AFM BioMed Conference

Barcelona

8-11 April 2025

https://afmbiomed.org/

IBEC R
Institute for Bioengineering of Catalonia

UNIVERSITAT DE BARCELONA

index

welcome 003
venue 005
program 006
invited speakers.....019
oral communications.....029
posters.....076
flash presentations.....130

Welcome

It is my great honor and pleasure to welcome you to AFM BioMed 2025 on behalf of the organizing committee. We are delighted to host this year's edition in the vibrant city of Barcelona, continuing the tradition of this esteemed conference dedicated to the application of Atomic Force Microscopy (AFM) in life sciences and nanomedicine. This event provides a unique platform to explore and address critical biological and biomedical challenges.

This year, the conference is proudly hosted by the Institute for Bioengineering of Catalonia (IBEC) and the University of Barcelona (UB), taking place in the Historic Building of the University of Barcelona—an inspiring setting for scientific exchange.
As always, we have prepared a high-quality technical program designed to engage and inspire. The conference features four key themes: Single Molecules; From Membranes to Cells; Multicellular Systems and Biointerfaces; and AFM Developments. We are honored to welcome Prof. Malgorzata Lekka as our keynote speaker, along with eight distinguished invited speakers from around the world.

In addition, the program includes contributed oral presentations, short flash presentations, and poster sessions, bringing together 160 participants from diverse scientific and geographical backgrounds.
I would like to express my heartfelt gratitude to the scientific and local organizing committees, session chairs, volunteers, sponsors, and all participants for their invaluable contributions in making this event possible.

I hope you find the conference engaging and productive.

Sincerely,
Marina I. Giannotti
Conference Chair

CHAIR

Marina I. Giannotti

ORGANIZING COMMITTEE

Marina I. Giannotti IBEC, CIBER-BBN, UB
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Lorena Redondo-Morata, Aix-Marseille Univ, INSERM, DyNaMo
Claire Valotteau (Aix-Marseille Univ., INSERM, DyNaMo)

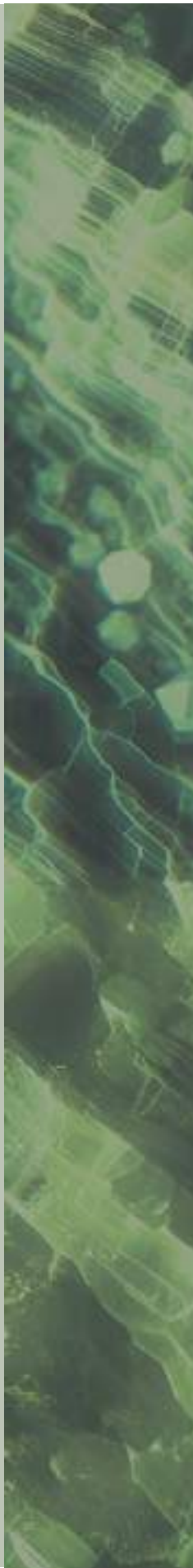
venue

University of Barcelona, Historical Building
Gran Via de les Corts Catalanes, 585
08007 Barcelona

program

8th april
AFM Biomed Conference 2025

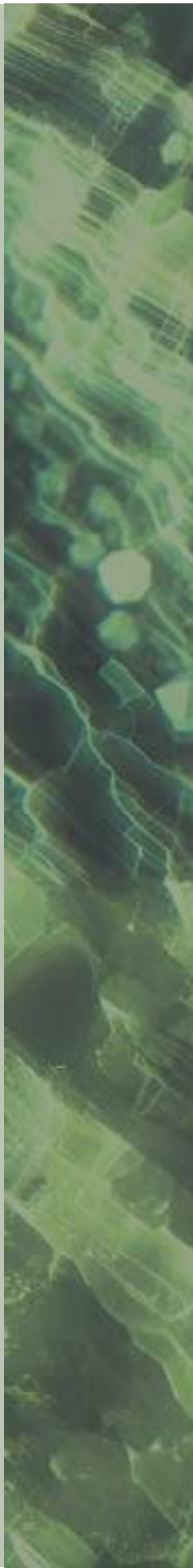
8.30h	Registration for the Bruker Workshop at AFM BioMed Conference 2025
9.00h	**Bruker Workshop at AFM BioMed Conference 2025**
13.30h	Registration for the AFM BioMed Conference 2025
14.20h	**Start of the conference · Welcome** Jordi Garcia · UB Josep Samitier · IBEC Daniel Navajas Jean Luc Pellequier
15.00h	**Exploring cell mechanics: implications for disease and therapy** · Malgorzata Lekka *Chair: Lorena Redondo-Morata*
16.00h	Coffee break + poster session I
16.30h	**Validation of contact mechanics models and data analysis approaches for nanoindentation measurements of soft samples** · Alessandro Podestà
16.45h	**In-Depth Biomechanical Investigation of Liver Sinusoidal Endothelial Cells Cultured on Substrates with Precisely Altered Stiffness Properties** · Dibakar Borah
17.00h	**Biomechanics of Macrophages During Interaction with Extracellular Stimuli · Massimiliano Galluzzi**
17.15h	**Ultrathin water layers on viruses and on virus models** · Alexander Bittner



8th April

AFM Biomed Conference 2025

17.30h	**AFM-based methods to investigate the correlation between macrophage viscoelastic behavior and phenotype in response to substrate stiffness** • Livia Angeloni
17.45h	**High speed AFM uncovers diffusion of LacY in membranes** • Oscar Domenech
18.00h	Welcome Reception at UB Gardens



9th April

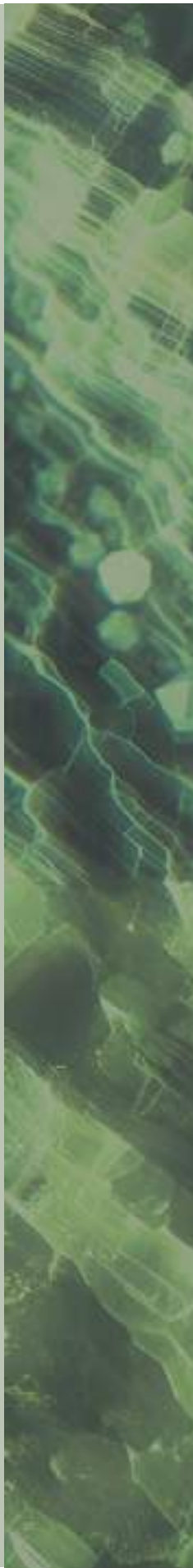
AFM Biomed Conference 2025

FROM MEMBRANES TO CELLS
Chair: Filomena Carvalho

9.00h	**Salbutamol attenuates the arrhythmogenic effect of aminophylline in cardiac organoids experimental model** · Martin Pešl
9.30h	**Highthroughput Biomechanical Characterization of Macrophage Polarization through Atomic Force Microscopy** · Beatriz Cantero Nieto
9.45h	**Changes in erythrocyte elasticity and morphology as potential predictors of survival in als** · Catarina Sousa Lopes
10.00h	**Characterization of the viscoelasticity of Red Blood Cells and rheological analogues with AFM** · Agathe Nidriche
10.15h	**A different metabolic regulation in favism erythrocytes induces surprising alteration of the cells? properties along the aging** · Simone Dinarelli
10.20h	**Interfacial Dynamics of Quantum Dot Adsorption on Cholera Vulgaris Cells Wall and Its Impact on Nanomechanical Architecture** • Varun Vyas
10.30h	Coffee break + posters I

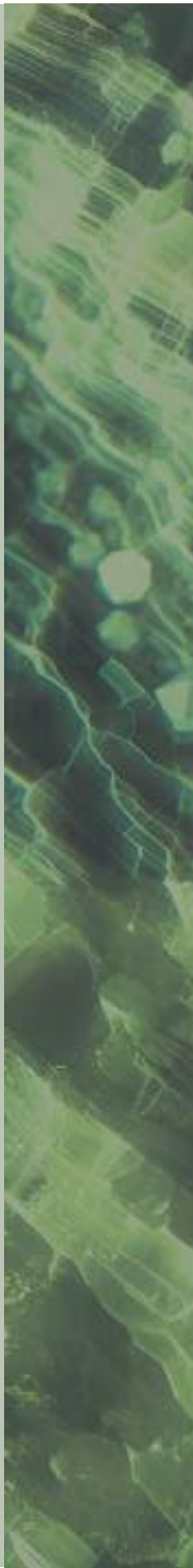
Multicellular systems and biointerfaces
Chair: Delphine Sicard

11.15h	**IRSp53 controls supracellular mechanical properties in epithelial tissues** • Alexander Cartagena



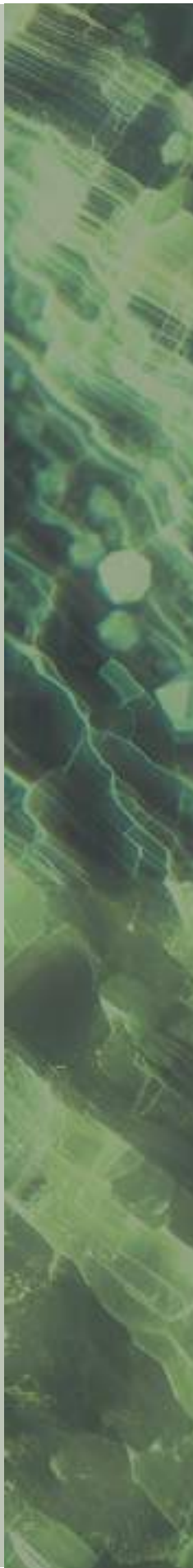
9th April
AFM Biomed Conference 2025

11.45h	**Characterization of Liquid/PAA and PEO Polymer brushes/Gold interfaces by 1D and 3D-AFM** • Dahlia SAAD
12.00h	**Scanning probe microscopy and raman spectroscopy investigation of cardiac organoids** • Federica Rigoni
12.15h	**Multiscale nonlinear mechanics of collagen i hydrogels under stretch** • Ignasi Jorba
12.30h	**Discovering multifactorial causality using AFM nanoindentation** • Jean-Luc Pellequer
12.35h	**Correlation between nanomechanical and chemical features of crosslinked hydrogels combining AFM with chemometric methods** • Grégory Francius
12.40h	**Nanomechanical biomarkers for non-communicable diseases** • Andreas Stylianou
13.00h	Lunch + poster session 1
	Single Molecules Chair: Fernando Moreno-Herrero
14.30h	**Sequence-dependent flexibility as a probe for biomolecular structure** • Nancy Forde
15.00h	**AFM studies of the molecular mechanism of genome organization** • Je-Kyung Ryu
15.15h	**AFM study to reveal the role of sulfation patterns of heparan sulfate in modulating the interactions between human papillomavirus and glycosaminoglycans** • Fouzia Bano



9th April
AFM Biomed Conference 2025

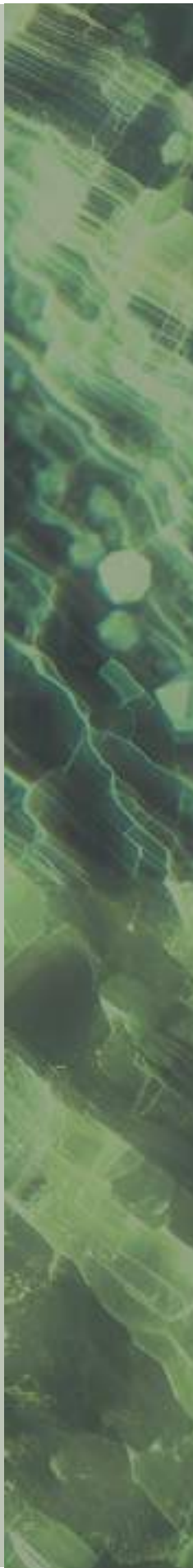
15.30h	**Interplay of glycans and adsorption-driven deformation in SARS-CoV-2 RBD variants onto surfaces with different hydrophobicity** • Antonio Miguel Bosch Fernandez
15.45h	**Study of plastocyanin conformational changes with pH by single protein unfolding** • Montserrat Sales-Mateo
15.50h	**Binding mechanism of oligopeptides on solid surface: Assessing the significance of single-molecule approach** • Joanne Lê-Chesnais
15.55h	**Atomic Force Microscopy reveals key insights into fibrillar species associated with Alzheimer's Disease** • Panangis Polykretis
16.00h	Coffee break + posters (1)
	AFM developments Chair: George Heath
16.30h	**Cellular stepwise confinement reveals different mechanical response in MEC-WT and MEC-KO1 under diverse pharmacological conditions** • Riccardo Campanile
16.45h	**Mechanical Properties at the Nanoscale of Cardiac Organoids Investigated by Scanning Probe Microscopy** • Tommaso Savoldi
17.00h	**Nanorheology and Nanoindentation Revealed a Softening and an Increased Viscous Fluidity of Adherent Mammalian Cells upon Increasing the Frequency** • Francisco Miguel Espinosa Barea



9th April

AFM Biomed Conference 2025

17.15h	**Overcoming challenges in the AFM mechanical nanocharacterization of soft heterogeneous biological materials** • Stylianos Vasileios Kontomaris
17.30h	**Microscale Biomechanical Characterization of Undecalcified Bone through AFM** • Nada Wahman
19.00h	**External event · El Drapaire**



10th April

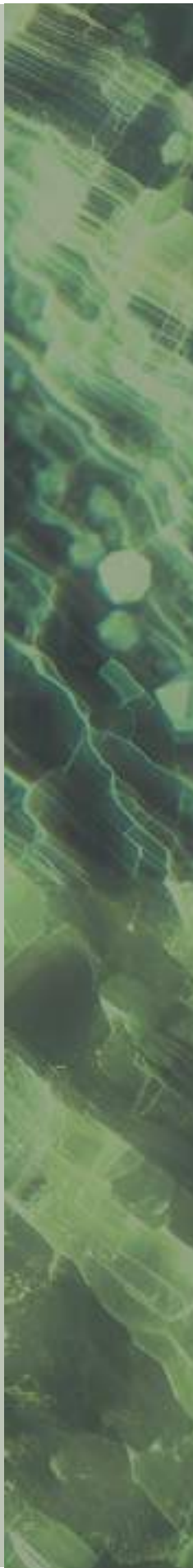
AFM Biomed Conference 2025

FROM MEMBRANES TO CELLS
Chair: Filomena Carvalho

9.00h	**Unlocking the Role of Fibrinogen-Erythrocyte Binding in Cardiovascular Diseases** • Filomena Carvalho (chaired by Martin Pešl)
9.30h	**Nuclear Stiffening and Structural Alterations Induced by Glycation in Diabetes** • Andra Dumitru
9.45h	**Nuclear Pore Complex plasticity on intact cell nuclei as seen by AFM** • Kassandra Gérard
10.00h	**Probing cell nanomechanics with the AFM** • Guillaume Lamour
10.15h	**Exploring the nanomechanics of Gaucher and Fabry disease models** • Marina Placci
10.20h	**Fibroblast extracellular-matrix mechanical properties in patients with Mucopolysaccharidosis by Atomic Force Microscopy** • Juan Manuel Casares-López
10.30h	Coffee break + posters (2)

AFM DEVELOPMENTS
Chair: George Heath

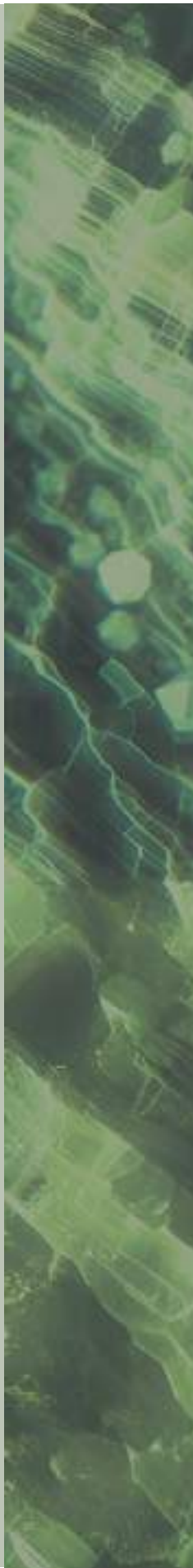
11.15h	**High-Speed Scanning Ion Conductance Microscopy** • Georg Fantner
11.45h	**Enhancing Large-Scale BioAFM Mechanical Characterization with SmartMapping** • Andre Körnig
12.00h	**Structural analysis of individual helical filaments by three-dimensional contact point reconstruction atomic force microscopy** • Wei-Feng Xue



10th April

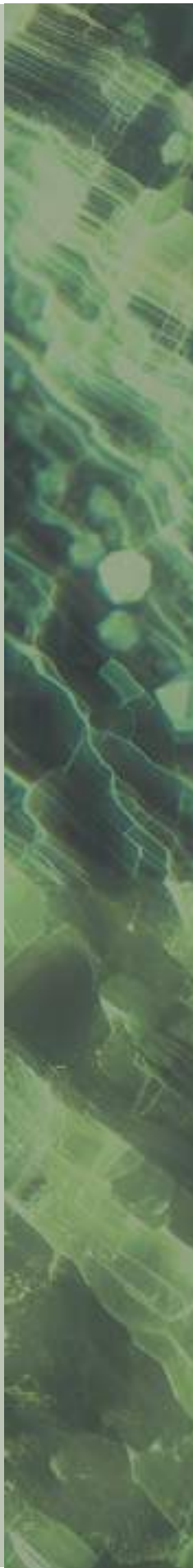
AFM Biomed Conference 2025

12.15h	**Use of AFM nanoindentation for extracellular vesicle detection and biogenesis analysis: a novel MATLAB-based approach** • Federica Collacchi
12.30h	**Control Algorithms for Long-Range HS-AFM to Study Leukocyte Tether Formation** • Lorenzo Villanueva
12.35h	**MEMS based AFM: Towards understanding unfolding dynamics of bacteriorhodopsin in sub-ms time resolution** · Suyambulingam Subramanian
13.00h	Lunch + poster session II
	SINGLE MOLECULES Chair: Fernando Moreno-Herrero
14.30h	**Exploring viral and long non-coding RNAs secondary structure using Atomic Force Microscopy** · Fernando Moreno-Herrero (chaired by N. Forde)
15.00h	**Observing Dynamic Conformational Changes within the Coiled-Coil Domain of Different Laminin Isoforms Using High-Speed Atomic Force Microscopy** • Franz Clemens
15.15h	**Surface active antibiotics in action: a real-time study of the killing mechanism using high-speed atomic force microscopy** • Sourav Maity
15.30h	**Visualization and analysis of P-glycoprotein dynamics by using HS-AFM** • Kanaoka Yui
15.45h	**Dynamic Interaction of Trigger Factor with the Ribosome Exit Tunnel During Cotranslational Folding** · Eider Nuñez



10th April
AFM Biomed Conference 2025

15.50h	**Photothermal infrared nanospectroscopy investigation of alteration in the secondary structure of Aβ1-42 aggregates induced by green tea active compounds** • Michal Czaja
15.55h	**Lipid membrane remodeling and repair** • Adai Colom
16.00h	Coffee break + posters II
	FROM MEMBRANES TO CELLS Chair: Filomena Carvalho
16.30h	**High-speed AFM reveals enzyme-induced membrane remodelling** • Abeer Alshammari
16.45h	**MurG ligase induces fluid regions in bacterial membranes** • Ignacio Casuso
17.00h	**To adhere or not to adhere: an approach to understand the initial biofilm formation** • Hannah Heintz
17.15h	**From Single Molecules to Tissues: Correlative BioAFM Serving as Core Facility** • Jan Pribyl
17.30h	**Unraveling lipid membrane dynamics and phase behavior using AFM** • Lorena Redondo-Morata
17.45h	**AFM on reconstituted bacterial outer membranes** • Corrin Blake
20.00h	**Conference Dinner · at Can Travi Nou**



11th April

AFM Biomed Conference 2025

AFM DEVELPOMENTS
Chair: George Heath

9.00h **AFM Image Filter Fight Club: Noise Vs Information** · George Heath (chaired by Georg Fantner)

9.30h **IR and microwave-Based Techniques for Analysing Molecular Signatures: A Novel Approach to Biomarker Detection** · Eric Lesniewska

9.45h **Enhancing atomic force microscopy with optical fibre interferometry** · Mário S Rodrigues

10.00h **Implementation of multiparametric corelative atomic force microscopy and optical nanoscopy to study polypharmacy in hepatic endothelium** · Bartlomiej Zapotoczny

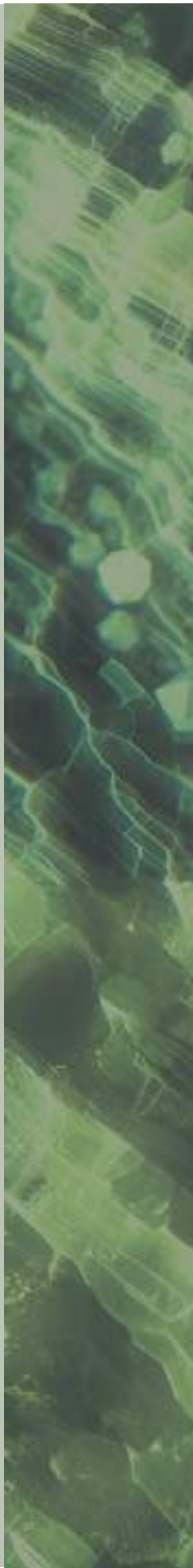
10.15h **Nanoscale dielectric imaging of cells and bacteria by scanning dielectric microscopy assisted by deep convolutional neural networks** · Mauricio Cano Galván

10.20h **Aqueous liquid/solid interface at the nanoscale: a comparison of the 3d-AFM mode to other experimental techniques and simulations** · Magali Phaner-Goutorbe

10.30h Coffee break + posters II

MULTICELLULAR SYSTEMS AND BIOINTERFACES
Chair: Delphine Sicard

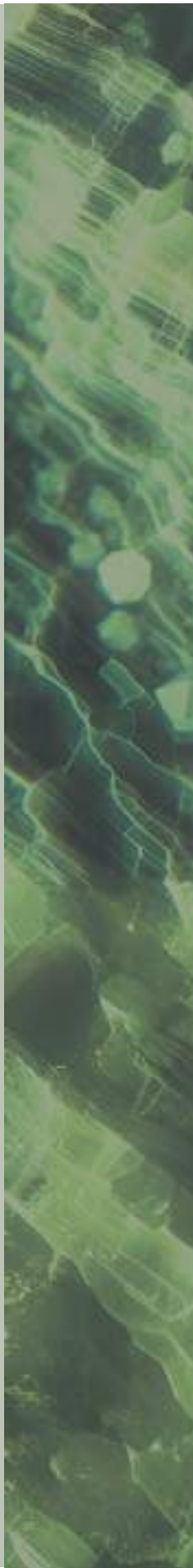
11.15h **Mechanobiology of Lung Tissue in Physiological and Pathological Conditions** · Delphine Sicard (Chaired by Alexander Cartagena)



11th April

AFM Biomed Conference 2025

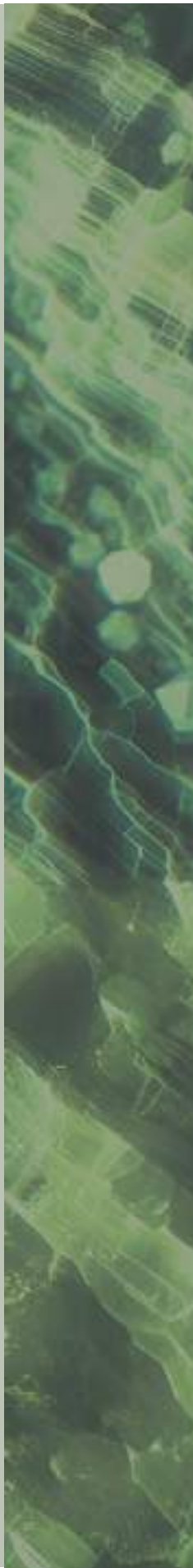
11.45h	**Exploring the biophysics of P. kessleri and S. cerevisiae cells in coculture using atomic force microscopy for the production of biofuels** • Simona Sebastiano
12.00h	**Understanding cancer aggregate migration; utilising Bio-AFM, biomechanics and molecular biology at the peritoneal boundary** • Jordan Turney
12.15h	**Mechanical properties of circulating tumour cells (CTC) and its clusters** • Yogesh Saravanan
12.30h	**Collagen fibril degradation in thoracic aortic aneurysms** • Bojin Marinov
12.35h	**Co-cultured aggregates in cylindrical cavities: fibroblasts influence the microenvironment of pancreatic cancer cells** • Isis do Vale Meira Lima
13.00h	Lunch
	FROM MEMBRANES TO CELLS Chair: Filomena Carvalho
14.30h	**Multifunctional Phenotyping of Individual Cancer Cells Using SICM Probes for Nanoscale Assays and Single-Cell Omics** • Petr Gorelkin
14.45h	**Key roles of N-terminal capping and fibrillation in the toxicity of phenol soluble modulins secreted by S. aureus** · Laura Bonnecaze
15.00h	**Transient effects on mechanical properties during cell division of Escherichia coli studied by high-speed in-line force mapping** · Christian Ganse



11th April

AFM Biomed Conference 2025

15.15h	**Quantitative imaging of bacterial cell wall with AFM: function of PBP1a synthase** · Laia Pasquina Lemonche
15.30h	**Sialic acid depletion leads to profound remodelling of glycocalyx architecture and mechanics in pancreatic cancer cells** · Andrew Massey
15.45h	**Effects of microbial glycolipids on phospholipid membranes using Atomic Force Microscopy (AFM)** · Yulia Fok
15.50h	**Bacterial Adhesion Strength is Linked to Cell Wall Age · Johannes Mischo**
16.00h	Coffee break
16.30h	**Square table** Chair: Felix Rico



invited speakers

Invited Speaker

GEORG FANTNER

EPFL, École polytechnique de Lausanne, Switzerland

High-Speed Scanning Ion Conductance Microscopy

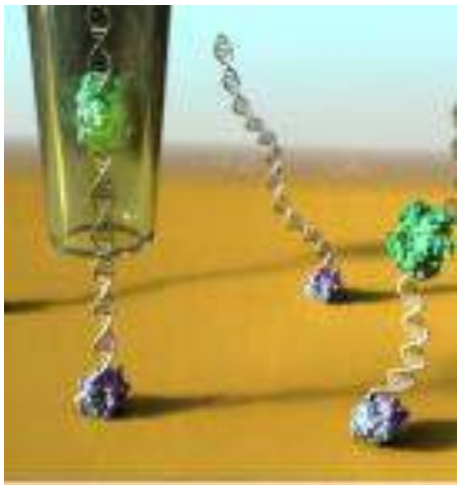
High-Speed Scanning Ion Conductance Microscopy

Georg E. Fantner, Barney Drake, Samuel M. Leitao, Zhara Dulabi, Jialin Shi, Aleksandra Radenovic, Vytautas Navikas

[1]École Polytechnique Fédéral de Lausanne, Lausanne, Switzerland

Georg.fantner@epfl.ch

Scanning ion conductance microscopy (SICM) has been around for decades [1], yet it has not received as much attention as other forms of scanning probe microscopy. Recently, this true non-contact technique has kindled renewed interest among biophysicists and biologists because it is ideally suited for label-free imaging of fragile cell surfaces where it achieves exquisite resolution down to the nanometer regime without distorting the cell membrane. SICM uses a glass nanopipette as a scanning probe and measures the current through the glass nanopore as a proximity detection of the sample surface [2]. The challenge to harness this technique for time resolved 3D nanocharacterization of living cells lies in the relatively slow imaging speed of SICM. In this presentation I will show how we apply what we have learned from high-speed AFM to the field of SICM. By reengineering the SICM microscope from the ground up, we were able to reduce the image acquisition time for SICM images from tens of minutes down to 0.5s while extending the imaging duration to days [3].



SICM, however, is much more versatile than just an imaging tool. I will also discuss our recent results using SICM as a single molecule characterization tool. We term this method scanning ion conductance spectroscopy (SICS) [4]. Using capillaries with exceptionally small nanopores, we can detect and manipulate single molecules in a repeatable and high throughout manner. Compared to other nanopore sensing techniques SICS has inherent temporal and spatial control of the DNA translocation through the nanopore. This greatly increases the SNR and enables detection of even single base gaps in a dsDNA strand. The ability to read the same molecule multiple times makes this technique well suited for biophysics and diagnostic applications.

References

[1] P. Hansma, B. Drake, O. Marti, S. Gould, and C. Prater, The Scanning Ion-Conductance Microscope, Science 243, 641 (1989).

[2] V. Navikas et al., Correlative 3D Microscopy of Single Cells Using Super-Resolution and Scanning Ion-Conductance Microscopy, Nat. Commun. 12, 1 (2021).

[3] S. M. Leitao et al., Time-Resolved Scanning Ion Conductance Microscopy for Three-Dimensional Tracking of Nanoscale Cell Surface Dynamics, ACS Nano 15, 17613 (2021).

[4] S. M. Leitao et al., Spatially Multiplexed Single-Molecule Translocations through a Nanopore at Controlled Speeds, Nat. Nanotechnol. 18, 1078 (2023).

Invited Speaker

MARTIN PEŠL

Masaryk University · Czech Republic

Salbutamol attenuates the arrhythmogenic effect of aminophylline in cardiac organoids experimental model

SALBUTAMOL ATTENUATES THE ARRHYTHMOGENIC EFFECT OF AMINOPHYLLINE IN CARDIAC ORGANOIDS EXPERIMENTAL MODEL

Pešl M.[1,2,3], Kabanov D.[4], Vrana Klimovič Š.[4], Beckerová D.[1], Ščurek M.[5], Brat K.[5], Bebarová M.[6], Rotrekl V.[6], Přibyl J.[7]

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[2] International Clinical Research Center, St. Anne's University, Hospital, Brno, Czech Republic
[3]First Department of Internal Medicine – Cardioangiology, Faculty of Medicine, Masaryk University, St. Anne's University Hospital, Brno, Czech Republic
[4]CEITEC MU, Masaryk University, Brno, Czech Republic.
[5]Department of Respiratory Diseases, University Hospital Brno, Faculty of Medicine, Masaryk University, Brno, Cz
. [6]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic.
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The aminophylline-salbutamol combination is widely implemented in clinical settings for the management of obstructive pulmonary disease. While the adverse effects, including arrhythmogenicity, of each bronchodilator are well-documented, the arrhythmogenic potential of their combined use remains largely uncharacterized. This study [1] aimed to delineate the proarrhythmic risk associated with aminophylline and salbutamol co-administration in vitro. Atomic force microscopy (AFM) coupled with cardiac organoids derived from human pluripotent stem cells (hPSC-CMs) was employed as previously described [2,3]. We assessed the chronotropic, inotropic, and arrhythmogenic responses of hPSC-CMs as reviewed [4]. Screened were responses to salbutamol monotherapy and aminophylline-salbutamol co-treatment. Heart rate variability (HRV) and beat rate variability (BRV) analyses enabled the detection of arrhythmic events in AFM-based hPSC-CM recordings. Results demonstrated a synergistic chronotropic and inotropic effect with combination therapy versus monotherapy. Crucially, salbutamol attenuated aminophylline's arrhythmogenic impact, likely through endothelial nitric oxide synthase activation mediated by beta-2 adrenergic receptor engagement. Findings were corroborated across two hPSC-CM lines (CCTL4 and CCTL12), indicating that salbutamol may confer cardiovascular protection when co-administered with aminophylline by mitigating its arrhythmogenic potential, in addition to providing bronchodilation.

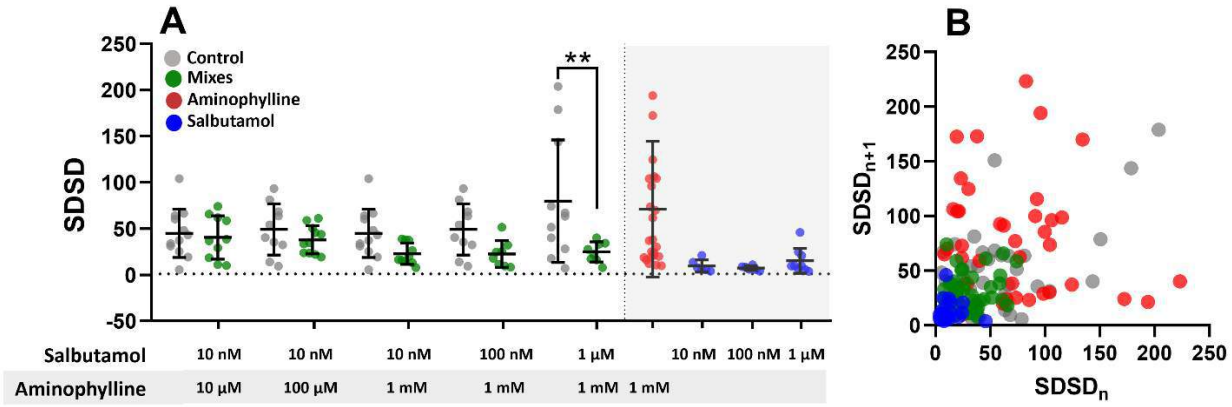

Figure 1. BRV analysis with combined salbutamol and aminophylline treatments. Scatter plots indicate means and SDs of SDSDs measured with mixed treatments and control (n=10 controls, n=10,11,10,8,8,24,6,6, and 9 for treatments; ordinary one-way ANOVA test) (A) SDSD results of cardiac organoids concentrations of salbutamol and aminophylline indicated in separate measurements of both treatments for comparison. (B) Poincaré plots of SDSD intervals.

References:

[1] Kabanov D, Vrana Klimovič Š, Beckerová D, Ščurek M, Brat K, Bebarová M, Rotrekl V, Přibyl J, Pešl M, Scientific Reports (2024) doi: 10.1038/s41598-024-76846-4
[2] Caluori G, Pribyl J, Pesl M, Oliver-De La Cruz J, Nardone G, Skladal P and Forte G Physiol. 2018 9:1121. doi: 10.3389/fphys.2018.01121
[3] Pribyl J, Pešl M, Caluori G, Acimovic I, Jelinkova S, Dvorak P, Skladal P Atomic Force Microscopy: Methods and Protocols, (2019) p. 343-353
[4] Kabanov D, Klimovic S, Rotrekl V, Pesl M, and Pribyl J. Micron, 155 (2022) 10.1016/j.micron.2021.103199

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Invited Speaker

NANCY FORDE

Professor of Physics at Simon Fraser University

Sequence-dependent flexibility as a probe for biomolecular structure

Sequence-dependent flexibility as a probe for biomolecular structure

Nancy R. Forde[1] and Alaa Al-Shaer[1]
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Collagen has been evolutionarily selected as the preferred building block of extracellular structures. Despite the inherent and surprising thermal instability of individual proteins at body temperature, collagen manages to assemble into higher-order structures that provide mechanical support to tissues. Sequence features that enhance collagen stability have been deduced largely from studies of collagen-mimetic peptides, as the large sizes of collagens have precluded high-resolution studies of their structure. Thus, there is a need for new methods – such as AFM imaging – to analyze the structure and mechanics of native collagen proteins.

In this presentation, I will describe how we use AFM imaging to investigate the response of collagen to changes in temperature. We have developed image analysis tools based on our chain-tracing software SmarTrace [1,2] that map the local bending stiffness of collagen along the backbone and correlate it with its local sequence [3]. Using these tools, we have characterized the sequence-dependent bending stiffness profile of collagen IV as a function of temperature and identified a putative initiation site for thermally induced unfolding [4]. In contrast to the canonical C-to-N terminal folding direction, we found an interchain cystine knot to enable folding in the opposite direction. While the work we have done applies sequence-dependent analysis to collagen chains imaged using AFM, the tools should find broad applicability to others imaging chain-like structures and wishing to quantify their mechanical properties.

References

[1] N. Rezaei, A. Lyons and N.R. Forde, Biophysical Journal, 115 (2018), 1457.
[2] M. Schneider, A. Al-Shaer and N.R. Forde, Biophysical Journal, 120 (2021), 2599.
[3] A. Al-Shaer, A. Lyons, Y. Ishikawa, B.G. Hudson, S.P. Boudko and N.R. Forde, Biophysical Journal, 120 (2021), 4013.
[4] A. Al-Shaer and N.R. Forde, bioRxiv 2024.10.02.616390.

Invited Speaker

ALEXANDER CARTAGENA-RIVERA

PhD Mechanical Engineering Stadtman Investigator at National Institutes of Health

IRSp53 controls supracellular mechanical properties in epithelial tissues

IRSp53 controls supracellular mechanical properties in epithelial tissues

Alexander Cartagena-Rivera[1*], Laila Ghorab[1], Andrew Massey[1], Amanda Bluem[1], Mazen Mezher[1], Stefano Marchesi[2], Andrea Disanza[2], Giorgio Scita[2]

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Epithelial morphogenesis during development and disease progression, including tissue shaping and patterning, occurs under a wide range of physicochemical conditions. However, the key molecular determinants and physical conditions that control multicellular tissue mechanics in epithelial morphogenesis remain elusive. Here we focused on IRSp53, a curvature sensing I-BAR containing protein known to link the plasma membrane to the actomyosin cytoskeleton. We depleted IRSp53 in two different epithelial models (MDCK cells as a model of kidney disease [1] and MCF10 DCIS.com cells as a model of breast cancer tumor progression [2]) to determine changes in mechanical properties using Atomic Force Microscopy (AFM). Using AFM nanomechanical mapping, we determined the changes in mechanical properties between wild type (WT) and IRSp53 KO monolayers observing a statistically significant decrease when IRSp53 is depleted in both epithelial models. This correlates well with observations that in dense monolayers, IRSp53 depleted cells show a more migratory and fluid state compared to WT controls. Next, we generated MCF10 DCIS cells 3D spheroids to investigate the mechanical behavior when IRSp53 is depleted. Using a tipless AFM approach [3], we observed a significant reduction in spheroidal surface tension when IRSp53 is absent. These observed effects are the result of reduced supracellular tension and disrupted organization of cell-cell junctions, which lead to decreased intercellular friction and enhanced local and dynamic cell rearrangements. Altogether, IRSp53 is a key regulator of supracellular mechanical properties in epithelial tissues providing a molecular basis for dysregulated epithelial morphogenesis observed in diseases including abnormal renal tubulogenesis and breast cancer ductal carcinoma progression.

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Chairs

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Unlocking the Role of Fibrinogen-Erythrocyte Binding in Cardiovascular Diseases

UNLOCKING THE ROLE OF FIBRINOGEN-ERYTHROCYTE BINDING IN CARDIOVASCULAR DISEASES

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Cardiovascular diseases (CVDs) remain the leading cause of death globally, often aggravated by atherosclerosis and its associated inflammatory processes. Erythrocyte hyperaggregation, driven by elevated fibrinogen levels, has been identified as a significant risk factor for cardiovascular events. The primary aim of this study was to explore the impact of fibrinogen-erythrocyte and erythrocyte-erythrocyte interactions in patients with carotid artery disease (CAD), essential arterial hypertension (EAH), chronic heart failure (CHF), or stroke. Using atomic force microscopy (AFM)-based force spectroscopy, we assessed the biomechanical properties of erythrocytes and atherosclerotic plaques, as well as the strength of fibrinogen-erythrocyte and erythrocyte-erythrocyte adhesion. Blood samples from CAD patients, taken before and after endarterectomy surgery, were compared to healthy controls. Results demonstrated that pre-surgical CAD patients exhibited stiffer, less deformable erythrocytes and stronger erythrocyte-erythrocyte adhesion, compared to controls. Increased plasma levels of total and γ' fibrinogen were also observed, correlating with increased cardiovascular risk. Six months post-surgery, erythrocyte deformability and cell-cell adhesion improved, and twelve months later, a reduction in erythrocyte aggregation and γ' fibrinogen levels indicated an improvement in the inflammatory process. Further analysis in patients with EAH, CHF, and stroke revealed that higher fibrinogen concentrations led to higher erythrocyte-erythrocyte detachment work, with the most significant effects observed in EAH and stroke patients [1-4]. Fibrinogen-erythrocyte binding forces were higher in EAH and CHF patients, despite a lower binding frequency [4,5]. Importantly, patients with elevated fibrinogen-erythrocyte binding forces at baseline had a significantly higher probability of hospitalization due to cardiovascular complications [5]. These findings highlight fibrinogen-erythrocyte interactions as potential biomarkers for early detection and prognosis of thrombotic events. Additionally, we incorporated a mathematical model developed to quantify erythrocyte-erythrocyte adhesion forces and changes in erythrocyte morphology [6]. AFM data showed that the work and detachment force required to overcome adhesion between erythrocytes increased in the presence of fibrinogen. The model successfully captured changes in erythrocyte morphology, the strong cell-cell adhesion, and slow separation between cells [6]. These findings provide important insights into the pathophysiological relevance of fibrinogen-induced erythrocyte aggregation, which can hinder microcirculatory blood flow. In conclusion, this study underlines the role of fibrinogen in promoting erythrocyte aggregation, a critical factor influencing blood flow and contributing to cardiovascular risk. These molecular interactions offer potential targets for future therapeutic strategies aimed at reducing erythrocyte aggregation and improving microcirculatory conditions in CVD patients.

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Chairs

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AFM Image Filter Fight Club: Noise Vs Information

AFM Image Filter Fight Club: Noise Vs Information

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Interpreting structures in AFM images is often challenging due to low signal-to-noise and tip convolution. Here, we present quantitative assessments of AFM image processing steps focusing on feature localization among other fundamental steps. We evaluate detection sensitivity, positional accuracy, precision, and overall resolution in response to denoising, deconvolution, and sub-pixel localization methods. We also examine the impact of pixel sampling and particle numbers on performance using the principles of localization AFM for quantification.[1] Our approach is applied to both simulated and experimental AFM images, with the goal of automating optimizations to reduce reliance on the user while significantly enhancing resolution, data throughput and accuracy in bio-AFM imaging. These workflows can be integrated into existing scripts, but are also implemented into a user-friendly interface, NanoLocz, an AFM and high-speed AFM analysis platform designed for seamless raw data import, automatic preprocessing, and efficient image analysis workflows.[2]

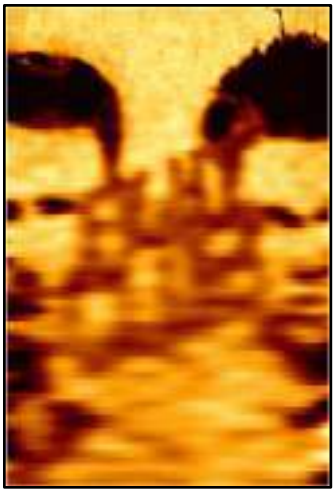

Figure 1. Fight Club image with progressively increasing noise and image filtering.

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Chair

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Exploring viral and long non-coding RNAs secondary structure using Atomic Force Microscopy

EXPLORING VIRAL AND LONG NON-CODING RNAS SECONDARY STRUCTURE USING ATOMIC FORCE MICROSCOPY

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RNAs fold into secondary and tertiary structures, which enable them to interact with proteins and perform complex cellular functions. However, most RNAs remain structurally uncharacterized, and the relationship between nucleotide sequence and final folded conformation is not yet fully understood. In this study, we employ Atomic Force Microscopy (AFM) to capture images of individual RNA molecules in both air and liquid environments to analyze their conformations. We first analyze the 5´-proximal regions in RNA genomes of Betacoronaviruses from four subgenera using a custom-built image analysis software based on volumetric measurements. We find a common domain organization and a dynamic arrangement of structural elements connected with flexible linkers across the four viral RNAs [1]. Next, we investigate the long non-coding RNA CONCR, which is involved in DNA replication and sister chromatid cohesion [2]. This much longer RNA presents a branched, flexible structure and wide conformational variability with a predominant morphology consisting of two distinct regions: a compact U-shaped domain and a larger, stem-like structure. To identify RNA ends, we implement a labelling method using poly-A tails of different lengths. This approach allows us to assign the U-shaped region to the 5´ ends and a branched, elongated domain to the 3' end, an architecture consistent with predictions from chemical probing experiments (Figure 1). Further analysis suggests that two interacting regions may undergo structural rearrangement, leading to an extended and super-extended conformation. Our findings provide insight into the selection of domains to study their structure further with cryo-EM and their potential interactions with helicase DDX11.

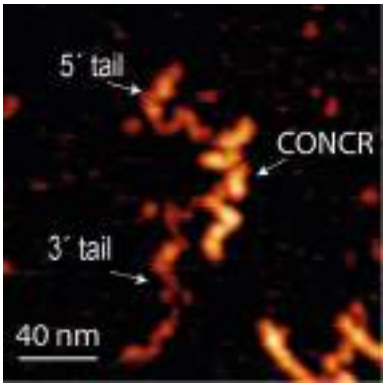

Figure 1. AFM image of lncRNA CONCR with its ends labelled with polyA tails of different length

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Chairs

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Multicellular systems and biointerfaces

Mechanobiology of Lung Tissue in Physiological and Pathological Conditions

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Mechanobiology is an emerging multidisciplinary field focuses on understanding how physical forces and mechanical properties related to extracellular matrix (ECM), cells and tissue contribute to signaling, development, physiology, and disease [1]. Recent progresses have been made to study mechanotransduction [2], but little is still known about mechanical changes in tissues, especially at microscale. To investigate mechanical properties, we have used atomic force microscopy (AFM) micro-indentation to characterize quantitively tissue stiffness.

