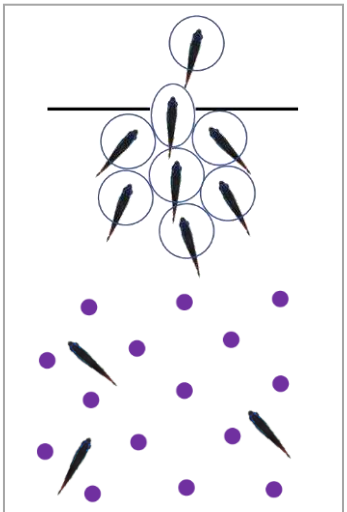

Figure 1: Top. Evacuation of a school of neon fish through a small aperture. Bottom. Perturbing a group of zebrafish with a lattice of obstacles.

In a second experiment, we perturbed a group of zebrafish with a periodic array of pillars and measured their collective behavior when varying the pillars density. When the density is low, the fish regroup with a typical inter-distance and a well-polarized state with parallel orientations, similar to their behavior in open water conditions. Above a critical density of pillars, their social interactions, which are mostly based on vision, are screened and the fish spread randomly through the aquarium, orienting themselves along the free axes of the pillar lattice. The abrupt transition from natural to artificial orientation happens when the pillar inter-distance is comparable to the social distance of the fish. We develop a stochastic model of the relative orientation between fish pairs, taking into account alignment, anti-alignment and tumbling, from a distribution biased by the environment. This model provides a good description of the experimental probability distribution of the relative orientation between the fish and captures the behavioral transition. Using the model to fit the experimental data provides qualitative information on the evolution of cognitive parameters, such as the alignment or the tumbling rates, as the pillar density increases. At high pillar density, we find that the artificial environment imposes its geometrical constraints to the fish school, drastically increasing the tumbling rate.

Altogether, these experiments show the importance of cognition in the collective behavior of fish with behavioral transitions occurring when the preferred social distance between fish is frustrated.

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Microtubule-based active nematic droplets: from patterns to motion

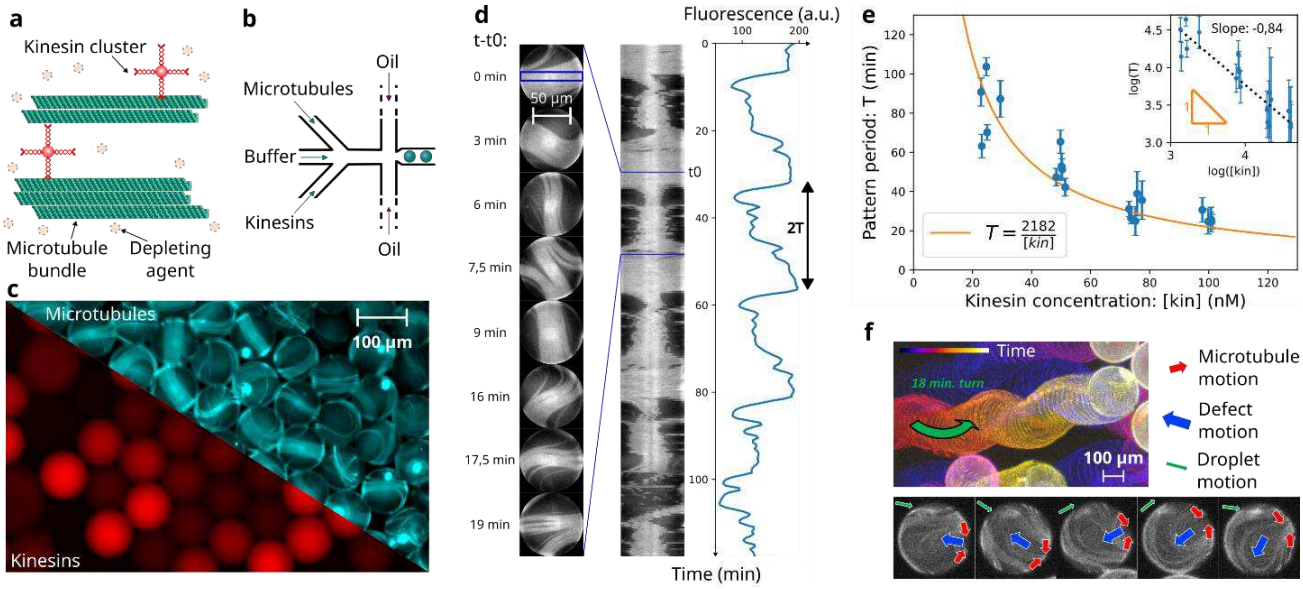
Romain Leroux*, Nicolas Lobato-Dauzier, Samuel Bell, Guillaume Sarfati, André Estévez-Torres, Jean-Christophe Galas

Laboratoire Jean Perrin (LJP), Institut de Biologie Paris-Seine (IBPS),
Sorbonne Université, CNRS, F-75005, Paris
*romain.leroux@sorbonne-universite.fr – 1st year PhD student

Known as the cytoskeleton, networks of active biopolymers dynamically shape the cell membrane. Described by active matter physics, the cytoskeleton has gained the attention of both theorists and experimentalists who developed filament-motor model systems that exhibit remarkable self-organizations [1, 2]. It is also a source of inspiration for the development of self-propelled objects in the biomimetic robotics field.

Here, we encapsulate a microtubule-kinesin based active matter inside water-in-oil droplets. The microtubule bundles spontaneously migrate to the oil-water interface and form an active nematic. Movements of the microtubules on the droplet surface are analyzed by following the movements of topological defects. Repeated sequences of movements are revealed by studying hundreds of droplets layered in a two-dimensional tissue. Once squeezed in between two parallel planes, the droplets acquire motility. Sinusoidal trajectories are observed that matches the nematic motion.

In addition to revealing the self-organization of the microtubule/kinesin system when constrained on a sphere, these results contribute to the engineering challenge of developing and controlling the motion of self-propelled micro-objects.



A spherically constrained microtubule/kinesin active matter exhibits periodic patterns of microtubule movement. (a) Scheme of the active matter formed by nongrowing microtubules bundled together by a depletion agent, and clusters of kinesin motors. (b) Scheme of the microfluidic device used to generate droplets with a fixed concentration of microtubules and a varying concentration of kinesin motor clusters. (c) Fluorescence two-color images of droplets showing microtubules (blue) and a reporter dye standing for the concentration of kinesin clusters (red). (d) Timelapse images showing a period of microtubule movement (left), kymograph showing repetitive sequences of microtubule movement (center), and plot extracted from kymograph, used to determine the pattern period (right). (e) Period of microtubule movement as a function of kinesin concentration, and *1/x* fit determined from a log-log plot (inset). (f) Squeezed droplets move. Sinusoidal trajectories match the periodic microtubule motion.

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Periodic structural collagens increase the resilience of the apical extracellular matrix

T. Sonntag[1,2]*, A. Erlich[3], C. Valotteau[1,4], R. Voituriez[5], N. Pujol[1,2]

[1]Aix Marseille Université, INSERM, CNRS, Turing Centre for Living Systems, Marseille, France
[2]CIML, Marseille, France
[3]Université Grenoble Alpes, CNRS, LIPHY, Grenoble, France
[4]DyNaMo, Marseille, France
[5]Laboratoire Jean Perrin, Sorbonne Université, Paris, France
*sonntag@ciml.univ-mrs.fr

In all multicellular organisms, the skin protects from potential threats and changes in the outside environment and maintains shape. The skin is composed of an apical extracellular matrice (aECM) surrounding an epithelium. If mechanical coupling between basal ECMs and cells and epitheliums have been well characterized, less is known about aECM. In the *C. elegans* worm, the aECM, or cuticle is mainly composed of different collagens arranged in different layers. One specific class of collagens stands out: it forms periodic circumferential structures called furrows along the whole length of the animal. Mutants in these collagens lead to a disordered aECM without furrows, and are therefore called furrow-less mutants. Interestingly, we have previously shown that furrow-less mutants present a persistent immune activation in their underlying epidermis and that their stiffness is different from wild-type worms [1,2]. We therefore hypothesize that the epidermis is capable of probing the mechanical state of the aECM to react to potential threats and that furrow acts as a damage sensor.

To further investigate the role of periodic furrow structures in mechanically coupling the aECM to the epidermis, we are developing a model describing the aECM as an elastic shell under pressure, and in parallel, tests to measure its resistance to stretching. The hydrostatic pressure inside the worms was modified by changing the osmolarity of the medium. We found that, when exposed to hypoosmotic shock, furrow-less mutants burst violently within minutes, while wild-type worms remained intact. By measuring the distance between furrows, we found that the aECM of a wild-type worm is stretched by up to 10% in hypoosmotic conditions. By modeling the worm as a thin elastic shell under pressure based on previous works [3,4], we are able to infer the hydrostatic pressure inside the worm from AFM force-indentation curves. As expected, low external osmolarities lead to higher hydrostatic pressure induced by osmotic shock, due to the water influx towards the interior of the cuticle. We found that furrow-less mutants have a higher hydrostatic pressure than wild-type worms. By using the Van't Hoff law to establish a relationship between external osmolarity and internal pressure in the worms, we are currently testing different saline conditions in which wild-type worms have the same pressure as furrow-less worms. We hypothesize that the absence of periodic furrows in the mutant decreases the stretching capacity of the cuticle which leads to rupture when increasing the internal pressure. Our comprehensive mechanical characterization of the worm skin will provide further insight into the dual role of periodic furrows: enhancing the matrix's resilience and serving as damage sensors.

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Phagocytosis via mannose receptor analysed using chemically functionalised and deformable particles

C. Pompili[1]*, S. Michelis[2], B. Dumat[2], J-M. Mallet[2], J. Fattaccioli[3], F. Niedergang[1]

[1]Institut Cochin, Inserm U1016 CNRS UMR8104 Université Paris Cité, Paris, France
[2]Laboratoire des Biomolécules, CNRS, ENS, Sorbonne Université, Paris, France
[3]Institut Pierre Gilles de Gennes, CNRS, ENS, Sorbonne Université, Paris, France

*chiara.pompili@inserm.fr

Phagocytosis is a cellular process that enables uptake and digestion of particles larger than 0.5 μm, playing a fundamental role in innate immune responses to pathogens and in tissue homeostasis. Phagocytic cells, such as macrophages, display a high variety of surface receptors that allow target recognition and internalisation. Engaged receptors induce signals triggering actin remodelling and phagosome formation. The orchestration of this process has been studied using opsonic receptors (i.e. Fc Receptor and Complement Receptor) as models. However, there are receptors whose phagocytic function is not fully understood. Here we focus on Mannose receptor, a C-type lectin able to recognise glycoconjugates with terminal mannose, fucose or N-Acetylglucosamine, expressed on microbial surfaces.

Our objective is to obtain a better knowledge of Mannose receptor by using deformable lipid droplets functionalised with fluorescent glycolipids bearing mannose. Two glycolipids conjugated to a matched pair of fluorophores to perform FRET were synthesised and characterised [1]. We showed that with FRET coupled to Fluorescence Lifetime Microscopy (FLIM), it is possible to study ligand clustering at the interface of the droplets. Besides, we assessed the specific recognition of these coated particles in primary macrophages. With this tool we are currently investigating, in living cells, Mannose receptor engagement and clustering to evaluate its possible importance in initiating particle internalisation. We are also interested in deciphering downstream signalling activation and actin dynamics that are important to coordinate membrane remodelling and engulfment. In conclusion, here we present our unique methodology with some recent findings regarding mannose-mediated phagocytosis.

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Control of lipid droplet dynamics in cells using engineered condensates

Chems Amari[1,3]*, Damien Simon[1,2], Theodore Bellon[1], Marie-Aude Plamont[1], Juliette Salvaing[3], Abdou-Rachid Thiam[2], Zoher Gueroui[1]

[1]Department of Chemistry, Ecole Normale Supérieure, CNRS. Paris, France
[2]Physics Department, ENS. Paris, France
[3]LPCV, CEA, UGA, CNRS. Grenoble, France
*chems.amari@ens.fr

Understanding the spatial and temporal regulation of lipid droplets (LDs) is crucial for unraveling their roles in cellular metabolism, stress responses, and cell fate [1,2]. However, the mechanisms governing LD dynamics and the tools to manipulate them in cells remain limited.

Here, we have developed ControLD (Controlled Trapping Of LDs), a novel approach to selectively confine LDs using engineered condensates. This method uses a novel system of artificial proteins which can undergo a liquid-liquid phase separation in cells, without perturbating the cell metabolism [3,4]. The trapping of LDs in these condensates contributes to their physical isolation from the rest of the cell, and their release into the cytosol can be induced within a minute time scale. Finally, we have found that the confinement of LDs affects their dynamics, as LD consumption during metabolic demand is greatly perturbed.

We anticipate that ControLD will provide a tool to manipulate LDs more effectively, which could be applied to study lipid droplets biology from an interdisciplinary perspective as well as create a new method to investigate their composition.

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Reverse haptotaxis: Cell migration towards low adhesion

Valentine Seveau de Noray[1,2,*], Xuan Luo[1,2], Ahmad Awada[1,2], Martine Pélicot[2], Marie-Pierre Valignat[1,2] and Olivier Théodoly[2].

[1]Aix-Marseille Université, Marseille, France.
[2] Laboratoire Adhésion et Inflammation, CNRS, Inserm, Marseille, France.
*valentine.seveau@inserm.fr

During the adaptive immune response, leukocytes use self-propulsion and self-steering mechanisms to reach the sites of inflammation. Here, we investigated the ability of T lymphocytes to orient themselves on different adhesive patterns. Adhesive haptotaxis has only been studied with mesenchymal cells and was always found to orient cells towards high adhesion. In this study we consider the case of amoeboid cells with a model of human effector T lymphocytes. Mesenchymal cells require adhesion to migrate whereas leukocytes can swim without adhesion [1]. Nevertheless, using optical micro-patterning [2], we show that lymphocytes are sensitive to the modulation of adhesion and can perform haptotaxis on a wide range of ICAM-1 gradients, including physiological conditions. Moreover, they display a unique phenotype of orientation towards low adhesion mediated by LFA-1, a novel mechanism that has been called reverse adhesive haptotaxis [3]. Existing models of mesenchymal adhesive haptotaxis, either passive (by tug of war mechanism [4]) or active (by integrin mechanotransduction [5]), can only explain orientation towards high adhesion. We therefore explore alternative dynamic mechanisms using mechanical and biochemical perturbations.

