Annual Meeting of GDR CellTiss, Paris-Mandres, Nov 13th-Nov15th

Monday, Nov 13th

12:30-13:30 *Welcome Lunch (Buffet)*

14:00-15:00 ***Mechanics of blastocyst morphogenesis***
Jean Léon Maitre, Genetics and Developmental Biology, Institut Curie, Paris.

15:00-15:15 ***In depth-spheroid phenotypic characterizations: effects of 5-Fluorouracil on cohesion***
Charlotte Rivière, Institut Lumière Matière, Lyon.

15:15-15:30 ***Magnetic nanoparticles as a tool to create, investigate and stimulate multicellular aggregates***
Gaëtan Mary, Laboratoire Matière et Systèmes Complexes, Paris 7.

15:30-15:45 ***Mechanical coordinates: designing geometrical microenvironments for the control mechanical waves in model tissues***
Vanni Petrolli, Laboratoire Spectrométrie Physique, LiPhy, Grenoble.

15:45-16:15 *Coffee break*

16:15-16:30 ***Cellular mechanisms regulating plant organ variability***
Antoine Fruleux, Reproduction et Développement des Plantes, ENS, Lyon.

16:30-16:45 ***Structure and dynamics of multicellular assemblies measured by coherent light scattering***
Benjamin Brunel, Laboratoire Spectrométrie Physique, LiPhy, Grenoble.

16:45-17:00 ***Micropatterns of BMP-2 and fibronectin on soft biopolymeric films regulate myoblast shape and SMAD signaling***
Thomas Boudou, Laboratoire Interdisciplinaire de Physique, LiPhy, Grenoble.

17:00-17:15 ***Drunken sailors in C. elegans embryos: what shall we do with Wnt ligands***
Pierre Recouvreux, IBDM, Marseille.

17:15-17:45 *Coffee break*

17:45-18:00 ***Engineered protein scaffolds to study the formation of membrane-less organelles in mammalian cells***
Marina Garcia-Jove Navarro, Département de chimie de l'ENS, Paris.

18:00-18:15 ***The effect of virulent factor on mechanical and structural properties of epithelial respiratory cells***
Christelle Angély, Biomécanique Cellulaire et Respiratoire, Institut Mondor de Recherche Médicale, Créteil.

19:00-20:00 *Diner*

20:00- *Drinks and free time*

Tuesday, Nov 14th

9:15-10:15 ***Dendritic cell migration: from microfluidics to in vivo imaging***
Ana-Maria Lennon-Duménil, Immunity and Cancer Institut Curie

10:15-10:30 ***Mechanics of phagocytosis***
Alexandra Zak, Laboratoire d'Hydrodynamique de l'Ecole Polytechnique.

10:30-10:45 ***Microrheology of astrocytes and glioma cells and contribution of intermediate filaments to their mechanics***
Charlotte Alibert, Compartimentation et Dynamique Cellulaires, Institut Curie.

10:45-11:00 ***Maskless Quantitative Multi-protein Photopatterning to orchestrate cellular microenvironment***
Pierre-Olivier Strale, Alvéole

11:00-11:30 *Coffee break*

11:30-11:45 ***Mechanics and force patterning in B-cell antigen extraction***
Paolo Pierobon, U932 – Immunité et cancer, Institut Curie, Paris.

11:45-12:00 ***The mechanical response of T cells during activation***
Anna Sawicka, LadHyX & Immunity and Cancer, CRI, Paris.

12:00-12:15 ***Disassembly-driven contraction of an F-actin network transports chromosomes in starfish oocytes***
Serge Dmitrieff, IJM, Paris.

12:15-12:30 ***Linking tensional force dynamics with actin architecture***
Tomas Andersen, Laboratoire Interdisciplinaire de Physique, LiPhy, Grenoble.

12:30-14:00 *Lunch*

14:00-18:30 *Fontainebleau: climbing and walking sessions*

19:00-20:00 *Dinner*

20:00-21:00 ***Poster session***

21:00- *Party with special guests*

Wednesday, Nov 15th

9:30-10:30 ***Membrane vesiculation without leakage: the advantage of asymmetric saturated-docosahexanoic phospholipids***
Bruno Antonny, Institut de Pharmacologie Moléculaire et Cellulaire, Nice

10: 30-10: 45 ***Assessing the translocation of Cell Penetrating Peptides using model membrane in inverse emulsions***
Simon Kulifaj, Analyse, Interactions Moléculaires et Cellulaires, UPMC, Paris.

10: 45-11:00 ***Ezrin enrichment on curved cell membranes requires phosphorylation or interaction with a curvature-sensitive partner***
Tsai Feng-Ching, Physico-Chimie, Institut Curie, Paris.

11:00-11:30 *Coffee break*

11:30-11:45 ***2D binding properties as function on the applied force and the interaction time of single domain antibodies binding tumor markers***
Cristina Gonzalez, ADHESION & INFLAMMATION LAB, Luminy, Marseille.

11:45-12:00 ***The Mechanosensitivity of Actin Bundles***
Emiko Suzuki, Institut Jacques Monod, Paris.

12:00-12:15 ***Impact of mechanics on actin disassembly by ADF/cofilin***
Hugo Wioland, Institut Jacques Monod, Paris.

12:15-12:30 ***T cell adhesion on engineered substrates: influence of ligand nano-clustering***
Emmanuelle Benard, Centre Interdisciplinaire de Nanoscience de Marseille.

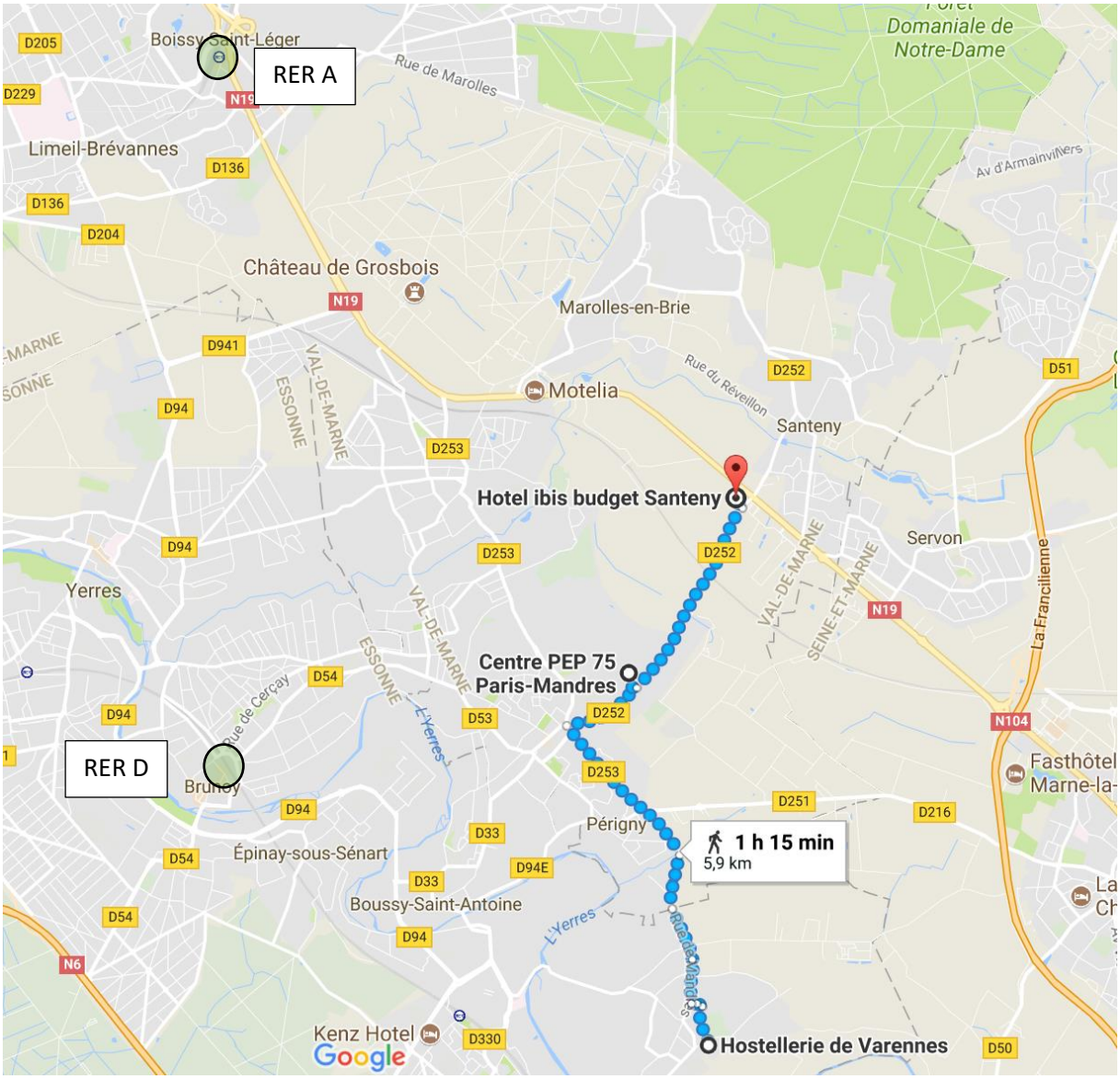
12:30-14:00 *Lunch (picnic)*

14:00- *End of the days*

Access to Paris-Mandres center “les PEP75”

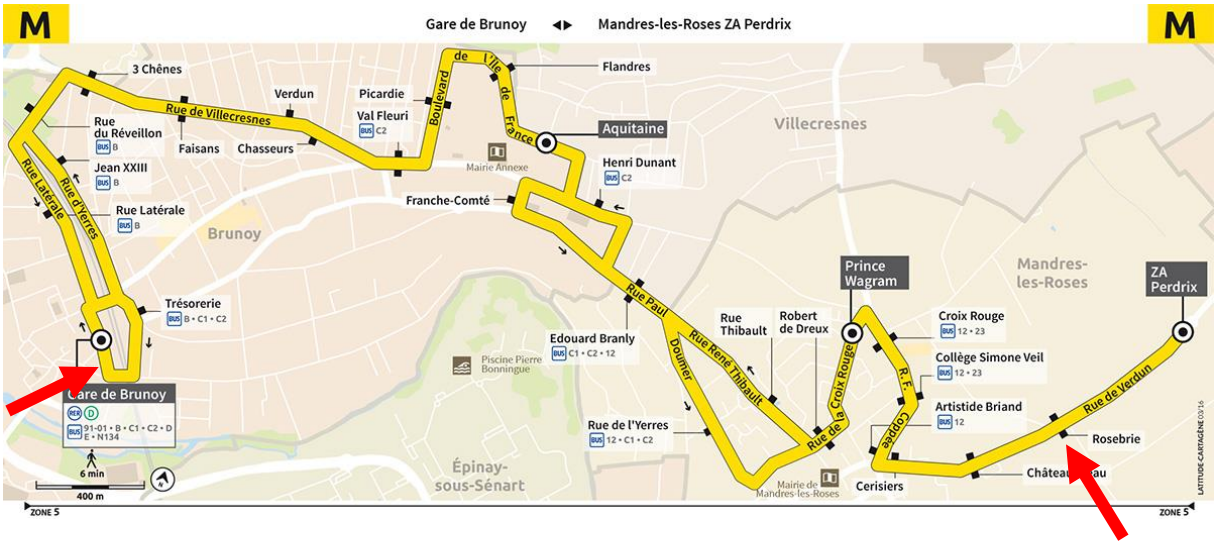
2 Rue du 8 Mai 1945, 94520 Mandres-les-Roses

Le centre Paris-Mandres est d'accès facile : on peut y arriver soit par le **RER D** (arrêt Brunoy, 52 min de marche si à pied, 4,2km) puis le bus M (arrêt Rosebrie, reste 250 m à pied) ou le bus C1 (arrêt Charles de Gaulle, reste 1.4 km à pied), soit par le **RER A** arrêt Boissy-Saint-Léger puis le bus 23 (arrêt Simone Veil, reste 450 m à pied). Les bus passent toutes les 15-20min. En fonction de l'heure de la journée, l'un ou l'autre de ces trois itinéraires est le plus court en temps, mais les distances à marcher sont différentes. Suivre la rue de Verdun jusqu'au numéro 98, puis prendre la rue perpendiculaire, vous y êtes !