In this talk, I will present our methodological study to minimize and balance the influence of sample preparation and AFM parameters in order to perform standardized AFM measurements on lung tissue. I will also discuss lung stiffness and mechanical changes in physiological and pathological conditions such as pulmonary arterial hypertension to establish correlations between tissue mechanical properties, lung cell behavior and respiratory function.

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Keynote speaker

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Exploring cell mechanics: implications for disease and therapy

EXPLORING CELL MECHANICS: IMPLICATIONS FOR DISEASE AND THERAPY

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Cell mechanics, i.e., the study of cell mechanical properties and behaviors, is pivotal in understanding fundamental biological processes and the progression of various diseases [1]. Dysregulation of these mechanical properties is often associated with pathological conditions, including cancer metastasis, cardiovascular diseases, and fibrosis, highlighting the importance of studying cell mechanics [2]. Cancer cells frequently display increased deformability compared to their healthy counterparts [3], facilitating their movement and invasion through tissues [4]. This reduced stiffness can be partly attributed to alterations in the actin cytoskeleton [5]. However, some research shows that the mechanical properties of cells change during processes like epithelial-to-mesenchymal transition (EMT) in cancer progression or stem cell differentiation. These findings suggest that mechanical cues are not merely passive cellular architecture consequences but active cell behavior and fate regulators. Conversely, the tumor microenvironment, particularly the ECM, tends to be stiffer than normal tissues, driving tumor progression [6]. Intertwined with biochemical signaling, these mechanical alterations contribute to the intricate interplay underlying disease development. Sophisticated microscopy techniques are indispensable for probing cell mechanics. Atomic Force Microscopy (AFM) stands out as a versatile tool in this field, enabling researchers to measure mechanical properties of cells, such as stiffness (Young's modulus) and viscoelastic behavior [7]. AFM quantifies cellular responses, offering insights into the underlying cytoskeletal structure and its dynamic changes under different conditions. Research using AFM and other techniques [8] has unveiled that altered cell mechanics are a hallmark of many diseases, notably cancer. Beyond measuring cell elasticity, AFM can uncover cell dynamic, time-dependent mechanical behavior, known as cell rheology [9]. This helps researchers to understand how cells respond to forces over time, providing valuable information about the viscoelastic nature of cells. Future perspectives in cell mechanics involve leveraging these advanced tools to unravel the interplay between mechanical properties and molecular signaling pathways. With the advent of machine learning and computational modeling, large datasets generated by techniques like AFM can be analyzed to predict cellular behaviors and disease outcomes. Techniques like AFM have significantly advanced our understanding, uncovering potential mechanisms and therapeutic targets [10]. Continued innovation in measurement technologies and data analysis promises to drive the field forward, offering new avenues for basic research and clinical applications.

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oral communications

Validation of contact mechanics models and data analysis approaches for nanoindentation measurements of soft samples

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In this work, we have validated, by means of finite elements simulations and experiments, the application of Hertzian contact mechanics models and corrections in the framework of linear elasticity, for the analysis of force vs indentation curves acquired by atomic force microscopy using spherical indenters [1].
We have systematically investigated the impact of both large indentations and vertical spatial confinement (bottom effect) on the accuracy of the nanomechanical analysis performed with the Hertz model for the parabolic indenter compared to the Sneddon model for the spherical indenter [2]. We carried out a comprehensive characterization of the corrections for the Hertz model proposed in the literature [2] in the framework of linearized force vs indentation curves, as well as a validation of a linearized form of the Sneddon model for the spherical indenter and of the combined correction for large indentations and bottom effect.
We have also developed a simple protocol to fabricate polyacrylamide samples with a controlled gradient of thickness; together with finite elements analysis methods, these samples represent a valuable tool for the validation of contact mechanics models and data analysis approaches.
Our results show that a suitably linearized and corrected Hertz model allows to accurately quantify the Young's modulus of elasticity of linearly elastic samples with variable thickness at arbitrarily large indentations using spherical tips.

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In-Depth Biomechanical Investigation of Liver Sinusoidal Endothelial Cells Cultured on Substrates with Precisely Altered Stiffness Properties

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Liver sinusoidal endothelial cells (LSECs) line the hepatic sinusoids and constitute a physical barrier between the blood and the liver parenchyma [1]. LSECs are a specialized type of endothelial cell that have unique feature – they are perforated with hundreds of nanoscopic (50-350 nm) transcellular pores called *fenestrations*. Fenestrations facilitate passive but size limited transport of macromolecules and solutes between blood and the hepatocytes. The porosity (the number of fenestrations per area of cell) of LSECs decreases with aging and is accompanied with multiple liver diseases. There is a need to develop re-fenestration strategies in defenestrated LSECs in order to restore full liver function. The literature data indicate that the porosity of LSECs decreases with liver stiffening [2]. This study implements novel AFM imaging modalities [3-4] to investigate whether controling the stiffness of the cells environment would affect LSEC porosity *in vitro*. Moreover, the correlation between cell stiffness and the deformability of fenestrations will be investigated.

Cells translate the perceived mechanical stimuli to biochemical signals that modulate gene expression, protein synthesis, cell proliferation, differentiation and migration [5]. Mechanotransduction of LSEC has not been reported so far. In this regard, the objective is to employ the combined knowledge of material science and life sciences to create a microenvironment that is conducive to the growth and survival of functional features in the cells. State-of-the-art findings show that the phenotype of hepatocytes, hepatic stellate cells, and LSECs are all directly improved by low stiffness [6]. The number of fenestrations increases when cultured on low stiffness substrate. The cells from diseased livers restored their functionality when placed in a soft environment. Therefore, targeting stiffness-modulated drugs can improve liver conditions. In this study, we try to examine the effect of substrate stiffness of LSECs *in vitro*. AFM was employed to assess the mechanical properties and topographical features of the LSECs. Our results indicate that culturing LSECs on a soft substrate affect the morphology of the cells and caused an increase in the porosity. AFM data will be discussed in accordance to quantitative fluorescence microscopy of selected cytoskeletal elements, endocytic properties of cells, gene expression levels and detailed gel surface characterization. Our studies indicate that reducing the elasticity of the culture substrate for LSECs may induce their partial refenestration – restoration of fenestrations in defenestrated LSECs.

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Biomechanics of Macrophages During Interaction with Extracellular Stimuli

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Macrophages are involved in every stage of the immune responses in the body tissues, including the resolution of the inflammatory reaction. The modulation of macrophages' behavior is closely linked with extracellular environment and physicochemical stimulation. Within these stimuli, macrophages are especially sensitive to surface properties, such as mechanical and geometrical characteristics, directly influencing adhesion, migration, and consequently their immune response.

In this investigation, we combine atomic force microscopy (AFM), finite element modeling (FEM) and quantitative biochemical approaches in order to understand the mechanotransduction from the extracellular matrix (ECM) and phagocytic particles into cellular response. The mechanical cues from the substrate are transduced into the cells through the formation of integrin-regulated focal adhesions and cytoskeletal reorganization, which in turn modulate cell biomechanics by decreasing cell stiffness.[1]

Surface topography and consequent biomechanical response impact the overall behavior of macrophages by increasing movement and phagocytic ability, without significantly influencing their inflammatory behavior. Along with ECM modification, the shape and stiffness of phagocytic particles also influenced the actin cytoskeletal organization, a fundamental process during phagocytosis. In particular, macrophages can exert internal forces able to bend and curl elongated particles with a stiffness of 150 KPa or less.[2]

Finally, we explore the biomechanical mapping of macrophages as a novel classifier to distinguish their activation status. Applying AI algorithms to different data maps we realize that mechanical data layer is very efficient to automatically distinguish macrophages' phenotypes.[3]

Our investigations suggest a strong potential of biomechanics as stimuli for the regulation of macrophage functions, as well as a novel classifier to determine their physiological status.

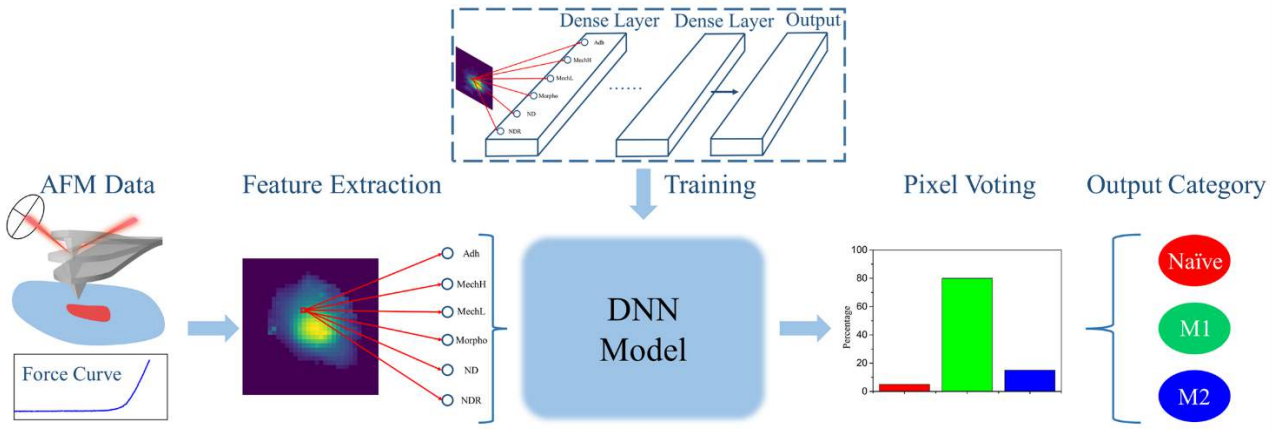

Figure 1. General processing pipeline for the training and prediction process of the neural network for biomechanics.

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Ultrathin water layers on viruses and on virus models

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Many viruses must "survive" in dry environments (air, soil) during transmission. How does this affect their surfaces?

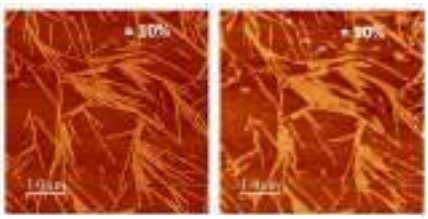

Figure 1. Tobacco Mosaic Viruses on Si wafer at humidity extremes

The surfaces of non-enveloped viruses, such as Tobacco Mosaic Virus (the role model for all viruses) [1,2,3], comprise only proteins, often in a highly symmetric structure, which protect the nucleic acid. They usually survive complete dehydration [4,5] but feature an ultrathin water layer (<5 nm) under ambient conditions (40% air humidity). AFM at variable humidity (Figure 1) shows a surprising and reversible reconstruction of the helical TMV capsid upon drying. A neutron reflectometry study gave further insight.

Enveloped viruses have a completely different surface, which is based on lipids. How can such viruses "survive"?

A simplified model of the influenza virus surface is based on hemagglutinin protein “spikes” in supported lipid bilayers. AFM, fluorescence correlation spectroscopy and neutron reflectometry prove that - under dry conditions - the spikes effectively block the otherwise irreversible restructuring of the lipid [6]. Under ambient conditions, a water layer on the lipid extends for 1 nm.

For a very rough model, it is possible to include also the important sugar moieties on the "spikes": Mannosylated gold nanoparticles, adsorbed on hydrophobic and on hydrophilic silane layers, are probed by hydrophobic and hydrophilic AFM tips. The mannose allows to retain ultrathin water layers (1 to 2 nm), but only at high air humidity (90%).

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AFM-based methods to investigate the correlation between macrophage viscoelastic behavior and phenotype in response to substrate stiffness

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Macrophages are innate immune cells responsible for the body's defense via pathogen clearance and wound healing. Thanks to their capability to polarize to pro-inflammatory (M1) or anti-inflammatory (M2) states, macrophages orchestrate the host response and coordinate the progression of several major diseases, such as sepsis, infection, atherosclerosis, fibrosis, and cancer. [1] Macrophages are mechanosensitive, [2] resulting in their ability to alter their polarization state in response to changes in the mechanical properties (e.g., elasticity) of the environment that can occur during the implant's lifetime and disease progression (e.g., fibrosis and cancer). However, the mechanotransduction mechanisms involved in physical cues–induced macrophage activation are still largely unknown. Mounting clues suggest the implication of cytoskeleton remodeling and cell mechanics, [3] highlighting the need to quantify specific macrophage mechanical parameters in "near-native" conditions of interest and correlate them with their cytoskeleton organization and biological function. In this work, for the first time, we used AFM-based force spectroscopy and microrheology (Nanowizard 4, Bruker) to quantify the elastic modulus and viscoelastic properties (storage and loss modulus) of primary human monocyte-derived macrophages i) in different polarization states (M0, M1, M2a) and ii) in response to changes in the mechanical environment (substrate elasticity in the range of 10-100 kPA) (Figure 1a). We correlated these quantitative cell mechanical parameters with the macrophage morphology, cytoskeleton organization (actin, intermediate filaments, tubulin, podosome formation, analyzed by immunofluorescence imaging), and polarization state (analyzed by qPCR). Our results showed a correlation between macrophage viscoelastic behavior and phenotype in response to both biochemical stimuli and changes in the substrate stiffness, paving the way for the possible use of quantitative macrophage mechanical parameters as biophysical markers for macrophage function and demonstrating the potential of AFM-based techniques to reveal novel insights into the mechanotransduction mechanisms underlying macrophages' stiffness-induced functional response.

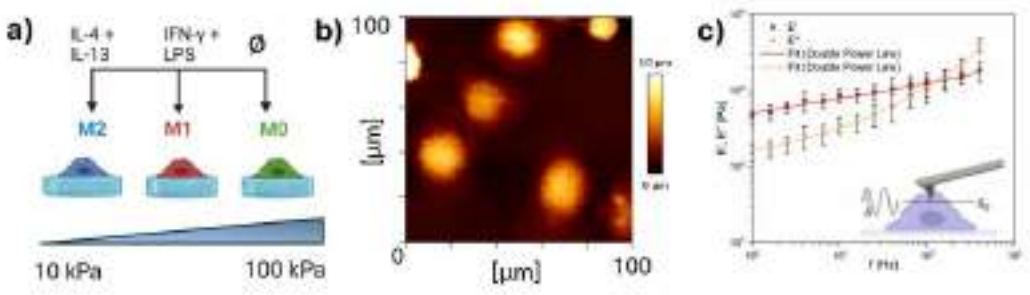

Figure 1. Primary human monocyte-derived macrophages were cultured on polyacrylamide gels with an elastic modulus of 10-100 kPa (a). AFM force spectroscopy-based methods were employed to image the macrophages (b) and analyze their viscoelastic behavior (c)

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HIGHTHROUGHPUT BIOMECHANICAL CHARACTERIZATION OF MACROPHAGE POLARIZATION THROUGH ATOMIC FORCE MICROSCOPY

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Macrophages play a critical role in innate immunity and are involved in a wide range of biological functions. Due to high plasticity, macrophages can be polarized into different phenotypes depending on the microenvironment, enabling them to perform specific functions during inflammation. This polarization capability underscores the importance of identifying biomechanical differences between various macrophage subtypes, as these variations could serve as valuable indicators for early diagnosis in various diseases Including chronic inflammatory diseases (such as rheumatoid arthritis, fibrosis, and atherosclerosis), neurodegenerative diseases, and cancer [1].

However, neither standardized measurement protocol or robust biomechanical analysis currently exists to efficiently explore these differences. In this context, atomic force microscopy (AFM) has emerged as an ideal tool, as it allows mapping cellular biomechanical properties with high spatial resolution under physiological conditions. To enable precise identification and differentiation of these phenotypes, we present a measurement protocol in which key experimental parameters are carefully controlled, along with the development of accurate analytical models and advanced data analysis techniques integrated with artificial intelligence. These results open new avenues for macrophage research and, more broadly, for highthroughput cell nanomechanical studies, projecting the AFM technique into real clinical applications.

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CHANGES IN ERYTHROCYTE ELASTICITY AND MORPHOLOGY AS POTENTIAL PREDICTORS OF SURVIVAL IN ALS

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Amyotrophic lateral sclerosis (ALS) is an aggressive neurodegenerative disorder affecting motor neurons[1–3]. It is associated with neuroinflammation and increased risk of thrombosis[4]. Erythrocytes have a fundamental role in binding to inflammatory mediators. Changes on erythrocytes have severe effects on cell functionality[5]. Erythrocyte aggregation, together with fibrinogen, have pathogenic implications in thrombus formation[6], promoting thrombotic events[5].

Using atomic force microscopy (AFM), we aimed to study the morphological, biomechanical and biophysical properties of erythrocytes from ALS patients. Elasticity studies were performed with an applied force of 300 pN before retraction and each force-distance cycle was acquired at 2 µm/s. We also evaluated changes on γ' fibrinogen plasma levels (an *in vivo* splicing variant of fibrinogen) as possible biomarker of ALS, testing its role as a predictor of disease progression and survival.

Our AFM results show that erythrocytes from ALS patients are thicker and have less membrane roughness than those from healthy blood donors[7]. In addition, they are less stiff than erythrocytes from the control group. Haemorheological parameters of ALS patients seem to be altered, potentially promoting changes on blood flow. Erythrocytes from ALS patients have higher tendency to aggregate and their blood is more viscous. These results may comprise prognostic value, given that erythrocytes are very adaptive cells, according to the different pathophysiological conditions[7]. Moreover, there is a link between γ' fibrinogen concentration and survival in ALS patients: patients with higher γ' fibrinogen plasma levels survived longer, and this finding was not influenced by confounders like age, gender, respiratory impairment or functionality (ALSFRS-R score)[4]. Early diagnosis through the identification of ALS biomarkers is a promising approach in ALS clinical research.

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Characterization of the viscoelasticity of Red Blood Cells and rheological analogues with AFM

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Despite their simple architecture, namely the lack of nucleus, organelles, a simple cytoskeleton with respect to other mammalian cells and a limited ATP activity, the range of bulk Young moduli of a Red Blood Cell **(RBC)** measured with AFM extends over several decades, from 50 Pa to 50 kPa. This is largely due to a multiplicity of cell fixation techniques and instrumental methods.
In that sense, we propose to study both the static behavior (Young modulus) and dynamics (complex shear modulus) of RBC from several donors with AFM, to decipher its viscoelastic behavior in a large range of frequencies. Furthermore, the ubiquitous use of the polyelectrolyte Poly-L-Lysine **(PLL)** in these studies to bind the cell to the substrate questions the impact of the PLL concentration and molecular weight on both the RBC shape and mechanical behavior [1]. We observe that the Young Modulus of a cell measured using low cantilever stiffness scales with the lowest values found in literature around 100 Pa. Concerning its dynamics [2], we find a viscoelastic behavior in adequation with previous work [3]. Moreover, using confocal imaging, RICM and AFM, we reckon that PLL alters the shape of live RBCs and stiffens the cell with increasing concentration, see Figure 1. However, a RBC attached to the substrate with PLL is about $2.5\mu m$ thick. Hence, the difficulty of those measurements arises because of indentations on the order of $1\mu m$ for forces as low as $50pN$. This challenges the AFM technique and the use of contact mechanics models for the characterization of those extremely soft samples. This is why we also study spherical indentation of RBCs with finite elements methods in order to make sense of our viscoelastic measurements in large indentation and deformation conditions.

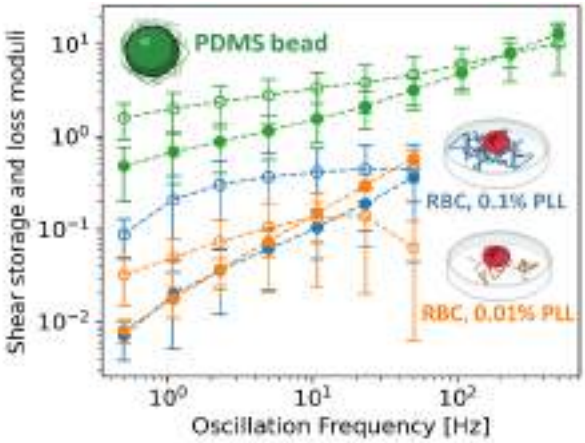

Figure 1. Shear storage modulus $G'(f)$, open markers, and shear loss modulus $G''(f)$, filled markers. Measurements are made for adherent RBCs with 150 kDa mw PLL concentrations of 0.01% (0.1mg/mL, in orange) and 0.1% (1mg/mL, in blue), indented with a $1\mu m$ spherical probe (Nunano Quest 5 TL, $k = 0.005N/m$). In green, comparison with 9.9:0.01 base:curing agent Sylgard 184 PDMS beads, indented with tipless cantilevers (Nanosensors TL-CONT , $k = 0.3\ N/m$.)

With this knowledge, within the frame of a project aiming at trapping and characterizing RBC under flow with optical tweezers [4], we attempt to produce polymeric RBC rheological analogues that we can tune, in order to mimic healthy and malaria-infected RBC, see Figure 1.

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Characterization of Liquid/PAA and PEO Polymer brushes/Gold interfaces by 1D and 3D-AFM

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Self-assembled or grafted onto organic monolayers bound to a solid surface, such as polymer brushes, can serve as detection layers for specific biomolecules in biosensors or as protective layers in tribological systems or lab-on-chip devices. For these applications, it is important to understand how the unbound section of the polymer brush is structured and how it interacts with liquid surroundings, especially at the nanometric scale. Our study aims to characterize this liquid/polymer brush interface using force spectroscopy, or 1D-AFM, and 3D-AFM, a mode developed by T. Fukuma and R. Garcia [1]. In this mode, 2D cross-sectional images in the (xz) plane are constructed using recordings of the cantilever's frequency shift Δf during the scans. These images represent the liquid organization at the interface. We are particularly interested in thiol-based polymer brushes deposited on a pure gold surface in a liquid medium, mainly used for applications in biosensors and tribology [2]. Three samples were studied: ultra-flat pure gold, pure Poly(acrylic acid) PAA, and pure Poly(ethylene oxide) PEO.
The polymers were observed in liquids with different pH levels and ionic strengths: in acidic HCl (pH = 5.5) and PBS (pH = 7.4) solutions and concentrations of 10^{-4}M and 2×10^{-1}M in NaCl. The force curves obtained with 1D-AFM measurements show the effect of the pH and the salt concentration of the different liquid environments on the length of the polymer chains. 3D-AFM measurements complement the obtained results, and our initial experiments suggest that this method is well-suited for visualizing the organization of the liquid molecules and ions on the polymer brush layer, and their dependence on variations in pH and ionic concentration (Figure 1).

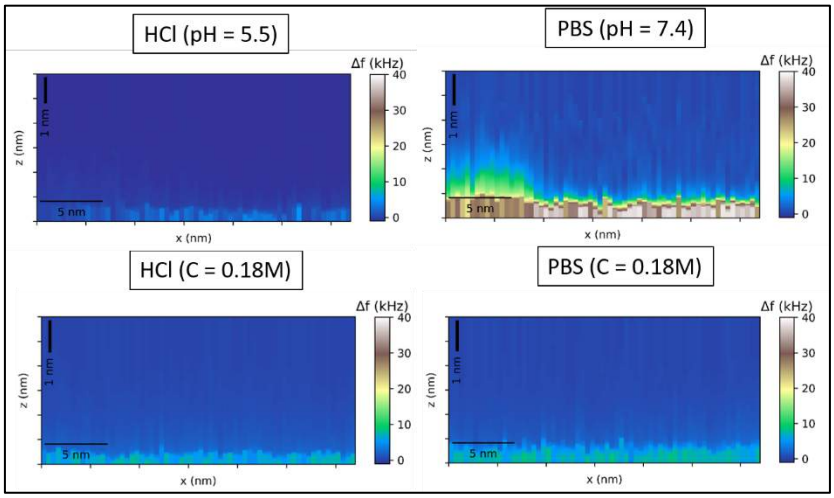

Figure 1. 2D (xz) cross-sectional images of the pure PAA sample characterized in different solutions by 3D-AFM.

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SCANNING PROBE MICROSCOPY AND RAMAN SPECTROSCOPY INVESTIGATION OF CARDIAC ORGANOIDS

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In recent years, several protocols have been optimized to produce from induced pluripotent stem cells (iPSCs) organoids, 3D complex structures, recapitulating organ multicellularity, geometrical organization and functionality. Organoids, become popular in neuroscience and oncology, have more recently reached the cardiac field [1]. Cardiac pathologies often include arrhythmic and fibrotic phenotypes, thus raising the need of in vitro electromechanical measurements, to allow the phenotypization of the in vitro tissue and a functional read-out of the capacity of a treatment to restore the physiological phenotype [2].
Scanning probe microscopy (SPM) combined with force spectroscopy represents an important tool to investigate the mechanics of cells and tissues in diseases [3]. On the other hand, Raman spectroscopy is a useful technique to obtain molecular and structural information of biological samples. In this work we combined SPM and micro-Raman methods in a mechano-chemical approach to correlate the mechanical properties (i.e. elastic modulus) obtained by SPM to the spectroscopical peaks which allows to recognize the presence of cardiomyocyte and collagen [4]. Our finding showed that the tissue stiffness increased in cardiac organoids affected by cardio-pathological disease, in agreement with increase in collagen levels detected.

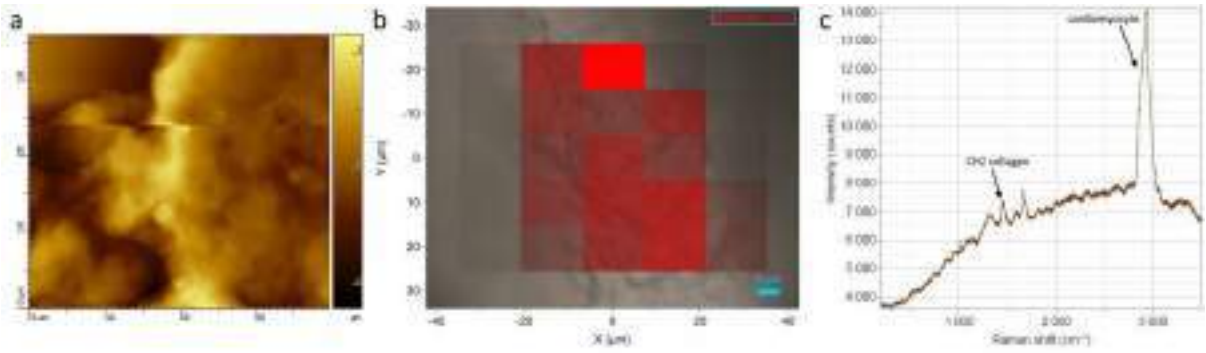

Figure 1. (a) AFM morphological image; (b) Raman map overlapped on the optical micrograph and (c) Raman spectrum of cardiac organoid.

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MULTISCALE NONLINEAR MECHANICS OF COLLAGEN I HYDROGELS UNDER STRETCH

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Recent studies have highlighted the role played by hydrogel mechanical properties as a key factor in directing cellular alignment (1). This is especially relevant in tissues like the myocardium, where anisotropic organization is crucial for its function. Moreover, the influence of strain on the myocardium's mechanical behavior is also critical. However, there is a lack of comprehensive understanding of hydrogel multiscale stiffness characteristics, particularly under strain conditions. To characterize the multiscale mechanical properties of collagen hydrogels using atomic force microscopy (AFM) and tensile tests with different crosslinking degrees and stretchs applied. To measure the micromechanical stiffness with AFM, a device to stretch samples compatible with simultaneous AFM measurements was first designed and built (Figs. 1A,B). To produce photocrosslinkable hydrogels, a 10 µL of Ru/SPS photocrosslinker (Advanced Biomatrix, #5248) was added to the collagen I (10 mg/ml) mixture. Neutralized mixture was then pipeted on top of the stretching device preparing gels of 50 µL volume and left it at 37 ºC for 30 min for thermal crosslinking. Afterwards, gels were illuminated with blue light (455 nm) for 0, 5 or 30 minutes. Then, the stretching device was placed on the AFM stage and the hydrogels were measured at strains up to 30% to extract the Young's modulus. In parallel, macromechanical properties of (un)crosslinked hydrogels were assessed by tensile testing. An analytical model modeling collagen I fibers as ideal springs connected through nodes was developed to correlate macromechanical properties with the microstructure (crosslinking degree) of the hydrogels. Collagen I hydrogels exhibited a baseline stiffness of ~100 Pa (uncrosslinked, control), ~900 Pa (crosslinked, 5 min), and ~1200 Pa (crosslinked, 30 min) (Fig. 1C). Notably, we report for the first time, AFM measurements of collagen I hydrogels under stretch. The Young's modulus displayed significant nonlinearity under strain, increasing ~3 to 5 fold at 30% strain (Fig. 1C), independently of the crosslinking degree. These findings align with previous studies on decellularized lung ECM under stretch (2). Moreover, the analytical model predicted with high accuracy the macromechanical properties measured by tensile test, giving crosslinking degree values (N), consistent with immunofluorescent image fiber analysis (not shown). This study presents the first report of nonlinear micromechanical behavior in collagen I hydrogels under strain (stress-hardening) with different crosslinking degrees. These findings offer insights into cellular alignment mechanisms in stiffness-patterned environments, crucial to the success of tissue engineering approaches.

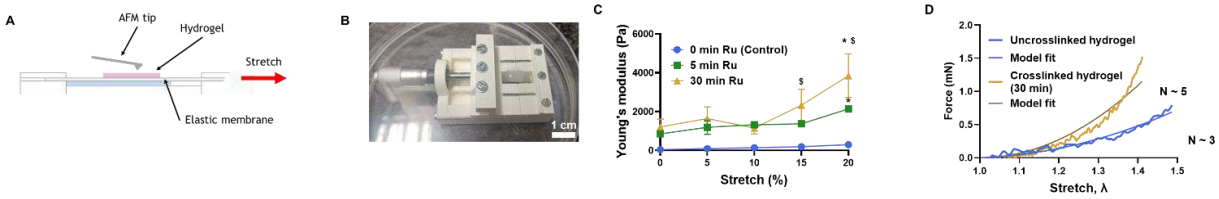

Figure 1. A) Schematics of the stretching device compatible with AFM. B) Photography of the stretching device with collagen I hydrogel. C) Micromechanical properties of collagen I hydrogels under strain with different crosslinking degrees. **, * statistical significance compared to 0% stretch. $ statistical significance compared to different crosslinking degrees. D) Analytical model correlates accurately the crosslinking degree (N) of the hydrogels with macromechanical properties by tensile tests.

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AFM studies of the molecular mechanism of genome organization

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Abstract. Chromosomal proteins such as structural maintenance of chromosomes (SMC) protein complexes, heterochromatin protein 1 (HP1), polycomb repressor complexes (PRC) are known to play a key role in genome organization. However, the molecular mechanism is not clearly understood. Particularly, universal mechanism for the genome organization should be debated. Here, using atomic force microscopy (AFM) and single-molecule fluorescence imaging, we show that chromosomal proteins induce microphase condensation along a DNA akin to the yeast cohesin SMC complex (Ryu et al. (2021)). Notably, particularly, they form clusters only on DNA longer than 1 kilobase pair. We propose that this phenomenon, termed bridging-induced phase separation (BIPS), involves DNA-protein-DNA bridging interactions. This study suggests that biomolecular condensation by chromosomal proteins may also be a fundamental principle underlying genome organization.

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AFM study to reveal the role of sulfation patterns of heparan sulfate in modulating the interactions between human papillomavirus and glycosaminoglycans

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Human papillomaviruses (HPVs) are small non-enveloped DNA viruses, several of which are responsible for cervical cancers. At the initial stage of infection, HPV16, the most prevalent cancer-causing type, interacts with glycosaminoglycans (GAGs) such as heparan sulfates (HS) [1]. The interaction of HPV16 virions with HS is important for both the initial attachment to host cells and for triggering a conformational change in the viral capsid termed structural activation which is critical to virus entry. In this context, the role of specific sulfation groups of HS in regulating these interactions is currently unknown.

Here, we used a combination of biochemical and biophysical assays [2-4], including atomic force microscopy (AFM) based single molecule force spectroscopy (SMFS) which measures the unbinding force between HPV16 coupled to an AFM tip and GAG chain (Figure 1). Our results showed that N-sulfation is crucial but alone insufficient for binding and structural activation of HPV16 and is likely aided by 6O-sulfation, whereas 2O-sulfation is dispensable [2]. Additionally, AFM-SMFS has allowed us to reveal that 6O-sulfation mechanically strengthens the interactions between heparin and HPV16 to facilitate binding. Altogether, these results find the direct involvement of sulfation patterns of HS in HPV16 binding and structural activation and reveal how the distinct sulfation groups of HS facilitate viral attachment to influence entry and thus will likely play a role in determining the tropism of HPVs.

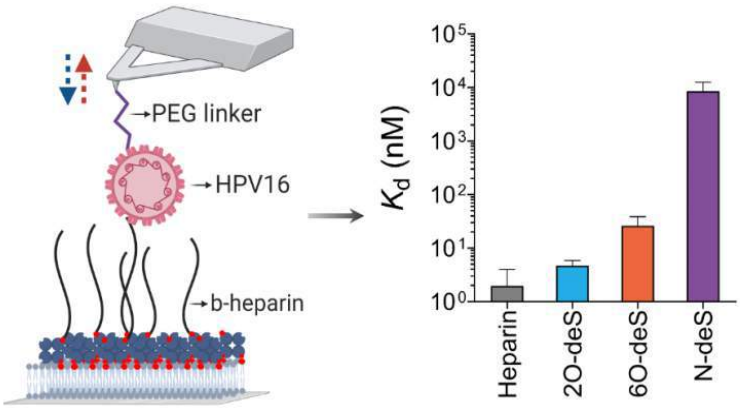

Figure 1. AFM-SMFS assay to measure the binding affinity between heparin and its derivatives, adapted from [2].

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Interplay of glycans and adsorption-driven deformation in SARS-CoV-2 RBD variants onto surfaces with different hydrophobicity

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Respiratory viruses, carried through airborne nanodroplets, frequently adhere to surfaces, including plastics and metals, making surface interactions critical in viral transmission. Our understanding of the interactions between viruses and materials remains limited, particularly in scenarios involving polarizable surfaces. In this study, we employ molecular simulations to examine the role of SARS-CoV-2 receptor-binding domain (RBD), including its glycan, across open and closed configurations and different variants on highly hydrophobic and highly hydrophilic surfaces.

Our findings reveal stable adsorption to hydrophobic surfaces of both open- and closed-RBDs with or without glycans. While the contact region of the closed-RBD shows no significant structural changes, the flexibility of the receptor binding motif (RBM) when it is in the open configuration (Figures 1 a,b), plays a role [1]. The residues involved in the contact with surfaces differ significantly from those interacting with its native biological interface, the ACE2 receptor (Figures 1c,d) [1]. Hydrophilic surfaces are clearly less prone to adsorption, especially in the close-RBDs. While glycans do not significantly influence the adsorption of open-RBDs on both types or surfaces, they play a pivotal role in the case of closed-RBDs, leading, due to their high flexibility, to unique glycan interactions with the protein and with the surface that can drive the adsorption [2]. For instance, glycans show the ability to anchor the protein even onto hydrophobic surfaces, despite also inducing repulsion to the protein. These findings show the complex interplay between RBD conformations, glycans, and surface properties, advancing our understanding of virus-surface interactions and could lead to current experimental efforts in designing virucidal surfaces and sensors.

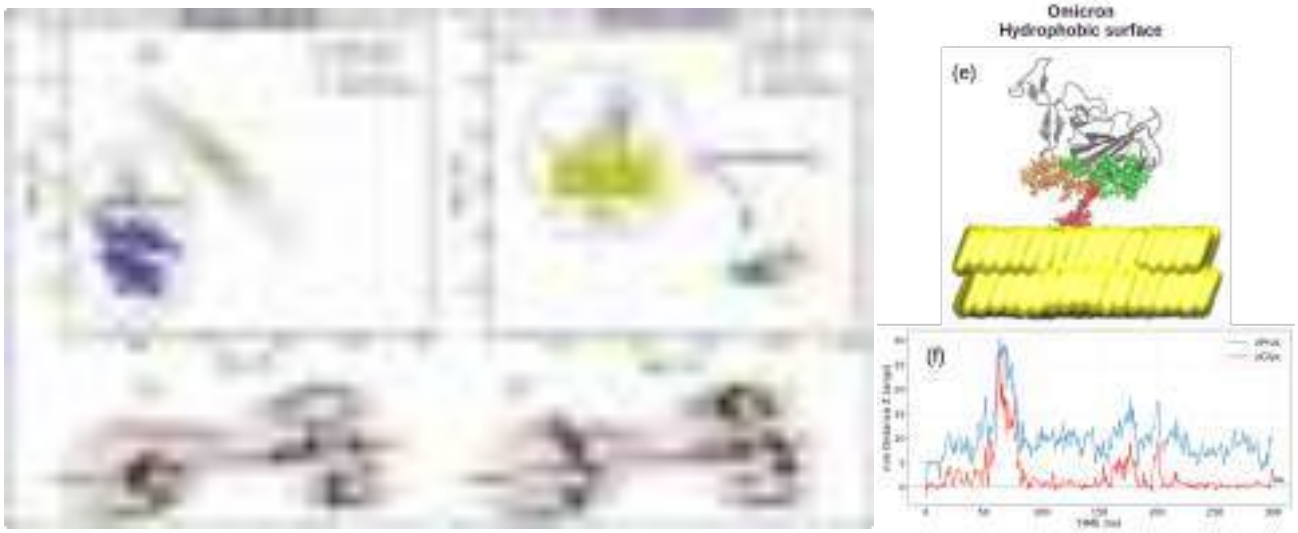

Figure 1. (a,b) Perpendicular versus parallel radii of gyration and (c,d) landing footprints of group 1 of the RBM (depicted in green in insets) onto the (a,c) ACE2 and hydrophobic surface (b,d). (e) closed-RBD with glycan snapshot. (f) minimum protein and glycan distance showing glycan anchoring to a hydrophobic surface.

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Cellular stepwise confinement reveals different mechanical response in MEC-WT and MEC-KO1 under diverse pharmacological

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Atomic Force Microscopy (AFM) has emerged as an essential tool for investigating the mechanical properties of single cells, enabling the determination of stiffness, mechanical characterization, and high-resolution imaging. However, living, low-adhering, and highly motile cells, such as leukocytes, present unique challenges for mechanical studies [1]. Traditional AFM approaches using sharp or colloidal tips are often unsuitable due to limited adhesion and insufficient force application, leading to probe slippage or cellular rupture.

To address these limitations, innovative strategies tailored to such cell types are necessary. In this study, we employed wedge-shaped cantilever tips [2] (Fig. A) to perform stepwise parallel confinement experiments (Fig. B) on two lymphocytic cell lines: MEC1, a wild-type model mimicking chronic B-cell leukemia, and MECKO, a derivative with knockout of the HS1 protein. HS1 is a cytoskeletal protein involved in actin dynamics and cellular signaling, playing a central role in leukemia progression [3,4]. By compressing cells up to 70% of their volume, we investigated their mechanical responses under basal conditions and pharmacological treatments targeting key signaling pathways [5]. These included Ibrutinib, a Bruton's tyrosine kinase inhibitor; Defactinib, a focal adhesion kinase inhibitor; and Cytochalasin D, an actin polymerization disruptor. The results revealed distinct mechanical behaviors between wild-type and HS1-knocked out cells, with significant differences in stiffness and viscoelastic properties both within the same cell line (treated versus untreated) and between the two cell models. Our findings underscore the mechanosensitivity of HS1, highlighting its role in mediating cellular adaptation to mechanical stimuli through interactions with the cytoskeleton and associated signaling networks. This study not only advances our understanding of the mechanotransductive functions of HS1 in leukemic cells but also demonstrates the effectiveness of AFM-based wedge confinement techniques for analyzing the mechanics of non-adherent, highly motile cells.

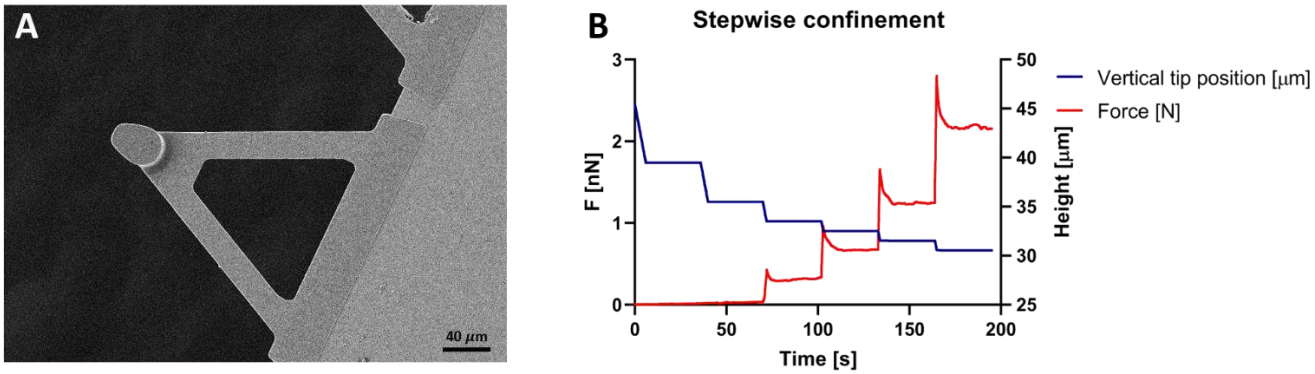

Figure 1. (A). Representation of a modified wedge cantilever and (B) force (red) and AFM tip position (blue) during stepwise confinement performed with wedge tip.

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Enhancing Large-Scale BioAFM Mechanical Characterization with SmartMapping

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Atomic force microscopy (AFM) is crucial for nanoscale mechanical property mapping, offering high-resolution characterization of stiffness, adhesion, and viscoelasticity. This capability is essential for understanding material behavior in complex structures like living cells, tissues, and biomaterials, thereby driving forward studies of cell behavior, disease progression, and drug treatments. Nevertheless, challenges such as sample roughness and limited lateral scanning range often hinder large-scale mechanical mapping, particularly for complex and heterogeneous specimens like biopolymers, hydrogels, and tissues.

We have developed a new SmartMapping tool that addresses these challenges by coordinating AFM head motors and XYZ-piezo movement. This innovation enables continuous, high-resolution mapping over extensive areas without user intervention, enhancing the precision and efficiency of AFM. We successfully tested this feature for comprehensive analysis of hydrogels with different stiffnesses (1 kPa and 50 kPa), analyzing centimeter-scale areas and creating detailed mechanical property maps. Additionally, we evaluated the applicability of the new feature for analyzing 2D cell populations seeded on hydrogels and highly corrugated 3D spheroid SKOV-3 model lines exceeding 100 µm in height. Furthermore, we assessed 600 µm thick vibratome-sectioned neuroblastoma tumors with very low stiffness (50-100 Pa) embedded in low-melting agarose gels, demonstrating that despite their roughness, such tissue samples can be effectively analyzed.

The new SmartMapping feature significantly advances AFM capabilities, enabling precise and efficient large-scale mechanical mapping. This development opens new avenues for studying a wide range of samples, from complex biopolymers to soft biological tissues, enhancing the scope and impact of automated AFM in biomedical and biomaterial research.

MECHANICAL PROPERTIES AT THE NANOSCALE OF CARDIAC ORGANOIDS INVESTIGATED BY SCANNING PROBE MICROSCOPY

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Nowadays, the design of advanced in vitro models, allowing effective recapitulation of the complexity of cardiac in vitro pathophysiology, is critical i) for the definition of the underlying mechanisms, ii) to test the efficacy of novel therapeutic treatments and iii) to move forward in the development of personalized medicine approaches [1]. The combination of induced pluripotent stem cell (iPSC) technology with microfabrication approaches, allowed the development of organoids, small dimension 3D structures, recapitulating organ multicellularity, geometrical organization and functionality. At this aim, we evaluated the feasibility to implement scanning probe microscopy (SPM) and force spectroscopy-based techniques to quantify the pro-fibrotic commitment in our cardiac organoid model and then relate the mechanical properties to pathological cardiac tissue remodeling. In this work, the mechanical properties of cardiac organoids were investigated at the nanoscale by SPM, and in particular atomic force microscopy (AFM). Force spectroscopy was carried out, after an accurate calibration of the probe mechanical response on a rigid Sapphire sample and a standard two-component polymer sample made of polystyrene (PS) and low-density polyethylene (LDPE). Local single force-distance (FD) curve and the FD curves mapping were carried out onto a surface of an organoid obtained by iPSC line from control or patient affected by known variant causing fibrotic deposition (FIB). Regarding the mechanical properties, obtained from the FD curves mapping down to 2x2 µm^2, a clear difference in the elastic modulus distributions on the surface of the organoids was observed, ranging from 25 to 2500 MPa, suggesting a different composition of the myocardium in response to the genetic background. The main experimental analysis focused on the elastic modulus investigation, obtained by Hertzian model [2], indicating that AFM can be a useful tool for phenotypization of fibrosis in cardiac organoids.