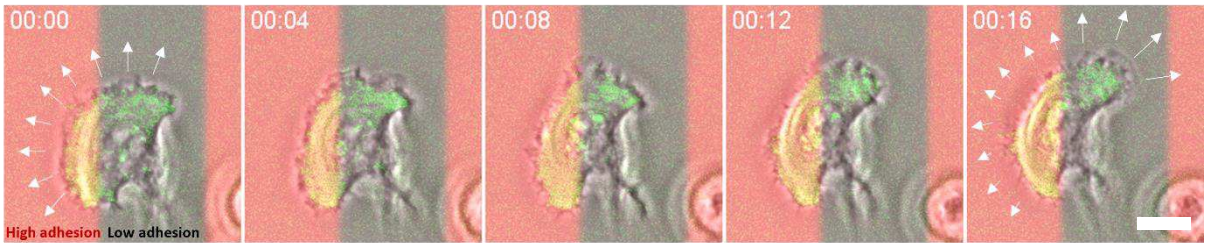

Figure 1 : Images sequence of effector T-cell reorienting towards the less adhesive zone after crossing an adhesive border. Red stripes and grey stripes are respectively the high and low concentration zones of ICAM-1. The adhesive footprint of the cell is shown in yellow/green. Time in minutes : secondes. Scale bar : 10µm.

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Geometry-driven organisation in living matter

Anna Erzeberger
EMBL, Heidelberg

The spontaneous generation of patterns and structures occurs in many living systems and is linked to biological form and function. Such processes often take place on domains which themselves evolve in time, and they can be guided by or coupled to geometrical features. The role of geometry in the self-organisation of functional structures however is not understood. I will discuss approaches and biophysical examples that illustrate how geometry directs spatial organization in different contexts and at different scales.

Effects of conformism in collective decision-making

A. Boussard[1]*, **A. Pérez-Escudero**[1]

[1] Research Centre on Animal Cognition (CRCA), CBI, Université Paul Sabatier, CNRS, Toulouse, France
*aurele.boussard@gmail.com

Abstract

When making a decision, individuals use their private information to evaluate the different options. In a group, they also can take advantage of other group members' private information, itself revealed by their choices. Bayesian estimation offers the tools to model decision-makers that are capable of balancing optimally these two sources of information [1, 2]. This framework is a powerful tool to understand how individuals in a group make decisions. Difficult decisions occur when the private information is uncertain. In such a situation, it is useful to rely on a group of potentially more informed individuals. One of the main indicators of the reliability of a group is its level of agreement (i.e. the proportion of group members that choose the same option). Groups formed by optimal (Bayesian) agents are most reliable when 100% of its members agree on the same choice, because when group members have accurate private information pointing towards the correct option they will naturally agree on it. However, human decision-making has significant biases that we must consider to understand how we should make and assess collective decisions. For instance, when individuals have access to the choices of others, they can conform to them, even if this contradicts their private information. This conformity bias leads individuals to put more trust in social than in private information [3]. We find that, when conformity is taken into account, 100% agreement stops being an indicator of high reliability, because it characterizes groups with high conformity, which are unreliable. Instead, the most reliable groups are those including a moderate proportion of disagreement, because it characterizes groups that don't get carried away in one direction without questioning it.

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Scaling of actin architectures

Alexandra Colin[1]*, Christophe Guérin[1], Anne-Betty N'Diaye[1], Benoit Vianay[1], Alex Mogilner[2], Manuel Théry[3], Laurent Blanchoin[1]

[1]CytoMorpho Lab, Laboratoire de Physiologie Cellulaire & Végétale, Interdisciplinary Research Institute of Grenoble, CEA/CNRS, Grenoble, France
[2]Courant Institute of Mathematical Sciences New York University, New York, USA
[3]Cytomorpholab, IPGG/ESPCI, Paris, France
*alexandra.colin@cnrs.fr

Cells constantly experience environmental changes requiring a fast adaptation of their structures in order to modify shape or type of movement for example. Understanding how cells adjust the size of their dynamic actin architectures in relation to overall cell size is a fundamental question. Due to their complexity, the relationship between the self-assembling properties of actin architectures and their ability to scale with cell size remains poorly understood. Decoupling these parameters to assess their relative contributions in a cellular context is extremely challenging, so we have developed a bottom-up approach to identify the key molecular mechanisms involved in the scaling of dynamic actin architectures. We have developed an experimental system in which polystyrene beads are propelled by an actin comet in a cell-sized microwell containing a limited amount of components. We have established the biochemical conditions to ensure bead movement over several tens of hours [1]. Subsequently, we studied how compartment size, biochemical conditions and number of beads in competition affect comet size and bead velocity at steady state. Thanks to this reductionist approach, we were able to establish some general principles controlling the scaling of actin architectures.

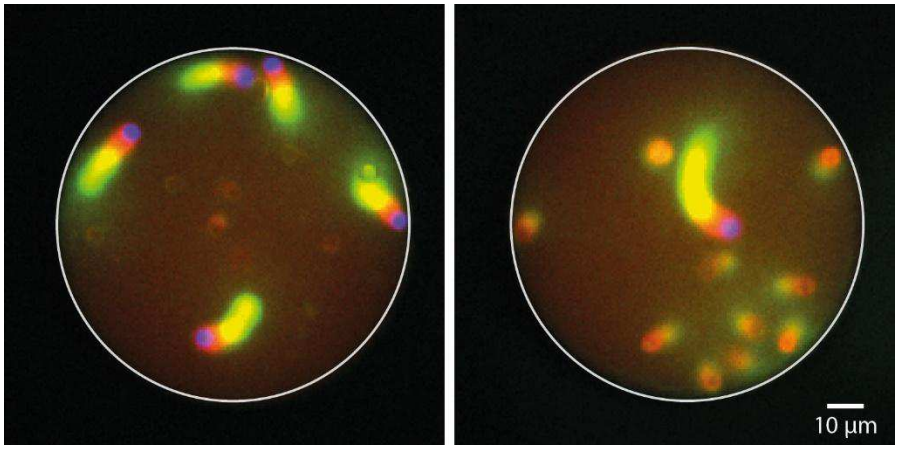

Figure 1: Examples of reconstitution of competitive actin networks in microwells, containing a limited amount of components.

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Wnt ligands mobility in C. elegans embryos

Pierre Recouvreux*, Pritha Pai, Valentin Dunsing, Remy Torro, Monika Ludanyi, Pauline Mélénec, Mariem Boughzala, Vincent Bertrand, Pierre-François Lenne

Institut de Biologie du Développement de Marseille

Aix-Marseille Université, CNRS, Turing Centre for Living Systems

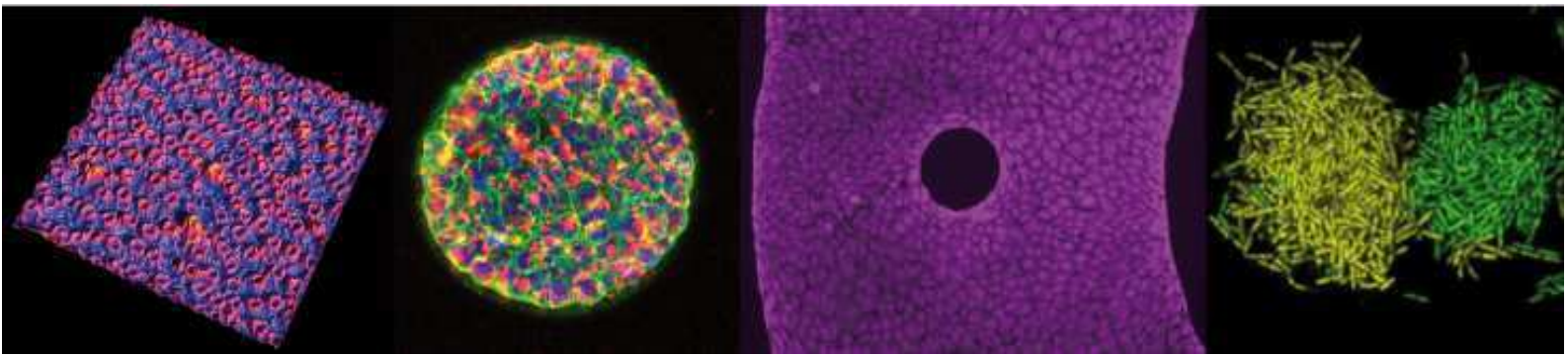
*pierre.recouvreux@univ-amu.fr

Multiple processes in animal development are regulated by signaling molecules that control tissue patterning and cell fates. However, the specific spatial and temporal dynamics of the spreading of these molecules often remain unknown, even though they determine the length scales and time frames of signal activity. The establishment of concentration gradients through morphogen diffusion is a commonly proposed mechanism, despite limited in vivo measurements of these gradients. More generally, the modes of morphogen dispersion in tissue are still unclear, with only a few exceptions. To address these gaps, we investigated the mobility of Wnt ligands, a conserved family of signaling proteins, in *C. elegans* embryos. We demonstrated that Wnts are expressed in the posterior half of the embryo with single-molecule Fluorescence In Situ Hybridization (smFISH). Additionally, through quantitative live imaging of endogenously tagged ligands, we observed their secretion and intercellular dispersion. Importantly, we measured that these ligands can diffuse throughout the tissue in a timescale shorter than the cell cycle. To quantify their diffusion, we determined the diffusion coefficient of Wnt ligands using Fluorescence Correlation Spectroscopy (FCS). Combining our experimental measurements with numerical simulations, we established that the diffusion of Wnt ligands is sufficiently rapid to polarize target cells in the anterior half of the embryo, even at a distance from the Wnt source. Consequently, our findings provide support for a diffusion-based, long-range Wnt signaling mechanism consistent with the dynamics of developmental processes.

approches quantitatives du vivant

Thursday May 16th, 2024

Poster Communications



ERK activation waves and curvature-driven mechanisms in collective cell migration

Zoé Barbier[1], Tsuyoshi Hirashima[2], Marine Luciano[1] and Sylvain Gabriele[1*]

[1] *Mechanobiology & Biomaterials Laboratory, Research Institute for Biosciences, CIRMAP, University of Mons, Place du Parc 20, B-7000 Mons, Belgium*
[2] *Level 9 T-Lab, National University of Singapore, 5A Engineering Drive 1, Singapore 117411*

Collective migration is a key function of many epithelial tissues, playing pivotal role in both physiological and pathological situations. Recent evidence suggests a correlation between extracellular signal-regulated kinase (ERK) activation waves and cell deformation, orchestrating collective cell migration. Specifically, anisotropic contraction induces the unidirectional propagation of ERK activation, which in turn guides multicellular alignment of polarity, facilitating long-range ordered migration. Furthermore, emerging evidence shows that local changes in matrix curvature can influence the migration of individual epithelial cells (curvotaxis). However, the precise mechanism by which local curvature changes modulate ERK propagation and contribute to coordinate collective movement remain unclear. Here we employ a photopolymerization technique to fabricate in soft hydrogels well-defined corrugation patterns of varying wavelengths, mimicking the multiscale curvature of human tissues. Our findings show that the local curvature induces uniaxial collective flow of MDCK cells along the corrugation axis, demonstrating a curvotaxis effect on collective migration. To elucidate the role of ERK waves in this process, we employed Förster resonance energy transfer (FRET)-based biosensors in MDCK cells and conduct time-lapse experiments lasting 24 hours on culture substrates featuring both flat and corrugated zones. Our results reveal that epithelial cells migrate faster on the corrugated part than on flat areas, suggesting regulation of directionality and migration speed by local curvature. Interestingly, we show that ERK signal is highly activated on corrugated parts, indicating a mechanism of intercellular communication responsible for the sustained transmission of directional cues facilitating collective cell migration on curved substrates.

Label-free metabolic imaging and energy costs in Chlamydomonas

Martine Boccara[1,2], Claude Boccara[1] and Benjamin Bailleuil[3]

[1]Institut Langevin, ESPCI Paris, PSL Research University, CNRS UMR 7587, 1 rue Jussieu, 75005 Paris, France
[2] ISYEB, Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université,EPHE, Université des Antilles, 57 rue Cuvier, 75005 Paris, France
[3]Institut de Biologie physicochimique, UMR 7141 CNRS / Sorbonne Université - 13 Rue Pierre et Marie Curie, 75005 Paris, France

We recently described a new non-invasive and non-destructive label free microscopic method that quantifies the dynamic metabolic activity of a cell [1, 3]. The method is label free as the contrast is due to interference between the illuminating beam (light-emitting diode, LED) and the one scattered by cell sub-micrometre structures (scatterers). The method delivers a tomographic image either using a static mode to show the morphology of the biological sample or dynamic mode, which highlights the metabolic contrast within a cell on a movie.

We successfully applied this method to diatoms under environmental stresses (iron or phosphate deficiency) [1]. The cells were immobilized in agar and a film was taken for a few seconds. We then computed the standard deviation of each pixel of the stack. We were able to show in diatoms that the detected dynamic signal was a metabolic signal as it was dependant on photosynthetic activity (signal values dependant of the LED wavelength and of the use of PSII inhibitors) [1] .

We were interested in correlating the dynamic signal within a cell with the energy consumption (expressed in ATPeq) for building macromolecules. We used an autotrophic model organism Chlamydomonas for which mutants are available. We used a mutant deleted of the chloroplastic gene encoding the large subunit of the Rubisco, ΔrbcL, this mutant is unable to fix atmospheric CO_2 and is devoid of pyrenoid [2] . We will present our results comparing the dynamic signal between wild type and ΔrbcL mutant in Chlamydomonas and tempting to associate dynamic signal to the cost in ATPeq consumption for building starch.

The method that we will describe in detail is easy to implement and could be very valuable for a variety of studies.

