

Week 01: Introduction

Alexandre Persat and Mahmut Selman Sakar

Lecture Overview

- Overall objectives
- Syllabus and grading
- Content and schedule
- How to read and critique research articles?

Instructor: Alex Persat

- BSc in Engineering, Ecole Polytechnique
 - MSc in Chemical Engineering, Stanford
 - PhD in Mechanical Engineering, Stanford
Stanford Microfluidics Lab, Prof. Juan Santiago
 - Postdoc in Molecular Biology, Princeton
Focus on Microbiology and Biophysics
 - “Visitor” in Bioengineering, Caltech
Focus on Microbiota
 - Associate Professor at the Global Health Institute
<https://www.p-lab.science/>
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Instructor: Selman Sakar

- Ph.D. in Robotics (Grasp Lab, University of Pennsylvania)
 - Single cell manipulation, magnetic actuation, bacteria actuation
- Postdoc in Bioengineering (M.I.T.)
 - Biological machines from living muscle tissue
- Research Scientist in Medical Robotics (ETH Zurich)
 - Bacteria-inspired microswimmers, robotic microsurgery
- Associate Professor at EPFL
 - Research: Microrobotics, mechanobiology, medical technology
 - Website: <https://www.epfl.ch/labs/microbs/>
 - Contact me for semester/ masters projects

Course Information

- Time
 - 13:00-17:00 Wednesdays
- Office and Email
 - Alex: AI 3138 and alexandre.persat@epfl.ch
 - Selman: MED3 2916 and selman.sakar@epfl.ch
 - Email us to arrange office hours
- Course Webpage
 - <https://moodle.epfl.ch/course/view.php?id=16743>
 - Check for content regularly

Course Material

- There is no textbook
- Lecture Notes
- Review Articles
- Research Articles

Background and Related Courses

- Biomaterials
- Physics of Cells
- Biomechanics of the musculoskeletal system

- Focus will be on cells, cell clusters and extracellular matrix (i.e., nano to mesoscales)

Grading

- Weekly assignments and class participation 20%
- Paper Presentation 20%
- Reports for 3 group projects 60%

Overall Objectives

- A new perspective to nature
- Nurturing interdisciplinarity and diversity
- Bridging programs in life sciences and engineering
- Intellectual feast

Tentative Schedule

Week	Date	Content
1	11/09	Introduction Crash course Mechanobiology of bacteria
2	18/09	Guest Lecture by Laure Leblanc Introduction to project 1 Image analysis tutorial
3	25/09	Mechanobiology of bacterial multicellularity Article 1 presentation
4	02/10	Animal cell mechanics Article 2 presentation
5	09/10	Adhesion and Mechanotransduction Article 3 presentation Project 1 session
6	16/10	Guest lecture on AFM - Georg Fantner Article 4 presentation Return project 1
Fall break – no lecture on 23/10		
7	30/10	Mechanobiology of cell groups Article 5 presentation Introduction to project 2
8	06/11	Tissue Mechanobiology Article 6 presentation
9	13/11	Guest lecture - Sangwoo Kim Project 2 session Article 7 presentation
10	20/11	Guest lecture – Eileen Gentleman Article 8 presentation Return project 2
11	27/11	Mechanobiology of cell growth Article 9 presentation Introduction to project 3
12	04/12	Mechanobiology of cancer Article 10 presentation
13	11/12	Mechanosensitive proteins Project 3 session Article 11 presentation
14	18/12	Mechanobiology with organoids Article 12 presentation Return project 3

Applications

- Developmental biology
 - Evolution and origins of life
 - Diseases and therapy: cancer, fibrosis, genetic disorders
 - Tissue engineering and regenerative medicine
 - Drug screening
 - Infections
 - Environment
-
- Review articles on Moodle

Crash Course on

- Cell and molecular biology (this week)

Weekly Assignments

- Form groups of 4-5 students
- Each group will present their paper during an exercise session
- Everyone else will send 2 questions on the articles by Sunday night every week
- We will forward selected questions to the presenting groups on Monday mornings
- Questions will addressed during the presentation by the presenting group on Wednesdays