BUS M

Be careful: buses M only in the early morning and late afternoon



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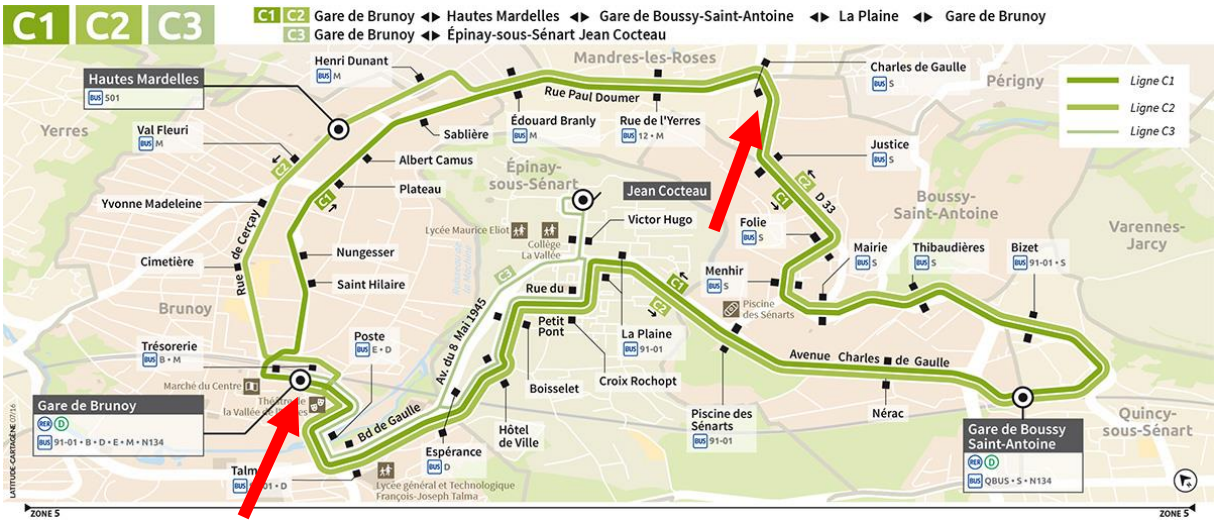

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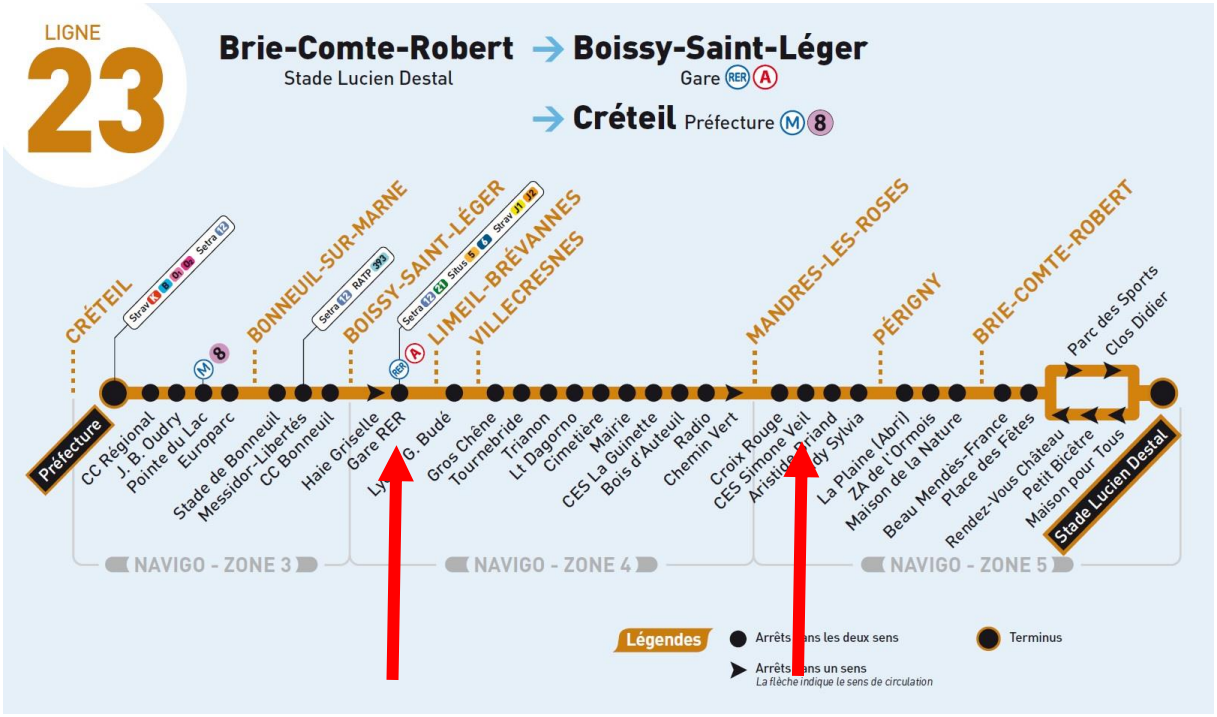
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Brie-Comte-Robert
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MANDRES-LES-ROSES
PÉRIGNY
BRIE-COMTE-ROBERT
Parc des Sports
Clos Didier
Préfecture
CC Régional
J. B. Oudry
Pointe du Lac
Europarc
Stade de Bonneuil
Messidor-Libertés
CC Bonneuil
Haie Griselle
Gare RER
G. Budé
Gros Chêne
Tournebride
Trianon
Lt Dagorno
Cimetière
Mairie
CES La Guinette
Bois d'Auteuil
Radio
Chemin Vert
Croix Rouge
CES Simone Veil
Aristide Briand
Sylvia
La Plaine (Abri)
ZA de l'Ormois
Maison de la Nature
Beau Mendès-France
Place des Fêtes
Rendez-Vous Château
Petit Bicêtre
Maison pour Tous
Stade Lucien Destal
NAVIGO - ZONE 3
NAVIGO - ZONE 4
NAVIGO - ZONE 5
Légendes
Arrêts dans les deux sens
Arrêts dans un sens
La flèche indique le sens de circulation
Terminus

Invited talks

Monday

Mechanics of blastocyst morphogenesis

Jean-Léon Maître

Genetics and Developmental Biology unit CNRS UMR3215, INSERM U934, Institut Curie, Paris

During pre-implantation development, the mammalian embryo forms the blastocyst, which will implant into the uterus. The architecture of the blastocyst is essential to the specification of the first mammalian lineages and to the implantation of the embryo. Consisting of an epithelium enveloping a fluid-filled cavity and the inner cell mass, the blastocyst is sculpted by a succession of morphogenetic events. These deformations result from the changes in the forces and mechanical properties of the tissue composing the embryo.

Using microaspiration, live-imaging, genetics and theoretical modelling, we study the biophysical and cellular changes leading to the formation of the blastocyst. In particular, we uncover the crucial role of acto-myosin contractility, which generates periodic waves of contractions, compacts the embryo, controls the position of cells within the embryo and influences fate specification.

Tuesday

Membrane vesiculation without leakage: the advantage of asymmetric saturated-docosahexanoic phospholipids

Bruno Antonny

Institut de Pharmacologie Moléculaire et Cellulaire, CNRS & Université Nice Sophia Antipolis, Valbonne

Phospholipids (PLs), the main components of biological bilayers, generally contain a saturated acyl chain at position sn1 of the glycerol backbone, whereas the acyl chain at position sn2 is either saturated, monounsaturated or polyunsaturated (omega-3 or omega-6) depending on the physiological context. How the acyl chain profile of PLs correlates with membrane properties is not well understood. By analyzing the effect of membrane shaping proteins on a comprehensive series of bilayers containing PLs with defined acyl chains, we show that asymmetric sn1-saturated sn2-polyunsaturated PLs offer the best compromise between maintaining membrane permeability at low levels while allowing membranes to be readily

vesiculated. Differences between polyunsaturated PLs indicate that the omega-6/omega-3 ratio impact on membrane mechanics.

Wednesday

Dendritic cell migration: from microfluidics to in vivo imaging

Ana-Maria Lennon-Duménil

Immunity and Cancer, U932 Inserm, Institut Curie, Paris

Introductory lecture to immunology.

Oral presentations

In depth-spheroid phenotypic characterizations: effects of 5-Fluorouracil on cohesion

Angélique Virgone-Carlotta[a], Manon Lemasson[a], Hichem C. Mertani[b], Jean-Jacques Diaz[b], Sylvain Monnier[a], Thomas Dehoux[a], Hélène Delanoë-Ayari[a], Charlotte Rivière[a]* and Jean-Paul Rieu[a]*

[a] Univ Lyon, Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière, F-69622, Villeurbanne, France

[b] Centre de Recherche en Cancérologie de Lyon, UMR INSERM 1052 CNRS 5286 Centre Léon Bérard, Lyon, France

**Senior authors have equally contributed to this work*

MultiCellular Tumor Spheroids (MCTS), which mimic the 3-Dimensional (3D) organization of a tumor, are considered as better models than conventional cultures in 2-Dimensions (2D) to study cancer cell biology and to evaluate the response to chemotherapeutic drugs . A real time and quantitative follow-up of MCTS with simple and robust readouts to evaluate drug efficacy is still missing. Here, we evaluate the chemotherapeutic drug 5-Fluorouracil (5-FU) response on the growth and integrity of MCTS two days after treatment of MCTS and for three colorectal carcinoma cell lines with different cohesive properties (HT29, HCT116 and SW480). We found different sensitivity to 5-FU for the three CRC cell lines, ranging from high (SW480), intermediate (HCT116) and low (HT29) and the same hierarchy of CRC cell lines sensitivity is conserved in 2D. We also evidence that 5-FU has a strong impact on spheroid cohesion, with the apparition of a number of single detaching cells from the spheroid in a 5-FU dose- and cell line-dependent manner. We propose an innovative methodology for the chemosensitivity evaluation in 3D MCTS that recapitulates and regionalizes the 5-FU-induced changes within MCTS over time. These robust phenotypic read-outs could be easily scalable for high-throughput drug screening that may include different types of cancer cells to take into account tumor heterogeneity and resistance to treatment.

Magnetic nanoparticles as a tool to create, investigate and stimulate multicellular aggregates.

Mary G., Mazuel F., Du V., Richard S., Gay C., Luciani N., Reffay M., Wilhelm C.

Laboratoire Matière et Systèmes complexes, UMR7057 CNRS & Université Paris Diderot, 10 rue Alice Domon et Léonie Duquet, 75205 Paris cedex 13.

Multicellular aggregates are currently used as a model for biological tissues: for biophysics, for drug screening, as tumor model systems or as building blocks for 3D bio-printing and tissue engineering. Magnetic nanoparticles are increasingly used in the biomedical field for diagnostic and therapeutic strategies, including imaging, hyperthermia and drug delivery. Recently, they have been proposed to develop new approaches to tissue engineering and manipulation, in which magnetic forces are used to manipulate single cells within a 3D construct.

We have developed a set of methods based on cell tagging with magnetic nanoparticles, to mold multicellular aggregates of chosen shapes (e.g. cylinders) and unprecedented sizes – up to a few mm in diameter – while preserving tissue integrity[1] (A).

The magnetism thus provided to the aggregate can be used as a tool to study and stimulate these model tissues. Subjecting magnetic cellular cylinders to tunable magnetic forces - through the use of an electromagnet - allowed us to investigate the rheological properties as a function of the aggregate maturation time (B).

In parallel, using a fix magnetic attractor, we have been able to assemble a 3D embryoid body from embryonic stem cells (ESCs). Then, by adding a second mobile magnetic attractor (C), we have demonstrated that a cyclic mechanical stretching of the embryoid body drives the ESCs' differentiation towards the mesodermal cardiac pathway[2].

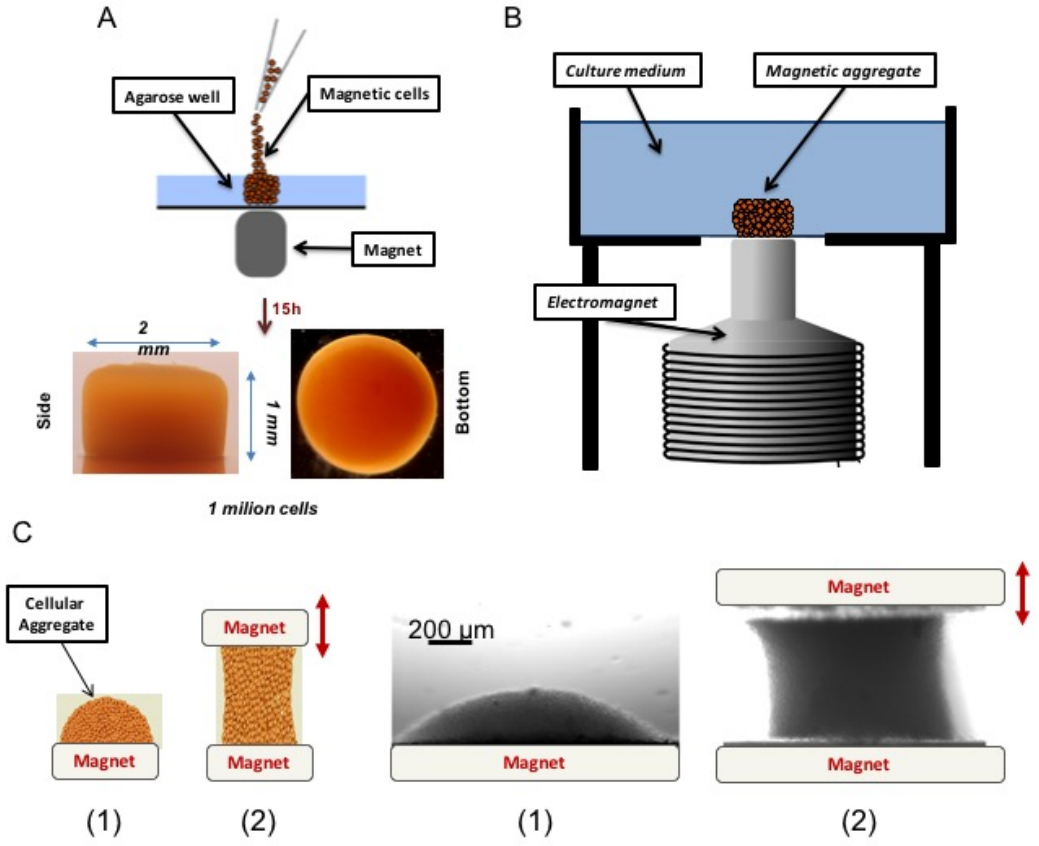

Figure: A) Magnetic molding method of cylindrical-shaped aggregate using magnetic cells. B) Magnetic Rheometer: a magnetic aggregate is stimulated remotely with an electromagnet. C) Magnetic stretcher: formation and cyclic stimulation of a embryoid body.

These new magnetic tools thus provide a promising all-in-one approach to create magnetic tissues, to stimulate them and to investigate their mechanical properties.

This work was supported by the European Union (ERC-2014-CoG project MaTissE 648779).

(1) *Mazuel et al. Phys. Rev. Lett.* **114**, 098105 (2015)
(2) *Du et al. Nature Communications* **8**, 400 (2017)

Mechanical coordinates: designing geometrical microenvironments for the control mechanical waves in model tissues.

Petrolli V. [1], Mandula O. [2], Herve L. [2], Allier C. [2], Moreau P. [1], Balland M. [1], and Cappello G. [1]

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2. CEA-LETI, MINATEC, Grenoble 38054, France

Correct development of organisms starting from single cells is an extremely complex and regulated process, whose characteristic landmarks are associated to spatial patterning of cell behavior. Precise and correct occurrence of these behavioral changes is generally attributed to signaling programs, but we recently discovered that mechanical tensions are responsible for many phenomena typical of morphogenesis, such as gastrulation, branching and buckling. [1] In particular, mechanical waves have been observed in expanding as well as colliding two-dimensional epithelial tissues. [2,3] These findings suggest that the presence of peculiar wave-like patterns might be of a more common nature, and that it might be of interest to fully understand morphogenesis and its guidance.