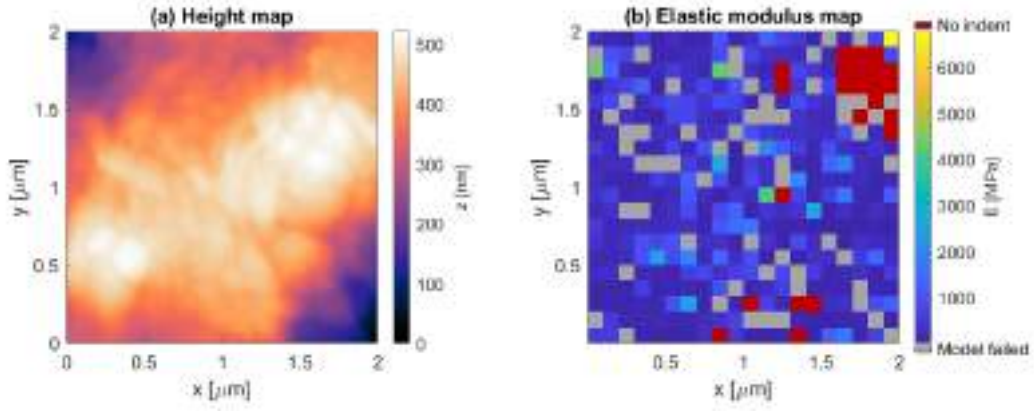

Figure 1. (a) AFM morphology and (b) elastic modulus map of a 2×2 µm^2 area of cardiac organoid.

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Nanorheology and Nanoindentation Revealed a Softening and an Increased Viscous Fluidity of Adherent Mammalian Cells upon Increasing the Frequency

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The mechanical response of a cell depends on the frequency or velocity at which the cell is probed [1,2]. The components of the cell which contribute to this property and their interplay are not well understood. Here, we integrated local deformation methods and theory [3,4] to develop a force microscopy approach to characterize frequency and velocity-dependent properties of living cells. We show that mammalian cells soften and fluidize upon increasing the frequency or velocity of the deformation. This behavior was independent of the method applied to deform the cell and the indentation values (25 or 1000 nm). At low frequencies (2-10 Hz) or velocities (1-10 µm/s), the response was dominated by the mechanical properties of the cell surface. At higher frequencies (> 10 Hz) or velocities (> 10 µm/s), the response became dominated by the hydrodynamic drag of the cytosol. Softening and fluidization did not involve any structural remodeling. It reflected a redistribution of the applied stress between the solid and liquid-like elements of the cell response as the frequency or the velocity was changed [5].

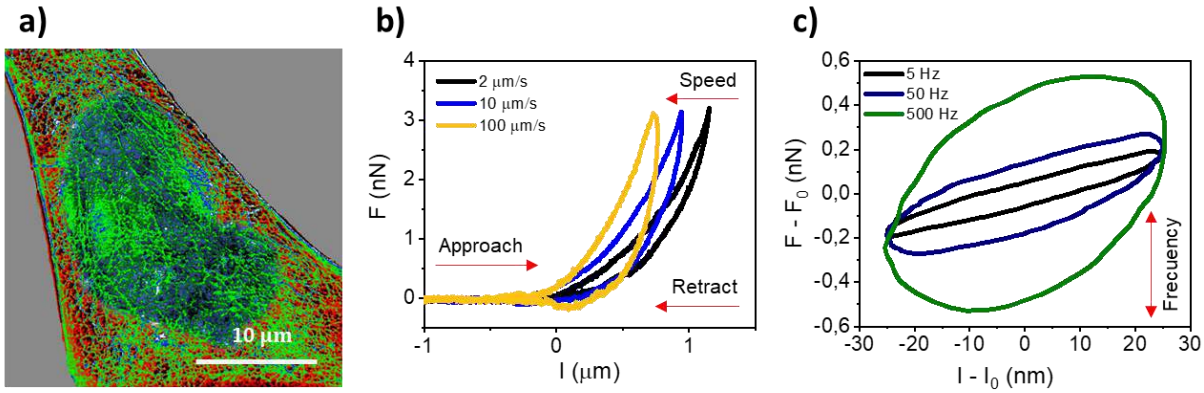

Figure 1. a) AFM nanomechanical map. b) Force-distance curve (nanoindentation) on a HeLa cell at different velocities. c) Oscillatory force-distance curves (nanorheology) on a HeLa cell at different frequencies.

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OVERCOMING CHALLENGES IN THE AFM MECHANICAL NANOCHARACTERIZATION OF SOFT HETEROGENEOUS BIOLOGICAL MATERIALS

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AFM nanoindentation has revolutionized biomedical sciences by enabling mechanical characterization of biological samples at the nanoscale [1]. Therefore, AFM is a promising tool for early disease diagnosis in various cases, such as cancer [2]. However, mechanical nanocharacterization in terms of Young's modulus is typically carried out using classic Hertzian equations, which assume the tested sample is homogeneous and isotropic [2]. Nevertheless, biological materials such as cells and tissues are highly heterogeneous; therefore, their mechanical properties are strongly depth-dependent [3]. In other words, the Young's modulus calculated at a specific point of interest depends on the indentation depth. Using a different indentation depth will result in a different Young's modulus value at the same point. The goal of this work is to demonstrate that accurate mechanical characterization of soft heterogeneous samples using AFM is feasible by extending the applicability of classic Hertzian equations through the weighted mean value theorem for integrals. We show that, in all cases (regardless of the indenter's shape or the finite thickness of the tested material), the applied force on the sample can be expressed in the following form [3, 4]:

$$F = \int_0^h E(y)\Omega(y)dy \tag{1}$$

where E(y) is a function related to the depth-dependent mechanical properties of the material, and Ω(y) is a function related to the indenter's geometry and the sample's thickness. In addition, let $E, \Omega: [0, h] \to R$ be such that E is continuous and Ω is integrable and does not change the sign on [0, h]. Then, there exists a number $\mu \in (0, h)$ such that [3, 4]:

$$F = E(\mu) \int_0^h \Omega(y)dy \tag{2}$$

The parameter $E(\mu)$ in equation (2) represents the overall or average mechanical properties of the tested sample in a specific domain. When recording the $E(\mu)$ parameters for different indentation depths, depth-dependent mechanical characterization becomes a straightforward procedure. The aforementioned approach has been applied to a variety of cases (fibroblasts, murine cancer cells, and lung tissues) to account for variations in the indenter's shape and sample thickness. In all cases, the depth-dependent mechanical properties of the tested samples were revealed. Accurate characterization of biological samples at the nanoscale is one of the most significant challenges that limits the applicability of AFM in real clinical settings. Recording the average mechanical properties of biological samples with respect to indentation depth will likely prove to be key for using AFM methods in clinical activities such as cancer diagnosis.

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Nuclear Stiffening and Structural Alterations Induced by Glycation in Diabetes

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Tissue homeostasis relies on the integration of mechanical and chemical signals across multiple scales to regulate cell fate and function. The nucleus plays a pivotal role in maintaining this balance, actively regulating cell phenotype and integrity through continuous feedback with the extracellular matrix (ECM) and cytoskeletal stiffness [1]. Glycation, a non-enzymatic modification of proteins by sugars or their derivatives, can disrupt this equilibrium. The resulting advanced glycation end-products impair protein function, leading to defects in molecular, cellular, and tissue mechanics [2]. While glycation of ECM proteins is well-characterized, its effects on intracellular proteins, particularly nuclear components, remain poorly understood in the context of mechanotransduction.

Using atomic force microscopy (AFM)-based nanoindentation and confocal microscopy, we show that methylglyoxal (MG), a major glycating agent elevated in diabetes and aging, disrupts nuclear mechanics. MG treatment stiffens the nucleus, decreases its fluidity, and compromises nuclear envelope integrity. Structural defects induced by MG include nuclear envelope blebbing, increased rupture susceptibility, and the formation of nucleoplasm-localized lamin aggregates (Figure 1A). This alters nuclear transport, as evidenced by YAP nuclear localization studies in response to mechanical cues. To enhance the physiological relevance of our findings, we used AFM to probe the viscoelastic properties of nuclei isolated from the hearts of mice recapitulating the type 2 diabetes phenotype (Figure 1B). Notably, these nuclei exhibited similar mechanical alterations, including increased stiffness and reduced fluidity, mirroring the *in vitro* changes observed using HeLa cells (Figure 1C,D). By linking MG-induced biochemical changes to nuclear mechanics for the first time, our findings suggest that glycation-driven stiffening observed *in vitro* may represent a mechanism for nuclear mechanical alterations *in vivo*. This study contributes to a deeper understanding of how glycation drives disease pathogenesis, particularly in conditions associated defective tissue mechanics.

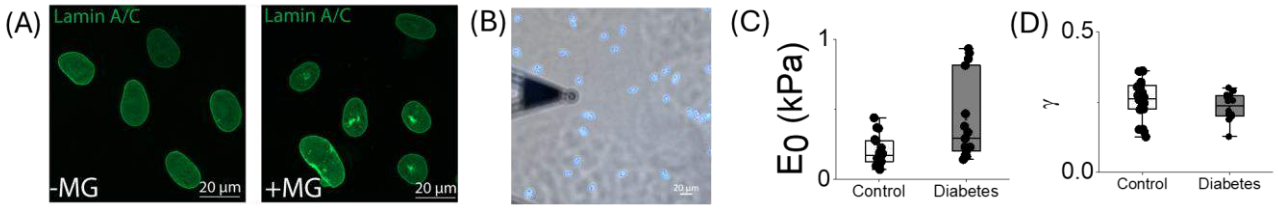

Figure 1. Figure 1. Nuclear envelope alterations and stiffening mediated by *in vitro* and *in vivo* glycation. (A) Maximum intensity projection confocal images illustrating features of HeLa Kyoto cells permanently transfected with lamin A/C-EGFP (green). After treatment with 500 µm MG for 3 days, aggregation of lamin A/C is observed in the nucleoplasm and an overall compromised nuclear morphology. **(B)** Epifluorescence image of Hoechst stained single nuclei extracted from mouse hearts probed by an AFM spherical probe. **(C)** Scaling modulus, E_0, and **(D)** fluidity, γ, of single nuclei from control and type 2 diabetes mice. Each dot represents the mean of three single force-distance curve performed on a nucleus. The median is represented as a horizontal line.

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Nuclear Pore Complex plasticity on intact cell nuclei as seen by AFM

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The Nuclear Pore Complex (NPC) is probably the largest multi-protein complex in eukaryotic cells. It exhibits a cylindrical architecture (8-symmetry structure) around a central channel filled with hydrophobic, unstructured proteins [1]. Passage through the nuclear pore enables proteins and RNAs to be transported selectively and directionally between the nucleus and the cytoplasm of cells and therefore plays a critical role in the regulation of gene expression [1]. Several techniques such as cryo-EM [2], AFM [2-4] and optical super-resolution [5] have been successfully used in the recent years to visualize and characterize the pores of a nuclear envelope. These studies have shown that the NPC is able to adapt its overall structure to the state of the cell exhibiting structural and mechanical plasticity in various contexts.

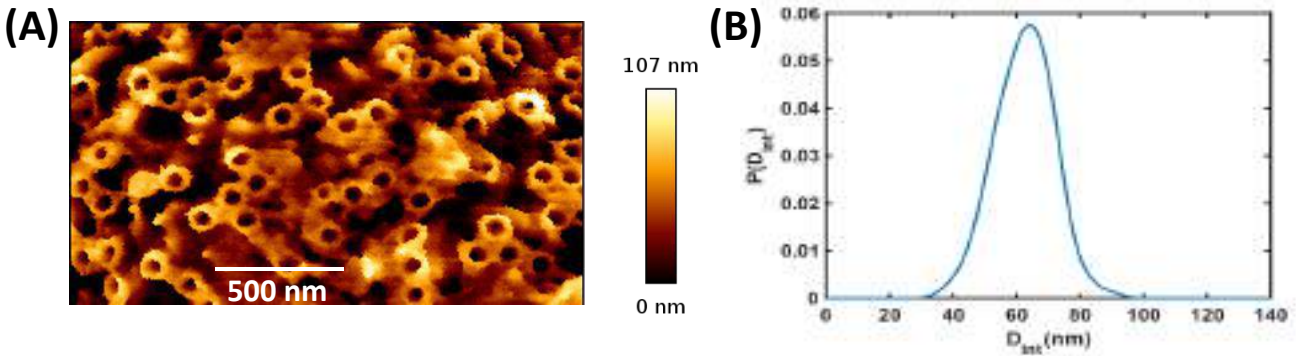

Figure 1. New experimental approach for AFM measurements of nuclear pore complexes on an intact cell nucleus. (A) Topographic AFM flatten image of a part of a hepatocyte mouse nucleus. (B) PDF of the of NPCs interior diameter with $\langle D_{int} \rangle$ = 62.4 ± 0.5 nm (standard error = $\frac{std}{\sqrt{N}}$ with N=378 the number of pores measured on 4 different nuclei).

Nevertheless, in most cases, the AFM studies have been carried out using a *Xenopus oocyte* nuclear envelop, opened and spread out on a surface, a configuration that facilitates 2D imaging but mainly provides access to the nuclear side of the NPC. Here, we implement a new method to visualize and precisely measure the internal diameter of NPCs by AFM on intact mouse nuclei (Fig. 1A) allowing to quantify the intrinsic plasticity of NPC (Fig. 1B). We use this approach to probe the effect of a natural biological rhythm, the circadian time clock, on nuclear pore plasticity in primary cell lines of mouse hepatocytes. We show in particular that the inner diameter of NPC is cycling though circadian time and we correlate this plasticity with several measured quantities such as for example the nucleus size, or the NPC density.

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PROBING CELL NANOMECHANICS WITH THE AFM

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Mechanical properties of cells play a key role in a broad range of biological processes, such as cancer, cell migration, cell differentiation in specialized tissue, and cell response to mechanical solicitation. Here, for each of these cases, we present four different types of experiments conducted with an atomic force microscope. (i) We characterize the stiffness and morphology of cancer cells cultured on micropatterns that constrain their geometry (**Fig. 1A**) and show how cortex tension combined with cell spreading affect the AFM measurement [1]. (ii) We exhibit a new AFM module that increases the spatiotemporal resolution of nanomechanical mapping (**Fig. 1B**), and we use it to characterize actin networks that act together to proper cell migration at the leading cell edge [2]. (iii) We map cells and cell junctions of a retinal epithelium during its maturation (**Fig. 1C**), and we show that cell somas undergo softening whereas cell junctions get stiffer over time [3]. (iv) In ongoing experiments, we use the AFM to perform fatigue testing on cardiac cells (**Fig. 1D**) and compare the dynamic response observed in wild-type cells versus mutant cells carrying a mutation in the desmin gene, a source of pathologies such as dilated cardiomyopathy. As desmin is an intermediated filament that synchronizes the contractile functions of cardiac cells, these data may contribute to deciphering the cellular mechanisms that link desmin mutations with clinical onset in patients.

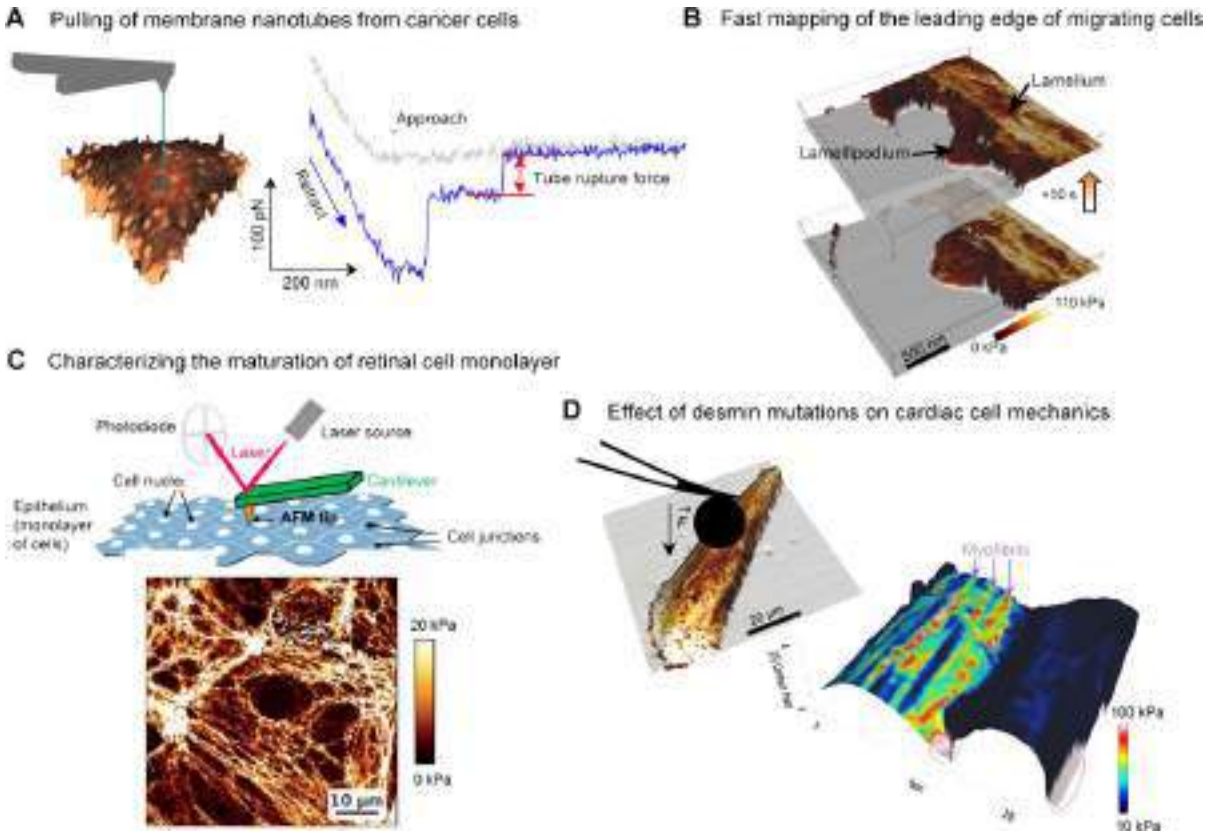

Figure 1. Four distinct systems in which we use AFM to unravel the links between cell functions and mechanics.

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Structural analysis of individual helical filaments by three-dimensional contact point reconstruction atomic force microscopy

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Atomic force microscopy (AFM) is capable of producing detailed three-dimensional topographical height images of bio-molecules with a high signal-to-noise ratio. This is a key capability of AFM, which enables the structural features of individual molecules to be studied without the need for ensemble averaging. This feature could offer structural analysis applications where heterogeneity of molecular populations, structural variations between individual molecules, or population distribution properties in general, hold important information. Here, a software tool Trace_y [1], designed to reconstruct the three-dimensional surface envelopes of individual helical filament structures from topographical AFM images by contact point reconstruction (CPR-AFM) [2, 3], is presented. The power of three-dimensional individual filament level structural analysis, and integrative analysis featuring AFM together with cryo-electron microscopy data is demonstrated on the structural analysis of individual helical amyloid protein fibrils where the assembly mechanism of heterogeneous, complex, and diverse fibril populations from a single amino-acid sequence due to structural polymorphism is not fully understood [4, 5]. Here, integrative structural analysis on amyloid fibrils formed from human Amyloid-β_{42} associated with Alzheimer's disease revealed that complex fibril populations assembled *in vitro* contain rare species that resemble *ex vivo* amyloid polymorphs from human brains [5]. The Trace_y software and the workflow presented here allows the structural information encoded in topographical AFM height images to be extracted and understood as 3D contact point clouds. This approach will facilitate the use of AFM in structural biology, in integrated methodologies together with other structural analysis tools such as cryo-electron microscopy to understand the structures and behaviours at individual molecule level.

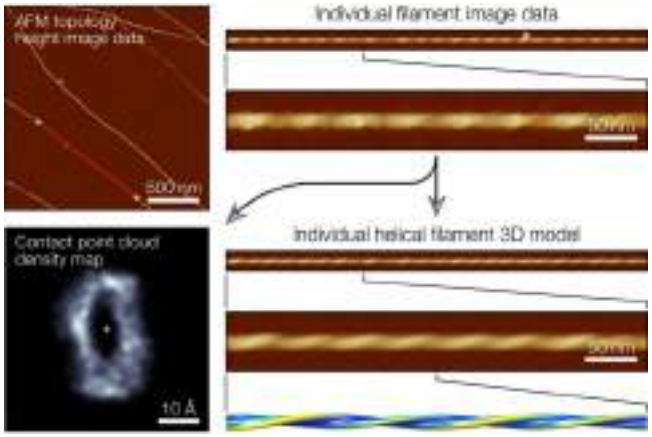

Figure 1. Trace_y is an open-source software designed to reconstruct the 3D surface envelopes of individual helical filament structures from topographical AFM height images by the reconstruction of 3D tip- sample contact point clouds, thereby facilitating the use of AFM in integrative structural biology.

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USE OF AFM NANOINDENTATION FOR EXTRACELLULAR VESICLE DETECTION AND BIOGENESIS ANALYSIS: A NOVEL MATLAB-BASED APPROACH

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Atomic Force Microscopy (AFM) is a powerful tool for studying biological specimens, offering high-resolution imaging and precise measurements of mechanical properties such as elasticity and adhesion [1] **¡Error! No se encuentra el origen de la referencia.** [3]. In this study, we focused on extracellular vesicles (EVs), which are lipid-bilayer-enclosed particles involved in intercellular communication and implicated as biomarkers in diseases like cancer [4]. EVs, including exosomes, microvesicles, and apoptotic bodies, differ in their biogenesis [5], yet the specific subtypes associated with cancer progression remain unclear. Moreover, the mechanical characterization of EVs under physiological conditions is challenging due to their nanometric size and complexity. We cultured MDA-MB-231 breast cancer cell line, from 3 to 14 days, and sorted EVs from conditioned medium by size using flow cytometry. To analyze their mechanical properties, we employed AFM to collect force–indentation curves on both live cells and isolated EVs, in liquid, deposited on mica substrates.
A novel MATLAB-based program was developed to process these curves, enabling accurate identification and localization of EVs independently of the traditional morphological data, leveraging the typical behavior of indentation curves on isolated vesicles [6].
This approach allowed us to obtain detailed elasticity and topography maps on isolated vesicles, thus directly visualizing the EV locations. Furthermore, we delved in the EV biogenesis on the cell membrane, revealing an increase in vesicle release—most pronounced between days 3 and 10 of culture (*Fig.1*) —likely driven by enhanced membrane budding activity. This trend correlated with significant changes in the elastic modulus of the cells. By leveraging nanoindentation data alone, our method provides a reliable and precise framework for studying EV behavior and mechanical properties in situ, and opens new opportunities for exploring cellular processes and the dynamic role of EVs in cancer progression with AFM.

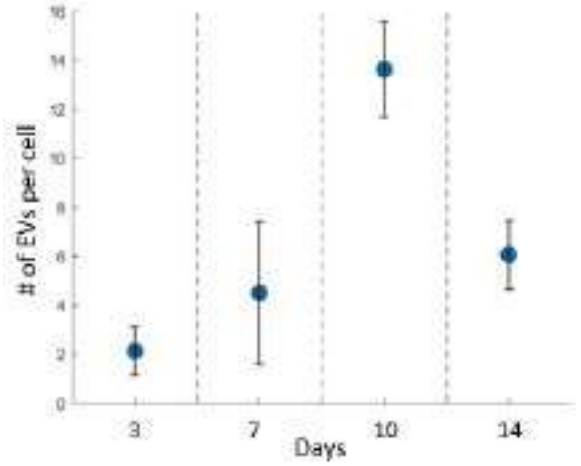

Figure 1. Temporal Evolution of the number of EVs-like curves of MDA-MB-231

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Observing Dynamic Conformational Changes within the Coiled-Coil Domain of Different Laminin Isoforms Using High-Speed Atomic Force Microscopy

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Laminins are trimeric glycoproteins with important roles in cell-matrix adhesion and tissue organization. The laminin α, ß, and γ-chains have short N-terminal arms, while their C-termini are connected via a triple coiled-coil domain, giving the laminin molecule a well-characterized cross-shaped morphology as a result. The C-terminus of laminin alpha chains contains additional globular laminin G-like (LG) domains with important roles in integrin-mediated cell adhesion. Dynamic conformational changes of different laminin domains have been implicated in regulating laminin function, but so far have not been analyzed at the single-molecule level. Here, we have used high-speed atomic force microscopy (HS-AFM) to investigate such dynamic conformational changes under physiological conditions at sub-second temporal resolution. While most laminin isoforms, including laminin-111 and -511, feature a stable S-shaped coiled-coil domain, the laminin-332 coiled-coil domains undergo rapid cycling between straight and bent conformations around a defined central molecular hinge [1]. Complementing the experimental AFM data with AlphaFold-based coiled-coil structure prediction enabled us to pinpoint the position of the hinge region, as well as to identify molecular rearrangement processes permitting hinge flexibility. Coarse-grained molecular dynamics simulations provide further support for a spatially defined kinking mechanism in the laminin-332 coiled-coil domain. Moreover, we show that coiled-coil unwinding within the hinge region makes laminin-332 susceptible to rapid protease digestion, identifying a novel conformationally-controlled mechanism of laminin degradation [2]. In additional experiments we observed the reversible unraveling of the C-terminus of the laminin-511 coiled-coil domain, temporarily exposing a ~25 amino acid stretch of the laminin α-chain. Using reconstructed laminin-511/integrin α6β1 complexes, we show that C-terminal coiled-coil unwinding disrupts the integrin α6β1 binding site located at the coiled-coil/LG domain interface of laminin-511, inducing rapid dissociation of the laminin/integrin complex [3]. Thus, HS-AFM can visualize molecular rearrangement processes within laminin coiled-coil domains and identify their role in regulating laminin function.

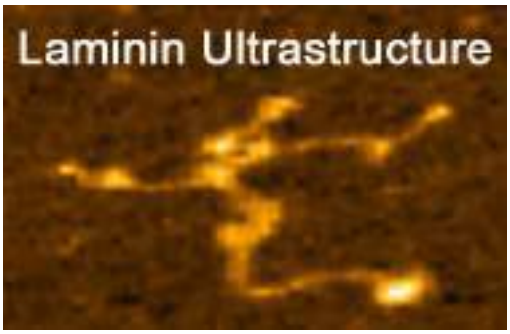

Figure 1. HS-AFM image of laminin-111

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SURFACE ACTIVE ANTIBIOTICS IN ACTION: A REAL-TIME STUDY OF THE KILLING MECHANISM USING HIGH-SPEED ATOMIC FORCE MICROSCOPY.

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Antimicrobial resistance (AMR) is one of the highest concerns in modern medical care. While a large number of potential drugs are introduced regularly, only a few reach clinical trials after years of preclinical testing. Therefore, increasing the preclinical screening efficiency and designing more efficient drugs are of utmost importance to combat AMR. To do so, understanding the mode of action of existing and new antibiotic candidates is essential in order to efficiently design novel antibiotics against multidrug-resistant bacteria. Despite this significance, the molecular mechanisms for many antimicrobials are still poorly understood. Due to their rapid activity at the nanoscale, a detailed mechanistic insight into the molecular interactions between antimicrobials and the target membrane is difficult to achieve. Thanks to high-speed atomic force microscopy (HS-AFM), it is now possible to follow (inter-/intra-) molecular interactions at sub-molecular spatial and millisecond temporal resolution [1-2]. Here, we investigate the molecular mechanism of different surface-active antibiotics and their interactions with membranes using HS-AFM. Our observations reveal the variety of pathways that individual antibiotics can follow to interact with the membrane/cell. We show that while certain antimicrobials spontaneously form pores on the membrane for lysing the cell, others induce global or local membrane thinning by spatially arranging the molecular assemblies at the membrane surface (Fig. 1). These results not only provide insight into the molecular mechanism of antimicrobials but also establish HS-AFM as a well-suited technique for studies of surface-active antimicrobials and their interaction mechanism at the membrane [3-6].

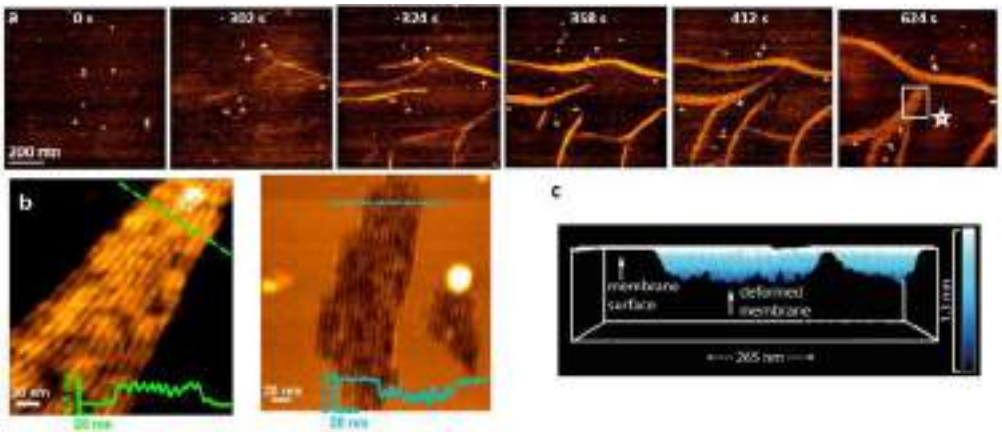

Figure 1. *The molecular mechanism of teixobactin mode of action captured by HS-AFM. (a) Snapshots of HS-AFM movie showing the assembly of teixobactin fibrils on top of a membrane. (b) High-resolution HS-AFM image of teixobactin fibril on top (left) and at the deformed membrane (right). (c) 3D rendered image of deformed membrane from b(right). Image reproduced from [6].*

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Visualization and analysis of P-glycoprotein dynamics by using HS-AFM

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P-glycoprotein (P-gp), one of the multidrug resistance transporters, is a membrane protein that transports a variety of drugs from the inside to the outside of cells via ATP hydrolysis [1]. Notably, P-gp plays a role in the efflux of chemotherapeutic agents, and its overexpression is recognized as one of the mechanisms by which cancer cells acquire drug resistance. Consequently, it is considered a critical factor that reduces the efficacy of chemotherapy in cancer treatment [2]. Understanding the correlation between the function and structural dynamics of P-gp is essential to elucidate the mechanisms underlying multidrug efflux and resistance. Structural information on P-gp has been revealed with such as Cryo-EM analysis. It is known that P-gp alternates between an Open conformation, where the nucleotide-binding domain (NBD) captures substrates from the intracellular side, and a Closed conformation, where the substrate is transported to the extracellular side [3]. By utilizing ATP hydrolysis, P-gp repeats the Open and Closed conformations, functioning like a pump to efflux substrates [4]. However, the dynamic transitions between these conformations during substrate efflux remain unclear. This study utilized high-speed atomic force microscopy (HS-AFM) to observe in real time provides insights into its dynamics and function.

Using HS-AFM, we observed P-gp samples reconstituted in nanodiscs (P-gp-ND) under nucleotide-free condition. With this free condition, the nucleotide-binding domain (NBD) exhibited significant opening and closing motions (Fig.1, Upper panel). Under conditions with 2 mM ATP, dynamics were observed in which the structure appeared to maintain an almost closed state (Fig.1, Lower panel).

Under nucleotide-free conditions, the NBD exhibited dynamic opening and closing motions, likely due to intrinsic protein fluctuations. In contrast, under ATP-bound conditions, the NBD displayed a concentration-dependent decrease in opening angle. This behavior suggests that ATP binding induces NBD dimerization (binding of the NBDs), leading to the Closed conformation, consistent with previous reports.

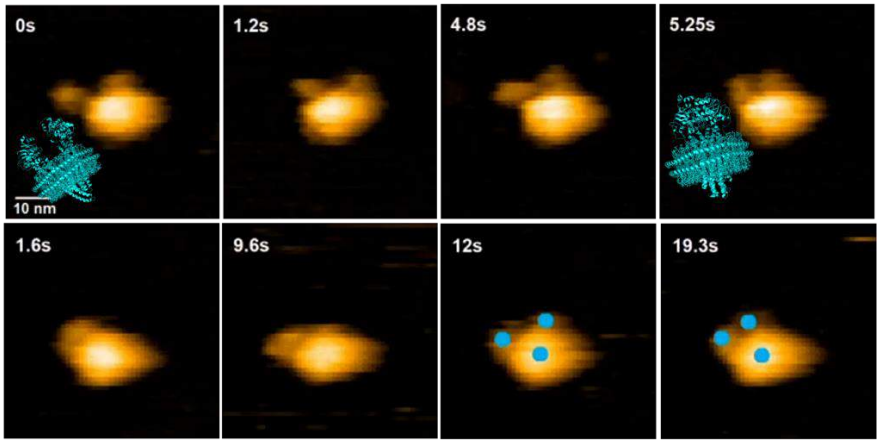

Figure 1. Dynamics of P-gp by HS-AFM observation, without ATP (Upper panel) and with 2 mM ATP (Lower panel)

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HIGH-SPEED AFM REVEALS ENZYME-INDUCED MEMBRANE REMODELLING

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Membrane-associated enzymes play crucial roles in maintaining cellular integrity and facilitating a wide range of essential dynamic processes. These enzymes are often involved in the synthesis and remodelling of phospholipid. Using high-speed atomic force microscopy (HS-AFM), we investigated the behaviour of a specific enzyme involved in phospholipid synthesis and its impact on membrane structure. Our study reveals that this enzyme can self-assemble into highly ordered arrays on membrane surfaces and selectively localize to regions of curvature. Its activity induces substantial membrane remodelling, driving the formation of distinct curvature patterns. Furthermore, we observed that the enzyme can sense and respond to membrane tension, reorganizing lipid bilayers to repair defects and maintain structural integrity. These findings highlight the enzyme's multifunctional role in sensing and inducing membrane tension, sorting lipids, and driving curvature changes. Such activities are critical not only for bacterial membrane dynamics but also for broader cellular processes across diverse organisms, shedding light on fundamental mechanisms of membrane remodelling and repair.

MurG ligase induces fluid regions in bacterial membranes

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Bacterial membranes have distinct components and are exposed to different environments, such as temperature variations, compared to eukaryotic cells. Microdomains of increased fluidity, known as Regions of Increased Fluidity (RIFs), have been observed in bacterial membranes and may act as platforms for the assembly of proteins. However, the exact mechanisms governing the organization of proteins in bacterial membranes are still not fully understood [1,2]. It is possible that protein assembly may not always rely on lipid segregation or pre-existing lipid domains, and other factors, such as electrostatic interactions between proteins, protein concentration, and lateral pressure, may be important.

This study used High-Speed Atomic Force Microscopy (HS-AFM) [3] and Fluorescence Microscopy to investigate the partitioning of MurG glycosyltransferases from Gram positive and Gram negative bacteria into lipid bilayers. Surprisingly, the MurG proteins were found to partition the membrane without requiring any preliminary lipid-induced partitioning. This study reveals important information about the membrane organisation of MurG proteins in phospholipid bilayers, providing insight into how these proteins help structure the bacterial membrane.

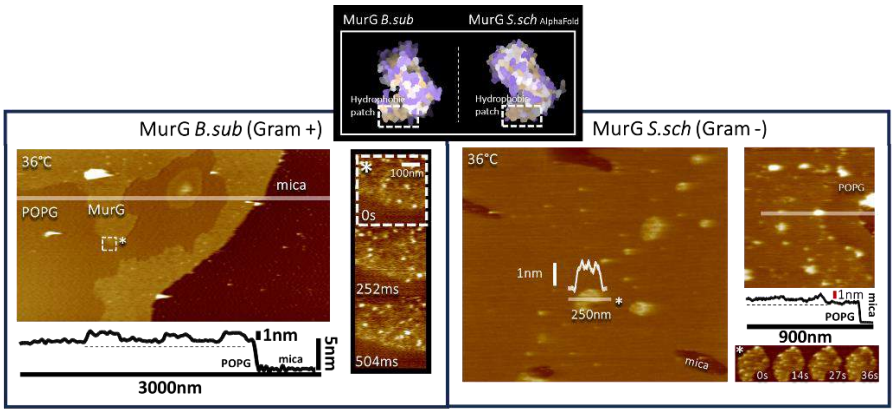

Figure 1. Partitioning of POPG bilayers by MurG proteins from Gram + and Gram - bacteria. On top, representation of the membrane interaction region of MurG proteins by AlphaFold. Bottom, afm images and topography profiles of POPG bilayers after addition of MurG protein: left, MurG Bacillus subtilis; right, MurG Salmonella schwarzengrund. The sequences show the diffusion of the proteins inside the partitions.

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To adhere or not to adhere: an approach to understand the initial biofilm formation

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The scientific community's understanding of the detailed mechanisms by which bacteria adhere (or do not adhere) to different surfaces is still far from complete. This discrepancy results from the need to reconcile two conflicting objectives: on the one hand, within the context of healthcare facilities (e.g., hospitals), it is imperative to prevent biofilm formation; on the other hand, within the domain of industrial microbiology (e.g., microbial electrochemical systems (MES)) it is crucial to induce it in accordance with the desired product. In addressing these two contradictory objectives, single cell force spectroscopy (SCFS) has been established as a research method. This methodology facilitates the characterization of properties, including the adhesion strength, by enabling the attachment of individual bacterial cells to diverse materials.
In the case of the Gram-positive bacterium *Staphylococcus aureus*, evidence has demonstrated that selectively structured surfaces [1], or alternatively hydrophobic/hydrophilic surfaces [2], can result in a reduction in bacterial adhesion. As previously mentioned, the formation of a stable and productive biofilm by the Gram-negative bacterium *Shewanella oneidensis* is an essential prerequisite for its use in an MES. Here, the experimental findings have indicated that the bacteria of the wildtype strain of *S. oneidensis* show a high degree of similarity in terms of adhesion strength to hydrophilic and hydrophobic surfaces. Interestingly, the adhesion of mutant strains of *S. oneidensis* to hydrophobic surfaces exhibited variations in comparison with one another [3].

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From Single Molecules to Tissues: Correlative BioAFM Serving as Core Facility

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Scientific Core Facilities are specialized service centers within research institutions that provide access to advanced technologies, equipment, and expertise. These centers are coupled with experts who provide training, technical support, education, and consultation to help researchers and students use the equipment effectively. We will use the story of our facility to show how unique it was to offer services focused purely on bioAFM and other specialized microscopies (e.g., Raman) in an area where electron microscopy or mass spectrometry laboratories routinely operate.
Only through intensive contact with colleagues, involvement in education, and convincing them to plan experiments could we use methods such as force microscopy in different modes to help deliver useful results in various bio-related projects. Examples include imaging single molecules [1], determining the effect of mechanical properties of the environment [2] or the effect of nanoparticles [3] on the growth and other properties of cells, and monitoring pathological phenomena in organs, such as the formation and treatment of fibrosis in liver tissue samples [4]. Figure 1 briefly illustrates selected examples of these applications.

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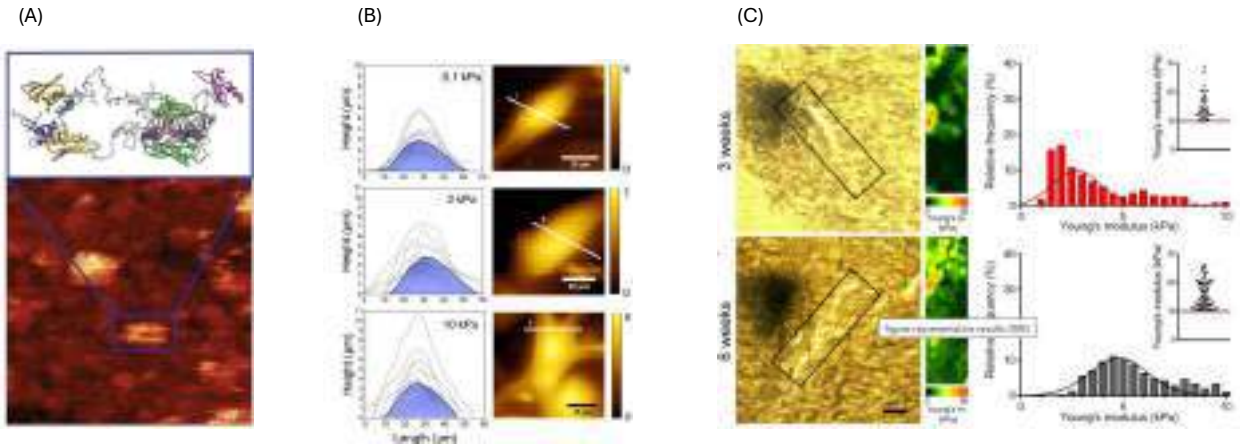

Figure 1. An example of the AFM application in the investigation of long RNA structure (A, adopted from [1]) is cell morphology changes affected by the different substrate stiffness (B, adopted from [2]) and collagen fibers formation in liver tissue slices (C, adopted from [4]).

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UNRAVELING LIPID MEMBRANE DYNAMICS AND PHASE BEHAVIOR USING AFM

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Synthetic lipid bilayers are crucial for modeling cell membranes, as they enable the controlled study of membrane properties and interactions in a simplified, reproducible environment. AFM-based force spectroscopy, in turn, is an ideal technique to investigate the mechanical properties of lipid bilayers at the nanoscale, their elastic mudulus [1], but also their deformation and rupture [2].

In lipid membranes, the ultimate lipid phase coexistence to be fully understood is transient nanodomains, often (confusedly) referred to as lipid rafts [3]. Based on current knowledge, microdomains in equilibrium are no longer considered suitable models for the biological structure that rafts represent. Multiscale spatiotemporal measurements of membrane mechanical properties can help to experimentally address different scenarios where membrane micro- and nanodomain formations provide theoretical support. AFM-based force spectroscopy can resolve the coexistence of domains at concentrations where height differences at domain boundaries are not detectable [4], providing an ideal approach for investigating the mechanical properties of lipid bilayers at the nanoscale. High-speed AFM imaging provides information about the dynamics of domain boundaries. Here, we will discuss several examples of non-equilibrium membrane fluctuations. First, the in situ conversion of sphingomyelin to ceramide. Ceramide is produced in cells from sphingomyelin by means of the enzymatic activity of endogenous sphingomyelinase, impacting the physicochemical properties of the membrane and inducing changes in the curvature, phase, segregation, and order (**Figure 1**). Then, we will discuss the effect of antimicrobial compounds. Mag2 and PGLa are two antimicrobial peptides that, upon their interaction with biomembranes, have been shown to gradually insert into the lipid bilayer as heterodimer clusters inducing several membrane perturbations, such as alterations in lipid packing, pore openings, and membrane disintegration. Using these examples, we will conclude that AFM measurements to explore the nanoscale mechanical properties and dynamic behavior of lipid bilayers enhance our understanding of membrane structure and function.

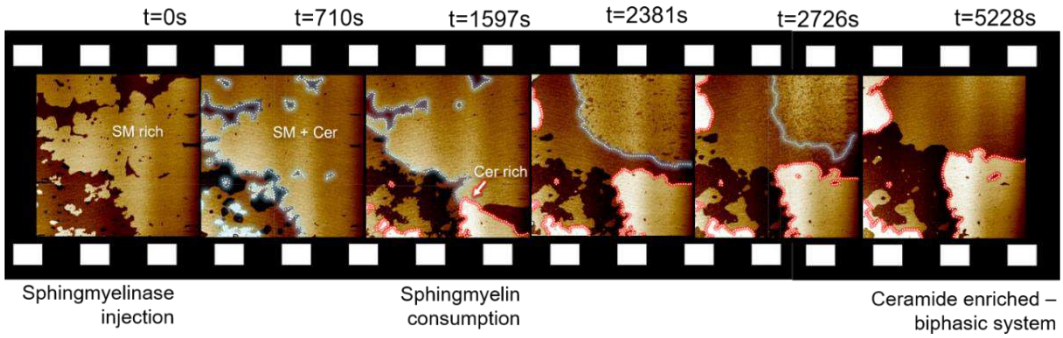

Figure 1. Fast nanomechanical mapping of a biphasic lipid membrane. After injection of sphingomyelinase, sphingomyelin-enriched domains evolve to ceramide-enriched domains, which impacts the lipid order, domain distribution and local mechanics.

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IR and microwave-Based Techniques for Analysing Molecular Signatures: A Novel Approach to Biomarker Detection

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In recent years, secreted extracellular vesicles (EVs), particularly from oncogenic cells, have garnered substantial attention due to their critical role in intercellular communication, especially within cancer progression. These nanometer-sized particles encapsulate unique protein and lipid compositions reflective of their cell of origin, serving as mediators in metastasis, treatment resistance, and immune evasion. Importantly, biomarkers within these vesicles are detectable in bodily fluids, presenting a promising, non-invasive avenue for early cancer screening and dynamic disease monitoring (Cordonnier et al., 2017) [1]. Furthermore, tracking fluctuations in circulating biomolecules offers new perspectives for proactive identification and tailored intervention in cancer progression (Vautrot et al., 2021) [2].