Project

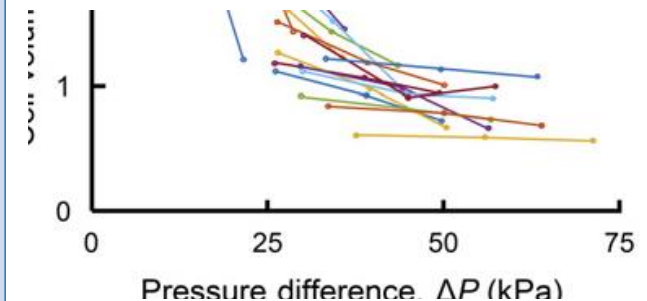
- 3 group projects on experimental mechanobiology
- Processing of images, data analysis, statistic, deduction

Mechanical stress compromises multicomponent efflux complexes in bacteria

Lauren A. Genova^{a,1}, Melanie F. Roberts^{b,1}, Yu-Chern Wong^b, Christine E. Harper^c, Ace George Santiago^{a,2}, Bing Fu^a, Abhishek Srivastava^{b,3}, Won Jung^a, Lucy M. Wang^{b,4}, Łukasz Krzemiński^{a,5}, Xianwen Mao^a, Xuanhao Sun^{b,6}, Chung-Yuen Hui^b, Peng Chen^{a,7}, and Christopher J. Hernandez^{b,c,7}

Significance

The field of mechanobiology examines how physical forces modulate cell physiology and has traditionally focused on eukaryotic organisms. Here we show that in bacteria, mechanical stresses can interrupt the structure and function of a molecular assembly used by Gram-negative bacteria to survive and grow in the presence of toxins. This work provides evidence that bacteria, like mammalian cells, can respond to mechanical forces through molecular complexes at the cell surface in ways that are relevant to growth. Our observations further suggest that mechanical forces may be used synergistically with other antimicrobials.

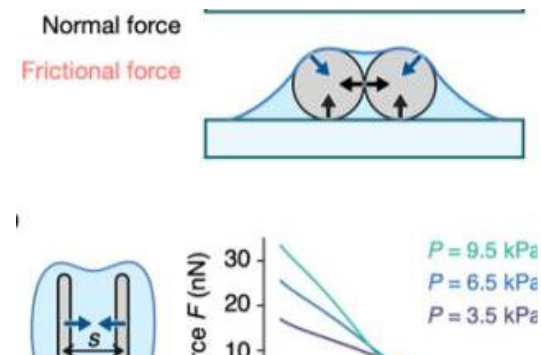


Article 2

Title: Capillary interactions drive the self-organization of bacterial colonies

Authors: Matthew E. Black^{a,1}, Chenyi Fei^{a,1}, Ricard Alert^{b,c,d}, Ned S. Wingreen^{a,e,2}, Joshua W. Shaevitz^{a,f,2}

Many bacteria inhabit thin layers of water on solid surfaces both naturally in soils or on hosts or textiles and in the lab on agar hydrogels. In these environments, cells experience capillary forces, yet an understanding of how these forces shape bacterial collective behaviors remains elusive. Here, we show that the water menisci formed around bacteria lead to capillary attraction between cells while still allowing them to slide past one another. We develop an experimental apparatus that allows us to control bacterial collective behaviors by varying the strength and range of capillary forces. Combining 3D imaging and cell tracking with agent-based modeling, we demonstrate that capillary attraction organizes rod-shaped bacteria into densely packed, nematic groups, and profoundly influences their collective dynamics and morphologies. Our results suggest that capillary forces may be a ubiquitous physical ingredient in shaping microbial communities in partially hydrated environments.