Here we intend to investigate the occurrence of mechanical waves in confined tissues and their correlation with the presence of boundaries. We use microfabrication techniques to confine the growth of model tissues to specific geometries, chosen for their ability to induce coherent long range cell alignment, and Traction Force Microscopy (TFM) to monitor tissue-substrate interaction in relation to the particular shape chosen. Longer observations carried out with lensless microscopy allow to study the dynamics of the system and correlate the wavelike phenomena observed to characteristic motion and jamming transition typical of epithelial cells.

1. Gjorevski, N. & Nelson, C. M. The mechanics of development: Models and methods for tissue morphogenesis. *Birth Defects Res. Part C - Embryo Today Rev.* **90,** 193–202 (2010).
2. Serra-Picamal, X. *et al.* Mechanical waves during tissue expansion. *Nat. Phys.* **8,** 628–634 (2012).
3. Rodríguez-Franco, P. Long-lived force patterns and deformation waves at repulsive epithelial boundaries. (2017). doi:10.1038/nmat4972

Cellular mechanisms regulating plant organ variability

How do organs form with consistent sizes and shapes, with substantial variability at the cellular level?

Current model is that a morphogen gradient spanning the organ provides cells with positional information that controls organ size. Nevertheless, recent evidences suggest that the simple interpretation of a global morphogen gradient is insufficient for size control. Moreover, a recently identified mutant showed less robust organ shapes than in wild type, and, counterintuitively, a more spatially homogeneous cell growth (Hong, 2016). We investigate the mechanisms enhancing or buffering cell variability and the consequences on reproducibility of organogenesis.

This talk will present a model for growth variability during the formation of 2D organs which is adapted to the morphogenesis of Arabidopsis sepals, studied by the team. We test different possible mechanisms entering into account in cellular variability. In a second time we determine how variability changes across scales, to understand how heterogeneous cells yield robust organs. We will confront these results to experimental observations.

* Hong L et al. (2016) Variable Cell Growth Yields Reproducible Organ Development through Spatiotemporal Averaging. Dev Cell 38, 15.

Structure and dynamics of multicellular assemblies measured by coherent light scattering

Benjamin Brunel[1], Carles Blanch[2], Romain Pierrat[3], Aurélien Gourrier[1], Jean-François Joanny[2,3], Rémi Carminati[3], Antoine Delon[1], Giovanni Cappello*[1]

Multicellular aggregates, or spheroids, represent an interesting model to study tumor response towards external stresses [1]. The question of cell organization and flows inside spheroids revealed to be a challenging topic as usual imaging techniques are limited in terms of penetration length and/or time resolution [2]. To answer this question, we developed a new method based on coherent light scattering:

1. From static light scattering, we deduce the average cells size and spatial organization (spatial auto-correlation).
2. From dynamic light scattering, we deduce cells displacements histogram as a function of time.

This technique gives statistical results over a large field of view, inside spheroids as thick as 400 µm. In comparison to standard microscopy, no dyes are needed and analysis does not require the usual processing (segmentation, tracking,…) which can sometimes lead to biases. Application to the study of osmotic pressure effect on spheroids showed that cells movements are significantly reduced under pressure (fig b,c). This 3D emergent property seems related to the role of extra-cellular matrix.

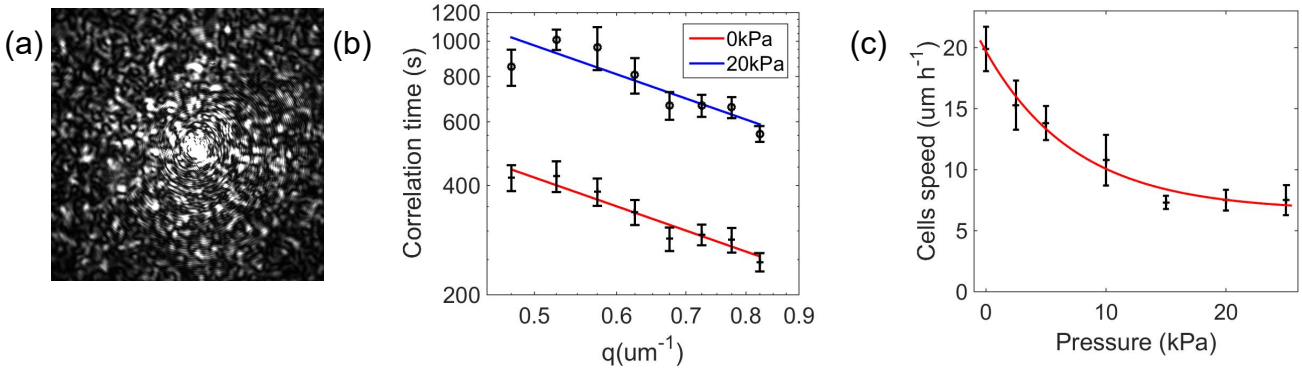

Figure: (a) Picture of a speckle pattern obtained by light scattered through a spheroid. (b) Intensity correlation time as a function of the scattering vector q for a spheroid without pressure (red) and with 20 kPa (blue). (c) Average cells speed as a function of pressure.

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3. ESPCI ParisTech, PSL Research University, CNRS, Institut Langevin, 75005 Paris
** Corresponding author: giovanni.cappello@univ-grenoble-alpes.fr*

[1] Monnier et al., *Methods,* **94,** 114-9 (2015)

[2] Chen et al., *Crit Rev Biomed Eng.,***41**, 393-403 (2013)

Signal mingle: Micropatterns of BMP-2 and fibronectin on soft biopolymeric films regulate myoblast shape and SMAD signaling

Vincent Fitzpatrick[1], Laure Fourel[2], Olivier Destaing[2], Flora Gilde[1], Corinne Albigès-Rizo[2], Catherine Picart[1] & Thomas Boudou[3]

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[2] Univ. Grenoble Alpes, INSERM, CNRS, Institute for Advanced Biosciences, 38700 La Tronche, France.
[3] Univ. Grenoble Alpes, CNRS, LIPhy, 38000 Grenoble, France

In vivo, bone morphogenetic protein 2 (BMP-2) exists both in solution and bound to the extracellular matrix (ECM). While these two modes of presentation are known to influence cell behavior distinctly, their role in the niche microenvironment and their functional relevance in the genesis of a biological response has sparsely been investigated at a cellular level. Here we used the natural affinity of BMP-2 for fibronectin (FN) to engineer cell-sized micropatterns of BMP-2. This technique allowed the simultaneous control of the spatial presentation of fibronectin-bound BMP-2 and cell spreading. These micropatterns induced a specific actin and adhesion organization around the nucleus, and triggered the phosphorylation and nuclear translocation of SMAD1/5/8 in C2C12 myoblasts and mesenchymal stem cells, an early indicator of their osteoblastic trans-differentiation. We found that cell spreading itself potentiated a BMP-2-dependent phosphorylation of SMAD1/5/8. Finally, we demonstrated that FN/BMP-2-mediated early SMAD signaling depended on LIM kinase 2 and ROCK, rather than myosin II activation. Altogether, our results show that FN/BMP-2 micropatterns are a useful tool to study the mechanisms underlying BMP-2-mediated mechanotransduction. More broadly, our approach could be adapted to other combinations of ECM proteins and growth factors, opening an exciting avenue to recreate tissue-specific niches in vitro.

Drunken sailors in *C. elegans* embryos: what shall we do with Wnt ligands ?

Pierre Recouvreux, Monika Ludanyi, Claire Chardès, Pauline Mélénec, Vincent Bertrand, Pierre-François Lenne

Institut de Biologie du Développement de Marseille, Aix-Marseille Univ., CNRS, Marseille, France

Animal development is the result of spatially and temporally controlled signalling events. A cell or a group of cells within a tissue receives information about their position in the tissue and subsequently takes appropriate decisions about fate, function or migration. Wnt signalling is such a pathway that control anteroposterior and dorsoventral patterning of embryos across species. While lot is known about the genetic pathway and the expression pattern of Wnt, the mode of action at a distance of this ligand still remains elusive. For example it has been proposed that Wnt form a concentration gradient that instruct the fate or the proliferation of a cell according to the local concentration of the gradient. However the Wnt protein itself has never been observed live. We use *Caenorhabditis elegans* as a model system to adress this question. We developed with CRISPR/CAS9 technique new strains expressing fluorescently labeled versions of the Wnt ligand and its receptor Frizzled. We aim at explaining the spatiotemporal characteristics of the establishment of Wnt signaling. To do so we combine advanced microscopy techniques (Lattice Light Sheet microscopy, Fluorescence Correlation Spectroscopy, ...) and numerical simulations in order to decipher the interplay between extracellular diffusion of the ligand, interactions with its receptor and tissue dynamics.

Engineered protein scaffolds to study the formation of membrane-less organelles in mammalian cells

Marina Garcia-Jove Navarro[1], Shunnichi Kashida[1], Racha Chouaib[2], Arnaud Hubstenberger[2], Dominque Weil[2], Zoher Gueroui[1].

[1] Chemistry Department, Ecole Normale Supérieure, Paris
[2] IBPS, Université Pierre et Marie Curie, Paris

Membrane-less organelles, containing both proteins and nucleic acids, are ubiquitous in cells and contribute to numerous important biological functions, including the storage and processing of mRNA and other biomolecules. Recently, novel multidisciplinary approaches have emerged providing paradigm-shifting advances about the origin of the biophysical properties of diverse membrane-less organelles. Indeed, the concept of phase transition in the cytoplasm has been proposed to describe the formation and dynamics of ribonucleoprotein granules throughout the cell cycle and development. Besides several age-related neurodegenerative diseases seem to be linked to aberrant phase transitions controlling organelle formation. However, how these supramolecular structures precisely assemble and disassemble in the cell, and how their function is spatially and temporally controlled remain questions unanswered by classical cell biology approaches. We proposed a novel strategy based on engineered protein scaffolds that assemble into micrometer-sized structures to reproduce the membrane-less organelle formation into the cytoplasm of mammalian cells. Such approach is an interesting tool to quantitatively assess the impact of biophysical parameters on the self-assembly process, but also how the condensation of biomolecules by phase separation can regulate functions in the cell.

The effect of virulent factor on mechanical and structural properties of epithelial respiratory cells

C. Angély[1], N.M. Nguyen [1], S. Dias[1], E. Planus[2], B. Louis[1], D. Ladant[3], A. Chenal[3] and D. Isabey[1]

1. Inserm U955 Eq13, Université Paris Est, CNRS ERL7240, Créteil, France
2. Centre de recherche Université Grenoble Alpes, Inserm U1290, CNRS Grenoble, France
3. Institut Pasteur, CNRS U3528, Paris, France

Background/Aims: During quite breathing, adults inhale around 12 000 liters of air containing particles, bacteria, virus and dust, meaning that the airway epithelium is permanently exposed to various aerocontaminants. In that frame**,** respiratory infections due to bacteria remain a major public health issue. Studying the effect of virulent factors is an appropriate way to model these respiratory infections. Accordingly, we study epithelial cell response to bacterial infection by using airway specific virulent factor with the ultimate aim to find new therapeutic targets. We presently explore the cellular and molecular mechanisms of virulent invasion in a cellular model of respiratory epithelial cells exposed to the adenylate cyclase (CyaA), a toxin produced by *Bordetella pertussis*, the causative agent of the wooping cough. Furthermore, CyaA is able to invade a wide range of eukaryotic cells using a unique mechanism that involves direct translocation of its catalytic domain to produce supraphysiological levels of cAMP [1]. By studying the changes in cell functions and cell mechanical properties in the course of an initial cell invasion by CyaA, we expect to assess the potential alterations of mechanotransduction processes induced by bacterial toxins.

Methods: First, we examined the functional effects of CyaA and more precisely, its toxicity on culture of A549 human alveolar epithelial cells by viability test. Then, we estimated the role of the toxin on repair and migration phenomenon by wound healing experiment. To better understand the behavior of the cytoskeleton (CSK) during the invasion of the toxin, we evaluated the role of CyaA on one of major components of CSK and adhesion of A549 cells by staining of actin fibers and focal adhesion (FA) points. The effects of CyaA on mechanotransduction was studied on A549 cells by a new force spectroscopy method which enables to assess the strength of multiple integrin bonds created in the early phase of cell adhesion by an Atomic Force Microscopy (AFM) [2]. Spherical probes of 6.6 µm in diameter coated with RGD peptide are used. This method provides complementary information on mechanical properties of CSK and adhesion properties between cells and matrix molecules. All these experiments are conducted with two monomeric species of CyaA [3], [4] (the active form CyaA and an enzymatically inactive form CyaAE5, i.e, unable to produce cAMP) to assess the role of cAMP on host cells after the translocation of the toxin [5].

Results: Concerning the viability test, we observe that CyaA affects the cell viability in a dose-dependent manner for short time exposure (from 15 to 60 min). At high CyaA concentration (> 0.5nM), the cell viability rate decreases of almost 30% after 60min of exposure to the toxin compared to the control condition. This result is especially marked for long time exposure (24 or 48h). In the case of an exposure to the inactive variant CyaAE5, no significant difference with the control condition was found, even for long time exposure. In the control case, the wound repair is complete after 36 h. In the case of pathogenic exposure, CyaA affects repair in a dose-dependent manner. Indeed, above 0.5nM, the cell monolayer fails to close. Regarding actin fibers and FA, we performed a quantification of the actin levels of fluorescence and the number of the FA points. We note that the levels of fluorescence and the number of FA points decrease drastically in dose-dependent manner. Indeed, after 60 min of exposure to CyaA at more than 0.5 nM, the actin level of fluorescence and the number of FA points decrease of almost 40% compared to the control. This method performed on A549 cells for 3 different CyaA concentrations (0.5, 5 and 10 nM) demonstrates that CyaA toxin significantly affects both cell adhesion (detachment forces are decreased) and cell mechanics (Young's modulus is increased). CyaA toxin (at 0.5 nM) assessed at three indentation/retraction speeds (2,5 and 10 µm/s) significantly affects global detachment forces, local rupture events and Young modulus compared with control conditions, while the enzymatically inactive form CyaAE5 has no effect.

