MECABIONIC

UNIVERSITÉ CÔTE D'AZUR

Mechanobiology across scales

from the molecule to the organism and back

KEYNOTE SPEAKERS

Thomas Surrey
CRG, Barcelona, SPAIN

Nicolas Minc
Institut Jacques Monod, Paris, FRANCE

Ewa Paluch
Cambridge University, UK

Ashley Nord
Structural Biology Center, Montpellier, FRANCE

Stanislav Y. Shvartsman
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Robert Arkowitz
iBV, University Côte d'Azur, Nice, FRANCE

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EMBL Heidelberg, GERMANY

Eric Honoré
IPMC, University Côte d'Azur, Nice, FRANCE

Vito Conte
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Cécile Leduc
Institut Jacques Monod, Paris, FRANCE

Rachele Allena
LJAD, University Côte d'Azur, Nice, FRANCE

Matteo Rauzi
iBV, University Côte d'Azur, Nice, FRANCE

Symposium

2 - 5 April 2024

Nice, FRANCE
University Côte d'Azur
Campus Valrose
LJAD Conference Room

ORGANISERS

Rachele Allena
Chloé Féral
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VILLE DE NICE

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INDEX

1. PRESENTATION

The mechanics of the living plays a paramount role in a plethora of biological processes. To dissect and understand the role of mechanics in living systems, an interdisciplinary community of scientists work together and develop/combine a large spectrum of technologies and tools.

The aim of the symposium is to gather researchers tackling the mechanics of the living from different scales. We believe this to be a unique opportunity to foster exciting and insightful discussions among an international and interdisciplinary community of experts focused on the understanding of the fundamental biophysical principles powering life from the molecule to the organism level.

As part of the thematic semester MECABIONIC on the mechanobiology at Université Côte d'Azur, "Mechanobiology across scales" will bring together junior and senior researchers from various fields including physics, biology, mathematics, computer science and engineering.

The program includes presentations of recent advances and breakthroughs in mechanobiology, scientific interactions among PhD students, postdocs and confirmed scientists as well as memorable social events.

This international symposium will take place between the 2nd and 5th of April 2024 in the city of Nice located in the beautiful region of the Côte d'Azur in the South-East of France

2. KEYNOTE SPEAKERS

Thomas Surrey
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4. VENUE

The conference will take place at the institute of mathematics Laboratoire Jean Alexandre Dieudonné located in the Campus Valrose of the Université Côte d'Azur in Nice (FR).

Address: 28 Avenue Valrose, Campus Valrose, LJAD, 06108 Nice, France.

The Campus Valrose is served by public transport, notably the tramway, and is close to Nice-ville station. Right in the heart of Nice, the Parc Valrose is a magnificent 10-hectare estate, remarkable for the richness of its flora and the beauty of its monuments. Its historical and heritage features make the Campus Valrose an exceptional place.

The Welcome Cocktail on April 2nd, 2024 will take place in the Grand Chateau on the Campus Valrose.

The Social Night on April 4th, 2024 will take place on the rooftop of the Hotel Aston La Scale in Nice (12 Av. Félix Faure, 06000 Nice).

5. HOW TO GET TO NICE

- By air: Flights to Nice land at the Nice International Airport. There are direct non-stop flights from New York to Nice that operate year-round. Paris, London and most European capitals also have non-stop flights to Nice.
- By train: Trains to Nice all stop at the main train station, Nice Ville. There's also the smaller Gare St Augustine and the Gare Riquier which handle domestic routes. More on Nice train stations.
- By bus: International Buses to Nice are handled by Eurolines but you'll get a better deal and a more comfortable bus using Flixbus. In either case, you'll be dropped off either at Nice Airport or the Gare Routiere Vauban where it's easy to catch a tram to the town center.
- By car: The main motorway (*autoroute*) that runs through the French Riviera is the A8 which extends all the way to Monaco and the Italian border.

6. HOW TO GET TO VALROSE CAMPUS

- By tram: Line 1, stop Valrose Université
- By bus: Line 11, stop Vallot Ligne 37 or stop Valrose

7. PROGRAM

TUESDAY APRIL 2nd

12h00 – 13h50	Registration at LJAD	
13h50 - 14h00	Opening remarks	
14h00 – 14h40	Thomas Surrey	chair: RAUZI
14h40 – 14h55	Aurélien Villedieu	
14h55 – 15h10	Nassim Anis Ahmine	
15h10 – 15h20	Joséphine Schelle	
15h20 – 16h00	Coffee break and meet the speakers	
16h00 – 16h40	Nicolas Minc	chair: HONORE
16h40 – 16h55	Jean-François Berret	
16h55 – 17h10	Urška Andrenšek	
17h10 – 17h25	Etienne Boulter	
17h25 – 17h35	OPTICS11	
17h35 – 18h30	Meet speakers and visit Valrose Park	
18h30 – 20h30	Welcome Cocktail at Grand Château	

WEDNESDAY APRIL 3rd

9h00 – 9h40	Nicoletta Petridou	chair: MINC
9h40 – 9h55	Daniel Gonzalez Suarez	
9h55 – 10h10	Qingsen Li	
10h10 – 10h20	Émilie Su	
10h20 – 11h00	Coffee break and meet the speakers	
11h00 – 11h40	Ashley Nord	chair: LEDUC
11h40 – 11h55	Sigolène Lecuyer	
11h55 - 12h10	Alberto Salvadori	
12h10 – 14h00	Lunch and meet the speakers	
14h00 – 14h40	Rachele Allena	chair: ARKOWITZ
14h40 – 14h55	Anotida Madzvamuse	
14h55 – 15h10	Nawseen Tarannum	
15h10 – 15h20	Line Chaar	
15h20 – 16h00	Coffee break and meet the speakers	
16h00 – 16h40	Eric Honoré	chair: SHVARTSMAN
16h40 – 16h55	Delphine Débarre	
16h55 – 17h10	Alaa Bou Orm	
17h10 – 17h25	Simon Hadjaje	
17h25 – 17h35	Mathieu Rivière	
17h35 – 18h15	Meet the speakers	
19h00	Free evening	

THURSDAY APRIL 4th

9h00 – 9h40	Stanislav Y. Shvartsman	chair: PALUCH
9h40 – 9h55	Tatiana Merle	
9h55 – 10h10	Raphael Clément	
10h10 – 10h20	Eleni Papafilippou	
10h20 – 11h00	Coffee break and meet the speakers	
11h00 – 11h40	Cécile Leduc	chair: LUTON
11h40 – 11h55	Nils Gauthier	
11h55 - 12h10	Pascale Monzo	
12h10 – 14h00	Lunch and meet the speakers	
14h00 – 14h40	Robert Arkowitz	chair: NORD
14h40 – 14h55	Thomas Alline	
14h55 – 15h10	José J. Muñoz	
15h10 – 15h20	Carlotta A. Lupatelli	
15h20 – 16h00	Coffee break and meet the speakers	
16h00 – 16h40	Vito Conte	chair: PETRIDOU
16h40 – 16h55	Erdem Karatekin	
16h55 – 17h10	Silvia Hervas-Raluy	
17h10 – 17h25	Stéphanie Torrino	
17h25 – 17h35	Jeanne Lefévère-Laoide	
17h35 – 18h15	Meet the speakers	
20h00 – 23h55	Gala dinner at Aston Hotel Roof Top	

FRIDAY APRIL 5th

9h00 – 9h40	Matteo Rauzi	chair: CONTE
9h40 – 9h55	Valentin Chalut	
9h55 – 10h10	Gildas Carlin	
10h10 – 10h50	Coffee break and meet the speakers	
10h50 – 11h05	Miroslav Vořechovský	chair: SURREY
11h05 – 11h15	Jakub Sumbal	
11h15 - 11h55	Ewa Paluch	
12h55 – 12h00	Closing remarks	

8. KEYNOTES ABSTRACTS

The role of mechanics during cell migration

Rachele Allena[1,2]

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[2] Institut Universitaire de France

Cell migration is a fundamental phenomenon involved in many biological processes such as embryogenesis, immunitary response and tumorogenesis. Besides the molecular and chemical mechanisms, mechanics has been shown to play a critical role during cell movement in different configurations. While experimentally quantifying the cell mechanical properties and assessing the mechanical conditions for a cell to efficiently migrate is still challenging and time consuming, *in silico* models constitute a powerful alternative to explore a large number of scenarios. In this talk, I will present the mathematical and mechanical approaches used to develop a series of numerical models that have enabled us to investigate cell migration over homogeneous and heterogeneous substrates, in presence of durotaxis, through rigid microchannels or over deformable micropillars. Taken together our results have confirm the influence of specific mechanical variables and establish the link between cell stresses, strains and migration efficiency.

Candida albicans morphogenesis at different scales

Antonio Serrano, Emily Plumb, Charles Puerner, Louis Chevalier, Priya Jaitly, Stephanie Bogliolo, Martine Bassilana and **Robert A. Arkowitz**[1]

[1] University Côte d'Azur/CNRS/INSERM, Institute of Biology Valrose (iBV), Parc Valrose, Nice, France

We have been focusing on the yeast to hyphal transition, as well as subsequent filamentous growth, both that of the main filament apex and filament branching, in the human fungal pathogen, Candida albicans. Filament branching results in an increase in the number of growth sites and may play a role in virulence in this fungal pathogen [1, 2]. We have been using live-cell microscopy to investigate these processes in C. albicans and to analyze morphological, as well as molecular, changes associated with these transitions. Our results indicate that filament branching is a distinct growth state. Specifically, we observe differences between branch and main filament growth, together with distinct distributions and/or levels of reporters for exocytosis, endocytosis and critical lipids. In contrast, the distribution and level of the key polarity small GTPase Cdc42 are similar between branch and main filament growth. We have also been investigating the biophysical characteristics of the cytoplasm using a micro-rheological probe [3] and observed a striking difference between these growth processes with respect to cytoplasmic crowding/viscosity, as well as in response to antifungal drugs. In summary, at the cellular scale, our results reveal that filament branching is a distinct growth process. Furthermore, at the sub-cellular scale, our results reveal differences in the cytoplasm during morphogenesis and response to antifungal drugs.

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[1] C.J. Barelle E.A. Bohula, S.J. Kron, D. Wessels, D.R. Soll, A. Schäfer, A.J.P. Brown, N.A.R. Gow, Asynchronous cell cycle and asymmetric vacuolar inheritance in true hyphae of Candida albicans, Eukaryotic Cell. 2 (2003) 398-410. doi:10.1128/EC.2.3.398-410.2003.

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[3] M. Delarue, G.P. Brittingham, S. Pfeffer, I.V. Surovtsev, S. Pinglay, K.J. Kennedy, M. Schaffer, J.I. Gutierrez, D. Sang, G. Poterewicz, J.K. Chung, J.M. Plitzko, J.T. Groves, C. Jacobs-Wagner, B.D. Engel, L.J. Holt, mTORC1 Controls Phase Separation and the Biophysical Properties of the Cytoplasm by Tuning Crowding. Cell. (174) 2018 338-349. doi: 0.1016/j.cell.2018.05.042.

The Border Zone in Myocardial Infarction: a mechanobiological analysis at the cellular and supracellular scales

Leon Hermans[1,2], Daniëlle Duffhues[1,2], Robert Passier[3,4], **Vito Conte**[1,2,#], Carlijn Bouten[1,2,#]

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[2]Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands
[3]Department of Applied Stem Cell Technologies, University of Twente, Enschede, The Netherlands
[4]Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands
[#]These authors contributed equally to the work and jointly supervised it.

