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Force microscopy is one of the pillars that sustain the advances in nanoscience and nanotechnology. In the recent years, force microscopy has experienced a boom in its application range from academia to industry. However, the technique or more precisely the methodologies associated with AFM face numerous challenges to bring together molecular resolution, quantitative mapping and high speed imaging. To overcome those challenges will require developing new scientific approaches as well as novel engineering solutions. In this context, the AFM is experiencing the evolution from the single to the multifrequency excitation and detection schemes. This transition is also stimulated by the emergence of new topics such as energy storage and nanomedicine.

The Multifrequency AFM conference series started in September 2008, this is about seventeen years ago. The topics covered by the conference have evolved to address current scientific and technology challenges at the nanoscale.

The 10th Multifrequency Conference is divided in three Symposia, the 4th Symposium of Cell and Soft Matter Nanomechancis, the 3rd Symposium on Solid-Liquid Interfaces and the Multifrequency AFM core sessions. The 10th MF conference represents an excellent opportunity to gather some perspective about the evolution of the field. The overall goal of conference series has remained unchanged: to create the environment where experts and newcomers alike interact, exchange and share information, expertise and knowledge about the science and technology of the new generation of force microscopes.

The long term vision of the Multifrequency AFM conference series is to contribute to the expansion of nanomechanical tools and methodologies in academy and industry.

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GENERAL INFORMATION

Poster Session

The poster session will be held during the conference on Wednesday 28th May at the building entrance from 16:30 to 18:00. Pins for mounting the poster on the board will be provided.

There will be a competition in the poster session for undergraduate students. The 1st prize is a trophy and a diploma, and the 2nd prize is a diploma.

Internet Access

Wireless internet access is available in the conference venue. Username and password will be provided at the registration desk

Dinner

The conference dinner will be held near the conference venue on Wednesday 28th at 19:30.

10th MULTIFREQUENCY AFM CONFERENCE SUMMARY

Monday 26		
	Room 1	Room 2
Afternoon	Symposium on Cell and Soft Matter Nanomechanics	
Tuesday 27		
	Room 1	Room 2
Morning	Symposium on Solid-Liquid Interfaces (Keynote James de Yoreo)	
	Symposium on Cell and Soft Matter Nanomechanics	Symposium on Solid-Liquid Interfaces
Afternoon	Symposium on Cell and Soft Matter Nanomechanics	Symposium on Solid-Liquid Interfaces
Wednesday 28		
	Room 1	Room 2
Morning	Multifrequency AFM Core Sessions (Keynote Nina Balke)	
	Multifrequency AFM Core Sessions	Symposium on Solid-Liquid Interfaces
		Multifrequency AFM Core Sessions
Afternoon	Multifrequency AFM Core Sessions	
	Poster Session	
	Multifrequency AFM Core Sessions (Plenary Peter Hinterdorfer)	
Thursday 29		
	Room 1	Room 2
Morning	Multifrequency AFM Core Sessions (Keynote Toshio Ando)	
	Multifrequency AFM Core Sessions	Multifrequency AFM Core Sessions
Afternoon	Multifrequency AFM Core Sessions	
Friday 30		
	Room 1	Room 2
Morning	Multifrequency AFM Core Sessions	

10th Multifrequency AFM Conference, May 26-30th, 2025			
Monday 26th May 2025			
Room 1	**4th Symposium on Cell and Soft Matter Nanomechanics**		
Time	**Duration (min)**	**Type**	**Presenter**
13:55 14:00	**5**	Welcome	Ricardo Garcia
14:00 14:25	**20+5**	Invited	Alba Diz-Muñoz
14:25 14:50	**20+5**	Invited	Jorge Alegre-Cebollada
14:50 15:10	**16+4**	Expert	Alexander Cartagena-Rivera
15:10 15:25	**12+3**	Oral	Annalisa Caló
Coffee Break: 15:30-16:00			
Room 1		Session moderator	Michael Krieg
16:00 16:25	**20+5**	Invited	Alexander Kabla
16:25 16:45	**16+4**	Expert	Jeanlex de Sousa
16:45 17:05	**16+4**	Expert	Felix Rico
17:05 17:20	**12+3**	Oral	Jaime Tejedor
End day 1			

10th Multifrequency AFM Conference, May 26-30th, 2025

Tuesday 27th May 2025

Room 1	3rd Symposium on Solid-Liquid Interfaces		
Time	**Duration (min)**	**Type**	**Presenter**
08:50-08:55	**5**	Welcome	Ricardo Garcia
09:00-09:30	**24+6**	Keynote	James de Yoreo

4th Symposium on Cell and Soft Matter Nanomechanics				**3rd Symposium on Solid-Liquid Interfaces**			
Room 1		Session moderator	Andra C. Dumitru	Room 2		Session moderator	Esther Lladó-Alarcón
09:40 10:05	**20+5**	Invited	Mingdong Dong	09:40 10:00	**16+4**	Expert	Matteo Chiesa
10:05 10:25	**16+4**	Expert	Michael Krieg	10:00 10:15	**12+3**	Oral	Markus Valtiner
				10.15 10.30	**12+3**	Oral	Gianlorenzo Bussetti
Coffee Break: 10:30-11:00				**Coffee Break: 10:30-11:00**			
4th Symposium on Cell and Soft Matter Nanomechanics				**3rd Symposium on Solid-Liquid Interfaces**			
Room 1		Session moderator	Jorge Alegre-Cebollada	Room 2		Session moderator	Angelika Kuhnle and Jeffrey Comer
11.00 11.25	**20+5**	Invited	Tomaso Zambelli	11.00 11.25	**20+5**	Invited	Marie P. Gaigeot
11:25 11:45	**16+4**	Expert	Marina I. Giannotti	11.25 11.45	**16+4**	Expert	Jeffrey Comer
11.45 12.00	**12+3**	Oral	José Pérez	11.45 12.00	**12+3**	Oral	Jian Gao
Break							
12:20 12:40	**16+4**	Expert	Andra C. Dumitru	12:20 12:45	**20+5**	Invited	Yingjie Zhang
12:40 12:55	**12+3**	Oral	Yoo Jin Oh	12:45 13:00	**12+3**	Oral	Zhen Tang
12:55 13:10	**12+3**	Oral	Victor G. Gisbert	13:00 13:15	**12+3**	Oral	Simone Benaglia

Lunch Break: 13:30-15:00

4th Symposium on Cell and Soft Matter Nanomechanics				3rd Symposium on Solid-Liquid Interfaces			
Room 1		Session moderator	Marina I. Giannotti	Room 2		Session moderator	Yingjie Zhang
15:00 15:25	**20+5**	Invited	Fernando Moreno-Herrero	15:00 15:25	**20+5**	Invited	Angelika Kuhnle
15:25 15:45	**16+4**	Expert	Shivaprasad Patil	15:25 15:45	**16+4**	Expert	Igor Siretanu
15:45 16:00	**12+3**	Oral	Livia Angeloni	15:45 16:00	**12+3**	Oral	
16:00 16:15	**12+3**	Oral	Andre Körnig				
16:15 16:30	**12+3**	Oral	Rafael Daza				
Coffee Break (16:30-17:00)							
End day 2							

10th Multifrequency AFM Conference, May 26-30th, 2025

Wednesday 28th May 2025

Room 1		General Session	
Time	**Duration (min)**	**Type**	**Presenter**
Room 1		Session moderator	James de Yoreo
09:00- 09:30	**24+6**	Keynote	Nina Balke
09:30- 09:55	**20+5**	Invited	Patrick Unwin
09:55-10:15	**16+4**	Expert	Kislon Voïchovsky

Coffee Break: 10:20-11:00

Multifrequency AFM sessions				**3rd Symposium on Solid-Liquid Interfaces**			
Room 1		Session moderator	Roger Proksch	Room 2		Session moderator	Kislon Voitchovsky
11:00 11:20	**16+4**	Expert	Hanna Cho	11:00 11:20	**16+4**	Expert	Esther Alarcón-Lladó
11:20 11:35	**12+3**	Oral	Daniel Martin-Jiménez	11.20 11.40	**16+4**	Expert	Christopher Kley
11:35 11:50	**12+3**	Oral	Marvin Hoffer	11.40 11.55	**12+3**	Oral	Florian Hausen
11:50 12:05	**12+3**	Oral	Daniel Rothhardt	**Multifrequency AFM sessions**			
12:05 12:20	**12+3**	Oral	Lara V. Fricke	12:05 12:20	**12+3**	Oral	Stefano Chiodini
Break				**Break**			

Multifrequency AFM sessions

Room 1		Session moderator	Hanna Cho	Room 2		Session moderator	Daniel Martín-Jiménez
12.30 12.55	**16+4**	Invited	Roger Proksch	12.30 12.45	**20+5**	Oral	Daniel Ebeling
12:55 13:15	**16+4**	Expert	Marti Checa	12.45 13.00	**16+4**	Oral	Jaime Colchero
13.15 13.30	**12+3**	Oral	Hans Gunstheimer	13.00 13.15	**12+3**	Oral	Miriam Jaafar
				13.15 13.30	**12+3**	Oral	Jorge Marques

Lunch break 13:30-15:00

Multifrequency AFM sessions			
Room 1		Session moderator	Carmen Munuera
14:55-15:20	**20+5**	Invited	Oleg Kolosov
15:20-15:40	**16+4**	Expert	Miguel Muñoz
15:40-16:00	**16+4**	Expert	Celia Polop
16:00-16:15	**12+3**	Oral	Husnu Aslan
16:15-16:30	**12+3**	Oral	Niklas Scheer
Poster Session 16:30-18:00 Moderators: Andra C. Dumitru, Christian Dietz, Daniel Martin-Jimenez, and Victor G. Gisbert			
18:15 18:30	**Awards ceremony** Andra C. Dumitru, R. Garcia		
18:30 19:25	**45+10**	Plenary	Peter Hinterdofer
Conference Dinner: 19:30-21:30 Buses leave at 21:15 and 21:45			

10th Multifrequency AFM Conference, May 26-30th, 2025

Thursday 29th May 2025

General Session

Room 1	Session moderator	Peter Hinterdorfer

Time	Duration (min)	Type	Presenter
09:00- 09:30	**24+6**	Keynote	Toshio Ando
09:30- 09:50	**20+5**	Invited	Georg Fantner
09:50-10:10	**16+4**	Expert	Shuai Zhang

Coffee Break: 10:20-10:50

Multifrequency AFM sessions | **Multifrequency AFM sessions**

Room 1		Session moderator	Neus Domingo	Room 2		Session moderator	Shiva Patil
10:50 11:15	**20+5**	Invited	Sonia Contera-Antoraz	10:50 11:10	**16+4**	Expert	Alvaro San Paulo
11:15 11:35	**16+4**	Expert	Kenichi Umeda	11:10 11:30	**16+4**	Expert	Amir F. Payam
11:35 11:50	**12+3**	Oral	Ignacio Casuso	11.30 11.45	**12+3**	Oral	Alberto M. Pérez
11:50 12:05	**12+3**	Oral	Jonathan D. Adams	11.45 12.00	**12+3**	Oral	Bartosz Pruchnik
12:05 12:20	**12+3**	Oral	Sidney Cohen	12.00 12.15	**12+3**		

Break | **Break**

Multifrequency AFM sessions | **Multifrequency AFM sessions**

Room 1		Session moderator	Nina Balke	Room 2		Session moderator	Christian Dietz
12.30 12.55	**20+5**	Invited	Gabriel Gomila	12.25 12.40	**12+3**	Oral	Michael Ruppert
12:55 13:15	**16+4**	Expert	Georg Gramse	12.40 12.55	**12+3**	Oral	Philip Schäfer
13.15 13.35	**16+4**	Expert	Neus Domingo	12.55 13.10	**12+3**	Oral	Ermes Scarano
				13:10 13:25	**12+4**	Oral	Vladimir Korolkov

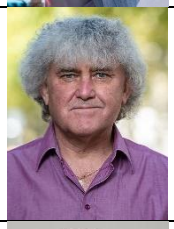
Lunch break 13:30-15:00

Multifrequency AFM sessions			
Room 1		Session moderator	Oleg Kolosov
Time	**Duration (min)**	**Type**	**Presenter**
15:00-15:20	**16+4**	Expert	Carmen Munuera
15:20-15:40	**16+4**	Expert	Cristina Gómez-Navarro
15:40-15:55	**12+3**	Oral	Andrea Cerreta
15:55-16:10	**12+3**	Oral	Sanket Jugade
End day 4			

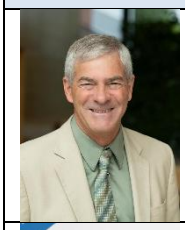
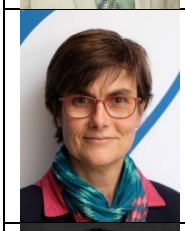
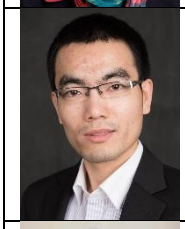
10th Multifrequency AFM Conference, May 26-30th, 2025			
Friday 30th May 2025			
General Session			
Room 1		Session moderator	Mingdong Dong
Time	**Duration (min)**	**Type**	**Presenter**
09:00 09:25	**20+5**	Invited	Adam Foster
09:25 09:50	**20+5**	Invited	Sergio Santos
09:50 10:10	**16+4**	Oral	Rubén Pérez
10:10 10:25	**12+3**	Oral	Ricardo Garcia
End Conference **Coffee Break: 10:30-11:00**			

10th Multifrequency AFM Conference Poster Sessions	
1	**Resolving the double layer and hydration structure of competing ions at solid-liquid interfaces** M. Olgiati *Institute of Applied Physic, Vienna, Austria*
2	**Dynamic and static degradation of perovskite oxide catalysts by electrochemical AFM** Muzaffar Maksumov *Institute of Energy Technologies (IET-1), Jülich, Germany*
3	**Effect of capillary condensation on non-contact Scanning Probe Microscopy** P. Sudersan *Universidad de Murcia, Murcia, Spain*
4	**Multimodal Multiparametric Investigation of Solid-Liquid Interface** Waqas Arshad *Ulster University, Belfast, UK*
5	**Systematic Multidimensional Quantification of Nanoscale Systems From Bimodal Atomic Force Microscopy Data** Chia-Yun Lai *Laboratory for Energy and NanoScience, Khalifa University*
6	**Evaluation of the Effects of O2 Plasma Etching on 3D Printed Polymers** Eider Berganza *Instituto de Ciencias Materiales de Madrid, Madrid, Spain*
7	**Magnetic Force Microscopy study of the hardening of rare earth-free core-shell ferrite-based nanoparticles** Esther Calle *Instituto de Ciencias Materiales de Madrid, Madrid, Spain*
8	**Structural Characterization of RNA combining AFM, Chemical Probing and Dynamic Fitting** Eva M. Martín-Cuevas *Centro Nacional de Biotecnología, Madrid, Spain*
9	**Torsional Mode AFM as versatile multifrequency mode: challenges and possibilities** Jaime Colchero *Universidad de Murcia, Murcia, Spain*
10	**Data-Driven Interpretation of Multifrequency AFM Observables for Improved Surface Property Assessment** Lamiaa Elsherbiny *Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates*
11	**Study of RNA condensation dynamics mediated by mycobacterial DNA-binding protein 1 (MDP1) using high-speed AFM** Nadia Shoukat *Nano Life Science Institute, Kanazawa, Japan*
12	**Viscoelastic Characterization of Polyurethane Coatings using Intermodulation Atomic Force Microscopy** Nick Wansink *Delft University of Technology, Delft, Netherlands*
13	**Integrating Work Function with Structural Characterization: Advanced nalysis of Cu–Ti Catalysts for Enhanced CO_2 Reduction** P. Atanasio *Sapienza University of Rome, Rome, Italy*
14	**Electron beam induced enhanced contrast in correlative in-situ single-pass heterodyne (H-) Kelvin probe force microscopy using piezo-resistive cantilevers** Prabhu Prasad Swain *Laboratory of Bio- and Nano-Instrumentation, Lausanne*
15	**Monochromatic Confocal Displacement Sensing in Atomic Force Microscopy** Ruben Guis *Delft University of Technology, Delft, Netherlands*
16	**Nonlinear Identification of Non-Smooth Dynamics in Atomic Force Microscopy**

	Santiago Mendoza Silva *Delft University of Technology, Delft, Netherlands*
17	**Short and sweet – crosslinking glycation stiffens diabetic titin** Alejandro Clemente-Manteca *Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain*
18	**Unmasking biomacromolecular conformational dynamics from AFM images with dynamic modes and molecular kinetics modelstitin** Antonio M. Bosch *Universidad Autónoma de Madrid, Madrid, Spain*
19	**Interplay of glycans and adsorption-driven deformation in SARS-CoV2 proteins onto surfaces with different hydrophobicity** Antonio M. Bosch *Universidad Autónoma de Madrid, Madrid, Spain*
20	**Highthroughput biomechanical characterization of macrophage polarization through atomic force microscopy** Beatriz Cantero Nieto *Institute for Bioengineering of Cataloni, Barcelona, Spain*
21	**Atomic Force Microscopy measurements of skin stiffness in neutrophil-depleted mice** Elías Herrero-Galán *Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain*
22	**Evidence of the bottom stiffness effect on atomic force microscopy-based cell mechanobiology** Francisco M. Espinosa *Instituto de Ciencias Materiales de Madrid, Madrid, Spain*
23	**Towards oil droplet based nanoindentation for cell mechanics** Giacomo Paccagnan *Laboratory of Biosensors and Bioelectronics, ETH Zürich, Zürich, Switzerland*
24	**Quantitative Biomechanical Profiling of Transformed Human Corneal Epithelial Cell** Indrianita Lionadi *Nanotechnology and Integrated Bioengineering Centre, Belfast, UK*
25	**T-cell immunomechanics: nanomechanical adaptations in tumor-like conditions** Judith Zubia Aranburu *Interdisciplinary Nanoscience Center, Aarhus, Denmark*
26	**Nanomechanical stimuli and activity of Ca ion channels in HeLa cells** Lazaro A. Dominguez *Instituto de Ciencias Materiales de Madrid, Madrid, Spain*
27	**Nanoscale dielectric imaging of cells and bacteria by scanning dielectric microscopy assisted by deep convolutional neural networks** M. Cano *Universitat de Barcelona, Barcelona, Spain*
28	**Impaired Nuclear Viscoelasticity upon MG-induced glycation** Mar Alcaraz-Hurtado *Louvain Institute of Biomolecular Sciences and Technologie, Louvain-la-Neuve, Belgium*
29	**AFM study of the adaptive mechanisms of blood cells under the influence of physical and chemical factors** Sergunova Viktoria *Negovsky Research Institute of General Reanimatology, Moscow, Russia*
30	**Morphology of neutrophils under the influence of serum from patients with infectious pathologies** Vladimir Inozemtsev *Negovsky Research Institute of General Reanimatology, Moscow, Russia*

MULTIFREQUENCY AFM SESSIONS			
	**Nina Balke** North Carolina State Univerisity	Small scale, big impact: Visualization of local electrochemical processes	**Wednesday 28th May Keynote Speaker**
	**Roger Proksch** Oxford Instruments Asylum Research	Frontiers in Accurate and Low Noise Atomic Force Microscopy using a new Interferometric Detector	**Wednesday 28th May**
	**Olog Kolosov** Lancaster Univerisity	Mixing 4-100 MHz ultrasonic frequencies in AFM for mapping nanosecond time scale electromechanics with nanoscale spatial resolution	
	**Peter Hinterdorfer** Johanes Kepler University Linz	Dynamic Multimeric Binding Strategies of Pathogens and Therapeutics: Lessons learnt from SARS-CoV-2 Variants of Concern	**Wednesday 28th May Plenary Speaker**
	**Toshio Ando** Kanazawa University	Past and Future of High-speed AFM for Dynamic Structural Biology	**Thursday 29th May Keynote Speaker**
	**Georg E. Fantner** École Polytechnique Fédéral de Lauanne	Correlative nanocharacterization by AFM and SEM	**Thursday 29th May**
	**Sonia Contera-Antoraz** University of Oxford	Nanoscale viscoelasticity of living and soft matter with AFM	
	**Gabriel Gomila** University of Barcelona	Probing the transversal and longitudinal electrical conductivity without physical contact by Scanning Dielectric Microscopy	

	**Adam Foster** Aalto Univerisity	Machine learning in Scanning Probe Microscopy	**Friday 30th May**
	**Sergio Santos** UiT The Arctic University of Norway	Exploiting Multifrequency AFM Observables for Object and Phenomenon Identification and Enhanced Material Characterization	

3rd SYMPOSIUM ON SOLID-LIQUID INTERFACES			
	**James J. de Yoreo** Johanes Kepler University Linz	An in situ look at interfacial structure and dynamics during nucleation and self-assembly	**Tuesday 27th May Keynote Speaker**
	**Marie P. Gaigeot** Université d'Evry	Modeling aqueous oxide interfaces and their HD-SFG non-linear spectroscopy	**Tuesday 27th May**
	**Yingjie Zhang** University of Ilinois	Integrating Microscopy and Spectroscopy to Unveil Liquid Structures Near Solid Surfaces	
	**Angelika Kuhnle** Bielefeld University	Solvation Layer Mapping at "Hydrophobic" Surfaces	
	**Patrick Unwin** University of Warwick	High-Throughput Multifunctional Scanned Probe Microscopy: A New Era in Electrochemical Materials Discovery	**Wednesday 28th May**

4th SYMPOSIUM ON CELL AND SOFT MATTER NANOMECHANICS			
	**Alba Diz-Muñoz** European Molecular Biology Laboratory	The missing mechanical link: how composite interfaces govern cell morphogenesis, immune migration, and stem cell fate	**Monday 26th May**
	**Jorge Alegre-Cebollada** Centro Nacional de Investigaciones Cardiovasculares	How do cells react to the viscoelasticity of the extracellular matrix?	
	**Alexander Kabla** University of Cambridge	Rheology of epithelial monolayers	
	**Mingdong Dong** Aarhus University	Shaping Cells with Patterns and Protein Crystals	**Tuesday 27th May**
	**Tomaso Zambelli** ETH Zürich	Intracellular mechanotransmission combining FluidFM and FLIM	
	**Fernando Moreno-Herrero** Centro Nacional de Biotecnología	Understanding DNA repair protein machines from single-molecule manipulation methods	

ORAL PRESENTATIONS

MULTIFREQUENCY AFM SESSIONS

Small scale, big impact: Visualization of local electrochemical processes

Nina Balke
North Carolina State University

Electrochemical energy storage is the key enabling component of electric vehicles and solar/wind-based energy technologies. The enhancement of energy stored requires the detailed understanding of ionic transport and electrochemical and electromechanical phenomena on *local* scales which are not accessible with current-based electrochemical techniques, such as voltammetry. Therefore, local probing of electrochemical and electromechanical behaviors on individual structural elements and heterogeneities, from grains to defects and further to individual atomic and molecular species, are invaluable. Force-based atomic force microscopies (AFM) can fill this gap and provided novel insights into local electrochemical processes on tens of nanometer and even molecular length under electrochemical control. I will highlight the development and application of in situ/operando force-based AFM methods to gain insight into the local charge storage mechanism in a variety of energy related materials.

In the first part of the talk, I will showcase how AFM can be used to investigate the structure and dynamics of the electric double layer for electrochemical capacitors. I want to highlight work on room temperature ionic liquids on model graphene electrodes since ionic liquids hold the promise of increasing the electrochemical stability windows for electrochemical capacitors. AFM was used to observe topological defects and show the existence of structural domains parallel to the solid-liquid interface towards a full picture of the double layer structure and their change with applied bias. In the second part of the talk, I want to highlight how AFM-based methods can be used to study ionic transport and local electrochemical reactions in supercapacitors and battery materials. Here, electro-chemo-mechanical coupling is the key to study ion insertion pathways and heterogeneities in local redox reactions. The first is demonstrated for the cation insertion into layered Ti_3C_2 electrodes based on their change in mechanical stiffness whereas the second is highlighted for proton insertion into WO_3 where the relationship between electrochemical current and electrode strain are discussed.

Investigating the role of collagen piezoelectricity in intrafibrillar mineralization

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Bone actively remodels its structure and composition in response to external loads by replacing old tissue with new [1]. Type I collagen, its fundamental building block, forms a fibrillar structure with a characteristic D-periodic pattern (~67 nm), serving as a template for mineralization. Osteoblasts secrete collagen to build a structural matrix, which is then reinforced by mineral deposition—both extrafibrillar and intrafibrillar—modulating bone's mechanical behavior. While collagen's role in mineralization is recognized, its precise mechanism remains unclear. Given bone's ability to adapt to mechanical loads, we hypothesize that collagen piezoelectricity—electrical charges generated along collagen fibrils under mechanical stress—drives intrafibrillar mineralization. To examine the hypothesis, we employed Piezoresponse Force Microscopy (PFM) to quantitatively analyze the correlation between the mechanical structure and piezoelectric properties of collagen fibrils at the nanometer scale. We compared collagen samples from healthy (wild-type, WT) and diseased (osteogenesis imperfecta, OI) bone models, as OI is associated with structural collagen mutations that lead to abnormal mineralization [2].

The quantitative PFM successfully identified an anisotropic and heterogeneous nature of piezoelectric properties in collagen [3-4]. WT collagen exhibited a periodic piezoelectric profile, with higher values in the gap region (0.31 ± 0.02 pm/V) than the overlap region (0.20 ± 0.01 pm/V). Interestingly, this piezoelectric profile corresponds to the periodic profile of mechanical stiffness in a mineralized collagen fibril. This implies that enhanced piezoelectricity in gap regions locally increase an electric potential under external loadings, facilitating mineral infiltration into the intrafibrillar space. In contrast, OI collagen lacked a clear periodic piezoelectric pattern, displaying similar values in the gap (.037 ± 0.03 pm/V) and overlap (0.32 ± 0.03 pm/V) regions, aligning with its homogeneous stiffness. This likely disrupts local electric interactions, reducing mechanical heterogeneity and making OI bone more brittle. While a direct link between collagen piezoelectricity and intrafibrillar mineralization remains unproven, the differing piezoelectric heterogeneity in WT and OI models highlights its role in modulating mineral infiltration and structural heterogeneity.

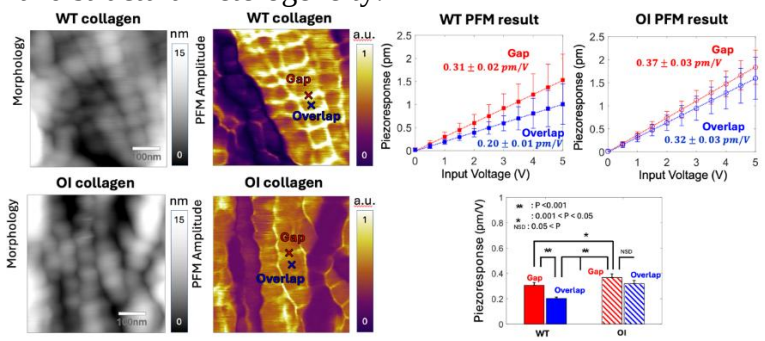

Figure 1: PFM results on wild-type (WT) and osteogenesis imperfecta (OI) collagen

[1] R.B. Martin, D.B. Burr, N.A. Sharkey, and D.P. Fyhrie, Skeletal tissue mechanics. Springer (2015).
[2] T. Li, S.-W. Chang, N. Rodrigues-Florez, M.J. Buehler, S. Shefelbine, M. Dao, and K. Zeng, Biomaterials **107**, 15–22 (2016).
[3] J. Kwon and H. Cho, Commun. Biol. **5**, 1229 (2023).
[4] J. Kwon and H. Cho, ACS Biomater. Sci. Eng. **6**, 6680–6689 (2020).

Bimodal atomic force microscopy with a torsional eigenmode for highly accurate imaging of grain orientation in organic thin films

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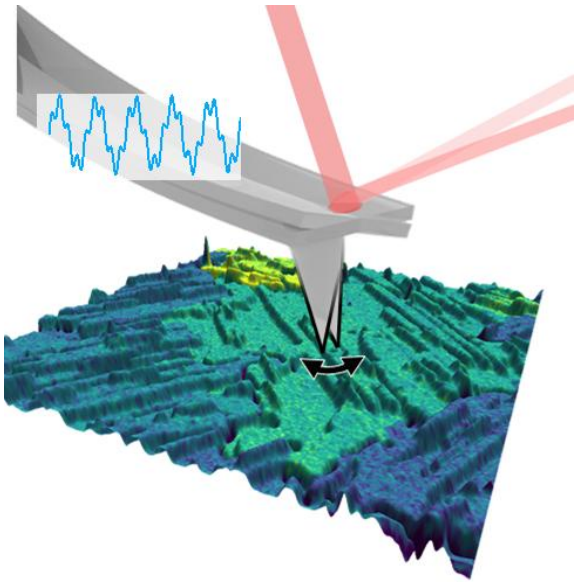
In organic electronics, the nature and spatial distribution of grains in polycrystalline thin films of small organic semiconductor molecules greatly impact the electronic properties of devices. Therefore, tools that accurately characterize organic films at the mesoscopic level are essential. To this end, we demonstrate here the power of a bimodal Atomic Force Microscopy (AFM) with a torsional eigenmode for highly accurate imaging grain orientations in organic thin films. The energy dissipated between tip and sample during scanning depends on the in-plane crystalline orientation of each grain. This fact alters the cantilever torsional observables, allowing grain orientation recognition. Remarkably, bimodal AFM with the torsional eigenmode has important advantages, such as high sensitivity in the applied forces, true molecular resolution, and multiple parameters for regulating the image contrast, making it competitive with other well-established AFM methods for grain detection in organic thin films, namely Friction Force Microscopy and Transverse Shear Microscopy.



R. Arilla, E. Barrena, C. Ocal, D. Martin-Jimenez, Nano Letters (2025).

Torsional force spectroscopy as a versatile tool to probe lateral interactions in biological and polymeric systems

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Torsional force spectroscopy (TFS) has emerged as a powerful technique for characterizing in-plane mechanical interactions at the nanoscale. Here, we demonstrate its versatility by probing both biological adhesion sites and inter- and intramolecular interactions in heterogeneous polymers. In the context of cellular mechanics, we utilized TFS to quantify the lateral forces of focal adhesions and cell-cell junctions in living cells. Our findings revealed local lateral forces in the range of 1.0–1.5 nN for focal adhesions, with slightly higher values at intercellular contacts (Fig. 1 a – f). Furthermore, a modified surface layer exhibiting reduced tip friction was observed next to retracting cell edges (Fig. 1 e – f, yellow arrows), highlighting the complex interplay between adhesion dynamics and cellular motility [1].

Moreover, we investigated the nanoscale mechanical interactions in annealed polystyrene-block-polybutadiene (SB) diblock copolymer films with TFS. By converting torsional observables into mechanical quantities—such as in-plane shear stress and dissipated energy—we mapped anisotropic tip-block interactions, revealing distinct interaction stages dependent on the relative shearing direction and indentation depth (Fig. 1 g – k) [2]. Our results demonstrate the broad applicability of TFS in resolving lateral force interactions in both biological and synthetic systems. By providing spatially resolved insights into focal adhesion mechanics and polymeric nanostructures, this method paves the way for advancements in mechanobiology and functional nanomaterial design.

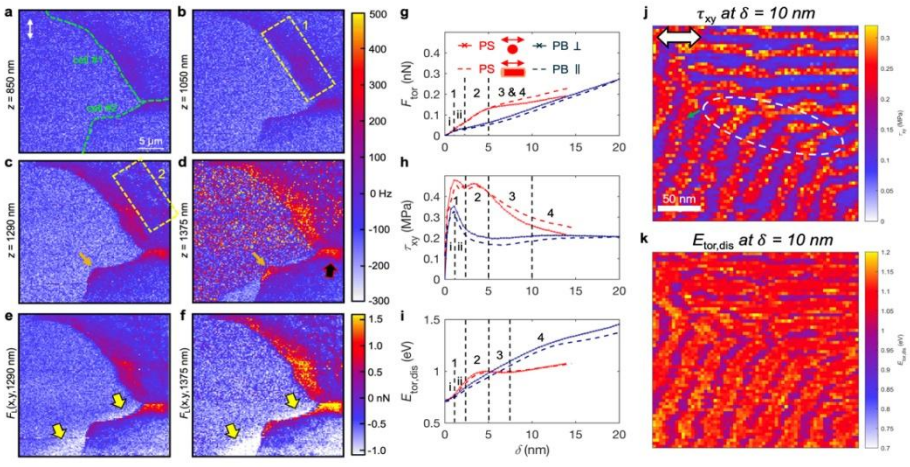

Figure 1. TFS on soft matter. a – f Torsional frequency and lateral force maps at the edges of two adjacent cells revealing enhanced interaction at the periphery and interface. g – k Force, shear stress and dissipated energy for in-plane interactions of a SB diblock copolymer.