Conclusion: Our results suggest that CyaA induces changes in cell function (viability and repair) and adhesion and mechanical properties of A549 are deeply modified by exposure to the active form of the toxin but not its enzymatically inactive variant. We find that CSK structure, mechanics and adhesion properties are significantly affected after cell exposure to CyaA. Altogether, our results show that the cAMP molecule plays a crucial role in the structural and mechanical properties during intoxication of respiratory cells.

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Mechanics of Phagocytosis

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The biological process of phagocytosis is one of the first steps of an immune response against microbial infection. This target-specific mechanism involves specialized cells, called phagocytes, among which are **neutrophils**. These foot-soldiers are able to internalize pathogens or dead cells into a dedicated compartment, the phagosome, and to digest them afterwards. During the creation of the phagosome, the cell surrounds the phagocytic target with a **phagocytic cup**. During this step, the neutrophil exerts some **forces** and **increases its tension** [1].

To investigate both cellular forces and stiffness, we use a **micropipette force probe** [2, 3] (Figure 1). This device allows handling a single neutrophil and bringing it in contact with a single target, here an opsonized polystyrene bead. We control the contact time between the neutrophil and its target, and we image the progression of the phagocytosis sideways under a high-magnification light microscope. The cell pushes or pulls on the opsonized bead, as witnessed by the bending of a flexible micropipette holding the bead. We can also impose this flexible pipette to indent the neutrophil thus measuring both **cellular tension and viscosity** according to time.

We observe that 12-μm diameter opsonized beads are not totally phagocyted. We investigate whether this is due to the target geometry or to limited membrane reservoirs. To quantify how the cell is able to actively mobilize these reservoirs, we indent the neutrophil while it is phagocyting a target. We measure both the **tension and the viscosity of the neutrophil** according to the phagocytic cup progression. This study will allow us to better understand the mechanical factors that regulate phagocytosis.

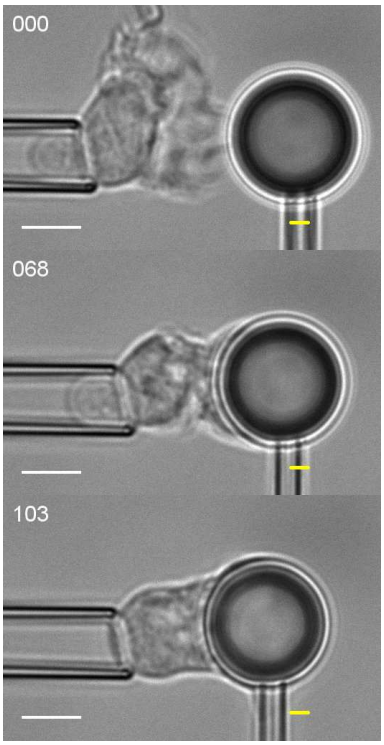

Figure 1: Chronology of events of a neutrophil forming a phagocytic cup on a 12-μm opsonized polystyrene bead. The yellow bar indicates the initial position of the flexible pipette. Aspiration pressure = 20 Pa. Time in seconds is upper left; scale bar = 5 μm.

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MICRORHEOLOGY OF ASTROCYTES AND GLIOMA CELLS AND CONTRIBUTION OF INTERMEDIATE FILAMENTS TO THEIR MECHANICS.

Charlotte Alibert, Bruno Goud, Atef Asnacios, Jean-Baptiste Manneville

Abstract:

Several studies show that cancerous tissues are stiffer than normal tissues, and the extracellular matrix seems to play a central role in this stiffening. However, at the scale of the individual cell, tumor cells appear to be softer than normal cells in a number of cancer types among which breast, pancreas, colon, and bladder cancers.
Here, we use two different microrheology techniques allowing us to probe the mechanical properties of cells at two different scales. The scale of the whole cell is probed with a single cell uniaxial rheometer, while the intracellular scale is probed with a set-up combining micropatterning and optical tweezers. These two techniques enable us to compare and correlate the internal visco-elastic properties with the mechanics of the entire cell.

We focus our study on the mechanics of astrocytes, the major glial cell type in the brain, and gliomas, brain tumors derived from astrocytes. We use two different grades of gliomas: an astrocytoma (grade III) cell line, and a glioblastoma (grade IV) cell line representing the most common and aggressive form of gliomas.

We first discriminate astrocytes and both types of gliomas based on their mechanical properties. We show that rat primary astrocytes are stiffer than glioma cells from both human and rat grade III and grade IV cell lines at both scales. Moreover, we bring out that mechanical differences exist between glioma cells grades, and that these differences depend on the probed scale. We explain the observed differences by changes in cytoskeleton composition and intracellular organization.

Next, since intermediate filaments (IFs) have been involved in the migration of glial cells and since the expression of IF proteins is modified in gliomas, we evaluate the contribution of IFs to the mechanics of gliomas and astrocytes. Focusing on three IF proteins (vimentin, nestin and GFAP), we show that the level of IF proteins correlate with the mechanical properties of the cells and that down-regulation of IF proteins lowers cell rigidity. Finally, using our intracellular technique, we are able to measure the force-deflection curve and the bending rigidity of IF bundles *in cellulo*.

Maskless Quantitative Multi-protein Photopatterning to orchestrate cellular microenvironment

Pierre-Olivier Strale, Alvéole

Cell biology is faced with significant challenges when attempting to create complex microenvironments to unravel intricate mechanisms involved in cell adhesion, cell polarity, cell migration etc... These challenges can be overcome by molecular printing which involves the controlled deposition of molecules on a substrate at the micrometer scale. These approaches have developed tremendously in the past few years and micropatterned substrates are now routinely used for biological research. To yield biologically relevant data, printed biomolecules should mimic the complexity of the in vivo microenvironment. Micrometer-scale gradients of multiple proteins are thus highly desirable.

Here we present a maskless quantitative multi-protein photopatterning solution which is based on the light-induced molecular adsorption of proteins (LIMAP) technology. This system combines a UV illumination module and a specific photoactivatable reagent (PLPP).

The combined action of UV-light and PLPP locally degrades antifouling polymer brushes allowing for the adsorption of proteins in a well-defined area.

PRIMO relies on a wide-field DMD-based projection system coupled to an epifluorescence microscope to project custom-defined patterns of UV light onto a cell culture surface. As a result, micrometer scale patterns are generated within seconds. The remaining background allows for the sequential patterning of multiple proteins. Controlled protein gradients of custom-defined shape can also be patterned. In addition, PRIMO technology allows micro-manufacturing by photopolymerization of UV-sensitive materials and protein patterning onto pre-existing 3D surfaces.

This technology empowers biomedical research in neurobiology, immunology, stem cell biology, oncology and tissue engineering.

Mechanics and force patterning in B-cell antigen extraction

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Efficient immune responses require the internalization, by B cells, of antigens presented on the surface of neighboring cells in lymph nodes. Two models have been proposed for the extraction of surface-tethered antigens by B cells: (1) spreading and contraction; (2) mechanical pulling on BCR-antigen complexes. These two cellular processes involve the actin-based molecular motor protein myosin II. We describe here a unifying model for antigen extraction by B lymphocytes, involving both global contractile forces at the periphery of the B-cell immune synapse and local pulling forces at its center. The peripheral contractile forces are dependent on a centripetal flow of myosin II, whereas the central pulling forces are generated by F-actin protrusions that form in a myosin II-dependent manner. We found that the peripheral contractile forces are pulsatile, providing a possible explanation for their role in favoring actin protrusion formation. Myosin II emerges therefore as global organizer of the cell-cell contacts and may be unexpectedly implied in other systems where receptors internalization is required.

The mechanical response of T cells during activation

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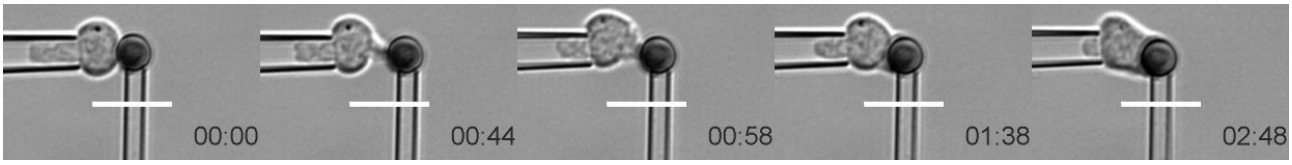
T cells activate upon antigen recognition on the surface of antigen-presenting cells (APCs). The interface between a T cell and an APC – the information that is read there, its integration, and the launch of the functional response of T cells – remains an active field of study. In particular, there is a growing interest in the mechanics of the process, as forces generated by T cells at the contact area with the APC were linked with the T cells biological function [1].

We measure forces that T cells exert perpendicular to the contact area (perpendicular to the immunological synapse plane), using the Micropipette Force Probe [2]. When a T cell pushes or pulls on its target, the holding micropipette bends, and its tip moves along the horizontal axis (figure below). Force developed by the cell is then directly proportional to the displacement of the micropipette measured in the microscope image. With this setup, we track also the morphology of the cell, as seen from the profile, after the contact with an activating bead.

Within first minutes of the activation process T cells follow a conserved sequence of events: they first push, then pull on their target. Both pushing and pulling forces change with the bending stiffness of the micropipette that holds the bead. This means that T cells adapt their mechanical response to the mechanical cues from the environment. Pushing forces start as soon as 30 s after the contact, marking them as one of the earliest events in the process. We will further study the role of cytoskeleton in the major changes in T cell morphology (figure below), to elucidate the mechanism of T cell activation.



Human primary CD4+ T cell, held in a micropipette, activated with an anti-CD3/anti-CD28 bead. Left to right: contact, pushing, collapse, spreading, pulling. Time in minutes:seconds, scale bar is 10 μm.

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The cell cortex under confinement: direct measure of thickness and dynamic response of the cell cortex subjected to compression forces

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Cell migration is central to many biological and physiological processes and happens in a variety of way. The different actin networks of the cell play different roles in migration either by actively generating forces or by influencing the mechanical properties of the whole cell. In the case of confined migration the cell cortex is often compressed between the outside elements (ECM, other cells…) and the cell nucleus. It has been shown that in constricted microchannels mimicking such environments a perinuclear branched actin network can start polymerizing [1]. Furthermore in vitro experiments on branched actin network have shown a response to confinement in both mechanical properties and polymerization dynamics [2]. Recently the observation of actin waves in 3D [3] also asks questions about the formation and physiological relevance for migration of such complexes structures of branched actin networks.

We developed a new tool to study the behavior of the cortex to understand these different types of activities and the mechanics behind complexes actin structures. We use super-paramagnetic beads under a controlled magnetic field: in this situation, the beads develop their own dipolar moment and are attracted to each other with a known force [4]. Thanks to the macropinocytosis ability of dendritic cell we can create a system where we have one bead inside the cell and one outside. We can thus confine the membrane and the cortex between these beads and track their position with a precision around 2nm.

This system allows for different measurements and tests upon the cell cortex. Due to the precision available in the tracking of the beads we can measure the thickness of the cortex at different levels of confinement. By combining our system with fluorescent microscopy we can observe a confined portion of cortex for signs of actin polymerization due to confinement. But we also record what seems to be the passage of actin waves between the beads. We can thus study the cortex and its dynamical features in different cases such as with various compressing forces or with drugs to affect the biochemical composition of the cortex.

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Linking tensional force dynamics with actin architecture

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The cellular tensional homeostasis relates to the regulation of dynamical forces that maintain the mechanic equilibrium in cells and cellular networks. It is these force dynamics and it's relation with actin organization what we are interested in exploring. A key limiting element in the understanding of the integration of force regulation in the cell mechanical sensing is the difficulty of coupling a deviation from an internal "tensional cellular homeostasis", with the active force measurement of its returning to equilibrium.
Using optogenetics combined with time resolved TFM on micropatterns as a strategy; we probed the time scales at which the mechanical homeostasis works by measuring the dynamics of cellular forces while submitting cells to different geometrical boundaries. This analysis could be done within the same cell thus preventing intercellular variability. Among the great strengths of optogenetics we find the possibility of performing precise transient and spatially signaling disruptions (1). This technique allowed us then to disturb the cellular mechanical equilibrium of cells constrained to different micropattern arrangements, thus affecting their actin organization, in a temporally controlled way. As a result, the force cellular profile showed a clear response to the light perturbations which enabled the analysis of the force time scales. Most importantly, it allowed us to tackle the question whether the cell architecture and actin orientation is affecting the cell force dynamics and its efficiency of pulling.

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Assessing the translocation of Cell Penetrating Peptides using model membrane in inverse emulsions

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Cell Penetrating Peptides (CPPs) are known to be able to cross cell membranes with a cargo through two different mechanisms: endocytosis and direct translocation. The molecular mechanism of the translocation is largely unknown. Our aim is to find the intermediate structures formed by CPP and protein, sugar, and lipid partners and measure the kinetics of the steps of the interaction of a CPP with a membrane. To assess the impact of the nature of the lipid on the translocation we use a model membrane in inverse emulsions.

Inverse emulsions are aqueous droplets into oil covered by lipids. At the interface between two adhering droplets a bilayer is formed. We monitor the translocation of fluorescently labeled CPP through this bilayer. The translocation of severals CPPs through negatively charged bilayer within tens of minutes has been detected. Nature of lipids and the asymmetry of the bilayer affect the translocation. We try to control the electrical potential in the droplet to measure the effects on translocation. We are currently improving the formation of the pairs of droplets with microfluidic devices.