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Synchronization of contractions in fibroblast microtissues

G. Cappello[1], O. Zajac[2], D. Matic Vignjevic[2], M. Balland[1], Thomas Boudou[1]*

[1]Lab. Interdisciplinaire de Physique (LIPhy), U. Grenoble Alpes, CNRS, F-38000 Grenoble, France.
[2]Institut Curie, PSL Research University, CNRS, F-75005 Paris, France
*thomas.boudou@cnrs.fr

During tumor progression, cancer-associated fibroblasts (CAFs) surround and actively compress cancer cells, which has been shown to regulate cancer cell proliferation [1,2]. Using microfabricated tissue gauges (µTUGs), we found that CAF microtissues contract periodically, on a timescale of a few minutes. We showed that the period and amplitude of these contractile oscillations are dependent on the cell density and myosin activity. By simultaneously assessing intracellular Ca^{2+} levels and local deformations, we demonstrated these contractions are driven by spontaneous intracellular Ca^{2+} oscillations, synchronized over the whole tissue, and we quantified the propagation speed of the calcium and contraction waves throughout the whole tissue. Periodic Ca^{2+} oscillations, and subsequent periodic contractions, require Ca^{2+} release from the endoplasmic reticulum and their propagation is regulated by mechanosensitive ion channels, possibly also by adherens junctions, but not by gap junctions.

Overall, these results highlight the chemical-mechanical coupling of CAFs, which is essential for the continuous compression and remodeling of the tumor niche, which in turn has a direct impact on tumor growth and morphology [3].

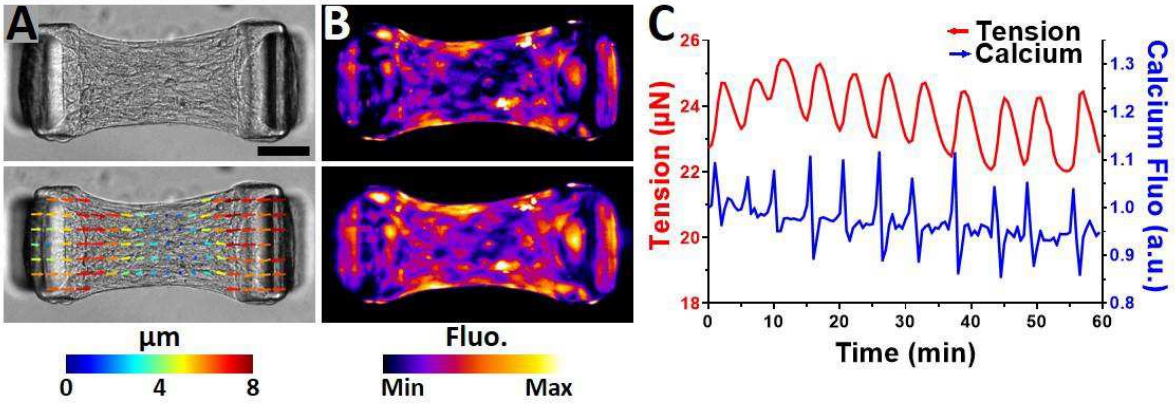

Figure 1: Spontaneous oscillations in CAF microtissues. (A) Displacement field induced by spontaneous contraction in a representative microtissue composed of human cancer-associated fibroblasts (CAF). (B) Calcium fluorescence intensities of the same tissue incubated with the calcium indicator Fluo-4 AM. (C) Temporal evolution of mechanical tension and fluorescence of calcium indicator in a representative microtissue. Scale bar is 100 µm.

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Soft extracellular matrix drives an endoplasmic reticulum stress-dependent S quiescence underlying molecular traits of pulmonary basal cells

[1]Institution, City, Country of First Author
[2]Institution, City, Country of Second and Third Author
[*]email address of presenting author

Cedric Chaveroux[1*], **Alice Nicolas**[2]
[1] : Centre de Recherche en Cancérologie de Lyon, Centre Léon Bérard [Lyon], Université Claude Bernard Lyon 1, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique
[2] : Laboratoire des technologies de la microélectronique, Commissariat à l'énergie atomique et aux énergies alternatives, Centre National de la Recherche Scientifique, Université Grenoble Alpes
* cedric.chaveroux@lyon.unicancer.fr

Soft microenvironment, either in 2D and 3D, preserves the characteristics of progenitors. However, the mechanism by which the mechanical microenvironment determines progenitor phenotype, and its relevance to human biology, remains poorly described. To address this point, we designed multi-well hydrogel plates with a high degree of physico-chemical uniformity to reliably address the molecular mechanism underlying cell state modification driven by physiological stiffness. Cell cycle, differentiation and metabolic activity could be studied in parallel assays, showing that the soft environment promotes an atypical S-phase quiescence and prevents cell drift, while preserving the differentiation capacities of human bronchoepithelial cells. These softness-sensitive responses are driven by defects in proteostasis and enhanced basal endoplasmic reticulum stress. The analysis of available single cell data of the human lung also showed that this non-conventional state coming from the soft extracellular environment is indeed consistent with molecular features of pulmonary basal cells. Overall, this study demonstrates that mechanical mimicry in 2D culture supports allows to maintain progenitor cells in a state of high physiological relevance for easily and robustly characterize the molecular events governing the progenitor biology in human tissues and their evolution across pathophysiological contexts.

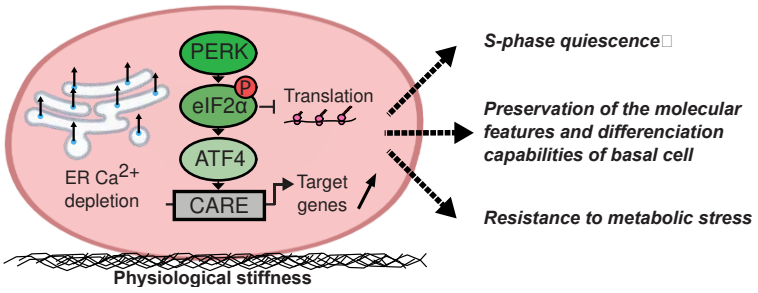


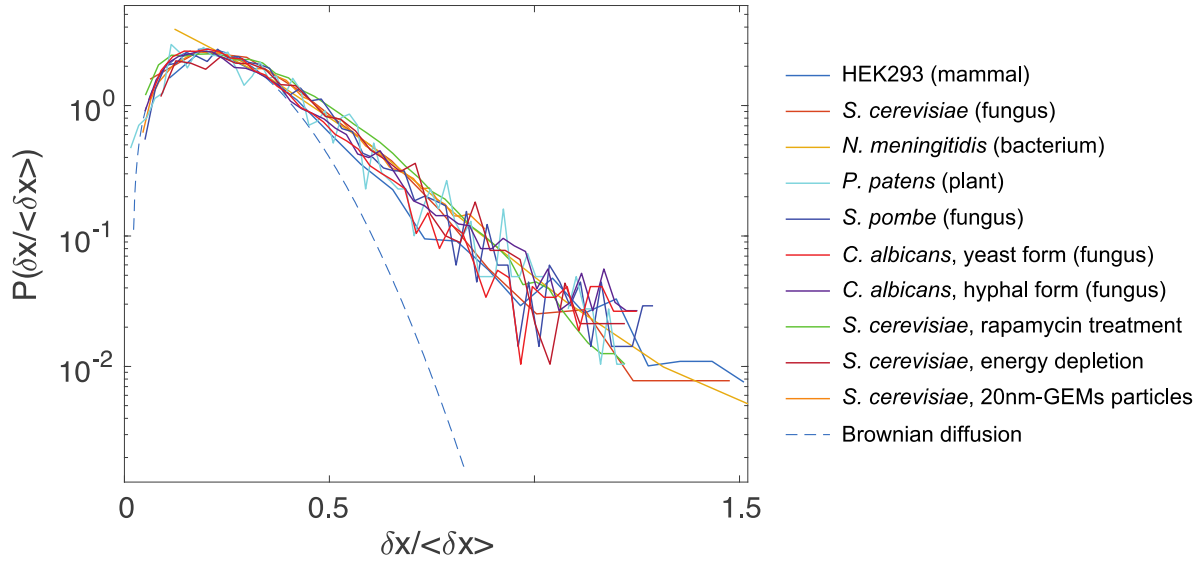
Figure 1: The progenitor state triggered by a soft microenvironment implicates the activation of a proteotoxic stress response.

Univsersal non-Fickian diffusion properties in the living

H. Kim[1], B. Alric[1,2], L. Le Blanc[1,3], L. Albert[1], N. Nguyen[1], L. Holt[4], E. Ilker[5] & **M. Delarue[1,*]**
[1]Laboratoire d'Analyse et d'Architecture des Systèmes, Toulouse, France
[2]University of Tokyo, Tokyo, Japan
[3]Institut Pasteur, Paris, France
[4]Insitute for Systems Genetics, New York City, USA
[5]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany

*mdelarue@laas.fr

The rheological properties of the living are far from being understood, even for fundamental systems such as a cytoplasm. We have shown, using genetically-encoded tracer nanoparticles of 40nm in diameter that the diffusion statistics of these particles in the cytoplasm of different organisms such as plants, animal cells, fungi or bacteria, and for various harsh chemical treatments such as energy depletion, display universal signatures, emphasized by our data showing a long exponential tail in the step size distribution, diverging from a typical Brownian motion.



In this poster, we will discuss the potential origin of this universality. We will show in particular that one needs to be careful when analyzing the statistics of diffusion and examining only the distribution of diffusion coefficients, as this metric is strongly influenced by the optical width of the imaging system, creating a strong statistical bias. We will propose a model based on the natural polydispersity in size in the cytoplasm to explain the various statistics observed in these different organisms.

Unraveling morphological and biomechanical responses to harsh environmental conditions in coccidian oocysts

J. El Husseiny[1,2*], J. Husson[2], R. Torro[1], L. Sedano[3], A. Silvestre[3], P.-H. Puech[1], A. Dumètre[1]

[1] Laboratory Adhesion and Inflammation (LAI), INSERM U1067, CNRS UMR 7333, Aix Marseille University, Marseille, France.
[2] LadHyX, CNRS, Ecole polytechnique, Institut Polytechnique de Paris, Palaiseau, France.
[3] INRAE, Université de Tours, UMR ISP, Nouzilly, France.
*jana.elhusseiny@univ-amu.fr

Coccidia are environmentally resistant parasites with significant implications for global health and economy due to severe tissue infections they can cause in humans and/or animals. Understanding the transmission and host infection by coccidia requires a close examination of the biomechanical responses of their oocyst form to environmental factors and disinfectant treatments.

Our study delved into the effects of a chlorinated household disinfectant and thermal treatment on the oocyst wall, with a focus on *Eimeria* species as a model of coccidia. For this, we employed fluorescence microscopy to quantify the morphological characteristics and autofluorescence of oocysts. To improve automated data processing, a StarDist model was trained to segment and classify oocysts. Complementing our microscopy-based investigation, we used microindentation techniques with a flexible micropipette as a spring to evaluate the rupture force of the oocyst wall under various treatment conditions.

Our microscopy findings show a significant reduction in autofluorescence and oocyst apparent area for both *Eimeria acervulina* and *E. tenella*, with the most dramatic effects found with combined disinfectant and thermal treatments, indicating impaired wall structure. Our innovative micromanipulation system quantified, for the first time to our knowledge, the rupture force of the oocyst wall, which revealed significant variations in *E. acervulina* in response to the same treatments, while *E. tenella* displayed less discernible differences.

This work sheds light on the varied effects of chemical and physical factors on coccidian oocyst features, providing important information about potential changes in responses of such parasites to environmental conditions. Future investigations will be performed to study the correlation between their infectivity and biomechanics.

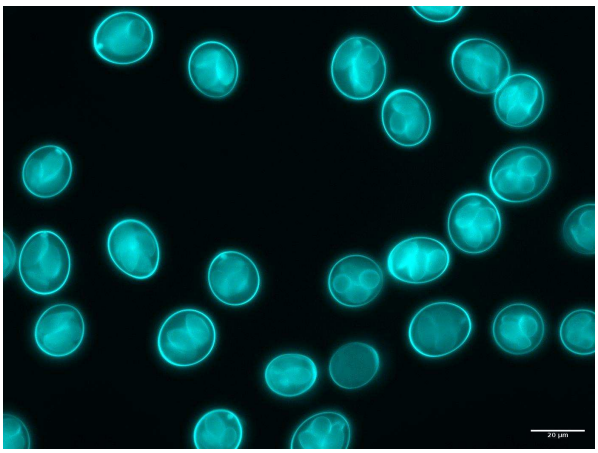


Figure 1: Autofluorescence of the wall of an *Eimeria tenella* oocyst under UV excitation, revealing structural properties

Aggregation dynamics in dense suspensions of paramecia

Dario Dell'Arciprete*, Alexis Prevost, Lea-Laetitia Pontani

Laboratoire Jean Perrin - Sorbonne Université - Paris, France
*email address: darioarci84@gmail.com

In Nature, one can find many examples of unicellular motile microorganisms that, despite their sizes and relatively simple structures, are able to perform a wealth of complex behaviors, can sense the environment and respond to many different types of taxes. For large assemblies of microorganisms, these taxes can lead to remarkable collective behaviors. For algae like *Chlamydomonas reinhardtii* (CR), phototaxis (the ability to respond to light gradients) can lead for instance to branched structures under a certain type of illumination [1]. For bacteria [2], eukaryotic cells like the amoeba *Dictyostelium discoideum* [3], and even CR [4], aerotaxis (the ability to respond to oxygen gradients) has also been shown to trigger propagating fronts towards the oxygen source.

Within this context of biological active matter, I will present some of our latest experimental observations related to another unicellular eukaryote motile organism, the ciliate *Paramecium tetraurelia*: when confined in a *quasi*-2D environment, we observed that a dense population of individual swimming paramecia shows (*i*) an aggregation process at short times (~ 2-3 min) resulting in the formation of clusters of arrested paramecia, followed by (*ii*) a dispersion process consisting in the breakdown of the aggregates in the form of a collective propagation front towards the open boundary of the system (within typically ~ 20 min).

I will discuss our observations within the framework of theoretical models for both aggregation and taxes induced collective motions.