To address the need for a rapid and non-invasive detection method, we have used AFM-IR spectroscopy to get molecular fingerprint and shown single-vesicle spectral differences. In parallel, we have developed a microwave-based approach for analyzing molecular signatures associated with cancer biomarkers. This last technique leverages the unique dielectric properties of biomolecules, which exhibit distinct impedance values when subjected to microwave frequencies. Our methodology involves non-destructive analysis of target molecules using a microwave sensing device that transmits signals through biofunctionalized biochips. Following standardized protocols for the preparation and characterization of extracellular vesicles, we captured target biomarkers onto gold-coated biochips functionalized to selectively bind specific molecules. These biochips, enhanced with a bioreceptive layer, ensure the selective capture and immobilization of target biomarkers directly from biological samples. By applying a range of microwave frequencies, we measured variations in signal amplitude and impedance, enabling the differentiation of biomarker signatures based on their electrical and dielectric properties (Ionescu et al., 2018) [3]. This approach offers real-time, label-free detection, providing a streamlined path from sample preparation to analysis and facilitating high-throughput biomarker screening.

The developed microwave-based biosensor successfully detected and distinguished the target biomarker within complex biological samples by leveraging its unique electrical and dielectric properties. Through precise signal mapping at specific microwave frequencies, we achieved high sensitivity in capturing the impedance variations associated with the biomarker. This enabled the simultaneous assessment of both topographical and electrical characteristics of the samples, providing a comprehensive profile of the biomarker's molecular signature. Our results demonstrated reproducible signal amplitudes under controlled parameters, confirming the sensor's capacity for consistent and accurate biomarker detection, thereby validating its utility for real-time, label-free analysis.

This innovative microwave-based sensing approach represents a significant advancement in non-invasive biomarker detection. By enabling rapid, sensitive, and selective evaluation of circulating biomarkers in minimal sample volumes, this method holds considerable potential for early cancer detection and monitoring. The technology's high-throughput capabilities make it a promising tool for personalized medicine applications, allowing for more precise tracking and management of oncogenic conditions over time. This work has been supported by the French National Research Agency: ANR-23-CE51-0003 AMACOP.

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Raizada G, et al. Raman spectroscopy of large extracellular vesicles derived from human microvascular endothelial cells. Anal Bioanal Chem. 2024 doi: 10.1007/s00216-024-05567-4.

Enhancing atomic force microscopy with optical fibre interferometry

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Measuring cantilever deflection with optical fibres [1, 2] offers the clear advantage of highly precise detector calibration, leading to increased accuracy in determining the cantilever's position and deflection. However, compared to laser beam deflection, this method might initially appear more complex and less user-friendly.

In this work, we demonstrate that the optical fibre strategy has additional advantages beyhond increased accuracy and can be implemented in a way that simplifies its use, even compared to laser beam deflection [3]. For instance, it allows for the straightforward acquisition of a clear cantilever spectrum in liquid, revealing several eigenmodes in what would otherwise be a *forest of peaks*. In the context of contact mechanics, we show that this approach enables the simultaneous and direct measurement of the indentation (δ) and cantilever deflection (Δx). Hence, the enhanced accuracy provided by the interferometric method benefits both axes of a force curve f(z). We present how the use of optical fibre eigenmodes mitigates time drifts and signal fluctuations. Finally, we present measurements of the viscoelasticity of cells.

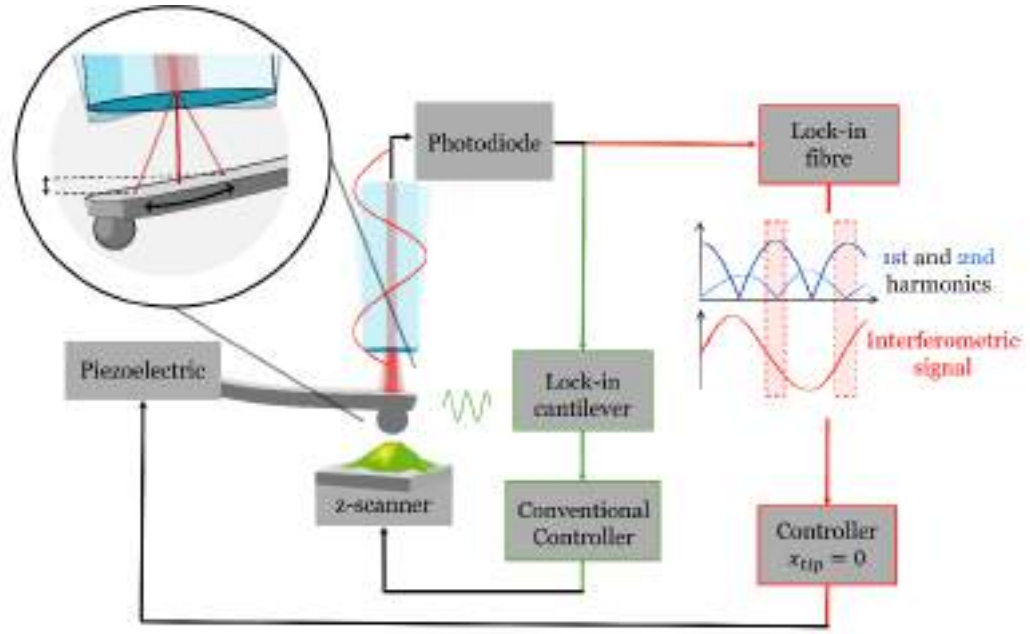

Figure 1. Interferometric AFM detection scheme using an optical fibre.

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IMPLEMENTATION OF MULTIPARAMETRIC CORELATIVE ATOMIC FORCE MICROSCOPY AND OPTICAL NANOSCOPY TO STUDY POLYPHARMACY IN HEPATIC ENDOTHELIUM

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Polypharmacy is defined as the concurrent use of five or more medications. Its social burden is soaring among elderly populations across Europe. Since detoxification is primarily facilitated by the liver, understanding drug interactions within hepatic cells is crucial. Our investigation focuses on liver sinusoidal endothelial cells (LSECs) due to their distinctive phenotype. LSECs possess fenestrations — transcellular pores ranging in diameter from 50 to 350 nm, i.e. being mainly beyond the resolution of conventional microscopy [1]. Fenestrations act like a canary in a coal mine, as their number and size respond quickly to physical and biochemical stimuli [2]. LSEC defenestration (significant decrease in the number of fenestrations), which often accompanies liver pathology and occurs gradually with age, impairs liver filtration and consequently hinders systemic detoxification.

To address polypharmacy, we have developed a multiparametric correlative microscopy platform based on atomic force microscopy (AFM). Over the past decade, we have advanced AFM imaging modalities to track dynamic changes in LSECs in response to drugs with a spatial resolution below 50 nm and on a timescale of less than one second [3,4]. Additionally, we have characterized the nanomechanical properties of LSECs in various liver pathologies [5], including a genetic model of liver inflammation [6]. Notably, we hypothesize a correlation between the deformability of fenestrations and the overall elasticity of the cell [6]. To investigate the effects of cytoskeletal changes on LSEC fenestrations at the nanoscale, we aim to integrate AFM with a super-resolution optical nanoscopy technique — structured illumination microscopy (SIM) — implementing a specialized design developed at the University of Bielefeld. The current stage of our study of polypharmacy on hepatic cells and the implementation of an innovative approach to correlative nanoscopy will be presented.

Acknowledgement:
This study was supported by the European Union's European Innovation Council (EIC) PATHFINDER Open Programme, project DeLIVERy, under grant agreement No. 101046928 and Hop-On Facility HORIZON-WIDERA program associated with DeLIVERy

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Exploring the biophysics of *P. kessleri* and *S. cerevisiae* cells in coculture using atomic force microscopy for the production of biofuels

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Microalgae are known in the bioproduction field for their ability to accumulate large quantities of lipids while growing on low-cost substrates [1]. However, their applicability on a large scale remains challenging because of low productivity issues, mainly due to their slow growth rate. To increase biomass production, one of the strategies explored is the coculturing of microalgae with other microorganisms that could interact in a symbiotic way with them [2]. For this, yeasts are ideal candidates as during their growth, they produce CO_2 that can be used by the microalgae, that on the other hand produce O_2 [3]. This symbiotic circle enables higher microalgae productivity, and thus enhanced lipid production. While examples of such co-cultures have been reported [4], fundamental questions remain as to whether the presence of another microorganisms has an impact on the cell's biophysical properties or on how cells interact with each other's. To address these questions, Atomic Force Microscopy (AFM) was employed to investigate how co-culture conditions modify cellular surface properties. The analysis focused on morphology and ultrastructure, including roughness measurements to assess surface structure changes and Young's Modulus to determine cell's mechanical properties. Physicochemical properties, such as hydrophobicity and surface charge, were also examined, as these reflect modifications in cell wall composition that can be influenced by growth conditions. Additionally, the interactions between the algae and yeast were probed using single-cell force spectroscopy technique with FluidFM to understand how the cells interact with one another and determine the nature and strength of the forces at play. So far, the results obtained show that the co-presence of the two microorganisms' species in the same environment induces significant changes of the cell surface properties. Specifically, microalgal cells exhibit significant alterations in both morphology and mechanical properties when co-cultured with yeasts in a nutrient-rich medium, which could be due to the production of exopolysaccharides (EPS) by the cells in these conditions. In contrast, yeasts do not present significant modifications of their nanomechanical properties. However, modifications in their surface ultrastructure indicate changes in the composition or organization of their cell wall. These observations suggest metabolic shifts in both cell types during coculture, which was further validated by Raman spectroscopy analyses. Overall, this ongoing study provides valuable insights into the behavior of cells in binary culture systems. AFM analyses offer a molecular-level understanding of the interactions between the two species, making these findings essential for advancing of co-cultures on industrial scale, particularly for the design of an economically and environmentally sustainable process.

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Understanding cancer aggregate migration; utilising Bio-AFM, biomechanics and molecular biology at the peritoneal boundary

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The mechanisms of cellular migration are fundamental, underpinning crucial biological processes in development and disease. A highly complex process, migration can occur as single cell, cellular cluster and/or multi-cellular aggregates, modulating vital physiological and pathological processes, including immune response, wound healing, cancer metastasis and embryogenesis. Multiple assays have evolved to study aspects of cellular migration *in vitro*, from microfluidics to optical microscopy and quantitative modelling, highlighting an adaptive process that depends on, and responds to, physical, molecular and chemical triggers.

In our lab, we are utilising BioAFM-based biomechanical analysis techniques in conjunction with optical microscopy and signalling perturbations (siRNA and Small chemical drug treatments) to better understand motility. This allows for the delineation of cellular and aggregate migration processes in the context of Ovarian Cancer and peritoneal metastasis.

Utilising mono and co-culture spheroids, we have optimised high throughput nano mechanics and rheological analysis to delineate Ovarian cancer cell colonisation and invasion to a matrix mimic. When ovarian cancer cells (SKOV3) are cultured together with a fibroblast cell line (MRC-5) component they are significantly softer than when in mono-culture spheroids (389.1±128.5 kPa vs 452.3±221.3 kPa). The outer proliferative zone of our spheroids transform (MET) and drive peritoneal adhesion. In a 3D *in vitro* model of this process, SKOV3 and MRC-5 co-cultures colonise a greater proportion of the matrix rich surface (4.84 mm rather than 1.3 mm SKOV3 only) in x,y – showing a 50% reduction in spheroid maximal height, and drive down into the substrate in z, with a single cell invasion maximal distance of 147.7 µm. Conversely, SKOV3 only cell spheroids only lose 10% of their height and exhibit a maximal single cell invasion to 73 µm.

Utilising the full range of BioAFM measurements we next aim to optimise our pioneering glycoform single molecule force spectroscopy approach, to identify ligand binding processes that are key to initial contact and subsequent drive through the matrix-rich environment. This will be achieved via the implementation of biochemically modified cantilevers and indenters of crucial integrin binding motifs (L1CAM, ICAM1, CA125, CD44, UPAI-1), that we hypothesise are important for the initial binding event to the mesothelial barrier.

This investigation combines novel multi-compartment, automated, large-scale BioAFM alongside high-content immunofluorescence to delineate the multiple stages of cell aggregate migration in cancer metastasis. Combining biophysical properties with epigenetic modification, transcriptome, bioinformatics and image analysis will provide new insights into multicellular aggregate architecture, cell migration/invasion, and fundamental cancer metastatic mechanisms.

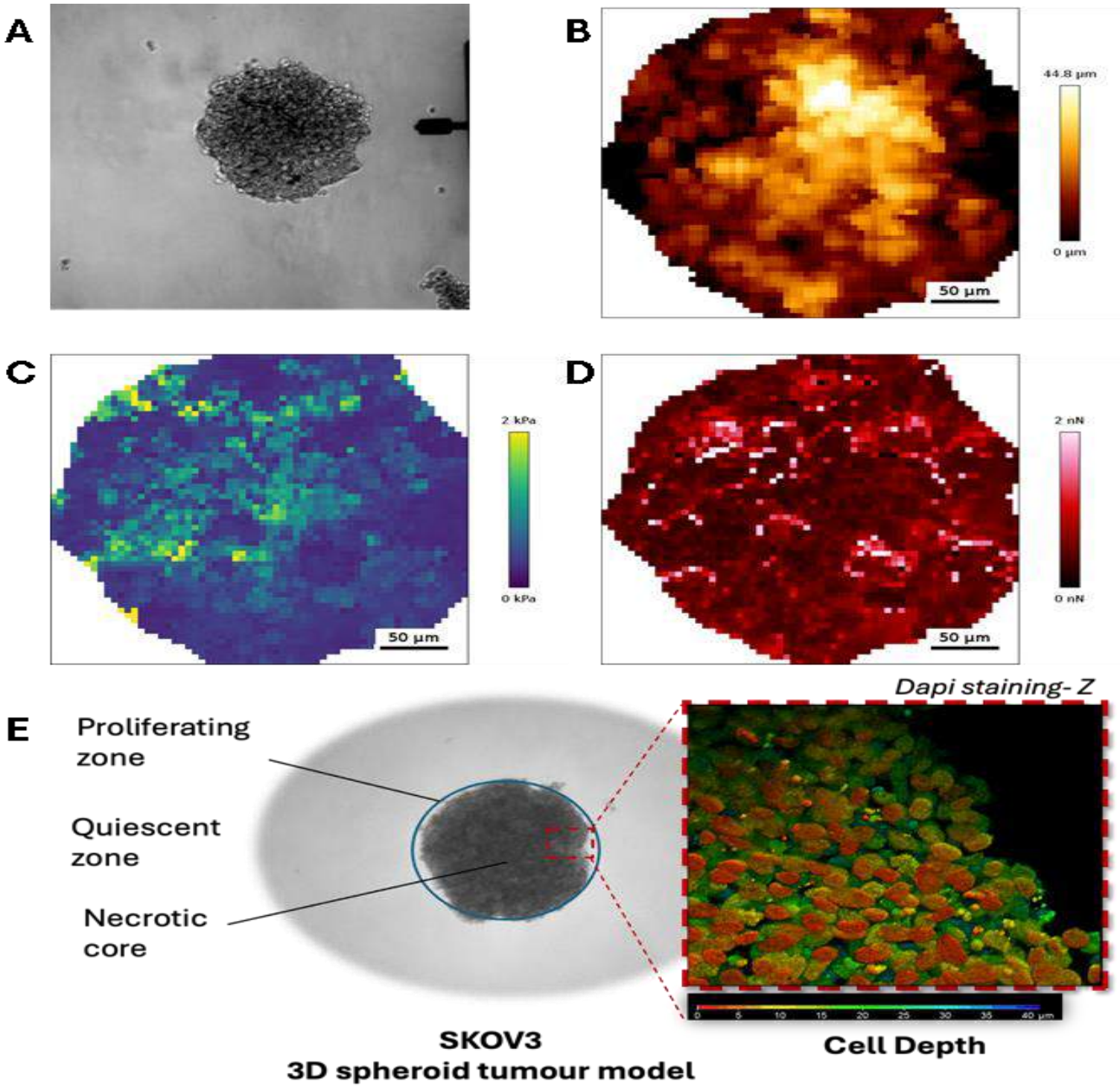

Figure 1. Graphical Abstract of BioAFM force maps collated, and CLSM fluorescence image. [A] Brightfield Image of a SKOV3 mono-culture spheroid, seeding density 2.5k cells, taken prior to nanoindentation experiments. [B] Combined topography channel of the piezo and stepper motors. [C] Upper-end Young's Modulus of the SKOV3 spheroid. [D] Adhesion channel showing the interaction with the indenter and the spheroid surface. All measurements were conducted using a Bruker JPK NanoWizard V multi-compartment BioAFM with a Bruker SAA SPH 5µm probe. [E] CLSM image of SKOV3 spheroid showing cell depth via a Hoechst nuclear stain.

Mechanical properties of circulating tumour cells (CTC) and its clusters.

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More than 66% of cancer-associated mortality is caused by tumour metastasis. This complex multistep process lays on the dissemination of tumour cells, known as circulating tumor cells (CTCs) that reach distant tissues via the bloodstream and initiate growth of secondary tumours. Recent studies have shown that CTCs clusters increase the metastatic potential by 50 times in comparison to single CTCs[1] and are able to pass through narrow capillaries, by deforming themselves whilst experiencing and resisting mechanical stresses at different rates[2]. Resources are being directed towards detection and phenotyping of these rare CTCs in the bloodstream, as they appear as potential non-invasive biomarkers for cancer diagnosis and prognosis[3]. Nevertheless, little is known about their mechanical properties.

Here, we explore the viscoelasticity of isolated CTCs and CTCs clusters from a cell line derived from a patient with colorectal cancer (CTC-44) using atomic force microscopy (AFM) viscoelastic measurements (Fig. 1). Our preliminary results, based on viscoelastic fits of force curves on individual CTCs, revealed that isolated CTC-44 has a stiffness scale factor (E_0) comparable to other circulating cells, such as leukocytes, while having a slightly more solid like behavior [4]. However, CTC-44 cells are significantly softer (10 times) than other cancerous colon cells[5]. To assess the time-scale mechanical response of CTC-44 we are currently carrying out AFM-based micro-rheology experiments, which provide a model free mechanical characterization, and allowing the detection of other mechanical responses, such as poroelasticity[6].

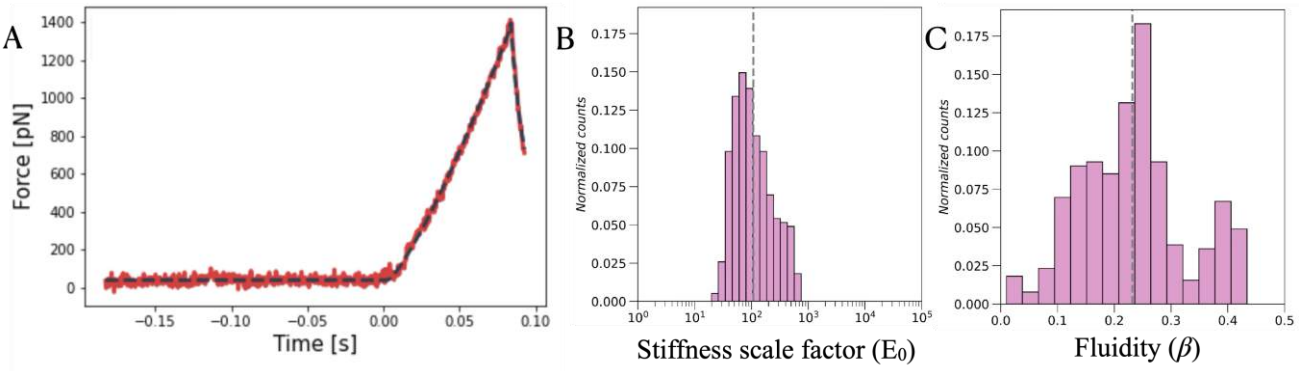

Figure 1. Viscoelasticity of circulating tumour cells (CTC 44), (**A**) Representative force vs time curve from AFM fitted using the Ting model. (**B**) Distributions of stiffness scaling factor (E_0) and (**C**) fluidity index (β) from Ting fitting.

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Multifunctional Phenotyping of Individual Cancer Cells Using SICM Probes for Nanoscale Assays and Single-Cell Omics

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Scanning Ion Conductance Microscopy (SICM) probes, employing micro- or nanopipettes, are ideally suited for performing nanoscale assays on cell surfaces. These probes facilitate a range of applications including patch-clamp recording from individual surface structures, iontophoretic delivery of reagents, and pressure micro-application to probe mechanical properties or deliver agents. The SICM methodology enables high-resolution imaging of uneven and convoluted cell surfaces by ensuring the pipette approaches from above, thereby avoiding surface dragging and potential damage [1].

In this study, we combined SICM imaging with fluorescence microscopy and nanomechanical mapping to observe changes in the mechanical properties of single cancer cells in response to various chemotherapeutic drugs targeting different components of the cytoskeleton [2,3]. We demonstrated that SICM probes can function as local biosensors measuring extracellular pH (pHe) [4], reactive oxygen species (ROS) [5], and various metabolites and electrochemically active compounds in living cells, organoids, and animal tumor models [6-8].

By integrating pHe data with mechanical properties and ROS levels, we could phenotype the heterogeneity of individual cells more precisely. Additionally, SICM probes were utilized for nanobiopsy, enabling single-cell omics analysis of pre-characterized cells. This multifunctional approach positions SICM as a unique tool for detailed phenotyping of individual cell heterogeneity and for verifying gene expression profiles of different subpopulations at the single-cell level, offering significant applications in cancer research.

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Key roles of N-terminal capping and fibrillation in the toxicity of phenol soluble modulins secreted by *S. aureus*

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The virulence of Staphylococcus aureus, a multi-drug resistant pathogen, notably depends on the expression of the phenol soluble modulins α3 (PSMα3) peptides [1], able to self-assemble into amyloid-like cross-α fibrils. Despite remarkable advances evidencing the crucial, yet insufficient, role of fibrils in PSMα3 cytotoxic activities towards host cells [2, 3], the relationship between its molecular structures, assembly propensities, and modes of action remains an open intriguing problem. In this study [4], combining Atomic Force Microscopy (AFM) imaging and infrared spectroscopy, we first demonstrated in vitro that the charge provided by the N-terminal capping of PSMα3 alters its interactions with model membranes of controlled lipid composition. N-terminal capping eventually dictates PSMα3 - membrane binding via electrostatic interactions with the lipid head groups. Furthermore, PSMα3 insertion within the lipid bilayer is favoured by hydrophobic interactions with the lipid acyl chains, only in the fluid-phase of membranes, and not in the gel-like ordered domains. Strikingly, our real-time AFM imaging emphasizes how intermediate protofibrillar entities, formed along PSMα3 self-assembly and promoted at the membrane interface, likely disrupt membrane integrity via peptide accumulation, and subsequent membrane thinning in a peptide concentration and lipid-dependent manner (Fig.1). Furthermore, an *in cellulo* approach enabled the identification of soluble intermediate species as the primary of cytotoxic activity and supported their characterization in complex mediums closely mimicking physiological conditions. Overall, our multiscale and multimodal approach sheds new light on the key roles of the N-terminal capping of PSMα3 and their intermediate self-assembling entities, rather than mature fibrils, in dictating deleterious interactions with specific lipid membranes, likely underscoring its ultimate cellular toxicity *in vivo*, and in turn *S. aureus* pathogenesis.

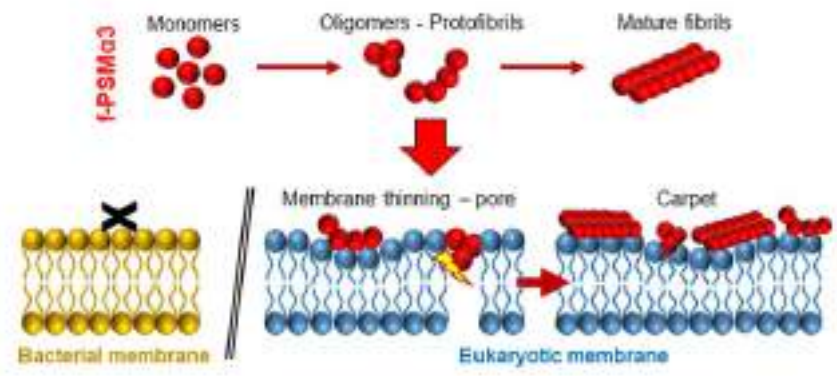

Figure 1. Schematic representation of the lipid-dependent deleterious activities of intermediate assemblies formed by N-terminal capping PSMα3, toxins secreted by S. aureus.

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Transient effects on mechanical properties during cell division of *Escherichia coli* studied by high-speed in-line force mapping

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High-speed atomic force microscopy (HS-AFM) is widely used to investigate biological samples ranging from single proteins to cells. The high frame rates (in the order of 10 frames per second), coupled with low invasiveness and high resolution make HS-AFM exceptionally suitable to study dynamics on a biologically relevant scale. Here, high-speed in-line force mapping (HS-iFM) is presented which records simultaneous topography and mechanical properties with independent resolutions. Using HS-iFM, living *Escherichia coli* cells were investigated by recording topography and mechanical properties during the cell division process with approximately 15s per frame. It was revealed that the division site gradually stiffened as the cell constricted locally. A thorough contact mechanical analysis revealed that the changes in stiffness are not likely to be influenced by the severe topographical deformation during division. The stiffness increase is hypothesized to be caused by localized tension and cell wall thickening. Furthermore, high-resolution imaging was achieved revealing nanometer sized dynamic pores on the outer membrane and formation as well as rupturing of bridges spanning the division site. A serendipitous observation of a bursting cell revealed the influence of Turgor pressure to the measured mechanical properties by a clearly defined sudden drop of elastic modulus concurrent with the bursting event.
HS-iFM revealed dynamics in the mechanical properties of the division process of E. coli, demonstrating mapping of mechanical properties coupled with high-resolution imaging at high speeds.

Quantitative imaging of bacterial cell wall with AFM: function of PBP1a synthase

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Streptococcus pnuemoniae (*S. pneumoniae*) is the leading cause of death by Pneumonia (1M cases annually) and is becoming resistant to existing antimicrobials. We urgently need to develop long-awaited new solutions. However, we first need to understand vital components of bacteria cells: their cell-wall composed mainly of peptidoglycan (PG). Here we will apply ultra-resolution microscopy [1-2] and software analysis to understand the basic framework of the cell-wall (*i.e.* PG).

After years of research using atomic force microscopy (AFM) on other Gram-positive bacterial species, the conclusion is that the PG is a highly porous heterogeneous hydrogel with four different architectures [2]. In this project, we apply the same tools to obtain the molecular architecture (on the order of 1 nm) of *S. pneumoniae* PG for the first time. We have previously explored *cocci*-shaped and *rod*-shaped cells. However, *S. pneumoniae* does not fit either of these shapes, it is classed as an *ovococci*. The shape difference is driven by the coordination of the Division and Elangosome machineries [1]. They are complex assemblies of synthesis and hydrolysis enzymes working together to create new PG. Here we use high resolution AFM in PeakForce^{TM} mode in liquid to image purified PG and whole cells to reveal the novel cell wall architecture from *S. pneumoniae*. Then, to study the effect of the division and elongasome machinery on cell shape and PG architecture, we repeat the experiment with a mutant lacking a synthase called PBP1a. Finally, we use custom-made image analysis tools (combining Gwyddion, FIJI and ChimeraX) to obtain quantitative comparisons between these samples (*e.g.* WT and the PBP1a mutant). This is bringing us one step closer to determine the function of individual enzymes involved in the cell wall synthesis pathway and how they create nanometric structures that drive the different shapes of bacteria. This will aid us in the future towards finding better strategies to combat infectious diseases resistant to antibiotics such as Pneumonia.

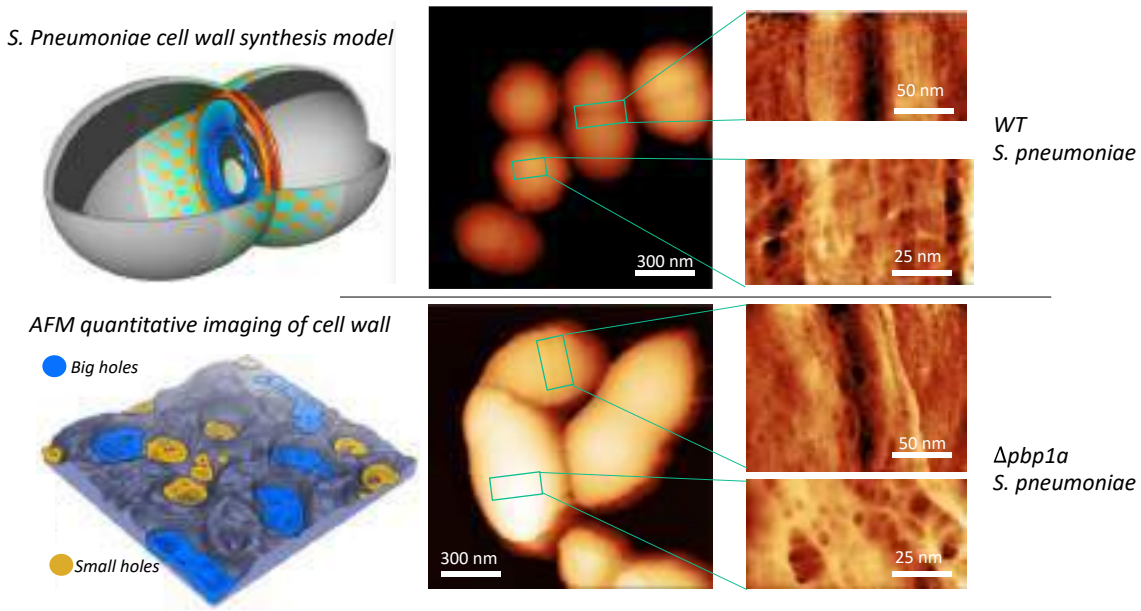

Figure 1. Top left corner, model of cell division in S. pneumoniae; right panels, AFM in liquid of whole bacteria cells (WT and Δpbp1a); Bottom left corner, 3D representation of an AFM image after using the custom-made software.

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Sialic acid depletion leads to profound remodelling of glycocalyx architecture and mechanics in pancreatic cancer cells

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Pancreatic cancer remains one of the most lethal malignancies due in part to issues with early detection and high levels of drug resistance [1-2]. Partly to blame for these issues is the glycocalyx, an extracellular structure found on most cells that is aberrantly glycosylated and has overexpressed biopolymers in cancerous cells [3]. In pancreatic cancer, this includes higher levels of mucin expression, increased sialylation, and more hyaluronic acid production [4]. Although there is considerable work detailing the biochemical role of these glycocalyx modifications, we sought to understand their role in the architecture and mechanical properties of the cell. The mechanical profiling of cells is an emerging field [5], yet the role of the glycocalyx from a mechanical standpoint remains less understood. To help elucidate these properties, we enzymatically degraded different components of the glycocalyx commonly found to be aberrantly expressed in pancreatic cancer (e.g., N-glycans, sialic acids, mucins, and hyaluronic acid) and visualized changes in the structure of the membrane via atomic force microscopy (AFM), confocal fluorescence microscopy and scanning electron microscopy. We observed a profound reduction in microvilli density and thickness that was the most consistent with sialic acid removal across all three cell lines investigated. Using AFM-based nanomechanical mapping, we investigated changes in cell surface mechanics and observed a significant reduction in the viscoelastic properties (elastic storage and viscous loss moduli) when removing sialic acid. This observation suggests that the cell surface softens and fluidizes in response to desialylation. In addition, preliminary cytokine expression data suggests that sialic acid removal leads to a pronounced pro-inflammatory response of cytotoxic $CD8^+$ T Lymphocytes, greater than removing other glycocalyx components. Lastly, a glycomics study also revealed unique changes in the structure of N- and O-glycans, with significantly more heterogeneity in the structure of N-glycans on pancreatic cancer cells, and O-glycans showing a particularly higher degree of sialic acid deposition. Future studies will attempt to translate *in vitro* observations of de-glycosylation with patient tissue viscoelastic data to highlight the role of glycocalyx modulation at an intratumoral level to better understand chemo and immune therapy resistance.

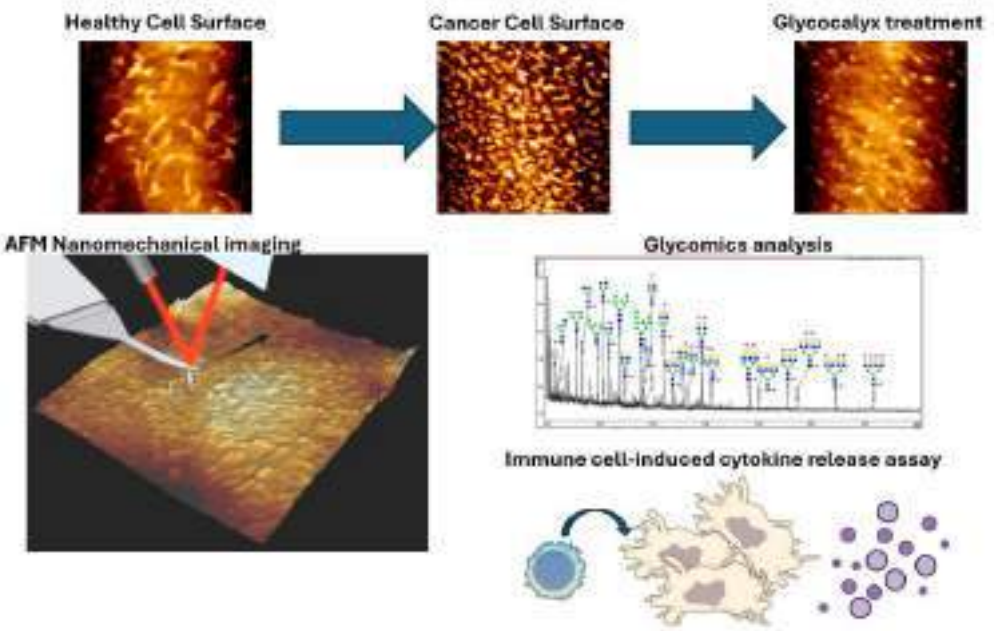

Figure 1. Increased density of glycocalyx components in cancerous cells leads to significant changes in cell surface morphology and mechanics, which are both reduced with removal of specific glycocalyx components (particuarly mucins and sialic acid).

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posters

	POSTERS 1 - 9 APRIL	
P1	Horacio V. Guzman	Unmasking biomacromolecular conformational dynamics from AFM images with dynamic modes and molecular kinetics models
P2	Matthew Brukman	Multi-Scale AFM Elucidates the Mechano-Transduction Driving Hair Follicle Regeneration
P3	João Belo	Quick and simple method to obtain viscoelastic parameters from force curves
P4	Jean-Luc Pellequer	Dual quantification for elasticity heterogeneity of soft materials
P6	Itzel Garcia Monge	Revealing the unbinding mechanics of hyaluronan? receptor interactions on live cells
P7	Dawid Lupa	Quantitative Nanomechanical Mapping Reveals Nanscale Heterogenity Of Inner Leaflet Of Inner Mitochondrial Membrane
P8	Carine Assaf	Development Of Atomic Force Microscopy Related Modes For The Study Of Plasma Membrane Repair
P9	Ana Svetic	From Liposome Fusion to Giant Plasma Membrane Vesicle Supported Plasma Membrane Models: Platforms to Study Extracellular Vesicle Internalization Dynamics in Breast Cancer
P10	Tim Kutz	Red blood cell membrane tension modulation by photo switchable molecules
P11	Mar Alcaraz Hurtado	Methylglyoxal-Induced Changes in Nuclear Viscoelasticity and Lamina Integrity

P12	Panagis Polykretis	The Impact of Actin Mutations on Neuronal Progenitor Cells studied by Atomic Force and Confocal Microscopies
P13	Manuela Brás	Quantification Of Mechanical Properties By Dynamic Force Microscopy Can Be A Novel Biomarker In Colorectal Cancer Disease
P14	Ellen Juel Pørtner	Viscoelastic differences between isolated and live cancer cell nuclei resolved with AFM microrheology
P15	Radka Obořilová	AFM Spectroscopy for the Study of Lipid Bilayer Stability and Morphology
P16	Martin Eduardo Villanueva	QCM-D and AFM combination: a ?marriage parfait? for experimental lipid membrane biophysics studies
P17	Devam Purohit	Quantifying and manipulating the molecular elasticity of Drosophila
P18	Sujal Kataria	From monolayers organized structures to perturbed mutistratified structures: interplay of cell-cell adhesion, cytoskeleton and viscoelasticty
P19	Samer Alokaidi	Patchy Adhesion of Staphylococcus aureus on Structured Surfaces Uncovered via Single Cell Force Spectroscopy
P20	Remy Ratajczak	Nanoscale electrical properties of biological nanofiber networks in Cable Bacteria
P21	Marcos Carrera	The influence of mechanical stretching on the conformational and ionization properties of linear poly(ethylenimine)
P22	Claudia Chitty	Morphometric quantification and visualisation of helical filament

		structures by integration and augmentation of cryo-electron microscopy data in simulation atomic force microscopy
P23	Javier Orradre Altabás	Stretching of hyaluronic acid at the single molecule level
P24	Laura Wolfthaler	Pulling Geometry as a Design Parameter for Coiled Coil-Based Molecular Force Sensors
	POSTERS 2 - 10 APRIL	
P25	Kubra Kelleci	Investigation of Effet the Mechanical Parameters of the Glioma and Keratinocyte Cells by using Finite Element Methods
P26	Jens Uwe Neurohr	Impact Of Geometry On Chemical Analysis For Photoelectron Spectroscopy Of Black Silicon
P27	Simone Dinarelli	Characterization of membrane budding and extracellular vesicles with AFM
P28	Mengjia Xu	FluidFM and electrophysiology
P29	Alexander Cartagena-Rivera	Ezrin is a major regulator of viscoelastic properties and force generation in T Lymphocytes during immunological synapse formation
P30	Vincent Fideli	Engineering bacterial traps to understand and inspire next-generation antibiotics
P31	Ekaterina Sherstyukova	Using Afm To Assess The Quality Of Whole Blood After Pathogen Reduction With Riboflavin And Uv Irradiation
P32	Viktoria Sergunova	AFM Study Of The Effect Of siRNA On Macrophage Phenotype
P33	Adrià Botet-Carreras	Cannabidiol interaction with neuronal model membranes

P34	Michal Swietlicki	The effect of DMSO on morphological and physiological traits of bacteria
P35	Giacomo Paccagnan	Understanding the biophysical role of Desmoglein 3 in Pemphigus Vulgaris via Fluidic Force Microscopy and Total Internal Reflection Fluorescence Microscopy
P36	Joshua Preston	Determining aortic wall nanomechanical properties in neonatal patients with coarctation of the aorta
P37	Konrad Szymanowski	Enhanced Understanding of Biomechanical Observations in AFM Studies Through Quantitative Analysis of Cytoskeletal Filaments from Fluorescence Imaging
P38	Nikolina Peranic	Nanomechanical Signature Of Dopaminergic Neurons
P39	Johannes Mischo	Characterization of the unique attachment organelle of Giardia duodenalis trophozoites by Single-Cell Force Spectroscopy
P40	Sukanya Das	Single-molecule force spectroscopy of a commercial thin film hydrogel
P41	Tom White	Nanomechanics of cell-derived matrices as a functional read-out in Collagen VI-related Congenital Muscular Dystrophies
P42	Izabela Swietlicka	Collagen Type I: morphological and molecular structure changes induced by different concentrations of ?-hydroxy ?-methylbutyric acid
P43	Jakub Máčala	Dual-Organoid Biosensor for Monitoring Cardiac Conduction Disturbances *In Vitro*
P44	Carlos Marcuello	Vipp-1 Mediated Membrane Remodelling Repair: Dynamics and

		Mechanics with Fast-AFM Imaging and Nanomechanics
P45	Yiwei Zheng	Influence of Internal and External Influences on the Peptide Assemblies Revealed by Atomic Force Microscopy
P46	Hirona Osaka	Analysis of the bundling of F-actin by ?-actinin
P47	Jae Won Jang	Spontaneously Directed Loop Extrusion in SMC complexes Emerges from Broken Detailed Balance and Anisotropic DNA Search.
P48	Jakub Hruska	AFM application across the life sciences field and its combination with special techniques
P49	Vincent Dupres	AFM automation for mechanical tracking of mammalian cells
P50	Harinderbir Kaur	Re-examining the topography of pentameric IgM-J chain using AFM

Unmasking biomacromolecular conformational dynamics from AFM images with dynamic modes and molecular kinetics models

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The adsorption of biomacromolecules onto AFM-substrates are crucial to understand and properly interpret AFM bioimages (tapping, multifrequency or high-speed modes). In particular, when the proteins have several domains with similar length-scales but slightly different conformations (e.g. RBD up or down of the SARS-CoV-2 spike). We have performed extensive simulations of the SARS-CoV-2 spike for various mutants and 2 modeled substrates1 (hydrophilic and hydrophobic, which can be extended to any surface-type via contact angles). Those data, helped us analyze and trained a simple dynamic mode decomposition2 (DMD) customized model for attending urgent 2D interpretations of more flexible domains in the spike's RBDs. Moreover, implementing VampNets3 together with our initial dynamic reconstruction could lead to the partial mapping of molecules below the substrates for experiments and thus to extend the predictability of states distributed on our adsorbed biomacromolecules. Our results allow us to reconstruct and predict with a minimal number of dynamic modes, the 2D domain motions of the SARS-CoV-2 spike adsorbed onto measured substrates. We show an extremely good agreement between RMSDs and Gyration radius between the HS-AFM and DMD-reconstruction.

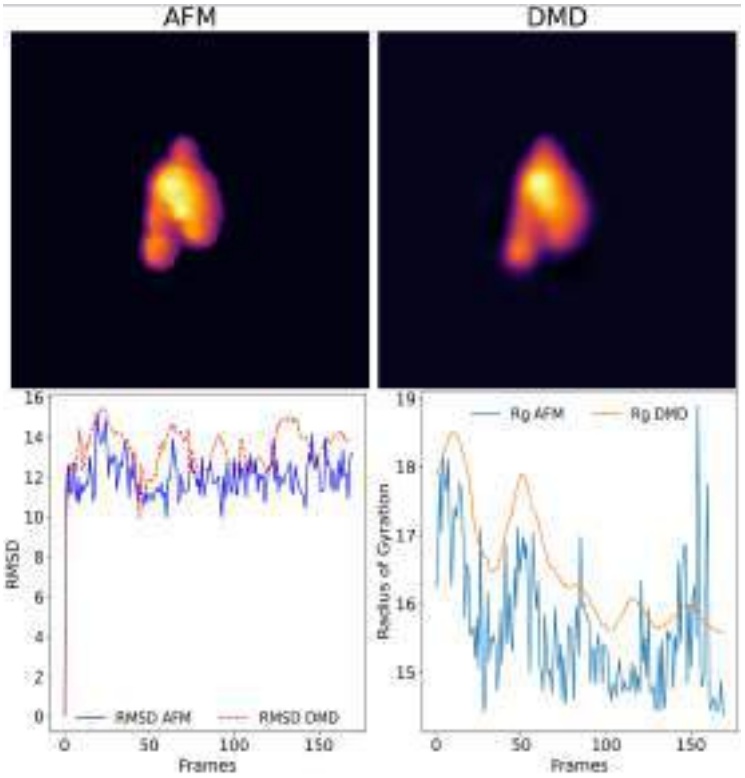

Figure 1. Reconstruction with a minimal number of dynamic modes of the 2D domain motions of the SARS-CoV-2 spike adsorbed onto measured substrates. We show an extremely good agreement between RMSDs and Gyration radius between the HS-AFM and the customized DMD-reconstruction

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Multi-Scale AFM Elucidates the Mechanotransduction Driving Hair Follicle Regeneration

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The rigid underlying matrix supporting mammalian skin triggers mechano-transduction in wounds to generate a tissue environment biased towards scarring and against regeneration, a phenomenon that also limits hair regrowth after a wound. One well-established model of mammalian regeneration is called Wound-Induced Hair Neogenesis (WIHN) and it requires an optimal tissue rigidity and a particular set of signal transduction pathways known as Wnt signaling.