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Exploring Atomic-Scale Properties of 2D $NiBr_2$ on Au(111) Using Multimodal Scanning Force Microscopy

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In recent years, two-dimensional (2D) van der Waals materials have gained prominence as a versatile platform for investigating low-dimensional quantum phenomena. Their reduced symmetry gives rise to electrical, optical, and magnetic properties that are markedly distinct from their three-dimensional counterparts. Among these, transition metal dihalides (TMHs) stand out as promising candidates for stabilizing ferro- or antiferromagnetic ordering in two dimensions, offering exciting opportunities for exploring magnetism at the atomic scale. [1]

Achieving epitaxial single-layer TMHs demands precise control over growth parameters and a deeper understanding of the interactions between TMHs and the substrate surface. In this study, we employ multimodal scanning force microscopy (SFM) to characterize $NiBr_2$ islands grown on Au(111) with atomic resolution. By simultaneously mapping the topography, magnetic forces, and local Kelvin potential, we uncover insights into the structural, electronic and magnetic properties of this system. This multimodal approach leverages the simultaneous operation of the first and second flexural modes of the cantilever, oscillating at amplitudes of 5 nm and 0.1 nm, respectively. The larger first-mode amplitude provides high sensitivity for detecting long-range magnetic forces and measuring the local Kelvin potential through sideband nulling. Meanwhile, the smaller second-mode amplitude, with approximately 40 times greater stiffness, enables precise detection of short-range chemical forces, allowing for atomic-resolution imaging of the sample topography.

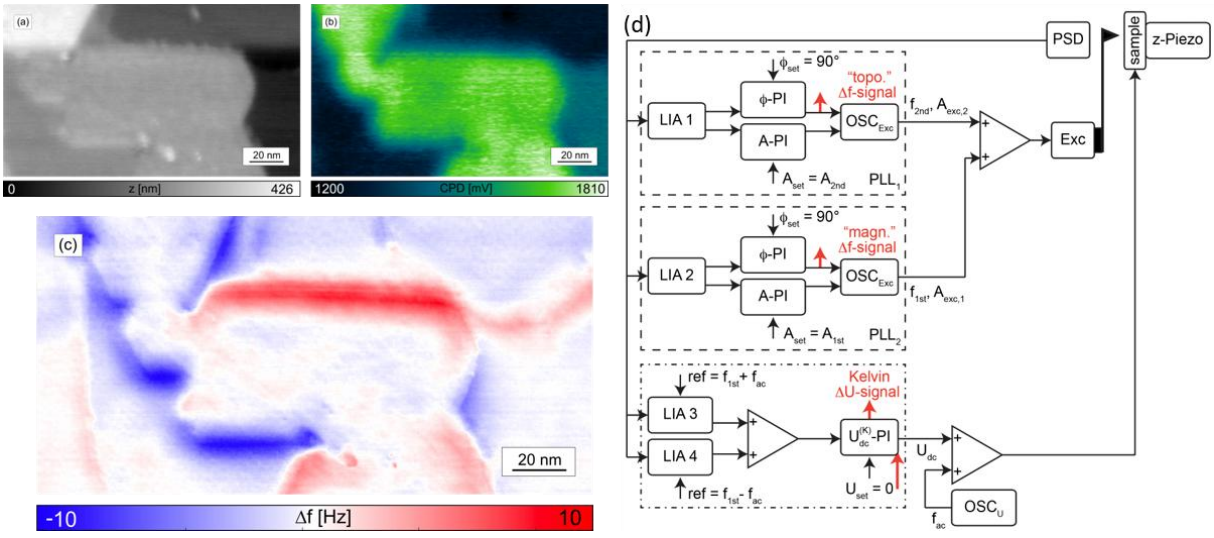

Figure 2: a) topographic b) KPFM c) MFM data over a monolayer high $NiBr_2$ island on Au(111) acquired simultaneously using multifrequency technique. d) Schematic drawing of the multifrequency technique.

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Characterization of polymeric coating using nonlinear dynamic atomic force microscopy

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Understanding viscoelastic properties is crucial for optimizing polymeric microstructures. Atomic force microscopy (AFM) has evolved into a powerful instrument for examining material properties at the micro- and nanoscale [1]. However, existing AFM characterization methods can suffer from insensitivity to viscoelastic material parameters [2] and it remains challenging to untangle the different viscoelastic properties from the measurement data. This presentation proposes a new approach that uses the AFM's nonlinear dynamic response to separately quantify the dissipative and conservative components of the tip-sample interactions. Through frequency sweeps on two polymeric coatings, we demonstrate that elasticity predominantly governs the strength of the nonlinearity, while viscosity influences the detuned nonlinear resonance frequency. Sensitivity analysis using a calibrated reference sample confirms the accuracy of our method. This method holds great potential for accelerating the development of polymeric coatings.

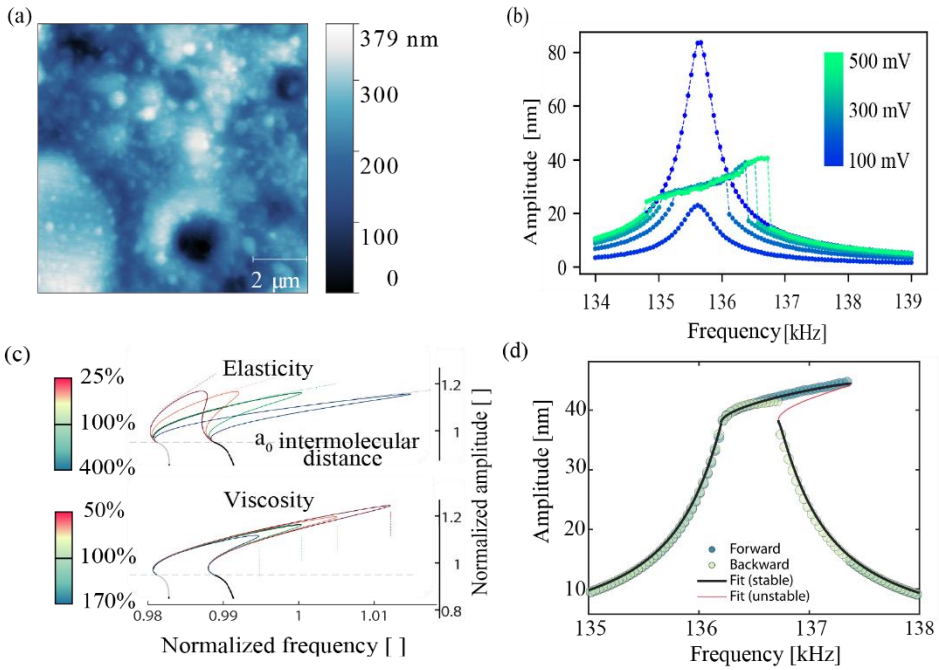

Figure 1: (a) Topography image of the coating material. (b) Forward frequency sweep of the experimentally measured amplitude response. (c) Influence of the sample's elasticity and viscosity on the amplitude-saturated branch. (d) Fit for a coating measurement.

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Electromechanical response of saddle points in twisted hBN moiré superlattices

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In twisted layered materials (t-LMs), an inter-layer rotation can break inversion symmetry and create an interfacial array of staggered out-of-plane polarization due to AB/BA stacking registries. [1-4] This symmetry breaking can also trigger the formation of edge in-plane polarizations localized along the perimeter of AB/BA regions (i.e., saddle point domains). [5] However, a comprehensive experimental investigation of these features is still lacking. Here, we use piezo force microscopy (PFM) to probe the electromechanical behaviour of twisted hexagonal boron nitride (t-hBN). For parallel stacking alignment of t-hBN, we reveal very narrow (width ~ 10 nm) saddle point in-plane polarizations (Fig. 1), which we also measure in the anti-parallel configuration. These localized polarizations can still be found on a multiply stacked t-hBN structure, determining the formation of a double moiré. Our findings imply that polarizations in t-hBN do not only point in the out-of-plane direction but also show an in-plane component, giving rise to a much more complex 3D polarization field.[6]

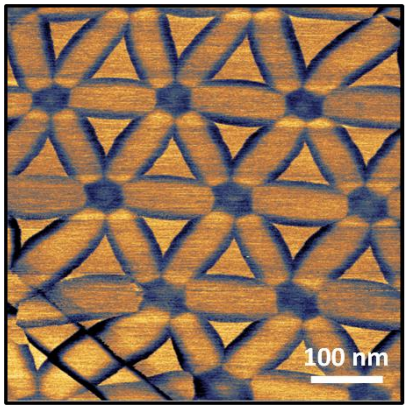

Fig. 1*: PFM amplitude map of a moiré superlattice in a twisted hBN sample.*

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Frontiers in Accurate and Low Noise Atomic Force Microscopy using a new Interferometric Detector

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Atomic Force Microscopes (AFMs) have become a standard tool for high resolution surface mapping of a wide variety of nanoscale samples. The vast majority of existing AFMs make use of an optical beam detector (OBD) that measures the bending of the flexible cantilever beam. Despite its popularity, accurate and reproducible mechanical measurements using this detection approach are challenging. Specific barriers to widespread accurate AFM include (i) highly inconsistent sensitivity calibrations, (ii) measurement noise floors significantly higher than thermal motion of the cantilever probes and (iii) uncontrolled mixing of vertical and in-plane forces acting on the tip. Component mixing inevitably complicates attempts at accurate mechanical measurements and can lead to enormous, and often unacknowledged uncertainties. In this presentation, we will demonstrate a series of new measurement workflows based around a new quadrature phase differential interferometer (QPDI) that routinely achieves a detection noise down to ≈5 fm/√Hz using standard commercial cantilevers (Fig. 1a)). The QPDI measurement remains linear and accurate for large deflections (>1 μm) down to sub-picometer thermal fluctuations. This improved noise and accurate calibration reveals details and features that have been hidden from view using conventional OBD measurements. In this presentation, we will show results for force measurements where the QPDI detector provides accuracy and low noise, clearly revealing previously hidden dynamics (Fig. 1b)).[2]. Even simple contact mode works better, resolving, for example, structures in twisted graphene bilayers that are not visible using an OBD detector (Fig. 1 c)).Finally, weak functional ferroelectrics, including high frequency (5G) filter design and manufacturing, beyond Moore's law computing materials such as HfO and HZO can be much more accurately and sensitively probed (Fig.1 d)).[2],[3]

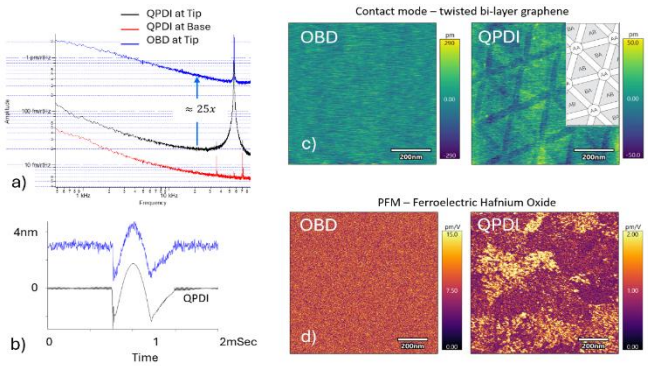

Figure 1. a) The noise amplitude spectra for the QPDI at the base of the cantilever (red), at the tip of the cantilever (black) and for an OBD detector at the tip of the cantilever (blue). b) 500Hz fast force curves plotted versus time for an OBD detector (blue) and for the QPDI (black). c) Contact mode images of twisted bilayer graphene measured with the two detectors. d) PFM images of

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On-demand generation of ferroelectric topologies via scan path engineering

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Atomic force microscopy (AFM) offers a unique platform for the precise manipulation of ferroelectric domains. Hierarchical assemblies of ferroelectric nanodomains, or superdomains, can exhibit exotic morphologies that underpin distinct behaviors and functionalities. Controlling these superdomains reliably is essential for achieving desired functional properties in ferroic systems. Here[1], we demonstrate how the scan path or trajectory of a biased AFM tip governs the final written domain structure in the ferroelectric $Pb_{0.6}Sr_{0.4}TiO_3$. By tailoring the scan trajectory, we elucidate a super-switching mechanism dominated by superdomain nucleation and super-boundary stabilization, enabling the formation of complex, topological polar structures such as center-divergent/convergent and flux-closure domains. (**Figure 1**). Through correlative piezoresponse force microscopy and optical spectroscopy, we confirm the topological nature and tunability of these emergent structures. Phase-field modeling validates the stability of these domains, highlighting the critical role of the scan path in stabilizing intricate defect states. This understanding opens the door to the on-demand design of topological domains with unprecedented precision, enabling the creation of arrays of tunable superdomains for neuromorphic circuits and other advanced nanoarchitectures. Our findings establish scan path engineering as a cornerstone for creating complex topological structures, redefining possibilities in nanolithography for ferroic materials.

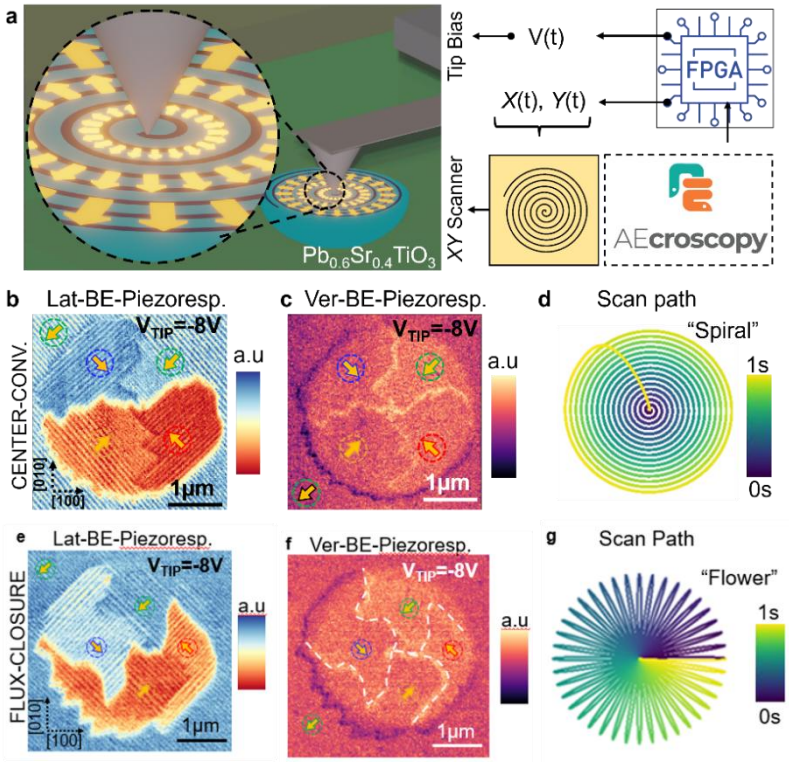

Fig 1. a Experimental setup used for the measurements **b** Lateral PFM of the written center-convergent structure. **c** Vertical PFM of the written center-convergent structure. **d** Spiral tip trajectory for the writing of the center convergent structure. **e-g** Same as b-d, but for a written flux-closure structure using a flower scan path.

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Chemical Bond Imaging Using Atomic Force Microscopy with an Adaptive Tunneling Current Feedback

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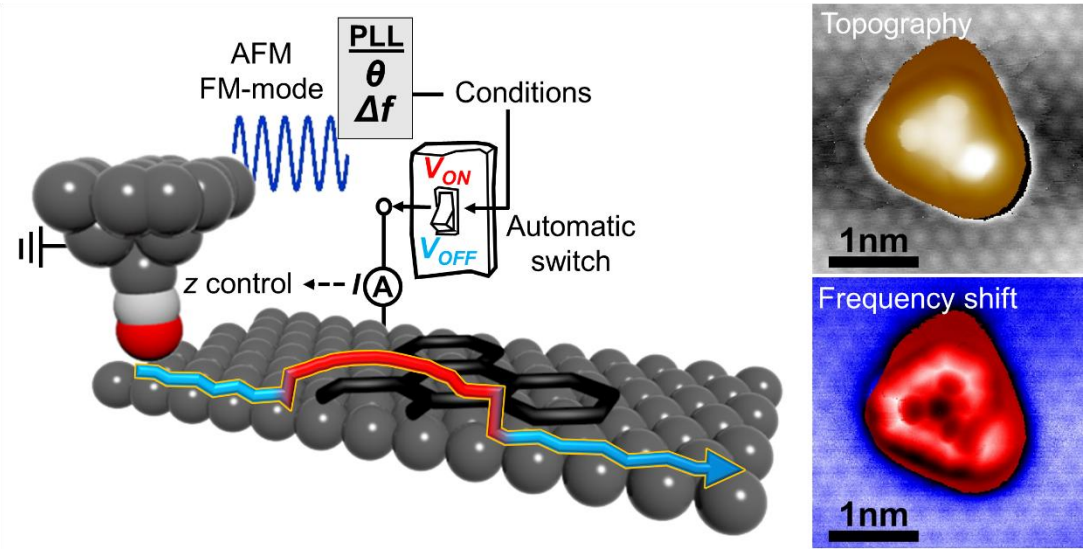
Low temperature atomic force microscopy (AFM) has become an invaluable tool for studies of molecules on surfaces. The high lateral resolution that is obtained by functionalizing the AFM tip with CO allows to visualize the chemical structure of individual organic molecules and to precisely determine their adsorption conformations, positions, and orientations, which is important for revealing on-surface reaction mechanisms. However, this so-called bond imaging method bears some technical difficulties and limitations that can be circumvented by employing customized operational modes. For quantitatively determining adsorption conformations of bulky molecules constant-current AFM is very useful. [1] Recently, we expanded this method by an adaptive feedback mechanism, which allows simultaneous imaging of molecules with submolecular resolution and the surrounding surface with atomic resolution (see Figure). [2] The approach enables to image adsorption sites of several adjacent highly mobile molecules in a single scan and could in the future contribute to autonomous scanning.



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Noise related to the chemistry of the sample imaged in Dynamic Atomic Force Microscopy

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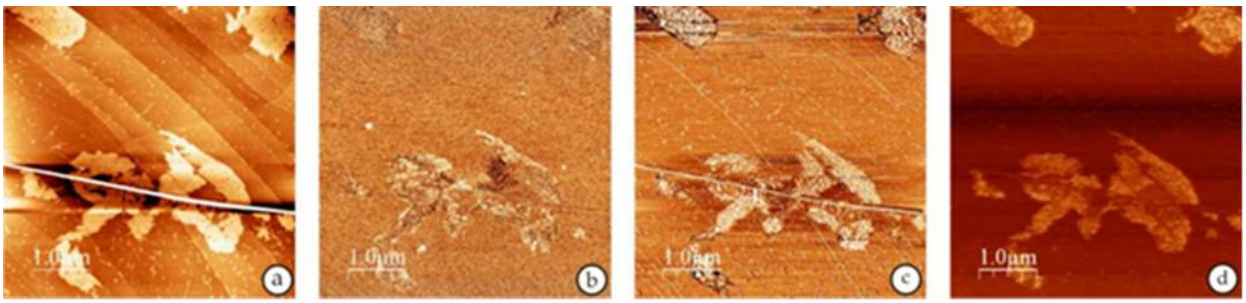
Generally either statistical or quantum noise limits the ultimate resolution. In the first case according to the dissipation-fluctuation theorem, noise is related to dissipation within a physical system. The second case, measurements at the quantum limit [1], is receiving increased interest also in the AFM community. Here, we measure and discuss noise in the tip-sample interaction for non-contact Dynamic Atomic Force Microscopy (nc-DAFM). We show that it is possible to acquire "noise" images with a well-defined pattern (Figure 1, [4]), different from topography or other acquisition channels. This noise is attributed to the interaction induced by liquid necks forming between tip and sample [3]. While previous analyses identified thermal noise as the dominant contribution to frequency fluctuations, experimental evidence reveals the presence of an additional and significantly stronger noise source when non-vanishing tip–sample interactions are present. This interaction-induced noise carries valuable information about the chemical nature of the sample, turning what is conventionally regarded as a limiting factor into an interesting signal.
Using different AFM techniques (Force Spectroscopy, Kelvin Probe Microscopy, 3D modes, etc), we analyze this noise for amphiphilic molecules (SDS) adsorbed on a graphite substrate. Our experiments show that the different chemical nature of these materials induce a different magnitude of the measured noise, leading to an image with "chemical" contrast (Figure 1), related to the nanoscale wetting properties of the sample [4].



Figures: Topography (a), Frequency Shift (b), Contact Potential (c) and Noise (d) images acquired simultaneously in nc-DAFM with topography feedback performed at constant oscillation amplitude.

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Magnetic Field Screening of 2D materials revealed by Magnetic Force Microscopy

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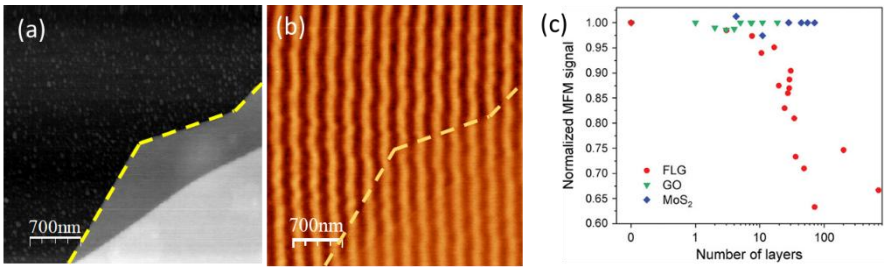
2D materials possess exceptional mechanical properties making them promising candidates for protecting nanostructures [1]. However, the magnetic field screening properties of 2D materials are largely unexplored. Graphene under ideal conditions should present a high diamagnetic response [2], but imperfections, such as defects or stacking faults, could result in a reduction of the diamagnetism. Despite this reduction, graphene diamagnetism would be expected to shield the magnetic field originated by a magnetic structure. On the other hand, graphene oxide (GO), a derivative of graphene, possesses oxygen-containing functional groups on its surface. These functional groups produce modifications on its electronic structure, increasing the effective mass of the charge carriers, opening a band gap, and therefore reducing its diamagnetism. As a result, we expect a significantly different behavior of the magnetic field when covering magnetic structures with graphene and with GO. Here we used Magnetic Force Microscopy (MFM) [3] to unveil the effects on the magnetic field of magnetic nanostructures when 2D materials are placed on top of them [4]. Due to the different electronic nature of the studied materials, electrostatic compensation can play an important role. Therefore, Kelvin Probe Force Microscopy (KPFM) with MFM [5] was used to avoid any possible influence of electrostatics.
Our work explores the ability of these 2D materials to shield the magnetic signal from different underlying magnetic structures. These findings suggest that 2D materials can offer effective protection while minimally affecting the underlying magnetic functionalities, important for data storage technologies and spintronics.



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Quantitative Nanomechanical Mapping at High Frequencies using Photothermal Off-Resonance Tapping AFM

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Multifunctional imaging, which combines the characterization of various sample properties such as elasticity and adhesion with topography, makes atomic force microscopy (AFM) a powerful tool for nanoscale surface analysis. However, most existing scanner-based methods for measuring mechanical properties are slow, limiting their use in fast spatial mapping of mechanical characteristics [1]. The primary source of this limitation is the piezo scanner, used to modulate the tip-sample distance. This can be overcome by direct cantilever actuation methods, such as photothermal excitation. By moving the cantilever's comparably smaller mass, higher actuation bandwidths are accessible, enabling fast approaches for AFM-based nanomechanical characterization.

This work extends the application of photothermal off-resonance tapping, a method previously employed for fast and gentle topographic imaging [2], to fast and quantitative nanomechanical property mapping. Simulations of laser-induced cantilever bending provide insights into the thermomechanical response, enabling the development of a calibration procedure that translates complex cantilever behavior into accurate nanomechanical measurements. Experimental validation using reference structures, polymeric and metallic samples demonstrate nanomechanical mapping at frequencies with tens of kilohertz. This approach, implemented on Nanosurf's DriveAFM [3], enables quantitative high-resolution nanomechanical within a one-minute measurement time.

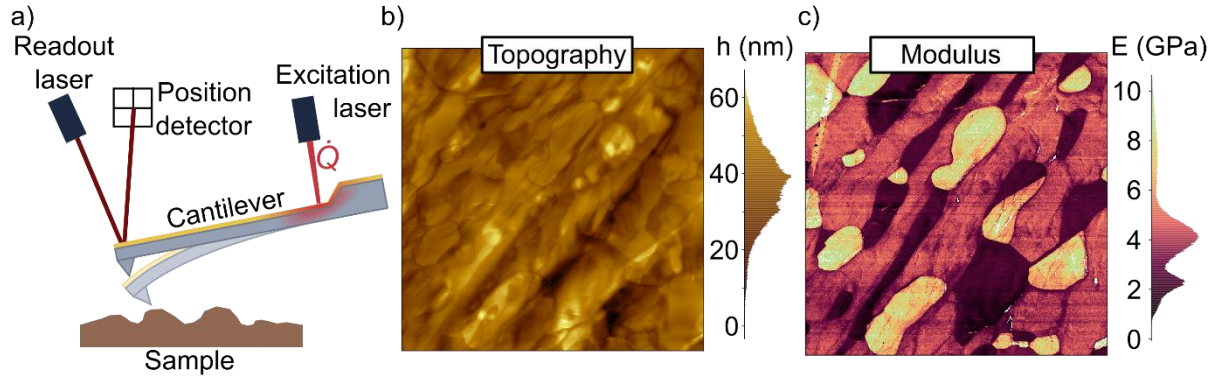

Figure 3: a) Schematic of photothermal off-resonance tapping for fast modulation of tip-sample distance. **b)** Topography and **c)** modulus of Field's metal acquired in 1 minute measurement time (25 kHz excitation frequency, 500 x 500 pixel, 10 µm image size) using photothermal off-resonance tapping.

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Characterization of magnetotactic bacteria by long-range Magnetic Force Microscopy

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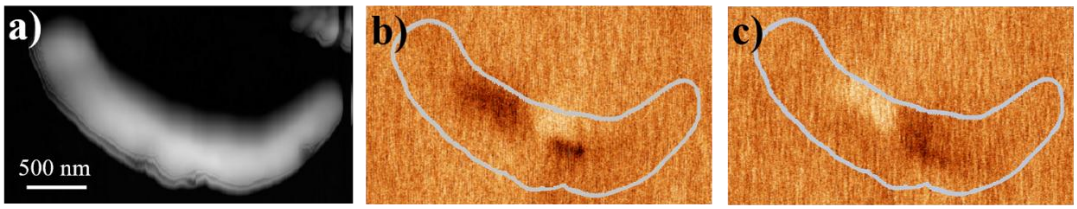
Magnetotactic bacteria (MTB) are microorganisms that biomineralize magnetic nanoparticles surrounded by a lipid bilayer membrane (so-called magnetosomes) forming a chain along the axis of the cell, which allows bacteria alignment under a magnetic field. Due to their properties, MTB have potential applications in medicine [1]. Despite the numerous advances and promising scenarios that MTB and biomagnetic materials in general disclose, challenges persist on multiple fronts including the characterization of individual elements. The majority of prevailing characterization techniques tend to provide an averaged collective behaviour rather than insights into individual features [2]. Scanning probe microscopies lead to new opportunities for the exploration of different set of properties of biomagnetic materials such as structural, mechanical, chemical, electrical or magnetic properties at the nanoscale.

This study makes use of magnetic force microscopy (MFM) to characterize individual MTB. As the characterization of these elements posed a significant challenge due to the magnetosomes' low signal and the relatively large size of the bacteria, customized MFM probes were developed through various strategies [3,4]. Furthermore, employing MFM imaging under an *in-situ* magnetic field provides an opportunity to gather quantitative data regarding the critical fields of these individual chains of nanoparticles, as depicted in the figure. This approach marks a substantial advancement in the field of MFM for biological applications, enabling the detection of magnetosomes under different conditions [4].



(a) Topography and MFM images of a single MTB after applying (b) positive and (c) negative saturating magnetic fields.

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Mixing 4-100 MHz ultrasonic frequencies in AFM for mapping nanosecond time scale electromechanics with nanoscale spatial resolution

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The superior spatial resolution of atomic force microscopy (AFM) in mapping nanomechanical and related properties, is not matched up by the sensitivity to short time scale processes. It is the slow response of contact mode and large characteristic resonance decay time of semi-contact modes, required for high force sensitivity, that limit time scales to, at best, μs range. Nevertheless, by using heterodyne principle, high frequency (HF) mechanical oscillations of the AFM tip and a sample can be converted to the low frequency (LF) force response via mixing at intrinsic tip-surface nonlinearity, using two adjacent frequencies. The easily detected resulting LF oscillatory force preserves the HF phase and time-scale nano-mechanical information, the principle of heterodyne force microscopy (HFM) [1]. The HFM benefits also include the ability to observe subsurface features [2], elimination of lateral forces and improved lateral resolution due to operation on the cusp of tip-surface force interaction.

Here we report the use of heterodyne HFM principle to explore the dynamics of nanoelectromechanical interaction in two-dimensional and polymeric materials – electrostatic heterodyne force microscopy, or E-HFM. In E-HFM the HF mechanical excitation at sub-10 MHz to 100 MHz frequency, f_{tip}, is applied to the base of the AFM cantilever traveling to tip practically unimpeded due to dispersive nature of flexural vibrations [3]. The electrical potential at the adjacent frequency f_{es} is then applied between the tip and the sample causing electrostatic or piezoelectric forces at the same frequency. Due to the nonlinearity of the tip-surface interaction, the force at the LF difference frequency $f_{diff}=f_{es}-f_{tip}$ is detected by the tip off the resonance (at a few kHz), or at the tip-surface contact resonance frequency (50-500 kHz). Principally, any phase differences occurring during electromechanical HF interaction can be detected at LF with a few to sub degree sensitivity, resulting in time scale sensitivity of sub-ns for 4 MHz HF range and 10 ps for the 100 MHz HF range. We report using the E-HFM to explore the dynamics of nanoelectromechanical response of suspended graphene membranes, and switching of PVDF ferroelectric domains (Fig 1b,c) with 5 ns time sensitivity, and a piezoelectric response in twisted bilayer graphene with ~ 1 ns time sensitivity observing some novel short time scale electromechanical phenomena (Fig.1d) not detected in the standard piezo-force microscopy.

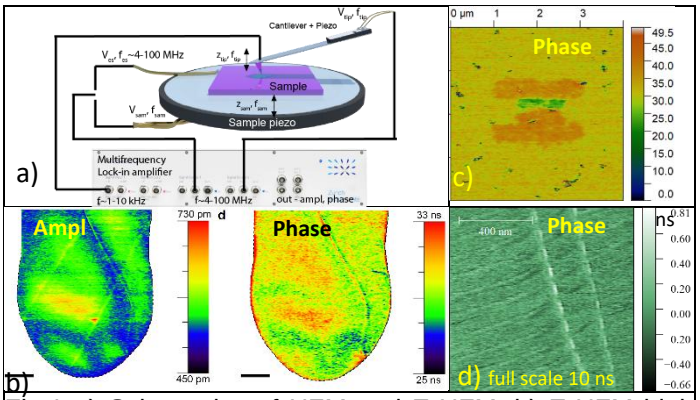

Fig.1 a) Schematics of HFM and E-HFM, b) E-HFM high frequency (~60 MHz) amplitude and phase (~response time) of suspended graphene membrane oscillation, c) E-HFM of piezoresponse of twisted bilayer graphene and d) PVDF ferroelectric domains (4 MHz HF excitation in c,d).