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Stiffening of suspended fibrous micro-tissues by active forces and compressive deformation

Gowthamy Sivakuru[1], Olga Vasiljevic[1,2], Thomas Charles[1,2], Jonathan Fouchard[1]*

[1]Laboratoire de Biologie du Développement, IBPS, Sorbonne Université, Paris, France
[2]Laboratoire Jean Perrin, IBPS, Sorbonne Université, Paris, France
*jonathan.fouchard@sorbonne-universite.fr

Soft fibrous tissues are three-dimensional tissues which serve as scaffolds for our organs at adult age, control transport in the extra-cellular space and participate in morphogenesis during developement. In these tissues, contractile cells live embedded in a porous fiber network called the extra-cellular matrix (ECM). These cells shape the ECM by renewing it (through synthesis and degradation) and by organizing it through traction forces transmitted to the ECM via specific transmembrane proteins. It is shown that such mechanical activity can generate rigidity gradients within the ECM network thanks to the non-linear mechanical properties of collagen fibers, the main component of ECM [1]. Conversely, because of their mechanosensitivity, cells can sense those gradients of mechanical properties and orient their shape and migration accordingly [2]. While large-scale patterns of cells and ECM have been recently observed in fibrous tissue, whether and how such cell-scale mechanical feedback is integrated at the population level to generate tissue-scale organizations and mechanical properties remains poorly understood.

To tackle this question, we culture soft fibrous micro-tissues suspended between parallel rods. This custom-made device permits to apply uniaxial deformations ranging from -50 % to +50 %, while measuring tissue-scale mechanical properties. The device can be coupled to confocal microscopy imaging to provide the cell-scale response to mechanical perturbations and evaluate forces generated at the single cell level.

Using this system, we study the tissue-scale stiffening generated by mesenchymal stem cells within a network of type-I collagen after days in culture, by comparing it to the response of actuated collagen gels. In addition, we demonstrate a stiffening of micro-tissue in response to controlled compressive deformation (Fig. 1) and measure the relative contributions of cell activity and ECM rearrangements in this process.

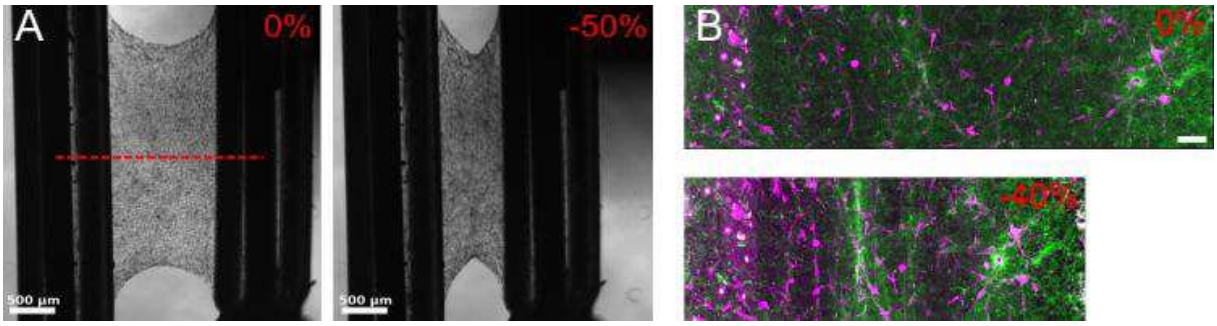

Figure 1: A suspended micro-tissue composed of mesenchymal stem cells (C3H/10T1/2 cell line) embedded in a network of type-I collagen is cultured for 3 days before being subjected to compressive deformation. A- Compression of micro-tissue imaged in bright-field. B- The same experiment imaged by confocal microscopy. Cell membrane is in magenta and ECM organisation is seen in confocal reflection microscopy.

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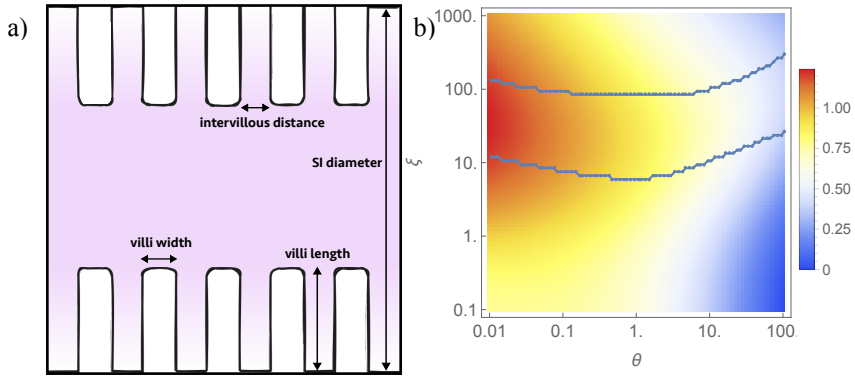
Are the intestinal villi optimally shaped for absorption?

Martin Garic[1]*, Rohan Vernekar[2], Dacil Yanez[2,3],
Stéphane Tanguy[3], Clément de Loubens[2], Claude Loverdo[1]

[1]Laboratoire Jean Perrin, UMR 8237,
Sorbonne Université, Paris France
[2]Laboratoire Rhéologie et Procédés, UMR 5520,
Université Grenoble Alpes, Saint-Martin-d'Hères France
[3]TIMC, UMR 5525,
Université Grenoble Alpes, La Tronche France
*martin.garic@sorbonne-universite.fr

The gastro-intestinal tract's primary function is absorbing nutrients, critical for sustaining metabolic activities and overall health. The small and large intestines are not smooth tubes. The inner surface of the small intestine is characterized by a myriad of small projections known as villi. On the other hand, the large intestine possesses crypts. These roughness magnify the absorptive surface of the small and large intestines.

Are the shape and arrangement of these roughness affecting absorption efficiency in the gastro-intestinal tract optimal for nutrient uptake? Focusing on how particles are absorbed between stationary villi, we show how villi spacing impacts absorption efficiency. We make approximate analytical calculations for the nutrient concentration and absorption flux and we find which inter-villi distance is optimal for absorption. We compare with physiological geometries observed in several animals.



(a) 2D cross-sectional view of the small intestine showing villi. The purple shows a concentration gradient as we go deeper between villi. (b) Phase space showing absorption as a function of dimensionless parameters. There exists optimal villi spacing that maximize absorption.

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Mechanics of the mesoderm during somitogenesis in chicken embryo

Pauline Gehan[1]*, Salomé Berland[1], Léana Lengagne[1], Isabelle Bonnet[1], Carles Blanch-Mercader[1], Karine Guevorkian[1]

[1]Physics of cells and cancer, Institut Curie, Paris, France
*pauline.gehan@curie.fr

During the early development of vertebrate embryos, mesoderm patterning and segmentation is a striking example of the complex interplay between biochemical pathways and cellular mechanics. Somites are multicellular structures arising from the periodical segmentation of the mesodermal tissue (called pre-somitic mesoderm or PSM) at its anterior part. During this process, under the action of opposing gradients of morphogens, progenitor cells gradually differentiate into somitic fate and undergo mesenchymal-to-epithelial transition (MET) from posterior to anterior of the PSM. This transition leads to the segmentation of the tissue and generation of somites. The signaling pathways involved in somitogenesis are well studied but little is known about their role in the mechanics of the PSM segmentation and the physical mechanisms of somite formation at the tissue level.

To bridge the gap between biochemical pathways and tissue segmentation, we focused on the mechanics of the pre-somitic mesoderm while it undergoes mesenchymal-to-epithelial transition. To do so, we used an *ex-vivo* approach in which we extract and divide the PSM of chicken embryos into several explants and assess their spreading dynamics on substrates coated with extracellular matrix. We observed a spatio-temporal dependence of the spreading dynamics as mesenchymal-to-epithelial transition progresses along the antero-posterior axis. With fluorescence microscopy and pharmacological inhibitors of acto-myosin activity, we showed that the transition from a non-wetting to a wetting behavior is mediated by a supra-cellular organization of actin around aggregates. The transition time between the two wetting regimes is regulated by an interplay between cellular contractility and cell migration along the antero-posterior axis during epithelialization. To obtain more quantitative insights of contractility and to probe cell-substrate interactions and cellular forces of cellular aggregates during spreading, we performed traction force microscopy experiments.

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Nuclear Pore Complexe plasticity

K. Gérard[1]*, D. Letkova[2], A. Salvetti[3], L. Lecoq[4], K. Padmanabhan[2], F. Montel[1] & C. Moskalenko[1]

[1] Laboratoire de Physique, CNRS UMR 5672, Ecole Normale Supérieure de Lyon

[2] Institut de Génomique Fonctionnelle de Lyon, CNRS UMR 5242, Ecole Normale Supérieure de Lyon, Université Claude Bernard Lyon 1
[3] International Center for Research in Infectiology, INSERM U111, CNRS UMR 5308, Ecole Normale Supérieure de Lyon, Lyon
[4] Institut de Biologie et Chimie des Protéines, Université Claude Bernard Lyon 1, Lyon

*kassandra.gerard@ens-lyon.fr

The nuclear pore complex is a selective and bidirectional biological pore that regulates exchanges between the nucleus and the cytoplasm [1]. It was shown that the nuclear pore complex dilates and constricts following external cues (energy depletion, osmotic stress, forces) [2] and developmental stages [3]. Here we show two other situations where we quantify its plasticity: under the effect of the circadian time and in interaction with two specific viral capsids: Hepatitis B virus (HBV) and Adeno Associated Virus (AAV). We use Atomic Force Microscopy (AFM) to probe the mechanical properties of those capsids and to visualize and quantify the size of nuclear pore complexes on purified adult mammalian cell nucleus. In addition, for the first time, we probe directly the interaction of AAV and HBV with those nuclei. Our preliminary results suggest different size of pores throughout different circadian time and different nucleus entry mechanisms for those two viruses.

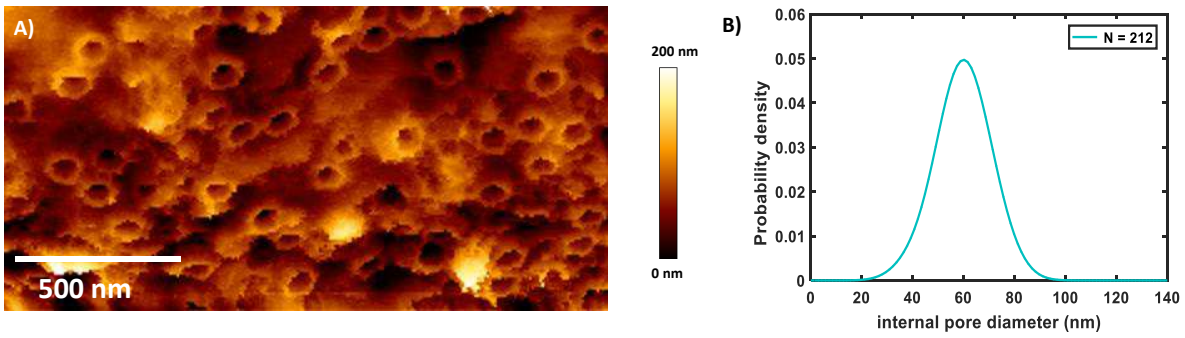

Figure 1: AFM imaging in buffer of nuclear pore complexes on a purified nucleus: A) AFM of a purified murine hepatocyte nucleus imaged in Peak Force mode in buffer. Some nuclear pore complexes are distinguishable. B) Probability distribution function of the nuclear pore complex internal diameter measured by AFM imaging in liquid of 4 different nuclei.

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Regeneration of branched actin filaments in response to mechanical stress

F. Ghasemi[1], LY. Cao[2], M. Mladenov[2], B. Guichard[1], M. Way[2,3], A. Jégou[1], G. Romet-Lemonne[1]

[1]Université Paris Cité, CNRS, Institut Jacques Monod, Paris, France.
[2]The Francis Crick Institute, London, UK.
[3]Department of Infectious Disease, Imperial College, London, UK.
Foad.ghasemi@ijm.fr

Branched actin filament networks play a crucial role in various cellular processes, such as cell motility and endocytosis. Branched actin filaments are formed when an activated Arp2/3 complex binds to the side of an existing filament (mother) and generates a new filament branching off the mother. When branched filaments push against the cell membrane, or as myosin motors pull on them, these filaments experience mechanical forces that affect their dynamics. Furthermore, different actin-binding proteins regulate the stability and the turnover of branches. Despite the importance of the disassembly of branched networks during their turnover, the mechanisms behind branch dissociation are not well understood. Here, we use a microfluidics-assisted TIRF microscopy approach to reconstitute single branched actin filaments from purified proteins *in vitro*. Thanks to microfluidics, we can grow branches, expose them to mechanical forces with different orientations, and examine their dissociation under precisely controlled mechanical and biochemical conditions. We show that the stability of branch filaments is determined by the magnitude of the applied force rather than the force orientation. Furthermore, contrary to the common consensus, we discover that the Arp2/3 complex remains bound to the mother filament upon branch dissociation. Unexpectedly, this surviving force-resistant Arp2/3 complex nucleates a novel branch filament, without requiring re-activation. Moreover, we find that the protein GMF, known for its role in accelerating debranching, regulates branch re-nucleation in a dose-dependent manner. We also quantify different reactions responsible for this re-generation mechanism. These new results shed light on how forces regulate branched actin networks. Our findings suggest that branch re-nucleation contributes to the reorganization of the network and provides a self-repair mechanism, helping branched networks to sustain and adapt to mechanical stress in the cellular environment.

Characteristics of proliferation in a perfused yeast assembly

M. Ghenni[1,2]*, P. Joseph[2], P. Duru[1], M. Delarue[2], O. Liot[1]

[1]Institut de Mécanique des Fluides de Toulouse, Toulouse, France
[2]Laboratoire d'Analyse et d'Architecture des Systèmes, Toulouse, France
*mathieu.ghenni@imft.fr

Bioclogging is a process that leads to the separation of biological particles from a fluid; it has many health or environmental applications and consequences [1, 2]. It results in cell assemblies with emerging properties related to the ones of the living organism composing it [3]: cells are deformable, can proliferate, consume nutrients, and die. These specificities might dramatically affect the clog structure and properties. Couplings between proliferation, clog growth and nutrient perfusion might lead to a nutrient-restricted environment. Bioclogging can thus be used to study the behavior of an assembly of cells under environmental constraints. Our goal will be to study the spatio-temporal characteristics of proliferation in a yeast assembly perfused with nutrients.