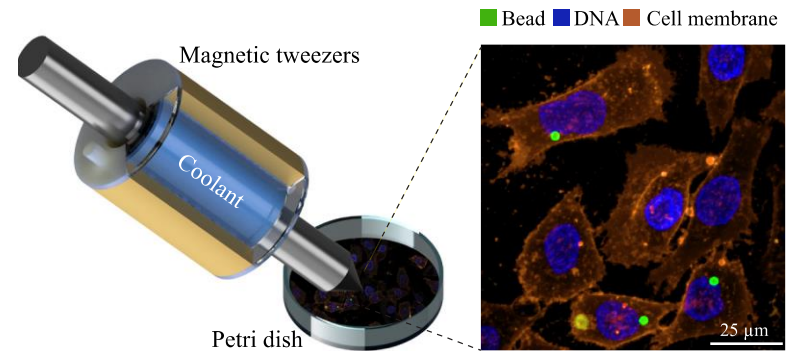


Article 3

Indentation induces instantaneous nuclear stiffening and unfolding of nuclear envelope wrinkles

Wentian Tang^a , Xin Chen^{b,c} , Xian Wang^{a,b} , Min Zhu^c , Guanqiao Shan^a , Tiancong Wang^a , Wenkun Dou^a , Jintian Wang^a , Junhui Law^a , Zheyuan Gong^a , Sevan Hopyan^{c,d,e,1,2} , Xi Huang^{b,c,d,1,2} , and Yu Sun^{a,f,g,h,1} 

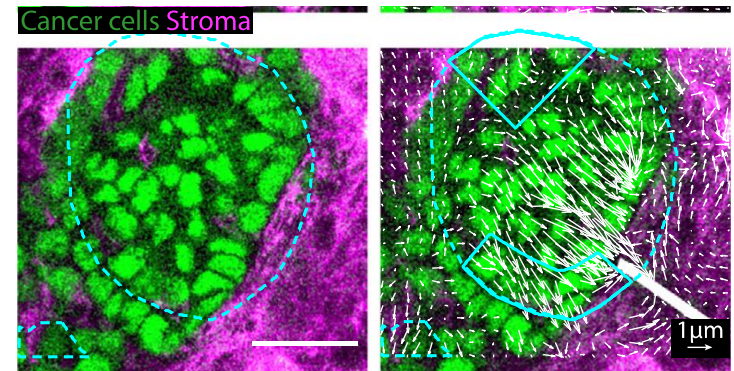
The nuclear envelope (NE) separates genomic DNA from the cytoplasm and regulates transport between the cytosol and the nucleus in eukaryotes. Nuclear stiffening enables the cell nucleus to protect itself from extensive deformation, loss of NE integrity, and genome instability. It is known that the reorganization of actin, lamin, and chromatin can contribute to nuclear stiffening. In this work, we show that structural alteration of NE also contributes to instantaneous nuclear stiffening under indentation. In situ mechanical characterization of cell nuclei in intact cells shows that nuclear stiffening and unfolding of NE wrinkles occur simultaneously at the indentation site. A positive correlation between the initial state of NE wrinkles, the unfolding of NE wrinkles, and the stiffening ratio (stiffness fold-change) is found. Additionally, NE wrinkles unfold throughout the nucleus outside the indentation site. Finite element simulation, which involves the purely passive process of structural unfolding, shows that unfolding of NE wrinkles alone can lead to an increase in nuclear stiffness and a reduction in stress and strain levels. Together, these results provide a perspective on how cell nucleus adapts to mechanical stimuli through structural alteration of the NE.



Article 4

Cancer-associated fibroblasts actively compress cancer cells and modulate mechanotransduction