The precise mechano-regulatory role of Wnt signaling and its contribution to hair regeneration has not yet been determined. We employed multiprobe AFM—ranging from blunt colloidal probes suitable for cell-scale measurements to home-made ultrahigh aspect ratio probes which can precisely measure forces at specific locations within cells—integrated with fluorescent optical microscopy to show that Wnt-mediated mechano-regulation impacts tissue mechanics during WIHN and dictates mechanosensitive cell behaviors in skin cells. [1]

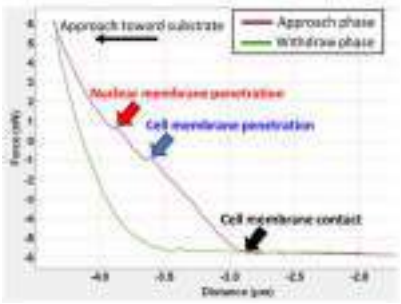

Figure 1. Resolving of puncture forces of cell and nuclear membranes via AFM

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Quick and simple method to obtain viscoelastic parameters from force curves

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The interplay between the mechanical properties of cells and their surroundings determines a plethora of key aspects in biological systems. In this context, measuring and correctly interpreting cell mechanical properties are essential steps. Cells are dynamic objects that should, at the very least, be considered viscoelastic. Atomic force microscopy (AFM) is routinely used to obtain a cell's Young's modulus, akin to a simple elastic response. This is, in part, due to the complexity of extracting additional information from force-distance curves, which often requires complex numerical algorithms [1] or strategies [2]. Here, instead, we provide analytical solutions that can be readily used to fit the standard linear solid (SLS) model to force-distance curves. We consider the two most commonly used indenter geometries - spherical and conical, and provide examples of curves acquired from cells and from rubber. Moreover, we demonstrate that considering the dynamic response of the indented objects allows for a different interpretation of adhesion.

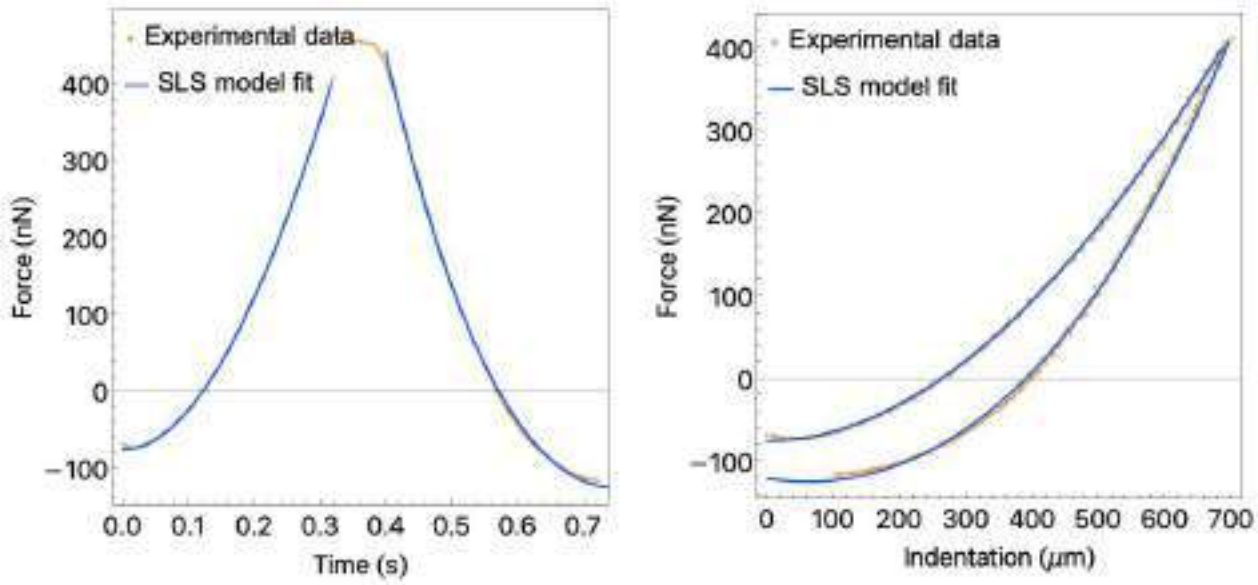

Figure 1. SLS model fit for approach and retract curves in time (left). Hertz model fit for approach and retract curves as a function of time(right).

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Dual quantification for elasticity heterogeneity of soft materials

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The analysis of structural stiffness allows us to elucidate the impact of material architecture and bonding on the elastic response to an applied force. The recently developed trimechanic theory aims to numerically quantitate the underlying restoring mechanics of the material under such an external load. In the theory, the restoring force is decomposed into three components: depth-impact (F_C), Hookean (F_H), and the force related to the shape of indenter (F_S). Combined with the model of three parallel connected springs (3PCS), the trimechanic theory transforms these force components or nano-mechanics to responding actions of elastic springs, as outlined in [1]. The trimechanic-3PCS model presents the elastic behaviors of material in a zone-wise expression, by which the elasticity parameters are explicitly deduced for each depth zone; thereby, one may observe the detailed delineation of nano-mechanical change with varying depth [1, 2]. This is one of dual aspects of analysis power of the trimechanic theory.

The other aspect of analysis power is optimizing the zone-wise parameters for the global representation of material elasticity. Thereby, we can obtain the global effective Young's modulus. The weighting factors were derived from the segmentation structure of the stiffness-depth curve, and its Euclidean norm, $\|\boldsymbol{w}\|$, was used as to score the elasticity heterogeneity of the material. The two analysis approaches have been applied to AFM (atomic force microscopy) indentation experiments on four polyacrylamide gels; each containing distinct acrylamide concentrations and bis/mono ratios. The results reveal that the four gel ingredients can be qualitatively distinguished by the trends of the global effective Young's modulus $\bar{E}$ and the apparent stiffness k_T with $\|\boldsymbol{w}\|$. The present study has shown that the acrylamide concentration possesses a significant influence on gel stiffness. However, the bis/mono-acrylamide ratio did not demonstrate such an effect in the same manner (manuscript in preparation).

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Revealing the unbinding mechanics of hyaluronan•receptor interactions on live cells

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Abstract. The extracellular matrix polysaccharide polymer hyaluronan (HA) and its cell surface receptors (e.g., CD44 and LYVE-1) are important mediators of cell adhesion and migration, and tissue mechanics. Whilst mechanical forces are important in these processes, methods are lacking to study the effect of mechanical forces on individual bonds of HA with its receptors directly on the surface of cells. We present an assay based on atomic force microscopy (AFM) to probe the frequency of bond formation (along with the receptor surface density) and the mechanical resistance of HA•receptor bonds to a force ramp on live cells [1].

We validate that HA•CD44 unbinding forces on cells are high compared to their relatively low binding affinity, and that bond rupture is dominated by a single energy barrier. Qualitatively, the data compare well with previous findings from experiments with purified, surface-anchored receptors [2]; quantitatively, however, clear differences are observed illustrating the importance of measuring interactions directly on cells [1]. Force spectroscopy on live cells is now being extended to probe the interaction of HA with LYVE-1, for which we have evidenced an unusual 'sliding' interaction of HA in the protein's binding grove using purified receptors (unpublished work). Overall, this project will provide a better understanding of the physical and molecular mechanisms underpinning immune cell adhesion in the blood and lymphatic vasculatures.

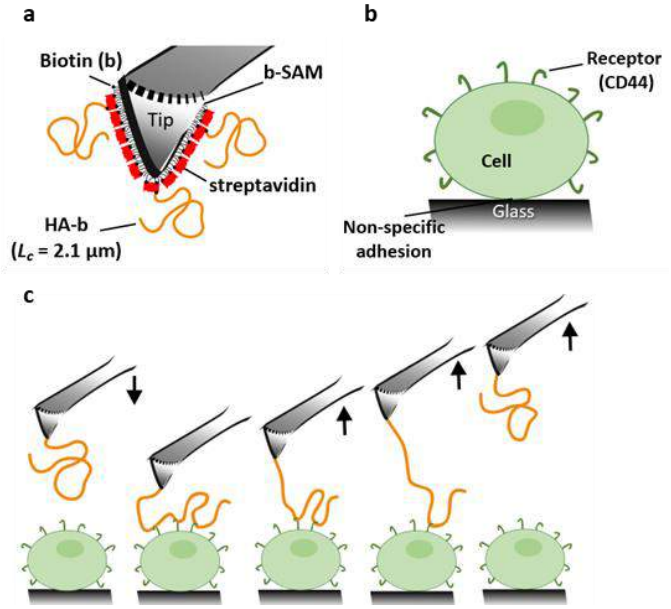

Figure 1. Schematic representations (not to scale) of single HA chain force spectroscopy on cells. (a) AFM probe presenting HA chains (here 840 kDa, corresponding to contour length L_c = 2.1 µm) anchored via their reducing end. **(b)** Live cells (here AKR1 CD44+) attached to a glass surface through non-specific adhesion. **(c)** Expected binding and unbinding processes. The AFM tip is lowered to the cell, enabling formation of one or more HA•receptor bonds, and when the tip retracts, unbinding forces are measured.

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QUANTITATIVE NANOMECHANICAL MAPPING REVEALS NANSCALE HETEROGENITY OF INNER LEAFLET OF INNER MITOCHONDRIAL MEMBRANE

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Membrane-bound organelles (MBO) are essential biological structures that regulate the fate of eukaryotic cells, playing a crucial role in maintaining cellular homeostasis. Among them, mitochondria stand out as particularly intriguing due to their dual lipid membranes: inner and outer, each possessing a unique composition distinct not only from other organelles but also from each other. Consequently, mitochondrial membranes exhibit complex and non-uniform nanoscale structures, most probably influenced by the formation of lipid domains/rafts with distinct topographical and nanomechanical properties [1]. To investigate these characteristics, we prepared both single-component (DPPC, DPPE, CL) and tertiary lipid monolayers (inner leaflet of the inner mitochondrial membrane) - on gold substrate using the Langmuir-Blodgett method. Topographical and nanomechanical properties of investigated systems were analyzed via high-resolution atomic force microscopy (AFM), working in PeakForce Tapping Quantitative Nanomechanical Mapping (PF-QNM) mode. By operating in a sub-nanometer indentation regime, QNM enabled the acquisition of force–distance curves for each pixel in the examined area, generating maps of elastic modulus, adhesion, dissipation, and deformation for both single-component (DPPC, DPPE, or CL) and mitochondrial lipid layers. This approach, in combination with computer image analysis, revealed that even single component systems are heterogeneous at the nanoscale, exhibiting topographically and nanomechanically distinguishable features. Application of masking procedure based on matrix algebra allowed us to determine the distribution of elastic modulus and adhesion force within lipid domains and their surroundings. For phospholipids, the depressed domains exhibited significantly higher stiffness than the continuous phase. In contrast, in cardiolipin-only systems, wrinkled domains protruding above the general plane were found to be more flexible. Importantly, these observations are independent of substrate effects and sample thickness variations, as both factors were accounted for. For the tertiary lipid system, mimicking the inner leaflet of the inner mitochondrial membrane, we observed distinct topographical and nanomechanical phase separation. This phenomenon is attributed to lipid sorting, where the lower phase is enriched in cardiolipin, a negatively charged lipid capable of interacting with DPPC or DPPE through electrostatic interactions and/or hydrogen bonding, thereby contributing to the overall stiffness of the inner leaflet. This interpretation aligns with our analysis of π–A isotherms, which revealed attractive intermolecular interactions as shown by the values of excess area per molecule and excess Gibbs free energy of lipid mixing.

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DEVELOPMENT OF ATOMIC FORCE MICROSCOPY RELATED MODES FOR THE STUDY OF PLASMA MEMBRANE REPAIR

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The plasma membrane is the biological barrier responsible for protecting the cell from changing external conditions [1]. This barrier is fragile, and the formation of micro-ruptures may occur, in particularly in cells submitted to biological or mechanical constraints such as muscle, epithelial, and endothelial cells. While normal cells can repair a mechanical membrane damage within a few seconds [2], mutations in key membrane repair genes induce defects in this physiological process, leading to cell death and the development of several pathologies: inhibition of the membrane repair processes leads to the development of muscular dystrophies [3], yet reinforced membrane repair abilities in cancer cells promote tumor invasion and metastasis [4].

Previous studies of membrane repair have allowed to identify some crucial proteins such as annexins (ANX), a large family of calcium (Ca^{2+})-dependent membrane-binding proteins involved in the repair mechanism. Nevertheless, accurate mechanisms responsible for membrane repair in living cells remain misunderstood. In particular, the membrane rearrangements that take place at the rupture site during membrane resealing remain unknown.

In this work, we use Atomic Force Microscopy (AFM) and its related imaging modes coupled to optical microscopy to obtain original information regarding membrane repair mechanisms thanks to the characterization of the topography of the cell membrane coupled to its mechanical properties. In addition, we develop a novel protocol to induce controlled mechanical damage to the cell membrane using the AFM tip, providing us with a unique window into membrane repair.

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From Liposome Fusion to Giant Plasma Membrane Vesicle Supported Plasma Membrane Models: Platforms to Study Extracellular Vesicle Internalization Dynamics in Breast Cancer

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Breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer-related deaths in women [1]. In present-day BC research, the majority of the focus lies in resolving the intricacies of metastasis, a process which first unfolds by creating a pre-metastatic niche, facilitated by extracellular vesicles (EVs) secreted by the BC cells [1]. EVs have gained importance as prospective biomarkers and nanocarriers, yet their internalization dynamics remain poorly understood. To address this mechanism, we decided to exploit supported lipid bilayers (SLBs) of increased complexity, designed to mimic natural plasma membrane (PM). Our SLB model systems were obtained from: i) fusion of liposomes with mixed lipid composition; ii) rupture of cell-derived Giant Plasma Membrane Vesicles (GPMVs). These bilayers provide a stable and reproducible platform for investigation of membrane morphology and associated biophysical properties, such as the presence of ordered lipid-raft domains and their role in EV uptake. In particular, SLBs from liposome fusion have been extensively studied in our laboratory [2], with specific attention on how different cholesterol levels influence membrane fluidity through formation of lipid-rafts of variable sizes and stiffnesses, and how this in turn affects EV internalization. With results from this simpler model, we then moved on to analyze a more complex model system: cell-derived GPMVs and their supported patches, which are hypothesized to retain the compositional diversity of native membranes [3]. To characterize both types of SLBs, Atomic Force Microscopy (AFM) high resolution imaging and high-speed AFM have been utilized. High-speed AFM was employed to explore the rupture dynamics of GPMVs and the formation of supported patches —a process that remains largely understudied. Furthermore, high resolution AFM was used to assess the morphological features of the patches and observe the presence of domains associated to lipid-rafts, consistent with those present in simpler, liposome fusion SLBs. This similarity in composition provides a foundation for comparison of model membrane dynamics and EV internalization, offering insights into how increased PM complexity may impact the process, which can then be extrapolated to a broader biological scale and implemented as valuable knowledge for future BC research.

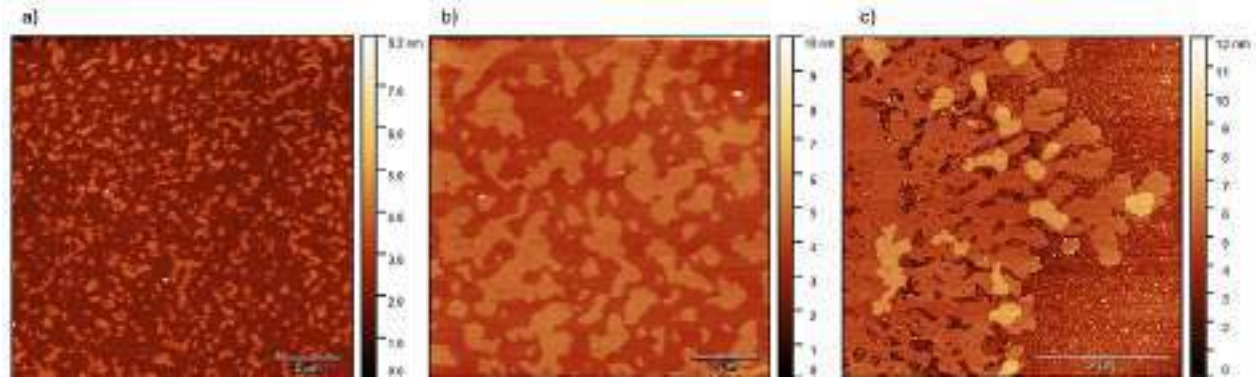

Figure 1. AFM topography images of liposome fusion SLBs at two different cholesterol concentrations: 7% mol% (low) (a), b) 33% mol % (high) (b), and cell-derived GPMV patches as a more complex PM model system (c). Lighter areas indicate lipid rafts, varying in size. Scale bars 2μm.

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Red blood cell membrane tension modulation by photo switchable molecules

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Cellular stiffness and surface tension are fundamental determinants of cell behavior and function [1]. However, the precise contributions of membrane and cortical components to overall cell mechanics remain unclear. Building upon our recently developed multi-modal approach, which combines atomic force microscopy (AFM), confocal spinning disk fluorescence microscopy (CSDFM), and micropipette aspiration, we investigated the mechanical properties of human red blood cells (hRBCs) as a model system, with a specific focus on membrane manipulation. By incorporating photo switchable azobenzenes into the hRBC membrane, we created a dynamic system to modulate membrane properties through light-induced conformational changes [2]. Comparisons were made between wild-type RBCs and those containing azobenzenes in both the cis and trans states. This approach enabled us to directly correlate changes in membrane conformation with alterations in mechanical properties. Our results demonstrate the feasibility of using photo switchable molecules to modulate cellular mechanics in a controlled and reversible manner. This approach not only advances our understanding of the contribution of the membrane to cellular tension but also establishes a novel platform for investigating the interactions between the membrane and cortex through precise modification of mechanical properties simultaneously with both AFM and micropipette aspiration.

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Methylglyoxal-Induced Changes in Nuclear Viscoelasticity and Lamina Integrity

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Diabetes mellitus is characterized by deleterious tissue stiffening, determining a particular mechanical fingerprint of the disease. Prolonged hyperglycemia leads to the accumulation of advanced glycation end-products, formed through the reaction of sugars with proteins, resulting in cross-linking that increases tissue rigidity and impairs organ function [1]. While extracellular matrix glycation and its effects are well-studied, the impact on intracellular organelles particularly the nucleus, remains poorly understood. Given the cell nucleus's role in mechanotransduction, glycation-induced mechanical alterations could have profound functional consequences [2]. Here, we investigate the effect of methylglyoxal (MG), a key glycating agent formed during glycolysis, on the mechanical properties of HeLa cells nuclei. We used Atomic Force Microscopy (AFM)-based nanomechanical spectroscopy to quantify the elasticity and fluidity of isolated nuclei and nuclear regions in MG-treated cells (Fig. 1A) [3].

We find that *ex-cellulo* treatment with 50 mM MG significantly increases nuclear stiffness and reduces fluidity, indicating that glycation directly alters nuclear mechanics (Fig. 1B, C). Chronic exposure to physiologically relevant MG levels (500 µM), produces similar effects in both isolated nuclei and nuclear regions of living cells, confirming that nuclear glycation directly alters its mechanical properties. Notably, these effects are reduced in Lamin-A/C knock-out cells, suggesting that lamin glycation is a key driver of the observed mechanical phenotype. Complementary confocal microscopy reveals a structurally compromised nuclear lamina in MG-treated nuclei, presenting features such as blebbing and nuclear envelope ruptures (Fig. 1D). By connecting MG-induced biochemical changes to nuclear mechanics, this work highlights a potential mechanism for diabetes-associated complications at the nuclear and cellular level.

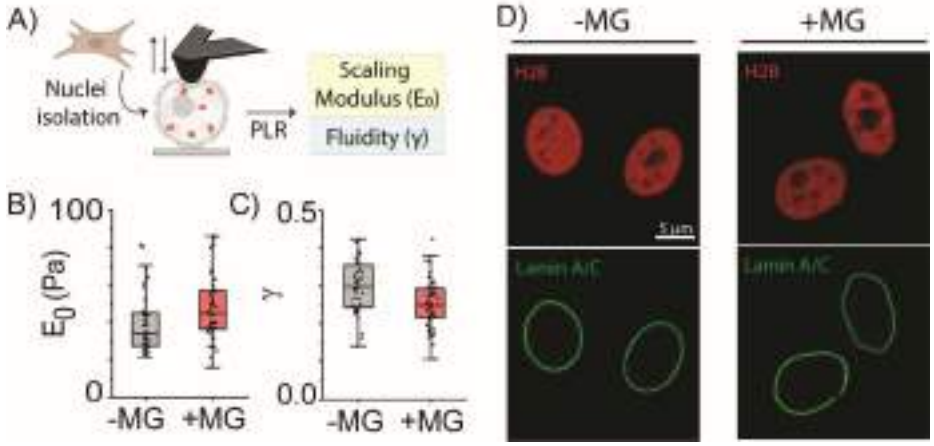

Figure 1. Glycation shifts nuclear mechanics as revealed using AFM-based mechanical spectroscopy. A) Schematical representation of the AFM-based mechanical studies. Isolated nuclei are probed to obtain its viscoelastic properties (scaling modulus, E_0, and fluidity, γ) by applying Power Law Rheology (PRL) model. **B-C)** E_0 and γ of the isolated nuclei before (-MG) and after 6 h incubation with methylglyoxal 50 mM (+MG). Each dot represents the mean of three single force-distance curve performed on a nucleus. The box represents the interquartile range and the whiskers the minimum and maximum data points. The median is represented as a horizontal line. **D)** Confocal images of isolated nuclei before (-MG) and after (+MG) 6h incubation with MG 50 mM. Nuclei extracted from HeLa Kyoto cells permanently transfected with histone H2B-mCherry and Lamin A/C-EGFP.

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The Impact of Actin Mutations on Neuronal Progenitor Cells studied by Atomic Force and Confocal Microscopies

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Actin is a vital component of the cellular cytoskeleton, essential to numerous cellular processes, particularly in the dynamic remodeling of cytoskeletal structures. Non-Muscle Actinopathies (NMAs) are autosomal-dominant disorders arising from heterozygous mutations in actin isoform genes, primarily affecting neuronal function [1-4]. The mechanisms driving these disorders remain poorly understood, prompting our investigation into Neural Progenitor Cells (NPCs) as a model to elucidate the cellular and molecular pathways involved. This study focuses on characterizing the mechanical and morphological properties of NPCs harboring mutations *ACTB*:p.R196H (β-actin) and *ACTG1*:p.T203M (γ-actin). We employed advanced biophysical techniques, including atomic force microscopy and confocal microscopy, to explore the relationship between cytoskeletal dynamics and cellular morphology. Our findings reveal that both mutations significantly alter the mechanical properties of the NPCs; specifically, the T203M mutation results in a 54% decrease in membrane stiffness compared to wild-type cells, while the R196H mutation induces stiffness reductions akin to actin depolymerization caused by Latrunculin A, yielding a mean apparent Young's modulus 67% lower than that of wild-type cells. Additionally, we developed a viscoelastic model that distinguishes between liquid-like and solid-like cellular components, highlighting distinct relaxation timescales for cytosolic and cytoskeletal structures. These variations correlate with the mutations' impact on actin polymerization capacity and reflect the phenotypic severity observed in patients with NMAs. Future research will involve differentiating NPCs into mature neurons to further investigate the physiological implications of these mutations on neuronal function. This work aims to enhance our understanding of NMAs and contribute to potential therapeutic strategies for affected individuals.

Acknowledgments

The research was funded by European Union – Next Generation EU, in the context of the Research Projects of Relevant National Interest (PRIN2022) panel promoted by the Italian "Ministero dell'Università e della Ricerca", Project MAS-NeurActin (Multifaceted Approach to the Study of Neuropathy-causing mutations of Actin) - prot. 2022Xj29r7.

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QUANTIFICATION OF MECHANICAL PROPERTIES BY DYNAMIC FORCE MICROSCOPY CAN BE A NOVEL BIOMARKER IN COLORECTAL CANCER DISEASE

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A novel approach to study colorectal cancer (CRC) is focused on the relationship between biochemical pathways and biophysical cues, which may contribute to disease understanding and therapy development [1]. Herein, we investigated the mechanical properties of CRC cells, namely, HCT116, HCT15, and SW620, using dynamic methodologies by atomic force microscopy (AFM). The dynamic methods allow the determination of elasticity, viscosity, and fluidity. AFM results were correlated with confocal laser scanning microscopy and cell migration assay data. SW620 metastatic cells presented the highest Young's and storage moduli, with a defined cortical actin ring with distributed F-actin filaments, scarce Vinculin expression, abundant focal adhesions (FAK), and no filopodia formation, which could explain the decreased migratory behavior. In contrast, HCT15 cells presented lower Young's and storage moduli, high cortical Tubulin concentration, less cortical F-actin, less FAK, and more filopodia formation, probably explaining their elevated migratory behavior. HCT116 cells presented Young's and storage moduli values in between the other two cell lines, high cortical F-actin expression, intermediate levels of total FAK, and abundant filopodia formation, possibly explaining them showing the highest migratory behavior.

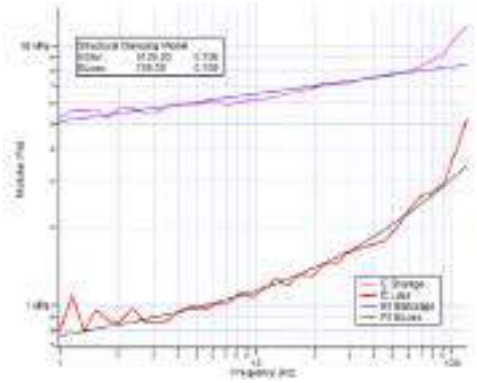

Figure 1. An example of the loss and storage modulus versus frequency obtained by frequency sweep. A power-law fit is applied to the storage modulus, whereas the structural damping model is used to fit the loss modulus. Both fit procedures result in a storage and loss modulus value at the lowest frequency (here, 1 Hz) of 5126 Pa and 750 Pa , respectively, and a power-law exponent of 0.106, in this example data set.

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Viscoelastic differences between isolated and live cancer cell nuclei resolved with AFM microrheology

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While isolated nuclei are commonly used in studies of nuclear mechanics, it has remained unclear whether their mechanical properties are equivalent to those of live cells. Our data resolves significant differences in the frequency-dependent mechanical response of MCF7 nuclei. Furthermore, through selective depolymerization of the actin cytoskeleton we attribute part of these differences to removal of the extra-nuclear matrix and a part to intrinsic changes in nuclei upon isolation.

Our findings are supported by viscoelastic modeling, which identifies the Jeffrey model as the most suitable according to the Bayesian information criterion. The analysis indicates that distinct components of the Jeffrey model correspond to the extrinsic and intrinsic nuclear elements, respectively. This highlights the Jeffrey model as an optimal minimal mechanistic model for nuclear mechanics.

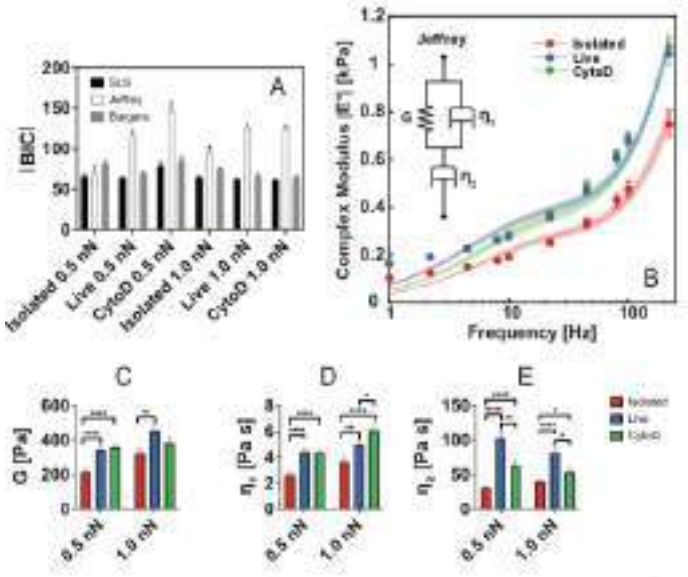

Figure 1. Viscoelastic circuit models of AFMMR data for cell nuclei. Three models (SLS, Jeffrey and Burger) were fitted to the magnitude of the complex modulus of nuclei (n=50). The Bayesian information criterion (BIC) is used for model selection (A). Fitting of the Jeffrey model to the mean stiffness |E*| for nuclei at 1.0 nN, with shaded regions indicating the standard error mean (B). The spring G (C), the dashpot η_1 in parallel with the spring (D), and a second viscous element η_2 (E). Error bars in (C-E) indicate the standard error on the mean.

AFM Spectroscopy for the Study of Lipid Bilayer Stability and Morphology

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Atomic Force Microscopy (AFM) allows visualization of nano-objects, biomolecules and cells while simultaneously investigating their mechanical properties. Force-distance curves derived from indentation experiments provide insight into the elastic and viscoelastic properties of macromolecules and living cells. Supported phospholipid bilayers can be another example of an object of study that serves as a mimic of cell membranes. And they can be used to simulate cellular processes in vitro. In addition to the usual measurements of mechanical properties, the penetration of the bilayer by an AFM tip with a small radius creates a characteristic peak in the force-distance curve. The location of this break is called a "rupture event" and provides valuable information about the phospholipid membrane, its thickness, fluidity or composition, etc. AFM can also capture protein-membrane interactions and the effects of specific agents on lipid bilayers or cell membranes, for example demonstrated here on pore-forming peptides with antimicrobial and anticancer potential. Or the effect of lipid oxidation on the structure and properties of lipid membranes and exploring the protective role of flavonoids against these changes.

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This work is supported by CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042 and European Regional Development Fund-Project „Innovation of Czech Infrastructure for Integrative Structural Biology" (No. CZ.02.01.01/00/23_015/0008175).

QCM-D and AFM combination: a "marriage parfait" for experimental lipid membrane biophysics studies

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Abstract. Cell membranes are key structures in defining life functioning in many biological processes, yet their complexity makes challenging to gain precise information at the molecular level in living organisms. In this regard, supported lipid bilayers (SLBs) are excellent biomimetic platforms that retain 2D order and lateral mobility. This makes them well suited to study lipid lateral interactions as well as interactions between lipid membranes and other molecules such as peptides, drugs, and nanoparticles. [1] The use of complementary surface-sensitive techniques, allows acquiring a detailed picture of both the structure and physicochemical properties of the membrane and its resulting interactions. [2]

In particular, the combination of quartz-crystal microbalance with dissipation monitoring (QCM-D) and atomic force microscopy (AFM) has shown to be very useful assess SLB formation and stability at. While QCM-D allows obtaining a mechanistic picture of SLB formation and phase behavior, AFM enables to resolve topographical features with nanoscale resolution and determining nanomechanical properties. We will show some examples of how this combination has helped elucidating several questions, from the impact of surface nanoroughness on the activation energy for vesicle rupture and organization of SLBs, to the effect quantum dots and peptides on the membrane structure and stability. [3-5]

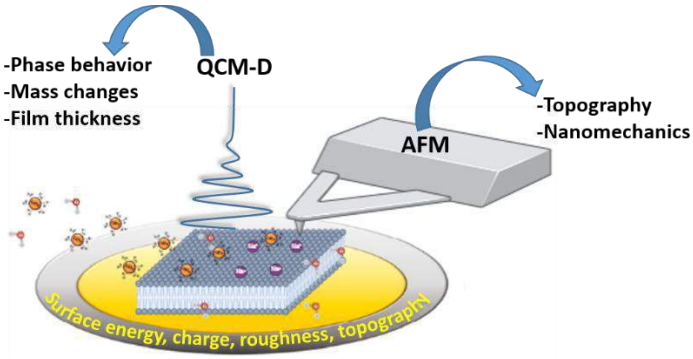

Figure. Example measurements on the interactions between SLBs and QDs by combining QCM-D and AFM.

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Quantifying and manipulating the molecular elasticity of *Drosophila* muscle

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The sarcomere is a structural and functional contractile unit of muscles. It comprises of actin filaments cross-linked at the Z-discs and centrally-linked myosin filaments. The actin and myosin overlap forms the A-band whereas the myosin-free zone forms the I-band. Titin is the third major element that spans half the sarcomere, linking actin to myosin filaments, and is the major source of passive muscle tension by storing elastic energy. In *Drosophila*, Sallimus (sls) is one of the titin homologs that spans the length pf the I-band; its N-term is anchored at the Z-disc and its C-term is anchored at the beginning of the A-band [1], [2] ,[3]. We have recently shown that deleting parts of the Sls I-band elastic PEVK domain reduces the I-band length and A-band length. This suggests that biomechanical feedback between the sarcomeric components allows some proportionate scaling of the filament length and possibly the sarcomere's active and passive forces [4]. However, how sarcomere A- and I-band lengths affect sarcomere mechanics is unknown.

Here we investigate how the Sls PEVK domain instructs the biomechanical feedback between the sarcomeric components. For this, (a) using AFM, we have mapped the viscoelasticity of wild-type and sls PEVK mutant sarcomere in the adult flight muscles and larval body wall muscles. (b) We are now quantifying the force-length relationship of these mutants by mechanically stretching the larva and inducing contractions using optogenetics. The identified differences suggest that titin's structure and sequence modulate the sarcomere's viscoelastic response, shedding light on muscle function.

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From monolayers organized structures to perturbed mutistratified structures: interplay of cell-cell adhesion, cytoskeleton and viscoelasticty

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Intestinal epithelial tissues are monolayered structures that display a high degree of organization and characteristic cell shapes. These properties rely on a complex interplay between cell polarity, junctional remodeling, and the actin cytoskeleton dynamics that regulate the monolayer mechanical properties. Knocking out Par6B in a human intestinal epithelial cell line derived from a human colorectal adenocarcinomacolonic (TC7) perturbs this organization and leads to multistatified epithelial structures. Immunostaining and confocal imaging allows us to dissect how Par6B KO impacts cell-cell adhesion and actin cytoskeleton, while atomic force microscopy (AFM) allows us to explore the mechanical properties of these structures. Our preliminary results, based on viscoelastic fits of force curves on cell aggregates, revealed that KO Par 6 mutation induces an overall decrease of the stiffness scale factor (E0). Mechanical maps also reveal heterogenous areas showing a high stiffness scale factor (E_0) and a more solid like behavior (Fig 1). To better understand these heterogeneities, we are implementing a direct correlation of the mechanical maps and structural observations.

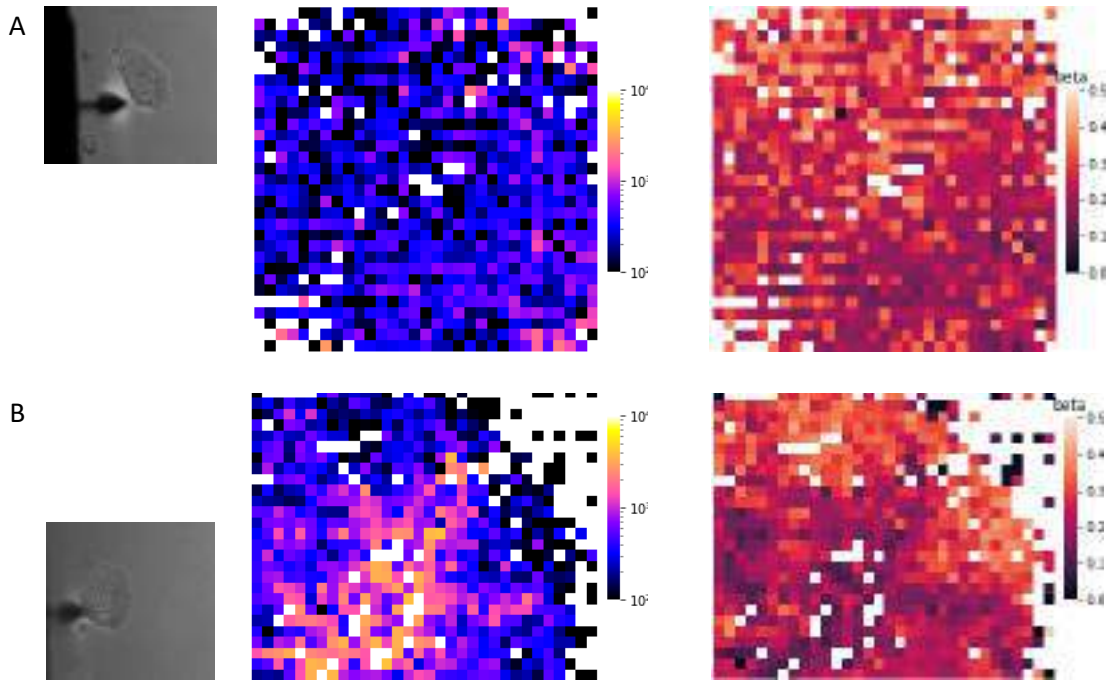

Figure 1. Nanomechanical characterization of (A) TC7 and (B) KO Par6 cells. KO Par 6 mutation induces heterogeneity in stiffness sacle factor (middle) and fluidity (right).

Patchy Adhesion of Staphylococcus aureus on Structured Surfaces Uncovered via Single Cell Force Spectroscopy

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Investigating bacterial adhesion at the single-cell level provides critical insights into biofilm formation and the influence of surface properties on microbial attachment. This study examines the adhesion behavior of *Stapylococcus aureus* on wrinkled polydimethylsiloxane (PDMS) surfaces using single cell force spectroscopy (SCFS) [1]. While conventional SCFS typically evaluates a single contact point, our approach—utilizing structured surfaces—enables mapping of adhesion across the lower portion of the bacterial cell envelope. This method reveals considerable variation in adhesion strength at different points on the cell surface, supporting the "patchy colloid" model originally proposed for *Escherichia coli*. Simulations, incorporating angle-dependent molecule-substrate interactions, suggest that localized adhesive "hotspots" on *S. aureus* may arise from surface roughness, chemical composition, and the clustering of specific adhesive proteins. These findings emphasize the significance of surface structuring in bacterial attachment and provide insights that inform the design of antimicrobial materials and enhanced models for bacterial surface interactions. By mapping adhesion across multiple contact points on individual cells, this work establishes a foundation for future research aimed at modulating bacterial adhesion on medical devices.

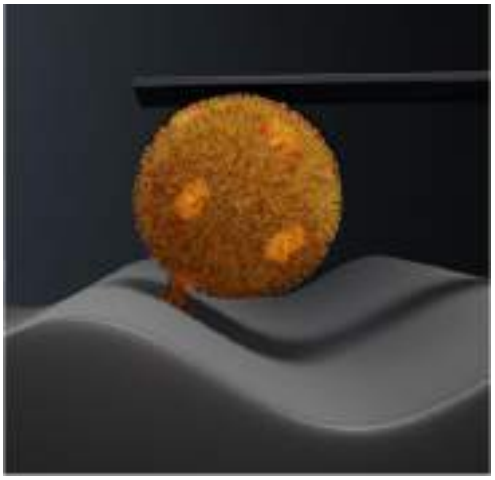

Figure 1. Model of the experimental setup for determining the adhesive force of a bacterium: The corrugated surface allows the lower part of the bacterium (diameter 1 micrometer) to be characterized using single-cell force spectroscopy. The results show that there are a few areas with high adhesive force on the surface of the bacterium.

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Nanoscale electrical properties of biological nanofiber networks in Cable Bacteria

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Cable Bacteria (CB) are a class of multicellular electroactive organisms that exhibit long-range electron transport over centimeter-scale distances, thereby extending the known length scale of biological transport by several orders of magnitude. When probed in a macroscopic setup using carbon or gold electrodes, complete bacteria behave as resistive biological wires with room temperature conductivities ranging up to 79 S/cm [1]. Temperature-dependent electrical measurements have shown that this conductivity can be described with an Arrhenius-type relation over a broad temperature range (− 195°C to + 50°C), demonstrating that charge transport is thermally activated with a low activation energy of 40–50 meV [2]. On a microscopic level, spectroscopy and (chemical) imaging showed that individual periplasmic fibers harbored in the characteristic ridge-like cell envelope (*figure 1A*) consist of a conductive metalloprotein core wrapped in an insulating protein shell [3]. However, the underlying conduction mechanism is still unclear.

Electrical scanning probe microscopy techniques such as conductive atomic force microscopy (C-AFM) and Kelvin Probe Force Microscopy (KPFM) are of great interest in characterizing isolated CB nanofibers directly at the nanoscale and can, therefore, contribute to disclosing the underlying conduction mechanism. Previous studies at X-LAB succeeded at localizing charge transport in these bacteria by introducing C-AFM, finding that electrical currents are mediated through a parallel network of conductive fibers embedded in the cell envelope (*figure 1*) [4]. Since the electrical properties of cable bacteria are sensitive to oxygen and humidity, recently, a novel integrated glovebox-SPM system has been installed in the lab. This upgraded setup allows us to further systematically investigate the local electrical properties of the nanofiber networks in cable bacteria in order to model the underlying electrical transport mechanisms.

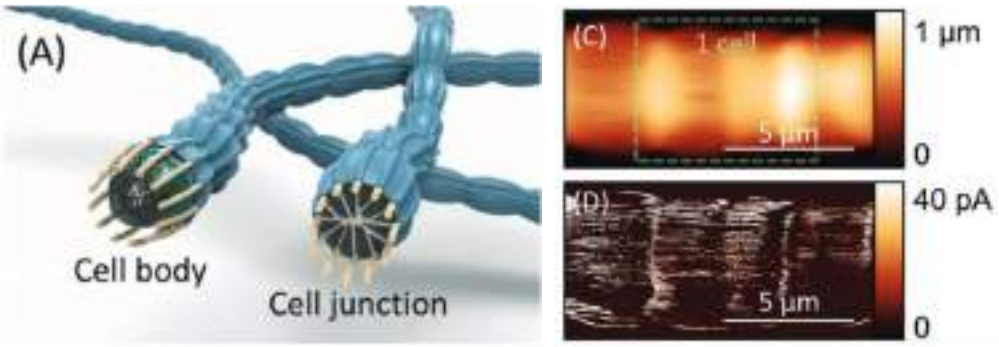

Figure 1. (A) Geometric model of Cable Bacteria, (C) AFM topography image, and (D) C-AFM current image. [4]

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The influence of mechanical stretching on the conformational and ionization properties of linear poly(ethylenimine)

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Poly(ethylenimine) molecules are polyelectrolytes broadly used to form polyplexes as gene delivery carriers. These molecules have several weak basic groups that can respond to physicochemical perturbations, a phenomenon known as charge regulation (CR). Here, we explore the possibility of inducing CR in linear poly(ethylenimine) (LPEI) by mechanical stretching owing to the coupling between the conformational properties of the molecule and its ionization state [1].

We explore this possibility using a combined experimental and theoretical approach. We measure the force-extension response of LPEI at different pH values using Atomic Force Microscopy-Single Molecule Force Spectroscopy (AFM-SMFS) [2]. We compare these experimental measurements with computer simulations using a minimal coarse-grained model (Fig 1, left) based on the Site Binding Rotational Isomeric State (SBRIS) model [3]. This model includes all possible rotations around the dihedral angles of the backbone, the acid/base equilibria of the basic groups in the molecule and explicitly couples them. Both experiments and simulation suggest that pH of the media moderately affects the mechanical response of LPEI (Fig 1, right) [4]. At the high and low force limits, the simulations reproduce well the experimental curves. However, we observe deviations between experiments and our computational model at the intermediate force regime, which is sensitive to specific interactions among chemical groups (*e.g.* hydrogen bonding). Interestingly, our simulations suggest that mechanical stretching can induce a charge regulation effect precisely at this intermediate force regime [5].

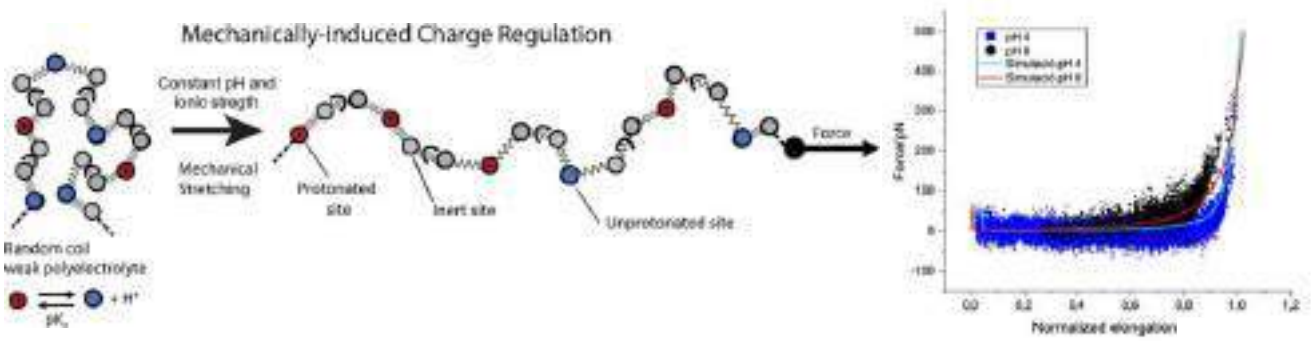

Figure 1. Left panel: schematics showing our coarse-grained model of LPEI. Right panel: force-extension curves of LPEI at pH values 4 and 8 measured using AFM-SMFS experiments (markers) and simulations (lines).