Myocardial infarction, commonly known as a heart attack, occurs when a local region of the heart muscle (myocardium) suffers from insufficient blood supply – for instance, due to a blocked coronary artery. This results in the local death of vast numbers of cardiomyocytes, the cells responsible for the myocardium's cyclic contraction that ensures heart beating. Lost cardiomyocytes are replaced by colonies of cardiac fibroblasts activated by the pathological pro-fibrotic signalling that follows infarction. Cardiac fibroblasts eventually stiffen the injured myocardial tissue through increased mechanical stress and collagen deposition, which preserves myocardial wall integrity but leads to scar formation and, ultimately, to adverse ventricular remodelling. Recent studies have highlighted the importance of the border zone, a transition region between the healthy non-infarcted myocardial tissue and the infarcted one. The border zone exhibits a hybrid physiological/pathological phenotype presenting impaired contractility due to cardiomyocytes there being still viable but hypocontractile. Importantly, the border zone can serve as a hub from which fibrosis expands over time from the infarcted regions into the non-infarcted ones, potentially resulting in fatal worsening of patient's prognosis. In this study, we build in vitro mimics of the infarcted myocardium from cardiomyocytes and cardiac fibroblasts derived from human pluripotent stem cells. Upon systematically quantifying the mechanobiological behaviour of these cardiac microtissues at the cellular and supracellular scales, we identify physical mechanisms that contribute to border-zone expansion.

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[1] Jackson, B. M. et al. *Extension of borderzone myocardium in postinfarction dilated cardiomyopathy*. Journal of the American College of Cardiology 40, 1160–1167 (2002).
[2] Yamada, S. et al. *Spatiotemporal transcriptome analysis reveals critical roles for mechano-sensing genes at the border zone in remodeling after myocardial infarction*. Nature Cardiovascular Research 1, 1072–1083 (2022).
[3] Sikander Hayat & Rafael Kramann. *Mapping the border zone in myocardial infarction*. Nature Cardiovascular Research 1, 978–979 (2022).

Mammalian Mechanoelectrical Transduction: Structure and Function of Force-Gated Ion Channels

Eric Honoré[1]

[1]Université Côte d'Azur, Centre National de la Recherche Scientifique, Institut national de la santé et de la recherche médicale, Institut de Pharmacologie Moléculaire et Cellulaire, Labex ICST, Valbonne, France.
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The conversion of force into an electrical cellular signal is mediated by the opening of different types of mechanosensitive ion channels (MSCs), including TREK/TRAAK K2P channels, Piezo1/2, TMEM63/OSCA, and TMC1/2. Mechanoelectrical transduction plays a key role in hearing, balance, touch, and proprioception and is also implicated in the autonomic regulation of blood pressure and breathing. Thus, dysfunction of MSCs is associated with a variety of inherited and acquired disease states. Significant progress has recently been made in identifying these channels, solving their structure, and understanding the gating of both hyperpolarizing and depolarizing MSCs. Besides prototypical activation by membrane tension, additional gating mechanisms involving channel curvature and/or tethered elements are at play.

Mechanics and dynamics of single vimentin filaments

Cécile Leduc[1]

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https://www.actindynamics.net/

Intermediate filaments (IF) are involved in key cellular functions including polarization, migration, and protection against large deformations. These functions are related to their remarkable ability to extend without breaking, a capacity that should be determined by the molecular organization of subunits within filaments. However, this structure-mechanics relationship remains poorly understood at the molecular level. I will present how, using super-resolution microscopy (SRM), we show that vimentin filaments exhibit a ~49 nm axial repeat both in cells and in vitro. Using an SRM compatible stretching device, we also provide evidence that the extensibility of vimentin is due to the unfolding of its subunits and not to their sliding, thus establishing a direct link between the structural organization and its mechanical properties [1]. In a second part, I will show recent results on the in vitro reconstitution of vimentin assembly dynamics. We show that vimentin filaments can spontaneously break without cofactors or post-translational modifications, but this fragmentation limits assembly only at very long-time scale (>18h) [2]. We also uncovered the mechanism responsible for fragmentation which involves subunit exchange. Our results show how vimentin self-repair to protect their integrity and provide new insights into the physical understanding of the intermediate filament length regulation.

REFERENCES

[1] F. Nunes Vincente, M. Lelek, J.-Y. Tinevez, Q.D. Tran, G. Pehau-Arnaudet, C. Zimmer, S. Etienne-Manneville, G. Giannone, and C. Leduc, Molecular organization and mechanics of single vimentin filaments revealed by super-resolution imaging, Science Advances *8* (2022) eabm2696. doi: 10.1126/sciadv.abm2696.

[2] Q.D. Tran, V. Sorichetti, G. Pehau-Arnaudet, M. Lenz M+, and C. Leduc, Fragmentation and entanglement limit vimentin intermediate filament assembly, PRX 13 (2023) 011014 doi: 10.1103/PhysRevX.13.011014.

How Large Cells Do It?
Mechanics of Mitotic Spindle Positioning in early embryos

Nicolas Minc[1]

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Life for all animals starts with a stereotyped 3D choreography of reductive divisions that specify cells fates, developmental axis and overall morphogenesis of early embryos. These division geometries are specified from the subsequent position and orientation of mitotic spindles. In animal cells, spindle position is commonly regulated by astral microtubules (MTs) that radiate from spindle poles and contact the cortex to apply forces that move and rotate spindles in place. However, in unusually large zygotes and early blastomeres, spindles are too small to contact the cortex, and appear to float in the cytoplasm. We used magnetic tweezers to displace and rotate mitotic spindles in live sea urchin embryos, and uncovered that the cytoplasm can impart viscoelastic reactive forces that move spindles, or passive objects with similar size, back to their original position. These forces hold spindles in the cell center, and are independent of cytoskeletal force generators, yet reach hundreds of piconewtons and scale with cytoplasm crowding. They increase with cell shape anisotropy, as a result of enhanced hydrodynamic coupling of the spindle with cell boundaries, which confers a stable centering precision to spindles as embryos develop. These findings suggest that bulk cytoplasm material properties constitute important control elements for the regulation of division positioning in early embryos and beyond.

Dynamics and Mechanics of the Bacterial Flagellar Motor: protein exchange dynamics and polymer dynamics

Ashley L Nord[1], Ruben Perez-Carrasco[2], Anaïs Biquet-Bisquert[1], Emilie Gachon1, Jasmine A. Nirody[3], Théo Pigaglio[4], Farida Seduk[4], Alessandro Barducci[1], Manouk Abkarian[1], Axel Magalon[4], Richard M. Berry[5], Francesco Pedaci[1]

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[5]Department of Physics, Clarendon Laboratory, University of Oxford, Oxford OX1 2JD, United Kingdom

For many bacteria, motility stems from one or more flagella, each rotated by the bacterial flagellar motor (BFM), a powerful rotary molecular machine. This membrane-spanning motor is self-assembled and made of more than 25 different proteins, and recent advances in 3D cryo-EM techniques have yielded stunning structural details. But, looking beyond such static pictures, we're interested in the relationship between function and structure, and particularly in BFM dynamics, which we probe via biophysical experiments. Here, we explore BFM mechanics in terms of protein exchange and polymer dynamics.

Torque in the BFM is provided by stator units, ion motive force-powered ion channels which assemble and disassemble dynamically. This exchange is mechanosensitive, with the number of engaged units dependent on the viscous load upon the flagellum. To explore the molecular mechanism underlying BFM mechanosensitivity, we measure stator binding and unbinding kinetics while dynamically varying the load on the motor. We find the stator units to behave as a catch bond, and this forceenhanced adhesion allows the cell to adapt to a changing mechanical environment, potentially playing a role in surface-sensing and biofilm formation [1,2].

The hook, a soft polymer at the base of the flagellum, acts as a universal joint, coupling rotation between the rigid rotor and flagellum. In multi-flagellated species, where thrust arises from a hydrodynamically coordinated flagellar bundle, hook flexibility is crucial, as flagella rotate significantly off-axis. But, the thrust also applies a significant bending moment. Thus, the hook must simultaneously be compliant to enable bundle formation yet rigid to withstand large hydrodynamical forces. It fulfills this double functionality via a dynamic increase in bending stiffness under increasing torsional stress. Such strain-stiffening allows the system to be flexible when needed yet reduce deformation under high loads, enabling high speed motility [3].

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Cell morphogenesis across scales, from molecular processes to cellular forces

Ewa Paluch[1]

[1] University of Cambridge

A precise control of cell morphology is key for cell physiology, and cell shape deregulation is at the heart of many pathological disorders, including cancer. Cell morphology is intrinsically controlled by mechanical forces acting on the cell surface, to understand shape it is thus essential to investigate the regulation of cellular mechanics.
In animal cells, shape is primarily determined by the cellular cortex, a thin network of actin filaments and myosin motors underlying the plasma membrane. We investigate how the mechanical properties of the cell surface arise from the microscopic organisation of the cortex, and how changes in these properties drive cell deformation. We have developed methods to investigate cortex composition and nanoscale architecture, and are exploring how the mechanical properties of the cortical network are regulated. Using a combination of cell biology experiments, biophysical measurements, quantitative imaging and physical modelling, we aim to understand the control of cell shape across scales.

Collective regulation of cell decisions

Camilla Autorino[1], Cristina Pallares-Cartes[1], Bernat Corominas-Murtra[2] and Nicoletta **Nicoletta Petridou[1]**

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Developing systems show an unmatched complexity due to a highly entangled regulation across different levels of organization. Accordingly, certain cell functions occur in response to cues spanning biological scales. Do cells perform certain functions in response to global cues propagated in the tissue they are embedded in? We address this question by using a physics-guided quantitative developmental biology approach combined with tailored in vivo engineering. Here we show that the first cell fate decisions in zebrafish, the specification of the meso-endodermal germ layer, rely on a mechanochemical feedback between morphogen signalling and the collective tissue material state. Using live imaging and statistical mechanics, we observe that when cells start receiving specification signals the tissue is operating close to a critical point in cell-cell adhesion strength triggering a tissue rigidity phase transition. Once cells start to specify, they also increase their cell-cell adhesion strength above the critical point and when the tissue rigidifies, specification signals are then quickly switchedoff. By blocking cell fate specification, we observe that the tissue stays at criticality, suggesting that tissue rigidification is instructed by morphogen signalling. Remarkably, when we genetically or optogenetically interfere with tissue rigidification and keep the system at criticality, cells continue specifying. We found that morphogen gradient formation is self-regulated by the tissue material state, where at criticality morphogens diffuse far and fast in the tissue, changing cell-cell adhesion strength. Once cell-cell adhesion strength increases above the critical point, it triggers tissue rigidification that hampers further morphogen diffusion. This uncovers a self-generated mechanism of morphogen gradient formation transcending biological scales, and proposes that cells use collective properties as a form of physical learning to exit pluripotency.form of physical learning to exit pluripotency.

Composite morphogenesis: how can a tissue fold and extend at the same time

Matteo Rauzi[1]

[1] University Côte d'Azur, CNRS, Inserm, iBV, Nice, FRANCE

During embryo development, epithelia can undergo different shape transformations. While these changes can be sequential, and thus driven by specific sequential cellular mechanisms, this is not always the case. A single tissue can undergo multiple simultaneous shape transformations resulting in a composite process. For instance, in vertebrates, during neurulation, the dorsal tissue folds forming the neural tube while elongating along the anterior-posterior axis separating the future head from the anus [1]. This raises an important question: how can a tissue undergo multiple simultaneous shape transformations if each transformation is per se driven by different and functionally specific cellular mechanisms? In addition, which signaling pathways are controlling composite morphogenetic processes? We use the protostome *Drosophila* and the deuterostome sea urchin *P. lividus* embryo as model systems and focus on the process of simultaneous tissue folding and extension resulting in the formation of an epithelial tube at the onset of gastrulation. By using advanced multi-view light sheet microscopy coupled to infrared femtosecond laser manipulation, optogenetics and quantitative big data analysis, we aim to shed new light on the evolutionary conserved signaling pathways, mechanisms and mechanics controlling and driving composite morphogenesis.