Ezrin enrichment on curved cell membranes requires phosphorylation or interaction with a curvature-sensitive partner

Feng-Ching Tsai, Aurélie Bertin, Hugo Bousquet, …, Evelyne Coudrier and Patricia Bassereau

Proper spatial localisation of proteins that connect cortical actin and the plasma membrane is essential for cell shaping and function. *In vivo*, the ERM protein ezrin is associated on membranes that are flat, or with positive or negative curvature. To assess the curvature-sensing of ezrin, we use cell biology and *in vitro* approaches that associate cryo-electron microscopy (cryo-EM) and mechanical measurements on model membranes containing PIP_2 and purified ezrin. We observe that ezrin (ezrinWT) and its constitutively phosphorylated mutant (ezrinTD) self-assemble in an anti-parallel manner, zipping adjacent membranes. Phosphorylation reduces ezrin intermolecular interactions, induces a conformational change, and facilitates ezrin binding to actin filaments, as shown by cryo-EM, and promotes ezrin binding to positively curved membrane. While neither ezrinTD nor ezrinWT senses negative membrane curvature alone, we demonstrate that their enrichment in negatively curved cellular membranes such as protrusions requires them to interact with curvature sensors, e.g. I-BAR-domain proteins. Overall, our work corroborates a role for ezrin, not as a curvature sensor but rather in the mechanical cohesion of membranes with actin.

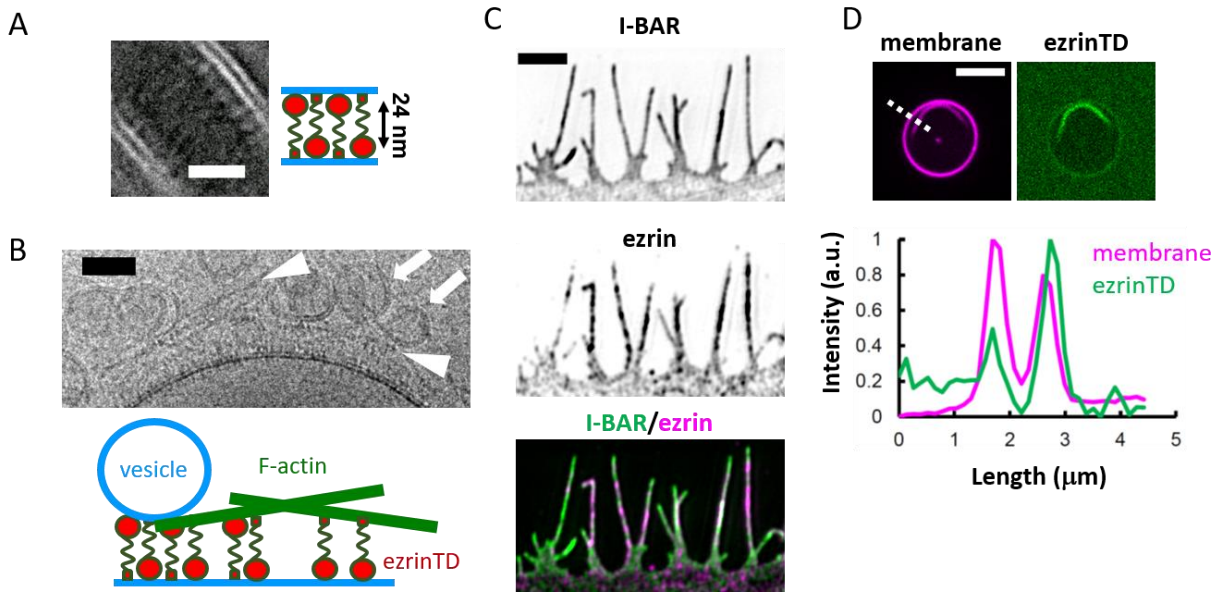

Figure 1. (A) Representative cryo-electron micrograph of PIP_2-membranes tethered by ezrinTD. Scale bar, 20 nm. (B) Representative cryo-electron micrograph of PIP_2-containing LUVs incubated with muscle F-actin in the presence of ezrinTD. Arrowheads indicate F-actin and arrows indicate tethered vesicles. (C) Representative structured illumination microscopy images of cellular protrusions of LLC-PK1 cells transfected with GFP-I-BAR domain and immunolabeled for endogenous ezrin. Scale bar, 2 μm (D) (Top) Representative confocal images of ezrinTD in IRSp53 I-BAR domain induced tubules. Scale bar, 5 μm (Bottom) Normalized fluorescence intensity profiles along the line drawn from outside the GUV towards the interior of the GUV, as indicated in the top image.

2D binding properties as function on the applied force and the interaction time of single domain antibodies binding tumor markers

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Therapeutic antibodies are now a common treatment of major diseases, especially in cancer. While capable to bind soluble antigens, antibodies do often bind their target at the interface between an immune cell and a target cell or a pathogen surface. While affinity is usually measured with one reactant in solution at least (*i.e.*, three dimensional environment or 3D), these measurements do not take into account the physical aspects of cell-cell interface (*i.e.*, 2D) that include force and relative motion of molecules constrained at surfaces, which can modify the interaction time available before binding. Our purpose is to look for links between 2D affinity or kinetics and cellular response. To quantify 2D binding properties, we perform kinetic measurements of five single domain antibodies (sdAbs) against the tumor marker HER-2 using a Laminar Flow Chamber (LFC). In the LFC, HER-2 is coated to a microbead surface and interacts with single sdAbs bound to the chamber bottom surface in the presence of flow. This allows us to measure at single molecular level the association kinetics (as the number of bond formed during a given interaction time between molecules) and the dissociation kinetics (as bonds lifetimes) under forces. Forces vary in the piconewton range and interactions times in the millisecond range.

Our results suggest to classify measured sdAbs in two groups. In one group, binding decreases non-linearly when the interaction time decreases and bond lifetimes are not affected by force. In a second group, binding decreases linearly with interaction time and bond lifetimes are modified by force.

Our anti-HER-2 sdAbs can be fused with an anti-CD16 sdAb, forming bi-specific antibodies able to recruit NK cells toward HER-2 positive breast cancer cells. We selected one sdAbs of each group and produced the corresponding bi-specific antibodies. We will perform NK-tumor cell cytotoxicity experiments with these, and try to correlate the tumor cell killing with 2D binding properties. This study provides a physical characterization of antigen-antibody interactions and could be useful for the selection of antibodies in therapeutics.

The Mechanosensitivity of Actin Bundles

Cells' ability to sense their environment is essential for many cellular processes including cell division, migration and morphogenesis. The actin cytoskeleton, which has been shown to be mechanosensitive, is organized into different architectures that carry out various functions within the cell. Filopodia, which are finger-like structures consisting of actin filaments bundled in parallel, emerge at the cell front and orient the cell in response to its mechanical environment. These actin filaments are elongated at their barbed ends by formins and Ena/VASP and cross-linked by the bundling protein fascin. These two machineries are thought to collaborate to design a unique type of actin network that governs filopodium dynamics, yet the exact mechanism by which these two key proteins synergize, and how mechanosensing is achieved in filopodia, are not well understood. Core questions such as: how actin filaments self-assemble in a bundle; how forces are transmitted along filopodia; how fascin and formin synergize to control the growth of actin filaments in filopodia remain to be addressed.

To tackle these questions, we use a microfluidics-based approach to reconstitute, *in vitro*, a minimal system to recapitulate the mechanosensitivy of actin bundles. We have probed the activity of the actin binding proteins formins and fascins in a large range of biochemical conditions : first on single filaments, then scaling up to bundles of several filaments. We have incorporated fluorescence polarization into the microfluidics setup to characterize the rotational behavior of actin filaments elongated by formin and bundled by fascin. This bottom-up approach allows us to understand actin bundle mechanosensitivity and brings us closer to obtaining a comprehensive description of force generation and transmission in formin and fascin generated bundles.

Impact of mechanics on actin disassembly by ADF/cofilin

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In the regulation of the actin cytoskeleton, disassembling networks is as important as assembling them. At the centre of the disassembling machinery is the family of actin-binding proteins ADF/cofilin. ADF/cofilin binds to the side of filaments forming domains that alter the conformation of actin monomers, increasing the filament helical pitch.

This change in structure leads to the severing of filaments thus accelerating the disassembly of networks. However how these shorter filaments then fully depolymerise to G-actin is not understood. A second important question is how ADF/cofilin targets specific actin filaments to disassemble. While biochemistry certainly plays an important role, it has also been proposed that mechanical stress could tune the effects of ADF/cofilin.

To tackle these questions, we use a microfluidic setup in which actin filaments are attached to the surface by one end or several points along their length. Through protein labelling and TIRF microscopy, we measure the depolymerisation dynamics at each end, ADF/cofilin binding and induced severing.

We discovered that ADF/cofilin not only accelerates the depolymerisation at the pointed end but also allows filaments to disassemble from their barbed end: in cells while ATP-G-actin should bind the barbed end to elongate filaments, ADF/cofilin synergies with Capping Protein to saturate filaments and put their barbed end into an unstoppable depolymerising state.

We then assessed the effect of tension, bending and twisting, mechanical stresses that occur in actin networks. While we observed no effect of tension, we found that filaments that cannot twist freely break faster: upon binding, ADF/cofilin puts filaments in an under-twisted state that promotes their severing.

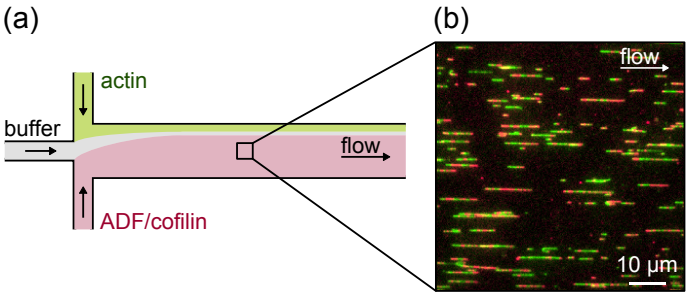

Figure 1: Microfluidic setup. (a) Sketch of the channel, solutions are injected from the three inlets at controlled flow rates. (b) Typical field of view. Actin filaments (green), covered with ADF/cofilin domains (red), are aligned with the flow.

Title: T cell adhesion on engineered substrates: influence of ligand nano-clustering

Authors

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Abstract

The interface between an Antigen Presenting Cell (APC) and a T-lymphocyte (T cell), sometimes called a synapse, plays a key role in sensitivity and precision of antigen recognition by T-cells. The importance of clustering of T cell receptors (TCR) is well established. In addition, it has recently been shown that the antigens, in the form of peptide Major Histocompatibility Complex (pMHC) recognized by TCR, are present on the membrane of APCs as defined submicronic clusters. The aim of this work is to study how such clustering of ligands influences T-cell membrane and actin organization.

To achieve this, we developed a novel hybrid system, where a synthetic substrate mimics the APC-membrane. The substrate consists of an array of sub-micrometric protein dots (diameter: 800±100 nm; spacing: 2μm), surrounded by a fluid supported lipid bilayer (SLB), which is optionally functionalized. The dots and the SLB are alternatively functionalized with molecules of anti-CD3 (targeting the TCR-complex), or ICAM-1 (ligand for the T-cell integrin LFA-1).

In T cells adhered to these substrates, local organization of TCR, the kinase ZAP-70 (one of the first molecules to be recruited to the TCR complex on activation), the actin distribution and membrane topography (measured using Reflection Interface Contrast Microscopy - RICM and Total Internal Reflection Fluorescence Microscopy - TIRFM) are impacted by ligand clustering. Colocalization of microclusters of both TCR and ZAP-70 with anti-CD3 dots is seen. The presence of ICAM-1 on the SLB does not appreciably perturb this organization, whereas B7 may have a slight impact. On ICAM-1 dots the TCR is not organized in micro-clusters.

The membrane of the adhering T cells exhibits a characteristic topography, when adhesive ligands (either ICAM-1 or anti-CD3) are present only in the dots but not on the SLB. Interestingly, the presence of an artificial polymer like polyethylene glycol on the SLB enhances the membrane topographical patterning, pointing to the repulsive role of membrane polymers in defining the topography of the synaptic interface.

Global parameters like cell area depend on the nature (ICAM-1 or anti-CD3) of the ligands present. On substrates with anti-CD3 (no ICAM), the cell area is the same on clustered and homogeneously distributed ligands as long as the average ligand density remains the same. However, in presence of ligands of LFA1, the global parameters are influenced by clustering or not of the TCR ligands. Specifically, the cell area is significantly increased when the same amount of anti-CD3 is clustered, rather than homogeneous.

Classically, the actin distribution in T cells adhering to a SLB functionalized with both ICAM-1 and anti-CD3, is in the form of a peripheral ring. However, on substrates patterned with dots of either ICAM-1 or anti-CD3 (no SLB functionalization), the actin is in form of colocalized dots. When in addition, the SLB is functionalized with the complementary ligand, the actin distribution becomes either homogeneous or peripheral. Dynamic imaging hints that TCR organizes the actin at early time and LFA-1 at late time, thus pointing to the crucial but different role of both in adhesion of T cells.

Posters

Studying the fundamental physical interactions of supported lipid bi-layers with incorporated transmembrane proteins

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For many years, supported lipid membranes on solid surfaces have been used as models of biological membranes and as a physiological matrix for the study of the structure and function of membrane proteins and receptors.[1] These primitive models of the cell membrane surfaces do not allow for biologically applicable lateral diffusion of both lipid membrane and trans-membrane proteins, or membrane deformation during fusion. In this investigation, we are developing model surfaces in which, a tethered polymer will act as a "spacer" between the solid substrate and the lipid membrane, providing a hydrated cushion for bio-chemically active membrane proteins.

Using self-assembly chemistry, we deposit a thin film (~3-10 nm) of hydrophilic Polyethylene Glycol (PEG) polymer layer on mica substrate, to cushion a lipid membrane. Through vesicle fusion, a lipid composition of 85% 1-Palmitoyl-2-Oleoyl-sn-glycerol-3-Phosphocholine (POPC) and 15% 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS), was injected onto the polymer within an aqueous buffer to form the cushioned membrane. We monitored the lateral diffusion of the lipids in the bi-layer using confocal fluorescence recovery after bleaching (FRAP). We have measured a lateral diffusion coefficient for our system of ~ 6 $\mu m^2/s$, which for solid surface supported lipids is ~10^{-3} $\mu m^2/s$. Therefore, we have developed a mobile lipid bi-layer surface suitable to incorporate trans-membrane proteins, without denaturing them.