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Scanning Thermal Microscopy of Electronic Devices

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One of the greatest challenges of modern society is related to energy consumption, dissipation and waste. A prominent example is that of integrated electronics, where power dissipation issues have become one of its greatest challenges. In this talk, I will discuss how to characterize energy dissipation in electronics, like heating in transistors based on 2D materials or in the conductive filaments of resistive random-access memories (RRAM), using spatially resolved thermometry. As the size of materials and devices shrinks to nanometer, atomic, or even quantum scale, it is more challenging to characterize their thermal properties reliably. Scanning thermal microscopy (SThM) [1] is an emerging method to obtain local thermal information of electronic devices [2, 3] by controlling and monitoring probe–sample thermal exchange processes. Gaining thermal insights of our electronics is essential to design energy efficient circuits and understand and optimize ultra-dense data storage.

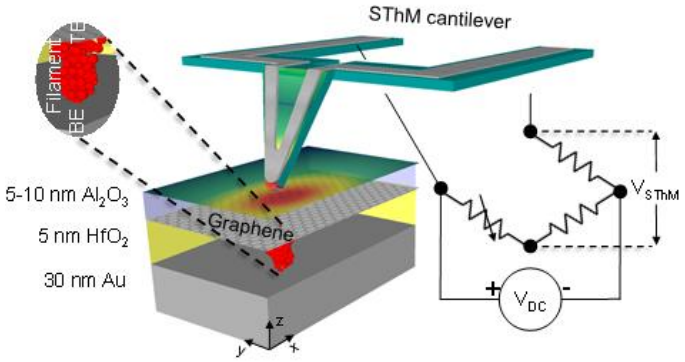

Figure 1. Thermal measurements of conductive filaments in RRAM memory devices. [4]

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Nucleation and early-stage growth of Li and Na anodes in ZESSBs at the nanoscale by synchrotron-PEEM and AFM

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Green, safe and high-performance batteries based on abundant materials are a key element in the transition to a carbon-neutral future. So-called zero-excess solid-state batteries (ZESSBs) are one of the most promising technologies that meet these criteria. However, their practical implementation presents challenges due to the uneven growth of alkali metal anodes. Innovative improvement strategies that enable this type of energy storage technology will only be achieved through a conceptual understanding of anode formation in ZESSBs.

Seven leading research institutions (UAM, NTNU, MUL, CEMEA, FHG-IKTS, CSIC-ICMM, UoS), two synchrotron radiation facilities (ESRF, ALBA) and two companies (AVL, HS), all from complementary research fields such as batteries, surface and material science, and multiscale modelling, have joined forces in the OPERA Horizon European project (https://horizon-opera.eu/) to address the challenge of ZESSBs. Our goal is to develop novel high-resolution operando and multiscale modelling methods to provide complementary insights into the structure, chemical composition, stress fields and defect formation during anode nucleation and growth in ZESSBs.

AFM is one of the tools we use to address this challenge. A new Park FX40 automatic AFM integrated in a glove box is dedicated to the investigation of alkali metal solid-state ion batteries. In this talk, I will present our ongoing investigations into the early stages of Li and Na anode formation in ZESSBs, simulating the current collector with a virtual electrode. Nanoscale analysis of the chemical composition, oxidation state, and morphology of anodes in two model systems (NaGdSiO and LiLaTaZrO) has been performed in situ using synchrotron photoemission electron microscopy (PEEM) and atomic force microscopy (AFM) in an inert atmosphere. We observed a 3D growth mode that maintains a constant grain aspect ratio and that growth is not determined by the structure of the electrolyte grain boundaries. Our findings highlight the virtual electrode method as a powerful tool to investigate the nucleation and early growth of anodes at the nanoscale, revealing how experimental conditions such as temperature, current, or conditions at the electrolyte interface can significantly influence the growth efficiency [1].

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Atomic Force Microscopy as a Multimetrological Platform for Energy Devices

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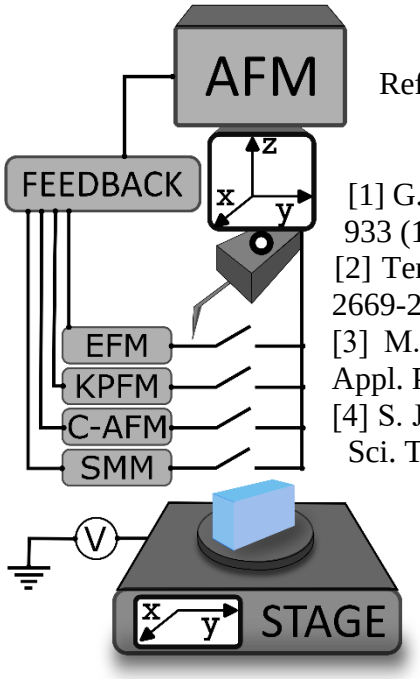
In this study, we present a comprehensive investigation utilizing Atomic Force Microscopy[1] (AFM) as a multimetrological platform for the characterization of novel energy harvesting devices, with a particular focus on optical nanomaterials - nanowires. Despite their challenging structure, AFM offers exceptional versatility in probing dimensional and functional properties of nanowires at the nanoscale. We demonstrate the capabilities of AFM measurements to provide an extensive understanding of the structural, electrical, and spectroscopic properties of nanowires using different operational modes, including Electrostatic Force Microscopy[2] (EFM), Kelvin Probe Force Microscopy[3] (KPFM), and Conductive-AFM[4] (C-AFM), in combination with various feedback mechanisms e.g. deflection, amplitude modulation and force. Our findings establish AFM as an invaluable metrological tool for the development of cutting-edge energy harvesting technologies and optical nanomaterials.



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Revealing thin coatings of cathode materials based on their mechanical contrast by contact resonance atomic force microscopy

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Contact Resonance Atomic Force Microscopy (CR-AFM) and Electrochemical Strain Microscopy (ESM) are two advanced AFM modes, utilizing contact resonance to significantly improve the signal-to-noise ratio. For active frequency locking, a phase-locked loop (PLL) or dual-frequency resonance tracking (DFRT) is used.

CR-AFM is particularly powerful for mapping mechanical property contrasts by analyzing the observables phase, amplitude, and frequency shifts. This is achieved by applying an external oscillation, typically induced via a piezo shaker or photothermal excitation and measuring the resulting changes in these observables. In contrast, ESM utilizes an applied AC electric field to excite mobile ions within an ionic conductive material, such as those found in battery cathodes. The ion motion induces cantilever oscillations, leading to an indirect excitation mechanism. ESM has been previously used to study battery materials. In a recent study we demonstrated that the ESM signal is also influenced by mechanical properties.[1]

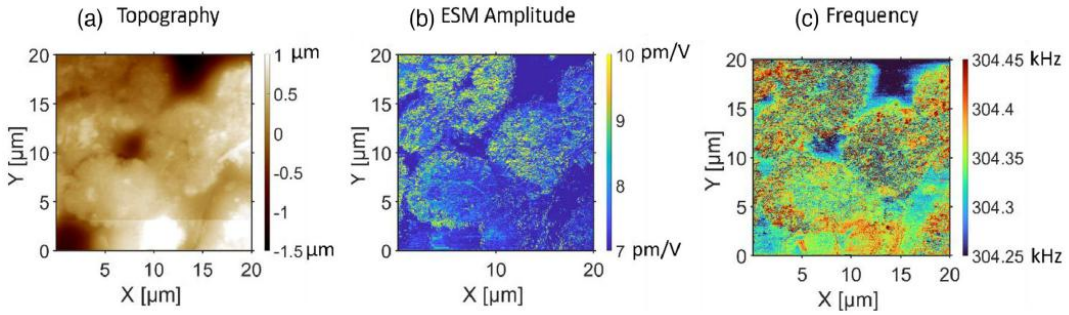

Figure 4: Topography, ESM Amplitude and Frequency-shift of NCM622 particles.[1]

While initial investigations primarily focused on larger grain structures, the next step involves a mechanical analysis at the nanometer scale. This is particularly relevant because many cathode particles are coated with an ultrathin layer, only a few nanometers thick, which poses a challenge for conventional AFM techniques. To address this, CR-AFM has proven to be particularly effective in visualizing these ultrathin coatings. In our recently submitted manuscript, we further demonstrate the precise visualization of delamination and variations in coating thickness, by actively locking the contact resonance frequency. These findings highlight CR-AFM potential as a highly sensitive tool for characterizing nanoscale mechanical changes in energy materials and the importance of actively tracking the contact resonances.

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Dynamic Multimeric Binding Strategies of Pathogens and Therapeutics: Lessons learnt from SARS-CoV-2 Variants of Concern

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Recent waves of COVID-19 correlate with the emergence of the Delta and the Omicron variant. We combined high-speed atomic force microscopy with single molecule recognition force spectroscopy to investigate, at single molecule resolution, the interaction dynamics of trimeric Spike with its essential entry receptor ACE2 [1]. Spike trimer undergoes rapid conformational changes on surfaces, resulting in arc-like movements of the three receptor binding domains (RBDs) that collectively screen a circular range of almost 360° degrees. Acting as a highly dynamic molecular caliper, it thereby forms up to three tight bonds through its RBDs with ACE2 expressed on the cell surface. The Spike of both Delta and Omicron (B.1.1.529) variant enhance and markedly prolong viral attachment to the host cell receptor ACE2, which likely not only increases the rate of viral uptake, but also enhances the resistance of the variants against host-cell detachment by shear forces such as airflow, mucus or blood flow. We uncovered distinct binding mechanisms and strategies employed by circulating SARS-CoV-2 variants to enhance infectivity and viral transmission.

The new SARS-CoV-2 variants are continuously emerging with critical implications for therapies or vaccinations. The 22 N-glycan sites of the Spike protein remain highly conserved among SARS-CoV-2 variants, opening an avenue for robust therapeutic intervention. Out of a lectin library, two lectins, Clec4g and CD209c, were identified to strongly bind to the Spike protein of SARS-CoV-2 [2]. We quantified unbinding forces and analyzed the number of bond ruptures between Clec4g/CD209c and the trimeric Spike protein. Multiple bond formations lead to stable complex formation, in which the number of formed bonds enhanced the overall interaction strength and dynamic stability of the lectin/Spike complexes. Equipped with suchlike molecular modalities, Clec4g/CD209c are multivalent efficient competitors in SARS-CoV-2 Spike binding to cellular ACE2.

We also determined the binding capacity of a molecularly engineered lectin cloned from banana, BanLec H84T, which was shown to display broad-spectrum antiviral activity against several RNA viruses. Our studies revealed that H84T-BanLec interacts with the Spike protein of the original viral strain, Wuhan-1 and several variants of concern (Delta, Omicron) [3]. Based on our force probing technique, dynamic molecular interaction patterns with accurate rupture force and length distributions were depicted. The complex multiple binding features between the dimeric H84T and trimeric Spike protein were analyzed with respect to the distribution of the glycosylation sites on the Spike. The capacity of lectins to block SARS-CoV-2 viral entry holds promise for pan-variant therapeutic interventions.

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Past and Future of High-speed AFM for Dynamic Structural Biology

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The introduction of high-speed (HS) AFM in 2008 [1] was a milestone in biophysics, enabling for the first time the direct, simultaneous observation of structure and dynamics of protein molecules [e.g., 2-4], even for intrinsically disordered proteins [5]. This advance has expanded the range of what can be observed and understood about proteins. The main reasons for this success are that such observations have long been desired and that there were no competing technologies to HS-AFM. We should bear in mind that these two aspects can serve as guidelines for the successful introduction of new measurement techniques. If we follow these guidelines, the pursuit of higher spatial resolution in in-liquid AFM at the expense of temporal resolution would not be a sensible choice because higher spatial resolution techniques already exist for biological molecules.

However, major technological advances in biophysics occur about every 20 years, and some of these advances render earlier techniques obsolete (e.g., Alphafold2 [6] or cryo-electron microscopy (EM) vs. X-ray crystallography). While averaging over many images is necessary, cryo-EM is becoming capable of analyzing time-evolving structures with a resolution of about 10 ms [7, 8]. On the other hand, recent improvements in HS-AFM [9] have already reduced its time resolution to 14 ms, and the introduction of smaller cantilevers will now further reduce it to < 10 ms. These different modalities of time-resolved microscopy may compete but are likely to complement. This is due to several problems arising from ensemble averaging in cryo-EM. Another recent trend in biophysics is the use of cryo-electron tomography (cryo-ET) [10, 11] for intracellular or in situ observation, while AFM (or HS-AFM) has been attempted to be used for intracellular observation [12]. Cryo-ET has already made great progress and is now transforming structural cell biology. It is therefore unlikely that in-cell AFM (or in-cell HS-AFM) will be able to compete with cryo-ET. I believe that it is better for HS-AFM to focus on single-molecule imaging (and fast force spectroscopy [13]) in order to best exploit the strengths of HS-AFM. In addition, recent computational methods are now allowing us to understand HS-AFM images of proteins from their atomistic structure [14]. This is another good reason to focus on single-molecule imaging.

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Correlative nanocharacterization by AFM and SEM

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Understanding complex structures when having only one set of information can be a daunting task. Therefore, a multifaceted view of the sample is becoming more and more important. Correlative microscopy is a great way to combine information from different imaging modalities. While some microscopy methods can be performed in-situ within one combined instrument (for example AFM and optical microscopy), others are generally performed sequentially in different instruments (for example light and electron microscopy – CLEM). In our work we combine the multiparametric imaging capabilities of AFM with the versatility of SEM and FIB (focused ion beam) within one instrument. Using custom self-sensing cantilevers, we have developed a compact AFM integrated in a commercial dual beam microscope for applications in materials and life science. In this presentation I will discuss technical developments[1,2] which enables correlative measurements of electrical properties (KPFM) inside an SEM, nanomechanical characterization correlated with SEM imaging as well as first results of 3D AFM/EM tomography of zebrafish retina.

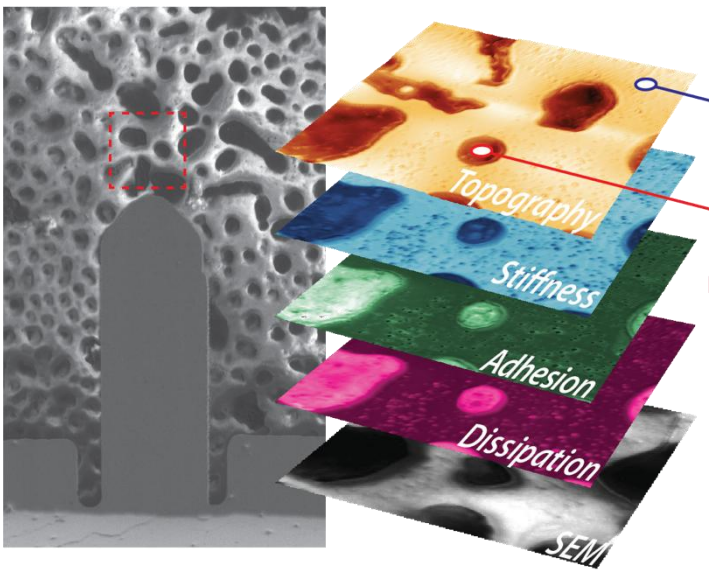

Figure 1: Correlative EM/AFM nanomechanical property measurements of industrial polymers

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Protein interaction, dynamics, and assembly at solid-liquid interfaces

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Proteins are the fundamental macromolecules in biological systems, governing nearly every aspect of cellular function and controlling the growth of hybrid biomolecular materials, such as bones. Their interactions, dynamic behavior, and ability to self-assemble into complex structures play crucial roles in maintaining life. Researchers have long sought to replicate nature's ability to construct hybrid materials by guiding the assembly of protein complexes with inorganic components. Despite advances in protein design that allow for the deliberate creation of synthetic protein-inorganic interfaces, the resulting assembly patterns often deviate from intended designs. This discrepancy suggests that critical interactions remain unaccounted for in existing design frameworks.

In this talk, I will present the efforts to understand how inherent interactions alter the structures that emerge during protein assembly at solid-liquid interfaces using in situ high-resolution and high-speed AFM and further characterize the tailored functions using functional AFM. First, I will discuss the assembly behavior of DHR10-MicaN protein nanorods, which feature a designed protein interface that complements the K^+ sublattice on mica.[1] High-speed AFM captured their in-plane dynamics as a function of KCl concentration. By integrating deep learning-based analysis and simulations, we quantitatively describe the epitaxial relationship between DHR10-MicaN and mica, as well as the role of colloidal forces, entropic effects, and interfacial hydration layers in governing protein mobility, assembly, and the resulting two-dimensional (2D) structures.[2-4] These findings highlight the need to incorporate the mentioned interactions into protein design frameworks for hybrid materials—both to enhance predictive accuracy and to better understand the associated biological processes, such as biomineralization. Second, I will discuss the epitaxial growth of highly ordered 2D silk fibroin (SF) on graphite.[5] Using in situ AFM and nano-FTIR, we demonstrated that these 2D lamellae retain the native silk nano-crystalline structure. Depending on concentrations, SF multilayers form either through direct assembly or via a two-step process, where an initially disordered monolayer crystallizes over time. Additionally, these films modulate the surface potential of graphite, further demonstrating the functional impact of the protein-graphite heterointerfaces in microelectronics and wearable devices. Finally, I will present the preliminary result of the binding of magnetic-field modulated protein-rare earth element ions for non-equilibrium separation.

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Nanoscale viscoelasticity of living and soft matter with AFM

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The dynamic shapes of biological cells and tissues emerge from a complex interplay of physics, chemistry and genetics, which determines--at each temporal and spatial scale--the mechanical properties that eventually form the adaptive structures of living organisms. Shape and mechanical stability of living organisms rely on precise control in time and space of growth, which is achieved by dynamically tuning the mechanical (viscous and elastic) properties of their hierarchically built structures from the nanometer up. It is now well-established that cellular behaviour crucially depends on the mechanical properties of the cells and their environments. Much attention has been directed towards the importance of the stiffness (i.e. the capacity of a material to elastically store mechanical energy) which has been the focus of most experimental research, however neither cells or the extracellular matrices are elastic. Biological systems dissipate energy (i.e. they are viscous) and hence they do not respond to mechanical deformations instantaneously, but present different time responses at different spatial scales that characterise their responses to external stimuli. Measuring viscoelasticity at the nanoscale has remained experimentally challenging [1,2,3]. I will present atomic force microscopy (AFM)- based techniques, developed in my lab, to measure and map the nano-viscoelasticity of living organisms, cells, membranes, collagen, ECMs, and tissue engineering matrices across the spatial and temporal scales, and chirp-based spectroscopic techniques to assess viscoelasticity from Hz to 100s kHz at the nano and micro scale [4]. I will also present tests for assessing which viscoelasic model better fits the experimental AFM results. Our results have uncovered, for example, that cell walls of plants [5] and extracellular matrices in tumours present almost perfect linear viscoelastic behaviour, and how different properties of cells are coupled, e.g. in the mechano-electrical coupling in neurons [6,7]; we are now extending these techniques to probe the capacity of ultrasound oscillations to modulate neuronal behaviour [7].

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Nanomechanical Sensing in Liquids with Dip-in Nanowire Probes

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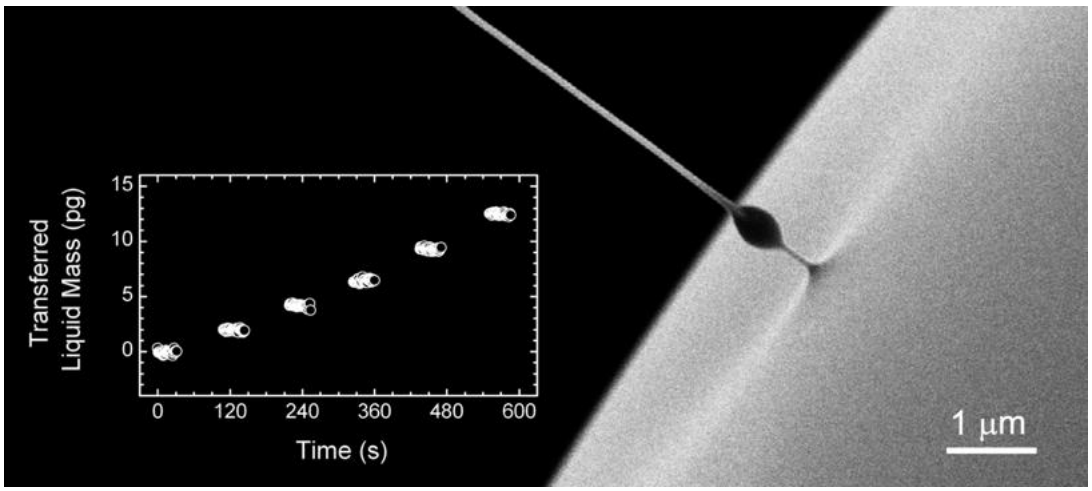
Liquids evolve along the outer surface of high aspect ratio nanostructures as a thin precursor film or as beads, so that nanowire-like structures can be used as suspended open nanochannels for liquid transport along its surface [1]. Nanomechanical sensing can be integrated into these platforms by reading out the changes in their resonance frequencies as a consequence of liquid flow or due to the presence of analytes. This contribution focuses on the use of Si nanowires as open nanochannel resonators following such approximation. The combination of open nanofluidics with nanomechanical sensing offers new perspectives for the study of liquid behavior at the nanoscale as well as for the characterization of nanoparticulate analytes dispersed in extremely small volumes of liquid solutions. Specific aspects of transduction and detection limits regarding nanowire resonators will be treated [2,3], and recent results regarding the transport of ionic liquids along Si nanowires will be detailed [4]. In addition, a demonstration of this approach for characterizing the mass of nanoparticles suspended in these liquids will be presented, highlighting its key performance advantages: high mass resolution, minimal sample volume requirements, and high-throughput [5].

Figure 1. Si nanowire immersed in an ionic liquid reservoir with the liquid spreading in response



to a DC voltage. Inset: measurement of the liquid mass accumulated after consecutive immersions.

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Advancing High-Speed AFM: DNA-Binding Protein Dynamics and Imaging Speed Enhancement

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High-speed atomic force microscopy (HS-AFM) is a powerful technique for visualizing biomolecules at the submolecular scale with a temporal resolution of 100 ms, making significant contributions to the understanding of various biological processes [1-3]. In this presentation, I will discuss the application of HS-AFM to DNA-binding proteins, specifically structural maintenance of chromosomes (SMC) complexes, alongside recent developments aimed at further accelerating the HS-AFM scanning speed.

Application: Human genomic DNA, spanning 2 meters, is tightly folded within a micron-sized nucleus, a dynamic process essential for chromosome functions such as cell division and gene expression. SMC complexes, including cohesin, condensin, and Smc5/6, play a pivotal role in shaping the genome's three-dimensional architecture. These ATPase motor proteins form ring-like structures that encircle DNA, yet their molecular mechanisms remain unclear. Using HS-AFM, we visualized the submolecular dynamics of Smc5/6, capturing one-dimensional diffusion along DNA and real-time structural transitions. ATPase mutant analysis suggested that the SMC complex initially engages DNA via its ATPase domain before transitioning to a stable DNA-bound state. These findings provide deeper insights into the DNA-binding mechanism of Smc5/6.

Development: To further enhance temporal resolution, we have been developing an advanced HS-AFM system with significantly improved imaging speed [4]. HS-AFM adopts an amplitude modulation scheme [5,6], with bandwidth-limiting devices including the Z piezo scanner, amplitude detector, and cantilever. To minimize conversion latency, we implemented a Hilbert transform-based architecture in the amplitude detector, reducing it to nearly zero [7]. For the Z scanner, a four-point mounted scanner eliminated the effect of halving the resonance frequency due to the shift in the center of mass caused by surface attachment, enabling a resonance frequency of 1.1 MHz [8]. Additionally, cantilever miniaturization using focused ion beam (FIB) technology yielded a cantilever with a resonance frequency of 3 MHz. Integrating these advancements, we successfully developed a system capable of imaging fragile biomolecules, such as actin filaments, at a speed ten times higher than the current setup.

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Multifrequency Atomic Force Microscopy and Cantilever-Based Sensors: Advancing Nanomechanical Spectroscopy

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Atomic force microscopy (AFM) has transformed nanoscale imaging and material characterization, providing insights into surface morphology, mechanical properties, and intermolecular interactions[1]. It operates by detecting microcantilever deflection as a sharp tip probes surface interactions, enabling high-resolution measurements in air and liquid. The cantilever's resonance frequencies and harmonics are key to extracting quantitative material properties[2]. AFM characterization techniques fall into two main categories: force–distance and parametric methods[3]. Force–distance spectroscopy measures tip-sample interactions during approach and retraction, revealing adhesion, stiffness, and dissipation[3]. Parametric methods, such as multifrequency AFM, excite and detect multiple eigenmodes to enhance sensitivity to viscoelasticity and dissipation, extending AFM's capabilities beyond single frequency imaging[4]. Cantilever-based sensors, inspired by AFM, are powerful tools for precision sensing in biomolecular detection, mass spectrometry, and environmental monitoring[5]. Their operation relies on resonance frequency shifts and mode coupling to detect nanoscale mass changes and mechanical properties. Advances in multimodal cantilever spectroscopy, including higher-order modes and nonlinear effects, have improved detection limits and broadened applications in nanomechanical characterization.

This study presents recent developments in wavelet-based AFM[6] and cantilever-based sensing, focusing on their applications in imaging, characterization, and mechanical property quantification of materials in both air and liquid environments.

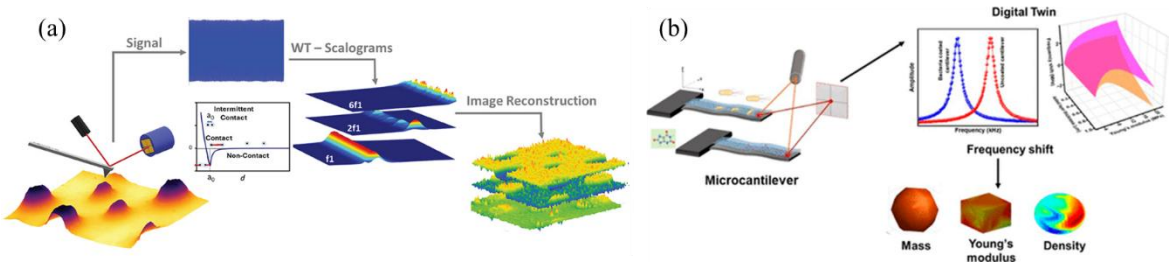

Figure 1. (a) Schematic of Wavelet-Based AFM[6], (b) Schematic of Schematic of microcantilevers for biosensing, mass detection, and characterization[5].

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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] 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.

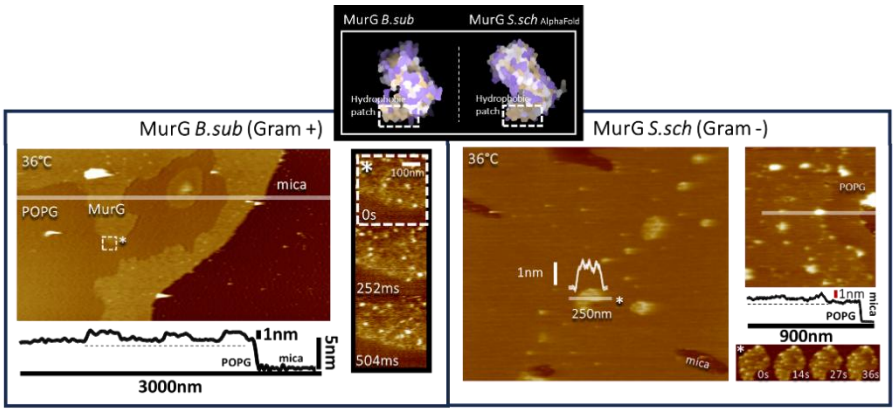

Figure. Comparison of the formation of partitioning in the POPG bilayer by two MurG proteins from Gram + and Gram - bacteria. On top, representation of the membrane interaction region of MurG proteins by AlphaFold. Bottom, Topographic images of POPG supported bilayer after addition of MurG protein - left MurG *Bacillus subtilis*, right MurG *Salmonella schwarzengrund* - and showing the diffusion of the protein in these partitioning.

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Suspended microchannel resonators for nanomechanical cell characterization and further beyond

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During the last decades important advances in the field of cell biology has been obtained thanks to the parallel development of nanomechanics and microfluidics. While nanomechanics has allowed expanding our knowledge on the cell structure and dynamic behavior, microfluidics has introduced innovative methods for the passive control and manipulation of cells with a high throughput. In this context, Suspended Microchannel Resonators (SMR) were developed to merge the best of these two worlds. Consisting on a cantilever or bridge-shaped resonator inside which a microfluidic channel has been integrated, these devices allow flowing a liquid sample containing the cells to be analyzed with a high throughput (up to 24.000 cells analyzed per minute [1]) while the resonance frequency of the structure is tracked over the time. Consequently, anytime a particle crosses the suspended structure, it produces a shift in the resonance frequency which is registered (Fig. 1a) and can be eventually related to mechanical properties of the cells as mass[2].

In this work we show how can we take advantage from different hydrostatic and hydrodynamic phenomena in SMR devices to obtain mechanical properties of cells related to stiffness [3] as well as for achieving the trapping of the particles at specific positions of the integrated channel (Fig. 1b). Moreover, in this work we also show how integrating metallic electrodes these devices can be used for the characterization of electrical properties of the sample under test (Fig. 1c) as well as trapping them at specific positions (Fig. 1b).

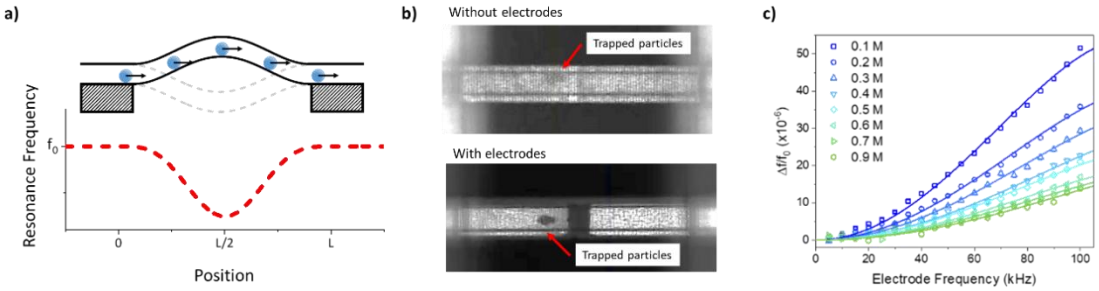

Figure 1. a) Schematic of the frequency shift produced in a SMR bridge-type resonator when a particle passes through it. **b)** Image of particles trapped in a SMR device as a consequence of (up) the appearance of some hydrodynamic forces and (down) application of a voltage in the electrodes. Device length: 500 µm. **c)** Relative mechanical frequency shift measured in the SMR device when a voltage is applied for samples with different electrical permittivity.

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Photothermal cantilever actuation across the frequency spectrum

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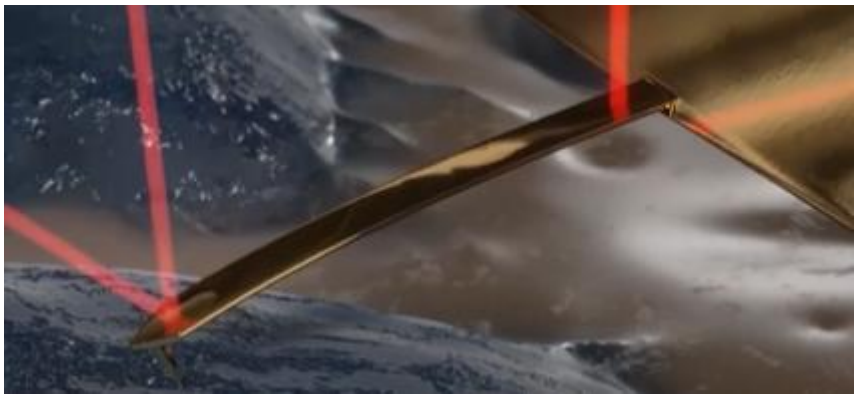
A fundamental function of an atomic force microscope is to modulate the distance between the tip and the sample. This modulation must be performed over a wide range of frequencies, from DC for feedback purposes, through to higher cantilever eigenmodes for advanced imaging techniques. Measurement speeds are often limited by the rate at which this modulation may be performed. A significant improvement can be achieved by moving the smallest and least massive object possible – in this case, by directly bending the cantilever itself. Photothermal actuation has emerged as one of the easiest and most flexible methods in which to directly actuate AFM cantilevers. In this presentation, we will cover some of the numerous ways in which photothermal actuation may benefit AFM operation, from feedback, through off-resonance operation, to higher and multiple eigenmode methods. The benefits of this approach extend beyond topographical imaging; here we will present some recent results towards high-speed and broad-frequency-range materials characterization.