REFERENCES

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Active passive strains during tissue morphogenesis

Daniel Alber, David Denberg, Eric Wieschaus, **Stas Shvartsman[1]**

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Tissue deformation during morphogenesis can occur actively through changes in constituent cell shapes and connectivities or passively in response to an outside mechanical driver. We use the rapidly deforming *Drosophila melanogaster* embryonic hindgut primordium as a model to examine the response of a presumed passive tissue to the actively deforming tissues bordering it. Employing light sheet fluorescence microscopy and computational image analysis methods, we extract cell kinematics and map strains with high spatial and temporal resolution. By integrating kinematics with surface and nuclear geometries, we evaluate the response of the hindgut to neighboring tissues, providing insights into the interplay between active tissues in the early embryo and their effects on other tissues undergoing significant strain.

Mechanisms of microtubule nucleation by γTuRC and of KIF2A-driven microtubule treadmilling

Cláudia Brito[1,*], Marina Serna[2,*], Gil Henkin[1,*], Pablo Guerra[3], Claire Thomas[4], Oscar Llorca[2,#], **Thomas Surrey**[1,5,6,#]

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During mitosis, a functional spindle requires high microtubule turnover. Such turnover is highlighted by the multiple functions of spindle poles, where microtubule minus ends are concentrated, and where microtubule nucleation and depolymerization happen side by side. How these seemingly antagonistic processes are coordinated during poleward microtubule flux is not understood. Here we present first cryo-electron microscopy structures revealing how the human γ-tubulin ring complex (γTuRC), the main microtubule nucleator in cells, changes conformation upon microtubule nucleation, explaing why it nucleates precisely 13 protofilament microtubules. We then present TIRF microscopy-based in vitro reconstitutions of the coordination of microtubule nucleation and minus-end depolymerization as seen at spindle poles, revealing that kinesin-13 KIF2A is a microtubule minus-end depolymerase, in contrast to its paralog MCAK. Due to its asymmetric activity, KIF2A still allows microtubule nucleation by plus-end growth from γTuRC, which in turn serves as a protective cap that shields the minus end against KIF2A binding. Efficient γTuRC-uncapping requires the combined action of KIF2A and a microtubule severing enzyme, leading to treadmilling of uncapped microtubules driven by KIF2A. Together these results provide insight into the molecular mechanisms of microtubule nucleation and its coordination with depolymerization at spindle poles consistent with their role in poleward microtubule flux.

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9. ABSTRACTS

Mechanobiology of the root hair cell

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Key Words: *Root hair, mechanotransduction, nucleus dynamics.*

Plant growth is controlled by genetic circuits but also by mechanical cues as the plants need to adapt to their mechanical environment. The plant roots are good systems to study such an adaptation since they grow in soils with many mechanical heterogeneities. In particular, we study the growth of the root hair cell, which is a single cell, from the root epidermis, exhibiting tip growth. Our aim is to investigate the effect of mechanical cues on root hair growth, and the associated mecanotransduction from the cell wall to the nucleus in growing plant roots. To this end, combining microfluidics and optical microscopy, we quantitatively characterize the growth of single root hairs by measuring, in different conditions and mechanical environments, their growth speed and the concomitant nuclear dynamics. In particular, using agar growth media of increasing young moduli leads to root hairs with decreasing growth speed and length, whereas growth duration is unaffected. Interestingly, the stiffness of the growth medium also affects nuclear dynamics by decreasing the distance between the nucleus and the tip of the root hair suggesting mechanotransduction from the root hair cell surface to the nucleus [1].
Eventually, having shown that the rigidity of the environment affects RH growth and nucleus dynamics, we sought to determine the effect of external mechanical resistance on the cell wall mechanics. We thus quantified the cell wall young modulus of living root hairs grown in medium of increasing stiffness using a bending experiment [2].

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WRINKLING INSTABILITY IN UNSUPPORTED EPITHELIAL SHEETS

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Key Words: *epithelial tissues, vertex model, wrinkling instability.*

We investigate the elasticity of an unsupported epithelial monolayer and we discover that unlike a thin solid plate, which wrinkles if geometrically incompatible with the underlying substrate, the epithelium may do so even in the absence of the substrate. From a cell-based model, we derive an exact elasticity theory and discover wrinkling driven by the differential apico-basal surface tension. Our theory is mapped onto that for supported plates by introducing a phantom substrate whose stiffness is finite beyond a critical differential tension. This suggests a new mechanism for an autonomous control of tissues over the length scale of their surface patterns.

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CYTOPLASMIC VISCOSITY IS A POTENTIAL BIOMARKER FOR METASTATIC BREAST CANCER CELLS

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Key Words: *Cell mechanics, Breast Cancer Cells, Active Microrheology*

Cancer is responsible for 25% of deaths worldwide. To stem the high mortality rate, it is essential to develop new diagnostic methods for the early detection of cancerous and metastatic cells. A common idea is that the metastatic potential of cancer cells correlates with their deformability, pointing to the possibility of using specific mechanical properties as biomarkers of malignancy and cancer aggressiveness [1]. These findings rely on measuring the apparent Young's modulus of whole cells using primarily atomic force microscopy. The present study aims to explore whether alternative mechanical parameters have discriminating features with regard to metastatic potential. Magnetic rotational spectroscopy (MRS) is employed in the examination of mammary epithelial cell lines, MCF-7 and MDA-MB-231, representing low and high metastatic potential, alongside normal-like MCF-10A cells. MRS utilizes active micron-sized magnetic wires in a rotating magnetic field to measure the viscosity and elastic modulus of the cytoplasm [2,3]. All three cell lines display viscoelastic behavior, with cytoplasmic viscosities ranging from 10-70 Pa s and elastic moduli from 30-80 Pa. Our findings indicate that MCF-10A normal breast cells exhibit the highest viscosity and elasticity, while MDA-MB-231 breast tumor cells with high metastatic potential display the lowest viscosity and elasticity. Importantly, our study highlights that Young modulus is not the sole characteristic affected by the breast cancer phenotype. To differentiate cells with low and high malignancy, viscosity emerges as the more discriminating parameter, as MCF-7 exhibits a 5 times higher viscosity as compared to MDA-MB-231. This study hence suggests that static viscosity, instead of the elastic or Young modulus, could be used as a potential marker for invasive and metastatic cancer cells.

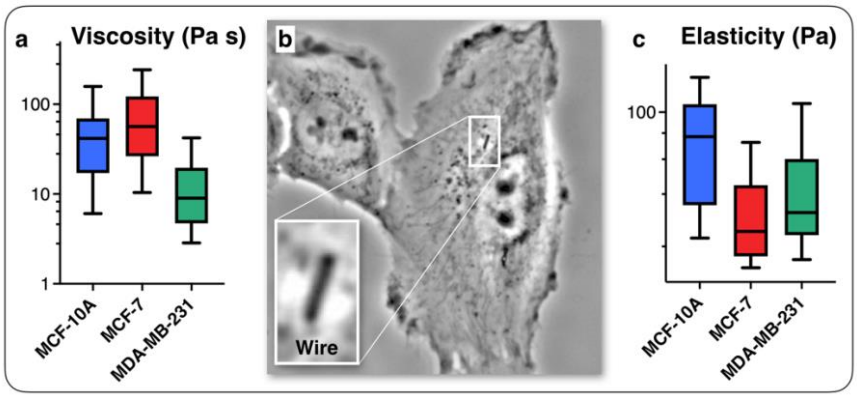

Figure 1 : **a)** Static viscosity for MCF-10A, MCF-7 and MDA-MB-231 epithelial breast cells. **b)** Phase-contrast optical microscopy images of MCF-10A cells showing an internalized magnetic wire (inset). **c)** Elastic modulus for MCF-10A, MCF-7 and MDA-MB-231 cells.

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Fluid-Structure Interaction of two-leaflet valves dynamics under flow

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Key Words: *Lymph, lymphatic valve, Fluid-Structure Interaction, Computer simulations.*

Abstract
The lymphatic system consists of initial lymphatic vessels that receive the lymphatic fluid and collecting lymphatic vessels that transport the lymph through the lymphatic network and its nodes[1]. The mechanism of pumping the lymph is achieved by a cycle of compression and relaxation of the lymphatic vessel wall, which is achieved by the surrounding muscles. The lymphatic valves enable the pumping of lymph in one direction, and prevent its reflux. A malfunction of this system can lead to conditions such as lymphedema, which is a serious bad side effect in some cases of cancer treatment of cancer and currently has no cure.

Methods and results
We are developing a fully coupled fluid-structure computational model to study the effects of various parameters on the dynamics of the valves using the Lattice Boltzmann method [2] to compute the fluid and the immersed boundary method to accomplish the fluid-structure interaction. The valves are modeled with a spring network, where adjacent points are interconnected with a Hookean spring, with the stretching force and the restoring force. This study contribute to better understand how the lymphatic system works if there is a valve that is not functioning normally in the lymphatic vessels, which represent bifurcations and junctions which come together to arrive at the drainage channel.
In figure 1, the left panel shows the pressure field developed in a T-junction geometry with the elasticity of the bottom valve is smaller than the top one. The two valves open under the pressure and adopt a steady shape. The right panel of the figure shows the variation of the rate flow as a function of the pressure gradient for the two cases: with and without valve. In the case of having no valve, the flow rate increases linearly with the pressure. Whereas for the case with the valves, the flow rate increases in a non-linear way with the pressure gradient, which reflects the effect of the presence of valves. Furthermore, the effect of the variation of the elasticity of the valve and the inlet speed are also investigated in such geometry.

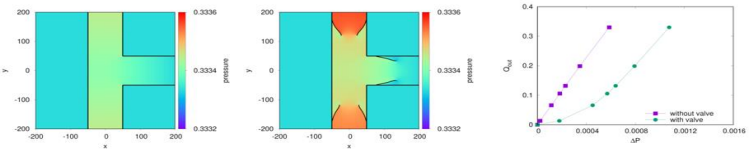

Figure 1: The pressure field in a T-junction geometry, and the variation of the flow rate as a function of the applied pressure gradient in presence or not of the valves at the inlets.

Conclusions
The present numerical method with the restoring and the stretching forces of the valve is simple and catches correctly the expected dynamics of the valve.
The developed numerical method will be used to further investigate the lymphatic valves dynamics encountered in the lymph pumping mechanism and to elucidate the causes and emergence of the lymphedema.

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EXPLORING CELL MECHANICS WITH LEGO® BRICKS

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Key Words: *Cell mechanics, LEGO®, experimental system, fun science.*

Mechanical forces are key regulators of cellular functions and cell behavior. These forces, originating both internally and externally, influence vital processes such as cell division, migration, and differentiation. Understanding how cells sense, transmit, and respond to these mechanical cues is crucial. Cell biologists are therefore increasingly compelled to use and possibly design devices to manipulate cells with mechanical forces. In this context, LEGO® bricks emerge as a novel and versatile option for designing and building such systems. Their modularity and precision make them suitable for constructing customizable and cost-effective experimental setups, providing an accessible platform for innovative research in cellular mechanics. Within this innovative framework, we have developed two systems. The first is a mechanical stretcher, crafted from LEGO® bricks and designed for cyclic uniaxial stretching of cells [1-2]. This system offers an unexpected way to study cell responses to mechanical strain. Complementing this, a magnetic tweezers system, also built using LEGO® components, including a LEGO® SPIKE™ intelligent brick to pilot the system, utilizes permanent neodymium magnets to apply forces to paramagnetic beads attached to cells. This setup enables application of mechanical forces to perform microbead rheology and assess cell mechanics upon force application. The use of LEGO® bricks in these systems aligns with the emerging trend towards creating more accessible and cost-effective research tools. This approach not only facilitates innovative scientific investigation but also promotes the principles of open and FAIR (Findable, Accessible, Interoperable, and Reusable) science [3]. By lowering financial and technical barriers, these systems empower a broader range of scientists, fostering inclusivity and collaboration in research. This democratization of scientific tools is a crucial step in expanding the reach and impact of scientific exploration.