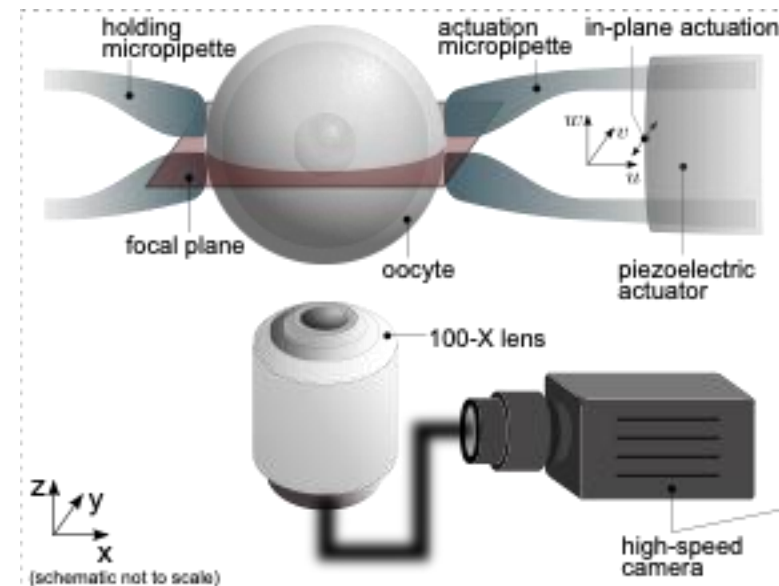
During tumor progression, cancer-associated fibroblasts (CAFs) accumulate in tumors and produce an excessive extracellular matrix (ECM), forming a capsule that enwraps cancer cells. This capsule acts as a barrier that restricts tumor growth leading to the buildup of intratumoral pressure. Combining genetic and physical manipulations in vivo with microfabrication and force measurements in vitro, we found that the CAFs capsule is not a passive barrier but instead actively compresses cancer cells using actomyosin contractility. Abrogation of CAFs contractility in vivo leads to the dissipation of compressive forces and impairment of capsule formation. By mapping CAF force patterns in 3D, we show that compression is a CAF-intrinsic property independent of cancer cell growth. Supracellular coordination of CAFs is achieved through fibronectin cables that serve as scaffolds allowing force transmission. Cancer cells mechanosense CAF compression, resulting in an altered localization of the transcriptional regulator YAP and a decrease in proliferation. Our study unveils that the contractile capsule actively compresses cancer cells, modulates their mechanical signaling, and reorganizes tumor morphology.



Imaging the subcellular viscoelastic properties of mouse oocytes

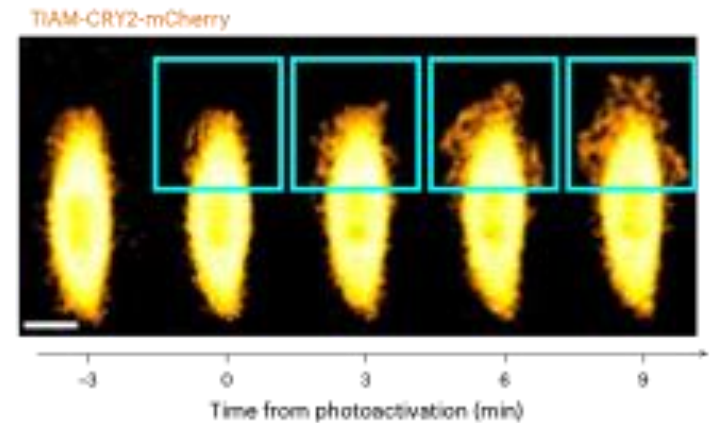
Guillaume Flé^a, Elijah Van Houten^b , Gaudeline Rémillard-Labrosse^c, Greg FitzHarris^{c,d}, and Guy Cloutier^{a,e,1} 

In recent years, cellular biomechanical properties have been investigated as an alternative to morphological assessments for oocyte selection in reproductive science. Despite the high relevance of cell viscoelasticity characterization, the reconstruction of spatially distributed viscoelastic parameter images in such materials remains a major challenge. Here, a framework for mapping viscoelasticity at the subcellular scale is proposed and applied to live mouse oocytes. The strategy relies on the principles of optical microelastography for imaging in combination with the overlapping subzone nonlinear inversion technique for complex-valued shear modulus reconstruction. The three-dimensional nature of the viscoelasticity equations was accommodated by applying an oocyte geometry-based 3D mechanical motion model to the measured wave field. Five domains—nucleolus, nucleus, cytoplasm, perivitelline space, and zona pellucida—could be visually differentiated in both oocyte storage and loss modulus maps, and statistically significant differences were observed between most of these domains in either property reconstruction. The method proposed herein presents excellent potential for biomechanical-based monitoring of oocyte health and complex transformations across lifespan. It also shows appreciable latitude for generalization to cells of arbitrary shape using conventional microscopy equipment.