Capturing Single Mitochondrial Activity by SPM-Based Noise Analysis

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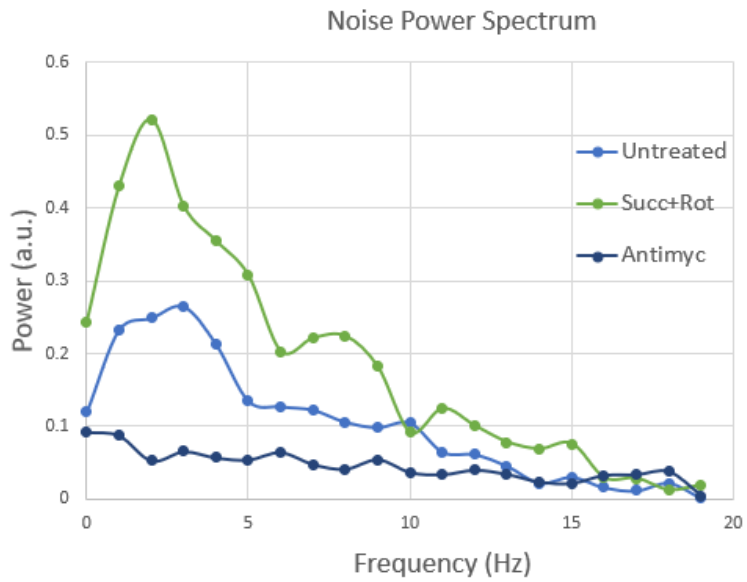
Often termed "the powerhouse of the cell", mitochondria are central to energy production in eukaryotic cells. Mitochondrial function is governed by an intricate combination of enzymes, membranes, ion transport mechanisms, and signaling functions all packed into a small organelle ranging from several hundred nm to several µm in size. Full understanding of mitochondrial function and dysfunction is elusive due to this complexity. In particular, no single technique is capable of monitoring and characterizing the full details of the energy production process. Various forms of microscopy have been used to study mitochondria. Whereas electron microscopy provides the highest resolution of their inner structure, it cannot observe real-time function. Optical microscopy, including various fluorescent techniques, can indirectly capture electrochemical activity and function of live mitochondria but only by adding dye molecules which perturb the system. Optical techniques also encounter resolution limitations. In this presentation, I will present a multi-faceted scanning probe microscopy-based approach to the study of mouse liver mitochondrial function. SPM offers several advantages in such studies: Firstly, the measurements can be made in buffer solution with viable mitochondria, so that their response to different additives can be directly observed as they occur. Secondly, the high resolving power allows us to obtain 3D images of individual mitochondria, which can simultaneously be characterized mechanically through fast force measurements. In addition, I will show that by measuring "noise spectra" (example in figure below) of the untreated, stimulated (Succinate + Rotenone), and inhibited (Antimycin A) mitochondria, we can monitor the organelle's activity, through the membrane potential (its driving force), and how it changes under different environments. All features of the noise power spectra – their broad envelope, sharper features, and integrated power can be related to the mitochondrial activity in a holistic way. This approach thus provides a nondestructive, real-time measure of the activity of single mitochondria and the corresponding morphological and mechanical changes.



Modification and application of active piezoresistive cantilevers for surface imaging with higher transverse modes of actuation

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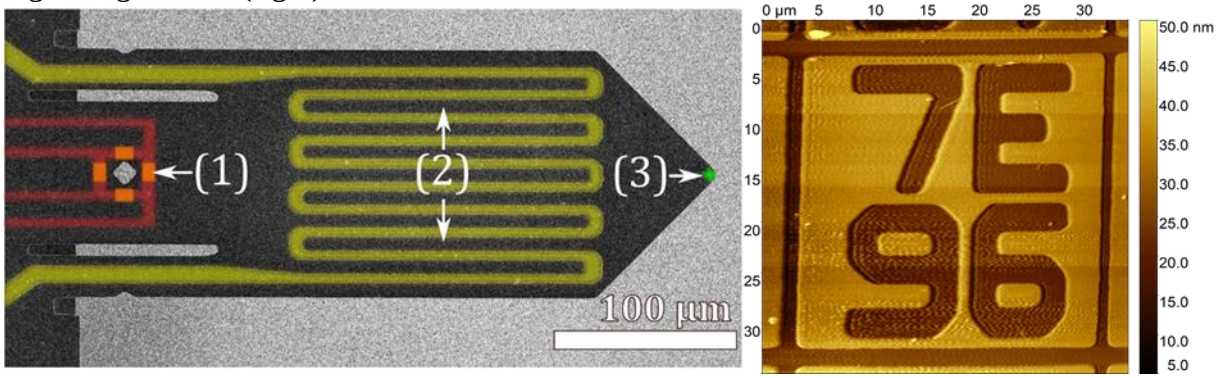
Active piezoresistive cantilevers are universal and versatile tool for multimodal scanning probe microscopy (AFM) – primary for atomic force microscopy (AFM) both in non-contact and contact modes. They consist of silicon cantilever body with resistive heater for actuation and piezoresistors for deflection detection [1]. Active piezoresistive cantilever-based probes are supreme in sensitivity, however their bandwidth is limited technologically to approx. 100 kHz due to required minimal size of the cantilever body.

To improve bandwidth we employ higher transverse eigenmodes of vibration. Sensitivity of those devices is inhibited in higher eigenmodes due to relatively high stiffness of the cantilever body and fixed placement of the piezoresistors. To counteract this effect, we additionally modify the cantilever by local milling with focused ion beam (FIB). We reduce stiffness locally in the proximity of mode shape arrows.

We show modelling and theoretical analysis of the active piezoresistive cantilever before and after modification. We perform sensitivity analysis on 2nd and 3rd eigenmode to prove the increase of sensitivity by the means of thermomechanical noise spectral analysis [2]. We present the imaging capabilities on the surface of SiO_2 20 nm relief calibration sample and SiC 1.5 nm steps sample [3]. We discuss on possibilities of modifications and improving sensor performance towards higher eigenmodes, enabling imaging in a bandwidth above 2 MHz.

Figures present active piezoresistive cantilever (left) and surface imaged with one actuated in higher eigenmode (right).



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Probing the transversal and longitudinal electrical conductivity without physical contact by Scanning Dielectric Microscopy

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Electrical conductivity of materials can be measured at the nanoscale for instance by means of conductive atomic force microscopy (c-AFM). However, c-AFM requires a good electrical contact between the conducting measuring probe and the sample and the presence of a second electrode to close the electrical circuit, which cannot always be set. To overcome this issue, alternative techniques such as scanning microwave microscopy (SMM) or scanning dielectric microscopy (SDM) have been developed, which, by using time-dependent electric fields, can access the conductivity of materials without the need of a physical contact with the sample. Here, the use of SDM to probe the conductive properties of materials at the nanoscale will be reviewd. Both instrumental and modelling aspects will be covered, showing that SDM can access both the transversal and the longitudinal conductive properties of materials at the nanoscale, and that it can be operated both in air environment, as well as, in an electrolyte environment. Recent applications of the technique to probe the conductive properties of electrically conductive cable bacteria [1] and of polymer materials in electrolyte gated organic transistors [2], [3] will be presented and discussed.

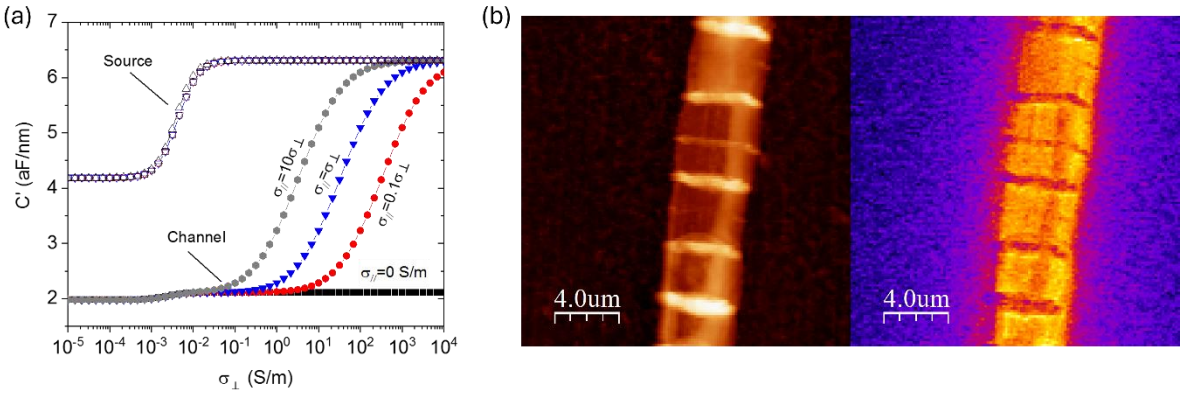

Figure 1. (a) Numerically calculated capacitance gradient (electric force) as a function of the transversal conductivity of an anisotropic polymer material in an Electrolyte Gated Organic Transistor for different longitudinal conductivities of the material. When the material sits on an insulating substrate the electric force feels both the longitudinal and the transversal conductivities. (b) Topography and electrostatic force images measured on a cable bacteria sitting on an insulating substrate. The high contrast of the electric force image reveals the longitudinal electrical conductivity of the cable bacteria.

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Low temperature multimode atomic force microscopy using an active MEMS cantilever

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High resolution atomic force microscopy is routinely performed at ultra-high vacuum and at low temperatures by employing a qPlus tuning fork sensor. Due to its intrinsic self-sensing capability, very high dynamic stiffness, and large Q factor, qPlus sensors are pre-dominantly used for obtaining atomic resolution [1]. However, microelectromechanical system (MEMS) based cantilever sensors offer several interesting features compared to conventionally used qPlus sensors. These include the ability to influence the sensor stiffness by direct design of the cantilever geometry, the ability to easily switch between higher eigenmodes of the cantilever and the ease of up scaling the fabrication process. In this work, we present a proof of concept for a MEMS microcantilever with integrated piezoelectric sensing and equipped with a focused ion beam deposited tungsten tip and demonstrate its capability to obtain scanning tunneling microscopy as well as high-resolution non-contact atomic force microscopy images on an atomically flat Au(111) surface at the fundamental and at an higher eigenmode of the cantilever. The results show that the effective signal-to-noise ratio in the AFM frequency shift image when using the MEMS sensor is comparable to the qPlus sensor at low imaging speeds and around 1.3 times better than qPlus sensors at high imaging speeds [2]. This opens up a number of opportunities for sensor improvement, for instance, finding the optimal cantilever stiffness, which is a trade-off between allowing small oscillation amplitudes, favoring a higher dynamic stiffness, and maximizing the sensitivity in the frequency shift channel, favoring a lower dynamic stiffness.

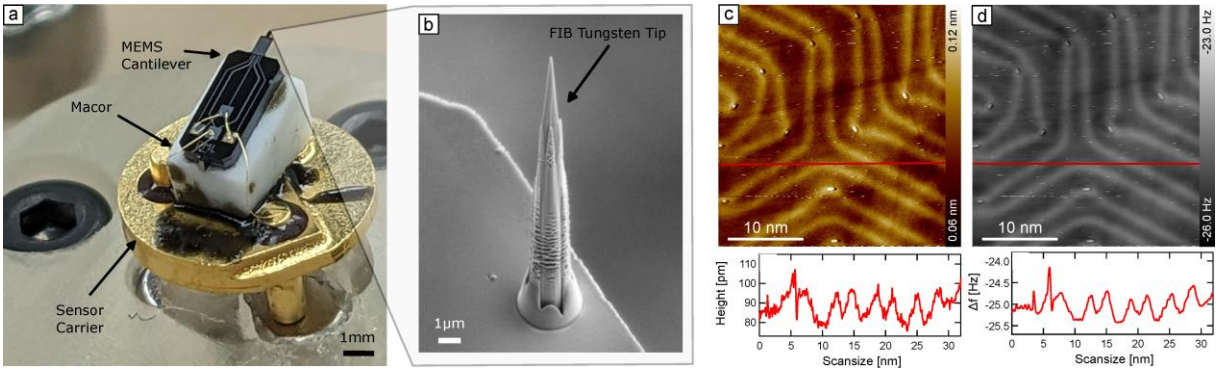

Figure: (a) Photo of the fabricated MEMS cantilever wire-bonded to a qPlus sensor carrier. (b) Scanning electron micrograph of the focused ion beam deposited tungsten tip. High-resolution LT-AFM/STM images obtained with the third mode of the piezoelectric MEMS cantilever of the Au(111) herringbone reconstruction showing (c) STM image with cross-section and (d) frequency-shift image with cross-section. Imaging conditions: It = 50 pA, V = 100 mV, image size = 30 × 30 nm, 600 × 600 pixel, raster time = 30 ms per pixel, scan speed = 1.67 nm s−1, A0 = 1.08 nm.

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Nanoscale infrared imaging and spectroscopy in organic and inorganic samples

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Infrared scattering-type Scanning Near-field Optical Microscopy (IR-s-SNOM) [1] and photothermal expansion-based AFM-IR [2] are state-of-the-art approaches to optical microscopy and spectroscopy bypassing the ubiquitous diffraction limit of IR-light to achieve a spatial resolution below 10nm with help of (bimodal) AFM. These rather new AFM-based methods not only enable imaging with high spatial resolution but even have the power to reveal chemical information of organic and inorganic materials on the 10nm scale. Exploiting the analytical power of nano-IR-spectroscopy, IR-s-SNOM can give insight into intermolecular interactions on the nanometer scale also in liquid with controlled solvation environment [3].

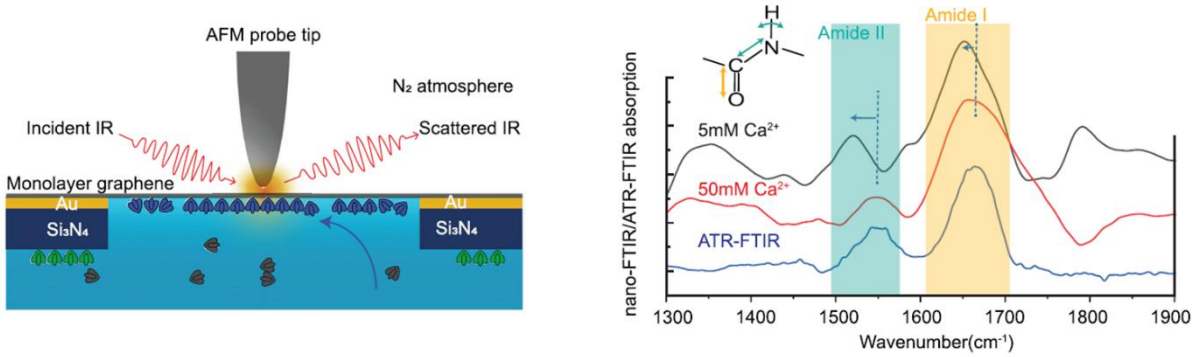

Fig. 1: Self-assembly process of S-layer proteins at the graphene-aqueous solution interface – Investigation using IR-s-SNOM in liquid and recording the amide I and II IR absorption bands reveals the noncovalent interaction between proteins and their response to the environment, including ionic strength and solvation (reorientation of protein lattice units, hydrogen bond formation with water molecules) [3].

We present the principles of the two infrared nano-imaging and -spectroscopy methods and describe their advantages and disadvantages on certain types of samples. Examples show how both methods are used in the field of material science, biomaterials, nanoparticles, nanostructured polymers, metal-oxides and else, with a few highlights of special experiments, like nanoscale pump-probe spectroscopy, near-field photocurrent measurements and others.

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Electrochemical Microwave Microscopy

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Research on catalysis and energy materials requires techniques with the potential to resolve elctrochemical processes with high lateral resolution. We introduce electrochemical scanning microwave microscopy (EC-SMM), a novel technique offering 16 ± 1 nm spatial resolution and atto-Ampere sensitivity for nanoscale electrochemical analysis (1,2). By applying EC-SMM to NiCo-layered double hydroxide flakes, we demonstrate its capability to pinpoint active catalytic sites and elucidate edge effects through localized impedance spectroscopy and cyclic voltammetry (2,3). Complementary numerical modeling of diffusion and migration dynamics further clarifies the atomistic mechanisms of ion intercalation and surface adsorption (2). Additionally, the development of innovative coaxial probes enhances measurement fidelity and extends EC-SMM's applicability to fields such as magnetics and bioparticle detection. This integrated platform provides a robust tool for advancing diagnostics and optimizing material performance across multiple disciplines.

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Cryogenic AFM with an integrated kinetic inductive electromechanical transducer

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We recently introduced electromechanical coupling through strain modulation of the kinetic inductance of a superconducting nanowire[1,2]. This coupling mechanism, which is the electrodynamic dual to conventional capacitive coupling, provides a compact and versatile solution for the detection of displacement in micro- and nano-mechanical systems.
We implement this detection principle on a micro-cantilever force transducer designed for low temperature atomic force microscopy. The transducer features a Si-N microcantilever with its first flexural eigenmode in the frequency range 5 − 10 MHz, coupled to a 4.5 GHz lumped-element superconducting resonant circuit.
We demonstrate force gradient sensing and imaging with electromechanical detection in a scanning system built inside a dilution refrigerator at 10 mK. Using the multifrequency pumping scheme known as back-action evasion, we realize efficient phase-sensitive readout of the driven cantilever motion[1,3]. Operating the AFM in the frequency modulation mode (FM-AFM), we investigate force gradient sensitivity through frequency shift vs tip-surface distance curves. We also investigate the speed, resolution, stability and robustness of feedback-controlled scanning while imaging a calibration test surface.

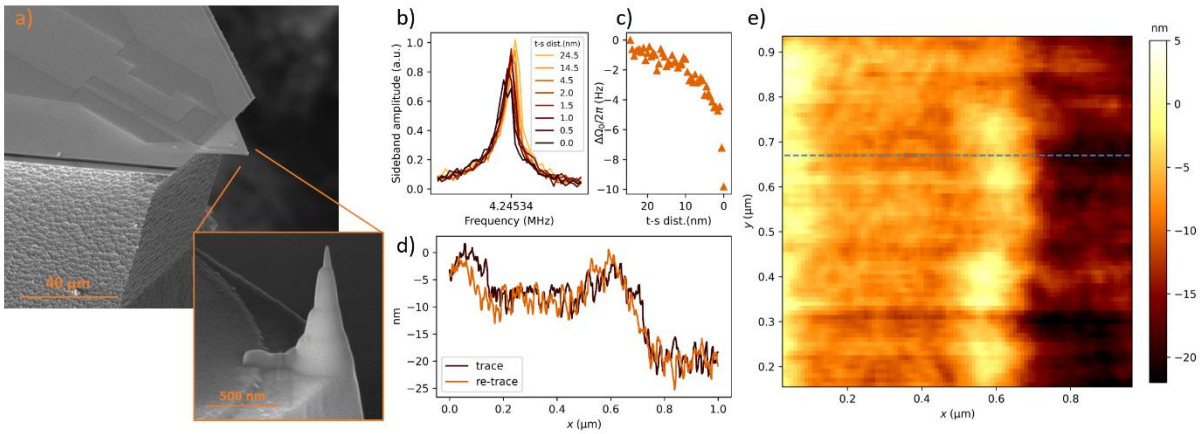

Figure 5: a) Side view of the electromechanical force transducer with integrated microwave microwave resonant circuit. The inset shows the AFM/STM conductive tip deposited at the cantilever apex. b,c) Motional sideband frequency shift vs tip-surface distance curves. d,e) FM-AFM on a test surface: d) raw data at the horizontal dashed line in the image e) showing surface topography with gaussian smoothing of a 1 × 1 μm scan area.

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Driving and Imaging Domain Wall Dynamics in Ferroelectrics using "Scanning Oscillator" Atomic Force Microscopy

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Piezo-response force microscopy (PFM) has been widely used to map piezo electric properties of ferroelectric materials. Further extension of this technique (switching spectroscopy – PFM, SS-PFM) in which an addition dc bias is applied to the AFM tip to induce nucleation and growth of new domains below the tip enabled imaging variations of ferroelectric switching properties across a surface with 10's nm resolution. It has been shown that domain nucleation is sensitive to several factors including defects, dopants, existing domains, etc. However, in these cases, very little information can be extracted about the bias driven dynamic motion of a domain wall itself, since the AFM tip is never positioned over a domain wall while its moving. Indeed, ferroelectric domain wall dynamics provides fundamental insights into the behavior of nanoscale interfaces that govern the macroscopic properties of ferroelectric materials. These domain walls, which separate regions of different polarization orientations, are highly responsive to external stimuli such as electric fields, mechanical stress, and temperature variations. Understanding their movement and interactions is crucial for optimizing ferroelectric-based devices, including non-volatile memories, sensors, and energy-efficient transistors. Moreover, domain walls exhibit unique electronic and transport properties that can be harnessed for novel nanoelectronic applications, making them a promising platform for next-generation functional materials.

To address the need to assess domain wall dynamics, we developed "Scanning Oscillator Microscopy" to visualize and quantify bias driven domain wall motion in [1]. Here, a combination of precisely timed high frequency AC bias (which drives piezoresponse to detect domain orientation) and larger amplitude low frequency AC bias (that modulates domain wall position) is applied to the tip during tip scanning. Notably, scanning and bias timings are set such that each measurement position receives exactly the same drive signal. The acquired data is then processed into a 3D data set of two spatial dimension (the scan area) and time (instantaneous domain map) to provide a movie (with effectively 1 ms time resolution) of domain wall position vs bias. Results of this approach applied to lead titanate with 180° domain walls will be presented. Notably, the antiparallel c+/c- domains show expansion and contraction under different voltage regimes. Since the voltage magnitudes explored were sub-coercive field, the domain wall motion was repeatable with several cycles. The overall displacement and velocities of the 180° wall depends greatly on its orientation relative to pre-existing large a- (in-plane) and c- (out-of-plane) domains and periodic a-c domain structures. The local curvature of the domain walls is also shown to affect their susceptibility to motion where regions of higher curvature in general show larger displacements. In addition, comparison between samples with varying levels of defect were studied to determine the correlation between domain wall motion susceptibility and defect concentration.

This work was supported by Center for Nanophase Materials Sciences (CNMS), which is a US Department of Energy, Office of Science User Facility at Oak Ridge National Laboratory.

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Organic Supramolecular Heterostructures

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Here we report on our recent progress in engineering and characterizing organic van der Waals materials – organic supramolecular heterostructures (OSH) with atomic force microscopy (AFM) [1–3]. Historically the area of molecular self-assembly on surfaces has been mostly limited to a single layered structures, but the introduction of organic supramolecular heterostructures offers a paradigm shift in this area by extending control of molecular self-assembly over second and further layers, potentially providing an elegant roadmap for precise engineering of organic crystals.

OSHs are layered organic materials that are stabilized by hydrogen bonds in plane and by van der Waals interactions and/or dipolar interactions between layers. They can be formed by, for example, growing sequential layers of bi- and mono-component two-dimensional supramolecular arrays stabilized by hydrogen bonding. The heterostructures are formed on layered materials such as hexagonal boron nitride (hBN), graphite or black phosphorus by depositing layers of cyanuric acid/melamine (CA.M), 5,10,15,20-tetrakis(4-carboxylphenyl) porphyrin (TCPP), trimesic acid (TMA) and terephthalyc acid (TPhA) and other molecules. We analyze OSHs under ambient conditions using ambient AFM and demonstrate the routine acquisition of images with resolution of 0.1 nm resolution in conjunction with conventional silicon probes (Multi75Al-G, NuNano350 or ArrowUHF). We have developed several approaches to characterize the multilayer heterostructures. These include 'through-the-layer' imaging and scratching experiments to reveal the underlying layer structure and/or substrate lattice.

AFM has confirmed a clear epitaxial arrangement between the layers of the heterostructure, although each layer can exhibit distinct symmetries, for example hexagonal (CA.M) and TMA, square (TCPP), linear (TPhA) arrangements. We demonstrate that heterostructure formation may be used to control the functional properties of supramolecular layers through a shift of the fluorescence peak position and a suppression of quenching for TCPP epitaxial layers.

The work will present examples of single molecule and sub-molecular resolution. In the latter case, we will discuss the opportunities and challenges in achieving and interpreting ultrahigh resolution AFM images of molecules in the ambient for the purpose of their unambiguous identification.

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Insights into MoS_2-based devices via operando KPFM

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Two-dimensional (2D) van der Waals materials have shown great potential for integration as components in electronic devices due to their unique electrical and optical properties [1]. Understanding the local behavior of these devices, often dominated by highly localized phenomena, is crucial for advancing in their application. Being 'all surface', this family of materials is particularly suited to be studied with local probe microscopy techniques and, taking a step further, within the device in its actual operational state [2-4].

In this talk, I discuss our recent approach to systematically study 2D-based devices under operando conditions, using Kelvin probe force microscopy (KPFM). Our primary focus has been on investigating the contact between the electrode and 2D material to obtain local information about the potential barriers. By employing a two-terminal configuration, we can effectively probe the voltage drops along the device through surface potential mapping (Figure). Based on the choice of electrode material, our findings reveal the ohmic and Schottky behavior of the contact, offering insights for optimizing 2D-based devices such as highly rectifying diodes or transistors with low contact resistance.

The study aims to contribute to the advancement of 2D material-based electronics by providing valuable insights into the fundamental aspects of device operation and optimization strategies.

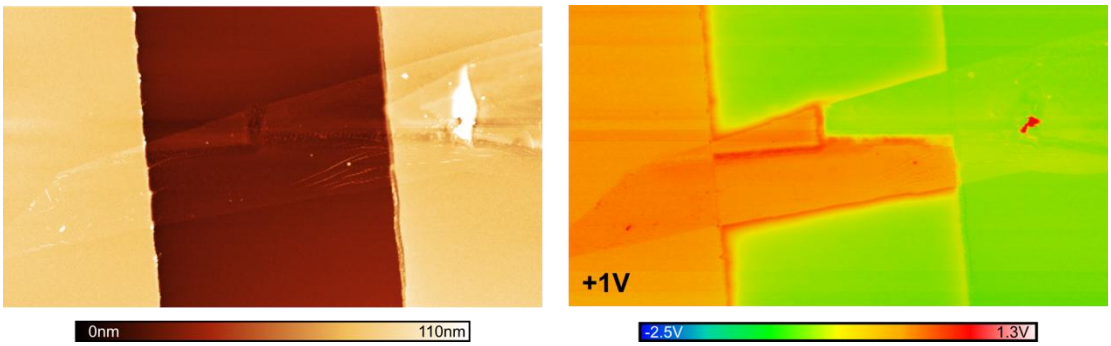

Figure: Simultaneous topographic (left) and surface potential (right) images of 1L-MoS_2 device. Measurements performed in operando conditions: source-drain bias of 1V.

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Mechanics of defective 2d materials

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Transition Metal Dichalcogenides (TMDCs), have been posed to play an active role in future flexible electronic devices due to their high mechanical strength and tunable electronic bandgap. One of the requirements of such application is mechanical reliability. The mechanical endurance of a materials is limited by either its ultimate tensile strength and fracture toughness, or its lifetime due to cycling loading .Here we evaluate the reliability of real life MoS2 where the presence of atomic defects is unavoidable. I will present our recent studies on mechanical response of MoS2 with systematically and controllably created atomic vacancies. We observe that atomic vacancies substantially modify the toughness, fatigue response and lubricity of MoS2.

Characterizing the electronic properties of graphene on silicon carbide via Atomic Force Microscopy

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The rising interest towards two-dimensional (2D) layered materials is motivated by their exotic behaviour and possible nanotechnological applications [1,2]. Such systems are formed by a single atomic layer such as graphene, boron nitride, transition metal dichalcogenides or by their stacking. The interplay of chemical and geometrical factors (the type of stacked layers, the out-of-plane relative alignment of their unit cells, the twisting angle, and the presence of strain or defects) leads to new material properties.
Stacking methods rely on the manual exfoliation and subsequent deposition of single layers at peculiar angles to obtain the desired behaviour. It has been reported the possibility of rotating the top layer of heterostructures by means of AFM [3], but the tuning of the geometry of such multilayers remains challenging. Silicon carbide (SiC) is a known precursor of graphene used to produce large-sized flakes of graphene via thermal decomposition. As a result of such a treatment, Si atoms leave the top layers of the crystal, leading to the formation of epitaxial single- or multi-layered graphene, whose electronical properties have been characterized also by AFM [4]. This reproducible way of obtaining graphene stacks on the top of a tunable wide-band-gap semiconductor makes it an interesting system to further characterize at nanoscale.
Here, we show several examples of AFM-based analysis of these structures. In particular, a mapping of the layer-dependent surface potential via heterodyne Kelvin Probe Force Microscopy (KPFM) on differently prepared SiC samples will be presented. Conductive AFM (C-AFM) experiments performed on the same areas show triangular and stripe-like domains to correlate to the number of stacked layers and to the KPFM signal. Finally, we demonstrate how electronic information about individual graphene flakes on insulating SiC can be obtained using scanning Microwave Impedance Microscopy (sMIM) to overcome the impossibility to apply a bias to graphene.

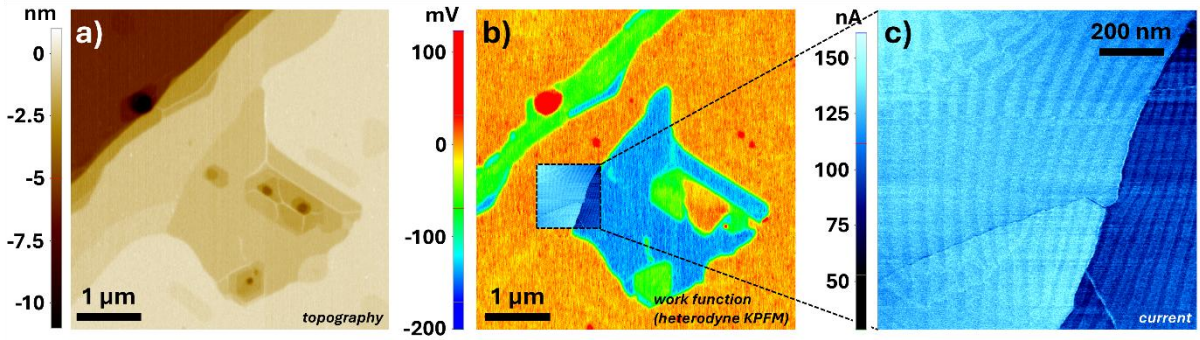

Fig. 1: a) topography and b) work function maps on the same area of a SiC sample; c) current map at constant voltage on an area of previous images. The sample was provided by Dr. Rejhon from Institute of Physics, Charles University, Prague, Czech Republic.

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Solid-like Behaviour of Confined Water in Graphene-Liquid Cells

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Graphene-liquid Cells (GLCs) are femto to nanoliter-sized water pockets sealed between two graphene sheets[1], offering a powerful platform for Liquid Phase Transmission Electron Microscopy of live biological specimens ranging from proteins to cells at ambient temperature[2,3]. The pressure inside a GLC with a height of 1 nm is estimated to reach 1 GPa[4], raising fundamental questions about the state of water under extreme confinement. The state of confined water influences the nanoscale structure, function, and reactions inside encapsulated biological samples. However, direct nanomechanical measurements of whether water remains liquid or transitions to an ice-like phase in such environments remain limited. To address this, we employ Atomic Force Microscopy (AFM) for nanomechanical measurements of water pockets up to 10 nm thick within suspended graphene layers. Using torsional cantilever excitation, we achieve stable imaging and observe a solid-like response to shear motion, suggesting ordered water layering. Additionally, Peak Force Tapping measurements reveal a stiffer response of solid-like water pockets than suspended graphene. By introducing spacer particles up to 100 nm in diameter, we investigate the mechanical behavior of confined water across varying pocket volumes. These insights advance our understanding of the nanomechanical properties of confined water, with implications for encapsulated biological systems.