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Persistent random walk model of cell migration over curved substrate

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Key Words: *Cell migration, Curvature, numerical model.*

Cell migration is a key process in tissue formation which occurs during embryo's development or regeneration. Among the numerous factors in the cell environment that impact cell behavior, substrate cell-scale curvature is a mechanical cue which redirects cells toward concave areas [1]. However, the process of change in directionality remains unknown: it may emerge from the activation of signal pathways by the curvature and/or the cell would be mechanically limited by the curvature.
Here, a persistent random walk (PWR) model adapted from [2] has been developed in order to assess the mechano-sensitivity of the cell to curvature. The action of the curvature is modelized by a force which triggers inner cell signal pathways and/or acts as an external passive force. Also, the sensibility to curvature can emerge from a local measure at the position of the cell (i.e. a gradient) or a global measure at the cell-scale (i.e. difference of curvature between the center and the cell membrane). Our study aimed to test all these hypotheses.

First simulations on wavy substrates show that cells behave differently depending on the hypotheses. Preliminaries results compared with experiments enabled to argue that, by a cell-scale measure of the curvature, the cell is mostly limited mechanically.

The PRW model is able to reproduce trajectories of migrating cells on curved surface. Further analysis will give more details about the activation of signal pathways. A recent numerical study showed as a proof-of-concept, that dynamic surfaces can guide cells in a given direction [3] and implementing dynamic surface with the PRW model will make emerge new methods to guide cells with topographical cues.

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β1 integrin contribution in collective migration induced by BMP2 in mammary epithelium

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Key Words: *Jamming/Unjamming, Epithelial-Mesenchymal Transition, Mammary Epithelium, Breast Acini, b1 integrin, BMP2*

During the neoplastic transformation, a solid-to-liquid transition referred to as unjamming transition can overcome the motility arrest of healthy, confluent epithelial cells, and promote tissue fluidization through large-scale collective cell migration. How cells control such transitions needs to be explored. Whether and how integrins, as cell-matrix receptors, participate in intercellular communications to support collective cell migration remains unexplored. BMP2 expression is elevated in several cancer tissues including breast and abnormal BMPR signaling has been reported in breast cancers. We previously showed the cooperation between integrin and BMP receptors to drive migration of isolated cells in 2D. Here we investigate integrin/BMPR cooperation in mammary epithelial cell model to explore the contribution of integrins in the promotion of collective migration. Our results identify a partial Epithelio-Mesenchymal Transition (EMT) of MCF10A cells in response to BMP2 stimulation with a sustained accumulation of E-cadherin at cell-cell contact. MCF10A cells adapt their shape through junctional plasticity which is associated with unjamming transition allowing collective network migration and cell cluster migration in 2D. BMP2-treated 3D breast acini undergo disorganisation of actin cytoskeleton and perturbation of the apico-basal polarity. Unjamming and acini-spheroid transitions are correlated with a perturbed distribution of β1 integrin at cell-cell and cell-ECM contacts. The fluidization of MCF-10A monolayer required β1 integrin to control collective migration. Mechanistically, our results suggest that β1 integrin might contribute to epithelial tissue cohesion through the building of mechanosensitive structures.

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Organoid growth on magnetically induced waves mimicking intestinal environment

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Key Words: *Gut-on-Chip, Organoids, Soft robots.*

The intestine, characterized by multi-scale curvatures (curvature of the tract, microstructures, …), undergoes deformations driven by a combination of muscular, and external movements, mainly related to digestion. Numerous severe and misunderstood pathologies, such as the Hirschsprung's disease, profoundly impact this intricate structural and mechanical properties. It is therefore a clear need to develop new physiological models and consequently more effective treatments.

Given the potential inadequacy of standard 2D in vitro and animal models for such tissue modelling, recent works in the last decade have led to a new promising in vitro approach, the Organ-on-Chip. Focused on partially reproduce the small intestine structural and architectural properties [1], the Gut-on-Chip technologies presented in the literature are mainly giving priority to the structural aspect than the movements. Indeed, folding and bending movements have not been yet well investigated while they are predominant for this organ functions. To address this aspect, we developed magnetic polymer membranes, inspired by the soft actuator technologies [2]

Through this work, we combined the use of magnetically actuated membranes (Fig. 1a) and hydrogel intestinal microstructure replicas (Fig. 1b) supporting mouse organoid growth. The magnetic membranes, composed of a NdFeB and PDMS mixture, achieve deformation amplitudes up to 1.7 mm and curvatures of about 1 mm^{-1} under a magnetic field of 86 mT. The fabricated microstructures are made of crosslinked Matrigel intestinal villi replicas [3] and are similar to the real microstructures, which in mice are ~ 450 µm high. Mouse intestinal organoids are grown in 3D (Fig. 1c) before being seeded on different substrate as 2D monolayers (Fig. 1d), with the aim of investigating cell response to dynamic deformations and mechanical stress on an in vitro reproduction of the intestinal wall.

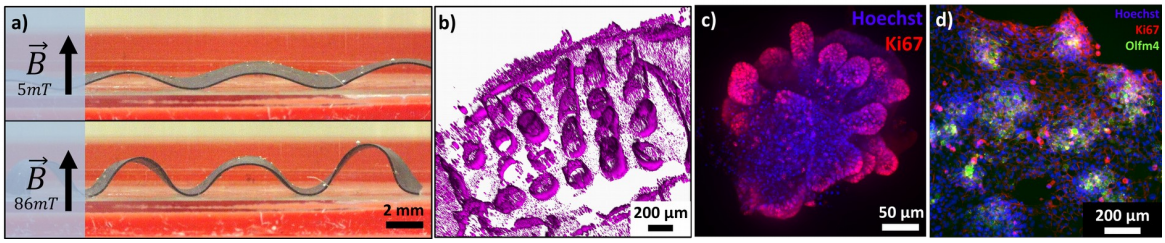

Figure 1 *(a) Magnetic membrane under a field of 5 (top) and 86 (bottom) mT. (b) Cross-linked Matrigel® villi with Caco-2 cells (purple, Hoechst, nuclei). (c) ISC (Intestinal Stem Cells) cultured as organoid in 3D Matrigel® domes (red, Ki67, proliferative cells; blue, Hoechst, nuclei). (d) ISC cultured as monolayers on 2D crosslinked Matrigel® (green, Olfm4, stem cells; red, Ki67, proliferative cells; blue, Hoechst, nuclei).*

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Locomotion in Trichoplax adhaerens

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Key Words: *epithelia, cilia polarity, locomotion, trichoplax*

Trichoplax, often referred to as the simplest living animal, is a marine, blob-like animal without any symmetries, body parts, or organs. It is essentially a flat aggregate of ciliated epithelial cells that feeds on green algae covering rock or sand on the seabed. While it does not have a muscular or nervous system, Trichoplax can move in seemingly Brownian or directed motion, using its cilia to crawl on the substrate. How it achieves persistent motion and changes directions is an open question. We developed molecular tools to tag components of the ciliary complexes, and analyzed the polarity of these complexes at the scale of the whole animal with single resolution, in normal and perturbed conditions. We find that in response to mechanical stimuli, Trichoplax is capable of remarkable polarity plasticity, and can re-orient its cilia in a coordinated manner within a few seconds to change directions. This exploratory work raises a number of questions on the sensing mechanisms used by Trichoplax to probe its environment, the conversion of these signals into coordinated ciliary beating reorientation, and eventually, into movement.

MECHANICAL REGULATION OF CELL ADHESION TO A SOFT WALL UNDER FLOW

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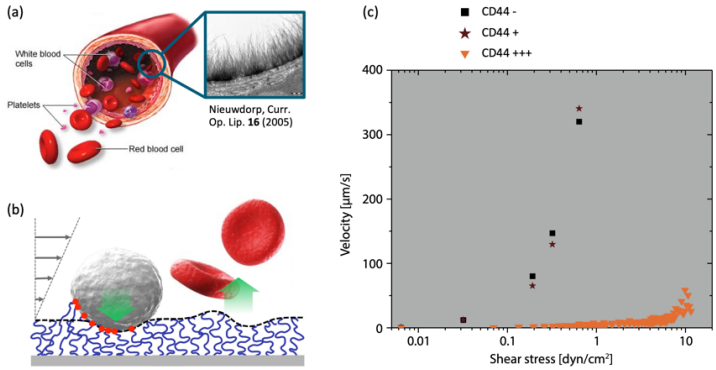
Key Words: *Cell adhesion, glycocalyx, in vitro model.*

Blood cell - vessel wall interactions are critical both for the flow of red blood cells, and for the control of white blood cell adhesion to the walls (e.g. at a site of inflammation). However, the biochemical and mechanical cues governing their thight regulation are still poorly understood, in particular because of the challenge of non-invasive investigation of cell-wall short-range interactions under flow in a complex environment. Using a home-built platform combining advanced biochemical surface functionalization, microfluidics and high-speed interferometric imaging [1], we have investigated experimentally the role of the softness of the vessel wall outer layer in the regulation of blood cell homing under flow. This brush, named glycocalyx and mainly composed of charged exopolysaccharides, is both thick (up to 1µm) and extremely soft (down to a few Pa in compression modulus). We have demonstrated that these peculiar mechanical properties induce a short-range repulsion of non-interacting cells, in good agreement with the theory of elastohydrodynamics that accounts for the effect of substrate deformation under hydrodynamic forces. We have thereby provided the first experimental evidence of this "soft biolubrication" effect at play at small scale [2]. On the other hand, we have shown that these same mechanical properties are a critical factor that stabilizes the homing of cells bearing specific receptors (CD44) for one of the main compound of the glycocalyx, hyaluronan (HA). Our results thus highlight the role of the glycocalyx as a gatekeeper for the adhesion to the blood vessel wall.



(a), the glycocalyx lining the wall of blood vessels is the outermost structure encountered by circulating cells. (b), schematic representation of stabilized attractive interactions of an activated white blood cell bearing CD44 receptors with a model glycocalyx under a shear flow, and of the repulsion of red blood cells with no specific biochemical interaction with the polymer brush. (c), velocity under flow of cells bearing different densities of CD44 receptors, showing superlinear increase of speed for CD44- cells and stabilized rolling for CD44++ cells, up to physiological shear stresses.

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Duchenne Muscular Dystrophy: A multiscale mechanical analysis using AFM and Ultrasound Elastography across developmental phases.