The model organism studied is the yeast *Saccharomyces cerevisiae*, which has well-known mechanical and biological properties, and is easy to cultivate. To this end, a quasi-2D microfluidic system has been developed. It consists of a channel ending in a single pore that retains the yeasts and generates a yeast assembly when a flow is applied. We couple this channel with an on-chip flow rate meter. First, we build a yeast clog of a few tens of microns in length, with a fixed pressure. Then we perfuse the clog with a culture solution to deliver nutrients to the yeasts. Due to proliferation, the clog length increases. It is then imaged under a microscope, enabling us to determine its length, motions within it, and proliferation characteristics using dedicated algorithms (see Figure 1).

Two growth regimes are observed for a proliferating clog, associated with two different clog states. In the first stage, clog growth is exponential, associated with quasi-uniform proliferation in the clog. After a few hours, the clog length-time evolution becomes linear-like. We see the appearance of two different zones, one proliferating, the other not. In addition, we can determine local proliferation rates in our clog. These results enable us to highlight the coupling between bio-reactive flow and proliferation within the clog. Proliferation leads to a decrease in flow rate, which in turn leads to a decrease in proliferation rate. A model has been developed to support these observations.

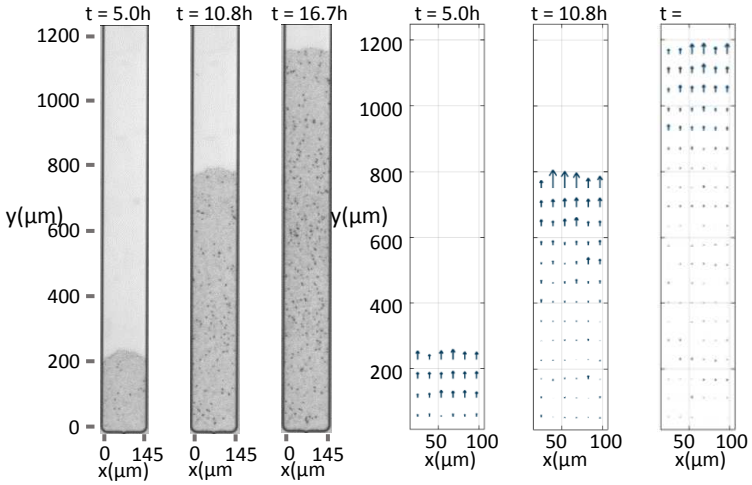

Figure 1 : Images of the yeast assembly at different times (left), and the corresponding displacement speed (right).

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A microfluidic platform for studying actin-based membrane remodeling

Lixin Huang[1]*, Rogério Lopes-Dos-Santos[1], Sid Labdi[1], Guillaume Laumour[1], Olek Maciejak[1], Michel Malo[1], Jacques Fattaccioli[3,4], Clément Campillo[1,2]

[1] Université Paris-Saclay, Univ Evry, CY Cergy Paris Université, CNRS, LAMBE, 91025 Evry-Courcouronnes, France;
[2] Institut Universitaire de France (IUF), 75231 Paris, France;
[3] Pasteur, Département de Chimie, École Normale Supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France;
[4] Institut Pierre-Gilles de Gennes pour la Microfluidique, 75005 Paris, France.

* lixin.huang@universite-paris-saclay.fr

Cell shape changes can be studied *in vitro* using biomimetic reconstituted systems, such as giant unilamellar vesicles (GUVs) coupled to reconstituted cytoskeletal networks. In particular, GUVs with controlled lipid composition allow the study of membrane deformation induced by actin network polymerization. However, studying the dynamics of these shape changes, on many GUVs with the possibility to sequentially add proteins in the assay is a major experimental challenge. To address these issues, we developed a microfluidic chamber to trap and filter GUVs by size and a protocol enabling the spatiotemporal track of the membrane and the surrounding actin network deformation. We characterize the filling of the chamber with GUVs and actin. Then, we monitor the symmetry breaking and the formation of actin comets on homogeneous GUVs. Furthermore, we follow membrane deformation sequences of phase-separated GUVs. Our microfluidic setup and experimental strategy allow for studying interactions between actin and lipid membranes and pave the way for studies on other mechanisms of membrane remodeling.

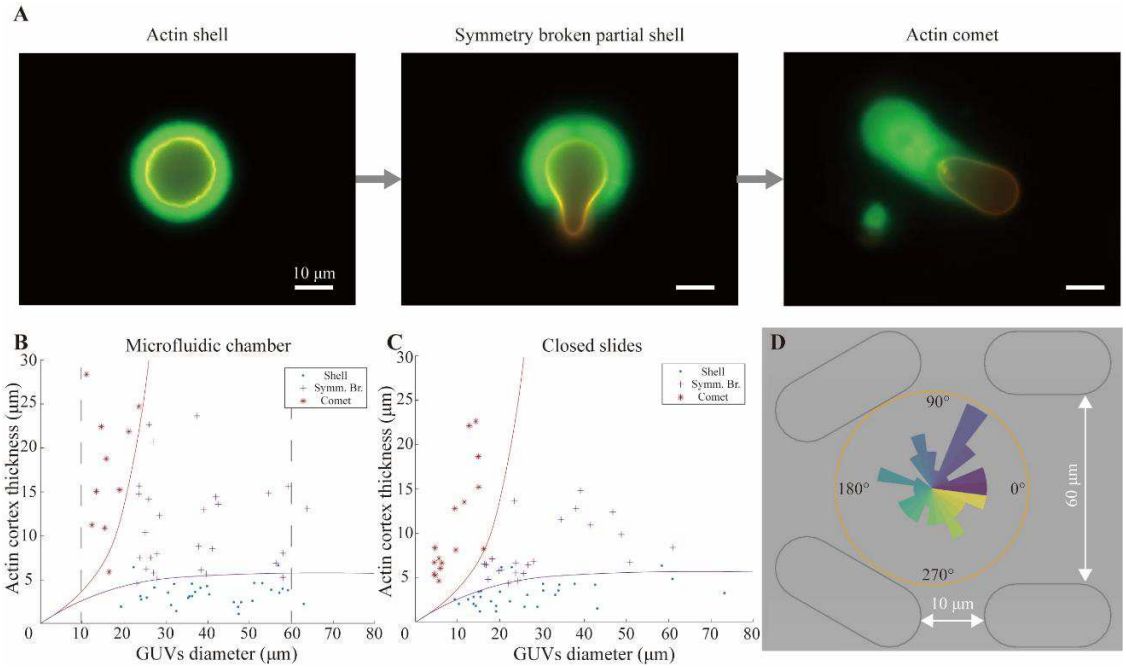

Figure 1: Actin network polymerization in microfluidic chamber on size-filtered GUVs.

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Yohalie Kalukula and Sylvain Gabriele
Mechanobiology & Biomaterials group, Research Institute for Biosciences, CIRMAP, University of Mons, Place du Parc, 20 B-7000 Mons, Belgium

Navigating long confinements requires a switch in cell morphology.

The migration of epithelial cells through narrow environments is a crucial process in tissue development, homeostasis, and diseases such as cancer. However, the mechanisms by which cells adapt to spatial confinement and whether a memory of the confinement can persist are still misunderstood. We investigated the transient migration of epithelial cells by using adhesive dumbbell-shaped micropatterns that lead to repeated back and forth migration events through confined spaces. By tuning the dimensions of the central narrow bridge that connect two squared-shape adhesive sites, we show that the crossing rate and the dynamics of transient migration are both controlled by a morphological switch triggered by the bridge aspect ratio. Indeed, epithelial cells on long and narrow bridges switch from an extended and slow morphology to a compacted and faster phenotype with a steady polarization state, raising the question about the existence of a polarization memory in confined cells. We demonstrate that the morphological switch can be controlled by tuning the area of the adhesive zones that connect to the bridge, allowing us to cancel or maintain the memory of confinement.

Stretching without breaking: How gut epithelium adapts to mechanical challenges to maintain its integrity

Krishnakumar V[1]*, Bernat-Fabre S[1], Cenac N[2], Krndija D[1]

[1]CBI, Toulouse, France
[2]IRSD, Toulouse, France
*Vishnu.krishnakumar@univ-tlse3.fr

Epithelia are specialized tissue barriers, protecting the organism's internal environment from external threats. The colon, uniquely exposed to periodic pressure and stretch from luminal contents (faeces) and muscular contractions, faces challenges to its epithelial monolayer and overall barrier integrity. While the mechanoadaptation of cell-cell junctions is well understood in in vitro models, the in vivo maintenance of intestinal epithelial integrity under diverse mechanical stresses remains largely unexplored. We characterised the effect of static pressure and stretch mediated by faeces on the colonic mucosa and observed a striking increase in junctional recruitment of the apical junctional complexes (ZO-1, E-cadherin and desmoplakin), and peri-junctional non-muscle myosin IIA (NMMIIA), indicating mechanoadaptation. To assess the dynamics of this response, we optimised colonic distension using an inflatable catheter to precisely control the duration and amplitude of the luminal pressure/stretch in vivo. Consistent with faeces-mediated stretch results, we confirmed junctional recruitment of ZO-1, plakoglobin (PG) and desmoplakin (DP) – PG and ZO-1 were recruited rapidly (5 min), whereas DP was more delayed (15 min). Simultaneously, a gradual decrease in apical cell area (20%) suggested an increase in apical cell contractility. Consistently, NMMIIA inhibition or depletion resulted in discontinuous tight junctions and a defective barrier upon mechanical challenge. Furthermore, in a mouse colitis model, colonic distension led to prominent fractures at the tight junction level. Collectively, our results indicate that apical cell-cell

junctions respond to in vivo mechanical challenges by reinforcement via myosin II-dependent recruitment of junctional proteins, highlighting the critical role of NMMIIA in this mechanoadaptive response. Our study provides new insights into the maintenance of epithelial integrity in both health and disease.

Confinement determines transport of a reaction-diffusion active matter front

N. Lobato-Dauzier[1*], A. Maitra[1,2], A. Estevez-Torres[1,3], J.C. Galas[1]

[1] Sorbonne Université, CNRS, IBPS, Laboratoire Jean Perrin, Paris, France
[2] CY Cergy Paris Université, CNRS, LPTM, Cergy-Pontoise, France
[3] Université de Lille, CNRS, LASIRE, Villeneuve d'Ascq, France
*nicolas.lobato-dauzier@sorbonne-universite.fr

Couplings between biochemical and mechanical processes have a profound impact on embryonic development. However, in-vitro studies capable of quantifying these interactions have remained elusive. Here, we investigate a synthetic system where a DNA reaction-diffusion (RD) front is advected by a turbulent flow generated by active matter (AM) flows in a quasi-one-dimensional geometry. Whereas the dynamics of simple RD fronts solely depend on the reaction and diffusion rates, we show that RD-AM front propagation is also influenced by the confinement geometry.

The reaction part uses the PEN DNA Toolbox [1] to produce exponentially a short single strand of DNA (ssDNA) from a template and enzymes (Fig. 1A). Our active gel is made of bundles of protein filaments (microtubules) driven by molecular motors (kinesins) (Fig. 1B), creating chaotic flows lasting for several hours [2]. The reaction mix and active gel are inserted in closed glass capillaries of varying width. An initial injection of ssDNA on one side triggers the RD-AM front (Fig. 1C). We first experimentally dissected the different components of the reaction-diffusion-advection process by knocking out reaction or advection and observed how RD-AM allows for faster transport over large distances, avoiding dilution (Fig. 1D). We then show how confinement impacts active matter flow: while changes in instantaneous flow velocities are small; correlation times are dramatically increased with decreasing confinement. RD-AM front speed increased 3 to 9-fold compared to a RD one (Fig. 1E).

This RD-AM experimental model provides a framework for the rational engineering of complex spatiotemporal processes observed in living systems. It will reinforce our understanding of how macro-scale patterns and structures emerge from microscopic components in non-equilibrium systems.

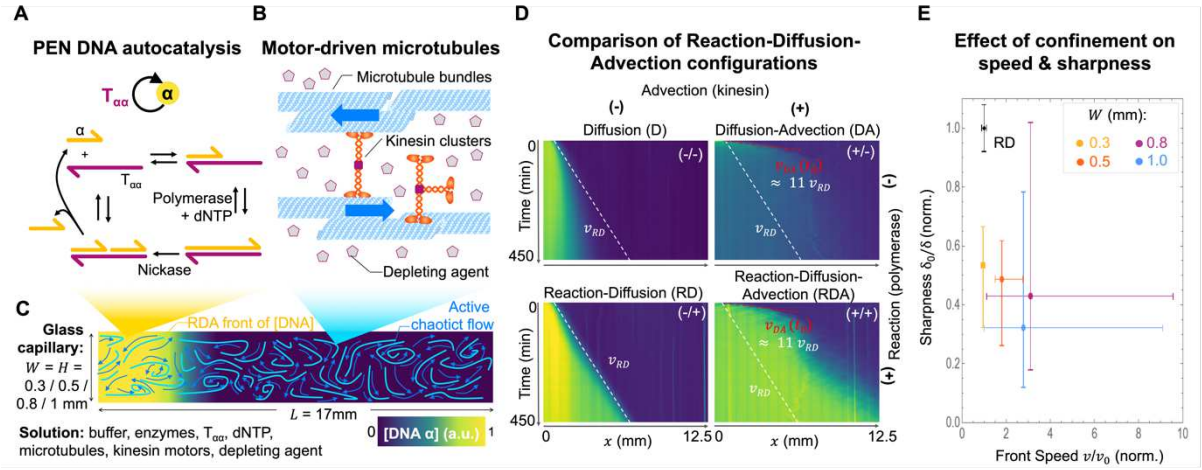

Figure 1 – Experimental setup and its building sub-systems. A: Chemical sub-system. ssDNA is amplified exponentially via a DNA template and enzymes. **B:** Mechanical sub-system. Microtubules bundles slide apart driven by kinesin motors. **C:** Experimental setup. Active chaotic flows interact with a [DNA] front in a closed glass capillary. **D**: Kymographs of DNA concentration of the RD-AM system in different reaction-diffusion-advection regimes. **E.** [DNA] front sharpness vs speed for varying channel widths. The dots and the bars correspond respectively to the mean and the extrema values.