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Morphometric quantification and visualisation of helical filament structures by integration and augmentation of cryo-electron microscopy data in simulation atomic force microscopy

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The structural analysis of polymorphous helical filaments such as amyloid fibrils requires an integrative structural biology approach that bridges near-atomic resolution ensemble averaged data derived from cryo-electron microscopy (cryo-EM) with individual filament level atomic force microscopy (AFM) imaging [1]. Here, we present a simulation atomic force microscopy (S-AFM) algorithm, implemented in the Trace-y software for the analysis of helical structures [2], that generates simulated AFM topographic images of helical filaments from their cryo-EM Coulomb potential density maps [3]. The algorithm can create augmented S-AFM datasets that incorporates noise and structural variability, which may serve as a vital resource for structural interpretation, quantification, classification, identification, visualization, and machine learning applications. By linking high-resolution structural information derived from cryo-EM with the ability of AFM imaging to visualise individual fibril morphologies and to quantify their structural properties within complex heterogeneous populations, this algorithm facilitates the integration of complementary information derived from AFM and cryo-EM. This integrative structural biology approach bridges experimental and computational methods, enhances the interpretability of AFM images, and has the potential to advance our understanding of amyloid polymorphism.

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Stretching of hyaluronic acid at the single molecule level

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Hyaluronic Acid (HA) is a weak flexible polyelectrolyte present in the extracellular matrix, a major structural component of biological tissues. As a weak polyelectrolyte, HA can modulate its charge in response to external stimuli, such as changes in the pH value and ionic strength conditions or due to the electrostatic interactions with other charged species and chain regions. This phenomenon is known as Charge Regulation (CR), defined as the capability of weak polyelectrolytes to modulate their ionization state as a response to the physicochemical environment.

Here, we report a study of the mechanical stretching of HA at the single-molecule level. In other words, we evaluate the interplay between CR and the effect of pulling the macromolecule with an external force [1]. With this aim, the present work has been carried out both experimentally by Single Molecule Force Spectroscopy using an Atomic Force Microscope (AFM-SMFS) under liquid environmental control [2] and computationally using Semi Grand Canonical Monte Carlo (SGCMC) simulations [3] with a coarse-grained model [4] (Fig. 1a).

The force-extension curves obtained from experimental data [5] suggest that the pH value and the ionic strength have a significant effect on the HA weak polyelectrolyte stretching (Fig. 1b).

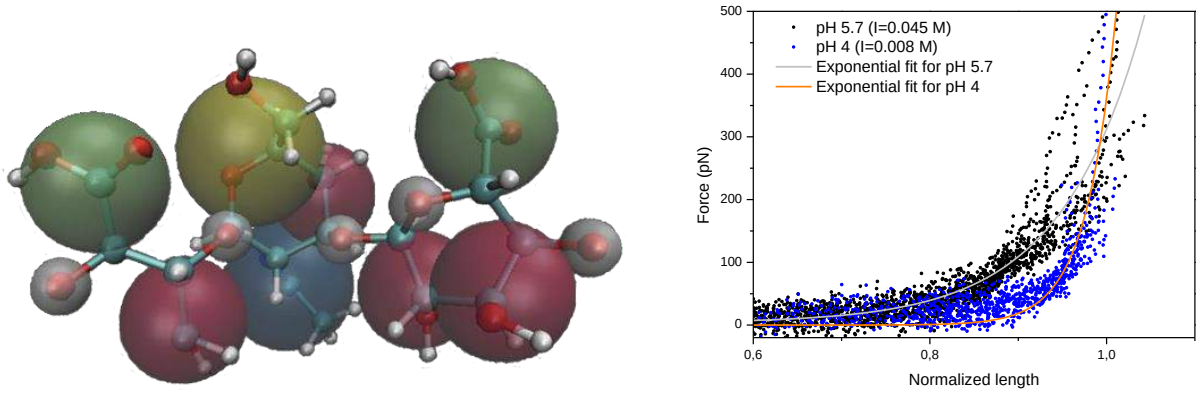

Figure 1. Left panel: Superposition of the HA coarse-grained model with the full atom molecule monomer. Right panel: Experimental force-extension HA data.

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Pulling Geometry as a Design Parameter for Coiled Coil-Based Molecular Force Sensors

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Molecular force sensors (MFSs) enable the quantification of piconewton-scale forces that govern numerous biological processes, such as cell adhesion, migration and differentiation. Conventional MFS platforms, such as double-stranded DNA, are well-established but require multistep chemical protocols to functionalize these MFS with cell adhesive ligands. To address this limitation, we developed a fundamentally new, protein-based MFS building block, based on heterodimeric coiled coils (CCs) [1-4]. Formed by self-assembling α-helices that intertwine into helical superstructures, CCs are essential components of cytoskeletal and extracellular matrix proteins where they serve mechanical functions.

In earlier work, we have shown that CC length, helix stability and hydrophobic core packing are key determinants of CC mechanics [1-4]. Here, we focus on the pulling geometry as a highly versatile parameter to tune CC mechanical stability while leaving thermodynamic and kinetic stability largely unaffected. Using atomic force microscope (AFM)-based single-molecule force spectroscopy (SMFS), we investigated loading geometries where force was applied parallel and/or perpendicular to the superhelix axis. Our findings reveal a pronounced dependence of the rupture forces on pulling geometry, for example, for the two different shear geometries possible for a heterodimeric 4-heptad CC [1,3]. These results underscore the importance of local helix stability and structural anisotropy in determining the mechanical response of CCs to applied forces.

We further present preliminary 2D cell culture experiments, using RGDS-functionalized 4-heptad and 5-heptad CCs as MFSs. While fibroblasts initially adhered on all MFS-containing and control surfaces, cell spreading behavior diverged after 60 to 120 min with observable differences in cell shape, spreading area and cytoskeleton organization. Future directions include the integration of higher-order CC assemblies for multiplexed ligand presentation and enhanced stability. Additionally, we aim to develop 3D cell culture systems based on MFS-containing hydrogels. Our findings contribute to the growing toolkit of MFSs, paving the way for innovative biomaterials capable of probing and modulating cell-matrix interactions with unprecedented specificity and versatility.

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Investigation of Effet the Mechanical Parameters of the Glioma and Keratinocyte Cells by using Finite Element Methods

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Nowadays, the biomechanics of living cells has been the focus of attention, as high-resolution techniques such as atomic force microscopy (AFM) allow the properties of living cells to be studied with nanoscale and precision. There are a lot of results showing that the mechanical changes in the cell or its extracellular matrix (ECM) are used as a biomarker of pathological changes [1]. Therefore, cell stiffness is an index that evaluates the status of cell cultures.

In this study, 2D axial biomechanical models of keratinocyte and glioma cells were made using the Finite Element Method (FEM modeling), and their mechanical properties were calculated. The keratinocyte cells modeled in the study are epidermal cells that make keratin, and glioma is a group of tumor cells formed in glial cells, the supporting tissue of the brain. The force-indentation curve was calculated using the Hertz model. Young's Modulus (E) values are compared with simulation outputs (Elasticity Value: Glioma, $\approx 10^4$ Pa, Keratinocyte $\approx 10^5$ Pa) at the same indentation depth, as well as cell-indentation studies given in the literature (Elasticity Value: Glioma, 11.4-33.1 kPa [2], Keratinocyte 1.1-210 kPa [3]). The simulation outputs are in good agreement with the Hertz model at low indentation depths, it is seen that the agreement decreases as the depth increases (Figure 1). The reason for this is understood to be caused by the stress (von Misses Stress) in the cell that occurs when the indenter presses on the cell surface in the 2D axial AFM indentation model. As the indentation increases in the model, the created mesh cannot dynamically follow the correctness of the solution.

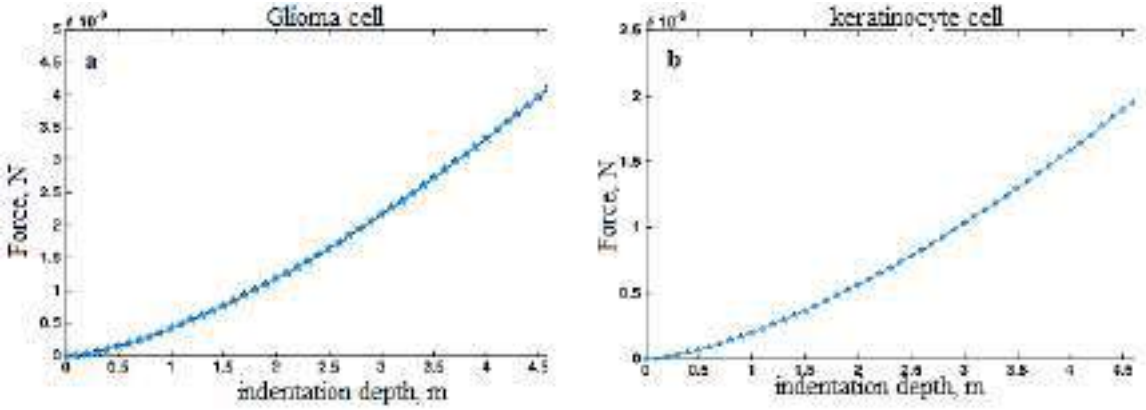

Figure 1. Force-indentation curves for (a) Glioma cell and (b) Keratinocyte cell

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IMPACT OF GEOMETRY ON CHEMICAL ANALYSIS FOR PHOTOELECTRON SPECTROSCOPY OF BLACK SILICON

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Nanorough surfaces such as Black Silicon (b-Si) are often classified by the root mean square (RMS) roughness. However, a single scalar value can only capture limited geometric information about the complex structured surface. As an important consequence, the RMS fails to predict the adhesive behavior of bacteria on b-Si [1], as well as chemical composition of such nanorough surfaces. For smooth surfaces, the chemical composition is analyzed using various spectroscopic techniques, a prominent example is X-ray photoelectron spectroscopy (XPS). However, this analysis becomes more complex for nanorough surfaces like b-Si due to the geometry's steep slopes, which mimic local variations in emission angles. A solution to this problem is offered by the so-called Minkowski functionals that provide a complete characterization of random geometric structures [2,3]. Here, we used the Minkowski tensors to correlate chemical data available via XPS with topographical information from Atomic Force Microscopy (AFM). This presentation will also highlight the significant impact of nanoscale geometries on chemical property analysis.

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Characterization of membrane budding and extracellular vesicles with AFM

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Extracellular vesicles (EVs) are membranous nanoparticles secreted by almost all cell types, are critical mediators of intercellular communication, reflecting the physiological and pathological states of their parental cells. EVs circulate in all body fluids, reaching distant cell targets and delivering different bioactive cargoes. As biological carriers, EVs influence their microenvironment altering cellular responses, being considered promising biomarkers for both physiological and pathological conditions. While Electron Microscopy (EM) is the gold standard for EV characterization, it often involves harsh sample preparation. Atomic Force Microscopy (AFM) offers a promising alternative, enabling high-resolution imaging of EVs without the need for extensive sample processing [1], [2], [3]. In the present study, we employed two human neuroblastoma SH-S5Y5 cell lines (Wyld Type and SOD1-G93A) to evaluate the efficacy of AFM against conventional EM in the characterization of membrane budding and isolated large vesicles-enriched fractions from control and oxidative stressed cells. Our results demonstrate substantial agreement between AFM and EM in characterizing EV morphology and distribution. However, AFM uniquely revealed subtle variations in membrane dynamics across different cellular regions. These findings suggest that AFM can provide valuable insights into EV biogenesis and trafficking, offering a powerful tool for understanding the impact of cellular stress on EV production and function.

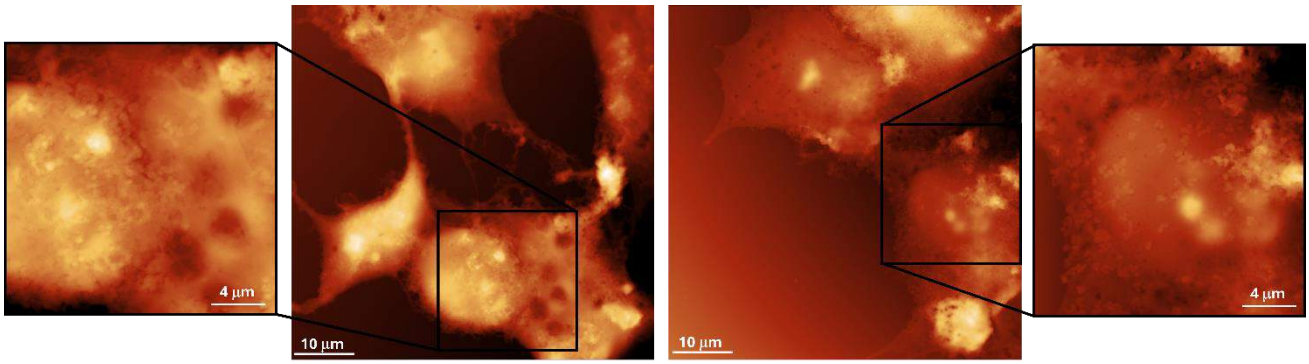

Figure 1. AFM images of membrane budding on WT (left) and SOD1-G93A (right) SH-SY5Y cells after exposure to oxidative stress.

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FluidFM and Electrophysiology

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FluidFM is a force-controlled pipette, comprising a standard AFM with microchanneled cantilevers [1], which makes it a versatile tool from single-cell biology to metal microprinting. By inserting an electrode in the FluidFM channel and a reference electrode in the physiological solution of the petri dish (eFluidFM), we combined AFM and scanning ion conductance microscopy (SICM) into a single technology [2]. This enabled simultaneous measurements of the ionic current and the cantilever deflection (force). We took advantage of the force signal to increase the scanning rate yet avoided unwanted interaction between the tip and cell membrane.
When it comes to another highlighted electrophysiological property, the membrane potential of cells, the patch clamp technique remains the gold standard for measurements. Our eFluidFM setup can be directly utilized as the patch clamp setup [3]. However, pyramidal tips seem not able to allow a stable and controlled tight seal interface between the cells and the probes (gigaseal), indispensable for an accurate measurement of the membrane potential. We tested different probe geometries, including cylindrical probes [4], funnel probes and nanosyringes, as well as surface modifications. Now we are able to maintain stable seal resistance when inserting the FluidFM tip into the cell, enabling whole-cell current clamp measurements, with precise force control. Furthermore, we have obtained promising results in achieving and maintaining stable gigaseal on the cell, suggesting the potential for force-controlled patch clamp experiments.

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Ezrin is a major regulator of viscoelastic properties and force generation in T Lymphocytes during immunological synapse formation

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The interface between a T Lymphocyte and an antigen-presenting cell, commonly referred to as the immunological synapse (IS), is essential for T cell activation. This process is initiated upon the engagement of T-cell receptor (TCR) binding to its specific antigenic peptide. Recent studies have shown that mechanical properties of T cells during activation play an important role in several cellular functions such as proliferation, migration, and cytotoxic activity [1-3]. The formation and maintenance of the IS are supported by force generation through the dynamic interaction of actomyosin and microtubule cytoskeletal networks [4]. However, the way this force generation influences the mechanical properties of T cells is not yet fully understood. Here, we used high spatiotemporal resolution atomic force microscopy mapping to quantify the viscoelastic response via common mechanical parameters (storage and loss moduli) across multiple timescales at the nanometer length scale. In addition, we used traction force microscopy to quantify the tractions stresses generated by T cells on soft silicone hydrogels during IS formation. Our findings show that T cells display structurally diverse viscoelastic properties at the nanoscale level during IS formation induced by CD3/CD28/LFA-1 co-stimulation. Particularly, we observed significantly higher elastic and viscous properties at the edge and central regions, while the peripheral transition region is softer and more fluid. These results align with changes in the actomyosin cytoskeleton architecture respective to those regions. Moreover, our results showed that cytoskeletal perturbations of different filamentous actin regulatory proteins in T cells lead to drastic changes in elasticity and fluidity of T cells, as well as changes in tractions stresses generated by T cells during IS formation. Interestingly, we observed profound softening, fluidization, and reduction in traction stresses when detaching the actin cortex from the plasma membrane by inhibiting Ezrin activity. Altogether, determining the relationships between key cytoskeletal structures at the IS and the cell's local and global mechanical properties is crucial for the maintenance and formation of IS, thus offering a deeper understanding of potential strategies to engineer T cells with enhanced activation and killing capacity.

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Engineering bacterial traps to understand and inspire next-generation antibiotics

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Antimicrobial peptides (AMPs) are a promising class of potent antibiotics that can act by disrupting bacterial cell membranes. The precise, molecular-scale mechanism underlying this disruption can be revealed using atomic force microscopy (AFM)[1]. However, many studies to date rely on phospholipid model membranes rather than live bacteria, thus avoiding the complexity of live bacterial cell envelopes [2,3]. Moreover, AFM studies on entire cells tend to focus on cells that are not or only slowly growing. Cell growth and division readily perturbs the adhesion of the cell to the AFM sample substrate, preventing routine measurement. To nonetheless study the bacterial cell envelope and its disruption over the entire cell cycle, more advanced immobilisation protocols are needed. Chemical immobilisation is easier to implement, but can alter cell viability and only allows a limited imaging time window[1]. Conversely, physical trapping is versatile and facilitates imaging for longer and over a greater range of buffers.

Here, we present developments in both bacterial immobilisation and our understanding of AMP activity on the outer membrane of living bacteria. Building upon previous work[4], we use microchannels to physically trap *Escherichia coli*. Additionally, we use AFM to demonstrate how a novel AMP compound progressively disrupts the membrane of live *E. coli* at high resolution. By understanding the mechanisms of action of these peptides on the bacterial surface, we help guide protein engineering approaches to improve AMP potency in future.

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USING AFM TO ASSESS THE QUALITY OF WHOLE BLOOD AFTER PATHOGEN REDUCTION WITH RIBOFLAVIN AND UV IRRADIATION

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Abstract. The use of whole blood from group O donors with low anti-A and anti-B antibody titers has gained increasing attention as a potential alternative to component therapy for transfusion in cases of massive blood loss [1-2]. Pathogen reduction technologies, such as riboflavin and ultraviolet (UV) irradiation, are being applied to enhance the safety of whole blood transfusions by reducing the risk of transmission of infectious agents [2-3]. This study aimed to evaluate the impact of pathogen reduction using riboflavin and UV irradiation on the biomechanical properties of blood cells. Whole blood from twenty-four male healthy donors of blood group O (I), with low anti-A and anti-B antibody titers, was divided into two groups: partially leukoreduced whole blood (control) and partially leukoreduced whole blood with pathogen reduction using the MIRASOL system (PR). The samples of whole blood were stored at +4-6°C for 7 days. NTEGRA Prima and NTEGRA BIO (NT-MDT SI, Russia) atomic force microscopes (AFM) were used to study erythrocyte morphology, cytoskeletal structure, and membrane elasticity on days 1 and 7 of storage. Hematological parameters and coagulation profiles were also assessed during storage. The dynamics of erythrocyte morphology changes were obtained using AFM. In both groups, a transformation of erythrocytes from discocytes to echinocytes was observed, probably due to the development of oxidative processes. No significant changes were observed in cytoskeletal structure or membrane elasticity, with the Young's modulus remaining at 5.5 ± 2 kPa in both groups. Thus, AFM allows for detailed analysis at the nanoscale, making it a unique tool for studying structural changes in cell membranes and cytoskeletal components that cannot be detected using traditional microscopy methods. The use of AFM data combined with the assessment of hematological parameters, coagulation, and clotting factors demonstrated that the presented method of whole blood preparation could serve as an alternative to component therapy, especially for patients with massive blood loss.

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AFM STUDY OF THE EFFECT OF siRNA ON MACROPHAGE PHENOTYPE

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Macrophages are key regulators of inflammatory and immune responses. Being highly versatile, macrophages can adopt various functional states, typically classified as classical (M1) and alternatively (M2) activated phenotypes [1]. IRF5 is directly associated with proliferation in the M1 phenotype. Although siRNA-based therapeutic agents are more expensive, they offer high specificity and allow for the blockade of the IRF5 gene [2]. Studying the biomechanical changes in macrophage membranes during polarization provides a deeper understanding of the adaptive mechanisms of these cells, as well as their role in inflammatory and regenerative processes. In this context, it is imperative to investigate the effect of genetic manipulations on macrophages' biomechanical and structural characteristics. In an experiment using atomic force microscopy (AFM), data confirmed morphological changes in macrophages after polarization. Significant changes in cell shape and surface topography were observed. Additionally, the AFM method enabled the detection of altered mechanical properties of macrophages, including changes in membrane stiffness, indicating the impact of polarization on their biomechanical characteristics. These results highlight the importance of AFM as a tool for detailed analysis of morphological and mechanical changes in macrophages in response to genetic interventions.

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Cannabidiol interaction with neuronal model membranes

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Cannabidiol (CBD) has experienced a notable rise in popularity due to its significant pharmacological benefits and minimal side effects. Some of the therapeutic effects of this molecule include reducing the severity of seizures in epilepsy [1], providing anxiolytic benefits by helping treat social anxiety disorder and insomnia [2], preventing cortical and hippocampal neurodegeneration, and offering anti-inflammatory and antioxidant properties. Additionally, CBD reduces the hyperphosphorylation of Tau protein and protects against neurotoxicity induced by the beta-amyloid peptide [3], among other effects. In this study, we investigated the interaction between CBD molecules and synthetic lipid bilayers that mimic the neuronal plasma membrane, aiming to understand the impact of CBD on the lipid membrane when administered to patients. Our monolayer experiments showed that as the lateral surface pressure increased, CBD and the lipids in the monolayer exhibited repulsive forces. However, when the mole fraction of CBD was increased, attractive forces between the molecules were observed. Additionally, atomic force microscopy images showed that the height and roughness of lipid bilayers were similar with and without CBD. However, Force Spectroscopy experiments revealed that the presence of CBD altered the lateral packing of the lipid bilayer.

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The effect of DMSO on morphological and physiological traits of bacteria

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Dimethyl sulfoxide (Me_2SO, DMSO) is a natural compound found in nanomolar concentrations in various environments and produced in large amounts anthropogenically. Widely used in industry, biochemistry, and cell biology due to its ability to dissolve polar and apolar compounds and penetrate biological membranes, DMSO is generally considered safe. However, previous studies [1] suggest that its toxicity depends on the dose and the organism's metabolic characteristics. This study evaluated DMSO's impact on proliferation, cytoplasmic membrane integrity and morphology of three bacteria species: gram-negative *E. coli* (ATCC 11303) and gram-positive *B. cereus* (ATCC 10876) and *S. aureus* (ATCC 12598). The MIC was determined using a microdilution method with resazurin dye [2]. Bacterial survival after 1-hour and 3-day incubation with 10, 15, and 30% DMSO [v/v] was assessed via the standard plate count method. Cell morphology was examined using classical simple staining with fuchsin and atomic force microscopy (AFM). Cytoplasmic membrane integrity was measured by the leakage of nucleic acids and proteins.

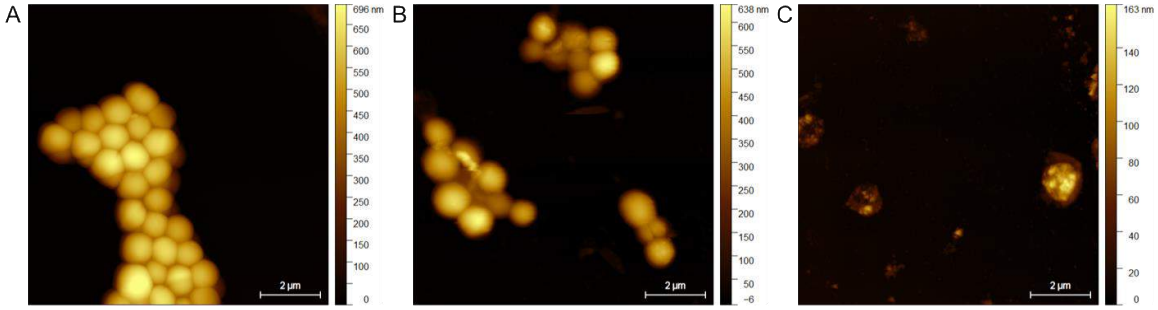

Figure 1. Sample AFM scans in hight mode of *S. aureus* bacteria not exposed to DMSO (A), after one hour exposition at 30% DMSO concentration (B) and effects of long-term (3-day) exposition at 30% DMSO concentration.

The MIC values for *E. coli*, *B. cereus*, and *S. aureus* were 13, 12, and 15% [v/v], respectively. Short-term exposure to 10-15% DMSO had little effect on survival. At 30% DMSO (~2×MIC), a slight (1.5- to 2-fold) decrease in bacterial counts was noted. After 3-day incubation, bacterial numbers decreased 2-3 times at lower concentrations, while at 30%, *E. coli* and *B. cereus* were reduced by 1000-fold, and *S. aureus* was completely eradicated. Prolonged DMSO exposure compromised membrane integrity, evidenced by increased extracellular nucleic acids (dsDNA, ssDNA, RNA) and proteins. AFM images indicate the cell wall structure damage (Figure 1). In conclusion, long-term exposure to DMSO or doses equal to or exceeding 2×MIC has cytotoxic effects, disrupting bacterial proliferation, transport across membranes and structure of cell envelope (cell wall and inner membrane).

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Understanding the biophysical role of Desmoglein 3 in Pemphigus Vulgaris via Fluidic Force Microscopy and Total Internal Reflection Fluorescence Microscopy

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The existence of a transcellular communication network is implicit in the remarkable complexity of tissue morphogenesis and regeneration. Through this network, individual cells perceive the environment and coordinate their biological activity in time and space. Studying biophysical properties in combination with biochemical network analysis is required to understand the fascinating capacity of tissue self-organization. In this framework, the role of desmosomal cadherin-type adhesion molecules in integrating tension forces into biophysical and biochemical networks is emerging due to their suggested outside-in signaling activity and critical role in bearing mechanical tissue stress. One report linking a specific biochemical signaling component to altered tissue stiffness, a well-known mechanical read-out, highly supported such activity in cells treated with PV autoantibodies capable of disrupting Dsg3 transadhesion.[1] Pemphigus is a unique group of autoimmune diseases and Dsg3 is the major target in PV. [2] Since the role of Dsg3 in mechanosensing and signaling has yet to be clarified, we are using PV as a model to further investigate the Dsg3 biophysical network. We are applying the innovative Fluidic Force Microscopy (FluidFM),[3] a versatile tool in single-cell biology[4] that consists of a force-controlled pipette based on AFM standards with microchanneled cantilevers to measure altered biophysical parameters upon disruption of Dsg3 transadhesion in human primary epidermal keratinocytes (HPEK). Our investigation into the Dsg3 signaling network includes measuring cell stiffness via indentation experiments and assessing cell-cell adhesion by detaching individual cells from their monolayers in control and Dsg3 antibody-treated cells. During the cell detachment process of control cells, Force vs Distance curves obtained with FluidFM reveals a series of events: the cantilever first reaches a peak force, followed by detectable minor jumps known as tether-breaking events. We are therefore integrating FluidFM with Total Internal Reflection Fluorescence Microscopy (TIRF) to enhance our understanding of the cell detachment mechanism at the cell-substrate interface and to elucidate the dynamics of observed tether-breaking events.

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Determining aortic wall nanomechanical properties in neonatal patients with coarctation of the aorta

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Coarctation of the aorta (CoA) is a congenital heart defect that accounts for between 5-10% of all congenital heart defect cases [1,2]. CoA is typically presented as a narrowing of the descending aorta at the aortic isthmus, where the ductus arteriosus inserts into the aorta, causing an obstruction of blood flow [3,4]. Patients with CoA are frequently diagnosed and treated within the first few weeks of life through surgical repair. However, even after successful surgical repair, these patients often experience a higher mortality rate than healthy age and gender matched controls [5]. This increased mortality rate is associated with the presence of persistent systemic hypertension in up to 68% of CoA patients post-successful surgical repair [6,7]. The mechanisms underlying the development of CoA and its associated persistent hypertension are yet to be fully understood. The aim of this study is to utilise atomic force microscopy (AFM) to characterise the nanomechanical properties of the aortic wall in CoA patients following childhood correction.

Aortic samples were collected from six neonatal CoA patients undergoing surgical repair at Alder Hey Children's Hospital, Liverpool, with the proximal end of the tissue labelled by the surgeon, with a stitch, to note the correct orientation. Samples were divided into three regions: the coarctation site, and a region proximal and distal to the coarctation site. Samples were cryosectioned to 5 μm and kept frozen at -80°C prior to AFM analysis. AFM was conducted on a Bruker Multimode AFM with a Si pyramidal tip with a nominal 8 nm radius and a 40 N/m flexural stiffness. Imaging was conducted with the Peakforce QNM modality. A minimum of three 1.5 μm images were collected per sample, focussing on the medial and adventitial layers. The AFM images demonstrate abundant collagen fibrils in the neonatal aorta. However, the collagen fibrils appeared more disorganised in the coarctation site compared to proximal and distal samples (Figure 1). The coarctation site also demonstrated a greater mean elastic modulus compared to both the proximal and distal groups in 4 of the 6 participants tested so far. In these patients, the mean elastic modulus of the coarctation site was 2778.9MPa (SD 835.50MPa), 3388MPa (SD 1177.19MPa), 3617.7MPa (SD 1549.67MPa) and 3974.53MPa (SD 2826.10MPa) respectively, compared to the proximal regions of 2047.2MPa (SD 485.11MPa), 3133.7MPa (SD 766.57MPa), 1386.9MPa (SD 172.83MPa), 2311.79MPa (SD 315.05MPa), and the distal regions of 2039.6MPa (SD 639.66 MPa), 2994.3MPa (SD 2135.71MPa), 1837.3MPa (SD 226.10MPa) and 2248.39MPa (188.44MPa). The increased elastic modulus and greater disorganisation of collagen fibrils in the coarctation site of these patients may indicate changes to extracellular matrix (ECM) composition as a result of CoA. This data provides an insight into potential mechanisms underlying CoA and its assosciated persistent hypertension after surgical repair, highlighting potential therapeutic targets in the ECM for further research.

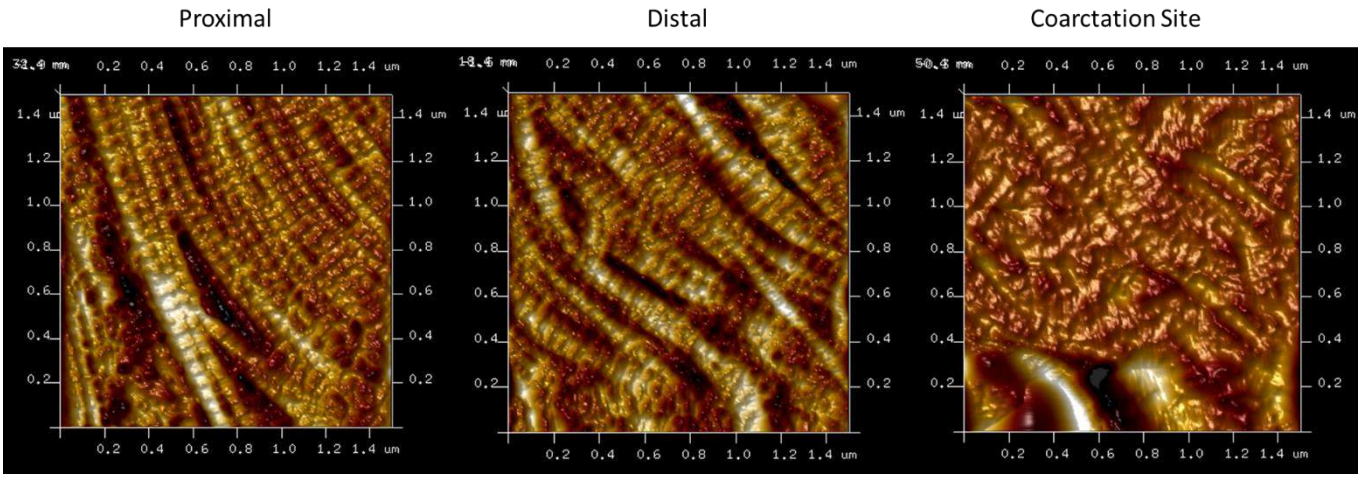

Figure 1. A comparison of collagen fibril structure in the proximal, distal, and coarctation site regions of the aorta in a patient with CoA

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Enhanced Understanding of Biomechanical Observations in AFM Studies Through Quantitative Analysis of Cytoskeletal Filaments from Fluorescence Imaging

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The advancement of fluorescence microscopy techniques has revolutionized cytoskeletal imaging, enabling detailed visualization of cellular structures. However, its application often remains qualitative, overlooking the potential for comprehensive quantitative assessment. This study emphasizes how quantitative fluorescence microscopy, utilizing tools like FilamentSensor2.0 [1], can significantly enhance the interpretation of data from force spectroscopy and force mapping modes of atomic force microscopy (AFM). Fluorescence microscopy data often exhibit high heterogeneity, with selected representative images failing to capture the full complexity of primary cell samples. Interpretation of such data can be prone to user bias and requires a high level of expertise. While AI and segmentation-based methods offer automation, they often lack the contextual detail provided by human expertise. Nonetheless, large-scale quantitative analysis provides a level of consistency and reproducibility that is invaluable for robust scientific conclusions.

We will demonstrate that by using free software such as FilamentSensor 2.0, it is possible to swiftly analyze extensive datasets, minimizing human error while preserving critical biological details. In the study, we selected liver sinusoidal endothelial cells (LSECs) in the murine model of systemic inflammation ($Mcpip1^{fl/fl}$ $LysM^{Cre}$) [2]. We reveal the distinct phases of LSEC defenestration characterized by changes in porosity, elastic modulus, actin filament abundance and fenestration deformability. We conclude that through the combined use of quantitative fluorescence imaging of actin filaments and AFM, we can identify potential key biomechanical parameters that influence LSEC functionality, including fenestration deformability under load and the role of cytoskeletal components in it.

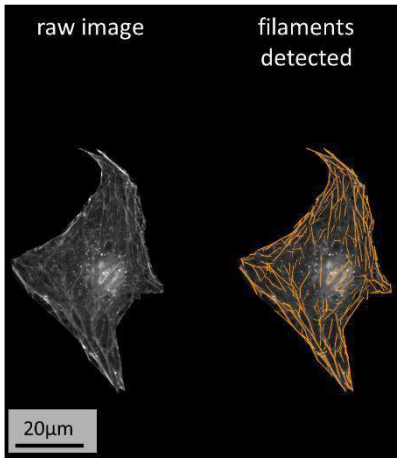

Figure 1. LSEC labeled for actin filaments (raw) with detected filaments marked in orange (filaments detected).

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NANOMECHANICAL SIGNATURE OF DOPAMINERGIC NEURONS

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The study of dopaminergic (DA) neurons is essential for unravelling the mechanisms of Parkinson's disease (PD) and developing effective therapeutic strategies. The versatility of the SH-SY5Y cell line in modelling DA neuronal properties and associated pathologies has established this neuroblastoma-derived model as a valuable *in vitro* tool [1]. Differentiation of SH-SY5Y cells using retinoic acid (RA) and the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) induces a pronounced dopaminergic phenotype [2]. These differentiated cells exhibit characteristics like DA neurons *in vivo*, including susceptibility to 1-methyl-4-phenylpyridinium (MPP^+)-induced toxicity, a hallmark of PD-related neurodegeneration.

Understanding the structural adaptations of differentiated SH-SY5Y cells is crucial for accurately mimicking the behaviour of DA neurons *in vivo*. While the biochemical and functional properties of these cells have been extensively studied, the mechanical properties remain significantly underexplored. Cellular mechanics influence a wide range of biological processes, including cytoskeletal organization, cellular signaling, and resilience to stress—all of which are integral to the pathology of neurodegenerative diseases like PD. Despite their importance, there is a critical lack of comprehensive studies linking nanomechanical changes to neuronal functionality and vulnerability in contexts of disease. Addressing this gap is essential to fully realize the potential of differentiated SH-SY5Y cells as a reliable model for DA neurons. ARTIDIS technology offers a solution to this unmet need by enabling high-accuracy characterization of the physical properties of biological systems. In this study, we present a comparative analysis of the nanomechanical properties of undifferentiated (control) and differentiated (treated) SH-SY5Y cells using ARTIDIS devices. This approach provides new insights into the structural adaptations underlying dopaminergic differentiation and their relevance to PD research.

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Characterization of the unique attachment organelle of *Giardia duodenalis* trophozoites by Single-Cell Force Spectroscopy

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Giardia duodenalis, a unicellular parasite, is a global causative agent of giardiasis, a gastrointestinal disease initiated by the adhesion of trophozoites to the human intestinal epithelium. This adhesion process is facilitated by the parasite's unique ventral disc, a microtubule-based, cup-shaped organelle. Yet, the underlying physical mechanism is still debated. We utilized fluidic force microscopy (FluidFM-based) single-cell force spectroscopy to quantitatively characterize the adhesion forces of *G. duodenalis* adhering to smooth glass surfaces, comparing their adhesion parameters with those of *Candida albicans* and human oral keratinocytes [1]. We observed gradual force increases when trying to detach *G. duodenalis,* with the maximum adhesion of 7.7 ± 4.2 nN (at pulling speed of 1 µm/s) shortly before detachment. When pulling speeds were increased to 10 µm/s, *G. duodenalis* adhesion forces rose to approximately 28.7 nN, demonstrating the mechanical resilience to tensile forces. These findings differ from the saw-tooth pattern of *C. albicans* and from the long cell interaction length of spreading keratinocytes [1].

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Single-molecule force spectroscopy of a thin film conducting polymer

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Abstract
The molecular bond ruptures from the surface of a thin film conducting polymer have been analyzed by force spectroscopy in scanning probe microscopy. The commercially available aqueous dispersions of poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) are frequently used as conductive and transparent coatings for optoelectronic applications.[1,2] We study the nature of the rupture forces when pulling an AFM nanotip out of contact from the surface and discuss the statistical distribution of rupture lengths and rupture forces in liquid environment. We determine the electrical current through these molecular chains by applying a small dc bias to a conducting AFM tip. The distributions of rupture lengths and current through the polymer chains give a picture on quantifying the current features at molecular levels.

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Nanomechanics of cell-derived matrices as a functional read-out in Collagen VI-related Congenital Muscular Dystrophies

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Collagen type VI-related congenital muscular dystrophies (COL6-RDs) represent a rare group of neuromuscular disorders with different severities of clinical manifestation. They are caused by autosomal mutations in any of the three major COL6 genes, leading to deficient or dysfunctional incorporation of collagen VI into the extracellular matrix of muscle and other connective tissues. [1]. Whilst no effective treatment is currently available for COL6-RDs, novel genetic therapies have emerged as a strategy to target different classes of mutation responsible for the disease. Developing new tools to assess the efficacy of these treatments is imperative for their clinical translation [2]. Atomic force microscopy-force spectroscopy (AFM-FS) applied to biological samples can provide information on the micro- and nanoscale mechanical properties of tissues, which regulate cellular behavior. Mechanical properties are largely determined by the organization and composition of the extracellular matrix (ECM), for which several *in vitro* models have been developed, including cell-derived matrices (CDMs). CDMs are decellularized, natural ECMs assembled by cells, that closely mimic the native *in vivo* microenvironment [3]. Here, we characterized the nanomechanical properties of CDMs obtained from primary skin fibroblast cultures of COL6-RD patients. We describe differences in the elastic modulus (*E*) among CDMs from patients with different clinical phenotypes, as well the restoration of *E* from genetically edited cells [4]. Results anticipate the potential of the nanomechanical analysis of CDMs as a complementary clinical tool to provide phenotypic information about COL6-RDs and their response to gene therapies.

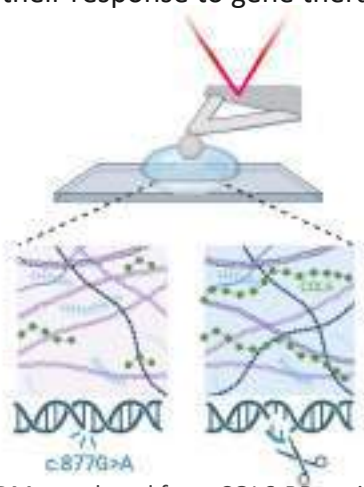

Figure 1. Nanomechanical analysis of CDMs produced from COL6-RD patient and genetically edited cells using AFM-FS. Created with BioRender.com.

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Collagen type I: changes in the morphology and molecular structure under the action of β-hydroxy β-methylbutric acid different concentrations

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Collagen type I is a key structural protein found in the extracellular matrix of various tissues and organs. β-Hydroxy β-methyl-butyric acid (HMB), a metabolite derived from the branched-chain amino acid leucine, has been shown to promote collagen synthesis [2]. However, the underlying mechanism of HMB-induced collagen synthesis remains unclear. This study examined the impact of different HMB concentrations on the molecular structure and morphology of collagen type I using FTIR spectroscopy and atomic force microscopy. Collagen fibres were incubated according to the protocol presented in [1] as a control group and in the presence of three HMB concentrations: 3 mg/mL, 30 mg/mL and 300 mg/mL. AFM scans were done on dry samples. The parameters describing the fibre morphology (hight, D-banding, cross section) were measured for each scan (Figure IA). FTIR spectra were recorded in the 4000-500 cm^{-1} range (Figure IB) at a resolution of 4 cm^{-1}. A single spectrum was obtained as an averaged signal from 40 measurements. After the acquisition, the spectra were corrected and normalised. The ratio of the surface areas of Amide II to Amide I and the area under the band corresponding to Amide III were determined to quantify the changes occurring in the collagen structure. In addition, second derivatives were calculated in the ranges related to the Amide I and Amide III bands to determine the changes occurring in the secondary structure of the examined collagen.

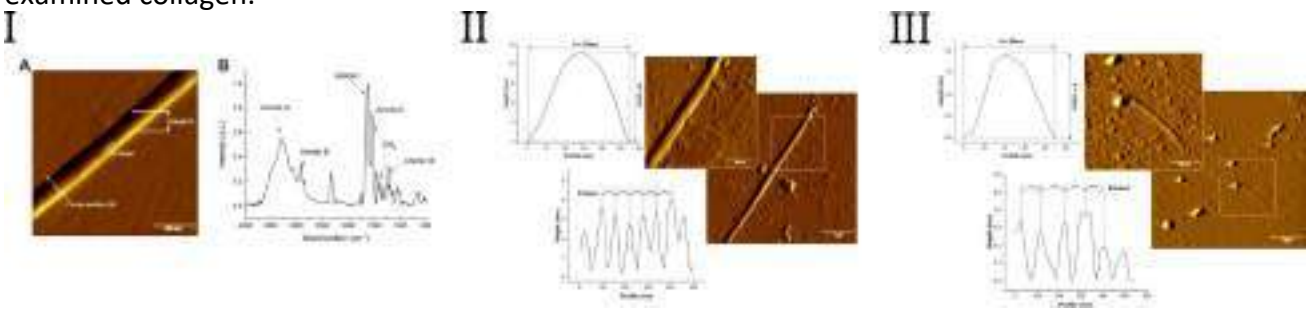

Figure 1. I. Collagen type I AFM image with height H, D-band and cross-section S (A) and its FTIR spectra with prominent bands (B), II. Collagen type I fibre polymerised in the presence of HMB at a concentration of 3 mg/mL; III. Collagen type I fibre polymerised in the presence of HMB at a concentration of 300 mg/mL

The findings demonstrated that low concentrations of HMB facilitated collagen type I biosynthesis, thereby enhancing fibre quality. In contrast, high concentrations of HMB disrupted collagen polymerization, leading to notable structural alterations. These changes were evidenced by a reduction in the intensity of the amide I, alterations in the amide III band, significant thinning of fibrils, and disrupted D-banding patterns, all of which suggest modifications in the secondary structure of collagen fibres. It could be then suspected, that the incubation of collagen with higher concentrations of HMB likely resulted in the disruption of hydrogen bonds critical for maintaining the triple-helix conformation and stabilizing secondary structures

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Dual-Organoid Biosensor for Monitoring Cardiac Conduction Disturbances *In Vitro*

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Atomic Force Microscopy (AFM) is traditionally employed as a high-resolution imaging tool to analyze surface morphology and mechanical properties by scanning a sharp tip mounted on a flexible cantilever. Robust feedback system and micromechanical transducers are responsible for the constant adjustment of tip-sample interaction; however, it can be utilized to monitor the contraction dynamics of cardiomyocytes.[1][2] Moreover, by distinguishing vertical and lateral contractile movements, AFM enables precise differentiation between focal and conductive arrhythmic contractions.

This work introduces an AFM-based biosensor using a dual-beating human pluripotent stem cell (hPSC)-derived organoid.[3] The high biosensor sensitivity allows detailed analysis of contractile behavior under pharmacological modulation with cardiomodulating drugs. Moreover, heptanol-induced arrhythmias were associated with inhibition of atrioventricular conduction, demonstrating the ability of the system to model mechanisms of conduction blockade. The dual-organoid system enhances model robustness by minimizing variability inherent in single-cell studies, thereby improving its translational relevance for cardiotoxicity assessment and precision medicine application in the area of cardiovascular health.