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Key Words: *Duchenne Muscular Dystrophy (DMD), Multiscale, Young's Modulus, Atomic Force Microscopy, Ultrasound Elastography.*

Duchenne Muscular Dystrophy (DMD) represents a severe, progressively debilitating genetic neuromuscular disorder that often results in premature mortality. The underlying cause of DMD is the absence of dystrophin, a critical protein that connects the contractile cytoskeleton to the muscle fiber membrane. To further the link between preclinical studies and therapeutic approaches, our team has developed the R-DMDdel52 rat model of DMD. This rat model displays various pathophysiological features like those observed in human DMD including severe and progressive skeletal muscle degeneration, increased fibrous tissue and fat infiltration, and changes in muscle fiber types highlighting the relevance of this model for studying DMD [1].
During a Ph.D. project, we conducted a detailed analysis of the mechanical properties of muscles affected by DMD using our rat model and compared to wild-type controls, employing a variety of techniques such as atomic force microscopy and ultrasound elastography. Our measurements spanned multiple biological scales — from myoblasts and myotubes to muscle fibers and the whole muscle. Additionally, these assessments were performed on rats at various developmental stages, specifically at ages of 1 month, 6 months, and 10 months, to understand the progression of DMD.
The study data indicates that DMD has a significant impact on muscle mechanical properties at all observed scales, suggesting a scale-dependent effect. Unexpectedly, even at the cellular level, in the myoblasts, the effects of dystrophin deficiency can be observed in the early stages of the disease. Additionally, this study highlights the pertinence of muscle fiber analysis in monitoring the DMD progression and identifies this biological scale as a potential focus for future evaluation of therapeutic strategies. Overall, this study enhances our understanding of how dystrophin deficiency affects muscle mechanics, providing insights across various biological scales and stages of the disease.

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Mechanically induced topological transition of spectrin regulates its distribution in the mammalian cortex

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Key Words: *Mechanoadaptation, Spectrin, Expansion Microscopy, Cortex*

The cell cortex is a dynamic assembly formed by the plasma membrane and the underlying cytoskeleton. As the main determinant of cell shape, the cortex ensures its integrity during both passive and active deformations by adapting cytoskeleton topologies through a yet poorly understood mechanisms. The spectrin meshwork ensures such adaptation in erythrocytes and neurons by adopting dramatically different organizations. Erythrocytes rely on triangular-like lattices of spectrin tetramers, whereas in neurons they are organized in parallel and periodic arrays. Since spectrin is ubiquitously expressed, we exploited Expansion Microscopy to discover that, in fibroblasts, distinct meshwork topologies can co-exist. We show through biophysical measurements (FRAP, FRET) and computational modeling that the diffused spectrin lattice, with the intervention of actomyosin, can dynamically transition into polarized cortical clusters fenced by actin stress fibers that resemble neuron-like periodic arrays. Spectrin clusters experience lower mechanical stress and turnover despite displaying an extension close to the contour length of the tetramer. Our study sheds light on the adaptive properties of spectrin, which participates in the protection of the cortex by undergoing mechanically induced topological transitions.

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Bio-mechanical difference between ascidian and jellyfish early embryogenesis

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Key Words: *Embryogenesis, Bio-mechanics, Ascidian, Jellyfish, Blastocoel*

Bio-mechanical forces of cells are important factors that lead to the emergence of early embryo shape. The early stages of embryogenesis can either be variable or stereotypical. Most deuterostomes, typified by humans, mice or sea urchin embryos have a variable cleavage pattern. Stereotypical cleavage patterns seem to be more common in protostomes. Even though we have been able to identify these different embryos based on their shapes early stages of embryogenesis (eg. 16-cell) for more than a century, we still do not know how these embryonic shapes emerge.

In order to determine how embryonic shape emerges we have been exploiting the ascidian embryo, an invertebrate chordate. Ascidians display an invariant cleavage pattern (a compact blastula) that is conserved within the clade. Cell biological and bio-mechanical properties of this embryo, such as alternate cell division or apical relaxation[1], seem to be important for maintaining 1) a compact blastula and 2) the invariant cleavage pattern. To measure these biomechanical properties, micropipette aspiration and force inference are used.

Micropipette aspiration allows an absolute measure of the apical surface tension of one cell at a time of the embryo, On the other hand, force inference allows a measure of the relative surface tensions of all membranes of the embryo[2], both apical and basolateral. Combining these two methods we aim to create an absolute spatiotemporal map of the surface tensions in an embryo for the first time. These maps will be used to build a model that could be tested by experiments and by simulations
.
These measures will be made on both normal and perturbed ascidian and also on a naturally non-steroptyped cleavage displayed by early embryo of the jellyfish Clytia

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MECHANICAL DESCRIPTION OF *DROSOPHILA* WING EXPANSION

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Key Words: *Biomechanics, morphogenesis, fluid-structure interaction.*

During its final transformation into its adult form, just after hatching from its pupal shell, an insect unfolds its wings within minutes. The wings expand rapidly from a compact, pleated structure to a rigid wing blade that allows the insect to fly. We study wing expansion in the fruit fly *Drosophila* (see Fig. 1a). We characterize the unfolding kinematics through a multi-scale approach using a combination of optical and electronic microscopy together with micro-tomography. Expansion is regulated by increasing internal pressure and injecting a fluid (hemolymph) into a folded deployable structure under hormonal control. We quantify the insect internal pressure *in vivo* during wing expansion (Fig. 1b) and investigate the mechanical properties of the tissue by performing tensile tests on folded wings and nano-indentation loading cycles (Fig. 1c). We show that the folded wing behaves as a viscoelastic material of shear modulus μ and viscosity η. Finally, we build a fundamental understanding of the kinematics and dynamics of wings expansion based on the wing structure and mechanical properties considering that the stress in the wing compensates for the increase in internal pressure during expansion (Fig. 1d). Our model also captures the dynamic of the wings expansion as shown Fig. 1e.

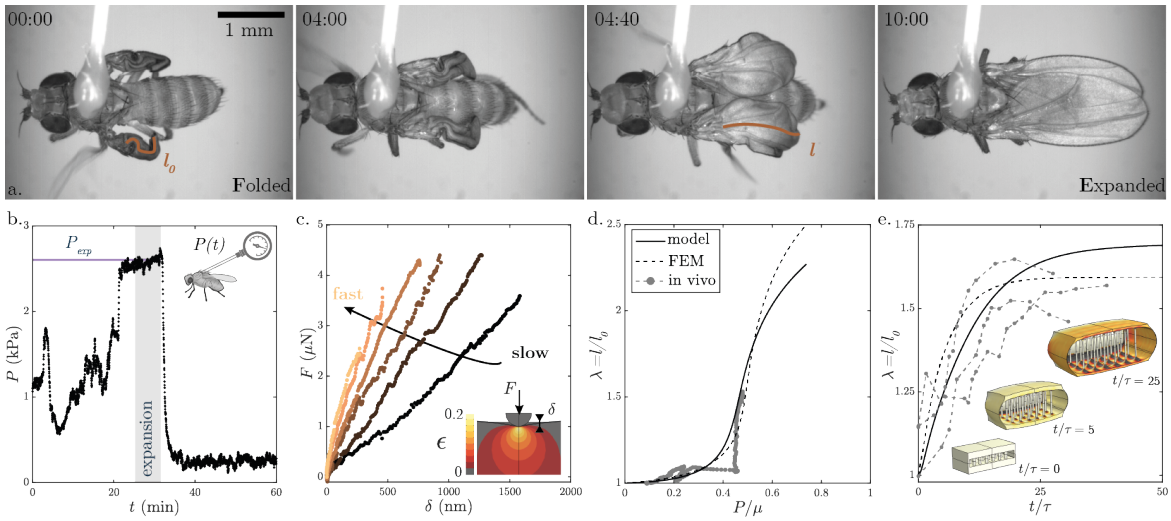

Figure 1: *Drosophila* wing expansion. (a) Snapshots of the wings expansion. **(b)** *In vivo* recording of the internal pressure $P(t)$ of a newly hatched fly. **(c)** Force-displacement curves obtained from loading cycles in nano-indentation, from slow indentation ($\dot{F} = 10^{-1}\,\mu$N/s, black) to faster ($\dot{F} = 10^{1}\,\mu$N/s, orange) described by a viscoelastic model. (Inset) FEM simulation of a spherical indenter imposing a force F on a viscoelastic sample undergoing deformation of strain ϵ and which surface deforms of a value δ. **(d)** In-plane deformation λ as a function of the applied pressure P normalized by the shear modulus of the material μ for an hyperelastic Gent material ($J_m = 20$). Pressure for the experimental data points is normalized by $\mu = 6$ kPa. **(e)** λ as a function of time normalized by the viscous time of the material τ. Time for the experimental data points is normalized by $\tau = 13$ s. Snapshots of FEM simulations at $t/\tau = 0$, 5 and 25 are shown. Experimental measurements, FEM and model are shown respectively with gray markers, dashed line and solid line in (d-e).

INTEGRATING RADIOMIC AND CELL MECHANOBIOLOGY TO PREDICT TUMOUR RESPONSE TO TREATMENT

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Key Words: *cancer mechanobiology, tumour growth, chemotherapy*

Cancer is a devastating disease that affects millions of people around the world, so research is vital to better understand its nature and to find new ways to prevent and treat it. Computational models have become an essential tool in the study of these such complex diseases.

We present a phenomenological model for analysing the behaviour of biological systems at different levels of complexity, from the cellular to the tissue levels. Tumoural and non-tumoural cells are primary interest herein for contribute directly to the mechanical properties of the tissue through the proliferation and death processes. Extracellular matrix also has a principal role in the cell-microenvironmental cross-talk therefore the tumour can promote to a better stage or keep growing. The biomechanical model is based on the mass and cellular balance equations coupled with elasticity. The multispecies model simulates the effect of the cellular processes that occur during tumour growth and shrinkage, namely proliferation and death.

The biomechanical finite element model of tumour growth starts from imaging data derived mainly from MRI sequences. This data comprises the geometry, the initial cellularity distribution and the tumour vasculature. At the end of the simulation, the results obtained are validated with a second set of imaging data obtained after treatment.

The study simulates three-month chemotherapy using real patient cases, and presents two distinct outcomes: in one of them, the tumour volume was reduced 20% and in the other one, the volume decreased 90%. One of the patients was classified as low-risk, whereas the other was classified as intermediate-risk. The model effectively reproduces these varying outcomes following the application of chemotherapy, facilitating the identification of cases in which the treatment may be effective.

Acknowledgments

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Membrane tension gradients, their relaxation by membrane flow, and exo-endocytic coupling

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Key Words: *Cell membrane tension, cell membrane flows, exocytosis-endocytosis.*

Many cellular activities, such as cell migration, cell division, phagocytosis, and exo-endocytosis, generate and are regulated by membrane tension gradients. Membrane tension gradients drive membrane flows, but there is controversy over how rapidly plasma membrane flow can relax tension gradients. We show that resistance to membrane flow spans orders of magnitude, depending on the cell type. For two cell types both specialized for calcium-triggered secretion, we find that membrane flow and tension equilibration is very rapid in one case, and unmeasurably slow in the other. In both cell types, stimulated exocytosis adds membrane to the cell surface through fusion of cargo-laden vesicles with the plasma membrane. The extra membrane is then retrieved more slowly through compensatory endocytosis. How exo- and endocytosis are coupled is not well understood.

In a neuronal terminal specialized for rapid synaptic vesicle turnover, we found that rapid membrane flow equilibrates membrane tension gradients within seconds over ~10 um distances. The rapid flow allows loose spatial coupling of exo- and endocytosis: membrane added at the active zone via exocytosis can be retrieved at a distant site via endocytosis.
By contrast, in neuroendocrine adrenal chromaffin cells secreting catecholamines, membrane flow is too slow to relax membrane tension gradients within minutes. In these cells, exo- and endocytosis sites overlap spatially: membrane added to the plasma membrane via exocytosis is retrieved locally.