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Machine learning in Scanning Probe Microscopy

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Scanning Probe Microscopy (SPM) has been the engine of characterization in nanoscale systems in general, and the evolution of functionalized tips as a reliable tool for high-resolution imaging without material restrictions has been a breakthrough in studies of molecular systems. In parallel, machine learning (ML) methods are increasingly being applied to data challenges in SPM. In particular, the success of deep learning in image recognition tasks has led to their application to the analysis of SPM images, especially in the context of surface feature characterisation and techniques for autonomously-driven SPM.

In this work, we explore the general potential for using ML approaches to aid in the analysis of Atomic Force Microscopy (AFM) and Scanning Tunnelling Microscopy (STM) images, along with the possibilities of introducing ML automation into experimental workflows. As an example, we build upon a deep learning infrastructure that matches a set of AFM/STM images with a unique descriptor characterizing the molecular configuration, and then develop a workflow that takes experimental images of complex molecular systems and revises initial ML structure predictions with neural network potential simulations [1]. In this context, we discuss the challenges of handling experimental data and possible data augmentation strategies. Beyond this, we show how ML approaches can be used actively during SPM experiments for construction of nanostructures through atomic manipulation, while also highlighting approaches towards automated construction of more complex molecular systems atom-by-atom and bond-by-bond [2].

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Exploiting Multifrequency AFM Observables for Object and Phenomenon Identification and Enhanced Material Characterization

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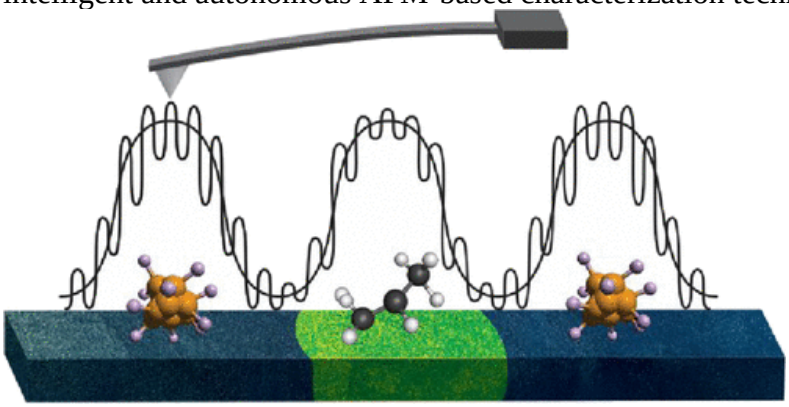
Multifrequency atomic force microscopy (AFM) generates multiple observables per pixel, capturing rich spectral and mechanical information beyond conventional single-channel imaging. While multifrequency AFM is traditionally employed for pixel-by-pixel quantification of material properties, its full potential extends beyond direct numerical extraction. We propose leveraging the inherent multifrequency data structure to first identify objects and distinguish physical phenomena before applying quantitative models.
By utilizing machine learning algorithms, we process multiple observables per pixel to classify material regions, detect transitions, and predict optimal force models in real time. This approach shifts the paradigm from direct parameter extraction to a two-step process: (1) object and phenomenon identification through data-driven classification and (2) subsequent analytical quantification using the most suitable force model. We demonstrate that this method improves contrast, enhances the reliability of material property retrieval, and reveals nanoscale interactions that remain concealed in raw AFM channels.
This framework establishes a more robust interpretation of multifrequency AFM data, enabling automated material classification and adaptive force modelling. It bridges the gap between high-resolution imaging and quantitative nanomechanical analysis, paving the way for more intelligent and autonomous AFM-based characterization techniques.



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A microscopic view on the interaction of the SARS-CoV2 Spike protein with surfaces with different hydrophobicity

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Surfaces play a key role in the transmission of respiratory viruses, that are carried through airborne microdroplets. Here, we explore with all-atom molecular dynamics (MD) simulations the interaction of the Spike (S1) protein for three different variants of the SARS-CoV-2 (Wild Type (WT), Delta and Omicron) with model surfaces of different hydrophobicity. We follow a multiscale approach, focusing first on the behavior of a single receptor-binding domain (RBD) in an open conformation [1] and, subsequently, scaling up to consider the whole S1 protein with different combinations of open and closed conformations for the three RBDs [2]. For the open RBD, our results show marked differences with respect to its interaction with the angiotensin-converting enzyme 2 (ACE2) receptor in the cell membrane. In particular, the protein's receptor binding motif (RBM) deforms significantly more (2-fold increase) upon adsorption on the inanimate surfaces without losing its secondary structure and exposes different amino-acids to form the contact. Hydrophobic surfaces favor, in general, a stronger interaction. Differences among the three variants are particularly important in the case of hydrophilic surfaces, where WT shows a stable distribution of H-bonds along the whole RBM contact region, while intermittent contact –with H-bonds concentrated on one of the RBM ends-- is found for the case of Delta and Omicron. This counterintuitive behavior, given the character of the mutations (increasing the presence of charged residues) in Omicron, can be traced back to the dominant role of the local protein flexibility in the adsorption process. Simulations for the whole S1 protein are consistent with this scenario while showing the interplay of open and closed configurations and the important role of glycans in the contact formation and evolution. These results can be relevant for the design of virucidal surfaces and help with the microscopic interpretation of AFM experiments characterizing the interaction of S1 with ACE2 [3].

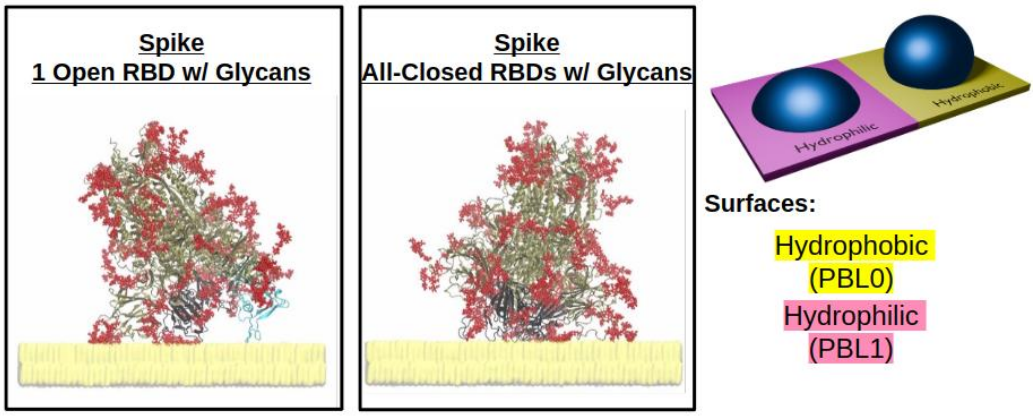

Figure 1: Snapshots from MD simulations of two configurations of the Spike S1 protein (open, closed, closed and three closed RBD conformations adsorbed onto a hydrophobic surface.

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ORAL PRESENTATIONS

3RD SYMPOSIUM ON SOLID-LIQUID INTERFACES

An *in situ* look at interfacial structure and dynamics during nucleation and self-assembly

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Interfaces play a critical role in nucleation and growth from solutions by altering the distribution of water and ions from that of the bulk, introducing an interfacial free energy that largely determines the free energy barrier to nucleation, and creating an entropic repulsion that acts as a volume exclusion force to drive colloidal assembly. The origin and characteristic length scales of these phenomena are inherently atomic-to-molecular but are manifest in ensemble dynamics and outcomes. Moreover, processes like nucleation and self-assembly arise from fluctuations, making the events that must be probed transient in nature. Consequently, in situ imaging techniques that can capture structure and its evolution, particularly at high speed and atomic-to-nm resolution, are required to build a quantitative picture of such processes. Here I use examples from high-speed, high resolution, and 3D AFM studies of interfacial structure, nucleation and biomolecular self-assembly to elucidate the mechanisms by which interfaces direct these processes, leading to unique pathways, materials, and morphologies. The results demonstrate the unique role of AFM methods in determining how ordered materials emerge at surfaces in solution by revealing the importance of surface charge, chemical gradients, and solvent organization near interfaces.

Rethinking Surface Wettability: The Counterintuitive Role of Water in Hydrophobicity Evolution of Graphitic Surfaces

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Surface wettability has traditionally been understood within a strict hydrophilic-hydrophobic dichotomy. However, recent findings challenge this binary perspective, particularly for graphitic surfaces, where water plays an unexpectedly complex role. Our study integrates Atomic Force Microscopy (AFM) force spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Density Functional Theory (DFT) simulations to investigate the temporal evolution of wettability on graphene and graphite. We demonstrate that, counterintuitively, the presence of water on these surfaces leads to a transition from initial hydrophilicity to long-term hydrophobicity. This transformation is driven by the organization of interfacial water layers, which form structured, ice-like networks near the surface, altering adhesion forces and passivating the material.
Findings from this work provide direct evidence that interfacial water, rather than airborne hydrocarbons, is the dominant factor influencing wettability shifts. The adsorption and structural rearrangement of water molecules impact the solid-liquid interaction energy, challenging the prevailing assumption that water alone cannot induce hydrophobic behavior. Additionally, our results underscore the need to move beyond static contact angle measurements, which fail to capture the dynamic and nanoscale complexities of surface interactions. Instead, we propose a paradigm where the wettability of graphitic surfaces is governed by the interplay between crystallographic surface structure and the phase behavior of nanoconfined water.

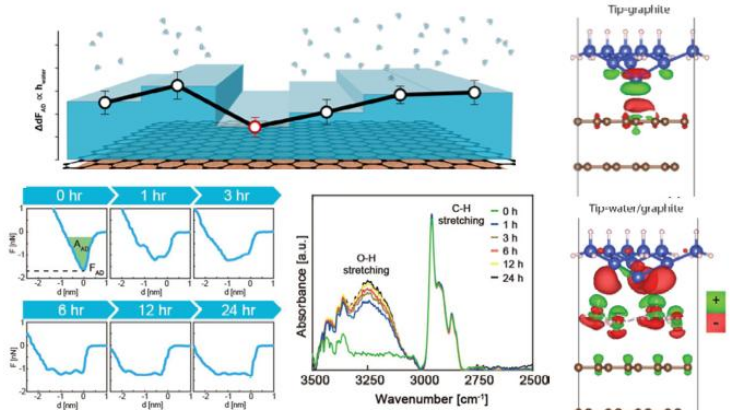

Figure 6 Graphical Abstract adapted from [1,2,3]

By rejecting the oversimplified hydrophilic-hydrophobic dichotomy, this work lays the foundation for a more nuanced understanding of surface wettability, with broad implications for nanofluidics, energy storage, anti-fouling coatings, and biomedical applications.

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Ion adsorption at charged interfaces: visualization and quantification of ion-specific effects and water structure

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Resolution on the hydration structure of solid/liquid interfaces has lately gained significant interest [1] due to the emerging electrochemistry-based technologies for green energy production and storage, whose working efficiency require clear understanding of the related processes at the atomistic scale.
At electrified (solid-liquid) interfaces, charged or dipolar molecules (e.g., ions, water molecules) can re-organize at the surface to screen its charge, but the screening mechanism can vary significantly depending on the type of ions supporting the electrolyte solutions and their concentrations. [2, 3] Such ion-specificity has often been directly attributed to hydration structure of ions in solution, which typically reproduced direct or reversed Hofmeister-like trends [4, 5]. Despite this rationalization, the driving forces leading to ion-specific behaviour at interfaces are not yet well understood, albeit important to determine the thermodynamic and kinetic efficiency of catalytic reactions [6].
In the present contribution, we will show how the energetics of a solid-liquid interface can be visualized experimentally with high-resolution AFM. This technique allows to visualize the populations of adsorbing of ions at charged surfaces like the one of muscovite mica [7]. Upon quantification of the surface coverage at equilibrium, different adsorption mechanisms could be more straightforwardly visualized for weakly and more strongly hydrated ions, respectively. This methodology highlights the possibility to outline a certain competitive behaviour of charged species at the surface. Understanding such competition as a function of type and concentration of ions allows us to unravel the interfacial thermodynamics directly from AFM data, which has been so far mainly exclusive to MD simulations.
The interfacial hydration structure was further elucidated with the aid of molecular dynamics (MD) simulations and surface-sensitive vibrational spectroscopies (SFG).

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Unraveling the Role of Step-Edge Confinement in Ion Adsorption and Molecular Ordering on Cu(111) in Acidic Media

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Copper is a crucial material for numerous technological applications, particularly in devices-production and -operation in liquid media, e.g. aqueous solutions. The adsorption mechanisms of ions on copper surfaces have been widely debated, yet only recently has research started to explore the role of surface defects in molecular self-assembly.
In this study, we chosen oriented step-ensembles on vicinal Cu(111) surfaces to investigate how physical confinement affects electrochemically induced molecular ordering. When Cu(111) is immersed in dilute H_2SO_4 and subjected to an appropriate potential, typical ordered molecular superstructures, e.g. of porphyrin molecules, form. However, the limited terrace width at vicinal Cu(111) surfaces introduces a confinement effect that could challenge this ordering process of the molecules. Our findings, however, reveal that, despite the physical constraints imposed by step edges, the overall electrochemical process still proceeds but simultaneously induces a restructuring of the substrate terraces such that the typical molecular ordering is again possible. Nevertheless, the initial terrace confinement plays a key role in controlling the orientation of the molecular superstructure and influencing the diffusion of copper atoms in the restructuring process.
These results provide insight in the relative energetics of ordered adsorption of the molecules vs. substrate rearrangement under electrochemical condition.

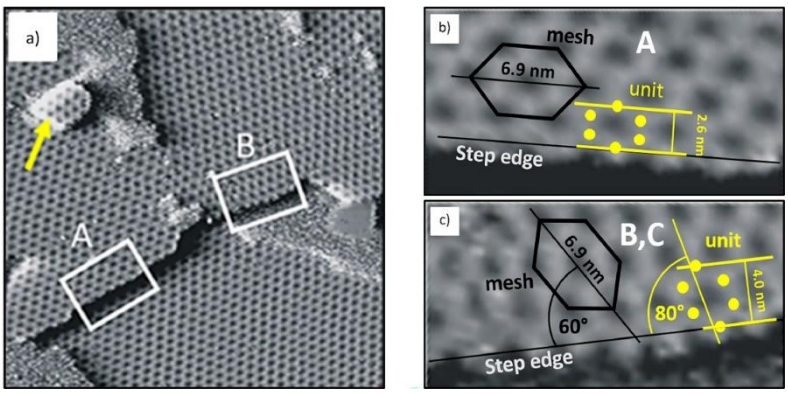

Figure: a) Note the growth of the Moiré covered island marked by the yellow arrow (101 nm × 101 nm, U_b = 169 mV, I_t = 1 nA). The white rectangles encompass portions of a step edge stabilized with Moiré-meshes of different orientation A or B, shown enlarged in panel b) and c), respectively. [1]

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Modeling aqueous oxide interfaces and their HD-SFG non-linear spectroscopy

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The detailed comprehension of the structure, dynamics and chemical reactivity at aqueous interfaces, at the molecular level, requires a synergy between experiments and molecular simulations. Non-linear optical SFG (Sum Frequency Generation) spectroscopies are one of the very few methods that can probe buried interfaces. The vast majority of HD-SFG experiments probe the optical response of the water in the thin layer of the interface,[1] while a recent new experiment has emerged to probe the optical response of the surface.[2] These signals however require an associated modeling at the molecular level in order to provide a definitive view on the structure of the top surface of a solid and of the water in direct interaction.[1-3]

In this presentation, I will review some of our theoretical works dedicated to the microscopic elucidation of aqueous interfacial oxides by combining DFT-based molecular dynamics simulations and theoretical HD-SFG spectroscopy.

I will in particular review some of our recent developments which are aimed to simplify the calculation of HD-SFG signals of aqueous interfaces, called the 'pop-model',[4] which advantages over current theoretical SFG calculations[3] will be highlighted. The 'pop-model' also opens the door to the inverse design of materials by spectroscopy, using machine learning techniques. I will also focus on recent theoretical works where we show that the organization of water in the interfacial layer (BIL, Binding Interfacial Layer) can be categorized into vertical and horizontal orders, with well-defined HD-SFG signatures of structural motifs that can be recognized through algorithmic graph theory.[5] These orders have enabled us to develop a molecular descriptor of hydrophobicity and hydrophilicity in the BIL.[6-7]

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Atomistic modeling of forces on AFM probes at liquid–solid interfaces

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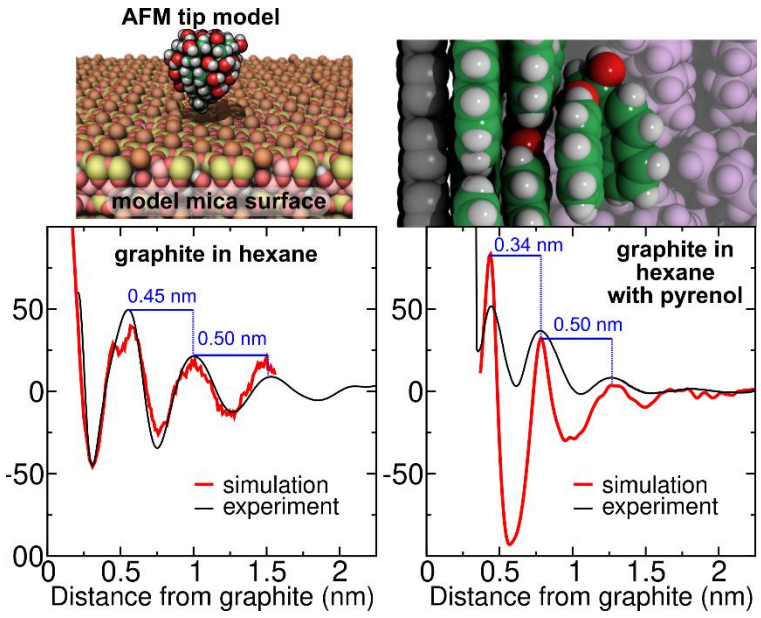
Molecular dynamics simulation has proven its utility in helping to interpret atomic force microscopy (AFM) data. However, the short time scale of these simulations (typically <100 μs), the limited transferability and interoperability of interatomic potential functions, and the lack of realistic structural models of material interfaces and AFM probes remain major challenges. The latter is the likely the most difficult challenge and will require advances in both computational and experimental methods. In this talk, I will describe simulation results in my group to address these challenges. Using enhanced sampling methods to overcome some time-scale limitations, we calculated the expected force on model AFM tip asperities and directly compared average force profiles to AFM measurements, obtaining quantitative agreement for systems such as mica in water and graphite in hexane. Likewise, we predicted how adding an aromatic compound (1-hydroxypyrene) to an alkane solution (hexane) changes the force profile at the graphite–solution interface, which was later confirmed by AFM experiments. The emergence of machine learning potentials trained on quantum chemistry data promises to overcome the limitations of existing interatomic potentials, expanding the range of materials that can be simulated. The structure of material interfaces (both substrates and AFM tips) depends on the details of their synthesis, as well as their history and the presence of contaminants. While purely computational approaches alone are unlikely to fully resolve the problem of characterizing these interfaces at the atomic level, simulations with machine learning potentials (or other reactive potentials), together with theory, can approximately model some synthesis processes and effects of the ambient environment to create complex interface models, including such aspects as native oxides and contaminant layers. Finally, I will discuss recent results showing how simulation predictions vary among different structural models of the AFM tip asperity and interatomic potentials.



Atomic-Scale Insights into Bias-Induced Local Anodic Oxidation on Silicon via Reactive Molecular Dynamics

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Precise control of bias-induced oxidation is crucial for fabricating next-generation nanoelectronic devices using local anodic oxidation (LAO) nanolithography. However, the atomic-scale physical–chemical interactions at the solid-liquid interface governing this process remain incompletely understood. Here, we use reactive force field molecular dynamics simulations to elucidate the reaction mechanisms and assess the effects of electric field strength, humidity, surface passivation, and crystallographic orientation on bias-induced oxidation. Our results reveal that oxidation primarily leads to the formation of Si–O–Si bonds while consuming H_2O, with –OH determining reaction rate. Parametric studies further reveal that both electric field strength and humidity significantly influence the oxidation process, each through distinct mechanisms. A comparison of silicon (100), (110), and (111) surfaces demonstrates distinct anisotropic oxidation behaviors, with the (111) surface exhibiting a high initial oxidation rate, while the (110) surface experiences enhanced oxidation under stronger electric fields. In addition, surface passivation by hydrogen or oxide layers significantly influences the nucleation and growth of oxidation sites, further affecting overall oxide formation. Our findings align well with theoretical and experimental studies, offering deeper insights into oxide growth mechanisms and providing valuable guidance for the fabrication of high-performance nanoinsulator gates using LAO nanolithography.

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Integrating Microscopy and Spectroscopy to Unveil Liquid Structures Near Solid Surfaces

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The structure of liquids near solid surfaces is crucial for many chemical, electrochemical, and biological systems. However, our ability to experimentally probe interfacial liquids has been significantly limited. Microscopy methods, mainly 3D atomic force microscopy (3D-AFM), enable angstrom-scale spatial resolution, but lack chemical sensitivity. Spectroscopy approaches, such as Raman scattering, infrared absorption, and X-ray absorption, can be sensitive to the interfacial chemical states, but lack spatial resolution. To overcome these technical limits, my lab has devoted efforts to integrating microscopy and spectroscopy methods to unravel the intricate structures of interfacial liquids. In this talk, I will discuss two approaches. One is to combine 3D-AFM with nanoparticle-enhanced Raman spectroscopy. This method has enabled us to thoroughly determine the structure of water–graphite interfaces under different gas environments and interfacial electric fields. Another approach is a new type of spectromicroscopy method that we have developed. Using a fabricated fiber probe, we deliver mid-infrared light to local spots at solid–liquid interfaces, and obtain spatially-resolved mid-infrared absorption spectra. I will discuss the applications of this spectromicroscopy technique in electrochemical and biological systems.

Charge-Modulated Hydration Structures at Solid–Liquid Interfaces: From Contact Electrification to Electrochemical Analogs

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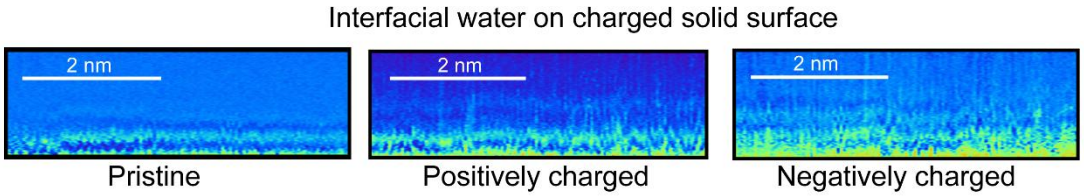
The widespread presence of aqueous solutions at solid surfaces and the growing understanding that water–surface interactions govern many interfacial properties have driven extensive research. Traditionally, water has been viewed as an inert solvent. However, recent studies show that water can directly participate in chemical reactions, challenging this long-standing assumption. In particular, interfacial water plays a central role in mediating electric fields, solvation effects, and chemical reactivity. For instance, contact electrification at solid–liquid interfaces can spontaneously generate reactive species such as hydrogen peroxide. The charge carried by microdroplets can create electric fields reaching several kilovolts per meter at microscopic scales—strong enough to drive electrochemical reactions. These discoveries highlight the need to better understand how surface charge alters interfacial water structure at the nanoscale. Despite significant efforts, key questions remain about how water molecules organize and orient near charged surfaces, and how their spatial structure varies across different electrostatic environments.

To explore how surface charge affects the structure of interfacial water, we employed three-dimensional atomic force microscopy (3D-AFM) to visualize hydration layers on solid surfaces with different charge states. Surface charge was modulated by rubbing conductive probes against various materials, and quantified using Kelvin probe force microscopy (KPFM). To minimize ion exchange across the interface and isolate screen effects, we introduced a monolayer of graphene between the solid and the liquid interface. We found that the hydration layers respond to surface charge in a layer-by-layer fashion, with changes in both interlayer spacing and molecular orientation. To simulate electrochemical conditions, we applied an external bias to graphite electrode in solution and tracked real-time changes in hydration structure. Notably, hydration layer oscillations induced by contact electrification resemble those observed in voltage-controlled electrochemical systems, suggesting a common underlying mechanism. These findings demonstrate that surface charge can dynamically modulate interfacial water structure through electrostatic interactions, coupled with intrinsic surface properties and solvent effects.



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Quantification of solvation forces with amplitude modulation AFM

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At a solid-liquid interface (SLI), liquid molecules form quasi-discrete layers extending several molecular diameters from the solid surface. This structuring arises from attractive solid-liquid interactions and the confining effect of the rigid surface [1]. Such layered organisation is crucial in adhesion, wetting/dewetting, slip, and friction. It also plays a key role in technological applications, including two-dimensional (2D) material-based energy devices, and biophysical processes like protein folding and molecular interactions.

Various experimental techniques, such as X-ray reflectivity, the Surface Force Apparatus, and Atomic Force Microscopy (AFM), particularly 3D AFM [2], have been used to probe these structures. A major advantage of AFM is its ability to provide both vertical and lateral resolution at the SLI with atomic scale. However, accurately converting AFM data into tip-sample forces remains challenging, as standard force reconstruction methods (FRMs) often yield inaccuracies due to their dependence on initial AFM parameters (e.g., free oscillation amplitude), the solvation force characteristic length, and the force functional form.

To overcome these limitations, we introduce a novel matrix-based FRM specifically designed for AM-AFM [3]. Unlike conventional approaches, our method reconstructs the full SLI interaction without loss of information due to experimental parameter selection or force type assumptions. We demonstrate its suitability with numerical simulations. We then show a direct application to 3D AFM experimental data at SLIs and soft biomolecular surfaces [4].

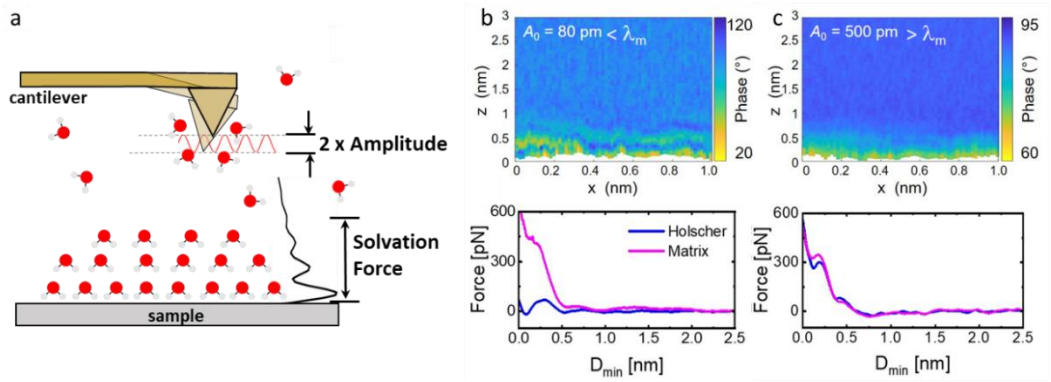

Figure: (a) Schematic of a cantilever oscillating at an SLI with water molecules on the tip and surface. The solvation force (black) shows hydration layers, and oscillation amplitude (A_0) is in red. (b, c) 3D AFM data for the SiOx/water interface: for A_0 larger than the decay length, λ_m, (c), both FRMs agree, otherwise the Holscher algorithm fails (b).

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Molecular-scale resolution mapping of electrified MoS_2-water interfaces by operando 3D-AFM

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Understanding the interfacial water structure on electrified surfaces, in particular few-layer transition metal dichalcogenides MoS_2, is relevant for advancing applications in biosensing, wearable electronics and catalysis. In this work, we implemented an operando 3D-AFM technique to probe the interfacial water structure on electrified surfaces with molecular-scale resolution. This method enabled to study the interfacial water structure of MoS_2 crystals under varying surface potential and electrochemical current density conditions. On pristine surfaces we observed the existence of two-to-four hydration layers. The interlayer distance (~0.3 nm) was independent of the surface potential (-0.6 to 1.9 VSHE) and the current density. More hydration layers were observed at high positive potentials. Operando 3D-AFM compatibilized simultaneous current density and interfacial water structure measurements.

Solvation Layer Mapping at “Hydrophobic” Surfaces

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Solid-liquid interfaces are omnipresent both in Nature and technology. Thus, understanding pivotal processes at interfaces almost always require a detailed knowledge of the interfacial structure. Still, the nature of the interface often remains elusive; and most relevant questions such as chemical composition, local structure or ion distribution are often difficult to answer.
Here, we study two prototypical systems, namely the graphite-water and the gold-water interface, using 3D AFM. We address the origin of the hydration structures on graphite [1] and report the formation of ubiquitous stripes both at the graphite-water [2] and gold-water [3] interface.

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Nanometer-Resolved Operando Photo-Response at the Semiconductor-Electrolyte Interface of Faceted Nanoparticles

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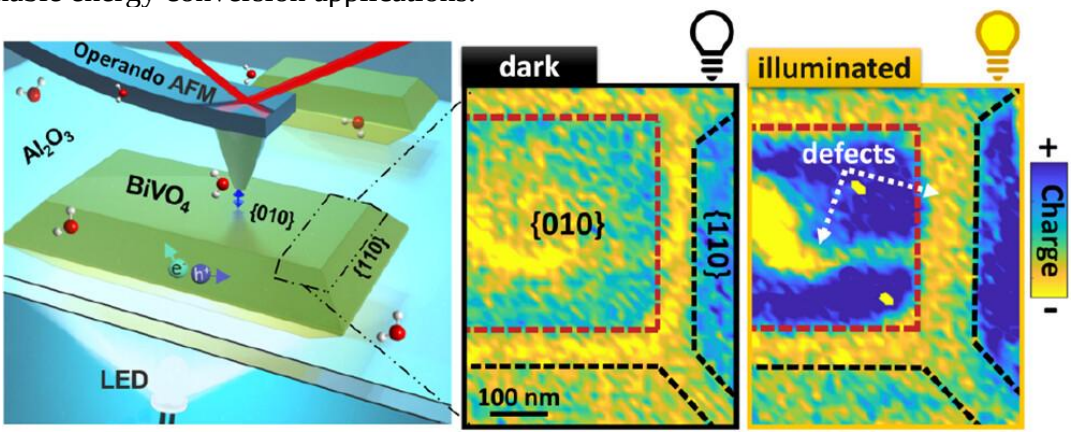
Understanding the nanoscale behavior of the semiconductor-electrolyte interface is essential for improving the efficiency of photocatalysts in energy conversion applications, such as hydrogen evolution, oxygen evolution, and CO_2 reduction reactions. This talk explores the facet-dependent photoresponse and charge dynamics of $BiVO_4$ nanoparticles (NPs) under operando conditions using advanced Atomic Force Microscopy techniques.

Our findings reveal significant differences in surface charge densities between {010} and {110} facets, with distinct pH-dependent behavior and positive charge accumulation under illumination. The measured surface charge is substantially stronger than previously reported for the surface photovoltage of $BiVO_4$ NPs in air. This highlights the critical role of the semiconductor interaction with ambient electrolytes of varying composition, demonstrating how tuning these interactions can optimize charge transfer across the solid–liquid interface for photocatalytic reactions.

High-resolution imaging further reveals a diverse range of surface defects, from atomic-scale vacancies and single-unit-cell steps to microfacets with varying orientations and degrees of disorder. These defects typically carry highly localized negative surface charge densities and exhibit an opposite photoresponse compared to adjacent facets.

By resolving these nanoscale interactions, we provide new insights into charge transport and recombination processes at the semiconductor-electrolyte interface. This understanding is key to optimizing $BiVO_4$-based photoelectrodes and enhancing their photocatalytic performance for sustainable energy conversion applications.



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High-Throughput Multifunctional Scanned Probe Microscopy: A New Era in Electrochemical Materials Discovery

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Electrochemistry is undergoing a renaissance, due to increasing recognition of its important role across science, from charge transport and electrified interfaces in biological systems to technological applications such as batteries, fuel cells, and electrolyzers. Electrochemistry also plays a crucial role in diagnostic and sensor technologies. Historically, visualizing processes at electrochemical interfaces has been a key scientific goal, and today, this objective is more relevant than ever. In this lecture, I will discuss the development and application of scanning electrochemical cell microscopy (SECCM), a technique we pioneered that uses a pipette meniscus to achieve high-resolution electrochemical-topography mapping at the nanoscale. SECCM, combined with complementary co-located microscopy, allows us to investigate and understand the intricate relationships between surface structure and electrochemical activity, providing deep insights into the mechanisms driving various electrochemical processes.