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Modelling and Inferring Protein Dynamics in Fission Yeast Mechanosensing

Enrico Lorenzetti*[1], Antoine Fruleux[2], Arezki Boudaoud[1]

[1]École Polytechnique, Palaiseau, France
[2]Université Paris-Saclay, Orsay, France
*enrico.lorenzetti@polytechnique.edu

Mechanical forces play an important role in determining the growth and the shape of a cell, yet they can also be a potential cause of damage. Indeed, cells are endowed with mechanosensors, i.e. receptors at the subcellular scale able to detect mechanical stimuli. In fission yeast, the transmembrane protein Wsc1 is such a mechanosensory. It stimulates glucan synthesis to reinforce the cell wall, the protective thin layer that surrounds the cell. Interestingly, Wsc1 clusters in the region of the cell wall where stress is applied.

This work aims at describing clustering by building a mathematical model based on reaction-diffusion equations that take into account the interactions between cell wall polysaccharides and Wsc1. To quantify the dynamical parameters of this model, we developed a new inference method for Fluorescence Recovery after Photobleaching (FRAP) experiments which only requires minimal hypotheses. Thanks to the flexibility of this method, it is possible to carry out the experiment with the Wsc1 protein as the cell wall is under compression and obtain accurate protein mobility estimations as a function of mechanical stress.

This study offers fresh methodologies for quantifying and comprehending intricate protein dynamics within cells and tissues.

Self-sustained velocity waves and pattern emergence in tissues

G. Marquez-Vivas[1]*, M. Dolega[2], C. Guilluy[2], P. Recho[1], T. Boudou[1], M. Balland[1], G. Cappello[1]

[1]Laboratoire Interdisciplinaire de Physique, Grenoble, France
[2]Institut pour l'Avancée des Biosciences, Grenoble, France
*genesis.marquez-vivas@univ-grenoble-alpes.fr

Multicellular arrangements govern a diverse range of morphogenetic events, relying on complex interplay between chemical and mechanical signals that contribute to supra-cellular organization. These mechanical cues can initiate various biological processes, including embryonic development, tissue repair, and regeneration. Recent studies have underscored the presence of long-range mechanical excitations at the supra-cellular level. These waves manifest in epithelial sheets independent of cell proliferation [1] and are correlated with oscillations in the forces exerted by cells on the substrate [2]. Remarkably, our recent observations indicate that they exhibit a distinct wavelength significantly larger than the cell size and a period several times shorter than the typical duration of a cell cycle [3]. Here, we confined epithelial monolayers using micropatterns and determined the critical dimension under which the whole monolayer migrates to and fro, and above which the monolayer oscillates as a standing wave. In the latter case, cells at wave nodes undergo repeated compression and stretching, while those at antinodes undergo alternating back-and-forth movements (Fig. 1). We hypothesize that periodic stretching may reorganize the cell cytoskeleton, a process dependent on the amplitude and period of the strain, and that cells positioned at different locations may thus experience varying mechanical stimuli at the timescale of cell division and differentiation. In this study, we investigate whether periodic mechanical stimulation induces spontaneous transcriptomic divergences between cells positioned at wave nodes and those at antinodes. To accomplish this, we utilized a photoactivatable strain of MDCK cells that transition from a dark state to red fluorescence upon activation with violet light [4]. These cells were cultured on micropatterns before illuminating specific locations of the monolayer. Consequently, we were able to sort cells from nodes and antinodes, and we are currently analyzing their respective transcriptomes. Overall, our findings may shed light on how mechanical signals propagate and translate into effective tissue formation.

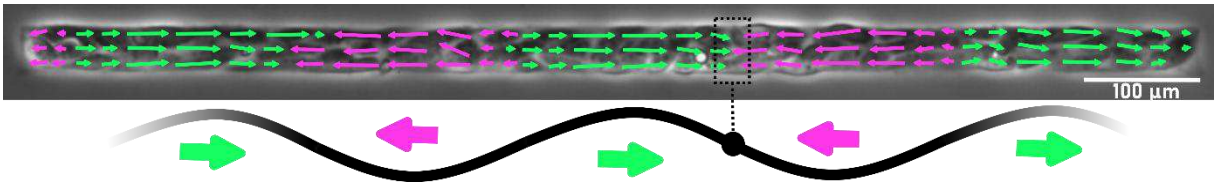

Figure 1: Confined epithelium displaying oscillatory motion. In the top panel, the relative velocity field is visualized using Particle Image Velocimetry. Velocities directed along the positive x-axis are depicted in green, while velocities along the negative x-axis are shown in pink. In the bottom panel, a standing wave representation, with a rectangle denoting a node within the wave.

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Design of vesicle prototissues with controlled mechanical properties and activity

Nishant Nair[1]*, Jordi Ignés[2], Francesc Sagués[2], Gladys Massiera[1], Laura Casanellas[1]

[1]Laboratoire Charles Coulomb, Université de Montpellier, France
[2]SOCSAM, Dept. Química-Física, Universitat de Barcelona, Spain
*nishant.nair@umontpellier.fr

Studying the mechanical properties of living cells and tissues is important for several biomedical applications as well as from a fundamental perspective. Given the inherently complex nature of these systems it becomes necessary to have simpler biomimetic cells and tissues which have a lower degree of complexity. Giant unilamellar vesicles (GUVs) are one of the widely used prototypes in such studies [1]. These are model systems for biological cells, containing an aqueous material inside a lipid membrane. Such individual GUVs can be assembled into prototissue structures by using receptor-ligand bonding (e.g. Biotin-streptavidin) [1].

In order to tune the mechanical properties of GUVs and their assemblies, an elastic material (agarose gel) can be encapsulated inside the vesicles [2]. By changing the elasticity of the gels (varying the agarose concentration), the mechanical properties of the vesicles and thereby the prototissues can be fine-tuned. In order to make prototissues akin to actual living cells, it would be necessary to provide vesicles the ability to display active dynamics [3]. For this purpose, we encapsulate an active nematic gel constituted of microtubules and kinesin motor clusters inside vesicles using the cDICE technique [4, 5]. This technique is suitable for optimally encapsulating the active material and for limiting the polydispersity of vesicles. Preliminary results of vesicle prototissues with controlled mechanical properties and activity will be presented in this poster communication.

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Quantitative analysis of the mechanical properties of healthy and cancer lung tissue for the design of mechano-mimetic culture substrates

F. Delebosse[1,2], D. Fuard[1], S. Soulan[1], C. Perrin[3,4], L. Chalabreysse[3,4], C. Migdal[2], A. Nicolas[1*]

[1]University Grenoble Alpes, CNRS, CEA/LETI Minatec, Grenoble Institute of Technology, Laboratory of Technology of Microelectronics, Grenoble, France
[2] Cell&Soft, Grenoble, France
[3] Department of Pathology, Groupement Hospitalier Est, HCL, Lyon, France
[4] University of Lyon, Université Claude Bernard Lyon 1, 69100 Lyon, France
[*]alice.nicolas@cea.fr

Mechanical properties of tissues are increasingly recognized as crucial in disease progression. Here we investigate the mechanical properties of normal and adenocarcinoma lung tissues from 18 patients using indentation-type atomic force microscopy. We show that these tissues exhibit a predominant linear elastic behavior. Microscale tissue stiffness and shape descriptors of stiffness texture are extracted from maps of the Young's modulus. Furthermore, a correlation between tissue composition and stiffness is performed. Combining these parameters with photolithography, stiffness-textured polyacrylamide hydrogels are engineered, resulting in culture substrates that mimic the tumor tissue's stiffness distribution. By culturing A549 cells on these hydrogels, the influence of substrate stiffness texture on cellular behavior is evaluated. The development of this versatile mechanomimetic platform reveals its potential applicability to other human tissues and is envisioned as an in vitro model to improve the predictability of drug screening.

Mechanotransduction in integrin-mediated phagocytosis

M. Depierre[1], A. Mularski[1], A. Ruppel[3], C. Le Clainche[2], M. Balland[3] and **F. Niedergang[1*]**

[1]Université Paris Cité, Institut Cochin, CNRS, INSERM, F-75014 Paris, France
[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)
[*]Florence.niedergang@inserm.fr

Phagocytosis is a mechanism of internalization and degradation of micro-organisms or cellular debris. This process is important for remodeling of tissues, disposal of dead cells as well as bacterial clearance. It depends on actin polymerization for particle engulfment and phagosome formation. In the organism, phagocytosis is mainly performed by professional phagocytes, such as macrophages. These cells are present in almost all tissues of the body and are thus exposed to environments with very different mechanical properties. Tissue mechanics can also be altered in some disease conditions. But how mechanical cues affect the ability of macrophages to phagocytose remains elusive. In addition, it is not known how phagocytosis by itself can influence how phagocytic cells interact with their environment.

We studied phagocytosis mediated by the complement receptor 3 (CR3, also known as $\alpha_M\beta_2$ or CD11b/CD18), which is a major phagocytic receptor for many pathogens and debris. It belongs to the integrin family of proteins and, as such, has mechanosensitive properties particularly relevant for mechanical studies.

We first found that the capacity of primary human macrophages to perform phagocytosis increased with the stiffness of their substrate. Using live traction force microscopy, we showed that phagocytosing macrophages displayed different contractile energy from non-phagocytosing cells, demonstrating that they have more dynamic interactions with their substrate. In addition, we found that phagocytosis led to a loss of F-actin adhesion structures, suggesting that macrophages become transiently less adherent during phagocytosis. Overall, these results highlight the existence of a novel mechanotransduction pathway in macrophages during integrin-mediated phagocytosis, with a connection between the dorsal side of the cells (phagocytosis) and their ventral side (adhesion to the substrate). We are investigating the molecular players that are involved in this unique process. Ultimately, this study sheds new light on a vital function of immune cells in the context of their environment.

Chirotaxis: matrix chirality modulates the cell migration speed.

Alexandre Remson[1, 2]*, Mathieu Surin[2], Sylvain Gabriele[2]

[1]Mechanobiology and Biomaterials Lab, University of Mons, CIRMAP, Research Institute for Biosciences, Belgium.
[2]Laboratory for Chemistry of Novel Materials, CIRMAP, University of Mons, Belgium
*Alexandre.remson@umons.ac.be

Chirality is a pervasive phenomenon in Nature, influencing essential processes like molecular recognition and self-organization [1]. Critical biological events, such as cell proliferation and migration, occur in interaction with the components of the extracellular matrix (ECM) [2]. While extensive research has explored the impact of physico-chemical properties of the cell microenvironment on cellular migration [3], the influence of the ECM chirality has been largely overlooked. To address this gap, we created precisely controlled culture surfaces coated with collagen I as a natural matrix or biomimetic matrices composed of collagen-mimetic-peptides (CMPs) with opposite chirality, using L vs. D amino acids. Circular dichroism confirmed a polyproline type II helix (PPII) conformation of the chains, with distinct chirality for the two biomimetic peptide surfaces. Here we show that D-surfaces hinder the complete spreading of epithelial keratocytes, rendering them less spread and more rounded, highlighting the sensitivity of keratocytes to ECM chirality. Notably, our findings demonstrate that cells migrating on D-surfaces exhibit reduced migration speed compared to those on collagen I and L substrates. To delve into the impact of molecular chirality on cellular mechanotransduction pathways, we characterized focal adhesions and employed specific inhibitors targeting collagen-binding integrin receptors during migration assays. This study sheds light on the previously overlooked influence of ECM chirality on cellular behavior and opens avenues for understanding the intricate relationship between molecular chirality and cell migration dynamics.

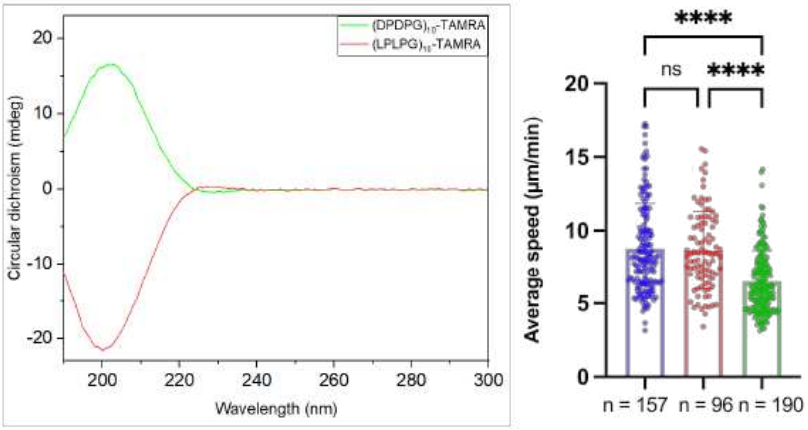

Figure 1: Left: Circular dichroism spectra of L and D peptides. Right: Average migration speed on collagen (blue), L-substrates (red) and D-substrates (green).

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Mechanical Stability of Multicellular Assemblies

Daniel Selma[1]*, Vladimir Misiak[1], Simon De Beco[2], Giovanni Cappello [1], Thomas Boudou[1], Martial Balland[1]

[1]Laboratoire Interdisciplinaire de Physique (LIPhy), France
[2]Institut Jacques Monod, France
*Daniel.selma-herrador@univ-grenoble-alpes.fr

Cell intercalation –or T1 transition– is a crucial process in morphogenetic events, where cells exchange positions by remodeling their cell-cell and cell-substrate adhesions. It allows a dynamic spatial redistribution of cells in parallel with keeping their integrity and collective cohesiveness as a tissue. Most of the experiments performed to assess this process are done *in vivo*, where mechanical readouts are indirectly retrieved from image analysis. Therefore, a complete understanding of cell intercalation is still lacking.

To address this gap, we designed a novel *in vitro* assay to simplify the system, by making a compromise reducing the biological relevance while allowing the imaging of specific molecular pathways and the quantitative measurement of forces involved. Practically, assemblies of four cells – cell quadruplets– were arranged *in vitro* to mimic the "*minimal tissue pavement*" that exists *in vivo*, i.e. the most basic structure that makes possible the T1 transition. To this end, a variety of extracellular matrix (ECM) architectures were micropatterned on treated glass coverslips. Subsequently, those patterns were seeded with Madin-Darby Canine Kidney (MDCK) cells, which self-organized into cell quadruplets owing to the optimization of the geometrical boundary conditions of the patterns. With such minimalistic system, *in vitro* cell intercalation events were recorded in two different patterns, although with a rare frequency. Thus, this approach allows the morphological characterization and the mechanical assessment of the dynamics of cell quadruplets with different bioarchitectures, over their lifetime and during the T1 transition.