Thus, the differences in membrane flow and tension equilibration can explain the very different exo-endocytosis coupling in these two cell types and likely represent adaptations to distinct membrane recycling requirements.

SUBSTRATE STIFFNESS IMPACTS EARLY BIOFILM FORMATION BY TUNING BACTERIAL SURFACE MOTILITY

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Key Words: *bacteria, surface motility, microcolonies, biofilms.*

Most bacteria live in surface-associated communities called biofilms. These spatially-organized communities are essential to the survival of bacteria in harsh environments, and are closely linked to antibiotic resistance in pathogenic strains. Biofilms stem from the colonization of substrates by bacteria, a promiscuous process that takes place on a wide range of living tissues and inert materials.

Here, we have studied experimentally the impact of substrate rigidity on bacterial adhesion and early biofilm development. By imaging and tracking adhering bacteria *in situ*, on polyacrylamide hydrogels of defined stiffness (1-100 kPa), we demonstrate that the opportunistic promiscuous pathogen *Pseudomonas aeruginosa* explores substrates differently based on their rigidity. This leads to striking variations in microcolony structure, strain mixing during co-colonization and phenotypic expression. Using simple kinetic models, we propose that these phenotypes arise through a purely mechanical interaction between the elasticity of the substrate and the type IV pilus (T4P) machinery, a contractile surface appendage that mediates surface-based motility ("twitching"). Our 1D model is based on a force balance between (i) a pilus that extends, attaches and retracts with a defined frequency; (ii) the deformation of the underlying substrate at the pilus tip upon retraction; and (iii) the friction force due to adhesion of the bacterial body when it is dragged across the surface at the other end of the pilus. The efficiency of pili activity is thus modulated by the deformability of soft substrates [1].

Together, our findings reveal a new role for substrate softness in the spatial organization of bacteria in complex microenvironments, with far-reaching consequences on efficient biofilm formation by *P. aeruginosa*, and possibly other microorganisms.

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ROLE OF NON-MUSCLE MYOSIN IN PERIPHERAL ACTIN NETWORK REMODELLING AT MITOTIC EXIT

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Key Words: *Mitosis, Actomyosin cortex, Cell shape.*

Cell shape is paramount to the function and life of eukaryotic cells and heavily relies on cytoskeletal architecture and dynamics. In particular, the subset of actin filaments located underneath the cell membrane, also referred to as peripheral actin, plays a key role in both global and local aspects of cell shape. Our current understanding of how differently organised actin networks are responsible for changes in cell shape is limited by the complexity, redundancy, and robustness of the actin interactome. Here, we use the cell shape transitions occurring at mitotic exit as a model to observe how active actin remodelling drives cell shape changes. While cells in mitosis are round and bear a uniform, contractile, meshwork-like cortex, interphase cells have distinctly organised subsets of sub-membranous actin, forming structures such as stress fibres, lamellipodia, and filopodia (Taubenberger et al., 2020). In this work, we focus on the role of non-muscle myosin in actin architecture remodelling at mitotic exit, through a combination of 2D and 3D live-cell microscopy and mechanical approaches. We look at non-muscle myosin II through the double lens of contractility and protrusivity (Betapudi, 2010; Salbreux et al., 2012) and seek to identify its role in the initiation of protrusions and the first establishment of lamellipodia as the cell exits its round state after mitosis. Overall, this work arches over molecular and cellular scales to provide an understanding of the actomyosin network remodelling occurring at mitotic exit.

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Novel cell stretching device for Mechanobiology and drug screening

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Key Words: *Mechanobiology, cell stretching, drug screening.*

Cells within the body are constantly subjected to mechanical stimuli during organ movement, development, and various functions. These mechanical stimuli play a crucial role in proper cell functioning, influencing processes like migration, proliferation, and differentiation. The cells sense such mechanical cues through a mechanism known as mechanotransduction. Anomalies in mechanotransduction can lead to various pathogeneses, including cancer, asthma, and heart disease. In the field of Mechanobiology studies, the advancement of in vitro technologies becomes imperative for applying physiologically mimicking mechanical forces to cultured cells and tissues, due to the intricate nature of in vivo biological systems. The increasing interest in Mechanobiology, along with existing technological gaps, has driven the development of a diverse array of tools in this field across research laboratories and companies. However, the majority of existing Mechanobiology tools suffer from issues of bulkiness and complexity, limiting their capacity to scale up effectively for diverse applications such as drug screening. To address these challenges, a novel cell stretching system has been developed to simplify the concept significantly. This innovative cell stretching device consists of two parallel membranes and an air chamber, which is connected to a vacuum pump. When the air chamber is vacuumed, atmospheric pressure deforms the two membranes, causing them to press against/support each other and create a flat, stretched region. The maximum stretching level is determined by the size of the membranes and the distance between them. The remarkably simplified cell stretching concept offers distinct advantages over existing devices. Its design can be upscaled to accommodate a large population of cells for protein and genetic studies. Moreover, it can be downsized and scaled up to a 96-well or 384-well plate format for applications such as drug discovery or screening. The proof of concept has been validated through the creation of a 3D printed prototype. The distinctively simplified concept, flexibility in both upscaling and downscaling, and the capability for straightforward mass production set apart this technology as the inaugural advancement in the Mechanobiology and Organ-on-a-chip field. It possesses significant potential for extensive research and applications, particularly in the fields of drug discovery and screening.

Elucidating the sensing and motion properties of a soil micro-swimmer through a biophysical approach

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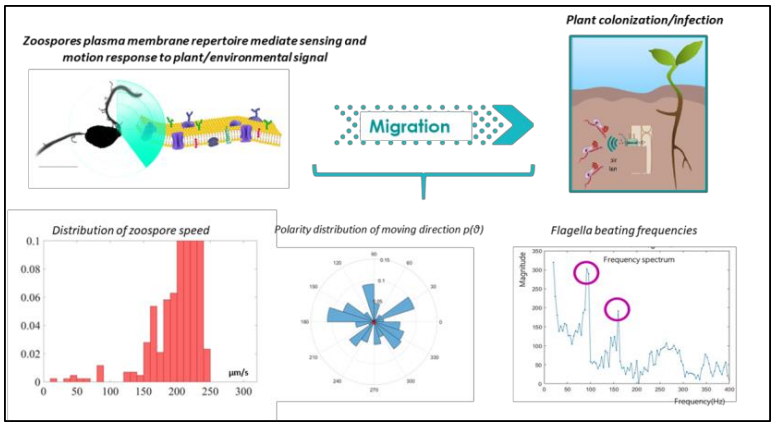
Key Words: *micro-swimmer, sensing, biomechanics.*

In soil ecosystems, the initial interaction phases between *Phytophthora* species and plant hosts are orchestrated by rhizospheric guidance factors acting upon the species' unicellular, biflagellate zoospores[1]. These micro-swimmers navigate the soil utilizing a combination of sensory and propulsion mechanisms[1,2], which are yet to be fully understood. In this context, a bio-physical approach was developed to identify those plasma membrane proteins that mediate zoospores interaction with the rhizosphere and govern the mechanics of their swimming responses to environmental stimuli, crucial for directing plant infection. Using LC-MS/MS analysis we initially characterized *Phytophthora parasitica* zoospores membrane composition, which resulted in an heterogeneous peptide profile differentially distributed between the cell body and flagella plasma membranes components. Subsequently, a microfluidic set-up was utilized for biomechanical analysis of the micro-swimmer response to distinct rhizospheric stimuli. The set-up allowed precisely quantification of various physical parameters, including speed, trajectory and directional change of zoospores swimming during stimulus response and facilitated observations at a reduced scale of their flagella beating frequencies. The set-up further enabled to discriminate zoospores specific stimuli response among other rhizospheric microbial species. Collectively, our findings shed light on the protein-mediated sensory and motion mechanisms of *Phytophthora* zoospores. This contributes significantly to understanding the complex network of rhizospheric interactions that drive oomycete dispersal in soil.



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DEVELOPING MECHANOBIOCHEMICAL MODELS FOR 2- AND 3-D CELL MIGRATION

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In this talk, I will present recent developments of mechanobiochemical models for 2- and 3-D single cell migration. These models seek to couple continuum mechanics models (viscoelastic, morphoelastic, hyperelastic) with bulk-surface reaction-diffusion models (geometric bulk-surface PDEs) for cell polarisation to study how single cells migrate through isotropic and non-isotropic environments. These theoretical and computational models are fit for experimental validation. Understanding single cell migration is fundamental to understand collective cell migration, which is a central process in tissue and organ formation, embryonic development, would healing and immune responses.

Mechanical regulation of morphogenesis and apoptosis

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Key Words: *compressive stress, apoptosis, morphogenesis*

During development and homeostasis, epithelia are constantly challenged by mechanical stress and have to resist or respond to these cues. As it can modulate cellular mechanisms, forces are involved in a large variety of processes such as embryonic development, tissue repair, aging and tumor development. Notably, apoptosis can be influenced by the cell mechanical environment and particularly compressive stress[1].

My research team demonstrated the significant role of apoptosis in shaping the developing leg disc of *Drosophila*[2]. The apoptotic pattern is driven by pro-apoptotic genes leading to a robust cell death pattern resulting in a formation of a fold. Maintaining a delicate balance in this process is crucial, as an excess or deficiency of dying cells could compromise tissue integrity or disturb fold morphogenesis. However, how the broad expression of pro-apoptotic genes leads to the death of only a subset of cells is unknown as no signaling targeting dying cells within these regions has yet been identified. In the context of our project, we took advantage of the *Drosophila* leg disc, allowing for *ex vivo* culture, to investigate the influence of external mechanical stress on morphogenesis and apoptosis regulation. Through strategic micromanipulations, we were able to tune the external mechanical stress naturally applied on the disc. Our findings indicate the crucial role of physiological compressive stress in both morphogenesis and the regulation of apoptosis.

Using advanced mechanobiological tools, including laser ablations, FLIM microscopy, and homemade state-of-the-art image analysis software[3] our investigation aims to unravel the impact of extrinsic forces on tissue and cell dynamics. Through this comprehensive approach, we aspire to gain a better understanding of mechanosensitive apoptosis, contributing to the broader comprehension of how mechanical cues sculpt tissue and regulate cellular behavior.

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UNRAVELING GLIOBLASTOMA HETEROGENEITY: INTRODUCING SP2G METHOD FOR IDENTIFYING INVASIVE SUB-POPULATIONS

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Key Words: *Glioblastoma, migration, micro-patterning, laminin, intra-patient heterogeneity*

Glioblastomas (GBM) are highly aggressive and debilitating primary brain tumors that lead to the destruction of the brain and the rapid death of the patients. GBM exhibit remarkable heterogeneity at various levels, including motility modes and mechanoproperties that contribute to tumor resistance and recurrence. It is now, well established that GBM invade the brain mainly by migrating along linear tracks such as the abluminal surface of the blood vessels. In previous works, we demonstrated that laminin –coated micropatterned lines could mimic these linear tracks and potentiate GBM motility (1-2). More recently, we used micropatterned grids that mimic better the brain blood vessel network and could unveil specific motility modes that single lines could not distinguish. This allowed identifying important players such as the formin FMN1 for GBM motility (3).
Here, we further improved our technique by combining micropatterned grids and spheroid spreading (SP2G for spheroid spreading on grids). This combination allows to rapidly image and analyze the various motility modes that can be found in GBM various cell lines. We set up a semi-automatic analytic pipeline that holistically dissects glioblastoma motile behavior by delivering quantitative outputs allowing a precise and unbiased quantification of migration and motility modes.
Importantly, SP2G allowed us to scrutinize the migratory behavior of diverse clones extracted from a heterogeneous population from a single patient. Our analysis revealed distinct motility modes exhibited by these clones, which we systematically categorized and correlated with molecular signatures identified through RNAseq, unveiling potentially crucial and motility-specific molecular players. The comprehensive intra-patient screening, sorting, and molecular characterization of the most motile clones provide a fresh perspective for further advancements and pave the way for a more targeted and efficient identification of pertinent treatments.