This approach enables the detailed study of complex interfaces by examining individual features such as steps, terraces, defects, wrinkles, crystal facets, grain boundaries, and single particles. I will showcase how SECCM and complementary co-located microscopy are used to tackle significant issues in electrochemical and interfacial science, including energy storage materials, electrocatalysis, corrosion, and synthetic membranes (2D materials).

Furthermore, SECCM supports high-throughput combinatorial experiments, with the ability to vary parameters across different spots. This capability is further enhanced by in-situ optical microscopy, which provides real-time visualization of the SECCM meniscus.

Looking forward, integrating SECCM with in-situ spectroscopy and machine learning will further accelerate the discovery and development of next-generation electrochemical materials.

Probing single metal ions at aqueous interfaces: imaging vs spectroscopy

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Atomic Force Microscopy (AFM) is a powerful tool to investigate solid-liquid interfaces *in-situ*, locally and with sub-nanometer precision. Details about the equilibrium molecular arrangement of the liquid at specific atomic locations of the solid are now routinely reported, also confirmed by computer simulations [1-4]. However, measurements often become more challenging when solutes or ions are present into the liquid and at the interface. For example, diffraction data [5] indicates clear adsorption sites and hydration configurations for metal ions at a range of solid-water interfaces, but AFM data remains limited. High-resolution images of adsorbed ions are sparse [6-8] and force spectroscopy data tends to show an interface exclusively dominated by the hydration landscape of the solid with the effect of ions either appearing indirectly or missing altogether [9-10]. Varying the ionic concentration does not significantly alter this picture. This apparent disconnect between AFM and other interface-sensitive techniques is problematic given ubiquity of aqueous interfaces in science and technology.

To explore whether this issue could be down to the imaging parameters usually used for high-resolution AFM, we systematically explore the impact of the operating amplitude and setpoint when acquiring high-resolution amplitude modulation images of mica in solutions of LiCl, NaCl, KCl, RbCl and $CaCl_2$, always at the same ionic strength. We compare the structures observed in each case with spectroscopy curves acquired immediately after. The results suggest that the ability to visualize adsorbed ions depends on both the type of ion investigated and the tip oscillation amplitude. For ions predominantly adsorbed in outer sphere coordination (Rb^+, K^+), relatively large imaging amplitudes (>0.70 nm) are needed, often giving rise to deviations from the traditional hydration layers oscillations [9-10] in the force spectroscopy curves. In contrast, ions able to adsorb in both inner and outer sphere coordination (Ca^{2+}, Li+, Na+) require smaller imaging amplitudes (<0.3 nm) and harsher setpoints to achieve consistent high-resolution images, presumably of strongly adsorbed inner sphere ions. Larger amplitudes are still possible, but interpretation of the contrast is less straightforward. Spectroscopy curves tend support this interpretation, with a longer-range monotonic component that dominates over hydration oscillations at larger amplitudes. The results go some way towards bridging the apparent gap between AFM and complementary techniques to explore ions at interfaces.

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Nanoscale interfacial energy mapping with electrochemical AFM

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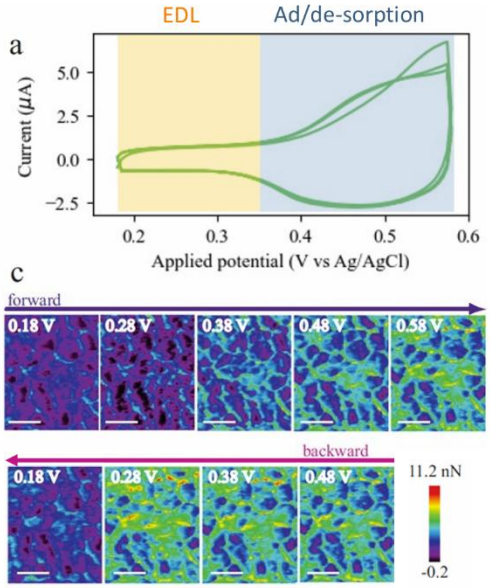
One of the challenges in achieving the energy transition is the conversion and storage of sustainable energy. Electrolysers can solve this issue as they convert green electricity to energy rich compounds such as hydrogen, hydrocarbons or alcohols produced from water and CO2. To make electrolyzers a scalable deployable technology we must find earth-abundant materials that are efficient and stable electro-catalysts, as only precious metals fulfill these requirements so far. But why/how are these good?

In recent years, more and more evidence has emerged on the intertwining of electrode inhomogeneities, local electrolyte structure and ion distribution, which all affect the nanoscopic electrochemical processes. All these are difficult to infer from macroscopic measurables. In-situ and operando Atomic force microscopy (AFM) is a powerful tool that has demonstrated with atomic resolution, that water structures itself at the interface with solids under equilibrium.

In this work, we use adhesion to probe the interfacial energy of the (biased) electrode/electrolyte under different electrochemical conditions. We track changes at the interface as a function of bias, and investigate the effect of different anions and cations. From adhesion imaging, we reveal that the water structure on even a simple Au film is highly inhomogeneous and dynamic. By disabling the vertical scan, we push the temporal resolution and track adhesion while running standard cyclic voltammograms.



Understanding and controlling these nanoscale phenomena provide us with many fascinating challenges, of direct relevance not only in energy storage but also other fields like nanomaterial fabrication.

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Nanoscale insights into electrocatalyst surfaces and electrochemical interfaces

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In key electrocatalytic reactions such as $CO_{(2)}$ electroreduction ($CO_{(2)}$RR) and oxygen evolution (OER), product selectivity and activity are strongly influenced by the nanoscale structure of electrode surfaces. Additionally, proton/electron transfer and reaction kinetics are highly sensitive to interfacial hydration structure, electrolyte/ionic composition, and surface-adsorbed species. Disentangling these structural and chemical factors, along with their dependence on applied potential, remains a fundamental challenge in electrocatalysis. In this talk, I will present our latest insights into electrode reconstruction during $CO_{(2)}$RR and OER, as well as reaction intermediates and interfacial hydration effects during CO_2RR. These findings are enabled by techniques including electrochemical atomic force microscopy (EC-AFM) and surface-enhanced infrared and Raman spectroscopy (SEIRAS, SHINERS). I will also discuss strategies for disentangling a catalyst's ensemble *vs.* local properties through nanoscale sensing of chemical and electrical properties.

Mechanical Insights on the Evolution of Functional Layers in Energy Materials

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In-Situ and *In-operando* Atomic Force Microscopy (AFM) are powerful tools to investigate functional layers in batteries and materials for energy conversion, such as electrolyzers. Especially, the temporal evolution of mechanical properties provides important insights into variations of the materials compositions or the dynamic behavior of the electrolyte.
In the case of batteries, the formation and growth of the solid electrolyte interphase (SEI) is important to understand to enhance the device performance and to avoid dendrite formation. The SEI has been studied on silicon [1] and metallic Lithium anode materials for Lithium-ion batteries in conventional electrolytes [2] and ionic liquids [3]. Various degrees of heterogeneity are found depending on the exact system under investigation. The valuable mechanical information obtained in addition to the morphology is critically discussed with respect to their reproducibility, as well as influences of the substrate and electrolyte.
Importantly, it will be demonstrated that the tip could interfere significantly with the material in contact, even under careful experimental conditions where this is less expected. Strategies to avoid tip artefacts in energy materials research by AFM are presented.
The ability to follow the dynamic behavior of the unique interfacial structures of ionic liquids as a function of applied electrode potentials has been investigated by friction force microscopy [4]. It is clearly found that the direction of switching the applied potential has a tremendous influence on the relaxation time of the interfacial structure. Implications of this behavior on applications of ionic liquids are discussed.

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ORAL PRESENTATIONS

4TH SYMPOSIUM ON CELL AND SOFT MATTER NANOMECHANICS

The missing mechanical link: how composite interfaces govern cell morphogenesis, immune migration, and stem cell fate

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Mechanics lay at the heart of much of physiology and pathology. My laboratory at the EMBL in Heidelberg studies cellular force transduction and mechanical signaling in animal cells and tissues by combining imaging and biophysical approaches with protein engineering. Our material science perspective has allowed us to obtain fundamental insights to enhance immunotherapy, gate stem cell differentiation and improve cancer treatment. In this talk I will show how animal cells exploit the remarkable biophysics that emerge through the coupling of polymer physics and membrane mechanics to regulate cell morphogenesis, immune migration, and stem cell fate, and show how our mechanical perspective can influence the clinic.

How do cells react to the viscoelasticity of the extracellular matrix?

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The mechanics of the extracellular matrix (ECM) determine cell activity and fate through mechanoresponsive proteins including YAP. Rigidity and viscous relaxation have emerged as the main mechanical properties of the ECM steering cell behavior. However, how cells integrate coexisting ECM rigidity and viscosity cues has remained poorly understood, particularly in the high-stiffness regime. In my talk, I will present our work using engineered stiff viscoelastic protein hydrogels showing that, contrary to previous models of cell-ECM interaction, substrate viscous energy dissipation attenuates mechanosensing even when cells are exposed to higher effective rigidity. This unexpected behavior is readily captured by a pull-and-hold model of molecular-clutch-based cell mechanosensing, which also recapitulates opposite cellular response at low rigidities. Consistent with predictions of the pull-and-hold model, we find that myosin inhibition can boost mechanosensing on cells cultured on dissipative matrices.

Aberrant glycosylation regulates the cell surface architecture and viscoelasticity of pancreatic cancer cells

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The cellular glycocalyx plays a crucial role in making pancreatic cancer one of the deadliest malignancies globally. It complicates early detection and reduces the effectiveness of conventional therapies. In pancreatic cancer, the components of the glycocalyx are often upregulated or abnormally glycosylated, promoting tumor progression through immune evasion, enhanced metastasis, and drug resistance. While these biochemical effects are known, the biophysical impact of the glycocalyx on cancer cells is less understood. In our study, we explored the structural and biomechanical effects of modifying the glycocalyx architecture in pancreatic cancer cells using various chemical compounds. We employed a recently developed Atomic Force Microscopy nanomechanical mapping method to visualize cellular mechanical heterogeneities in a high spatiotemporal context. This new method leverages recent advancements in viscoelastic analysis via discrete modified Fourier transform (Z-transform). Our new approach allows for the viscoelastic inversion of high-resolution spatiotemporal data at rates which are orders of magnitude faster (more than 37,386-fold) than optimizing a traditional rheological model for each pixel. In addition, the method utilizes model-free viscoelastic quantities, such as material retardance and relaxance. Our nanoscale multi-timescale viscoelastic measurements revealed that 2D adherent human pancreatic ductal adenocarcinoma cells have reduced elastic storage modulus and viscous loss modulus compared to healthy pancreatic ductal epithelial cells. Additionally, we observed a progressive reduction in both moduli with metastatic progression, a hallmark of cancer metastasis. We further investigated the architectural and biophysical effects of glycocalyx architectural modulation in pancreatic cancer cells. Perturbations of hyaluronic acid (HA), sialic acid (SA), mucins, and N-glycans through enzymatic treatments led to significant architectural remodeling of the cell surface. Interestingly, removal of SA and mucins resulted in a softer and more fluid cell surface, while removal of HA softened and increased viscosity. In addition, preliminary cytokine expression results suggest that SA removal leads to a pronounced pro-inflammatory response of human 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 SA deposition. Our findings suggest that the glycocalyx of human pancreatic ductal adenocarcinoma cells fundamentally regulate extracellular surface architecture, mechanical properties, composition, and function, thereby promoting tumor progression and metastasis by acting as a physical barrier to antitumor responses.

Mechanical mapping of soft materials and cells with colloidal AFM probes through advanced models

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Colloidal spherical probes, with radii ranging from 100's nm to 10' um, are ideal for mechanical mapping of soft samples and living cells, as they guarantee minimum sample damage, small pressure, and well-defined indentation geometry. Nevertheless, current theoretical models for data interpretation, including those available in commercial software tools, are not accurate for this probes when large indentations are considered, since they do not properly account for the bottom contact effect. Large indentations occur very often in force curve-based experiments with colloidal probes on very soft materials, like soft polymers, hydrogels, and living cells, and therefore there is the need to develop and test more advanced theoretical models than the existing ones. Here, we present a force indentation analytical model that considers accurately the bottom contact effects for the case of a spherical probe. The model is valid in the full range of indentations and sample thicknesses, as shown by numerical calculations, and has been experimentally validated with the mapping of the Young's modulus of soft hydrogels (Y = 3 kPa) and ultra-soft cells (M0, M1 and M2 macrophages) (Y < 1 kPa). We will also present a software tool integrating this (and other analytical models with bottom effect correction) with the aim to achieve large scale and automatic quantitative mechanical cell phenotyping at high throughput using colloidal probes, approaching on the fly mechanical quantitative analysis.

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Abstract Title: Rheology of epithelial monolayers

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Epithelial tissues are crucial during embryonic development and in adult organisms, forming essential physiological barriers within the body. These tissues frequently undergo and even instigate significant deformations while maintaining mechanical integrity. This presentation explores the dynamics of epithelial tissues under various strains and strain rates, emphasizing modelling strategies. Experimental investigations of in-vitro suspended epithelial monolayers of Madin-Darby Canine Kidney cells [1] reveal that these materials exhibit complex visco-elastic rheology [2,3] and active contractile behaviours [4], leading to tensioning and spontaneous curling at free edges [5]. At large deformations, the rheology becomes highly non-linear and rate dependent, a behaviour associated with the recruitment of intermediate filaments as load-bearing components. This regime is particularly relevant when exploring the initiation of tissue rupture, which results from the interaction between the material's non-linear rheology and the collective dynamics of linker populations at junctions [6]. Our findings shed light on the complementary roles of key cytoskeletal networks in maintaining mechanical integrity and barrier function of epithelial tissues.

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Biomechanical Insights into the Proteomic Profiling of Cells in Response to Red Light Absorption

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Photobiomodulation (PBM) is a promising non-invasive therapy for tissue repair, but its underlying cellular mechanisms are not fully understood. In this study, the biomechanical and proteomic responses of three cell types – keratinocytes (HACAT), fibroblasts (L929), and osteoblasts (OFCOLII) – exposed to red light (633 nm) are investigated using atomic force microscopy (AFM) and mass spectrometry-based proteomic analysis. Red light absorption resulted in cell-type-specific changes in viscoelastic properties, with fibroblasts exhibiting increased fluidity, reduced stiffness, and enhanced motility. Conversely, keratinocytes exhibited intensity-dependent responses, while osteoblasts appeared to be relatively insensitive to irradiation conditions. Proteomic profiling identified key signaling pathways involved in immune response, ATP production, and stress regulation. The immune and ATP pathways are strongly linked to the modulation of viscoelastic properties, particularly in fibroblasts, while weaker correlations were observed in keratinocytes. Cytoskeletal remodeling, primarily within the F-actin network, is identified as the main driver of mechanical alterations, with additional contributions from microtubules and intermediate filaments. These findings provide new insights into how red light absorption modulates cellular viscoelasticity through cytoskeletal remodeling, with potential applications in optimizing light-based therapies for tissue regeneration and disease treatment.

Mapping cell mechanics in health and disease

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Cells exhibit mechanical heterogeneity influenced by the cytoskeleton's composition, organization, and cellular state. This is affected during diseases like cancer, where malignant cells have been shown to be softer than normal ones, To obtain a mechanistic understanding of cell mechanics in heath and disesase, it is important to map the viscoelasticity across the cells body and correlate it with information about the cytoskeleton state. We have established an approach based on cell culture on micropatterns and averaging of atomic force microscoscopy mechanical maps, together with traction force and polarization-resolved fluorescence microscopies. We applied this approach to normal and malignant cancer cells. Our results suggest that cell softening in cancer cells correlates with the cytoskeleton order at the molecular scale.

High-Throughput Nanorheology of Living Cells Powered by Supervised Machine Learning

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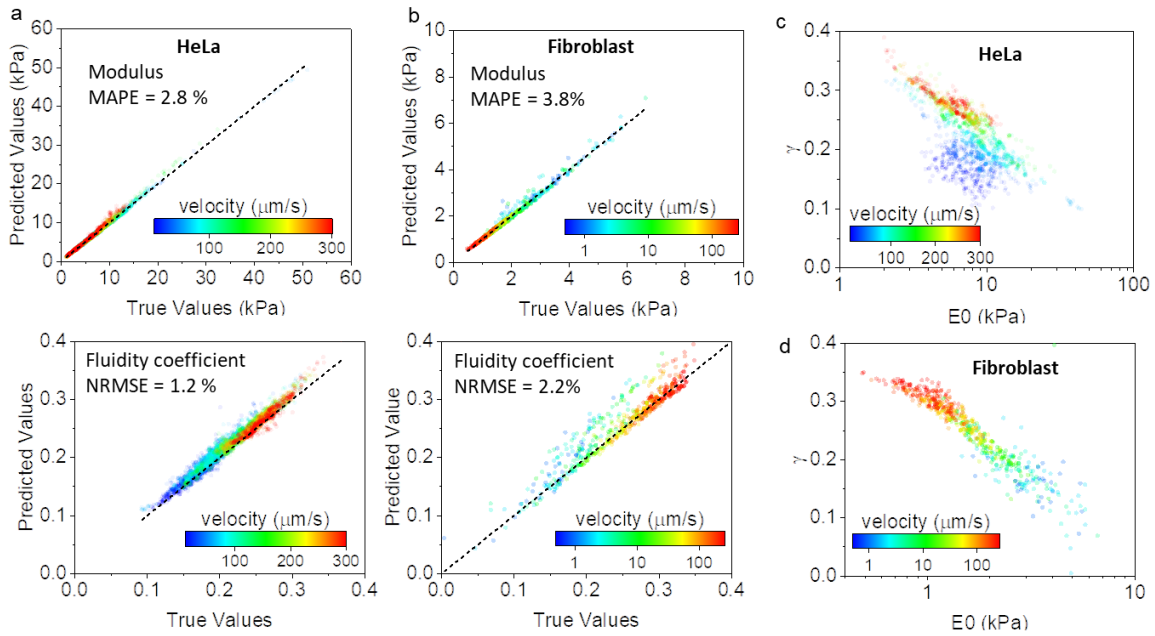
Atomic force microscopy (AFM) is extensively applied to measure the nanomechanical properties of living cells. Currently, the analysis of AFM-nanoindentation data is performed by model fitting. Model fitting is slow, data intensive and prone to error. Therefore, AFM applications on cell biology are limited by the low throughput of the technique. Here, we develop a supervised machine learning regressor for transforming AFM force-distance curves into nanorheology behaviour. The method reduces the computational time required to process a force volume of a cell made of 2.62 x 105 curves from several hours to minutes. In fact, the regressor increases the throughput by 50-fold. The training and the validation of the regressor are performed by using theoretical curves derived from a contact mechanics model that combined power-law rheology with bottom-effect corrections and functional data analysis. The regressor predicts the modulus and the fluidity coefficient of mammalian cells with a relative error below 4%.



Shaping Cells with Patterns and Protein Crystals

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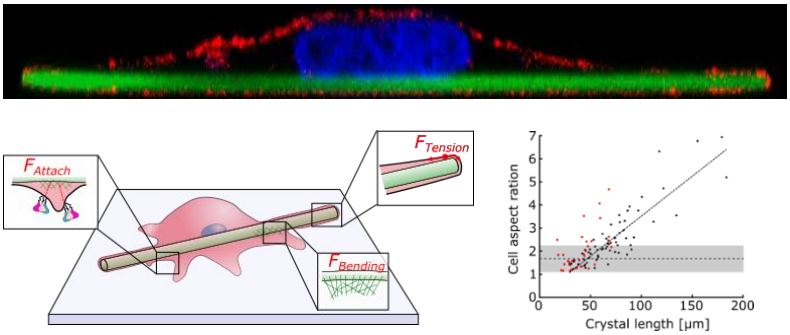
Mechanobiology explores how mechanical forces shape biological systems, from molecules to tissues. This talk highlights our recent work integrating extracellular protein micropatterning with intracellular force generation to control cell mechanics. By varying the geometry of protein micropatterns, we regulated single-cell shape, spreading, and migration. Specifically, the aspect ratio of patterns influenced transitions from confinement to elongation and affected cell division dynamics. We also employed in cellulo-grown protein crystals as internal sources of tensile force. These crystals interact with adhesion forces to drive cell elongation, rotation, and fusion, while also influencing cytoskeletal organization and crystal growth. To further quantify these interactions, we apply Atomic Force Microscopy approach provides new insights into how cells integrate mechanical cues from both internal and external environments.



Dissecting Cytoplasmic Heterogeneity: A Quantitative Assay for Measuring Organelle-Specific Contributions to Cellular Mechanics

Michael Krieg, Frederic Catala-Castro, Santiago Ortiz-Vasquez, Verena Ruprecht, Paolo Frigeri.

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The ability to sense and respond to mechanical information is pivotal for tissue organization, organismal development and neuronal function. Research over the last decades has informed us that most if not all cells are mechanosensitive, and that this mechanosensitivity arises both, from the cell surface and their intracellular organelles. A key finding is that the cytoplasm is extremely heterogenous and that different organelles contribute to the cellular mechanoresponse. Even though it is well understood how the mechanical properties of the plasma membrane and the actin cortex endow the cell with the ability to measure forces, measuring how the nucleus, mitochondria and the ER respond to forces is still a formidable challenge [1].
The reason for this is the difficulty to simultaneously apply and measure mechanical stresses at a region of interest, with high spatial and temporal resolution directly inside the cell, without the confounding perturbation of other structures. While techniques such as atomic force microscopy (AFM) and micropipette aspiration provide valuable mechanical insights, they primarily probe the cell surface and lack direct access to intracellular mechanics, Brillouin microscopy and FRET-tension sensors provide excellent readout of the stresses, even on the level of a specific molecule, but cannot be used to manipulate cellular structures. In this talk, I will focus on our efforts to develop a versatile optical tweezer technique to characterize the intracellular rheology of various biosystems. This technique uses a single, time-shared laser beam to measure both, stress and strain, from which we obtain the viscoelastic response function of the intracellular compartment in real time [2]. To demonstrate this TimSOM (Time-Shared Optical tweezer Microrheology) technique, I will showcase results how the viscoelasticity of the cytoplasm, nuclear interface, and nucleoplasm of zebrafish cells change in various conditions.
To further understand how the nucleus is involved in mechanosensitive processes, I will show unpublished results from a dual-trap assay to determine the mechanics of nuclear positioning, together with creep compliance measurements and nuclear calcium imaging to understand the mechanosensitive response of the nucleus to large deformations. Using this toolbox, we observe that the actin cytoskeleton together with KASH domains in the LINC complex work together to contain and dampen external stresses thus controlling nuclear positioning and regulating mechanosensation pathways.
This work highlights the importance of understanding the mechanical intricacies at the cellular level, shedding light on how specific structures can contribute to cellular behaviour within the organism. Furthermore, this research provides a novel perspective on the importance of viscoelastic properties in understanding the functionality of cells within complex biological systems.

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Intracellular mechanotransmission combining FluidFM and FLIM

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Studying the interactions between biochemical and mechanical cues in the cellular behaviors necessitates creating tools enabling in situ monitoring and quantifying such small forces in single-cell levels while preserving cell viability and avoiding influences on the ongoing cellular events. Mechanosensory complexes at the cellular level are such a demanding situation in which the dynamic interconnectivity of the cytoskeleton, nuclear envelope, and nucleoskeleton is yet to be understood.

FluidFM combines a hollow microchannel cantilever with conventional AFM and has proven to be a promising tool in biological applications, especially in cell mechanics. [1] On the other side, fluorescence lifetime imaging microscopy (FLIM, [2, 3]) is advantageous for probing molecular environments using fluorophores reporting membrane tension changes through their fluorescence lifetime changes. FluidFM enables to manipulate intact cells with simultaneous screening of molecular responses. FluidFM micro-manipulation of single cells includes but not limited to quantitative injection of impermeable molecules and extraction of biomarkers, transcriptomes, and other biomolecules into and from cytosol (or nucleus) of living cells. This provides noticeable evidence on the single cell dynamics and therefore numerous biological processes in a spatially defined fashion.

In this lecture, I am presenting our results on the manipulation of the cellular membrane tension while monitoring the activation of Piezo1 mechanosensitive ion channels via calcium imaging [4] as well as on the dynamic mechanosensory of essential components of the linker of nucleoskeleton and cytoskeleton (LINC) complex by in situ 3D stress mapping over the nuclear envelope.

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Interaction and regulation in redox partner proteins of the respiratory and photosynthetic electron transport chains

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Protein-mediated electron transfer (ET) reactions play a crucial role in cellular respiration and photosynthesis. In living systems, these ET reactions occur between protein pairs, with at least one having the ability to diffuse freely. This applies to small electron-carrying proteins such as plastocyanin (Pc) and ferredoxin in photosynthesis, as well as cytochrome c (C*c*) in the mitochondrial electron transfer chain. The exceptional efficiency of these processes arises from the optimization of reorganization energy and a high turnover rate. In this context, the complexes formed between redox proteins must maintain a balance between specificity and binding strength while remaining transient to ensure a sustained and efficient electron transfer process. Charge exchange over several nanometers through the aqueous solution has been observed for these complexes in electrochemical scanning tunnelling spectroscopy experiments, suggesting that direct contact may be needed [1,2]. Using force spectroscopy, we investigate interprotein interactions in appropriately oriented cytochrome c_1 (Cc_1, subunit of cytochrome bc_1)-C*c*, and photosystem I (PSI)-Pc complexes [3,4]. We also explore various factors that influence the interaction, including post-translational modifications, redox state, and environmental conditions (ions, pH). Our findings enhance the nanoscopic understanding of interactions between proteins involved in electron transfer and their regulatory mechanisms.

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Decoration strategies for DeepTip™ AFM probes and examples of their use for the characterization of biological systems

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DeepTip™ atomic force microscopy probes produced by the company Bioactive Surfaces are available in a variety of materials, elastic properties of the cantilevers and geometries of the tips. All DeepTip™ probes, however, are characterized by a high surface density of amine groups compatible with most crosslinking chemistries employed for the decoration of the probes. This contribution illustrates how this versatility can be exploited for the characterization of biological systems at the molecular and cell scales with two selected examples. As a first example, the interaction between the Pru p 3 protein and its natural substrate, phytosphingosine, is characterized with single-molecule resolution. The Pru p 3 is one of the major allergens of peach and its interaction with phytosphingosine seems to be a critical step for the recognition of the protein by the immune system. Phytosphingosine was bound to the AFM probe through the amine group of the molecule through the usage of a sulfhydryl crosslinking chemistry. A variation of the same crosslinking chemistry was employed to bind the Pru p 3 protein to a functionalized substrate and the interaction between protein and substrate was determined from the recorded force-distance curves. As a second example of the use of DeepTip™ probes for the characterization of biological systems, the intensity of the adhesion of porcine adipose-derived mesenchymal cells (pADSCs) on decellularized cardiac patches was measured. Decellularized cardiac patches are considered a promising treatment for the regeneration of the heart function after an infarct. However, the combined implantation of patches and stem cells does not result in a successful therapy at present, being assumed that the cells are not retained by the material upon being implanted. In this context, tipless AFM probes were decorated with a biotinylated anti-CD29 antibody, that recognizes specifically a membrane protein of the pADSCs, and used to attach a single cell to the cantilever. The intensity of the interaction between single cells and the decellularized cardiac patches was obtained from the force-distance curves recorded using an atomic force microscope.

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Mechanical and Structural Alterations of Cell Nuclei 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 key role in this process, by actively interacting with the extracellular matrix (ECM) and the cytoskeleton to maintain cellular integrity[1]. Glycation—a non-enzymatic modification of proteins by sugars or their metabolites—disrupts this balance by impairing protein function, leading to defects in cellular and tissue mechanics[2]. While ECM glycation is well-characterized, its impact on intracellular proteins, particularly nuclear components, remains poorly understood in the context of mechanotransduction.

We investigate how glycative stress from methylglyoxal (MG), a reactive glucose metabolite elevated in diabetes, modulates nuclear mechanotransduction. Using atomic force microscopy (AFM)-based nanoindentation, we show that MG stiffens the nucleus, reduces fluidity, and disrupts the nuclear envelope of HeLa cells (**Figure 1A**). Combined AFM-confocal experiments reveal that glycated nuclei behave like rigid structures that fail under high force rather than deforming gradually. Functionally, MG alters nuclear transport, as shown by the increased nuclear localization of the Yes1-associated transcriptional regulator (YAP1) on stiff substrates compared to controls. To extend these findings to a more physiologically relevant context, we used AFM to probe the viscoelastic properties of nuclei isolated from the hearts of mice recapitulating the type 2 diabetes phenotype (**Figure 1B**). These nuclei present similar mechanical alterations, including an increased scaling modulus and reduced fluidity, mirroring the *in vitro* effects observed in HeLa cells (**Figure 1C, D**).

Our results suggest that glycation-driven nuclear stiffening disrupts mechanotransduction, potentially driving mechanical dysfunction in disease. These findings provide new insights into how metabolic stress alters nuclear mechanics, with implications for diabetes and other conditions characterized by defective tissue mechanics.

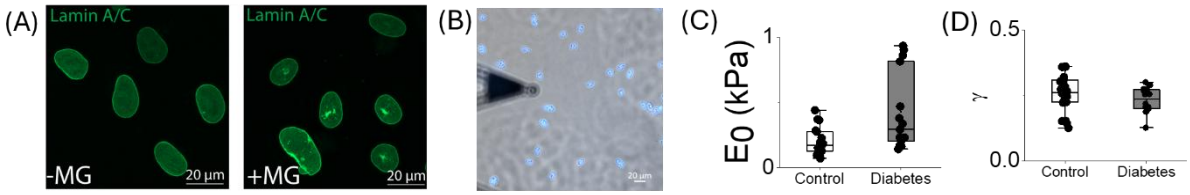

Figure 1. Nuclear envelope alterations and stiffening mediated by *in vitro* and *in vivo* glycation. (A) Confocal images illustrating features of HeLa cells permanently transfected with lamin A/C-EGFP (green). After treatment with 500 µm MG for 3 days, aggregation of lamin A/C is observed in the nucleoplasm and an overall compromised nuclear morphology. **(B)** Epifluorescence image of Hoechst-stained single nuclei extracted from mouse hearts probed by AFM. **(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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Plant-derived anti-HER2 antibody suppresses trastuzumab-resistant breast cancer with enhanced nanoscale binding

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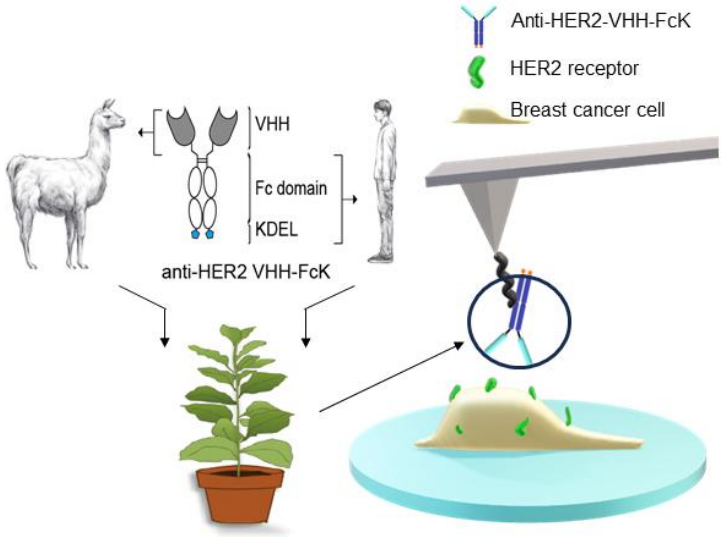
Traditional monoclonal antibodies like trastuzumab encounter limitations when treating HER2-positive breast cancer, particularly in cases that develop resistance. This study introduces a plant-derived anti-HER2 VHH-FcK antibody as a potent alternative for overcoming these limitations. A variety of biophysical techniques, *in vitro* assays, and in vivo experiments uncovers the antibody's nano-scale binding dynamics with transmembrane HER2 on living cells. Single-molecule force spectroscopy reveals the rapid formation of two robust bonds, exhibiting approximately 50 pN of force resistance and bond lifetimes in the second range. The antibody demonstrates specific affinity to HER2-positive breast cancer cells, including those that are trastuzumab-resistant. Moreover, in immune-deficient mice, the plant-derived anti-HER2 VHH-FcK antibody exhibits superior anti-tumor activity, especially against tumors that are resistant to trastuzumab. These findings underscore the plant-derived antibody's potential as an impactful immunotherapeutic strategy for treating trastuzumab-resistant HER2-positive breast cancer.