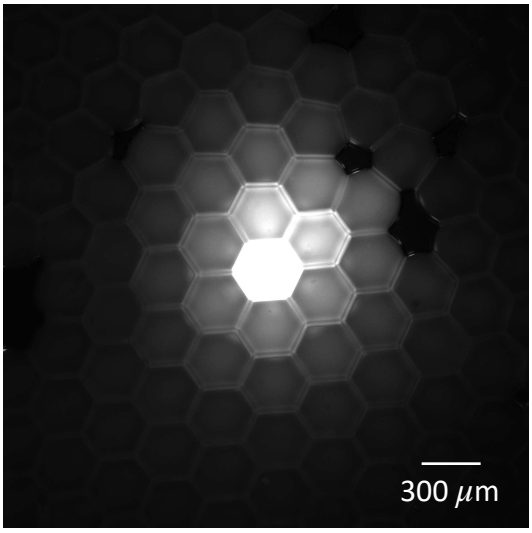
Molecular transport in 2D biomimetic tissues

Cécile Vincent[1]*, Sapna Ravindran[1], Alexis Prevost[1], Lea-Laetitia Pontani[1] , and Elie Wandersman[1]

[1]Laboratoire Jean Perrin, UMR 8237, Institut de Biologie Paris Seine, Sorbonne Université/CNRS, Paris, France
*cecile.vincent@sorbonne-universite.fr

In tissues, cells that are in direct physical contact with each other can exchange molecules via protein clusters called gap junctions, which form nanometric channels across the membranes of adjacent cells. Molecular transport through gap junctions has been observed in biological tissues but the precise physical mechanisms who control it are still unclear. To address this end, we have developed a simplified biomimetic approach : tissues are mimicked with 2D arrays of aqueous droplets bathing in an oil and lipid phase. Once brought into contact, the droplets connect by lipid bilayers called Droplet Interface Bilayers (DIBs) [1]. Transmembrane nanopores can be inserted in the bilayers to mimic the gap junctions.

Using a biomimetic system and epifluorescence microscopy, we have quantified the diffusion of calcein across the DIB network, as the membranes are decorated with inert transmembrane proteins (alpha-Hemolysin, forming a simple nano hole) at various concentrations. Our results are directly confronted with statistical physics models of molecular transport based on continuous time random walks. Finally, we will present the synthetic biology methods we are currently developing to insert mechanosensitive proteins in the DIB membrane, to obtain a stress dependent molecular permeability, as observed in certain types of gap junctions.



Fluorescence image of a 2D Droplet Interface Bilayer network, the lipid membrane of which are decorated with inert αHL pores. A fluorescent droplet loaded with calcein was placed at the center at t=0. The image shows the diffusion of calcein at t=15 hr.

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Artificial Touch Mechanoreceptors : design, functionalization and mechanical response

P. Tapie, A. Prevost, L.-L. Pontani and E. Wandersman*

Sorbonne Université, CNRS, Institut de Biologie Paris-Seine (IBPS), Laboratoire Jean Perrin (LJP), 4 place Jussieu, F-75005 Paris, France.
*elie.wandersman@sorbonne-universite.fr

Touch mechanoreceptors are neural cells embedded in the skin whose plasma membrane is decorated with mechanosensitive transmembrane nanopores [1,2]. Upon mechanical excitation of the skin, the ion permeability of these mechanosensitive nanopores is modified and yields to an ion flux across the mechanoreceptor's membrane, which can be the precursor of an action potential. If the physiologies of touch mechanoreceptors are well characterized, the precise physical mechanisms yielding to membrane depolarization remain to be unraveled. The existence of a static mechanical response threshold is unclear, as well as the relationship between a dynamical mechanical excitation amplitude and frequency and the pore open/closed time distribution. Experimental measurements of ionic currents in cultured mechanoreceptors under mechanical stimulation are usually performed in vitro. The mechanical stimulation is however localized, intrusive and does not reproduce the in vivo conditions such as the existence of a surrounding soft layer, which transmits all stresses. In addition to this, the stimulations likely produce a spatially inhomogeneous response of an unknown number of pores.

To address these questions, we mimic the functioning of touch mechanoreceptors by lipid vesicles embedded in an agarose gel, mimicking the surrounding skin [3]. This system is produced using a very simple fluidic method which can be performed with a usual micro-pipette in melt agarose, yielding, upon gelation to a pseudo-vesicle trapped in the gel (a vesicle with an oil cap on top). We have imaged the trapped vesicles using confocal fluorescence microscopy and we provide evidence of the lipid membrane presence and integrity. Furthermore, we will show that transmembrane nanopores can be inserted in the membrane, using synthetic Biology protein expression methods. To probe their insertion, we have measured the transport properties of fluorescent markers across the membrane of such nanoporous vesicles, using epifluorescence microscopy. Last, we will show that the vesicles can be mechanically deformed by indenting the gel surface and discuss how stresses anisotropy and fluctuations can encode tactile signals.

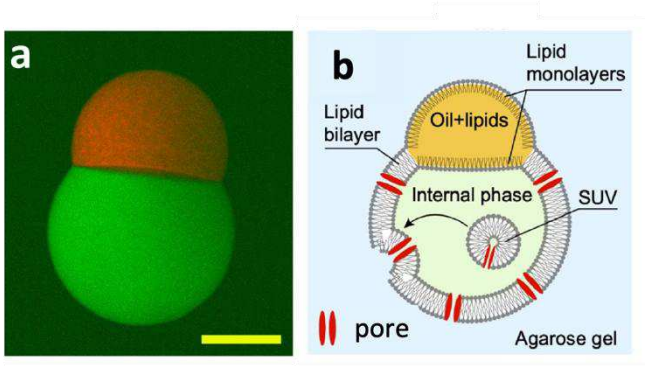

Figure 1: a) Confocal microscope image of a pseudo-vesicle (inner aqueous phase in green contains carboxy-fluorescein, oil cap in red contains Nile Red fluorophore) embedded in an agar gel. Scale bar 200 µm. b)Sketch of the pseudo vesicle, and method to insert transmembrane nanopores via Small Unilamellar Vesicles (SUV) insertion.

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approches
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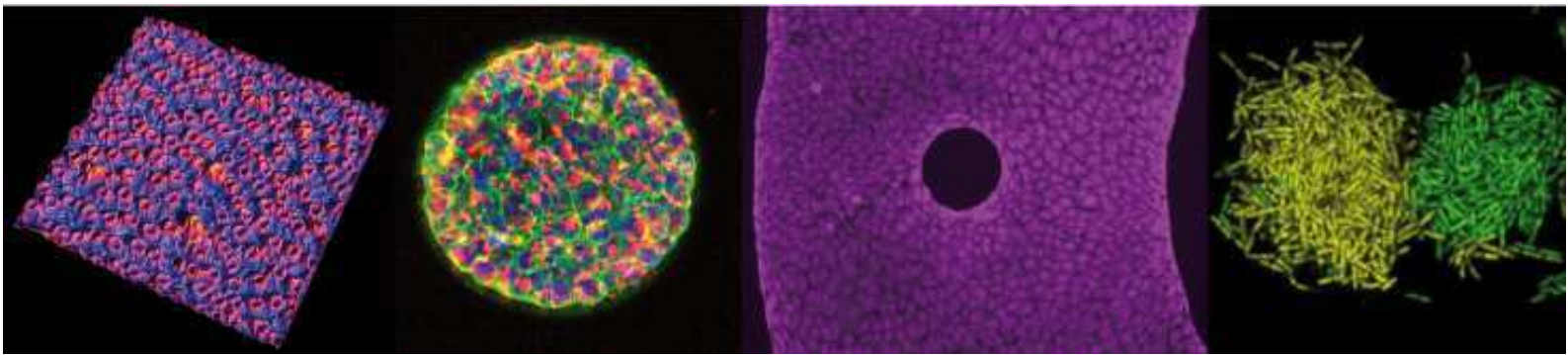
Friday May 17th, 2024

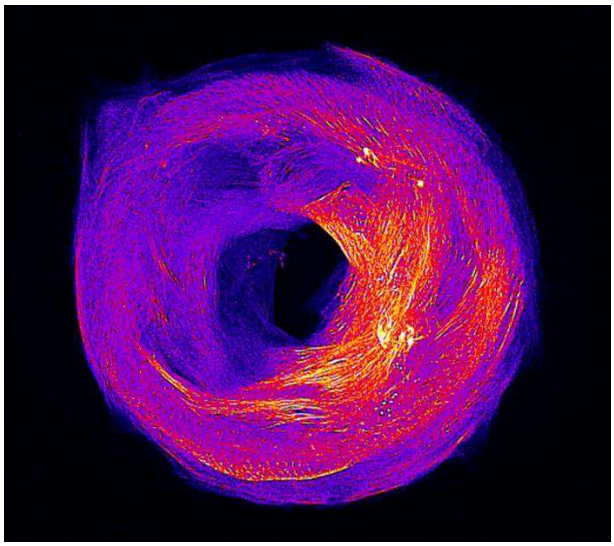
Oral Communications



Collective rotations : experiments and theory

Daniel Riveline
Université de Strasbourg, CNRS, IGBMC, France
https://riveline.igbmc.science

Cells, tissues and organs can rotate spontaneously *in vivo* and *in vitro*. These motions are remarkable for their robustness and for their potential functions. However, physical mechanisms coordinating these dynamics are poorly understood.
I will present two examples of spontaneous rotation with experiments synergized with theory. In a first study, we show that cell doublets rotate in a 3D matrix and we identify mesoscopic structures leading the movement. Our theoretical framework integrates consistently cell polarity, cell motion, and interface deformation. We also report that the Curie principle is verified in its symmetry rules. In a second experiment, we show that rings of epithelial cells can undergo spontaneous rotation below a threshold perimeter. We demonstrate that the tug-of-war between cell polarities together with the ring boundaries determine the onset to coherent motion. The principal features of these dynamics are recapitulated with a Vicsek model. Altogether both examples could set generic rules to quantify and predict generic motion of tissues and organs.



A developmental system's view on tooth identity evolution

Sophie Pantalacci[1]*, Klara Steklikova[2], Océane Chanel[1], Claudine Corneloup[1], Laurent Guéguen[3], Marie Sémon[1]

[1]LBMC/ENS Lyon, Lyon, France
[2]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic
[3]Univ Lyon, Université Claude Bernard Lyon 1, CNRS, Laboratoire de Biométrie et Biologie Evolutive, F-69100, Villeurbanne, France
*sophie.pantalacci@ens-lyon.fr

Serial organs are similar organs found at different places in the body, such as fore/hindwings in insects or different teeth in mammals. They can have different shapes depending on their position in the body, associated with different functions. This identity is specified during development by a handful of specifically expressed genes which bias the developmental trajectory towards a specific final shape.
This final shape can evolve quite rapidly between species as organ function changes. Therefore it is often difficult to identify one organ from its shape only, without knowing its position in the body or having access to its phylogenetic history. This is well illustrated by anterior and posterior wings in insects (e.g. elytra/wings; wings/haltera) or lower and upper molars in mammals. If there is no conserved identity in terms of final shape, would the developmental system nevertheless show a conserved identity?

The molar model, with its well-known phylogenetic history, years of developmental genetics and a physico-chemical model of tooth morphogenesis, is an ideal system to tackle this question. We focused on the recent drastic change in upper molar shape seen in the mouse lineage, resulting in the marked differentiation of the two molars as compared to the ancestral situation still seen in hamster. We quantified and compared the spatio-temporal dynamics of these 4 developmental systems, with in situ markers, transcriptomic timeseries and scRNAseq. Our results point to a morphogenetic identity of lower and upper molars, although no clear identity is visible in the adult tooth. The early spatio-temporal dynamics of the sequential process of cusp formation shows features specific to each tooth, reminiscent of the so-called “tribosphenic” molars of early mammals. A conserved transcriptomic identity is peaking at the “tribosphenic stage” and is supported by a conserved dose of transcriptional regulators, some also specify jaw identity. Interestingly, this ancestral identity was exaggerated in mouse, in relation with the new phenotype and enhanced differentiation of the two teeth.

We conclude that this morphogenetic identity was likely conserved throughout evolution since early mammals, constraining the early morphogenetic trajectory of developing lower and upper molars. Although it poorly constrains the final shape, it served as a basis to evolve the sharp shape differentiation of mouse molars.

An in vitro epithelial model to measure and manipulate the mechanics of cell-cell junctions

V.Misiak[1]*, T.Boudou[1], G.Cappello[1], M.Balland[1]*

[1]LiPhy, Saint-Martin d'Hères, France
*vladimir.misiak@univ-grenoble-alpes.fr

At the heart of tissue dynamics lies the ability of cells to change their shape, rearrange and divide while maintaining cohesiveness. For instance, cell intercalation, a process by which four cells rearrange their topology by remodeling their contacts, has been shown to be a major process in the reshaping of tissue in multiple living systems [1]. Previous research revealed a lot on the molecular machinery that regulates the remodeling of cell–cell contacts [1,2]. There is still a lack of approaches to dynamically probe the mechanical properties of a rearranging system.

Our project aims to answer two unanswered questions: How are stresses distributed within epithelial architecture, and how are these forces dynamically regulated to ensure cellular reorganization during morphogenesis while preserving tissue integrity?

Employing a bottom-up approach, we designed a minimal four-cell system mimicking in vivo epithelial pavement using micropatterning (Figure 1.a-c). Despite the geometrical constraints imposed by the adhesive micropattern this minimal four cell system (cell quadruplets) can rearrange as in cell intercalation. By observing this elementary epithelial model and combining cell segmentation, traction force microscopy and monolayer stress microscopy, we unravel the link between its mechanical and morphological properties (Figure 1.d). We quantify differences in mechanical anisotropy between intercalating and non-intercalating systems, suspecting a link to central junction remodeling mechanisms. Optogenetic coupling enables precise perturbation of cell-cell adhesions [3] and contractility [4], highlighting the central junction's mechanical stability role. Our ongoing research focuses on correlating topological changes with cohesive forces in our quadruplets.