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ON THE INFERENCE OF GROWTH ON DEVELOPMENTAL TISSUES

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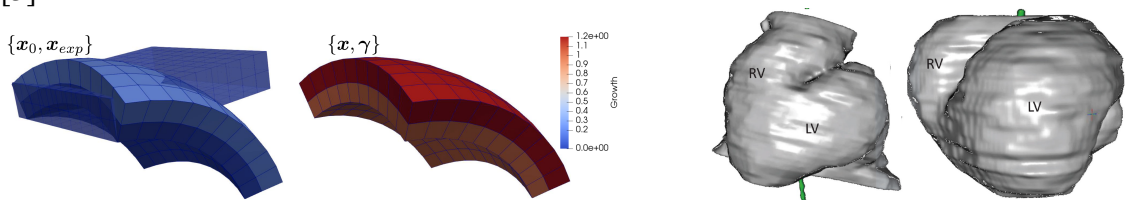
Key Words: *growth, inverse methods, development, tissues.*

During development, tissues adopt a series of shape that strongly depend on the temporal distribution of growth, which in many cases is the result of a feedback chemo-mechanical process. This growth determines the functionality and final shape of the organ or organism, but is not directly measurable, other than from indirect methods or local proliferation rates [1].

We here present an indirect method that allows inferring the growth field from two 3D shape descriptions, $\boldsymbol{x}_1$ and $\boldsymbol{x}_2$. Our methodology aims at computing new domain positions $\boldsymbol{x}$, boundary conditions (reactions $\boldsymbol{r}$) and growth $\boldsymbol{\gamma}$ that best match the measured shapes on consecutives instants. As usually encountered in inverse methods, the solution is not unique. We adopt an iterative regularisation process the furnishes a suitable set of unknowns ($\boldsymbol{x}$, $\boldsymbol{r}$, $\boldsymbol{\gamma}$) [2], even when the positions $\boldsymbol{x}_1$ and $\boldsymbol{x}_2$ are measured on partial subsets of the domain, e.g. at the visible boundary.

We demonstrate our results with a set of synthetic examples, which are currently being applied to early heart development [3], which exhibits high inhomogeneities on the growth distribution, but that under aberrant developmental processes can lead to fatal malformations [3].



(a) (b)

Figure 1. Left: Synthetic bilayer with differential growth. In red, computed deformed shape and growth patterns. Right: embryonic mouse heart at stage E9.5 [3].

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Characterisation of the nonlinear rheology and rupture dynamics of epithelial monolayers

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Key Words: *Tissue rheology, fracture, nonlinear viscoelasticity*

Tissues' structure is inherently hierarchical, spanning the sub-, uni- and multi-cellular levels. This creates a complex multiscale problem, where deformation applied at the tissue level leads to stress at the cellular and subcellular levels [1]. Despite the importance of tissue integrity, their nonlinear rheology and fracture behaviour remains an elusive topic in biomechanics. Previous experimental data shows that epithelial monolayers possess a stiffening behaviour [2]. This peculiar mechanical behaviour could be attributed to the Intermediate Filaments (IF), which are in turn coupled to the desmosomes at the cell-cell junction allowing the transfer of the stress across cells [3]. Therefore, when a macroscopic stress is applied to an epithelial monolayer, this highly nonlinear behaviour may be originated by the interfaces (at the desmosomes) or the IF. This creates open questions, such as (i) how we can use the rheology to capture the underlying architecture (ii) what component of the cytoskeleton is responsible for the complex viscoelastic response and (iii) what can be learnt from the molecular dynamics close to rupture about healing, remodelling and catastrophic failure. To address this, we propose a novel approach combining rheological models with stochastic models of adhesion to discern the subcellular elements responsible for the nonlinear response. Our approach aims to develop a physically based model that explains subcellular mechanisms underlying macroscopic tissue responses near rupture. Two rheological networks have been developed, exhibiting distinct behaviours under different loading protocols (strain ramp, strain cycles, stress creep), offering testable hypotheses to understand tissue material architecture and pinpoint the subcellular causes of nonlinear responses. Understanding the nonlinear rheology and fracture behaviour of tissues is a complex yet crucial aspect of biomechanics research with applications in bioengineering and biomimetics.

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BIOMECHANICS OF FAST ACTUATION IN MIMOSA PUDICA

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Key Words: *plant movements, osmotic flows.*

Despite an absence of muscles, plants move. Plant movements span timescales ranging from tenths of seconds to several hours, and rely on a rich variety of physical mechanisms[1]. The "touch-me-not" plant, *Mimosa pudica*, folds its leaflets and petioles in a couple of seconds in response to external stimuli such as touch, brutal light and moisture variations, or electric currents. This movement is reversible, and the leaf resets in a dozen of minutes.

At the base of every mobile unit lies a flexible, hinge-like bulge called pulvinus. This pulvinus acts as the motor organ. Fast, osmosis-driven water transport across the pulvinus is the textbook explanation for *Mimosa pudica*'s fast motions[2]. This organ-wide water transport received only limited direct experimental evidence, however. Our goal here is to better understand the physics of fast actuation in pulvini and, thus, to test the current consensus.

We experimentally quantify three aspects of the phenomenon: (i) the kinematics of the motion (with high-speed imaging), (ii) the mechanics of the pulvinus (developed torque, macroscopic rigidity), and (iii) the osmotic swelling dynamics. Our preliminary results lead us to question the textbook scenario and to elaborate an alternative mechanism based on local water redistribution from cells to neighboring air cavities.

Figure: Fast movement of a *Mimosa pudica* leaf. The main pulvinus, at the base of the leaf, is triggered by electrical stimulation at t = 0 and bends in seconds.

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Actin based motility unveiled: how chemical energy is converted into motion.

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Key Words: *Cellular motility, actin-based motility, large strains multi-physics, finite elements.*

The chemo-mechanical motor of several physiological and pathological processes in biological systems is a polymerization process, which converts chemical energy into mechanical work. The chief component in this activity is actin, a multi-functional protein forming filament in the cell cytoskeleton [1]. In a recent publication, a thermodynamically consistent continuum-mechanics formulation was proposed, stemming from continuity equations that account for actin chemical kinetics. We have suggested [2] that the volumetric expansion exerted after the phase change from monomeric to a cross-linked network of actin filament ultimately converts chemical energy into motion.

In this note, unpublished results will be presented for the first time. The main novelty is the application of Helmholtz free energies with no entropic contributions. The model manifests itself in macroscopic descriptors of biochemical and biological details of the relevant processes, thereby resulting in sufficient generality to be appropriate for several biological systems, targeting cellular motility.

Numerical simulations of Listeria pathogens, with data taken from biological literature, show that the main features of actin-based motility are captured with remarkable accuracy. Fluorescent imaging and quantitative analysis describing the cellular force transducer elements, like the cytoskeleton, will validate our computational models.

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ROLE OF MICROTUBULE ACETYLATION IN GLIOBLASTOMA MECHANOTRANSDUCTION DURING MIGRATION AND INVASION

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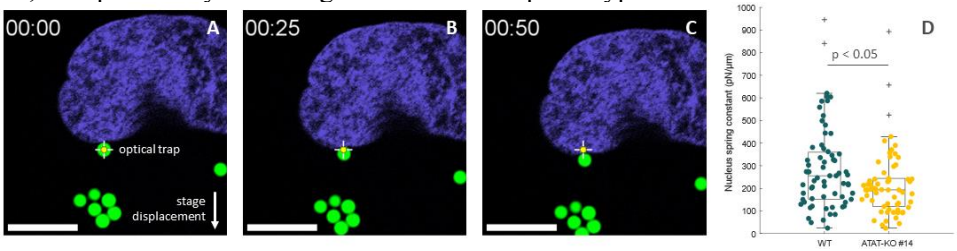
Key Words: *Glioblastoma multiforme, Microtubule, Nucleus, Mechanotransduction, Optical tweezers*

Glioblastoma multiforme (GBM) is the most lethal primary brain tumour, with a median survival of 14 months only. The main factors for this poor prognosis are the invasive character of GBM cells and their resistance to treatments. Indeed, even after surgical resection of the primary brain tumour and treatment with radio- and chemotherapy, recurrence inevitably occurs from cancer cells that invaded further in brain tissues. In this context, the mechanical properties of GBM cells and of their subcellular components could modulate the invasive character of GBM cells.

Microtubules form one of the major cytoskeletal networks. They are essential for cell mesenchymal migration. Microtubule acetylation, a post-translational modification catalysed by ATAT1, has been shown *in vitro* to confer mechanical resistance and stability to microtubules [1], and to promote migration in astrocytes, the healthy counterpart of GBM cells [2,3]. The mechanical properties of microtubules regulate mechanotransduction from the cell membrane to the nucleus, which is the largest organelle of the cell and thus a physical limiting step in migration through confined microenvironments. In ATAT1-KO GBM cells, where microtubules are significantly less acetylated, we have measured a lower nuclear stiffness through optical tweezers experiments, compared to WT GBM cells. Moreover, our preliminary results show that microtubule acetylation is increased after irradiation, thus potentially favouring a more invasive phenotype.



A, B, C: Confocal imaging of an internalised bead (green) close to the nucleus (blue). The bead is optically trapped and indents the nucleus as the microscope stage is linearly displaced (time indicated in the top left corner in seconds). The relative displacement of the bead and the nucleus yields a spring constant value used as a proxy for nuclear stiffness.
D: The nucleus spring constant is significantly reduced in ATAT1-KO GBM cells compared to WT GBM cells ($p = 0.034$).

Understanding the interplay between microtubule acetylation, GBM invasion and viscoelastic properties of both microtubules and the nucleus is needed to find new therapies for this lethal disease. Further experiments will focus on the mechanical effects of radio- and chemo-therapy, which could play a critical role in the resistance of GBM to treatment.

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Optical tweezers-based mechanical characterization of mitochondria

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Key Words: *Mitochondria, Cytoskeleton, Mechanotransduction, Optical tweezers, Rheology.*

Widely acknowledged as the cellular energy powerhouses, mitochondria are dynamic organelles capable of sensing and integrating mechanical and metabolic cues. Previous *in vivo* studies have examined the influence of external forces applied to the cell on mitochondria mechanical response [1], but a direct assessment of their mechanical properties within living cells is still lacking. To address this question, we used an optical tweezers-based intracellular micromanipulation technique with endocytosed beads [2], to probe the mechanical and rheological properties of single mitochondria and their microenvironment in RPE-1 cells. Our novel approach involves applying controlled forces to deflect single mitochondria, enabling the direct measurement of their effective stiffness and relaxation times (Figure). Additionally, we investigated the influence of the cytoskeleton in the mechanical properties of single mitochondria and local regions within the mitochondrial network, using pharmacological drugs. Preliminary experiments were also conducted to explore correlations between the morphological and structural properties of mitochondria and their mechanical properties. Our results show that the rheological properties of the mitochondrial microenvironment are not correlated with the morphology but with the density of mitochondria, particularly when cytoskeleton integrity is impaired. Further experiments should provide valuable insights into the complex interplay between mitochondria morphodynamics, mechanics and subcellular interactions.