Figures: Schematic overview of mechanism of action for a plant-derived anti-HER2 camelid monoclonal antibody and single-molecule and nanometric biophysical assessments.

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Unraveling the Mechanical Landscape of the LmrP Multidrug Transporter by Single-Molecule Atomic Force Microscopy

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Multidrug efflux pumps are essential for bacterial drug resistance, yet their conformational dynamics and mechanical stability remain poorly understood. A major challenge is studying their conformation at the single-molecule level under physiologically relevant conditions and without labeling. Here, we use single-molecule force spectroscopy (SMFS) [1,2,3] to examine the energy landscape of the multidrug transporter LmrP from *Lactococcus lactis* within its native membrane [4]. In this way, the structure of the LmrP is stabilized by maintaining its natural lipid environment and cellular components [5]. Our results show that LmrP unfolds through a hierarchical, force-dependent pathway, revealing key energy barriers associated with its transport cycle. The total unfolding energy directly reflects the equilibrium between outward- and inward-open conformations, allowing us to quantify their relative populations in a physiological context. This approach also demonstrates how ligands, membrane composition, and environmental factors influence LmrP's conformational landscape, providing deeper insights into its function and regulation.

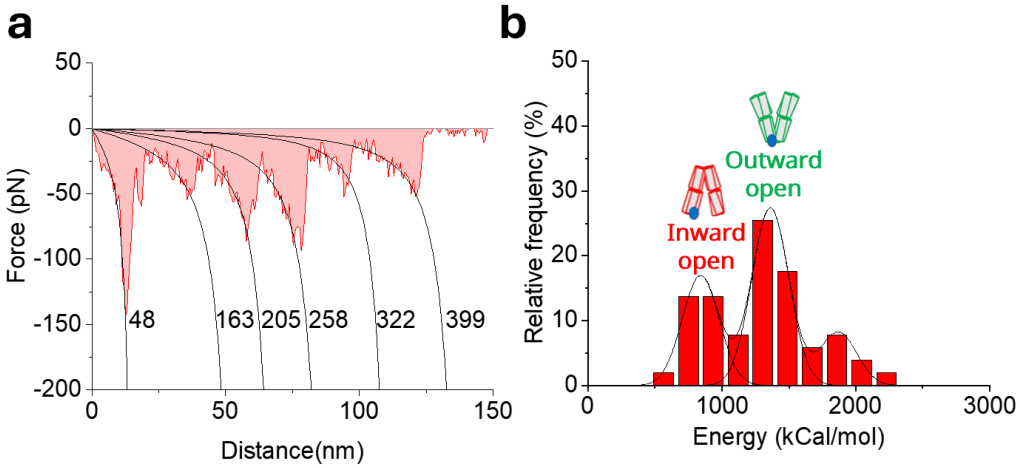

Figure 1. (a) Force-distance curve during the unfolding of the LmrP, with each peak corresponding to the unfolding of one segment of the protein. (b) Histogram of the unfolding mechanical energy as measured by SMFS, showing two distinctive populations corresponding to two different states of the LmrP transporter.

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Understanding DNA repair protein machines from single-molecule manipulation methods

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Single-molecule manipulation methods allow the real-time study of DNA-protein interactions, avoiding the averaging effects of bulk experiments. These methods provide insights into dynamic processes, such as conformational changes and transient interactions, that are often hidden in ensemble measurements. They enable the detection of rare or intermediate states, which are crucial for understanding complex mechanisms like DNA replication, repair, and transcription. Additionally, single-molecule approaches can reveal heterogeneity within a population, helping to distinguish different functional states of DNA-protein complexes.

In this talk, I will describe two single-molecule manipulation methods, magnetic tweezers and optical tweezers combined with confocal microscopy, that we employ to study DNA-RNA-protein interactions involved in the repair of double-strand breaks (DSB). These processes are essential for the viability of all organisms. Specifically, I will focus on the activity of helicases involved in DSB repair via the homologous recombination pathway, as well as the roles of Ku, APLF, and the long non-coding RNA NIHCOLE in the formation of the synaptic complex during the non-homologous end-joining pathway.

Soft Glassy Rheology of single cells with pathogenic Huntington's aggregates

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There is a strong correla on between the mechanical proper es of cells and various diseases and this is emerging only recently. There is growing consensus among various groups that cell's mechanical response is akin to So Glassy Materials. These, in physics are conven onally described using me scale invariant power law. Atomic force microscopy (AFM) has been widely used to measure single cell's apparent Young's modulus by trea ng it as a fully elas c object. More recently, quan ta ve characterisa on of the complete viscoelas city of single cells has become possible. We performed AFM-based nano-indenta on experiments on hemocytes isolated from third instar larvae to determine their viscoelas city and found that live hemocytes, like many other cells, follow a scale free power-law rheology (PLR) akin to so glasses. Further, we examined the changes in the rheological response of hemocytes in the presence of pathogenic protein aggregates known to cause neurodegenera ve diseases such as Hun ngton's disorder and amyotrophic lateral sclerosis. Our results show that cells lose their fluidity and appear more solid-like in the presence of certain aggregates, in a manner correlated to ac n reorganisa on. More solid-like cells also display reduced intracellular transport through clathrin-mediated endocytosis (CME). However, the cell's rheology remains largely unaffected and is similar to that of wild-type (WT) hemocytes, if aggregates do not perturb the ac n organisa on and CME. Moreover, the fluid-like nature was significantly recovered when ac n organisa on was rescued by overexpressing specific ac n interac ng proteins or chaperones. Our study, for the first me, underscores a direct correla on between parameters governing glassy dynamics, ac n organisa on and CME[1,2].

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Investigating the correlation between macrophage mechanical properties and function in response to biochemical and physical cues

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Macrophages are key innate immune cells that, thanks to their capability to polarize to pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes, orchestrate the host response to biomaterials and coordinate the progression of several major diseases, such as atherosclerosis, fibrosis, and cancer. [1] Macrophages were demonstrated to be mechanosensitive, [2] meaning they can adjust their polarization state in response to changes in the mechanical properties of their environment, such as elasticity, which can vary during disease progression and implants' lifetimes. However, the mechanisms through which physical cues trigger macrophage activation remain poorly understood. Emerging evidence points to the role of cytoskeleton remodeling and cell mechanics in macrophage mechanotransduction, [3] highlighting the need to quantitatively measure macrophage mechanical parameters under near-native conditions and correlate them to cytoskeleton organization and biological function. In this study, we used AFM-based force spectroscopy and microrheology (Nanowizard 4, Bruker) to measure the elastic modulus and viscoelastic properties (storage and loss modulus) of primary human monocyte-derived macrophages i) in different biochemically induced polarization states (M0, M1, M2a) and (ii) in response to varying substrate stiffness (ranging from 10 to 130 kPa) (Figure 1a). Biochemically-induced pro-inflammatory macrophages exhibited lower elastic modulus and higher viscosity than anti-inflammatory macrophages on all substrates. When cultured on soft substrates, macrophages showed higher elastic modulus and lower viscosity compared to macrophages on stiff substrates, which correlated with a higher pro-inflammatory gene expression (as measured by qPCR) and a different cytoskeleton organization (as observed by immunofluorescence imaging). Notably, these differences in cell mechanics were independent of cell morphology, as quantified via morphometric analysis. Overall, our results suggest that macrophage mechanical properties could serve as biophysical markers of macrophage function and highlight the potential of AFM-based techniques to unravel new insights into the mechanotransduction mechanisms driving macrophage responses to mechanical stimuli.

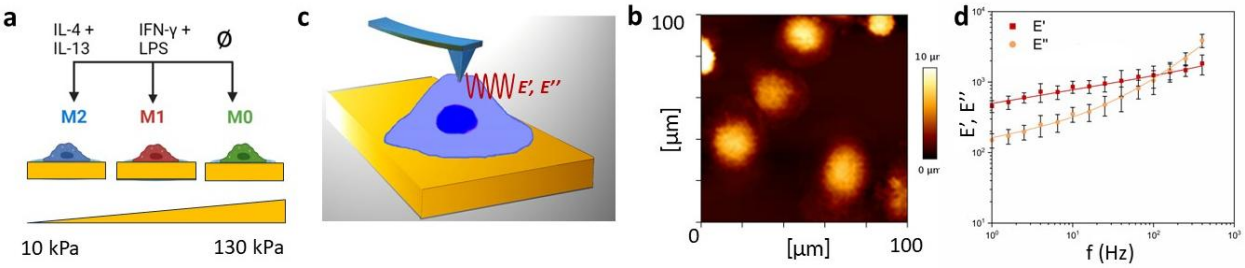

Figure 1. Primary human monocyte-derived macrophages were cultured on polyacrylamide gels with an elastic modulus of 10-130 kPa (a). AFM force spectroscopy-based methods (b) were employed to image the macrophages (c) and analyze their viscoelastic behavior (d)

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Enhancing Large-Scale BioAFM Mechanical Characterization

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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.

Application of atomic force microscopy for the identification of the Pgp protein in the lumen of blood vessels

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P-glycoprotein (Pgp) is a transporter protein in brain blood vessels that regulates the crossing of biomolecules, including drugs, through the blood-brain barrier (BBB). In adults, impaired Pgp activity reduces the efficacy of neuroprotective and anticonvulsant drugs by affecting their transport and retention in the brain, making treatments for brain protection and seizure control less effective. While this issue is well-documented in adults, no studies have examined Pgp function in newborns, leaving it unclear whether similar drug inefficacy occurs in neonatal brains. This gap in knowledge is critical, as neonatal brain injuries remain a major concern in the treatment of great prematures with limited therapeutic options. In this context, therapeutic hypothermia is the only specific treatment for moderate hypoxic-ischaemic brain injury in these patients, leaving most affected newborns without a specific therapy since, for instance, antiepileptic drugs are ineffective in nearly half of cases. These challenges highlight the urgent need for research into the possible function of Pgp in newborns and the possibilities opened for the development of a more effective neuroprotective and anticonvulsant strategies for this vulnerable population.

In this study, atomic force microscopy was employed to investigate the presence and location of the Pgp protein in the endothelial cells of neonatal rat blood vessels. The methodology follows a multifactorial approach that starts with the characterization of the neonatal rat aortic tissue through atomic force microscopy. Subsequently, functionalized DeepTip^{TM} AFM probes were decorated with anti-Pgp antibodies and the interaction between these sensor molecules and Pgp immobilized on a functionalized substrates was validated *in vitro*. Finally, the lithography mode was utilised to identify the presence of this protein in both endothelial cell cultures and neonatal rat aortic tissue.

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POSTERS

Resolving the double layer and hydration structure of competing ions at solid-liquid interfaces

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In the framework of clean energy technologies, electrochemical production of hydrogen from water and conversion of CO2 into valuable green fuels are of great interest, despite their kinetic and thermodynamic limitations. Overcoming these limitations requires an accurate molecular-level description of the transition states taking place at the solid-liquid interface.
The chemistry of the supporting electrolyte was shown to affect the activities of catalytic reactions [1]. The weakly-hydrated cations, for example, seem to act as proton donors through water hydrolysis, which can alter the proton availability (i.e., kinetics) of HER [2] or buffer the interfacial pH to an ideal value for CO2 reduction in acidic media [3].
More recently, the effects of additions of organic cations derived from quaternary ammonium salts has been investigated [4]. For example, the size of the quaternary ammonium salt was found to provide selectivity towards CO2 reduction intermediates [5], thus affecting the reaction pathways. Additions of tetrabutylammonium ions to alkaline electrolytes were also shown to controversially promote the HER despite its physisorption and site-blocking effect [6].
This opens the question on how the organic ions are structuring and/or competing with (for example) alkali ions within the electric double layer under high surface charging regime. For this purpose, we investigate the structure of electric double layers consisting of ionic mixtures on the surface of muscovite mica. The mixtures consist of alkali ions (namely, Cs^+) and quaternary ammonium salts with different hydrophobic character [tetraethylammonium (TEA^+) or tetrabutylammonium (TBA^+)]. Competition between the ions is investigated with high resolution AFM imaging and force spectroscopy, while the interfacial hydration structure is more deeply investigated with three-dimensional AFM (3D-AFM). Such understanding provides insights for tuning and optimizing the chemistry of electrocatalytic solutions.

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Dynamic and static degradation of perovskite oxide catalysts by electrochemical AFM

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A fundamental understanding of degradation mechanisms under static and dynamic conditions is essential to develop catalysts for the oxygen evolution reaction (OER), the bottleneck in efficient electrochemical water splitting. Perovskite oxides are novel class of OER electrocatalysts [1-2], however, the differences in their degradation and stability in alkaline electrolyte are not yet fully understood. To address this, epitaxially grown $La_{0.6}Sr_{0.4}CoO_3$ (LSCO), $La_{0.6}Sr_{0.4}FeO_3$ (LSFO) and $La_{0.6}Sr_{0.4}MnO_3$ (LSMO) were compared by employing electrochemical atomic force microscopy (AFM) during cyclic voltammetry (CV) and chronoamperometry (CA).
Electrochemical AFM results, mapping the topography and friction force of materials during the first CV showed distinctly different and irreversible degradation paths for perovskites. Continuous topography and friction force measurements over prolonged cycling, as well as post-catalysis analyses, further supported the observed dynamic degradation mechanisms, specifically bulk degradation in LSCO and LSMO, and surface passivation in LSFO. Consequently, the Co-based perovskite exhibited reduced stability and activity loss, whereas the Fe-based perovskite demonstrated improved stability. A comparison with steady-state OER conditions showed that electrochemical AFM during CA detected a short delay of morphology and friction force changes relative to the start of electrochemistry.
The results elucidate how electrochemical AFM can differentiate degradation mechanisms under dynamic and static conditions in alkaline environments as well as between transition metals in perovskite oxides. Thereby, the conclusions contribute significantly to the understanding of perovskite degradation at the solid-liquid interface.

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Effect of capillary condensation on non-contact Scanning Probe Microscopy

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It is known that under humid conditions, an AFM tip close to a surface causes the spontaneous condensation of a water meniscus between the tip and the surface [1,2]. Interestingly, the resulting capillary interactions have both conservative and dissipative components that depend on the nano-scale contact angle of water with the surface. Typically, capillary force causes the tip to snap to contact. However, when the cantilever is oscillated (as in DAFM), not only does the tip avoid snap-in, but rather, it manages to avoid any hard contact with the surface altogether. The temporary dissipative interaction experienced by the tip due to condensation, which occurs only when the instantaneous tip-sample distance during an oscillation cycle is below a particular length, causes the cantilever's oscillation amplitude to pulsate and avoid contact. This is shown by experimental amplitude-distance curves made on hydrophilic (mica) and hydrophobic (HOPG) samples, as well as numerically simulating the cantilever's non-linear harmonic response to such interactions (Figure 1). Operating the DAFM at constant phase using a phase locked loop (PLL) allows separating the conservative and dissipative parts of the total interaction, where, the "frequency shift" channel contains information related to the local van der Waals and capillary interaction, while, the "amplitude" channel is related to the purely dissipative component of the capillary interaction. In the manner, the local nano-scale wetting property of the surface can be mapped out, while avoiding tip-sample contact. This approach is tested by performing measurements on interdigitated electrode samples, where the gold electrodes were selectively functionalized with thiols. Such a model sample provides us with micro-scale hydrophobic (thiol-coated gold) and hydrophilic (glass) regions, whose wetting properties can be characterized in a single AFM scan image.

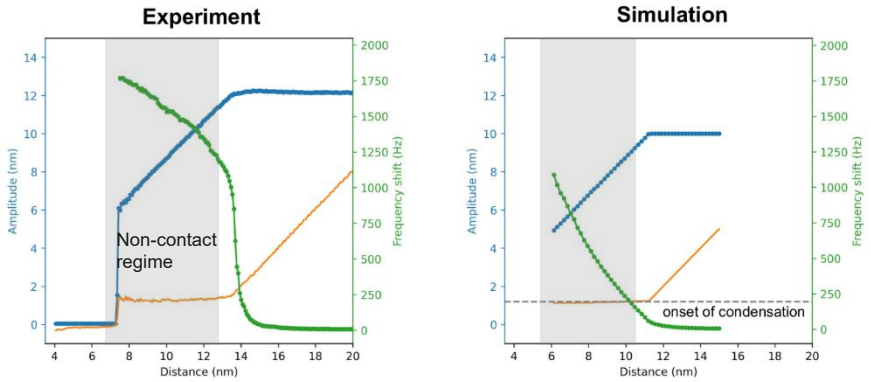

Figure 1 – Experimental vs simulated interaction-distance curves on mica. Orange curves show that the tip-sample distance at the lower turning point of oscillation is not zero in the "non-contact" regime

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Multimodal Multiparametric Investigation of Solid-Liquid Interface

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Understanding the molecular-scale organization of water at solid-liquid interfaces (SLI) is crucial for diverse fields, including nanofluidic, electrochemistry, and biomaterials science [1]. At the SLI, water molecules form complex hydration structures that influence interfacial phenomena such as wettability, adhesion, ion transport, and lubrication. These interactions play a fundamental role in biological systems, energy storage devices, and the performance of functional surfaces [2]. In this study, we employ atomic force microscopy (AFM), alongside contact angle measurements and quartz crystal microbalance (QCM) techniques, to probe hydration structures, interfacial forces, and wettability on various substrates, including Mica, highly oriented pyrolytic graphite (HOPG), and single-walled carbon nanotubes (SWCNTs). AFM force mapping provides high-resolution insights into force distributions perpendicular to the interface. Contact angle measurements complement this by characterizing surface wettability, while QCM detects interfacial mass and dissipation changes associated with hydration effects. By integrating QCM and AFM data, we also estimate slip length [3], which is a key parameter in nanofluidic transport. To explore the effect of ionic composition on interfacial hydration, we conduct experiments using three different electrolyte solutions—magnesium chloride ($MgCl_2$), potassium chloride (KCl), and sodium chloride (NaCl)—at varying concentrations. These salts influence hydration structures through ion-specific interactions with surfaces, affecting water layering, surface charge screening, and slip behavior at the interface. This multi-technique approach enables a detailed investigation of how surface chemistry, topography, and wettability influence hydration structuring at the nanoscale. The findings contribute to a broader understanding of interfacial water behavior, with implications for nanofluidic transport, energy storage, and biomolecular interactions at solid-liquid interfaces.

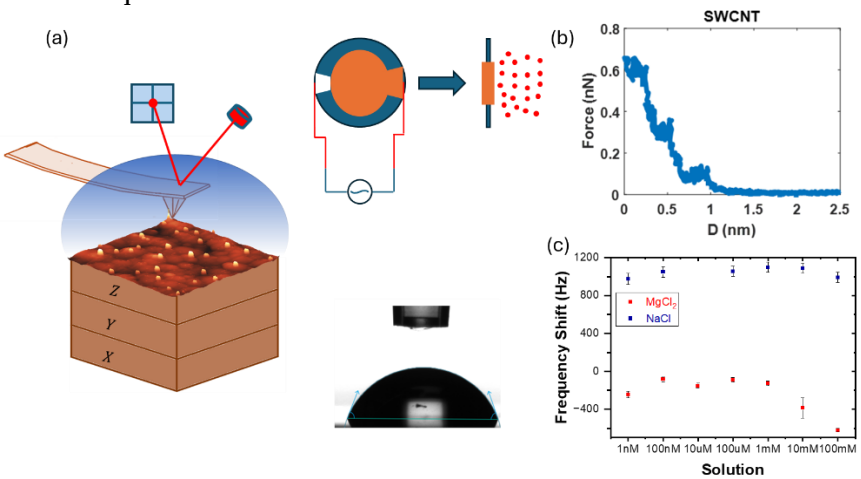

Figure 1. (a) Schematics of AFM, QCM and Contact Angle **(b)** AFM Results with water on SWCNT **(c)** QCM Results on Gold using MgCl2and NaCl

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Systematic Multidimensional Quantification of Nanoscale Systems From Bimodal Atomic Force Microscopy Data

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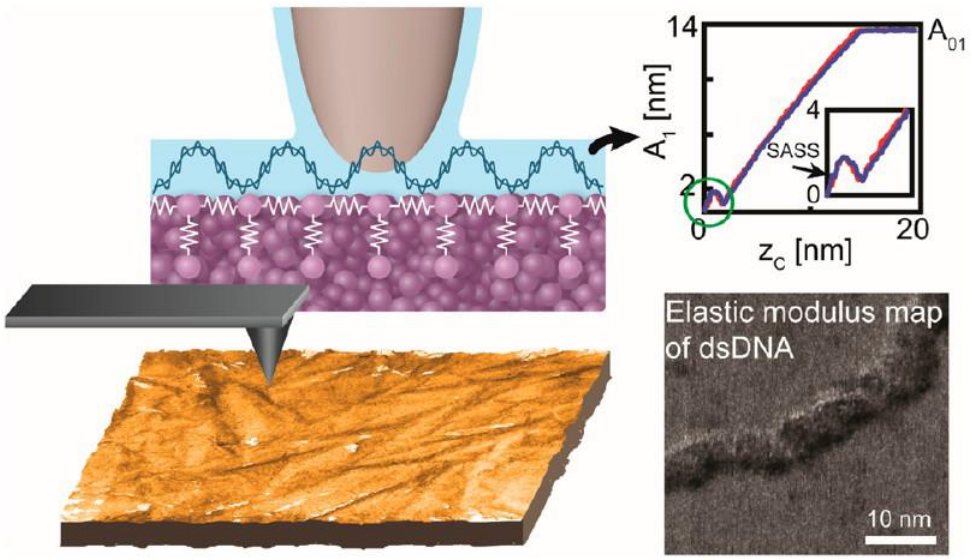
Here we explore the raw parameter space in air in bimodal atomic force microscopy (AFM) in order to enhance resolution, provide multiparameter maps, and produce suitable transformations that lead to physically intuitive maps general enough to be recognized by the broader community, i.e., stiffness, Hamaker constant, and adhesion force. We further consider model free transforms to enhance the raw parameter space in the form of alternative and more intelligible contrast maps. We employ highly oriented pyrolytic graphite, calcite, polypropylene, and dsDNA on mica to demonstrate a systematic form of parameter expansion. The proposed methodology to enhance and interpret a larger parameter space introduces a methodology to tractable multidimensional AFM from raw bimodal AFM maps.



Evaluation of the Effects of O_2 Plasma Etching on 3D Printed Polymers

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Two-photon polymerization (2PP) has revolutionized 3D microfabrication, enabling sub-micrometer resolution for applications in optics, biomedical engineering, metamaterials, and microrobotics.[1] Despite its versatility, further miniaturization is challenged by the diffraction limit and material constraints. Amongst the several strategies proposed to overcome these limitations, post-processing techniques such as pyrolysis and O_2 plasma etching are the most accessible and convenient alternatives (Fig. 1a).[2] While pyrolysis induces isotropic shrinkage and transforms polymeric structures into carbonized materials, drastically changing the material properties, O2 plasma etching enables controlled feature refinement and selective removal of sacrificial supports, circumventing the resolution limitations of this laser writing technique and facilitating the printing of suspended components. This study explores the changes induced by plasma etching treatments on the surface roughness, mechanical properties via bimodal Atomic Force Microscopy (Fig. 1b), which in combination with Raman spectroscopy, shed light on induced changes in material composition of 3D microstructures.[3] The results offer key insights for the design of 3D microstructures with controlled physical properties, to fit the targeted application.

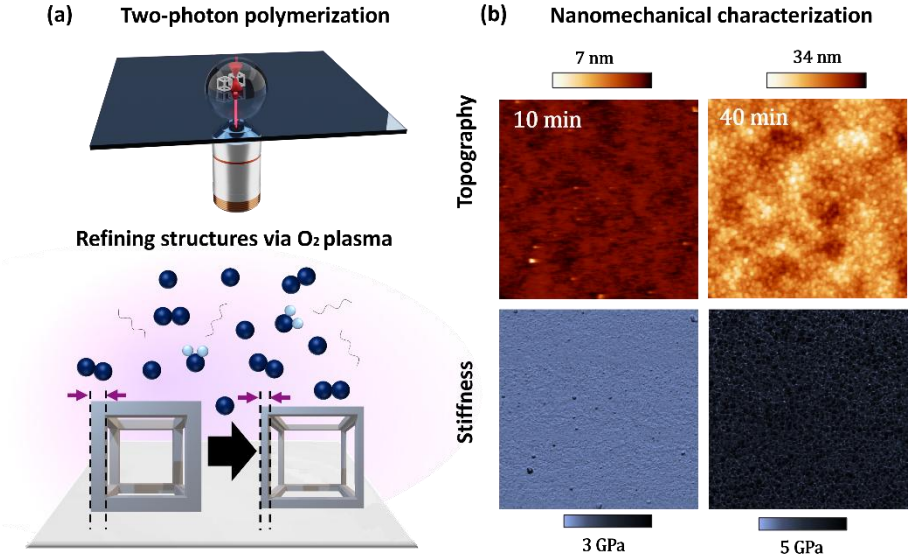

Fig. 1. (a) Sketches showing the two-photon polymerization and O_2 plasma etching to achieve fine features. (b) Nanomechanical characterization of 3D printed surfaces exposed to plasma. Images are 2x2 μm^2.

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Magnetic Force Microscopy study of the hardening of rare earth-free core-shell ferrite-based nanoparticles

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The development of sustainable, rare-earth free permanent magnets has become an active topic of research in the recent years due to its significant implications for automotive and biomedical applications. In that sense, ferrite magnetic nanoparticles combining hard and soft magnetic materials offer a promising route for designing new rare-earth free permanent magnets with enhanced magnetic properties. A versatile, widely used technique for local characterization of magnetic nanostructures is Magnetic Force Microscopy [1,2]. It enables relatively high spatial resolution, simplicity in operation as well as sample preparation, and the capability to apply in situ magnetic fields to study magnetization process [3]. In this work we present a novel synthetic strategy to efficiently design magnetic nanostructures nanoparticles (10-20 nm) by virtue of a multi-shell onion structure in which hard magnetic materials (cobalt ferrite) and soft magnetic materials (manganese ferrite) are carefully layered. These unique structures enable strong multiple exchange couplings at the interface of hard and soft magnetic layers [4], enhancing the nanoparticle magnetic properties by strain-induced magnetic anisotropies. Variable Field-Magnetic Force Microscopy (VF-MFM) has been used to measure hysteresis loop at single NP level in both in plane and out of plane field configurations. This has experimentally confirmed the change from superparamagnetic to ferromagnetic behaviour, as well as the hardening despite of the reduction in amount of hard material.

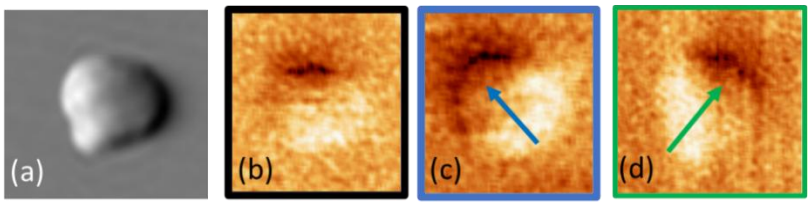

Figure: (a) Topography image of an individual multishell (4 layers) nanoparticle (~ 20 nm size); MFM images acquired at room temperature at different magnetic fields (b) remant state (c) + 700 Oe (d) -700 Oe.

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Structural Characterization of RNA combining AFM, Chemical Probing and Dynamic Fitting

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The secondary and tertiary structures adopted by single-stranded RNA molecules play a crucial role in determining their function in cellular processes. However, the majority of 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 investigate RNA secondary structure and conformational dynamics, as it enables direct visualization of structural heterogeneity at the single-molecule level. Moreover, we integrate AFM imaging with advanced image analysis and Molecular Dynamic simulations to gain insights into the secondary structure of RNAs. We apply a dynamic fitting approach based on [1][2], leveraging coarse-grained molecular dynamics simulations to validate chemical-probing-derived secondary structures against AFM experimental images.
As a proof of concept, we apply this methodology to the 5′ proximal region of SARS-CoV-2, demonstrating its ability to resolve structural features and conformational flexibility (Figure 1). We further extend this approach to longer RNAs with more complex architectures, incorporating modifications such as 5′ and 3′ tagging with poly-A tails and targeted oligonucleotide-induced structural disruption. Our work underscores the advantages of AFM in capturing global RNA conformations, which, when combined with high-resolution techniques such as chemical probing, can provide deeper insights into RNA structural organization and function.

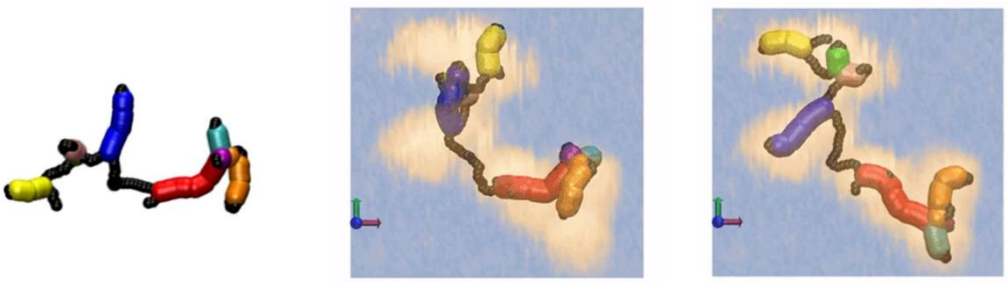

Figure 1 – Dynamic Fitting of the 5’ proximal region of Sars-CoV-2. Coarse-grained model obtained from published Secondary Structure [3]

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Torsional Mode AFM as versatile multifrequency mode: challenges and possibilities

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Generally, in Dynamic Atomic Force Microscopy (DAFM) the tip is oscillated normal to the sample surface, allowing to measure the Force Gradient $F'(z)$ of tip-sample interaction in this direction, as well as dissipative processed related to the motion normal to the surface. If the tip is oscillated laterally -so called "torsional mode"- it should be possible to determine lateral force gradients $F'(x)$, where x is the lateral scanning direction, as well as dissipation associated with lateral displacements [1,2]. In fact, while typical multifrequency operation using some of the multiple normal resonance modes of the cantilever allows to acquire a variety of interesting data ("use each resonance mode for a different measurement"), all normal modes are essentially accessing the same (normal) force gradient $F'(z)$. Therefore, "lateral multifrequency operation" should open a "new dimension" for DAFM.

While this mode has been proposed quite long ago, we believe it is used surprisingly little. One problem of torsional modes may be associated to the correct calibration of lateral/torsional oscillation amplitude. In this work we will show that accurate calibration of oscillation amplitude in torsional mode is fundamental for the correct analysis and interpretation of data in experiments involving lateral DAFM. In fact, we will discuss that surprisingly this mode has a very high sensitivity (in our case close to 1pm!). If this extreme sensitivity is not taken into account, it may be detrimental for a variety of applications, since very large lateral oscillations may be induced, resulting in uncontrolled experimental conditions and even tip wear.

This study focuses on the development and implementation of advanced methods to calibrate the oscillation amplitude for operation in torsional modes. A combination of theoretical and experimental approaches is employed to enhance the accuracy and repeatability of oscillation measurements. Essentially, we propose a calibration procedure where the typical DAFM electronics is uused to transfer the thermal noise of the cantilever motion from its resonance frequency to a much lower frequency within the typical bandwidth of the corresponding data acquisition electronics of a scanning force microscopy system [3]. This method enables a precise "lock-in" type calibration of very small lateral oscillation of the tip, which should allow a variety of well controlled and precise DAFM experiments accessing dissipation, forces and force gradients in the lateral direction.