The next step will be to incorporate membrane proteins in the cushioned membranes, followed by force measurements with the Surface Forces Apparatus (SFA). We aim to specifically examine Synaptotagmin 1 (syt1) protein, which is a synaptic vesicle anchored membrane protein that acts as the calcium sensor for neuro-transmission, to observe how binding is affected by the curvature, fluidity and deformity of the lipid bi-layer.[2]

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Long and Short Range Interactions in the Regulation of the Amoeboid Motility

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Dictyostelium is considered to be a solitary amoeba while food is available and turning into a social cell upon starvation. We are showing here that, even when food in plentiful, *Dictyostelium* cells interact with each other through multiple mechanism both by contact and through secreted factors to regulate their motility and spreading.
We created small colonies using micro-stencils and tracked the cell trajectories as they spread out in order to better investigate the onset of these collective behaviors[1]. From data analysis and simulation of an elementary model, we demonstrate that contact interactions act to speed up the early population spreading by promoting individual cells to a state of higher persistence, which constitutes an as-yet unreported contact enhancement of locomotion. a phenomenon we called CEL (Contact enhancement of Locomotion). Our findings also suggest that the current modelling paradigm of memoryless active particles may need to be extended to account for the history-dependent internal state of motile cells.
At longer time, the average cell speed is reduced by secreted Quorum Sensing factor (QSF) that accumulates in the media and acts at long range to prevent cells to drift too far apart from the others[2]. The cAMP-independent response to this unidentifed, high-molecular-weight molecule includes a reduction of the cell movements, in particular through the down-regulation of a mode of motility with high persistence time. Using indirect estimation of the QSF concentration and mathematical analysis of the cells' response, we demonstrate that the QSF production is under negative feedback: the secretion rate decreases linearly as a function of the concentration, which gives the cells a way to detect when they exceed a density threshold. The combination of CEL and QSF effects on cell motility might result into an optimization of space utilization.

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Microfluidic contraction flow of artificial tissues

Y. Meriguet, G. Massiera, L. Casanellas

The aim of this study is to develop an artificial cohesive tissue and determine its spatiotemporal dynamics under microfluidic flow. The artificial tissue is obtained by the assembly of human ghosts (red blood cells from which hemoglobin has been released [Dodge1962]) and mediated by the presence of wheat proteins.

By imposing a constant flow, we study the passage of the ghost aggregates through a microfluidic constriction. Our velocimetry results show that, depending on the aspect ratio between the cell aggregate and the constriction size, the tissue adopts different strategies in order to advance through the constriction: aggregate reorientation, flow localization, or cell deformation.

The comparison of our results with experiments performed with living cell aggregates under comparable flow conditions [Tlili2015] may enable to have a deeper insight on the effect of internal cell activity on tissue mechanics as well as the role of cell adhesion, which are both essential in embryogenesis and tumor metastasis processes [Lecuit2007].

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In vitro study of T cell migration on substrates with modulated adhesiveness

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Our defense against pathogens relies on the complex and sophisticated orchestration of leukocyte recruitment. This process is mediated by external cues among which the role of adhesion molecules is still only partially deciphered. In this project, patterns of adhesion molecules will be created using light-induced molecular adsorption (LIMA). The first part of the project is dedicated to adapting the LIMA technique to functionalize substrates for specific adhesion molecules ICAM-1. ICAM-1 molecules have been patterned on antifouling surfaces to achieve specific binding and patterned proteins have been quantified using fluorescent antibodies. In the second part of the project, substrates with different adhesiveness have been created, allowing us to investigate the relation between substrate adhesiveness and T cell migration properties.

Title: The impact of actin binding protein on branched networks force generation.

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Saccharomyces Cerevisiae or budding yeast is a model organism in biology. Its genome and metabolism is being thoroughly investigated as the cells are easily manipulated in the laboratory. Yeast, as many organisms, interacts with the environment through endocytosis and exocytosis. In contrast to mammalian cells, its endocytosis follows only one pathway – Clathrin Mediated endocytosis (CME). Moreover, its actin networks are an essential part in yeast's CME mechanism and dynamics. For these reasons, we will use yeast to decipher the role of actin networks in endocytosis.

Actin is regulated by a large number of actin binding proteins (ABPs). Mutations or deletion of these proteins can trigger major changes on the endocytosis phenotype. For this project, we will study *in-vitro* the mechanics and assembly dynamics of branched actin gels assembled by Arp2/3 machinery. An innovative *in-vitro* approach, developed in the laboratory, allows us to probe the force generated by the actin gel by using lab-made magnetic micro-cylinder force sensors. These cylinders give us the possibility to probe the elasticity, the plasticity and the assembly process, in presence of opposing forces, of dense cytoskeletal networks. The advantage of the method are the range of attainable forces and its high throughput.
This study will use cellular extracts of wild type or specific mutant yeasts to precise the role of these proteins in the mechanisms of actin growth . Its role can be verified by adding the ABP in branched networks formed from a minimal set of purified proteins to validate the experiments made with the extracts.

In this manner by controlling the ABPs present and the stress applied during network growth, we can examine the role of each protein in the mechanics of the gel in order to compare the relation between physical measurements *in-vitro* (between two cylinders) and observations *in-vivo* (during endocytosis). We thus aim at understanding the phenotypes observed in mutants and wild-type yeast as well as the role of different actin partners in the force generation by the Arp2/3 machinery.

MODULATION OF MEMBRANE RIGIDITY BY THE ESCRT-III COMPLEX

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The ESCRT-III complexes is an evolutionary conserved membrane scission machinery and it is essential in many cellular processes such as cytokinesis, multivesicular bodies formation, HIV release and nuclear membrane repair. Membrane scission by ESCRT-III is accomplished by constricting the negatively curved membrane present inside the neck connecting two membrane-delimited compartments. In Homo Sapiens there are at least 12 ESCRT-III proteins, called CHMPs. However, only a subset of them, namely CHMP4B, CHMP2A/B and CHMP3 appear to be strictly required in all these processes, indicating that they might constitute the minimal scission machinery. Several CHMP2B mutations have been reported to cause neurological disfunctions; however the function of this protein is still unknown. We investigated the mechanical properties of CHMP proteins polymer and found that they can modulate membrane rigidity in a subunit-specific fashion, providing a possible molecular mechanism explaining the pathogenic property of the mutated CHMP2B. Our results indicate that modulation of membrane rigidity is an important aspect of ESCRT-III function and assign a novel function for CHMP2B.

GDR 3070 : Physique de la cellule au tissu

Combination of micropatterning and magneto-active substrates for local mechanical stimulation of single cells

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Cells can sense and respond to their physical environment by translating a mechanical cue into an intracellular biochemical signal[1]. Yet, how a cell senses local mechanical stimulations is still under investigation. The cellular response to an external mechanical stimulation has been studied with various static and dynamic systems, so far limited to global deformations[2,3] or to local stimulation through discrete substrates[4,5].

Here we aimed at generating fibronectin micropatterns on very soft (20 kPa) polydimethylsiloxane (PDMS) substrates containing magnetic micro-pillars. We introduced a novel technique for patterning fibronectin on soft and sticky PDMS by using polypropylene sheet as an intermediary step before directly curing PDMS on top of it.

To mechanically stimulate the obtained single cells, we used electro-magnets to move the embedded magnetic micropillars which in consequence locally deformed the PDMS substrate. We characterized the magnetically-induced surface stress and the resulting cell shape and forces. The combination of our micropatterning approach with magneto-active substrates thus represent a new tool to study mechanotransduction in single cells, and complement existing techniques by exerting a local and dynamic stimulation through a continuous soft substrate.

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Active Membrane-Quake Detection in Red Blood Cells by Back Focal Plane Interferometry

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Fluctuations of the red blood cell membrane exhibit both thermal and active components [1,2]. For a better detection of these fluctuations, we use our custom designed microscope with the back focal plane interferometry, a simple yet very powerful optical technique for nanometric displacement observation [3]. Combining it with the wavelet analysis, we reveal single events, membrane-quakes, in the thermal background of the fluctuations. These events can be identified as active and are related to the single protein binding and unbinding processes within the cytoskeletal scaffold, and therefore to the ATP-consumption [2]. The use of the visually more adapted wavelet transform unveils both time and frequency information about the membrane activity, while the classical power spectral density analysis complements it with the physical parameters of the system. We present our preliminary results of the red blood cell membrane activity in the normal and ATP-depleted conditions.

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Study of the gamete interaction during mammalian fertilization

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Mammalian fertilization is the process by which a spermatozoon and an oocyte meet and interact to produce a new being. The oocyte is a large round cell surrounded by the Zona Pelucida (ZP), a glycoproteic envelope that the fertilizing spermatozoon has to cross to reach the oocyte membrane and fuse with it.

If several spermatozoa succeed to cross the ZP and fuse with the oocyte, more than one paternal genetic material are incorporated into the egg. This process, called polyspermy, leads to fertilization failure. One well-documented mechanism ensuring monospermy is the calcium induced hardening of the ZP, occurring after sperm/egg fusion [1]. However, we found that in some cases, one or several sperm can cross the ZP and doesn't fertilize, even if it is a fertilized oocyte or not. This observation shows that a spermatozoon, normally able to fertilize after the ZP crossing, does not necessarily fuse.

Our goal is to better understand the gamete interaction mechanism by determining why these spermatozoa are unable to fuse. The only currently known factors mandatory for fusion in mammals are: three proteins (CD9 and Juno on the egg, and Izumo1 on the sperm) and one specific flagellum beating mode. [2]

We study each one of these factors during an in vitro fertilization: dynamics of the 3 proteins and beating of the flagellum. Our hypothesis is that the post-fusion remodeling of the egg plasmic membrane could have as consequences that, neither the oocyte membrane, nor the sperm one are anymore able to interact properly with each other to fuse.

Preliminary results show, by immunostaining, that a release of Juno from the egg membrane occurs after fusion, resulting in protein coverage of the sperm heads localized in the vicinity of the oocyte. We also observe that spermatozoa crossing the ZP have not necessarily the specific beating leading to fertilization.

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Mapping drug mechano-sensitivity in tumour spheroids with brillouin light scattering

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Aims and/or Background: Mechanical properties are key players in tumour physiology, but their exact role in growth, invasivity and response to drugs remains largely unknown due to the lack of characterisation techniques. Standard microscopy techniques are limited by the photon mean free-path in the imaging depth they can achieve to ~100 μm. Besides, the use of fluorophores or tags alters normal cell functions and eventually kills cells, hindering the study of drug kinetics over standard therapeutic time scales. Most importantly, they provide a contrast that does not reveal mechanical properties. In this work we implement a novel quantitative, label-free microscopy technique based on Brillouin light scattering (BLS) to decipher the link between mechanical properties and drug efficacy.

Methods: To understand the physics of tissues and to accelerate the translation of novel therapeutics to the clinic, it is necessary to define biological models that recapitulate closely the complex mechanics of tumours. Multicellular spheroids (MS) are an apt tumour model that captures the spatial gradient distribution of mechanics and biological factors, and resistance to drug penetration. For demonstration, we monitored with BLS the mechanical properties of MS formed from a colorectal cancer cell line HCT116 during a 3-days chemotherapy with 5-fluorouracil (5-FU).

Results: We captured BLS maps with 10 μm resolution in the equatorial plane of the MS to probe the distribution of mechanical properties and monitor in-depth drug sensitivity. Our images reveal a clear variation in the rigidity and viscosity from the outer rim to the core of the untreated MS. In addition, the mechanics across the centre of the spheroid during the 5-FU therapy show the radial action of the drug starting in the outer regions of the MS from the first day of exposure to reach the core in about 3 days. This observation is consistent with live/dead assays by epifluorescence microscopy.

Conclusion: Such results, which cannot be observed by any other existing modality, demonstrate the ability of BLS to image quantitatively drug efficacy on *in vitro* models using mechanical properties as the contrast mechanism, without tags, and with an unprecedented imaging depth in ~300-500 μm objects. Our approach should shed light on the link between mechanics, structure and biological functionality, thereby offering innovating solutions for the understanding and control of tumors and design of anti-cancer drugs.

Synaptotagmin interactions with membranes: measuring the force of calcium triggering of neurotransmission

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A critical step of neurotransmission is the rapid and synchronized fusion of synaptic vesicles with the pre-synaptic plasma membrane of the neuron upon calcium entry, allowing the release of neurotransmitters immediately upon arrival of an action potential. A calcium binding protein anchored in the synaptic vesicle, the Synaptotagmin-1 (Syt1), has been identified as the calcium sensor of this process, being able to accelerate fusion by more than four orders of magnitude in presence of calcium. Despite this crucial role, the molecular mechanism involved remains unclear.

The cytosolic domain of Syt1 consists of tandem Ca2+- binding C2 domains (C2A and C2B) attached to the membrane via a juxtamembrane linker domain. Different sites of the protein are responsible for its specific roles. First, the interaction of the polybasic region of the C2B domain with the anionic lipid PIP2 is needed for the initial docking of the synaptic vesicle at the plasma membrane. Second, upon calcium binding, the aliphatic loops on each C2 domains partially insert into the membrane, enabling the SNARE proteins to complete membrane fusion.

While the mechanistic details described above are mostly well-accepted, importantly, the binding energies of Syt1 with membranes have never been reported. Therefore, the aim of our work is to measure the energetics of the major membrane binding sites of Syt1, i.e. the polybasic motif and the calcium-loop insertions of C2B and C2A. For this we use a Surface Force Apparatus (SFA), a device that provides a direct measurement of the interaction force between two surfaces as a function of their separation distance, with nanoscale resolution. This system has successfully been used to measure the energy of the SNARE proteins assembly, but the previous experiments did not include Syt1. In our set up, both surfaces are coated with a lipid bilayer. One of them mimics the synaptic vesicle membrane, on which we bind Syt1. The opposing bilayer mimics the inner leaflet of the plasma membrane and contains PIP2 and PS lipids. We will present results obtained with various lipid compositions and relevant mutations of the protein and how these interactions are impacted by the presence of calcium. Ultimately, we plan to provide a complete mapping of the energetics of the critical membrane interaction sites of Syt1.

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RESEARCH INTERESTS

Plants display great developmental plasticity in response to the environment while maintaining their core, functional structures. I am interested in how plant cells integrate a plethora of signals to coordinate and adapt growth and development to an ever-changing environment. My research methods include advanced quantitative imaging techniques and biomechanical micro measurements as well as traditional techniques like genetics and molecular cloning.