Optogenetic generation of leader cells reveals a force–velocity relation for collective cell migration

During development, wound healing and cancer invasion, migrating cell clusters feature highly protrusive leader cells at their front. Leader cells are thought to pull and direct their cohort of followers, but whether their local action is enough to guide the entire cluster, or if a global mechanical organization is needed, remains controversial. Here we show that the effectiveness of the leader–follower organization is proportional to the asymmetry of traction and tension within cell clusters. By combining hydrogel micropatterning and optogenetic activation, we generate highly protrusive leaders at the edge of minimal cell clusters. We find that the induced leader can robustly drag one follower but not larger groups. By measuring traction forces and tension propagation in clusters of increasing size, we establish a quantitative relationship between group velocity and the asymmetry of the traction and tension profiles. Modelling motile clusters as active polar fluids, we explain this force–velocity relationship in terms of asymmetries in the active traction profile. Our results challenge the notion of autonomous leader cells, showing that collective cell migration requires global mechanical organization within the cluster.

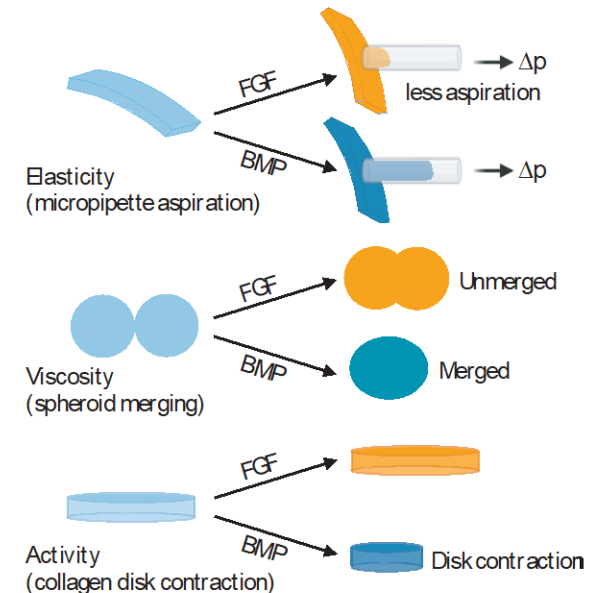


Article 7

Morphogens enable interacting supracellular phases that generate organ architecture

Sichen Yang[†], Karl H. Palmquist[†], Levy Nathan, Charlotte R. Pfeifer, Paula J. Schultheiss, Anurag Sharma, Lance C. Kam, Pearson W. Miller, Amy E. Shyer^{*‡}, Alan R. Rodrigues^{*‡}

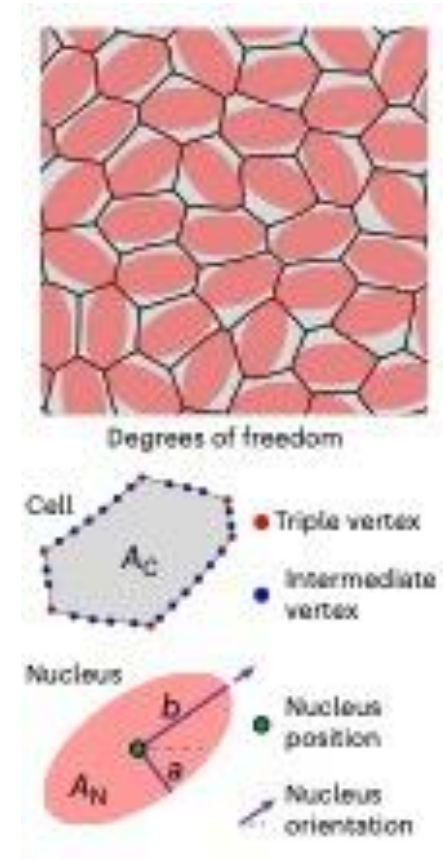
During vertebrate organogenesis, increases in morphological complexity are tightly coupled to morphogen expression. In this work, we studied how morphogens influence self-organizing processes at the collective or “supra”-cellular scale in avian skin. We made physical measurements across length scales, which revealed morphogen-enabled material property differences that were amplified at supracellular scales in comparison to cellular scales. At the supracellular scale, we found that fibroblast growth factor (FGF) promoted “solidification” of tissues, whereas bone morphogenetic protein (BMP) promoted fluidity and enhanced mechanical activity. Together, these effects created basement membrane-less compartments within mesenchymal tissue that were mechanically primed to drive avian skin tissue budding. Understanding this multiscale process requires the ability to distinguish between proximal effects of morphogens that occur at the cellular scale and their functional effects, which emerge at the supracellular scale.