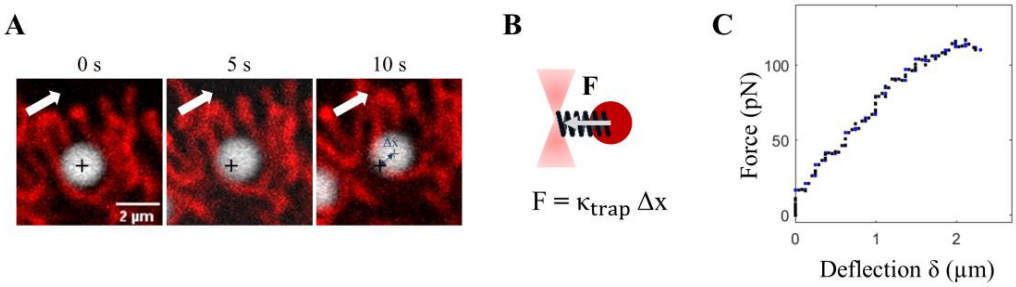

Figure: *Measurements of the* ***effective stiffness of mitochondria*** *in living cells. A. Images showing a typical deflection experiment of a mitochondrion in RPE-1 cells. A mitochondrion (red) is deflected by moving the cell towards the top-right direction (white arrow) which displaces the bead (grey) away from the trap centre (grey cross) of a distance Δx. B. Scheme of the bead in the optical trap. The force F exerted on the mitochondrion is deduced from the bead displacement Δx after calibration of the optical trap stiffness κ_{trap}. C. Example of force-deflection curve. The effective stiffness is obtained by a linear fit of the force-deflection curve at low force.*

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A 3D VERTEX MODEL REVEALS MECHANISTIC COUPLING OF PATTERNING AND PROLIFERATION IN ORGANOIDS: BIOLOGICAL INTERPRETATION

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Key Words: *reaction-diffusion system patterning, mechano-signaling coupling, organoid, branching morphogenesis.*

Branching morphogenesis is an evolutionarily conserved mechanism that increases the functional area of organs, including mammary gland. Branching morphogenesis of mammary gland is tightly regulated by local paracrine signals, however, how numerous microenvironmental signals are integrated in the epithelium to result in specific patterns of the gland is poorly understood. Here, we have used an organoid system, employing primary mammary organoids, that create spontaneous branches upon stimulation with fibroblast growth factor 2, with integrative computational modeling that couples 3D vertex model with activator-inhibitor system. Based on predictions from the computational model, we untangled the roles of patterned and isotropic proliferation on branching of organoids. Using pharmacological disturbance experiments, we identified the underlying intracellular signaling pathways – ERK and AKT – that control nascent branch emergence and isotropic organoid proliferation, respectively. Furthermore, we demonstrated that while ERK and AKT drive two distinct morphological processes, they are functionally intertwined. We further used the computational model to demonstrate that the connection of ERK and AKT signaling is indirect via the mechanics of proliferation-driven increase in tissue volume affecting patterning (Fig. 1). Taken together, we have identified ERK and AKT as key pathways for mammary branching morphogenesis, driving epithelial branching (patterning) and stratification (isotropic proliferation), respectively, two processes indispensable for proper mammary gland development. Furthermore, our interdisciplinary approach demonstrates the strength of computational modeling for solution of biological problems.

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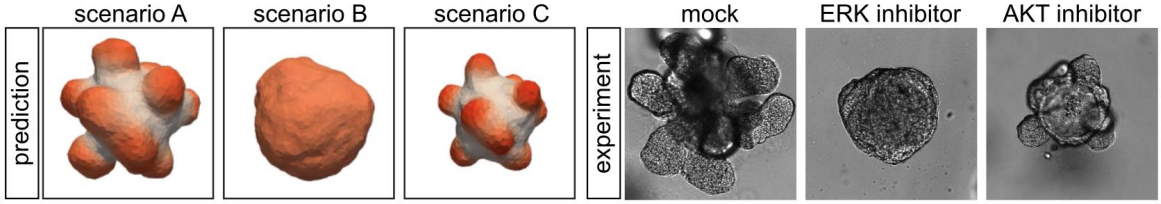

Fig. 1 Comparison of computational predictions and experimental data.

MECHANICAL REGULATION OF CELL DIVISION ORIENTATION: INVESTIGATING THE ROLE OF NUCLEAR MITOTIC APPARATUS PROTEIN

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Key Words: *cell division orientation, mechanical force, NuMA*

Cells in our body constantly experience a variety of mechanical forces from their environment. Responding to forces is crucial to shaping and maintaining tissues, with errors in the process contributing to failure in embryogenesis and diseases such as cancer[1,2]. One cellular process regulated by force is division orientation, which is determined by the position of the mitotic spindle. For example, stretching a tissue causes most divisions to align with the stretch axis[3], and spindle-associated cortical proteins may be important in this process. Nuclear mitotic apparatus protein (NuMA), which is key to spindle positioning, has been implicated in mechanosensitive spindle orientation. However, the mechanistic details remain uncharacterised, especially in a tissue context. Therefore, we utilised the *Xenopus laevis* animal cap tissue, to which tensile forces can be applied externally, to understand the role of NuMA in mechanosensitive spindle orientation. Using GFP-tagged NuMA, we show that cortical localisation of GFP-NuMA is dynamic and sensitive to mechanical stretch. Furthermore, we use mathematical modelling of spindle movements to show that amplifying microtubule-pulling forces at sites of experimentally observed cortical NuMA, as opposed to other pulling regimes, orients the spindle in a manner that most closely matches experimental data. Additionally, using morpholino-targeted knockdown of *Xenopus* NuMA we show that reduced NuMA levels disrupt division orientation according to stretch and cell shape. Interestingly, our data suggest that mechanosensitive spindle orientation through NuMA is an effect of direct force sensing rather than sensing changes in cell shape. By comparing two tissue stretch regimes, we also demonstrate that NuMA responds specifically to anisotropic tension to orient cell divisions. Overall, using live tissue imaging and mathematical modelling, our results indicate that NuMA is vital to orient the mitotic spindle according to an external force.

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Mechano-dependent sorbitol accumulation supports biomolecular condensate

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Keywords: Biomolecular condensate; Mechanobiology; Cell metabolism; Glucose metabolism; Sorbitol

Abstract

Biomolecular condensates regulate a wide range of cellular functions from signaling to RNA metabolism, yet, the physiologic conditions regulating their formation remain largely unexplored. Biomolecular condensate assembly is tightly regulated by the intracellular environment. Changes in the chemical or physical conditions inside cells can stimulate or inhibit condensate formation. However, whether and how the external environment of cells can also regulate biomolecular condensation remain poorly understood. Increasing our understanding of these mechanisms is paramount as failure to control condensate formation and dynamics can lead to many diseases. Here, we provide evidence that matrix stiffening promotes biomolecular condensation in vivo. We demonstrate that the extracellular matrix links mechanical cues with the control of glucose metabolism to sorbitol. In turn, sorbitol acts as a natural crowding agent to promote biomolecular condensation. Using in silico simulations and in vitro assays, we establish that variations in the physiological range of sorbitol, but not glucose, concentrations, are sufficient to regulate biomolecular condensates. Accordingly, pharmacologic and genetic manipulation of intracellular sorbitol concentration modulates biomolecular condensates in breast cancer – a mechano-dependent disease. We propose that sorbitol is a mechanosensitive metabolite enabling protein condensation to control mechano-regulated cellular functions. Altogether, we uncover molecular driving forces underlying protein phase transition and provide critical insights to understand the biological function and dysfunction of protein phase separation.

Contractile forces from the epiblast drives hypoblast motion during avian gastrulation.

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Key Words: *Passive deformation, tissue morphogenesis, gastrulation, hypoblast, patterning.*

In amniotes, interactions between the epiblast (i.e. the pluripotent embryonic territory) and the hypoblast (i.e. the extraembryonic endoderm) define the site of primitive streak formation, where gastrulation takes place. These interactions have been extensively studied at the molecular level and the current models posit that the displacement of the hypoblast relative to the epiblast contributes to the emergence of the site of primitive streak formation [1]. Indeed, the driving forces underlying the morphogenesis of the epiblast and the hypoblast have been reported to be different. Whereas contractile forces drive the remodeling of the epiblast and primitive streak formation [2], epithelial spreading and migration have been proposed to underlie hypoblast morphogenesis [3]. However, it remains unclear how epiblast and hypoblast dynamics integrate, as they were not imaged simultaneously. Live imaging transgenic quails, we show that the counter-rotating flows associated with primitive streak formation in the epiblast can be concomitantly observed in the hypoblast. Using microsurgery, we demonstrate that a mechanical coupling between the two layers enables the contractile forces generated in the epiblast to be transmitted to the hypoblast. We thus conclude that the hypoblast passively deforms under the action of the forces that deform the epiblast and not from active migration, as previously proposed. As morphogens secreted by the hypoblast regulate embryonic axis formation in the epiblast, one needs to reconsider previous models in which migration of the hypoblast relative to the epiblast controls embryonic axis formation in avian. In sum, our work redefines how hypoblast morphogenesis takes place in avians and illustrates how mechanical coupling between tissues contributes to embryonic patterning.

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A 3D VERTEX MODEL REVEALS MECHANISTIC COUPLING OF PATTERNING AND PROLIFERATION IN ORGANOIDS: MATHEMATICAL FORMULATION

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Key Words: *reaction-diffusion system patterning, mechano-signalling coupling, cell proliferation.*

Inspired by the work Okuda and his collaborators [10.2142/biophysico.12.0_13], we developed a three-dimensional computational model to reveal the interaction between patterning and proliferation in epithelial tissue. The geometrical representation is based on Voronoi cells that define individual cells and their lateral, apical and basal faces. The viscous-damped system of forces controlling the shape of the multicellular tissue is derived from a potential featuring both volumetric and surface terms. Equipped with the cell rearrangements via cell-neighbour exchanges, the model allows for cell division while following the cell cycles (Fig. 1).

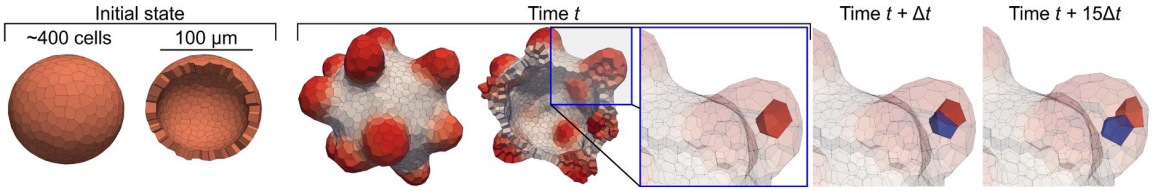

Fig. 1 Cell division during pattern-driven growth from an initial single-layered spherical organoid.

Pattering is controlled by the interplay of morphogens (activator and inhibitor) in a reaction-diffusion system [10.1007/bf00289234]. The formation of patterns controls the cell proliferation in which the local concentration of activator plays a role of a mitogen. The cell growth rate is modelled via Hill equation, in which the exponent plays a central role in the presented coupling between patterning and proliferation. We have used our model to decipher mechanistic and signaling events underlying fibroblast growth factor 2 (FGF2)-induced branching morphogenesis of mammary gland organoids. We identified a feedback loop between the formation of patterns, influencing the rate of cell proliferation and consequently the tissue mass increase. This feedback loop critically impacts the subsequent patterning through a diffusion-reaction system of morphogens. Importantly, the interplay between proliferation and patterning in silico accurately captures observed variations in the shapes of organoids growing in vitro upon different intensity and dynamics of FGF2 signaling or perturbation of downstream signaling pathways. In a complementary contribution, we present integration of the model with biological data that demonstrates its strength to predict organoid morphognesis.

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