EDUCATION

2009-2014 PhD student at Universiteit Utrecht, the Netherlands

2005-2009 BASc at Hogeschool van Arnhem en Nijmegen, the Netherlands

PROFESSIONAL EXPERIENCE

2014-current Postdoctoral and EMBO fellow at RDP, ENS de Lyon, France
"Shaping with water": investigating the role of turgor pressure during plant shoot morphogenesis.

2009-2014 PhD student at Universiteit Utrecht, the Netherlands
Transcription factor movement and tissue patterning in Arabidopsis root meristem.

Role of Myosin 1b in actin network assembly by in vitro approaches

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The actin cytoskeleton is the dynamic architecture of living cells made of several structurally and functionally distinct arrays of actin filaments. Establishment and maintenance of a polarized network of growing filaments require links between the barbed ends of filaments and the membrane. Actin-binding and membrane-associated proteins link the growing barbed ends of filaments to the membrane to control both their polarity and dynamics. Cell shape changes require forces that are generated on membranes by the combined action of actin filament polymerization and myosin motor activity. Despite a wealth of information on myosin activity, the role played by these proteins of myosin 1 family on the architecture and dynamics of cellular actin networks remain unclear.

Myosin 1b (Myo1b) is a single-headed membrane-associated motor that bind actin filaments. It comprises an N-terminal motor domain coupled to a C-terminal tail homology 1 domain (containing a lipid-binding domain) by a neck region that binds Calmodulin. A new mechanism can be envisioned for Myo1b-induced membrane deformation. Myo1b could act as an active tether between polymerizing actin filaments and the membrane. The force generated by actin polymerization could be transduced by Myo1b into membrane tubulation. If the actin filaments are nucleated by formins and if myosins help orientating elongated filaments, a polarized actin network could be observed. The polarization will depend on the equilibrium between processive elongation by formins and sliding of the filaments by myosins motor activity. Alternatively Myo 1b may control the arp2/3 actin network required for the membrane deformation.

The goal of this project is to determine the mechanism by which membrane-associated Myo1b activity controls the elongation and orientation of growing actin filaments to shape a polarized network of actin filaments that can deform the membrane. To this aim, we developed, new *in vitro* microscopy assays with pure proteins.

Formin's processivity under applied force

Formin, a key actin regulator, is involved in a number of pathologies. It is able to keep tracking the barbed end of actin filaments for a finite time, while accelerating actin polymerisation in the presence of profilin. In cells, formin's behaviour is precisely regulated by different factors, including local actin/profilin concentration, mechanical forces and other actin associated proteins. Clarifying the mechanism of formin's processive movement is essential in order to understand formin's behaviour. However, so far, how formin's processivity responds to various chemical or mechanical conditions is still unclear.

Here, we investigated how formin dissociates from the barbed end for different actin/profilin concentration and/or for various applied pulling forces, in order to answer 1) whether profilin has an impact on formin's processivity; 2) how formin's processivity responds to applied forces.

We found that formin's dissociation rate (k_{off}) from actin barbed end has a positive correlation with its elongation rate (V_{elong}) at fixed profilin concentration. However, when the concentration of profilin is increased, k_{off}/V_{elong} is decreased. These results indicate that formin's processive movement is improved by the presence of profilin.

Also, we found that formin's processivity is very sensitive to applied pulling forces. Formin's dissociation rate increases exponentially with force, even at relatively small force (<10 pN) is applied. Moreover, we found that force has a dominant impact on formin's processivity, for all actin/profilin concentration that we tested, irrespective of the elongation rate.

Finally, our study of formin's processivity will contribute to develop a more comprehensive model to describe formin's behaviour, especially how formin responds to mechanical forces in cells.

Biophysical approach of the mucociliary function: Mucus rheology and beating coordination

The mucociliary function of the bronchial epithelium ensures the continuous clearance of the respiratory system, which relies on two main elements: mucus and cilia beating coordination.

We perform here a rheological characterization of mucus samples extracted from ALI (Air-liquid interface) cultures of bronchial epithelium. Our approach combines macro- and micro-rheology techniques with the aim of quantifying the mucus viscoelastic properties at different length scales (from the size of bronchial cilia up to the scale on which mucus is transported). This specific methodology allows us to compare samples corresponding to different patient pathologies.

In addition, we will describe our method to quantitatively characterize the coordination between cilia and how density and spatial distribution influences this coordination and consequently the mucus motion, required for the mucoliary clearance.

Quantitative FRET to study mechanotransduction

Alexis Coullomb, Cécile Bidan, Aurélie Dupont – LIPHy, Grenoble

Fluorescence Resonance Energy Transfer (FRET) imaging techniques allow us to measure in real time and in live cells distances between two fluorophores. Thanks to ingenious constructions implying spring-like proteins or kinase substrates, FRET efficiency can be used to monitor molecular forces or the activity of a signaling proteins respectively. Unfortunately, most measurements are only qualitative, and it is hard to compare results between cells, experiments and laboratories.

Here we propose a method using alternating laser excitation (ALEX FRET) to quantitatively measure the FRET efficiency and fluorophores stoichiometry. We correct for the cross-talk of donor fluorophores into the acceptor channel, and for the direct excitation of acceptor fluorophores during the excitation of donors. Two other factors remain to be determined: the gamma factor, that corrects the differences in fluorophore's quantum yields and photon detection efficiencies, and the beta factor, that accounts for the differences in excitation intensity and absorption cross-section of the fluorophores. These two factors are more challenging to estimate, and we will discuss the different ways to evaluate them.

Single-cell leukocyte mechanics: force generation and rheology

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Leukocytes are very soft cells that perform many diverse functions: they adhere, crawl, transmigrate, kill, phagocytose or interact with other cells. During their activation, leukocytes both generate mechanical forces and change their viscoelastic properties (i.e. they stiffen/soften, get more or less viscous). We have developed micropipette-based setups to quantify single-leukocyte mechanical properties and monitor them over time while a leukocyte gets activated by a relevant stimulus.

We use this approach in diverse contexts involving leukocytes: activation of T lymphocytes, phagocytosis of a target by a neutrophil, or transmigration of a lymphoblast across an endothelial monolayer. We measure forces generated by T lymphocytes[1-3] and perform microrheology experiments with a profile microindentation setup[2,4,5] (Figure 1), These mechanical measurements shed a new light on how cell mechanical properties evolve over a short period of time (seconds), how they adapt to the stiffness of their environment, and how intracellular signaling is involved.

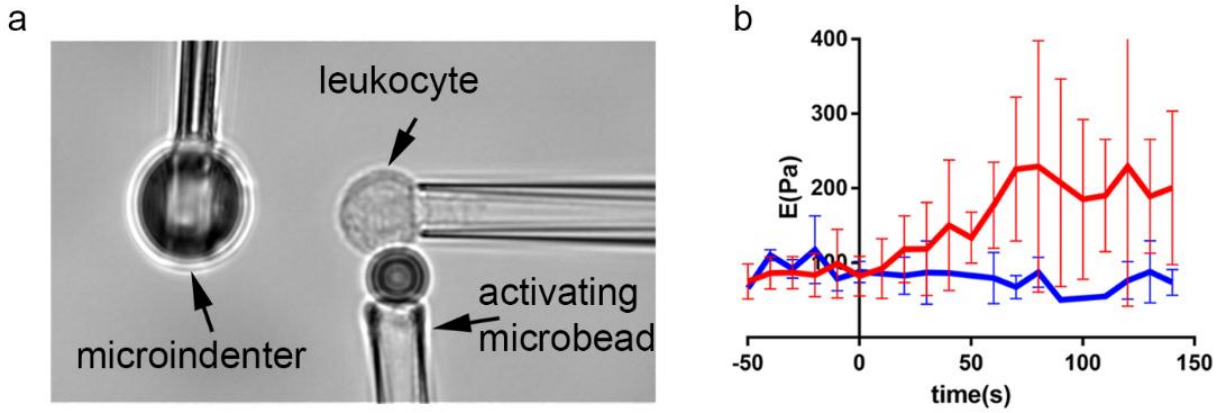

Figure 1. (a) Microindentation setup coupled to an auxiliary micropipette bringing an antibody-covered microbead in contact with a leukocyte help by a micropipette. Cell viscoelastic properties of the leukocyte can be monitored over time during cell activation. (b) Time evolution of the leukocyte Young's modulus its activation (red curve). Cell-bead contact occurs at time t=0. The curve in blue corresponds to a non-activating bead.

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CELL MECHANICS ACROSS SCALES AND GENE EXPRESSION

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In multicellular organisms, cells generate and undergo mechanical forces that propagate through tissues. Using genetically encoded molecular tension sensors, we seek to understand how mechanical forces propagate through scales and the molecular mechanisms by which they activate genetic programs.

We show that in adherent migrating cells, various proteins of adhesion complexes and the nuclear envelope can undergo tension changes that exhibit non-trivial relationships between each other and with cell scale forces. Moreover, these tension changes associate with changes in enzymatic activities and post-translational modifications of specific proteins, and ultimately gene expression.

These results provide insight into the complexity of the cell composite mechanics and its connection with major signaling networks involved in development and disease.

A Mathematical Model of the Liver Circadian Clock Linking Feeding and Fasting Cycles to Clock Function

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To maintain energy homeostasis despite variable energy supply and consumption along the diurnal cycle, the liver relies on a circadian clock synchronized to food timing. Perturbed feeding and fasting cycles have been associated with clock disruption and metabolic diseases ; however, the mechanisms are unclear. To address this question, we have constructed a mathematical model of the mammalian circadian clock, incorporating the metabolic sensors SIRT1 and AMPK [1]. The clock response to various temporal patterns of AMPK activation was simulated numerically, mimicking the effects of a normal diet, fasting, and a high-fat diet. The model reproduces the dampened clock gene expression and NAD+ rhythms reported for mice on a high-fat diet and predicts that this effect may be pharmacologically rescued by timed REV-ERB agonist administration. Our model thus identifies altered AMPK signaling as a mechanism leading to clock disruption and its associated metabolic effects and suggests a pharmacological approach to resetting the clock in obesity.

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QUANTITATIVE STUDY OF THE CONTACT BETWEEN B CELLS AND ANTIBODIES-FUNCTIONNALIZED OIL DROPLETS USING MICROFLUIC TRAPS

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In the immune system, B cells have the function to produce antibodies after the binding of B cell receptors with antigens presented at the surface of other cells, like dendritic cells for example [1]. This specific recognition leads to B cell spreading and antigen accumulation at the contact of the two cells [2]. In previous works, the kinetic of the antigen accumulation has not be fully studied [3]. Moreover, the B cell mechanical forces implied in the recognition processes are not known.
In our work, we used antibodies-functionnalized oil droplets as antigen presenting cells. As liquid objects, they have the advantage of allowing antibody accumulation at their surface and potentially deformation, permiting force measurement. We put them into contact with B cells using a dedicated microdevice with adapted microfluidic traps to see the « time zero » of contact and also to increase the statistics of contact. Results from the kinetic studies and persperctive on future developments will be presented.

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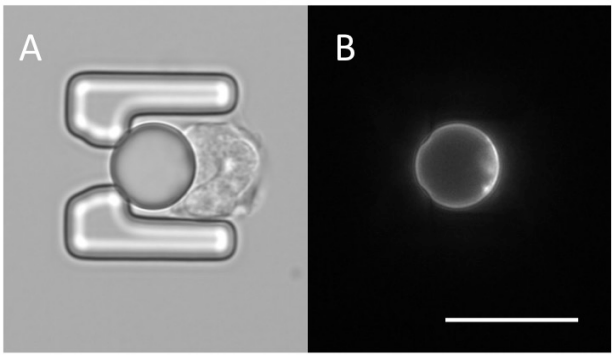

Image showing a B cell in contact with an antibodies functionnalized oil droplet in a microfluific trap. The trap has been first loaded with the functionalized oil droplet and the B cell has been loaded in secund. The time of the image is 7 minutes after contact. (A) Microscope image in bright field. (B) Microscope image in fluorescence of the droplet showing an accumulation of streptavidin at the contact between the droplet and the cell. (Bar=20µm)

Anisotropic actomyosin organisation driving of morphogenetic flow in three-dimensions

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Claire Lye[3] Bénédicte Sanson[3]

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[3]*PDN, University of Cambridge, United Kingdom*

Morphogenesis is a three-dimensional process during which an organism undergoes complex deformations to acquire a given shape and organisation. The genetic patterning of Drosophila embryos and the way this regulates key molecules and complexes, such as actomyosin, is well described. How the motor Myosin II generates local mechanical action is understood, however, the way this is integrated at the scale of the embryo to drive morphogenetic movements is still to be characterised. Axis extension in Drosophila is a good model system for this, since it involves the deformation of the whole of the embryonic epithelium. It is dependent on a well-characterised anisotropic myosin recruitement pattern in the germband tissue, where actomyosin organises in oriented supracellular cables through a planar-polarisation mechanism.

In order to resolve the stresses and deformations produced at the scale of the whole embryo, we develop a novel finite element technique which allows us to solve the three-dimensional mechanical balance resulting from a given global distribution of myosin-generated prestress. Our prediction of local mechanical behaviour is based on a rheological law recently validated for cortical actomyosin (1,2) and extend to the case when myosin generates an anisotropic prestress (3).

Numerical simulations confirm that the planar-polarised arrangement of myosin in the germband can trigger embryo-scale flows similar to those observed experimentally. Interestingly, this mechanical behaviour is shown not to rely necessarily on cell intercalation, but rather on the anisotropy of myosin action, which can entail cell elongation as well as intercalation. We also show that the mechanical balance that leads to axis extension towards the posterior of the embryo is crucially dependent on the embryo's geometry, including the presence anteriorly of the cephalic furrow, which can act as a guide for morphogenetic movements.