Acknowledgment:

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Vipp-1 Mediated Membrane Remodelling Repair: Dynamics and Mechanics with Fast-AFM Imaging and Nanomechanics

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Cell membrane repair is vital for cellular survival, but the molecular mechanisms remain largely unclear. [1]. This study investigates the polymerization dynamics of vesicle-inducing protein in plastids 1 (Vipp1), a key protein for membrane repair in photosynthetic microorganisms [2] using fast atomic force microscopy (Fast-AFM) to observe Vipp1 behavior on supported lipid bilayers (SLBs). Previous research utilized stiff mica surfaces as the support for SLBs [3]. Here, we employed softer polydimethylsiloxane (PDMS) surfaces to determine if substrate rigidity influences Vipp1 polymerization and membrane repair.

Similar to observations on mica, Vipp1 filaments formed at low salt concentrations on PDMS. Interestingly, Vipp1 ring structures were observed to bind to membrane defects enlarging them before releasing at high salt concentrations. Furthermore, we explored Vipp1 behavior on SLBs mimicking the bacterial membrane composition. Results showed Vipp1 ring fraction preferentially polymerizes at the boundaries of negatively charged lipid domains (Fig. 1a) leading to a softening of the membrane nanomechanical properties (Fig. 1b).

These findings provide valuable insights into the molecular triggers and dynamics of bacterial membrane repair at the nanoscale.

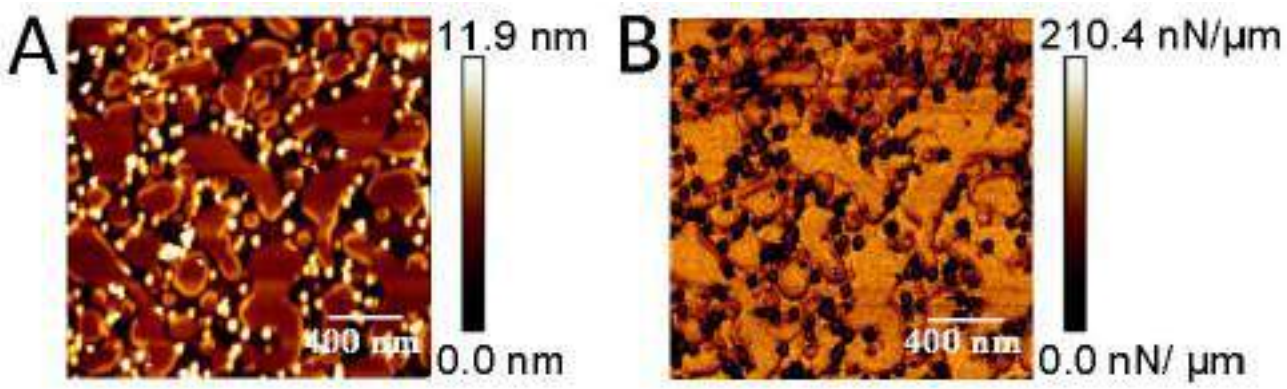

Figure 1. (**a**) AFM topography image of E.coli extract lipid after injection of Vipp-1 (ring fraction) on soft PDMS. It shows Vipp-1 polymerization at the surroundings of the lipid patch. (**b**) correlated nanomechanics of the stiffness map.

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Influence of Internal and External Influences on the Peptide Assemblies Revealed by Atomic Force Microscopy

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The self-assembly of peptides into nanostructures is a fundamental process with significant implications for materials science, nanotechnology, and biomedical applications. It is governed by a combination of intrinsic properties and external environmental factors. In our study, we investigate a short peptide that exhibits curved assembly on surfaces and displays distinct chiral assembly behavior. Using our custom-built Atomic Force Microscopy (AFM) system, we reveal that chirality plays a pivotal role in determining the directionality of assemblies, with a complete reversal in the in-situ growth direction observed when the peptide chirality is altered. Prolonged incubation times further enhance these chiral features, leading to the formation of more stable and ordered fibril structures. We also examine the effects of both internal factors, such as amino acid mutations, and external conditions, including surface properties and solution environments, on peptide assembly. Mutations within the peptide sequence significantly modulate the interaction with surfaces, resulting in diverse assembly morphologies.

Our results provide critical insights into the mechanics of peptide self-assembly, emphasizing the roles of chirality, incubation time, and environmental factors. These findings contribute to a deeper understanding of peptide assembly behavior and offer valuable strategies for designing controllable peptide-based nanomaterials in various applications.

Analysis of the bundling of F-actin by α-actinin

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SYNPO2La is thought to be important with respect to structural development and function of cardiomyocytes[1]. It is also known to interact with α-actinin and F-actin, but the specific role of SYNPO2La is not known. Recently, negative staining electron microscopy has revealed that SYNPO2La may facilitate F-actin bundling by α-actinin. However, it was unclear how SYNPO2La, α-actinin, and F-actin interact to bundle F-actin. High-speed atomic force microscopy (HS-AFM) is a scanning probe microscope that can directly visualize biological samples such as proteins with spatial and temporal resolutions on the nanoscale and millisecond order[2], and has contributed to the elucidation of the structural dynamics of biomolecules[3].

In this study, we analyzed the complex with SYNPO2La by HS-AFM imaging; when SYNPO2La was observed by HS-AFM, a disordered region was observed around the globular structure. Furthermore, this disordered region was observed to bind to α-actinin, which cross-links F-actins by binding to α-actinin with both ends of the homodimer entering between two F-actins(Fig1). On the other hand, in the presence of SYNPO2La, α-actinin/ SYNPO2La was found to bind to the top surface of the closely apposed F-actin(Fig2). SYNPO2La cross-links F-actins, while α-actinin, SYNPO2La, and α-actinin/ SYNPO2La complexes cross-link F-actins, respectively, and F-actin are more densely and at more free angles than the F-actin/α-actinin cross-linked structure. F-actin, and at the same time, SYNPO2La changed the cross-linking method of α-actinin and promoted the networking of F-actin.

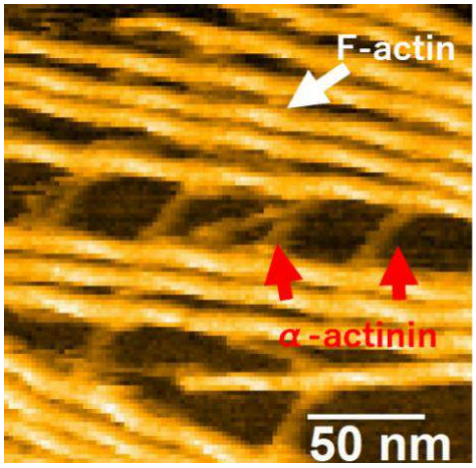

Figure 1. HS-AFM image of F-actin/α-actinin.

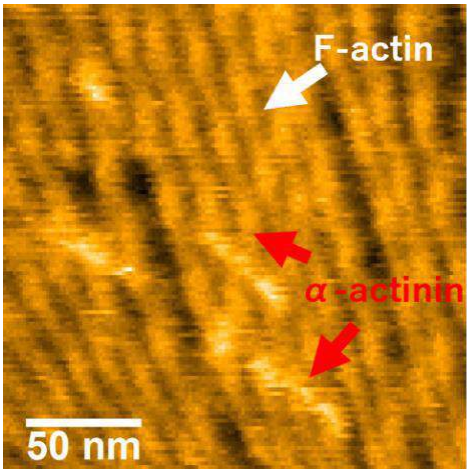

Figure 2. HS-AFM image of F-actin/α-actinin/SYNPO2La.

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Spontaneously Directed Loop Extrusion in SMC complexes Emerges from Broken Detailed Balance and Anisotropic DNA Search.

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DNA loop extrusion by structural maintenance of chromosomes (SMC) proteins, including cohesin, condensin, and the SMC5/6 complex, is fundamental to genome organization. However, the molecular mechanism by which these complexes achieve persistent directionality while minimizing backward steps remains unclear. Here, we used atomic force microscopy (AFM), including high-speed AFM, to directly visualize the structural dynamics of the yeast condensin SMC complex during DNA binding and extrusion. Our AFM studies reveal a directional bias in the hinge domain, which extends orthogonally to the bound DNA, suggesting a built-in geometric constraint that facilitates rectified loop extrusion. Computational simulations incorporating these structural constraints further support a self-rectifying mechanism that operates even in the absence of explicit directional cues. By combining high-resolution AFM imaging with simulation, we uncover an overlooked anisotropic search mechanism that may be a conserved feature of the SMC family. These findings provide crucial insights into the biophysical principles governing genome organization.

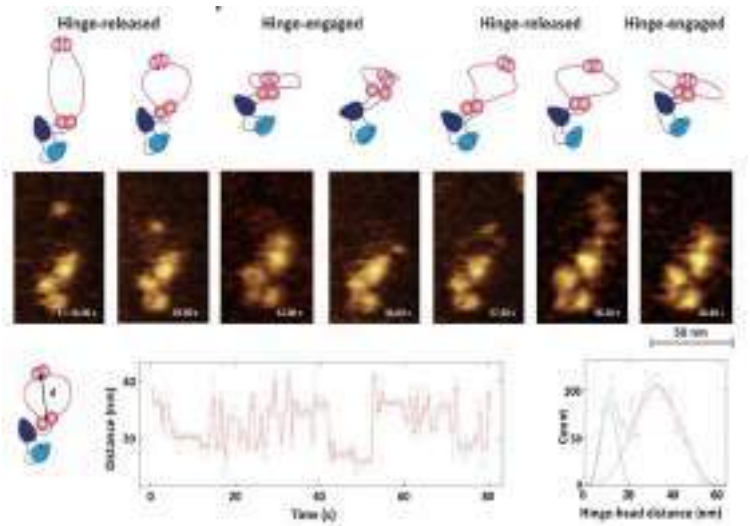

Figure 1. High-Speed AFM Captures Dynamic Conformational Changes in Condensin During DNA Loop Extrusion

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AFM application across the life sciences field and its combination with special techniques

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These approaches enhance the obtained data by integrating AFM (topography, mechanics) with information from other techniques within the same region of interest. It provides key insights into the structural and biomechanical behaviour of biological systems in their native state and physiological environments, enabling a deeper understanding of complex processes such as biomolecule imaging and cell mechanics.

AFM's versatility is further enhanced through its combination with other techniques, such as Raman spectroscopy, fluorescence microscopy, and scanning electron microscopy (SEM). While Raman spectroscopy provides precise chemical characterization, fluorescence microscopy enables the specific localization of desired interactions. Moreover, the correlative AFM-in-SEM enables precise *in-situ* data correlation, combining nanoscale topography with different information from SEM channels. Additionally, advanced AFM techniques, such as Fluid AFM, enable nanomanipulation, including single-cell nano-injection, expanding its applications in biomedical research. These combined methodologies provide a deeper understanding across a wide range of life sciences, including cellular processes, biomaterial interactions, and disease-related mechanical alterations, making AFM an excellent and useful tool for modern biological and biomedical research.

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AFM automation for mechanical tracking of mammalian cells

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In recent years, the study of mammalian cell mechanics has gained significant attention for its role in understanding key physiological and pathological processes, such as stem cell differentiation or cancer cell migration [1,2]. Atomic Force Microscopy (AFM) is a technique commonly used to measure the mechanical properties of biological samples, including cells and tissues, due to its ability to achieve nanometric resolution under conditions that closely mimic the physiological environment. While AFM has demonstrated its effectiveness in assessing various mechanical parameters, such as stiffness, elasticity, adhesion, and viscoelasticity, its application remains limited by its low throughput. Such a limitation partly explains why AFM is not established as a diagnostic tool in the biomedical field [3].

To address this challenge, we have developed an automated system that integrates image-based cell detection with advanced algorithms to enhance data quality. This system actively monitors experimental parameters and performs non-contact cantilever calibrations, enabling prolonged, unattended operation. By minimizing the need for user intervention, our approach significantly improves AFM's efficiency, making it a more viable tool for large-scale biomedical applications.

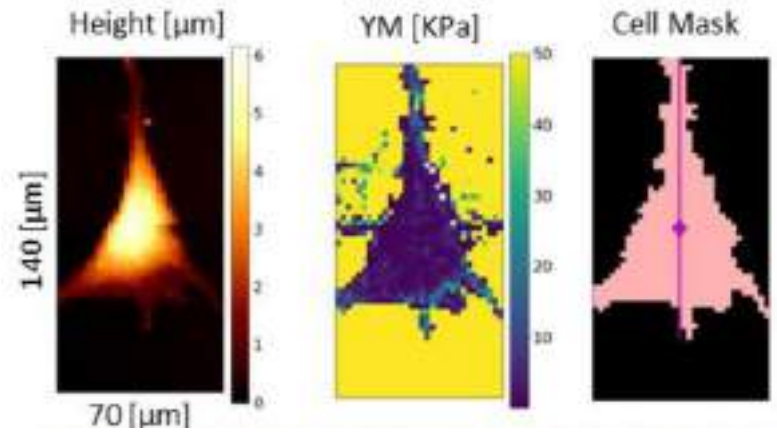

Figure 1. AFM automation allows to measure the topography (height, left) and mechanical properties (Young´s modulus, center) of a migrating NIH3T3 fibroblast. In our experiments, optical microscopy images are continuously acquired to estimate the centroid of single cells. This centroid position is used to establish the location in the corresponding AFM measurement (cell mask, right).

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Re-examining the topography of pentameric IgM-J chain using AFM

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IgMs are the first antibodies produced by the body in response to initial exposure to an antigen. IgM-Js are pentameric complexes composed of five protomers, each made of two heavy and two light immunoglobulin chains, and linked by a joining (J) chain [1]. In addition to pathogen recognition, the importance of IgM in complement activation is well known [2]. A textbook representation of IgMs consists of a planar and symmetrical pentamer (Fig. 1). However, recent negatively-stained electron microscopy (EM) [3], as well as cryo-EM structures, revealed an incomplete symmetry with the J chain partially occupying the space of a missing sixth protomer (Fig. 1).
IgMs have previously been imaged by atomic force microscopy (AFM) and their topographic interpretation has been performed according to the model with 5-fold symmetry presumed at the time [4, 5].
In this work, we imaged both the full-length and the Fc fragments of IgM-J. The Fc fragment comprised only three terminal domains of the heavy mu chains. Following their recombinant expression and purification detailed in [6], Fc and IgM molecules were imaged on mica under different conditions (mica with and without $NiCl_2$ precoating, in ambient and in liquid conditions).
We present high-resolution topographic images of Fc and full-length IgM molecules obtained with an image-processing tool, the Laplacian Mask [7]. We show that this method gives more promising results than the successful Laplacian Weight method [8], especially for samples imaged in liquid environment.

Figure 1. Left, a textbook representation of symmetric pentameric IgM molecule. The heavy chains are drawn in blue and the light chains in orange. The J chain is symbolized with a black loop in the center (image from wiki). Middle, a cryo-EM structure (6KXS, [9]) of a pentameric IgM (represented in colored ribbons) with the J chain (represented in CPKs). Right, a crop of a corrected ambient AFM image of 500 nm with 512 pixels of a Fc pentamer containing a J chain.

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flash presen-tations

High speed AFM uncovers diffusion of LacY in membranes

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The dynamic behavior of membrane proteins within lipid bilayers is essential for understanding key biological processes, including signal transduction, molecular transport, and cellular communication [1]. High-speed Atomic Force Microscopy (HS-AFM) has revolutionized the study of these processes, offering high-resolution imaging and real-time observations of molecular dynamics. In this work, we have studied lactose permease (LacY) from *E. coli* as a model transmembrane symporter embedded in lipid bilayers formed with polar lipid extract of *E. coli*. We observed protein entities freely diffusing along lipid surface (Figure 1) and we were able to estimate a 2D translational diffusion coefficient. This value is substantially lower than values obtained for other proteins using different techniques, such as fluorescence lifetime imaging microscopy [2] or fluorescence recovery after photobleaching [3], indicating that the mica surface is restricting the lateral movement of the protein within the lipid bilayer. By directly visualizing membrane protein diffusion in situ, AFM can bring light into the mechanisms underlying protein function, membrane organization, and cellular dynamics.

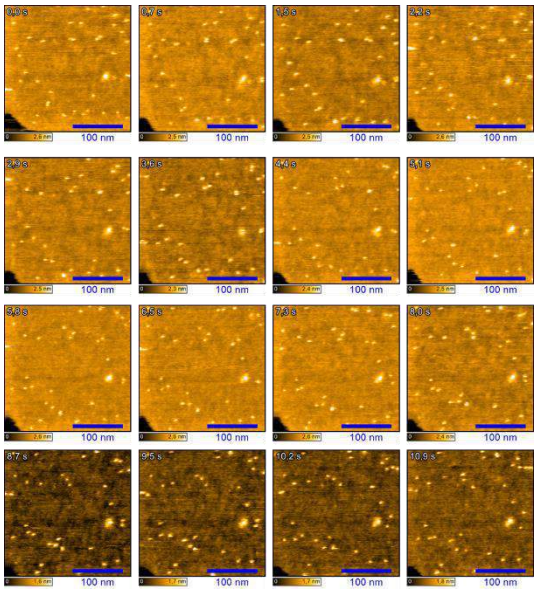

Figure 1. Time- lapse HS-AFM images of LacY proteins embedded in *E.coli* polar extract bilayer.

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A different metabolic regulation in favism erythrocytes induces surprising alteration of the cells' properties along the aging

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Favism uniquely arises from a genetic defect of the Glucose-6 Phosphate Dehydrogenase (G6PD) enzyme and results in a severe reduction of erythrocytes' (RBCs) reducing power that impairs the cells' ability to respond to oxidative stresses. After exposure to fava beans or a few other drugs, the patients experience acute hemolytic anemia due to RBCs' lysis.
Here we compare biochemical, biophysical, and ultra-morphological properties of normal RBCs and cells from favism patients measured along cellular aging employing a combination of AFM, nanomotion sensor, microRaman and biochemical assays [1,2].
It is known that along the aging, the cells' structural and functional properties degrade [3]. However, the present study revealed intrinsic differences in G6DP-deficient cells that result in a peculiar pattern of structural, functional and ultra-morphological alteration that are also associated to distict microRaman evolution observed during the aging. In particular, the initial higher fragility and higher presence of structural/morphological alterations of favism cells develop, at long aging times, into a stronger resistance to external stresses and into a higher general resilience to lysis [1].
This surprisingly higher endurance against cell aging has been related to a metabolic regulation that permits, to G6PD defective cells, a lower energy consumption in environmental stress conditions. Our results provided a direct and coherent link between the RBCs' metabolic regulation and the cell properties that would not have been possible to establish without an investigation performed during aging.

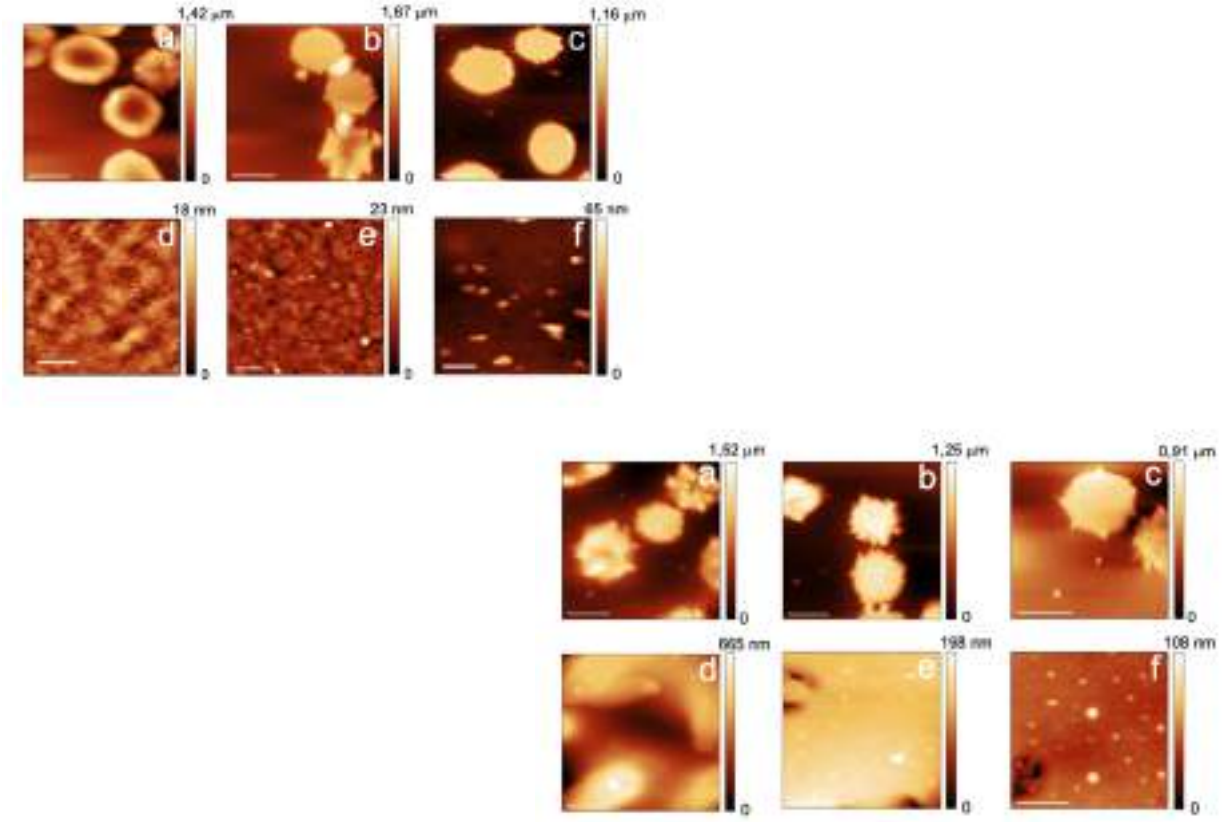

Figure 1. Aging pattern at the meso- and nano-scale, as observed in normal (upper) and in favism (lower panels) RBCs.

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Interfacial Dynamics of Quantum Dot Adsorption on *Cholera Vulgaris* Cells Wall and Its Impact on Nanomechanical Architecture.

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Chlorella Vulgaris is one of the most abundant microalgal species that populate aquatic environments [1,2]. The constant release of various nanoparticles (NPs) including Quantum dots (QDs) into the aquatic ecosystem induces toxic effects on microalgae which disrupts biological organization at the lowest of trophic levels. This study focuses on the adsorption dynamics of differentially PEG functionalized (–COOH or -NH2) CdSe/ZnS QDs at the cell wall interface. Interfacial dynamics of adsorption were largely driven by the electrostatics of QDs and microalgae under different pH environments. Confocal measurements confirmed that the adsorption -NH2 group functionalized QDs over algal cell wall at pH 4.5 & 6.2. Further studies with AFM revealed large differences in cell wall roughness as a function of QD exposure at different pH in MES buffer. Multiparametric nanomechanical investigations also brought forth unique concentric nanomechanical patterns hidden within unilaminar structure of algal cell wall. Finally, it was at pH 6.2 with QD treatment a significant decrease in nanomechanical properties like cell stiffness and Young's modulus was visible. In LC media, the stability cell wall architecture was reflected by the presence of chitosan-like hair-like fibers, and at different pH with QD administration such features were not visible. These changes in the various nanomechanical properties suggest that the toxic effects of QD begin with disruption of the cell wall integrity and pH-driven electrostatics plays a major role in the interfacial dynamics of QD adsorption.

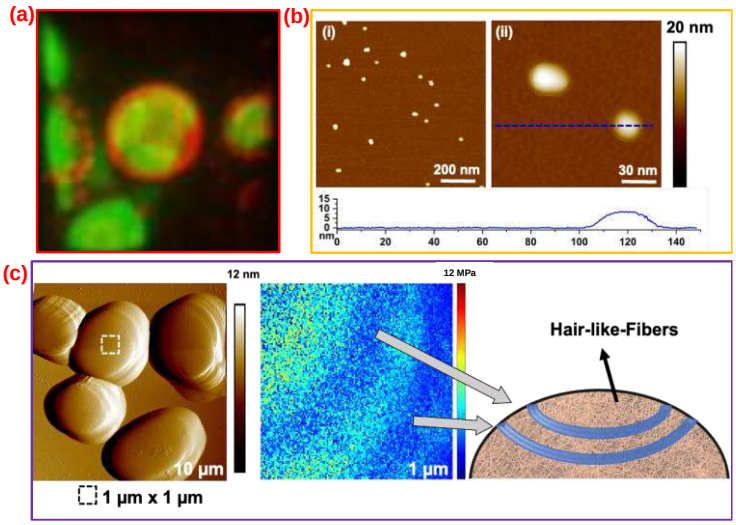

Figure 1. (a) 3D fluorescence image QDs adsorbed on algal cell wall. (b) AFM images with their cross-sections. 1 μm^2 sized AFM image of QDs in air (i), with a 150 nm x 150 nm sized image on the bottom right. (ii) A cross section of the QD surface is marked with dotted blue line can be observed at the bottom of the two images.(c) Representative algal topography with 1 µm x 1 µm white square boxed region selected for force curve measurements. After Young's modulus analysis of apical region of microalgae, one can observe circular ring like nanomechanical features. Two circular zones of lower modulus are graphical illustrated and are marked grey arrows.

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Discovering multifactorial causality using AFM nanoindentation

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In the scientific methodology, measurement is the cornerstone. Measurement is a process of quantification in which facts are often derived. From these facts, modern biology attempts to build a causal chain that aims to describe the effect of a stimulus on its physiological consequence. These ideal steps are sometimes hindered by the so-called operational aspect, which many of us are familiar with and most of us ignore. The absolute fallacy of the operational aspect is to assume that we know exactly what we are trying to measure.

A recent piece of research illustrates the blunt face of operational aspects. We, including Christian Godon, Jean-Marie Teulon, and Michael Odorico, performed initial nanoindentation experiments on 4-day-old living roots of *Arabidopsis thaliana*. It was concomitantly observed that the external primary cell wall of the root became stiffer with a root growth arrest phenotype when the root was grown under low phosphate conditions [1]; and was later attributed to the presence of $Fe^{2/3+}$ and Al^{3+} in the growth medium [2]. When we repeated these measurements for the development of the novel tri-mechanical nanoindentation analysis framework [3], we observed no more effect of the low phosphate condition and 20 µM or more of Fe^{2+} did not affect either the stiffness or the growth of the root.

The mystery was solved by Thierry Desnos, and his team in Cadarache, by measuring the metal content of the agar powder used to grow plant seedlings. The absence of Aluminum was a surprise and it was suggested to simply replenish the missing concentration of Al^{3+} to the agar medium to recover the previous phenotype. To cut a long story short, had we followed this correction step, we, including Harinderbir Kaur, would have missed a key element of the Arabidopsis root growth arrest phenotype, where we found that Al^{3+} triggers a physiological response (organic acid exudation) that enhances the toxic effect of Fe^{2+} on the primary cell wall of the seedling [4]. Thus, despite “accurate” measurements, the biological causal chain always remains more complicated than anticipated.

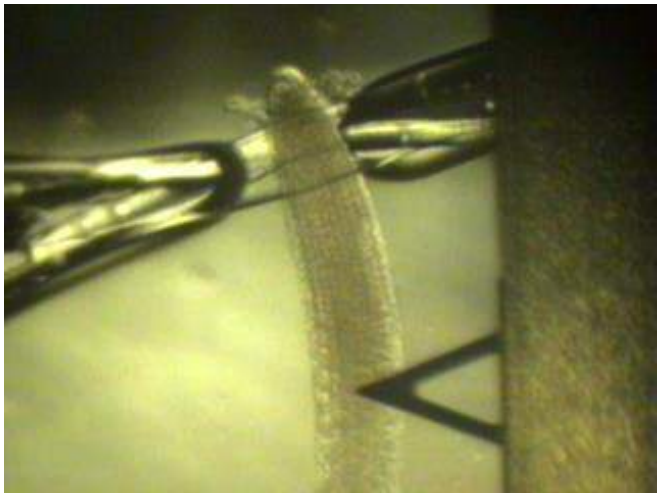

Figure 1. Root tip of an *Arabidopsis thaliana* seedling fixed on a glass slide using a silicon-based pressure-sensitive adhesive. The triangular shape of a PNP cantilever is located near the root transition zone, approximately 500 µm away from the root tip. Nanoindentation is performed on living seedlings, which presents a series of challenges as previously outlined [5].

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Correlation between nanomechanical and chemical features of crosslinked hydrogels combining AFM with chemometric methods

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Treatment of severe skin infections like acne require antibiotic prophylaxis that is a common dermatological condition involve in antibacterial resistance.[1] Indeed, such global health issue led to an increasing interest in alternative therapies like phage therapy.[2] This method uses bacteriophages—viruses that specifically target and destroy bacteria causing skin infections. A key challenge is selecting safe and effective phages for therapeutic use, as well as incorporating them into hydrogels as therapeutic to release them locally in a controlled manner.[3] Hydrogels, which can absorb and slowly release active substances, will protect the phages from dehydration and inactivation while ensuring prolonged, targeted release on the skin.[3] In this study we focus on the mechanical and chemical properties of hydrogel and their impact on phage infectivity. Furthermore, modulating and controlling the mechanical properties of hydrogels, and in particular exponentially growing polyelectrolyte multilayer films, have been a major challenge, given their importance for a wide range of applications including tissue engineering, implantable biomaterials and drug delivery systems. In this work, we investigated the crosslinking reaction of hydrogels based on the association of poly(allylamine) (PAH) and hyaluronic acid (HA) with either 1,4-butanediol diglycidyl ether (BDDE) or divinylsulfone (DVS) at different concentrations and its impact on phage storage and infectivity after release. [4-5] We combined atomic force microscopy (AFM) and infrared spectroscopy (ATR-FTIR) to address the correlation between chemical and mechanical properties resulting from the crosslinking reaction and the impact on phage uptake and release into the crosslinked hydrogels. We deciphered how the affinity of each cross linker (DVS and BDDE) to alcohol and amino chemical functions drives the chemical features of the hydrogels and strongly impact on phage infectivity. Indeed, these features can be described and explained by a Bayesian Positive Source Separation method (BPSS).[5] These results are of high importance for pharmaceutical and cosmetics industries especially in the formulation of hydrogel and the targeted application.

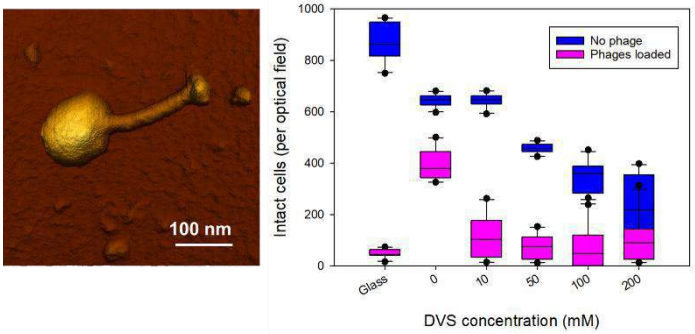

Figure 1. AFM image of isolated phage and infectivity profile after release from crosslinked hydrogel

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NANOMECHANICAL BIOMARKERS FOR NON-COMMUNICABLE DISEASES

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Non-communicable diseases (NCDs) represent a significant global health challenge, placing substantial burdens on healthcare systems worldwide. Among the ten most common NCDs are cancer and chronic respiratory diseases, which, together with cardiovascular diseases and diabetes, account for over two-thirds of global deaths. Early detection and accurate monitoring of NCDs are essential for effective management and improved patient outcomes. However, conventional biomarkers and imaging techniques have limitations, emphasizing the need for discovering novel, more precise and more reliable biomarkers that can be also used for prognosis and early diagnosis. Mechanical biomarkers, which utilize the mechanical properties of tissues and cells, are emerging as a promising approach for NCD detection and monitoring [1]. In this study, we used Atomic Force Microscopy (AFM) to identify unique nanomechanical fingerprints (NMFs) for highly desmoplastic cancers (i.e., breast, pancreatic, sarcoma) and pulmonary fibrosis. The goal was to determine whether AFM-based NMFs can serve as novel biomarkers for these two NCDs and to compare their NMFs. Additionally, we investigated the potential application of these NMFs for disease state characterization (diagnosis) and treatment monitoring. The NMFs characterizing specific tissue states were assessed and evaluated as potential biomarkers using tissues from murine tumor models as well as from patients with pulmonary fibrosis. We further analyzed these unique NMFs using biopsies from mice with cancer and pulmonary fibrosis undergoing treatment with approved pulmonary drugs, such as Tranilast and Pirfenidone. These mechanotherapeutic agents alter the tissue mechanical properties by targeting the TGF-β pathway leading to reduced collagen type I expression [2]. AFM data were complemented with histopathological staining using Picrosirius Red and polarized microscopy. Moreover, the feasibility of applying these techniques to assess NMFs in human tissue biopsy samples was explored. Finally, in vitro studies were conducted to correlate the observed changes in NMFs with alterations in the nanomechanical properties of cancer and lung cells. The results revealed that AFM-based measurements are sensitive enough to detect subtle changes in NMFs during the progression of both cancer and pulmonary fibrosis. Changes in NMFs were also correlated with variations in collagen content. Notably, the NMFs of these two NCDs exhibited significant similarities, underscoring the potential of AFM-based nanomechanical measurements as biomarkers for diagnosing and monitoring the treatment of various NCDs. Future applications may include their use as prognostic biomarkers to support the development of novel personalized treatments.

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STUDY OF PLASTOCYANIN CONFORMATIONAL CHANGES WITH PH BY SINGLE PROTEIN UNFOLDING

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Plastocyanin (Pc) is a copper-containing redox protein involved in the photosynthetic electron transfer (ET) chain, transferring electrons from cytochrome b_6f to Photosystem I. In previous works, ET between Pc and photosystem I (PSI) was studied using electrochemical scanning tunneling microscopy (ECSTM) [1]. Studies using a Pc-coated Au (1,1,1) electrode have shown that the electrochemical response depends on the electrolyte pH. In particular, electrochemical impedance spectroscopy (EIS) evidences a reversible change in the total resistance (Rt) in the coated electrode with the pH. Suggesting a reversible arrangement of the Pc film at the electrode surface.

Following these results, we explored the existence of Pc conformational changes at the electrode surface due to the pH change. Protein unfolding using single molecule force spectroscopy (SMFS) can provide a hint of the Pc film conformational changes when pH is modified. In the current work, we look at Holo and Apo Pc unfolding at pH 4.5 (Pc physiological pH) and pH 7.8, from where we extract the frequency of positive events, and the distance and force where the probe-protein contact is ruptured. Here, we observe an increase of protein maximum extension with the increase of pH (Figure 1). Returning to its original values as pH decreases, indicating a reversible dependance with the pH. In all experiments, a similar breaking force (around 110 pN) was measured. At higher pH, an increase of number of events (Figure 1) -as well as multiple events- are observed. These results are aligned with the obtained using EIS. Altogether, our results support the assumption of a conformational change in the protein structure with the pH, modifying the monolayer characteristics.

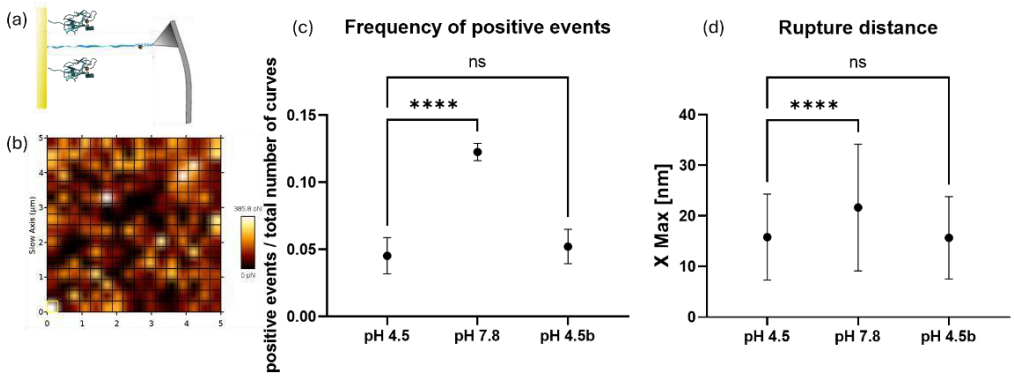

Figure 1. (a) Schematic representation of protein unfolding. (b) Rupture Force Map example of Holo Pc in ammonium acetate 50 mM at pH 4.5. (c) Frequency of unfolding events at different pH for Holo Pc in ammonium acetate 50 mM. (d) Rupture distance at different pH for Holo Pc in ammonium acetate 50 mM.

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Binding mechanism of oligopeptides on solid surface: Assessing the significance of single-molecule approach

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Oligopeptides, composed of short chains of amino acids, exhibit a remarkable capacity to interact with inorganic surfaces, including metals and oxides and other minerals. This unique property has shown great promise in a range of fundamental and applied topics, especially in the context of the origin of life [1], biomineralization [2] and biomaterials research [3]. Advances in combinatorial biology have enabled the identification of peptide sequences with strong affinity for various inorganic surfaces. These interactions are similar to molecular recognition processes involving biological ligands - receptors and are commonly studied *via* AFM-based force spectroscopy and the single-molecule level. Herein, we focus on the peptide/gold interface owing to its significance in various topics and its suitability to perform experiments both in model and real conditions. Through adsorption tests, a molecular-level understanding of the interface was achieved by combining experimental and theoretical studies, uncovering adsorption behavior that is highly sequence-dependent. Single-molecule force spectroscopy (SMFS) offers an alternative to these ensemble-averaged measurements, providing insights into interaction modes that may remain accessible with ensemble-average methods. While this technique is well-established for studying molecular recognition between biological entities, probing the interaction between biomolecules and mineral surfaces is particularly challenging. Indeed, this challenge arises from the numerous solid surface sites fully accessible to the biomolecule functional groups, introducing an additional level of intricacy compared to traditional ligand-receptor configurations. We show here that the development of a rigorous methodology to sorting interactions of interest is an essential step when probing single interaction events. The thermodynamic and kinetic aspects of the peptide adsorption are investigated using both static and dynamic force spectroscopy measurements. Specifically, we show the possibility of providing a reasonable estimate of the peptide free energy of adsorption $\Delta_{ads}G°$ by analyzing adhesion work fluctuations on the basis of the Jarzynski equality, and by using a parametric Gamma estimator. This approach [4] provides a robust method for examining the various factors influencing peptide adsorption and assessing their impact on $\Delta_{ads}G°$, offering an alternative to exploring adhesion forces, which can sometimes lead to misinterpretations.

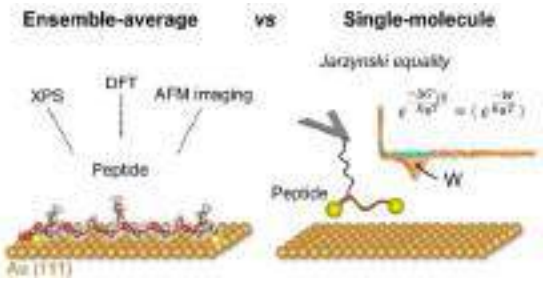

Figure 1. This presentation addresses the complementarity and potential discrepancies between single-molecule and ensemble-average approaches to probe the binding mechanism of oligopeptides on inorganic solids

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Atomic Force Microscopy reveals key insights into fibrillar species associated with Alzheimer's Disease

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Alzheimer's disease (AD) is the most common form of dementia and is characterized by the aggregation of two proteins in the parenchymal brain: The amyloid-β peptide, which forms extracellular plaques, and the hyperphosphorylated tubulin-associated unit (Tau), which creates intracellular neurofibrillary tangles [1,2]. However, the precise pathogenic mechanisms of AD, including the factors that trigger protein aggregation and the underlying molecular processes, remain largely unclear [1,2]. We employed Atomic Force Microscopy (AFM) to investigate fibrillar species associated with Alzheimer's disease (AD), focusing on the *in vitro* aggregation processes of the amyloid-β peptide 1-42 ($A\beta_{1-42}$) and the characterization of fibrillar structures in cerebrospinal fluid (CSF) samples. Our research demonstrates that AFM imaging is a powerful tool for monitoring $A\beta_{1-42}$ aggregation process, enabling comprehensive morphological characterization of the fibrillogenesis throughout its entire timeline [3]. This methodology facilitated the identification of critical structural features of the aggregation species, including the formation of distinctive protofibrils during the early aggregation stages [3]. These protofibrils subsequently evolve into complex macromolecular networks, providing valuable insights into the aggregation process. We employed surface-enhanced Raman spectroscopy (SERS) to monitor the progressive changes in the secondary structure of the aggregation species during fibrillogenesis, determining when β-sheet begin to dominate over random coils conformation [3]. These findings underscore the importance of early aggregation phases in understanding the molecular pathophysiology of AD and identifying potential therapeutic targets.

AFM analysis performed on crude CSF samples from AD patients and individuals with other neurological conditions, revealed peculiar fibrillar species characterized by the regular repetition of dimeric globular units along the longitudinal axis, which exhibit significant structural similarities to fibrin protofibrils (Figure 1). This finding suggests a potential link to blood-brain barrier disruption [4], and raises important questions about their role in the etiology of AD, as well as their potential utility as biomarkers for brain damage and disease progression [5]. Collectively, our findings highlight the critical role of AFM in elucidating the morphology and dynamics of amyloid aggregates in AD, paving the way for future advancements in diagnostics and therapeutics.

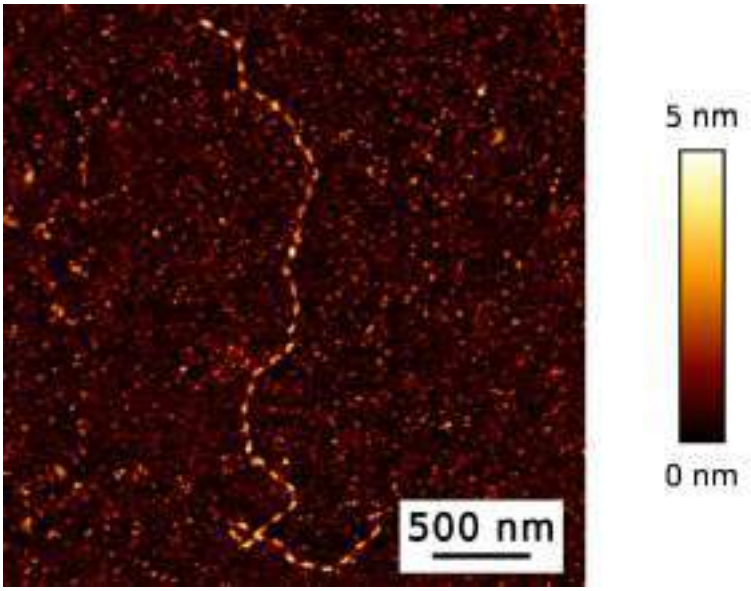

Figure 1. Tapping-mode AFM height image, acquired on the crude CSF of an AD patient deposited on mica, showing a fibril characterized by the regular repetition of dimeric globular units along the longitudinal axis (colour-coded height bar is shown beside).

Acknowledgments

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Microscale Biomechanical Characterization of Undecalcified Bone through AFM

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Bone stiffness is vital for understanding bone health, disease, and assessing fracture risk due to its direct correlation to strength and mechanical durability [1]. Traditionally, the stiffness of bone is quantified through bending, compression, and tensile tests, which measure resistance to deformation by applying mechanical forces. However, these techniques give bulk measurements and fail to characterize microscopic changes in bone stiffness important for understanding bone cancer disease states such as osteosarcoma. Here, we present a novel method based on atomic force microscopy (AFM) for micro-nanoscale biomechanical characterization [2] of undecalcified bone sections.

We developed an improved tape-transfer method [3] for sectioning and mounting bone cryosections, preserving their native form and mechanical properties during AFM studies. This method enables exact correlation of measured areas to same-slide microscopy imaging, allowing direct comparison of bone morphology to mechanical differences. In a pilot study, we analyzed femur sections from 7-week-old female mice, comparing stiffness at the single-cell level across different tissue types. AFM experiments revealed significant stiffness differences between undecalcified bone (120 ± 65 kPa), bone marrow (0.5 ± 0.7 kPa), and muscle (11 ± 4 kPa). Additionally, cartilage chondrocytes showed lower Young's modulus values than their extracellular matrix. Aged femur from 70- to 114-week-old female mice showed a gradual decline in bone stiffness, potentially corresponding to loss in mineral density and structural changes in bone. Our method utilizes AFM to determine the Young's modulus of tissue and cellular properties of undecalcified bone at the microscale. This technique can be transformative in studying bone health and abnormalities such as bone metastasis and osteoporosis, deepening the understanding of bone disease mechanisms.