A nuclear jamming transition in vertebrate organogenesis

Sangwoo Kim ^{1,5,6}, Rana Amini^{2,6}, Shuo-Ting Yen ², Petr Pospíšil ²,
Arthur Boutillon ², Ilker Ali Deniz² & Otger Campàs ^{1,2,3,4} 

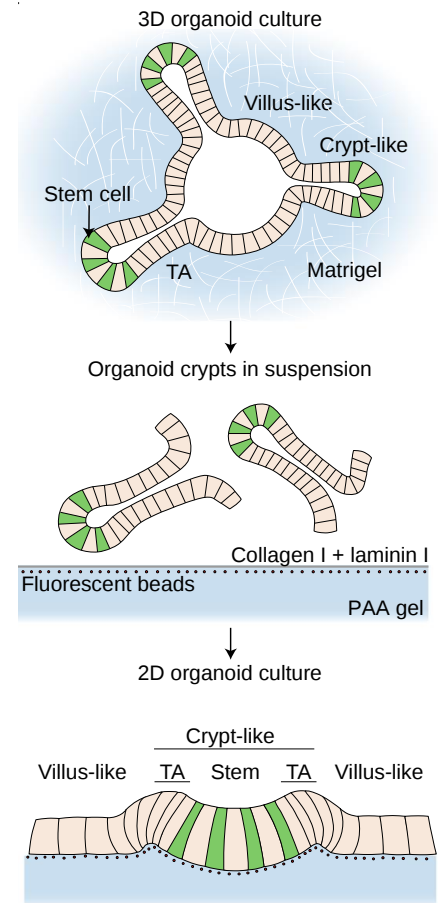
Jamming of cell collectives and associated rigidity transitions have been shown to play a key role in tissue dynamics, structure and morphogenesis. Cellular jamming is controlled by cellular density and the mechanics of cell–cell contacts. However, the contribution of subcellular organelles to the physical state of the emergent tissue is unclear. Here we report a nuclear jamming transition in zebrafish retina and brain tissues, where physical interactions between highly packed nuclei restrict cellular movements and control tissue mechanics and architecture. Computational modelling suggests that the nuclear volume fraction and anisotropy of cells control the emerging tissue physical state. Analysis of tissue architecture, mechanics and nuclear movements during eye development show that retina tissues undergo a nuclear jamming transition as they form, with increasing nuclear packing leading to more ordered cellular arrangements, reminiscent of the crystalline cellular packings in the functional adult eye. Our results reveal an important role of the cell nucleus in tissue mechanics and architecture.