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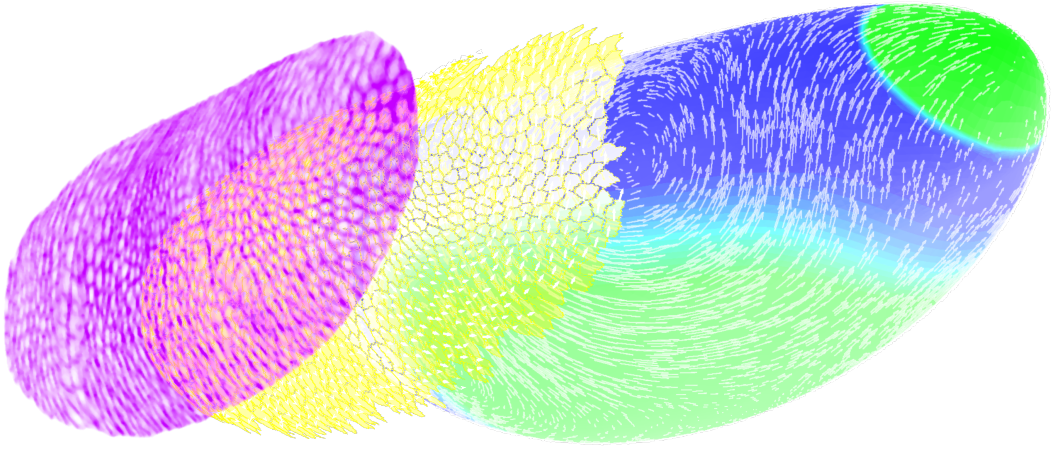
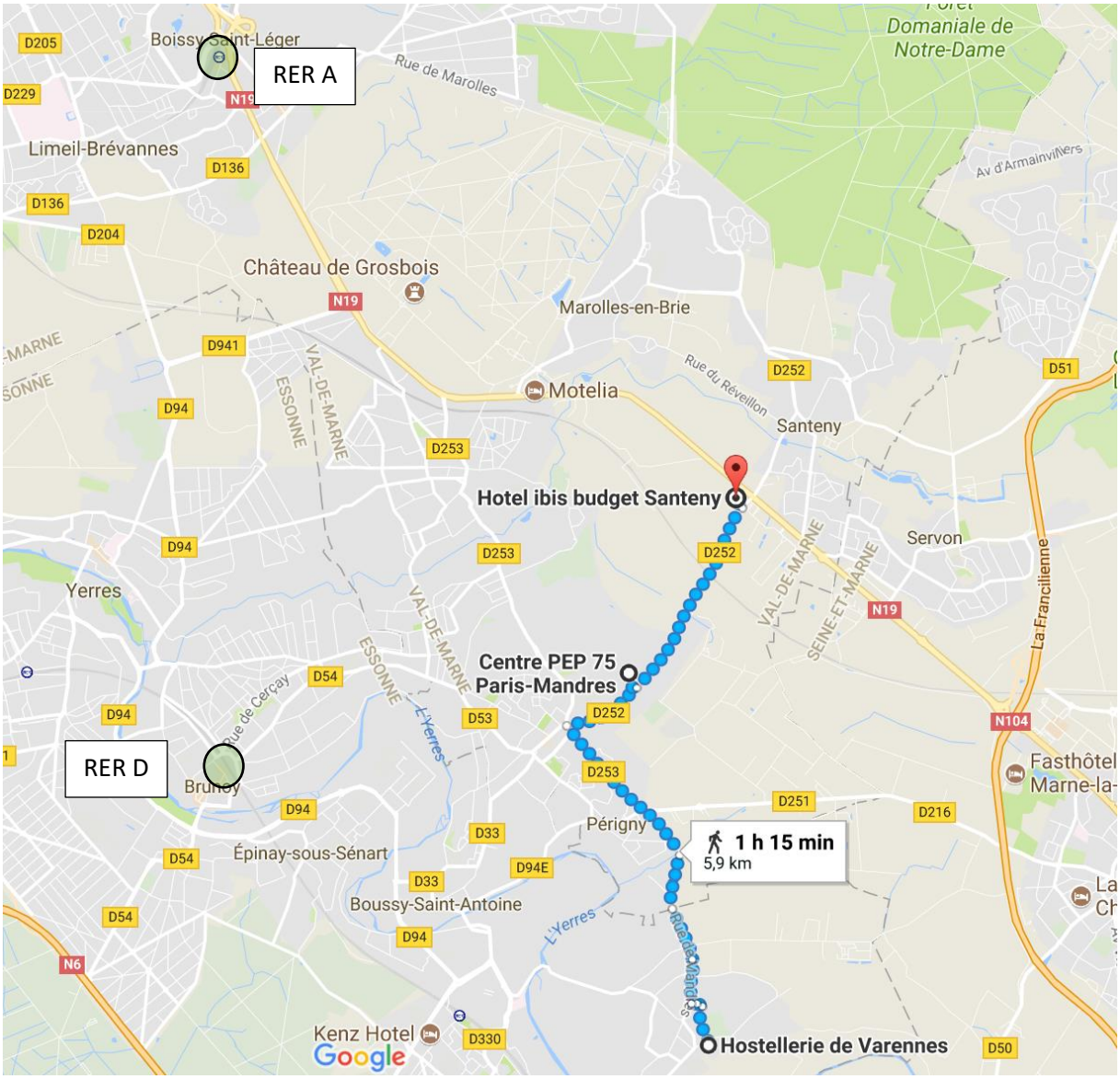


Figure 1: Myosin distribution (colour-coded in green on right-hand side image) and a mechanical model are predictive of a morphogenetic flow (arrows, right-hand side image) comparable to the experimentally observed flow (central image), obtained from the tracking of the dynamics of cell contours (left image).

Access to Paris-Mandres center “les PEP75”

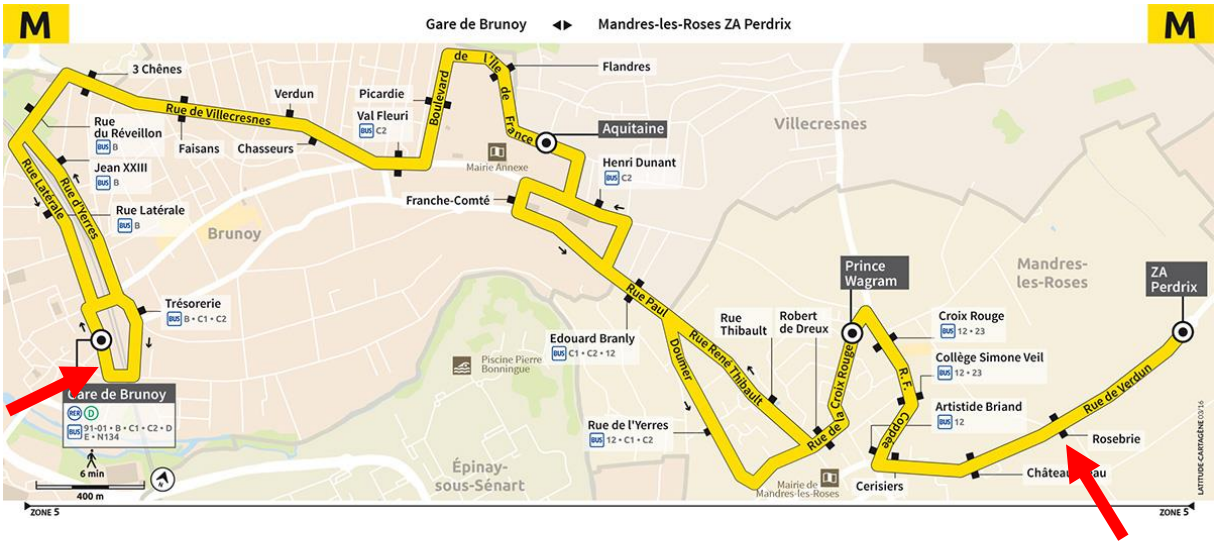
2 Rue du 8 Mai 1945, 94520 Mandres-les-Roses

Le centre Paris-Mandres est d'accès facile : on peut y arriver soit par le **RER D** (arrêt Brunoy, 52 min de marche si à pied, 4,2km) puis le bus M (arrêt Rosebrie, reste 250 m à pied) ou le bus C1 (arrêt Charles de Gaulle, reste 1.4 km à pied), soit par le **RER A** arrêt Boissy-Saint-Léger puis le bus 23 (arrêt Simone Veil, reste 450 m à pied). Les bus passent toutes les 15-20min. En fonction de l'heure de la journée, l'un ou l'autre de ces trois itinéraires est le plus court en temps, mais les distances à marcher sont différentes. Suivre la rue de Verdun jusqu'au numéro 98, puis prendre la rue perpendiculaire, vous y êtes !



BUS M

Be careful: buses M only in the early morning and late afternoon



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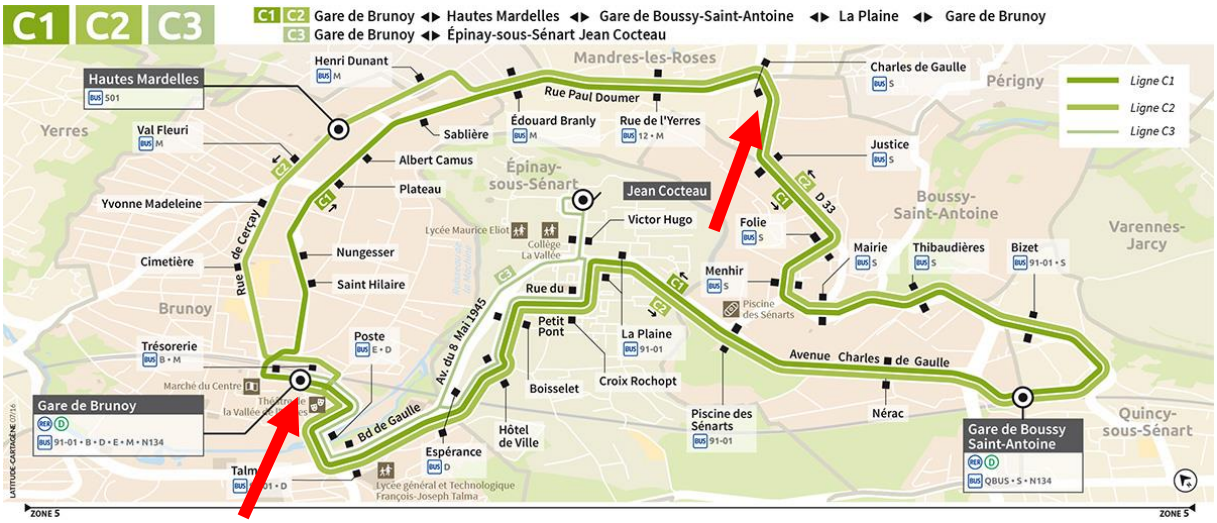
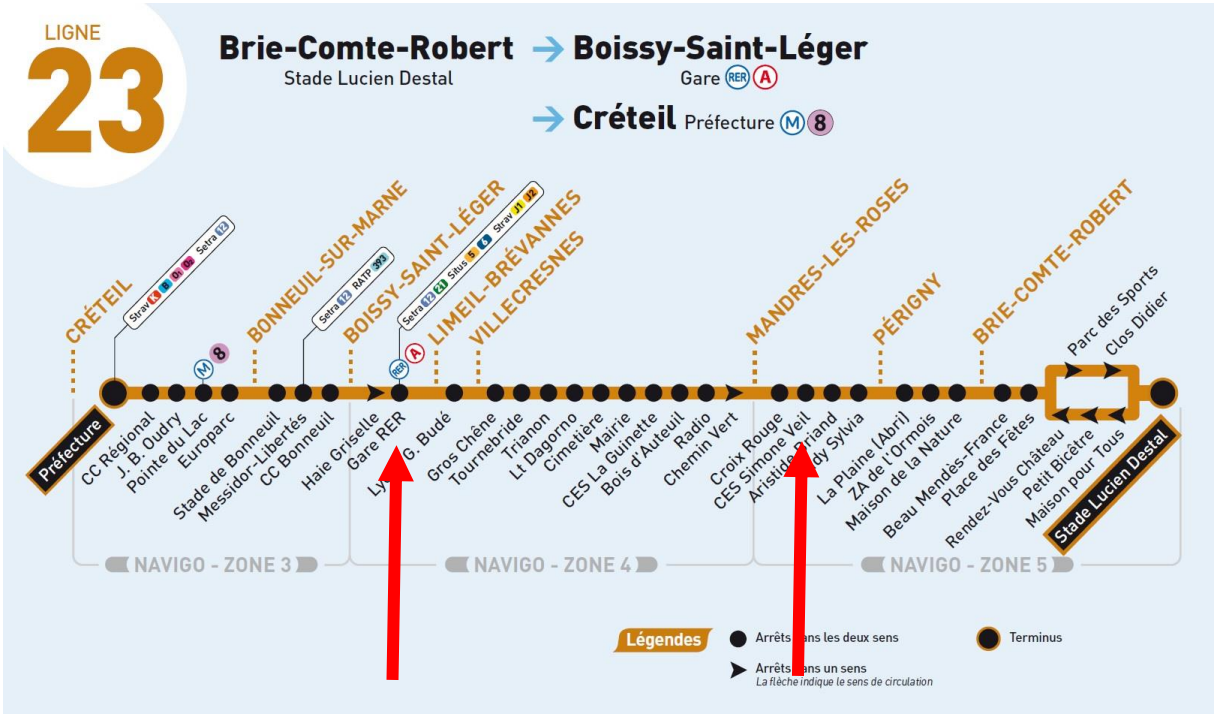


BUS 23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BONNEUIL-SUR-MARNE
BOISSY-SAINT-LÉGER
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VILLECRESNES
MANDRES-LES-ROSES
PÉRIGNY
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CES La Guinette
Bois d'Auteuil
Radio
Chemin Vert
Croix Rouge
CES Simone Veil
Aristide Briand
Sylvia
La Plaine (Abri)
ZA de l'Ormois
Maison de la Nature
Beau Mendès-France
Place des Fêtes
Rendez-Vous Château
Petit Bicêtre
Maison pour Tous
Stade Lucien Destal
NAVIGO - ZONE 3
NAVIGO - ZONE 4
NAVIGO - ZONE 5
Légendes
Arrêts dans les deux sens
Arrêts dans un sens
La flèche indique le sens de circulation
Terminus