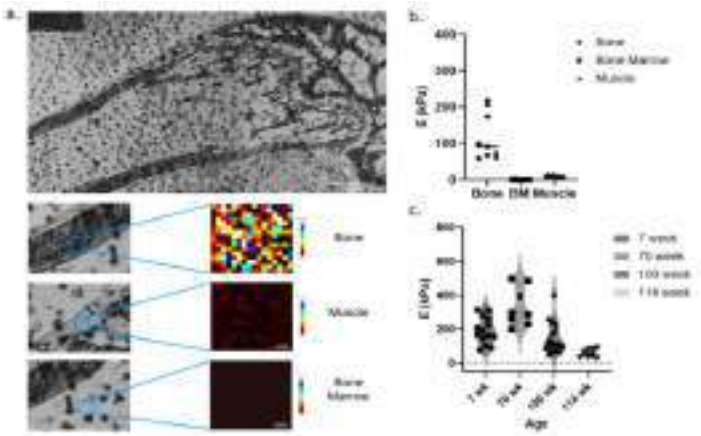

Figure 1. (a) Brightfield image of undecalcified femur from a 7-week-old female mouse, overlaid with AFM stiffness heatmaps. Zoomed-in regions highlight tissue-specific AFM stiffness heatmaps for undecalcified bone, bone marrow, and muscle. (b) Quantification of Young's modulus [E] for undecalcified bone, bone marrow, and muscle from 7-week-old female mice. (c) Young's modulus measurements of undecalcified femoral bone in female mice aged 7, 70, 110, 114 weeks shows a decline in tissue stiffness.

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Exploring the nanomechanics of Gaucher and Fabry disease models.

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Gaucher disease (GD) and Fabry disease (FD) are lipidosis caused by mutations in the genes encoding different lysosomal enzymes. In GD, acid β-glucosidase deficiency leads to glucosylceramide (GlcCer) accumulation; while in FD, the expression of α-galactosidase is altered leading an aberrant accumulation of globotriaosylceramide (Gb3) in cells [1][2]. Despite the genetic, biochemical, and medical levels of both diseases are well characterized, how GlcCer or Gb3 accumulation in membranes alters cell biology and biomechanics processes is poorly understood.

In this work, supported lipid bilayers (SLBs) were used to study the implications of GlcCer and Gb3 over the cell membrane nanomechanical properties by atomic force microscopy (AFM) and force spectroscopy (AFM-FS). For this, liposomes composed of different combinations of dioleoyl-phosphocholine (DOPC), sphingomyelin (SM_E) and cholesterol (Chol), with and without GlcCer or Gb3, were synthetized to simulate the excess of each glycosphingolipid in GD or FD, respectively. SLBs were generated following the Vesicle-fusion Method onto mica surfaces. Their structural, topographical and nanomechanical properties were characterized. The breakthrough force (F_b) was determined as an indicator of the lateral interactions between lipid molecules [3][4]. Our results show that both GlcCer and Gb3 fully segregate from DOPC SLBs into domains. However, in DOPC:SM_E:Chol SLBs, GlcCer generally leads to an increased packing and mechanical resistance of the membrane domains; while Gb3 generally affects the phases distribution leading to a phase inversion on the bilayer (Fig. 1). Finally, membrane's results were compared with overall nano-mechanics of cells from healthy vs GD and FD patients.

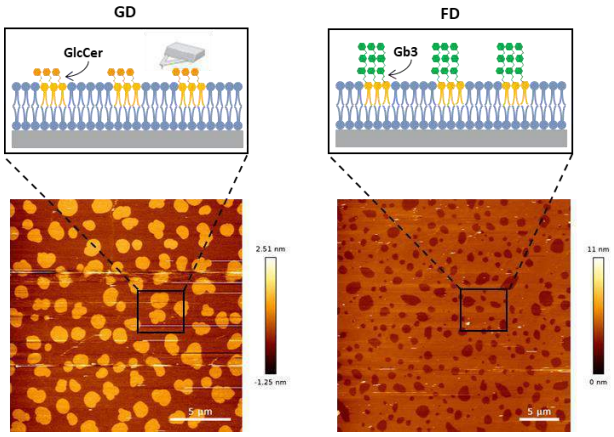

Figure 1. Differential phase distribution of DOPC:SME:Chol ± GlcCer or Gb3 supported lipid bilayers.

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Fibroblast extracellular-matrix mechanical properties in patients with Mucopolysaccharidosis by Atomic Force Microscopy

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Mucopolysaccharidosis (MPS) is a group of rare diseases characterized by the accumulation of glycosaminoglycans (GAGs), affecting the cell membrane, intracellular space and extracellular matrix [1]. Atomic force microscopy (AFM) is a key multifunctional tool in quantifying essential parameters of biological samples to study cell adhesion, tissue mechanics, immune response or adhesion forces between biomolecules, such as proteins, DNA or cell membrane ligands [2,3]. For instance, in the case of cancerous cells, it has been shown that they have a different stiffness than healthy cells [4]. The principal aim of this study is to evaluate the elasticity of the extracellular matrix of fibroblasts from MPS patients and to identify possible mechanical alterations, through changes in the morphology and Young's modulus of the extracellular matrix from the affected cells and to establish this parameter as a biomarker of the disease. *In vitro* analyses were done with the decellularized extracellullar matrix pre-stored in a phosphate-buffered saline (PBS) solution and the presence and crystallization of these salts were considered in the examination of the results. Morphology evaluation was complemented by using an optical profilometer, as it can be observed in Figure 1. Statistically significant differences in elastic modulus were found between MPS patients and healthy controls, demonstrating the potential of AFM to physically characterize and detect abnormalities in biological tissues, favoring the development of personalized diagnostics and treatments.

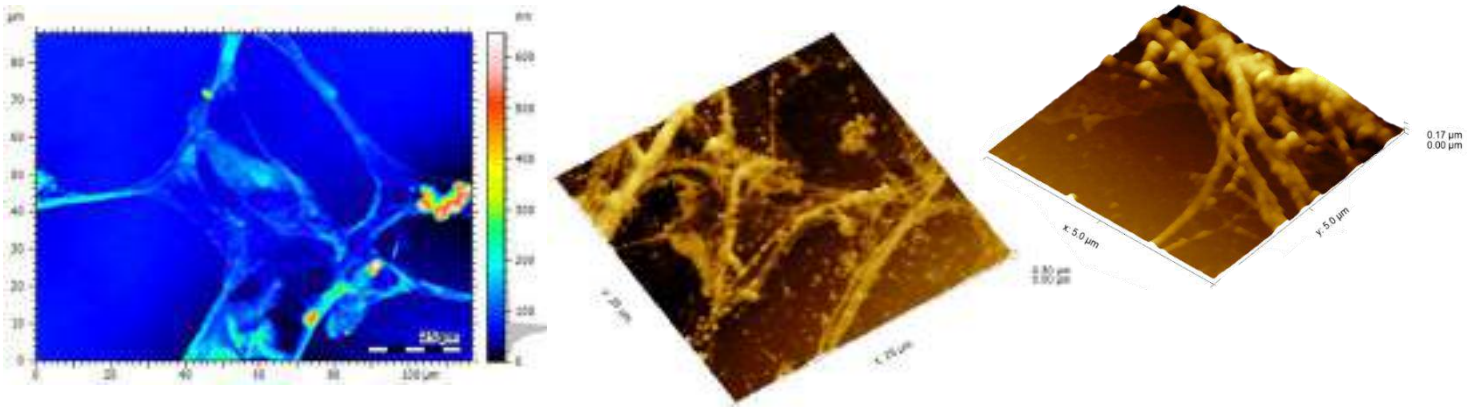

Figure 1. Optical profilometry (left) and AFM (middle and right) images of the PBS salts and extracellular matrix.

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Control Algorithms for Long-Range HS-AFM to Study Leukocyte Tether Formation

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The adhesion and rolling of leukocytes along the endothelium during immune responses rely on the formation of long membrane tethers (8 nm to ~40 µm) at high velocities (~mm/s) [1] over millisecond timescales (2–5 ms) [2]. However, conventional High-Speed Atomic Force Microscopy (HS-AFM) systems, with a range of ~1 µm to 40 µm, are inadequate for studying these processes [3].

To address this limitation, we developed a custom 3D-printed stage integrating a long-range vertical piezoelectric actuator with a range of ~60 µm and velocities up to 3 mm/s, coupled with a HS-AFM head. The extended range and high speed, while enhancing capabilities, pose challenges in data acquisition, as the increased force curve travel length elevates total data volume and acquisition time.

To overcome these challenges, we designed a novel control algorithm that optimizes the force-curve acquisition process by:

1. Minimizing the duration of non-essential phases (approach) through maximum piezo velocity, while maintaining precise control of critical phases (contact and retraction).
2. Implementing a predictive algorithm that dynamically estimates the sample's topography based on prior data, reducing redundant measurements and further improving efficiency.

Our algorithm achieved a significant reduction in the duration of individual force curves, decreasing total approach trace time 5 times compared to that at constant approach velocity. This improvement facilitates high-resolution, long-range force mapping and enables the study of membrane tether formation under physiologically relevant conditions.

These advancements in HS-AFM instrumentation and control algorithms pave the way for more comprehensive investigations of leukocyte mechanics and other fast, long-range biological phenomena, broadening the application of HS-AFM in biomedical research.

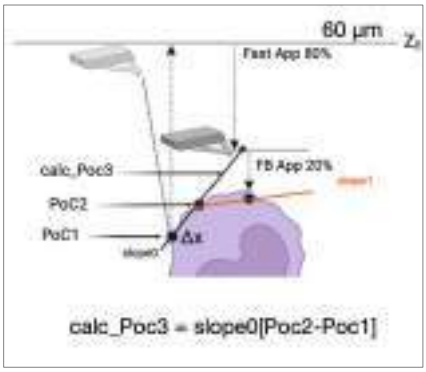

Figure 1. The algorithm uses an 80% fast approach and 20% slower force-feedback approach, estimating the Point of Contact (PoC) from the average of differences between previous PoCs, $PoC_{i-1} = \frac{1}{N}\sum_{n-1}^{N}(PoC_{i-n} - PoC_{i-n+1}) + PoC_{i-1}$, where N is number of previous PoCs used.

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MEMS based AFM: Towards understanding unfolding dynamics of bacteriorhodopsin in sub-µs time resolution

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Atomic Force Microscopy (AFM) has revolutionized the study of biomolecules, offering unparalleled insights into complex biological processes like protein unfolding by capturing real-time dynamics with high precision. Recent advancements in microfabrication, such as modifying cantilevers using focused ion beam (FIB) techniques, have achieved remarkable time resolution of 1 µs by increasing the probes resonance frequency [1], enabling near real-time observation of bacteriorhodopsin (BR) unfolding. However, further downsizing cantilever to achieve higher resonance frequency is constrained by the optical diffraction limit of laser-based detection, underscoring the need for alternative methods. Emerging approaches, including AFM probes based on quartz or MEMS, address these challenges, but not all probes offer required time and force resolution for studying biological processes (Figure 1a). This study focuses on MEMS and optomechanical devices as promising solutions to expand faster speed measurement capabilities of AFM probes in biomolecular research. Our setup involves MEMS-AFM probes from Vmicro company, which overcome the limitations of traditional cantilevers by offering higher resonance frequencies by one order of magnitude (≈ 15 MHz, Q = 600 in air) [2]. We investigate BR proteins in dry conditions with samples prepared on mica substrate and dried to preserve structural integrity. Experimental force spectroscopy performed on dry BR membrane taking advantage of the special ability of BR to maintain structural and functional stability in dry environments. Measurements done on a large number of sites revealed sawtooth patterns indicative of protein unfolding (figure 1b). This foundational work with MEMS probes paves the way for enhanced resolution and applicability to physiologically relevant environments using optomechanical probes in close future, offering GHz-range frequencies and temporal resolution in the nanosecond range by coupling optical cavities with mechanical resonators [3-4].

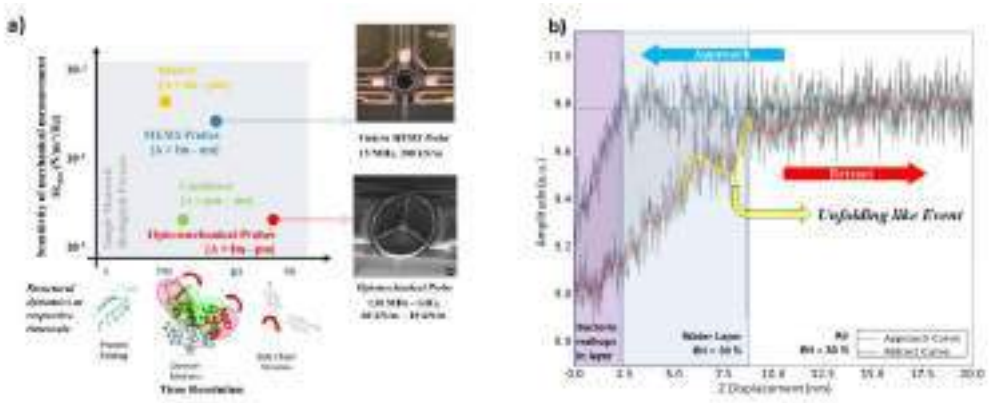

Figure 1. (a) Overview of AFM probe technologies for dynamic mode: force gradient resolution and temporal resolution of various probe technologies are depicted. Ranges of vibration amplitudes of each probe technology are indicated, with representative images for probes used in this work [2–4]. (b) Representative amplitude-displacement curves obtained with a MEMS probe (13.6 MHz) on dry BR samples (30% RH). During approach (blue curve), the probe oscillates at free amplitude until contacting the BR membrane, followed by a sharp amplitude decrease due to membrane compression. During retraction (red curve), sawtooth-like amplitude patterns (highlighted in yellow) are observed, indicating protein unfolding events. The probe then returns to free amplitude after detachment.

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Dynamic Interaction of Trigger Factor with the Ribosome Exit Tunnel During Cotranslational Folding

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Trigger Factor (TF) is a molecular chaperone essential for the co-translational folding of nascent polypeptides. However, the dynamic and possibly multivalent interaction of the TF with Ribosome-Nascent Chain Complexes (RNCs) is not clear. In this study, we explore the dynamics of TF's interaction with RNCs using high-speed atomic force microscopy (HS-AFM) and molecular dynamics (MD) simulations. We observe that TF binds specifically to RNCs, not empty ribosomes, interacting with ribosomal protein L23 and, less frequently, with L17 near the ribosomal exit tunnel. These interactions suggest that TF plays a role in stabilizing nascent polypeptides as they emerge from the ribosome, facilitating proper folding during translation. Interestingly, although TF is a dimer under physiological conditions, we observe that it binds to the ribosome as a monomer. In contrast, the dimeric form of TF is more stable, acting as a reservoir of molecules that can be recruited as needed.

Our findings also highlight the flexible nature of the TF monomer, which in solution reversibly transitions from an extended to a more compact conformation, driven by hydrophobic collapse as confirmed by MD simulations. This flexibility likely allows TF to bind to various targets. While previous studies suggested a 1:1 stoichiometry of TF binding to the ribosome, we find that multiple TF molecules can bind to a single ribosome during translation. These real-time HS-AFM observations provide new insights into TF's role in co-translational folding and highlight its dynamic interaction with the ribosome.

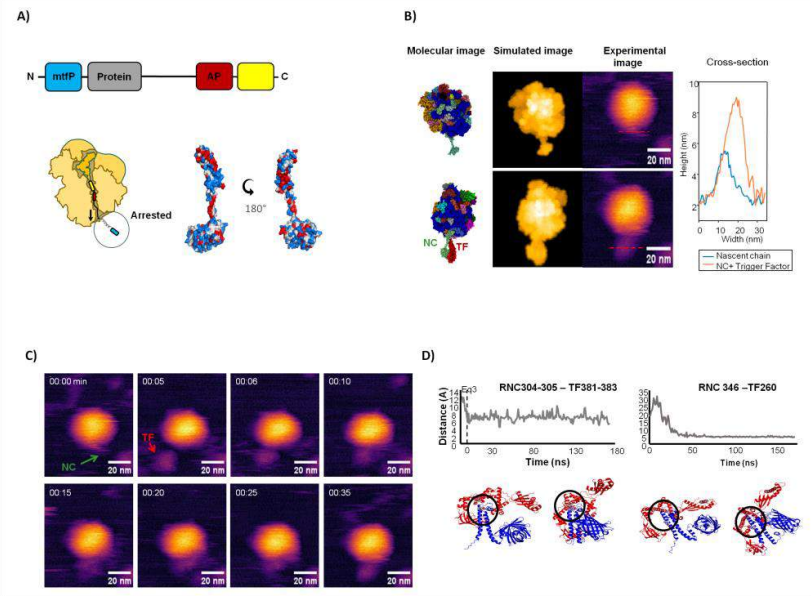

Figure 1. **A)** Schematic of the nascent chain used for the TF binding assay: includes a blue mtfp fluorescent protein, grey α-helices (A and B) from the KCNQ channel, a red SecM arrest peptide, and a yellow mcpVenus protein (not translated). The AlphaFold structure of the accessible part of the nascent chain is shown, colored by hydrophobicity (red for hydrophobic, blue for hydrophilic, white for neutral residues). **B)** Diagram of TF-RNC interaction, displaying molecular images of the ribosome complex with and without TF binding. Simulated AFM images from BioAFMViewer (ref) [4] are matched with experimental data, along with a graph of cross-sectional height and width for the nascent chain alone

versus TF-bound. **C)** Sequential frames show TF binding to RNC in physiological buffer (RNC at 2 nM, TF at 1 µM), where TF remains bound for 30 seconds. **D)** Molecular dynamics simulations detail the TF-NC interaction, showing decreasing distance between residues 304-5 from RNC 381-383 from TF, and residue 346 from RNC with 260 from TF over time and the initial and final frames, highlighting binding stability and dynamics.

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PHOTOTHERMAL INFRARED NANOSPECTROSCOPY INVESTIGATION OF ALTERATION IN THE SECONDARY STRUCTURE OF $A\beta_{1-42}$ AGGREGATES INDUCED BY GREEN TEA ACTIVE COMPOUNDS

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Abnormal protein aggregation is a pathological process related to many serious disorders including neurological diseases. In particular, the presence of fibrillar aggregates of the amyloid β peptide ($A\beta_{1-42}$) is a molecular marker of Alzheimer's disease [1]. Although the general structure of $A\beta_{1-42}$ fibrils is well described, most techniques are based on the study of bulk samples, providing only averaged information. For a better insight into the processes related to protein misfolding, and aggregation, single-molecule level studies are required.

During the past 20 years, a novel analytical approach called molecular nanospectroscopy has been developed and continuously improved [2]. These techniques combine the nanoscale spatial resolution of scanning probe microscopy (usually AFM) and the chemical sensitivity of vibrational spectroscopy (Raman or FTIR). Among many applications, molecular nanospectroscopy can be used to investigate a secondary structure of the protein and study the process of abnormal protein aggregation [1-3].

In the presented study, we applied photothermal infrared nanospectroscopy, also referred to as atomic force microscopy-infrared (AFM-IR) spectroscopy, to investigate the interaction between $A\beta_{1-42}$ and epigallocatechin gallate (EGCG), a natural flavonoid which is present in green tea. The obtained AFM-IR spectra allowed us to profile the secondary structure of the individual protein aggregates and qualify the influence of the EGCG on the ongoing aggregation process. Moreover, we performed the nanoscale chemical imaging of the individual Aβ fibrils visualizing the distribution of particular protein structures within the investigated aggregates. Exemplary results are presented in Figure 1.

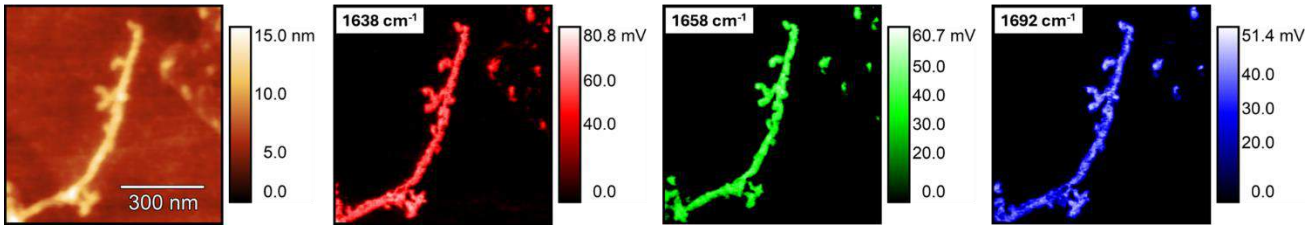

Figure 1. Results of AFM-IR chemical mapping of a single $A\beta_{1-42}$ fibril. Three absorption maps collected for different light wavenumber in amide I spectral range correspond to a specific secondary structure: the parallel β-sheet (1638 cm^{-1}), the unstructured coil (1658 cm^{-1}), and the anti-parallel β-sheet (1692 cm^{-1}).

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Lipid membrane remodeling and repair

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Membrane remodeling and **repair** functions are indispensable processes for all cellular life, as membrane is constantly subjected to various stresses, including mechanical forces, oxidative damage, and pathogen attacks. These functions are executed by proteins such as **Vipp1** in photosynthetic plastids[1], and ESCRT-III[2] in eukaryotic organisms. Using **FAST-AFM** (*Fig. 1a,c*) and **CRYO-EM** (*Fig. 1b*), we observe Vipp1 polymerizing into sheets and spirals from **membrane defects**. Real-time imaging reveals spiral convergence into central rings crucial for membrane budding (*Fig. 1a* and *c*). Our study emphasizes the *role of membrane defects in Vipp1 polymerization and underscores the importance of ring structures in lipid membrane interaction and repair*. Examining Vipp1's morphological shifts between polymers, we identify helical filament architecture and twisting as key factors for transitioning between planar and 3D forms (Fig. 1c). The structural parallels with ESCRT-III hint at broader implications for understanding geometric changes in ESCRT-III filaments during the shift between 2D and 3D configurations[3]

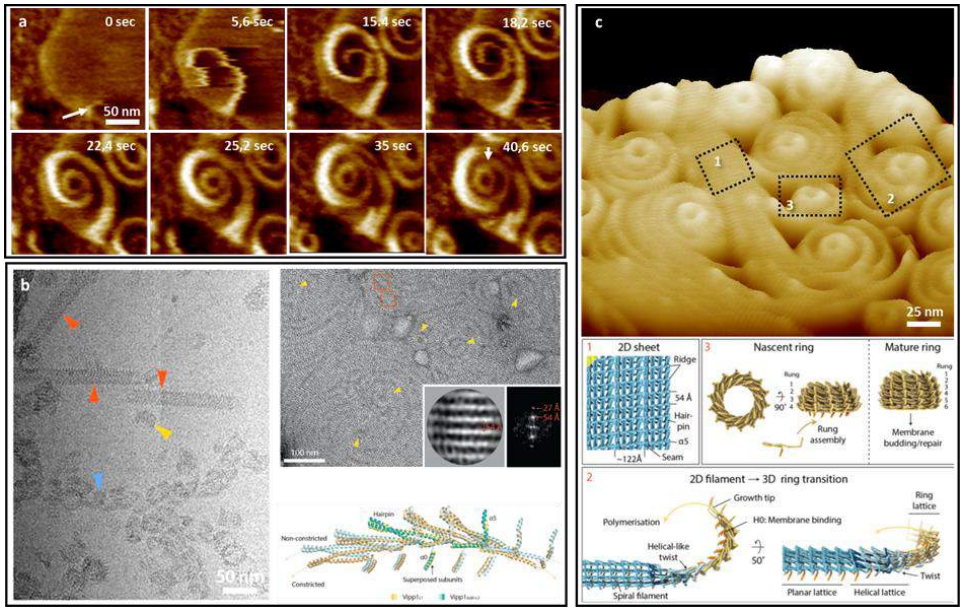

Figure 1 a) Fast-AFM images showing ring biogenesis from a spiral. **b)** Cryo-EM reveals Vipp1 helical filaments. NS-EM exhibits 2D planar spirals and sheets on a lipid. **c)**AFM image (top) and model of Vipp1 planar sheet, spiral and 3D ring biogènesis.

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AFM on reconstituted bacterial outer membranes

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Antimicrobial resistance represents a growing health threat: more and more bacteria develop resistance against currently available antibiotics, with the consequence that currently routine diseases and medical interventions can become major health risks. This risk is particularly pronounced for Gram-negative bacteria, since they contain an additional layer of protection in the form of the outer membrane. Besides this medical importance, interest in the outer membrane has been stimulated by recent scientific discoveries such as the mechanical role of the outer membrane, its remarkable lack of fluidity, and its phase-separated nature. Atomic force microscopy (AFM) has played a key role in such discoveries. In this context, we have established and will here briefly report on AFM as a crucial tool to resolve outer membrane organisation of living bacteria [1,2,3].

For all its advantages, however, AFM on living cells comes with the disadvantages that their surfaces represent substantial complexity and heterogeneity and that – to gain further scientific understanding – there is limit scope to vary physicochemical properties of the membrane without affecting bacterial viability. To address this, we set out to complement the experiments on living cells with AFM experiments on reconstituted bacterial outer membranes. Here we describe the development of such model membranes, including key characteristics such as the presence of trimeric outer membrane proteins (OmpF) and membrane asymmetry between phospholipids in the inner leaflet and lipopolysaccharides (LPS) in the outer leaflet. The inclusion of LPS and outer membrane proteins is critical, as they are largely responsible for providing mechanical strength and impermeability to many antibiotics [4]. Such model membranes have the additional advantage that they are amenable to (conventional and high-speed) AFM experiments for high in-plane resolution, and to scattering methods for high out-of-plane resolution, of which we will here present the first results.

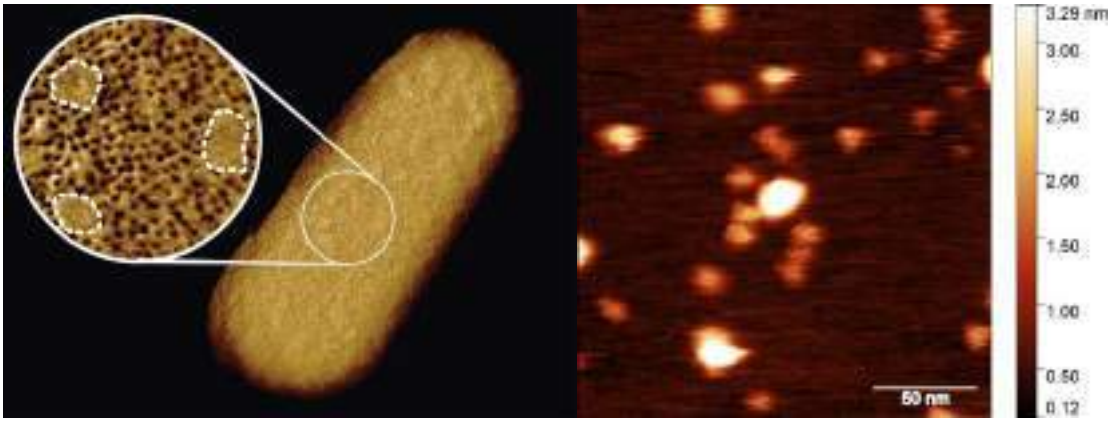

Figure 1. Left: AFM image with close-up, acquired on living *E.coli*.[1] Right: AFM image taken on a Nanoracer, showing the OmpF trimer structure within a supported lipid bilayer.

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Nanoscale dielectric imaging of cells and bacteria by scanning dielectric microscopy assisted by deep convolutional neural networks

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The computational demand for processing electrical images in Scanning Dielectric Microscopy (SDM) is particularly high, sometimes requiring weeks or even months to complete due to the complexity of the models employed and the high volume of data to process. This creates a need for methods that can accelerate the data processing workflow, and artificial intelligence can be an adequate tool for this purpose. Techniques such as Multilayer Perceptrons have been previously used to model and replicate the processes involved in SDM data simulations. This work proposes the use of Convolutional Neural Networks (CNNs), known for their proficiency in image processing, in combination with a point-by-point Deep Neural Networks (DNN). This model allows for the consideration of point-specific electrical and topographical data, while also taking into account information from its surroundings resulting in a better contextualization of each point within the image.

This proposed neural network is trained using data derived from calculations of experimental data and has a high level of accuracy when replicating the calculation outcomes, even when trained with small datasets. By training this model with data curated for its intended application, it is possible to obtain pre-trained models that only require fine tuning for accurate predictions, which reduces even further the amount of data that is needed and thus the total execution time. Application of the methodology to eukaryotic and bacterial cells are demonstrated showing the potential of this approach for high throughput nanoscale dielectric imaging of biological samples.

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AQUEOUS LIQUID/SOLID INTERFACE AT THE NANOSCALE: A COMPARISON OF THE 3D-AFM MODE TO OTHER EXPERIMENTAL TECHNIQUES AND SIMULATIONS

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A large number of phenomena occur at the aqueous solution/solid interface, in the first few nanometers of liquid in contact with the solid: catalysis reactions, bio-recognition on biosensors, tribology, water recovery, etc. The fundamental understanding of these processes requires molecular-scale knowledge of the physicochemical behavior of solid surfaces in contact with liquids and the structure of liquids at the interface.

Most conventional techniques such as IR spectroscopy, X-ray diffraction, NMR, etc. are unable to directly characterize the local structure of the liquid with high spatial resolution. The surface force apparatus (SFA) was the first technique to probe the structure of the interfacial solution between two lipid bilayers deposited on microscopic mica surfaces immersed in an aqueous solution [1]. Since then, the second harmonic generation (SHG) technique has provided vibrational spectra of molecules adsorbed or reactive at the interface [2,3]. However, all these techniques are limited to large, atomically smooth surfaces and cannot achieve molecular lateral resolution. In parallel, molecular dynamics (MD) simulations have been widely used to study the interface at the molecular scale [4-7]. It has been shown that the interface generally consists of a compact layer of oriented water molecules, and that the hydration layer varies as a function of facets for Ag or Au surfaces, for example [5]. In addition, the presence of ions from the aqueous solution and their adsorption onto the solid surface can also have an impact on the hydration layer [6]. Previously, we have shown that the organization and dynamic properties of Na+ and Cl- ions and interfacial water molecules on the salinized silica surface depend on the structure of the silane monolayer and the charge of the silane molecule head groups. We have also observed that the overall properties of water are recovered at about 0.7 nm above the silane monolayer [7].

In comparison, three-dimensional atomic force microscopy (3D-AFM) offers new opportunities to experimentally study liquid-solid interfaces at the molecular scale, in both lateral and normal directions to the interface [8-10]. Previous work has shown that water molecules adjacent to a membrane interact strongly with the head groups of adsorbed molecules [10]. The unique ability to visualise hydration structures has also been reported [8].Our aim is to compare 3D –AFM to other techniques and MD simulations for potential applications in biotechnology and nanomedicine.

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Collagen fibril degradation in thoracic aortic aneurysms

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Thoracic aortic aneurysms (TAAs) are defined as a ballooning over 5.5 cm of the thoracic aorta. Congenital heart conditions such as bicuspid aortic valve (BAV) elevate TAA risk, however, in many cases TAAs undergo without any underlying congenital or genetic disease. These are referred to as idiopathic degenerative aneurysms (DA). Previous work has shown that despite the presence of a similar diameter aneurysm to DA, BAV patients appear to have a more structurally intact dilated aorta with stiffer tissue and compact elastin fibres [1]. Here, we sought to determine ultrastructural and nanomechanical changes in the collagen fibrils of TAA tissue from BAV and DA patients. Aortic wall tissues were obtained from 10 BAV patients with mean age of 62.2 ± 14.06 years (7 males and 3 females) and 10 DA samples with mean age of 69.8 ± 9.93 years (8 males and 2 females) (P=0.241). The tissues were cryosectioned to 5 µm and imaged with atomic force microscopy (AFM). The morphology of the adventitial, medial and intimal layers of the tissue were examined with a Si tip with 8 nm radius and a 40 N/m flexural stiffness. Collagen fibril organisation and mechanical properties were determined with the PeakForce QNM modality [2]. Abundant collagen fibrils were identified in both groups. However, in DA tissue the collagen fibrils clearly demonstrated signs of degradation with altered D-periods and decreased elastic modulus. The whole aortic tissue of DA patients demonstrated a 62% decrease in elastic modulus compared to BAV aortic tissue ($p<0.0001$). We also observed layer-specific changes in elastic modulus. For DA tissue, the tissue stiffness was lower by 37 %, 30 % and 42 % in the inner, middle and outer layers respectively ($p<0.0001$). The D-period was found to be increased and more variable in the DA group relative to BAV, (Figure 1B, $p<0.05$). The heterogenous distribution of D-periods was more pronounced in the middle layer. Previous research has demonstrated that diseased aortic tissue has decreased extensibility and tends to stiffen over time, which could lead to aortic dissections and other catastrophic aortopathies, thus decreasing the chances of patient's survival drastically [3]. Our study with AFM PFQNM demonstrates structural and biomechanical differences of the collagen fibrils in BAV and TAV-DA, providing unique insights into the nanoscale degradation processes associated with this disease. These findings underscore the potential of AFM as a powerful tool for studying extracellular matrix alteration at a nanoscale in TAA and may aid the development of future diagnostic and therapeutic strategies targeting the collagen matrix.

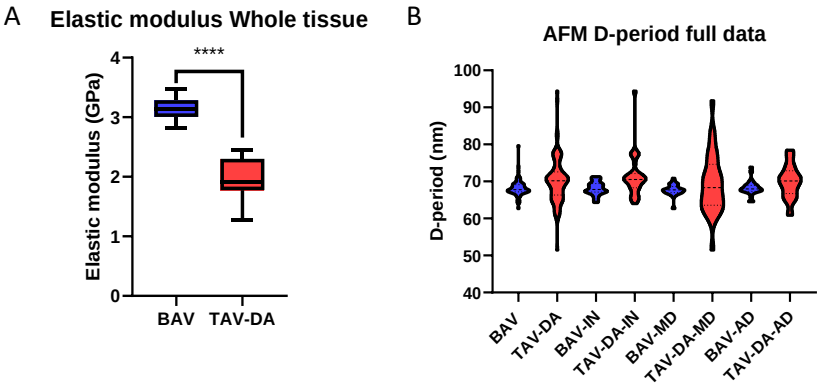

Figure 1. A) Elastic modulus difference between DA and BAV in whole tissue B) Layer specific D-periodicity difference between DA and BAV; IN-Inner layer, MD-middle layer, AD-outer layer; (*) $p<0.05$, (**) $p<0.01$, (****) $p<0.0001$.

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CO-CULTURED AGGREGATES IN CYLINDRICAL CAVITIES: FIBROBLASTS INFLUENCE THE MICROENVIRONMENT OF PANCREATIC CANCER CELLS

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Pancreatic ductal adenocarcinoma is a malignant disease characterized by a desmoplastic stroma, i.e., a dense fibrotic tissue that surrounds the tumor and contributes to drug resistance [1]. The stroma can affect the stiffness of the tumor microenvironment (TME) and drive tumor growth, mainly due to the proliferation of fibroblasts [2]. However, the rheological properties of three-dimensional aggregates that mimic the TME under the influence of fibroblasts remain poorly understood [3]. To address this, cylindrical cavities were designed in PolyHema-coated Petri dishes as confined spaces to cultivate multicellular aggregates (monotypic and heterotypic) and measure their mechanical properties. In this study, we used human pancreatic cancer cells (originated from a pancreatic carcinoma, PANC-1), and normal dermal fibroblasts (NDF). The monotypic aggregates consisted of PANC-1 cells, while the heterotypic ones were composed of both PANC-1 and NDF cells. Atomic force microscopy (AFM) was employed to determine the viscoelastic properties of these samples, such as the loss and the storage moduli, at different time scales. As a result, the AFM measurements revealed that heterotypic aggregates were more stable and exhibited significantly higher rigidity compared to monotypic ones. This increased rigidity is likely due to extracellular matrix proteins secreted by fibroblasts, which strengthens the aggregate structure [4]. These findings suggest that fibroblasts in the stroma may play a significant role in pancreatic ductal adenocarcinoma progression and its resistance to mechanical stresses and therapies.

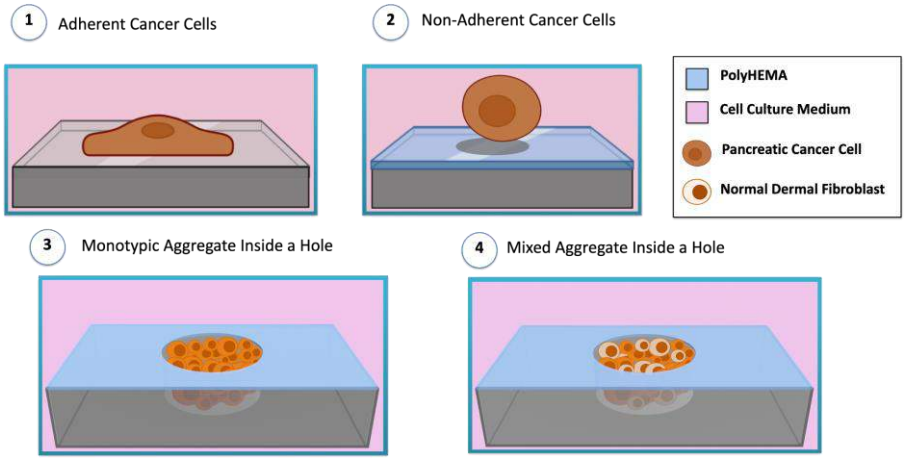

Figure 1. Representation of the samples used in this work to study the rheological properties of cancer cell aggregates. PANC-1 and NDF cell lines were used in four different preparations: adherent (uncoated dishes) and non-adherent PANC-1 cells (in PolyHEMA-coated dishes), monotypic, and heterotypic aggregates (both in PolyHema-coated dishes).

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Multimodal Biomechanical Analysis of Cell Spheroids: Combining AFM and Complementary Techniques

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Cell spheroids are a simple yet powerful model system for studying biochemical and biophysical intercellular interactions, including those occurring during tissue morphogenesis and regeneration. Describing mechanical phenomena at the level of multicellular model systems is essential for understanding tissue mechanics and its fundamental principles in health and disease. Although AFM has been routinely applied in cell biomechanics studies [1], spheroids present a more complex system where only surface cells are accessible for probing with a standard AFM setup. Mechanical analysis of cell spheroids as a whole requires larger-scale techniques, such as parallel-plate compression. Interestingly, combining data from AFM and parallel-plate compression enables the estimation of parameters beyond the capabilities of either technique alone. In this study, we analyzed spheroids composed of mesenchymal and epithelial cells, integrating data from both techniques with microscopy, experiments on spheroid behavior under different conditions, and modeling. We observed higher surface tension in mesenchymal spheroids compared to epithelial ones, as well as increased bulk modulus and relaxation time. These latter two parameters align with the bulk poroelastic behavior of spheroids and the higher cell density and ECM content in mesenchymal spheroids. Our results demonstrate that interactions between cells and the ECM, along with cellular contractility, determine the mechanical properties of multicellular aggregates [3]. This study was supported by the Russian Science Foundation (grant No. 23-74-10113, https://rscf.ru/en/project/23-74-10113/).

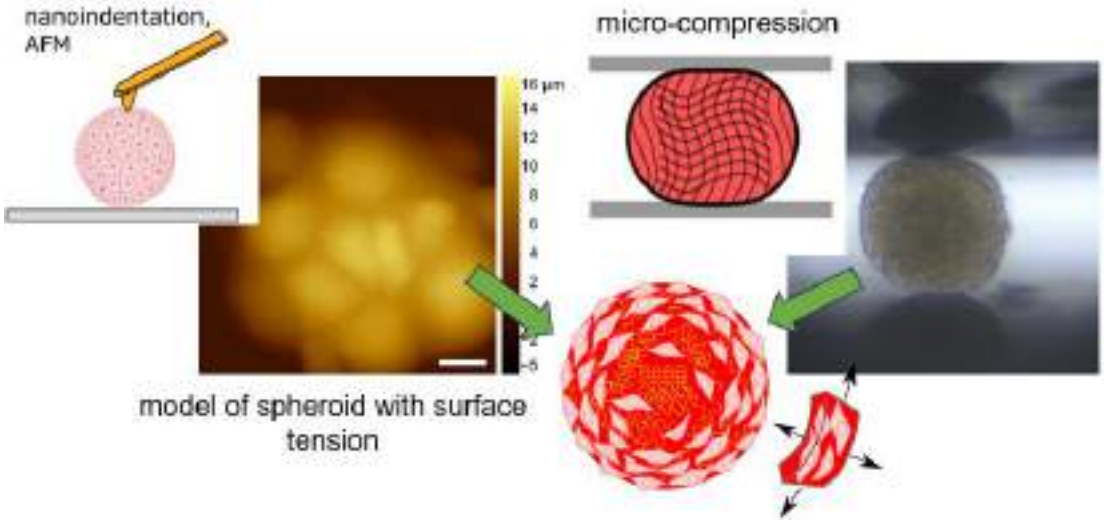

Figure 1. Combination of AFM with micro-compression experiments and modelling allows profound description of spheroid biomechanics, providing data on surface and bulk properties (surface tension, bulk modulus, and relaxation).

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

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Microbial glycolipids (MGs) are biosurfactants comprising a hydrophilic saccharide moiety coupled to a hydrophobic fatty acid. Their antimicrobial properties are raising an increasing interest as they might be bio-based alternatives to antibiotics, as well as offering potential anti-biofilm or wound healing properties [1], offering biodegradability and low ecotoxicity. Antimicrobial activity involves changes in and rupture of the cellular membrane inducing lysis. likely through saccharide-membrane interactions followed by penetration driven by the lipophilic moiety [2]. However, their mechanisms of action are still not well understood. The current project aims to study the effect of microbial glycolipids on supported lipid bilayers using atomic force microscopy (AFM) to elucidate the underlying molecular mechanisms at the nanoscale. By performing both imaging and force spectroscopy measurements, we are able to access a wide array of membrane properties including topography, dynamics, breakthrough forces and elasticity.

First, we observed the dynamic evolution of two types of model phospholipid (PL) membranes after injection of two distinct MGs (sophorolipid C18:1 (SL) and glycolipid C18:1 (GL). We characterized the effect of MGs on the (nano)mechanical and structural properties of membranes through membrane solubilization or expansion (Figure 1). Second, supported lipid bilayers were formed by mixing PLs and MGs at various ratios to form vesicles in suspension and were characterized using a similar approach. These insights into the effect of MGs on model membranes at the nanometre scale can contribute to a better understanding of their biological activity, as well as their potential use as nanocarriers.

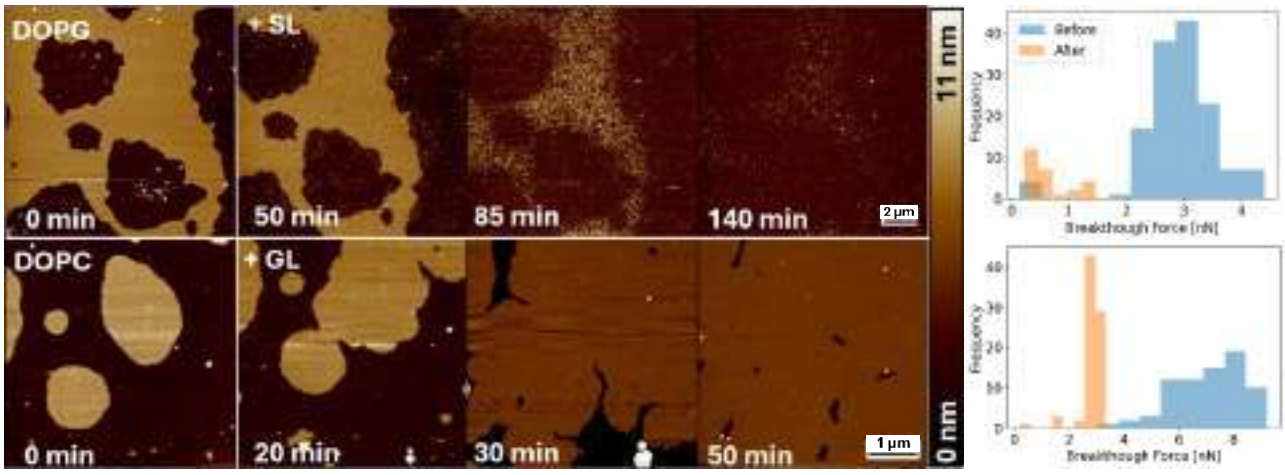

Figure 1. AFM images showing the evolution of model membranes (DOPG – top, DOPC – bottom), after injection of MGs (sophorolipid C18:1 (SL) – top, glycolipid C18:1 (GL) – bottom) showing membrane solubilization (top) and expansion (bottom), and evolution of the Breakthrough Forces before and after injection (right histograms).

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Bacterial Adhesion Strength is Linked to Cell Wall Age

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Initial bacterial contact formation is a critical precursor to biofilm formation, a process that can have beneficial or detrimental outcomes depending on the context. To understand this initial adhesion step, we investigate the adhesive properties of individual bacterial cells using single-cell force spectroscopy. The interaction is primarily mediated by macromolecules on the bacterial cell wall, whose composition and spatial distribution vary significantly between cells and influence adhesion behavior. Our studies on *Staphylococcus aureus* revealed distinct regions of high adhesion on the cell surface, contributing to heterogeneity in adhesive strength [1]. Additionally, bacterial cell division reveals newly synthesized cell wall structures, constituting approximately 33–50% of the surface, leading to further heterogeneity within individual cells [2]. We combine high-resolution Atomic Force Microscopy imaging of single *S. aureus* cell wall morphology with blunt tip force spectroscopy using a tip change technique to investigate the influence of cell wall age on the adhesion capability of individual cells.

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