Mechanical compartmentalization of the intestinal organoid enables crypt folding and collective cell migration

Carlos Pérez-González^{1,2,3,11}, Gerardo Ceadá^{1,2,11}, Francesco Greco⁴, Marija Matejčić¹, Manuel Gómez-González¹, Natalia Castro¹, Anghara Menendez¹, Sohan Kale^{4,5}, Denis Krndija³, Andrew G. Clark³, Venkata Ram Gannavarapu³, Adrián Álvarez-Varela^{6,7}, Pere Roca-Cusachs^{1,2}, Eduard Batlle^{6,7,8}, Danijela Matic Vignjevic³✉, Marino Arroyo^{1,4,9}✉ and Xavier Trepac^{1,2,8,10}✉

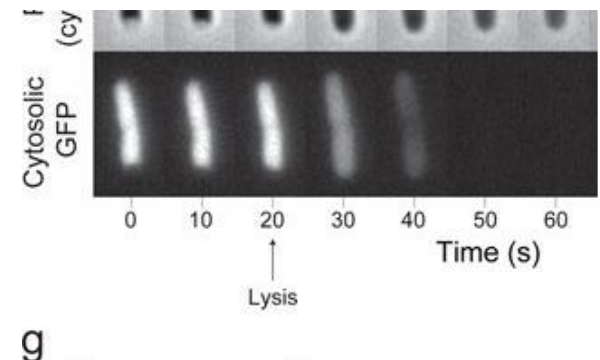
Intestinal organoids capture essential features of the intestinal epithelium such as crypt folding, cellular compartmentalization and collective movements. Each of these processes and their coordination require patterned forces that are at present unknown. Here we map three-dimensional cellular forces in mouse intestinal organoids grown on soft hydrogels. We show that these organoids exhibit a non-monotonic stress distribution that defines mechanical and functional compartments. The stem cell compartment pushes the extracellular matrix and folds through apical constriction, whereas the transit amplifying zone pulls the extracellular matrix and elongates through basal constriction. The size of the stem cell compartment depends on the extracellular-matrix stiffness and endogenous cellular forces. Computational modelling reveals that crypt shape and force distribution rely on cell surface tensions following cortical actomyosin density. Finally, cells are pulled out of the crypt along a gradient of increasing tension. Our study unveils how patterned forces enable compartmentalization, folding and collective migration in the intestinal epithelium.



The outer membrane is an essential load-bearing element in Gram-negative bacteria

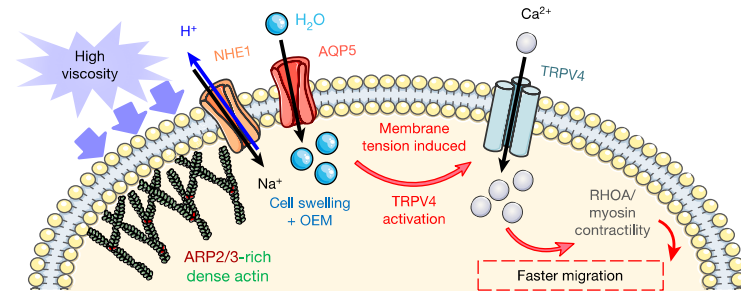
Enrique R. Rojas^{1,2,7}, Gabriel Billings³, Pascal D. Odermatt^{1,4}, George K. Auer⁵, Lillian Zhu¹, Amanda Miguel¹, Fred Chang⁴, Doug B. Weibel^{5,6,7}, Julie A. Theriot^{2,8,9,10}, and Kerwyn Casey Huang^{1,8,10,11,*}

Gram-negative bacteria possess a complex cell envelope consisting of a plasma membrane, a peptidoglycan cell wall, and an outer membrane. The envelope is a selective chemical barrier¹ that defines cell shape² and allows the cell to sustain large mechanical loads such as turgor pressure³. It is widely believed that the covalently cross-linked cell wall grants the envelope its mechanical properties^{4,5}. Here, we demonstrate that the stiffness and strength of *Escherichia coli* cells are largely due to the outer membrane. Compromising the outer membrane, chemically or genetically, greatly increased deformation of the cell envelope in response to stretching, bending, and indentation forces, and induced elevated levels of cell lysis upon mechanical perturbation and L-form proliferation. Both lipopolysaccharides and proteins contributed to outer membrane stiffness. These findings overturn the prevailing dogma that the cell wall is the dominant mechanical element within Gram-negative bacteria, instead demonstrating that the outer membrane can be more stiff than the cell wall and that mechanical loads are often balanced between these structures.