This bottom-up approach offers insights into epithelial mechanical stability during morphogenesis, potentially informing complex tissue reorganization mechanisms like cell intercalation.

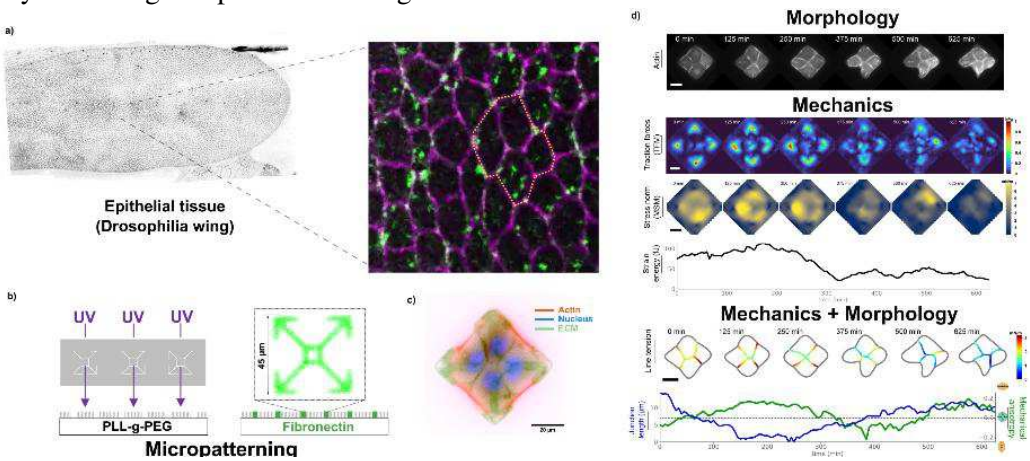


Figure 1: a) Images of drosophilia wing and outline of an elementary tissue pavement in drosophilia extracted from Etournay et al. 2015 elife and Oxford Press 2015 b) Scheme of the micropattening process used to get the ECM geometry needed to reproduce a cell quadruplet. c) Immunostaining of a cell quadruplet with actin in red, nucleus in blue and fibronectin in green. d) Multiple figures showing the coupling of morphological and mechanical readouts for one cell quadruplet. Scale bars are 20 µm.

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Self-organized wave-like beating of actin bundles driven by myosin X motors

Ludivine Chaix[1]*, Antony Lee[1], Pascal Martin[1]

[1]Physique des Cellules et Cancer, Institut Curie, Paris, France
*ludivine.chaix@curie.fr

Large assemblies of biological filaments and molecular motors can exhibit emergent meso- and macro-scale behaviors that are not present at the single-molecule scale. As an example of self-organization in motor-filament systems, our group has recently shown that a wave-like beating behavior can emerge in vitro in an assembly of polymerizing actin filaments and myosin motors [1]. Despite the different molecular identity of the filaments and motors at work, the beating waveforms in this artificial system mimic those observed in eukaryotic flagella. Our minimal reconstituted system provides a powerful platform to study a fundamental question: how can motor activity be coordinated in space and time depending on the shape of the filament bundle, resulting in periodic wave-like oscillations?

In our assay, we used surface micropatterning of a nucleation promoting factor to control the geometry of actin polymerization into parallel filament networks. We studied how these filaments self-assembled in the presence of myosin X, which is involved in vivo in filopodia assembly and cargo transport along these parallel networks of actin filaments. We found that, as observed previously with heavy-mero myosin II and myosin V [1], myosin X can also drive actin-filament self-assembly into wave-like beating bundles. As observed previously with myosin V, myosin X preferentially binds to the curved and dynamic tips of the bundles, which is suggestive of a general feedback mechanism based on curvature control of myosin affinity to actin. In addition, we observed motor streaming along the filaments, corresponding to processive motor motion towards the barbed ends of the actin filaments. Using FRAP experiments, we characterized how motor binding-unbinding kinetics and transport dynamics couple to dynamic shape changes associated to the beating phenomenon of the actin bundles. Altogether, our experiments are expected to shed light on coordination mechanisms in active motor-filament systems.

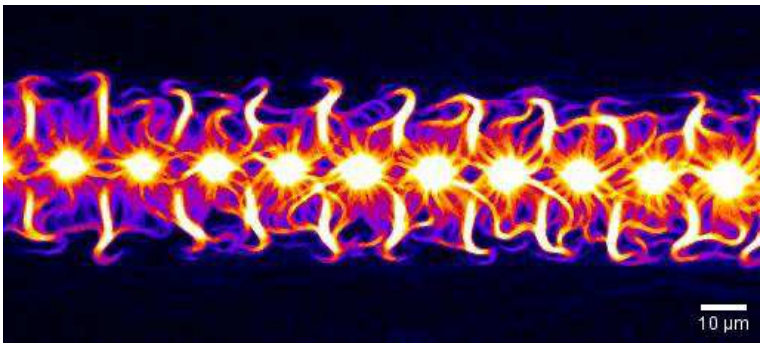

Figure 1: Fluorescently labelled actin filaments, growing from disks patterned with a nucleation-promoting factor, self-organize in the presence of myosin X motors into bundles that exhibit wave-like beating.

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Molecular dissection of a genetic AND gate enables predictive design and control of its response function

L. Troisi[1,2], V. Hakim[2], M. Morel[1]*

[1]PASTEUR, ENS Chemistry Department, Paris, France
[2]LPENS, ENS Physics Department, Paris, France
*mathieu.morel@ens.psl.eu

The rapid progress of synthetic biology have led to the development of synthetic genetic circuits, which have evolved from simple transcriptional cascades to much more sophisticated architectures, paving the way for novel therapeutic strategies [1]. Synthetic gene circuits are commonly engineered using well-described building blocks, promising a highly reliable behavior for high-specificity cell targeting and tunable response. However, implementing circuits in mammalian cells remains challenging and precise response functions are still hard to predict. A major effort is needed to streamline network design, coupling model-based prediction with systematic measurement of circuit responses, in order to optimize existing circuits or create *de novo* functional networks [2].

To this aim, we have developed an experimental platform combining microscopy-based cytometry, pixel-correlation analysis and thermodynamic modelling (Fig.1), in order to build a predictive model of genetic AND gates based on the dimerization of two independent proteins, a DNA binding domain and a transcription activator, into a synthetic transcription factor that trigger an output gene [3].

To build a robust theoretical model, we dissected the molecular mechanism in simpler events. First the cis-activation of the output promoter by a monomeric transcription factor, then the binding competition of active/inactive factors on the said promoter, and eventually the full AND gate including the dimerization of the transcription factor. For each part, varying molecular parameters (number of binding sites, strength of the core promoter, dimer affinity) allowed us to confirm insights on how this system works and reinforced the parameter search of our model. From this establishment of a comprehensive model, we successfully predicted the response function of AND gates with novel affinity mutants of the dimer. We now aim to predict the design of circuits combining competitive AND gates and allowing advanced functionalities such as complex cell-state classifiers, multi-output switches or oscillators.

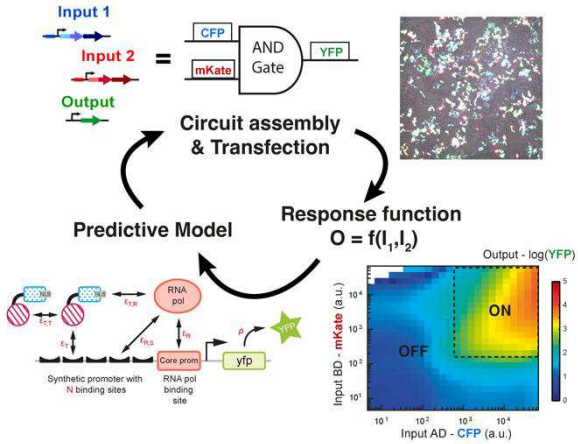

Figure 1: Design-Build-Test-Learn platform for predicting genetic AND gates response function.

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Investigating the dynamic response of nucleolus to static mechanical compression

Yuthika Shetty*, Lydia Pernet, Monika Dolega

Institute for Advanced Biosciences, Grenoble, France
*yuthika.shetty@univ-grenoble-alpes.fr

Extrinsic biomechanical cues are highly regulated to influence cell fate and function under normal physiological conditions. Individual cells have the ability to perceive and respond to extracellular chemical and mechanical stimuli. These stimuli trigger complex intracellular signaling pathways that have evolved to help maintain cellular homeostasis. Nucleolus, a prominent sub-nuclear organelle, is the central hub of cellular stress response pathways [1]. Due to its dynamic properties, the nucleolus has been shown to rapidly respond to various extracellular stressors [2,3,4]. A previous study within our team revealed that static mechanical compression of epithelial monolayer triggers changes in the regulation of genes encoding ribosomal proteins, suggesting a potential mechanical regulation of the ribosome biogenesis process [5]. Here, we investigate the nucleolar state when exposed to a static compressive force. Live cell imaging was performed to observe the dynamic response of the nucleolus to mechanical stress and the possible biomechanical triggers involved. Nucleolin, a multifunctional nucleolar protein, translocates out of the nucleolus into the nucleoplasm during mechanical stress response. Functional consequences of this phenomenon was examined using Click-IT RNA imaging assay which revealed a significant reduction in the rate of transcription of rDNA. Further investigation of the sequence of events during mechanical stress response revealed an immediate and rapid loss of nuclear volume and dynamic changes in the histone modifications of the chromatin. These results suggest a possible mechano-regulation of ribosomal biogenesis in the nucleolus during mechanical stress response triggered by the mechanical deformation of the nucleus.

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The Min system drives polar secretion of the outer membrane adhesin Ag43 in *E. coli*

Dario Dell'Arciprete[1,2], Yankel Chekli[3], Bianca Audrain[3], Thierry Mora[1], Vincent Croquette[1,2], David Bensimon[1,2], Jean-Marc Ghigo[3], Jean-François Allemand[1,2], Arnaud Gautier[4], Christophe Beloin[3], Nicolas Desprat[1,2,5,*]

[1] Laboratoire de Physique de l'Ecole Normale Supérieure, ENS, Université PSL, CNRS, Sorbonne Université, Université Paris Cité, 75005 Paris, France.
[2] Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Ecole Normale Supérieure, CNRS, INSERM, PSL Research University, 75005, Paris, France
[3] Genetics of Biofilms, Institut Pasteur, rue du Dr. Roux. 75015 Paris, France.
[4] Sorbonne Université, Ecole normale sup\'erieure, Université PSL, CNRS, Laboratoire des Biomolécules, LBM, 75005 Paris, France.
[5] UFR de physique, Universit\'e Paris Cit\'e, 10 rue Alice Domon et Leonie Duquet. 75013 Paris, France.on, City, Country of First Author
[*]email address: nicolas.desprat@phys.ens.fr

Surface proteins are essential components of bacteria-environment interactions. Akin to their cytoplasmic counterparts, polar localization of surface proteins is required in many contexts such as cell infection or biofilm formation. However, live imaging studies of Outer Membrane Proteins (OMP) in the Gram-negative bacterium *E. coli* have revealed that OMP are inserted homogeneously on the lateral surface. Despite passive advection to bacterial poles by addition of new cell wall material and reduced polar diffusion, only old proteins accumulate efficiently but slowly at bacterial poles. Here we show how the dynamics in the periplasmic space between the inner and the outer membrane actively localize Ag43, an *E. coli* OMP involved in cell-cell adhesion, to bacterial poles. The periplasmic dynamics was captured by a Fokker-Planck equation that account for inhomogeneous drift and diffusion. We further demonstrated that the dynamics of periplasmic Ag43 is coupled to the Min system, whose cytoplasmic components are known to oscillate from pole to pole during the cell cycle. Our results suggest that different pathways co-exist for OMP positioning and that the Min system not only organize cytoplasmic components but also contributes to the organization of OMP on the cell envelope.

Mechanics and polarity in B cells

P. Pierobon[1,2]*, J. Pineau[2], L. Pinon[3], A-M Lennon-Dumenil[2], J. Fattaccioli[3]

[1]Institut Cochin, CNRS UMR8104, INSERM U1016, Université Paris Cité, Paris, France
[2]Institut Curie, INSERM U932, and PSL, Paris, France
[3]Ecole Normale Supérieure, Institut Pierre Gilles de Gennes, CNRS UMR 8640, Paris, France
*paolo.pierobon@inserm.fr

B cells serve as the primary producers of antibodies within the immune system. To elicit an effective immune response, they must recognize and internalize antigens through a specialized signaling platform known as the immune synapse. The formation of this structure involves the generation of forces and the polarization of cellular structures. In our research, we employ microfluidics and micro-fabricated tools to investigate the forces present at cell-cell contacts and the cellular rearrangements triggered by antigen recognition. Recently, we introduced functionalized oil droplets as a novel antigen presenting tool, revealing an unexpected role for microtubules in limiting F-actin polymerization, thus facilitating the formation and maintenance of a distinct immune synapse. Our ongoing work expands upon these methodologies to explore the interaction of these cells with the micro-environment of lymph nodes and the influence of mechanical factors on their immune function.

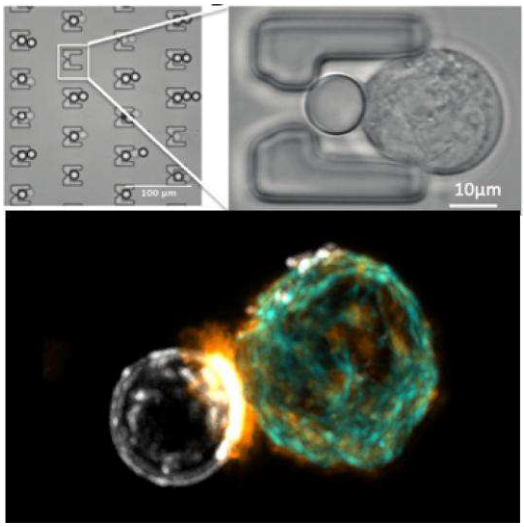

Figure 1: Top: Microfluidic traps to study the encounter of a B cell and an antigen coated droplet. Bottom: 3D rendered example of an encounter between a droplet (left, stained actin) and a B cell (right, stained actin)

References

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