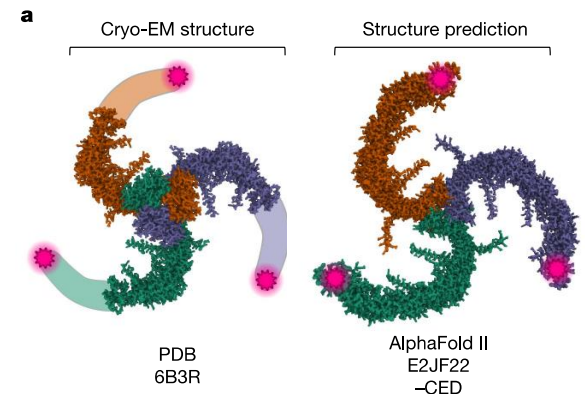
Extracellular fluid viscosity enhances cell migration and cancer dissemination

Cells respond to physical stimuli, such as stiffness¹, fluid shear stress² and hydraulic pressure^{3,4}. Extracellular fluid viscosity is a key physical cue that varies under physiological and pathological conditions, such as cancer⁵. However, its influence on cancer biology and the mechanism by which cells sense and respond to changes in viscosity are unknown. Here we demonstrate that elevated viscosity counterintuitively increases the motility of various cell types on two-dimensional surfaces and in confinement, and increases cell dissemination from three-dimensional tumour spheroids. Increased mechanical loading imposed by elevated viscosity induces an actin-related protein 2/3 (ARP2/3)-complex-dependent dense actin network, which enhances Na⁺/H⁺ exchanger 1 (NHE1) polarization through its actin-binding partner ezrin. NHE1 promotes cell swelling and increased membrane tension, which, in turn, activates transient receptor potential cation vanilloid 4 (TRPV4) and mediates calcium influx, leading to increased RHOA-dependent cell contractility. The coordinated action of actin remodelling/dynamics, NHE1-mediated swelling and RHOA-based contractility facilitates enhanced motility at elevated viscosities. Breast cancer cells pre-exposed to elevated viscosity acquire TRPV4-dependent mechanical memory through transcriptional control of the Hippo pathway, leading to increased migration in zebrafish, extravasation in chick embryos and lung colonization in mice. Cumulatively, extracellular viscosity is a physical cue that regulates both short- and long-term cellular processes with pathophysiological relevance to cancer biology.



Direct observation of the conformational states of PIEZO1

PIEZOs are mechanosensitive ion channels that convert force into chemoelectric signals^{1,2} and have essential roles in diverse physiological settings³. In vitro studies have proposed that PIEZO channels transduce mechanical force through the deformation of extensive blades of transmembrane domains emanating from a central ion-conducting pore^{4–8}. However, little is known about how these channels interact with their native environment and which molecular movements underlie activation. Here we directly observe the conformational dynamics of the blades of individual PIEZO1 molecules in a cell using nanoscopic fluorescence imaging. Compared with previous structural models of PIEZO1, we show that the blades are significantly expanded at rest by the bending stress exerted by the plasma membrane. The degree of expansion varies dramatically along the length of the blade, where decreased binding strength between subdomains can explain increased flexibility of the distal blade. Using chemical and mechanical modulators of PIEZO1, we show that blade expansion and channel activation are correlated. Our findings begin to uncover how PIEZO1 is activated in a native environment. More generally, as we reliably detect conformational shifts of single nanometres from populations of channels, we expect that this approach will serve as a framework for the structural analysis of membrane proteins through nanoscopic imaging.



How to read and present the articles?

- Motivation
- Experimental and Computational Techniques
- Main results
- Mechanistic explanations

- Criticism: clarity of writing, presentation of results, significance

How to read and present the articles?

- Make sure you go through supplementary information
- Cited work could help a lot
- Google technical keywords
- Email us if you cannot figure out a key step

Next steps

- Form groups by Monday and choose a representative
- Try to diversify with respect to sections
- Self organization on Moodle
- Do not forget to explore the review articles