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Data-Driven Interpretation of Multifrequency AFM Observables for Improved Surface Property Assessment

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This work explores how machine learning techniques can help identify and separate different types of surface forces in Atomic Force Microscopy (AFM) experiments. AFM measurements are often complicated by the simultaneous presence of multiple interactions, such as van der Waals forces, electrostatic forces, and magnetic forces. Accurately distinguishing these forces is vital for understanding nanoscale properties and behaviors in materials research [1].
A synthetic dataset was first created using a damped mass model to simulate cantilever motion in AFM under various force regimes. This controlled approach enabled the generation of different power-law forces—commonly exponents of 2, 3, or 4—to represent van der Waals, electrostatic, and magnetic interactions. The classification models were then trained in MATLAB's Classification Learner App, using key AFM observables like amplitudes, phases, and virials. Different algorithms, including Support Vector Machines (SVM) and neural networks, were tested for their ability to categorize the power-law exponents with high accuracy [2].
While the classification performed very well on synthetic data, applying the trained models to real AFM measurements introduced challenges. Experimental data can be noisy and inconsistent, leading to potential misclassifications. By carefully normalizing amplitudes (dividing them by the free amplitude) and phases (dividing by 180°), the models were better able to generalize to real-world measurements. Tests on graphene samples, which should mostly exhibit van der Waals interactions, showed that normalization significantly improved the model's accuracy in predicting a power-law exponent of 2.
To further confirm the presence of magnetic forces (power-law exponent ~4), Magnetic Force Microscopy (MFM) imaging was used. The MFM results supported the model's predictions by revealing the expected dipole-dipole interactions. Overall, this study demonstrates that combining AFM observables with robust machine learning and careful data preprocessing can substantially improve the identification and analysis of complex surface interactions at the nanoscale.

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Study of RNA condensation dynamics mediated by mycobacterial DNA-binding protein 1 (MDP1) using high-speed AFM

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, can enter a dormant state, contributing to treatment challenges. During dormancy, Mtb undergoes genome condensation, a process reportedly facilitated by Mycobacterial DNA-binding protein 1 (MDP1) [1], which is highly abundant in dormant cells. MDP1 function is known to be regulated by post-translational modifications (PTMs) [2]. While MDP1's role in DNA compaction is established and RNA regulation during dormancy is reported [3], the mechanism of MDP1's interaction with RNA remains limited. This study aimed to address two key questions: Does MDP1 induce RNA condensation, if yes, then how do PTMs affect MDP1's RNA condensation activity? To answer these questions, we investigated the dynamics of MDP1-mediated RNA condensation and the influence of PTMs on this condensation utilizing high-speed AFM (HS-AFM). We examined the interaction of *E. coli* total RNA with two variants of MDP1: native *M. tuberculosis* MDP1 (nMDP1-mtb), characterized by a high degree of PTMs, and a recombinant *E. coli*-expressed variant (eMDP1-mtb) with minimal PTMs. HS-AFM observations revealed a PTM-dependent difference in RNA condensation behaviour. nMDP1-mtb induced robust RNA condensation, forming “cotton ball-like” condensates. In contrast, eMDP1-mtb exhibited RNA binding but failed to promote significant RNA condensation. These findings demonstrate that MDP1 induces RNA condensation and PTMs are critical for its activity, suggesting a role for PTMs in regulating RNA configuration and function during mycobacterial dormancy. This study highlights the need for further investigation into the fate and function of RNA during Mtb dormancy, a relatively unexplored area with potential implications for the development of novel therapeutic strategies against tuberculosis.

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Viscoelastic Characterization of Polyurethane Coatings using Intermodulation Atomic Force Microscopy

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Understanding the nanoscale viscoelastic properties of polymeric coatings could explain their macroscopic behaviour and the root cause of their failure. A possible application of this knowledge is in the development of long-lasting protective coatings for the aerospace industry or in detecting the end-of-life and required maintenance of such coatings. [1] Intermodulation AFM (ImAFM), a powerful dual-tone excitation method enabling nanomechanical characterization of samples, can provide new insights in this respect [2].
In this study, the viscoelastic properties of a polyurethane coating have been determined by ImAFM using the Derjagin, Muller, Toropov (DMT) viscoelastic model. By exploiting the experimental force quadratures, both dissipative (viscous) and conservative (elastic) components of the tip-sample force are obtained. A sensitivity analysis of the DMT model reveals that each component exhibits distinct and predictable dependencies on either viscosity or elasticity. This technique enables the separation and quantitative determination of viscoelastic properties, resulting in values that agree with bulk values obtained from macroscale dynamic mechanical analysis. By leveraging the DMT viscoelastic model, this nanoscale viscoelastic characterization method enables fast, accurate, and quantitative measurements. This could potentially provide a direct link between the nanoscale viscoelastic properties and the macroscale mechanical performance of polymeric coatings.

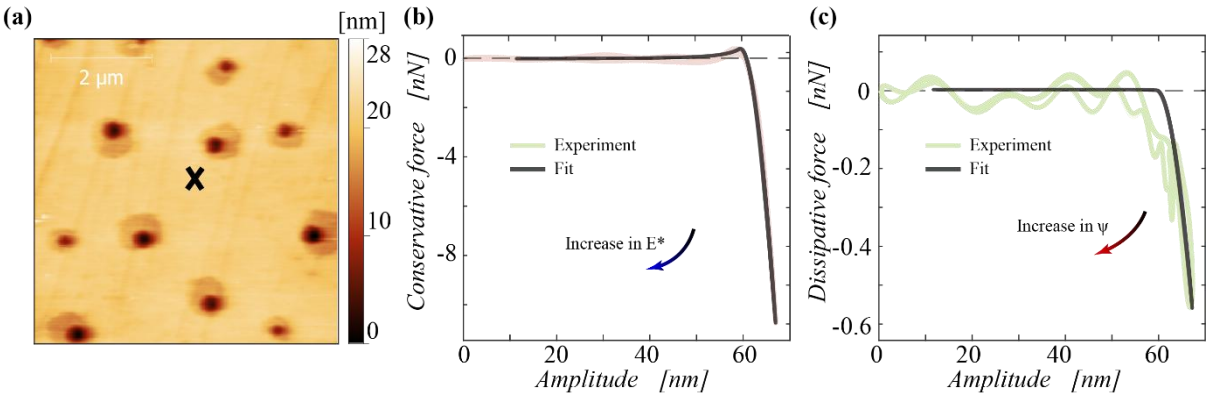

Figure 1: (a) Topography image of the coating polyurethane (PU). Experimental and fitted (b) conservative and (c) dissipative tip-sample forces by Intermodulation AFM on PU.

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Integrating Work Function with Structural Characterization: Advanced nalysis of Cu–Ti Catalysts for Enhanced CO_2 Reduction

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CO_2 reduction is a critical challenge in mitigating climate change, driving research into a diverse array of catalysts.[1] Among these, copper-based electrocatalysts have garnered noteworthy attention due to copper's ability to reduce CO_2 to hydrocarbons, or oxygenated hydrocarbons, at significant rates.[2] In particular, the preferentially exposed (110) facet of copper has been shown to be selective for producing oxygenated hydrocarbons, effectively reducing CO_2 to C_2 products such as acetaldehyde, ethanol, and acetic acid.[3]

In this study, we investigate a Cu-Ti alloy electrocatalyst sputtered onto FTO, aiming to understand potential benefits of bimetallic catalysts. Metallic heteroatoms in alloys are theorized to modify electronic and structural properties of copper, potentially stabilizing the active (110) facet and enhancing the catalyst's performance. Moreover, alloying can have a beneficial effect on the onset potentials of reactions and products selectivity.[4] We explored Ti contents in the range 2-10% and performed extensive characterizations to elucidate the relationship between catalyst's structure, along to its chemical and physical properties, with its performance, to further investigate its effect in CO_2 reduction.

Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDX) allowed to examine catalysts morphology, dimensions, and the accurate %at of Ti. X-ray Diffraction (XRD) confirmed the presence of the desired (110) peak and revealed strain induced by titanium incorporation. Investigations conducted using Atomic Force Microscopy (AFM) techniques, including mechanical AFM and Kelvin Probe Force Microscopy (KPFM), provided crucial insights into the functional properties in particular the work function of the cathode, an important parameter for cathodic activity. The ability to map at the nanoscale the minimum thermodynamic work necessary to remove an electron from a surface is an information that will provide a deep insight over the effect of the variation of titanium.

This preliminary study aims to shed light on the mechanistic aspects of CO_2 reduction over bimetallic copper-based catalysts and to assess the potential role of titanium as an alloying element, potentially paving the way for enhanced electrocatalyst performance in CO_2 conversion processes and future further studies for in operando analysis.

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Electron beam induced enhanced contrast in correlative in-situ single-pass heterodyne (H-) Kelvin probe force microscopy using piezo-resistive cantilevers

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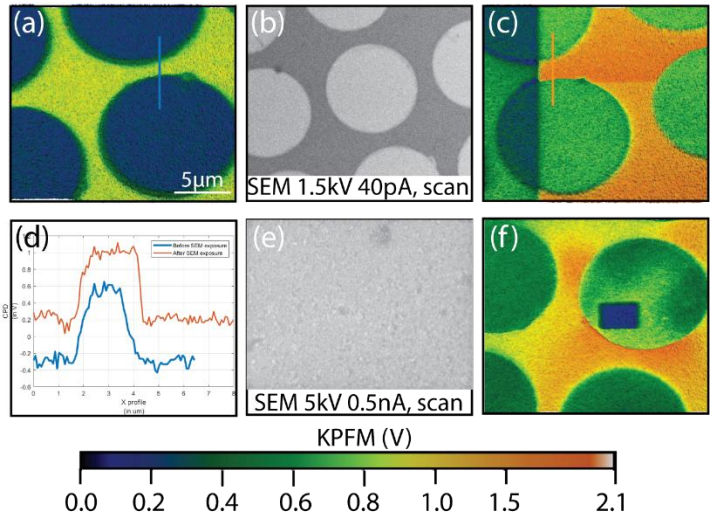
In this work, we perform single-pass Kelvin probe force microscopy (KPFM)[1] using piezo-resistive cantilevers[2] inside the chamber of a focused ion beam-scanning electron microscope (FIB-SEM) for in-situ analysis. Multilayer MEMS self-sensing cantilevers[3] allow separate channels for sensing and applying voltages for electrical measurements using atomic force microscopy (AFM). Traditionally, such self-sensing cantilevers show intrinsic capacitive crosstalk pronounced at higher frequencies. For improved resolution and sensitivity, we use heterodyne (H-) KPFM[4] configuration to perform surface potential measurements in a single-pass. A cantilever tip-scanning AFM[5] integrated inside the FIBSEM aids us in correlative analysis. Using H-KPFM configuration at higher eigen-mode provides us a large, easy separation of topography and KPFM signal, bringing better sensitivity and faster imaging. The influence of electron beam and ion beam on the sample surface can be evaluated through the contact potential difference (CPD). Low dosage exposure aids in clearing of the contaminants from the sample surface, and allows an enhanced contrast in the exposed area. Such correlative analyses can be useful in high-resolution imaging of point defects in 2D hetero-structures and layered semi-conductor devices.



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Monochromatic Confocal Displacement Sensing in Atomic Force Microscopy

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Optical detection methods have emerged as powerful tools for measuring nanoscale displacement and velocity. But achieving both high sensitivity and speed in determining sample position remains challenging.
Here we introduce Monochromatic Confocal Displacement Sensing. In this purely geometrical method, a laser beam is focused onto a sample. The reflection is collected on a detector. An axial displacement of the sample results in a focal shift at the detector amplified by the ratio between the objective and collector focal lengths squared. By blocking part of the beam with a pinhole, this focal shift is converted into a change in optical amplitude. Such a system can be designed for a broad sensitivity range, and the detection speed is only limited by the photodetector bandwidth, giving the technique potential in High-Speed AFM [1]. Sensitivities similar to an optical lever or optical interferometer can be reached [2].
We show and experimentally verify a model for the sensitivity of such a confocal system. With a 20X microscope objective and a collector lens with a focal distance of 25cm, we resolve the thermomechanical motion of an AFM cantilever, achieving a sensitivity of 6.4 pm/√Hz. Finally, we demonstrate the use of Monochromatic Confocal Displacement Sensing by imaging Blu-ray disc structures in contact mode.

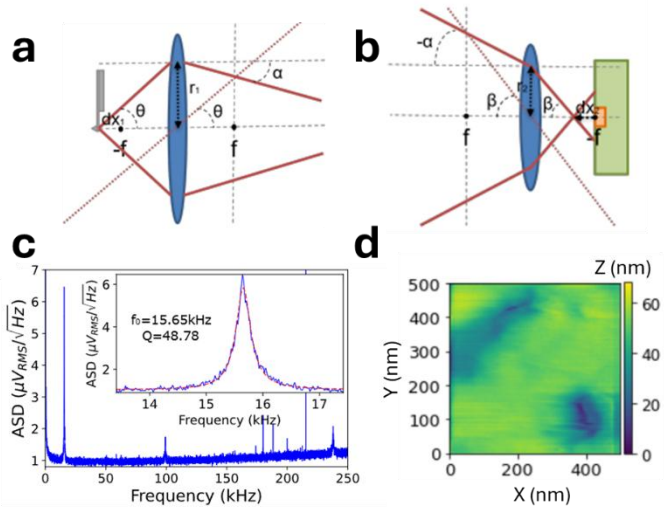

Figure 1: (a). cantilever displacement w.r.t focus leads to a change in convergence angle. (b). This results in a change in focal shift and optical power on the detector. (c). Thermal spectrum of AFM cantilever. (d). Confocal AFM image of Blu-ray disc structure.

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Nonlinear Identification of Non-Smooth Dynamics in Atomic Force Microscopy

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Force reconstruction in Atomic Force Microscopy (AFM) data represents a significant challenge due to the complex dynamic interactions between the AFM tip and the sample, which are both highly nonlinear and non-smooth [1]. Recent advancements in machine learning highlight its potential to extract detailed information, particularly data-driven algorithms for identifying dynamical systems [2]. This study employs recent advancements in data-driven machine learning to preserve and retrieve the non-smooth characteristics of AFM dynamics. To achieve this, we combine nearest neighbor data clustering and Hybrid Sparse Identification of Nonlinear Dynamics (SINDy) to accurately reconstruct force-distance relations from data. This methodology recovers non-smooth equations of motion for various AFM models, including Derjaguin-Muller-Toporov (DMT) and DMT with Kelvin-Voigt. Furthermore, the algorithm precisely estimates the intermolecular distance, demonstrating its effectiveness in predicting the transition between the attractive and repulsive dynamical regimes. This method has the potential to improve the force reconstruction fidelity, offering a robust tool for advanced nanomechanical studies.

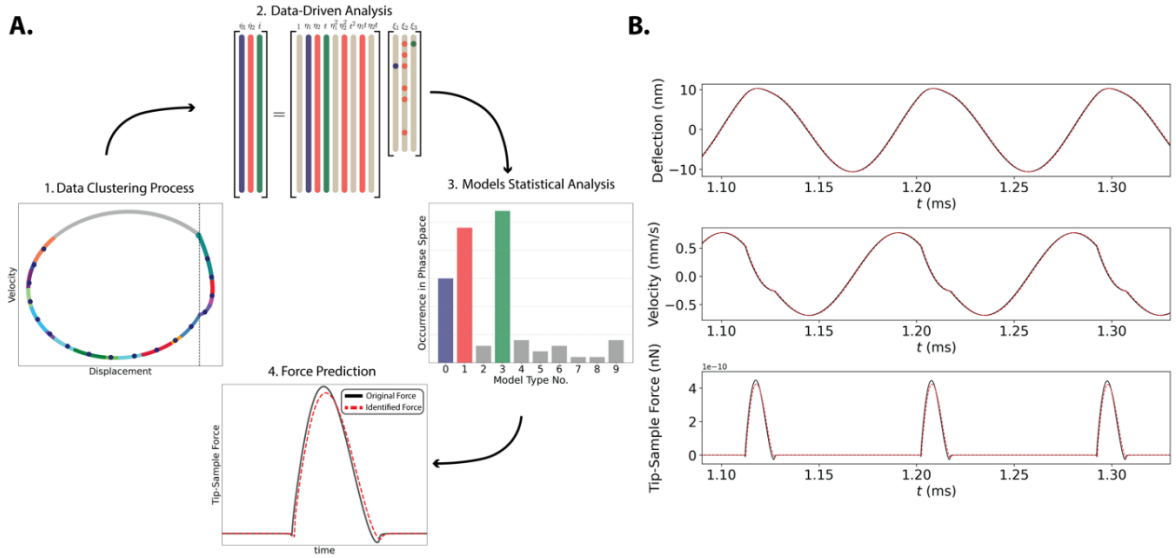

Figure 1: A. Schematic of the Hybrid SINDy Identification Process, from clustering the time-series data of state variables for a localized analysis to applying a data-driven SINDy approach followed by a statistical evaluation of multiple identified models for predicting tip-sample forces. B. Steady-state response prediction: Comparison of the cantilever state vectors displacement, velocity, and the tip-sample force between data generated by the DMT model with Kelvin-Voigt (black) and the data-driven identified dynamics from the simulated non-smooth equations of motion found with the SINDy algorithm (red).

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Short and sweet – crosslinking glycation stiffens diabetic titin

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Myocardial stiffening is an early feature in diabetic patients, leading to the development of diabetic cardiomyopathy and heart failure [1]. While the mechanism responsible for extracellular matrix stiffening has been described, the molecular basis of cardiomyocyte stiffening remains poorly understood. To address this question, we investigate the mechanical modification of cardiomyocytes by glycation, a highly prevalent posttranslational modification in diabetes, capable of producing non-crosslinking and crosslinking modifications in biomolecules [2]. This work focuses on the effect of this modification on the mechanical properties of titin, a protein that plays a pivotal role in the mechanical properties of cardiomyocytes [3].

In order to explore the extent of glycating modifications in titin and cardiomyocyte mechanics, we first demonstrate increased levels of non-crosslinking modifications in titin extracted from obese mice using mass spectrometry. In addition, using this same technique, we have been able to detect crosslinking glycations for the first time in the native protein extracted from obese mice. Furthermore, we have substantiated the efficacy of magnetic tweezers as a tool for detecting crosslinking glycations using recombinant protein. This approach has enabled the quantification of the proportion of crosslinking glycations in native titin from obese and healthy mice. Finally, we have tested mutant recombinant titin using single molecule force spectroscopy AFM to reveal the spatial distribution of residues undergoing crosslinking modifications. These results suggest that nanomechanical alterations in titin, induced by crosslinking glycations, may play a pivotal role in the increased myocardial stiffness observed in individuals with diabetes

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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 substrates [1] (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 decomposition [2] (DMD) customized model for attending urgent 2D interpretations of more flexible domains in the spike's RBDs. Moreover, implementing VampNets [3] 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.

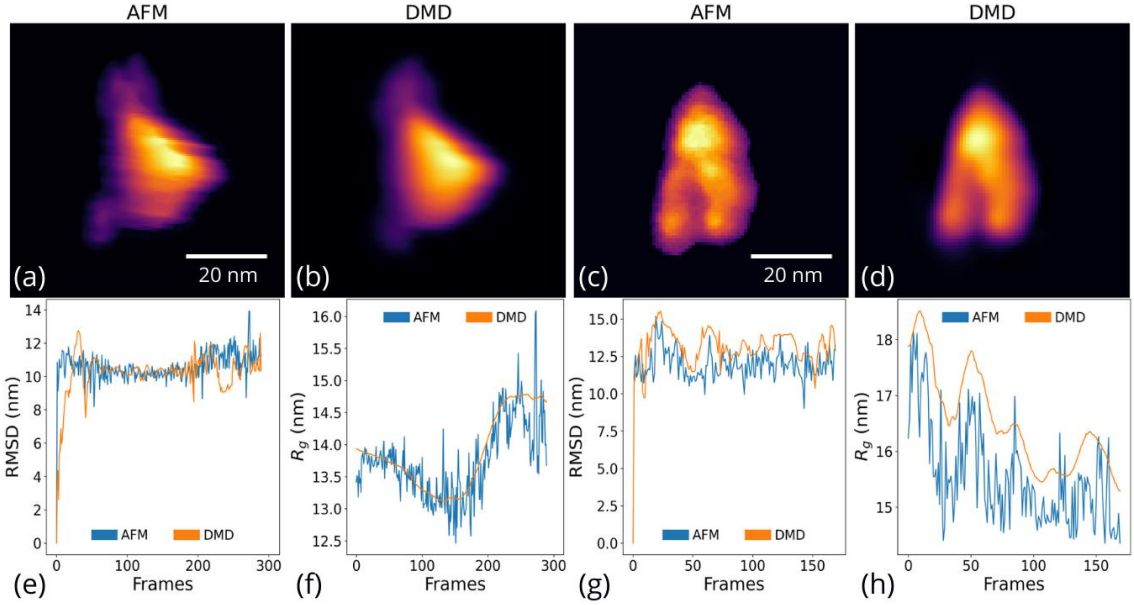

Figure 1. DMD applied to HS-AFM images of the SARS-CoV-2 spike protein. WT variant (a) AFM experiments and (b) DMD reconstruction. The Omicron variant is shown in (c) Experiments and (d) DMD reconstruction. (e, f) RMSD and Rg vs. number of frames for AFM and DMD WT variant, respectively. (g, h) similar to (e-f) applied to the data for Omicron.

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Interplay of glycans and adsorption-driven deformation in SARS-CoV2 proteins onto surfaces with different hydrophobicity

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Using all-atom molecular dynamics (MD) simulations, we examine the interactions of the receptor-binding domain (RBD) and S1 Spike (S1) protein of the SARS-CoV2 in open and closed conformations across hydrophobic and hydrophilic surfaces. In our previous works, we have shown that hydrophobic surfaces are more prone to adsorb the RBDs in its open configuration and that the influence of glycans in the adsorption is negligible [1]. Recently, we have focused our work on understanding the impact of glycans on the adsorption of closed RBDs. We have considered two opposite glycan configurations relative to the RBD center of mass, namely, one glycan at the RBM and the other in the "head" of the RBD. By computing the percentage of hydrogen bonding with the surface during the simulations (Figure 1A), the glycans are inducing the most H-bonds with the surfaces, highlighting the crucial role of glycans in surface adsorption [2]. On the other hand, glycans have shown to mainly adsorb in a 2-dimensional (parallel to the surface) fashion in the presence of hydrophobic surfaces (PBL0), while for hydrophilic surfaces (PBL1), 3D fluctuations are their main contributions (Figure 1B). We finally compared the RBD results with the S1 and found consistent configurations at the contact regions for both open and closed models. Potentially, our study will help to understand how glycans can adsorb to surfaces of different polarities.

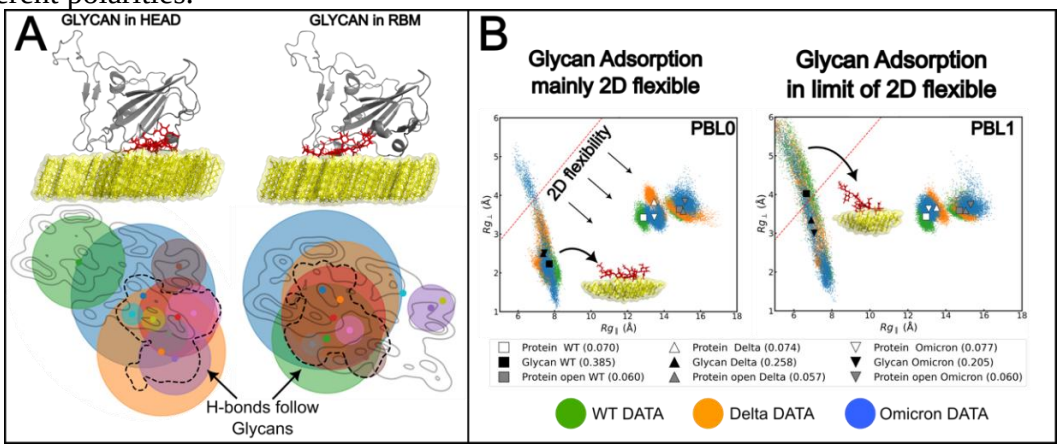

Figure 1: (A) Two configurations considered for closed-RBD simulations (top), and the corresponding H-bond circle representations. The size of the circles represents the percentage of H-bonds during the simulations. Discontinuous black lines represent the position of the glycans (bottom). (B) Position contours of three RBDs in the S1S (top), and the contact histograms of each RBD to the surface. Colors of contours and histograms are consistent and represented in the bottom view of the S1S.

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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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Atomic Force Microscopy measurements of skin stiffness in neutrophil-depleted mice

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Among the different strategies of biological systems for their defense against environmental threats, physical barriers that confer mechanical protection and prevent microbial entry is one of the most evident. The innate immune system is considered the main force of defense against invading pathogens once they have entered the body. Immune leukocytes are particularly committed to this mission, and neutrophils constitute a paradigmatic example of highly migratory leukocytes that rapidly detect and colonize areas of infection in order to trigger a diverse response that includes an avid phagocytic capacity. Interestingly, neutrophils also populate non-infected tissues, acquiring different phenotypes and functions. Recently, a population of neutrophils has been reported to produce extracellular matrix in the skin, reinforcing its structural and mechanical properties and contributing to shield the organism from external challenges even before they occur [1]. Here, we apply Atomic Force Microscopy to measure the stiffness of the skin of neutrophil-depleted mice in comparison to wild-type counterparts in order to determine the physical effect of neutrophils in this tissue and their mechanical contribution to this primary barrier of defense.

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Evidence of the bottom stiffness effect on atomic force microscopy-based cell mechanobiology

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Atomic force microscopy (AFM) is the leading method for characterizing the nanomechanical properties of cells. These properties are determined through model fitting. Semi-infinite contact mechanics models predict that force depends on the mechanical properties of the cell, indentation, and tip geometry [1-4]. In contrast, finite-thickness rheological models suggest that force should also depend on the rigidity of the substrate, although this effect has never been experimentally observed. If it were present, it would make cells appear stiffer than they actually are. In this study, we designed a force-distance curve experiment to investigate the influence of substrate rigidity on the measured forces and apparent moduli obtained by AFM. Data fitting using a semi-infinite power-law rheological model showed an increase in the apparent modulus with increasing force. However, this behavior was identified as an artifact that disappeared when a bottom-effect correction model was applied. Our findings demonstrate that the force applied to a cell is intrinsically influenced by the stiffness of the substrate, whereas the true mechanical properties of the cell remain unchanged.

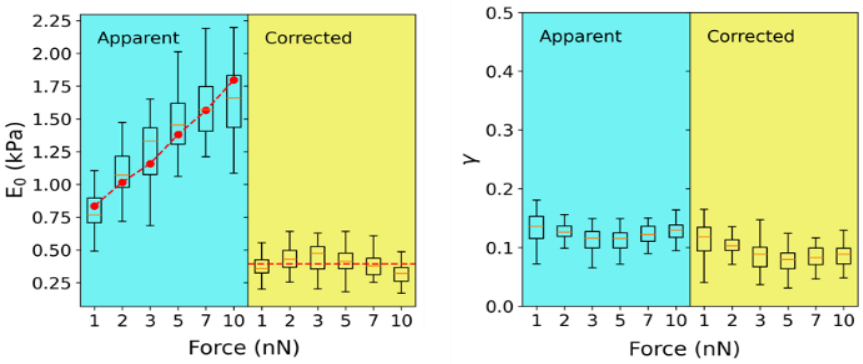

Figure 1. Apparent and corrected nanomechanical parameters of HeLa cells cultured on a Petri-dish**.** Modulus and fluidity coefficients obtained over a cytoplasmatic region.

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Towards oil droplet based nanoindentation for cell mechanics

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Atomic force microscopy (AFM) indentation techniques have significantly advanced the mechanical characterization of living cells[1]. However, classical AFM indentation presents limitations, including difficulty in rapidly changing indenters and limited flexibility in adjusting indenter geometry. FluidFM, an AFM technique based on hollow cantilevers[2], partially addressed these issues by enabling reversible capture of spherical beads for indentation[3]. Nevertheless, the bead capturing process remains tedious and time-consuming.

In this study, we propose and characterize an innovative approach leveraging FluidFM's capability to generate spherical oil droplets[4], thus removing the necessity for manual bead handling. For indentation-based mechanical assessments, precise knowledge of the indenter's geometry is essential, alongside a thorough understanding of the interactions between the indenter and the cell during the contact phase. Therefore, to accurately control and characterize the geometry and size of these oil droplets in real-time, we implement a self-excited cantilever strategy. Cantilevers are maintained in resonance via a feedback loop that dynamically tracks resonance frequency changes induced by droplet size variations. To enhance the stability and precision of this self-excitation process, an Arduino-based control system is integrated within the feedback loop. This system applies a time shift, amplitude correction, and saturation to the signal from cantilever deflection to the piezo actuator, maintaining consistent resonance conditions.

Employing oil droplets as dynamic indenters offers multiple advantages: automatic droplet creation, rapid size tuning for adjustable measurement resolution, and the use of soft, deformable probes that more closely mimic biological materials' properties—providing gentler, more physiologically relevant indentations, similar to biomembrane force probes.

By comparing our experimental data with established contact mechanics models, we aim to develop a robust, versatile platform to advance cellular mechanics studies.

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Quantitative Biomechanical Profiling of Transformed Human Corneal Epithelial Cell

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Abstract- The mechanical properties of cells, influenced by the interaction between cortical surface mechanics and cytoskeletal viscoelasticity, show distinct variations throughout different stages of the cell cycle[1,2]. This study uses a correlative multimodal approach, combining bimodal atomic force microscopy (AFM), force mapping, and load-rate analysis, to examine the biomechanical characteristics of human corneal epithelial cells (HCE-T) in both their mitotic (dividing) and interphase (resting) stages. Our findings reveal that these mechanical differences are driven by the dynamic roles of the cortex and cytoskeleton during cellular reorganization, rounding, and alterations in adhesion and viscoelastic properties. These changes, alongside the complementary roles of microtubules and actin, highlight a shift in the mechanical properties of the cytoplasm during cell division. Quantitative microscopy[3] demonstrates significant differences in stiffness, viscosity, adhesion, and loading rate responses between the cell cycle stages, reflecting the complex frequency and time-dependent behavior of cellular components. This novel approach provides a valuable platform for understanding cellular transformations and offers important insights for applications in mechanobiology and biomedical engineering.

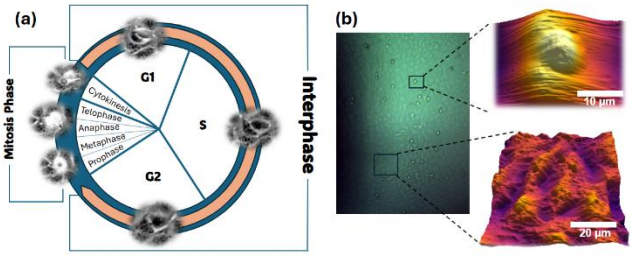

Figure **1**. Different cell cycles captured by phase contrast images (**a**) which then imaged by AFM for quantification (**b**).

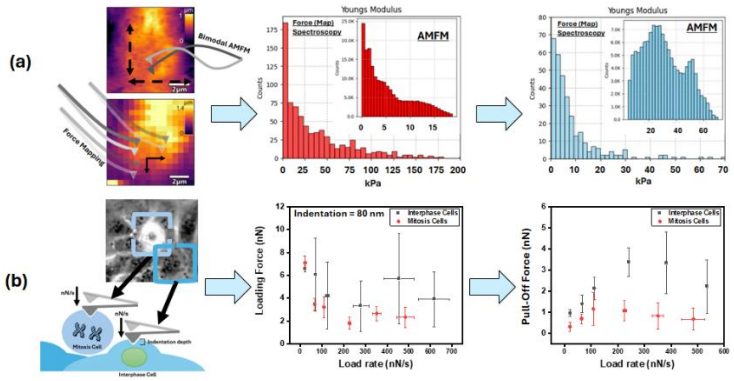

Figure **2**. Correlative multimodal approach combining bimodal AFM and force mapping (**a**) with load-rate analysis (**b**) to examine the biomechanical characteristics of different cell cycles.

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T-cell immunomechanics: nanomechanical adaptations in tumor-like conditions

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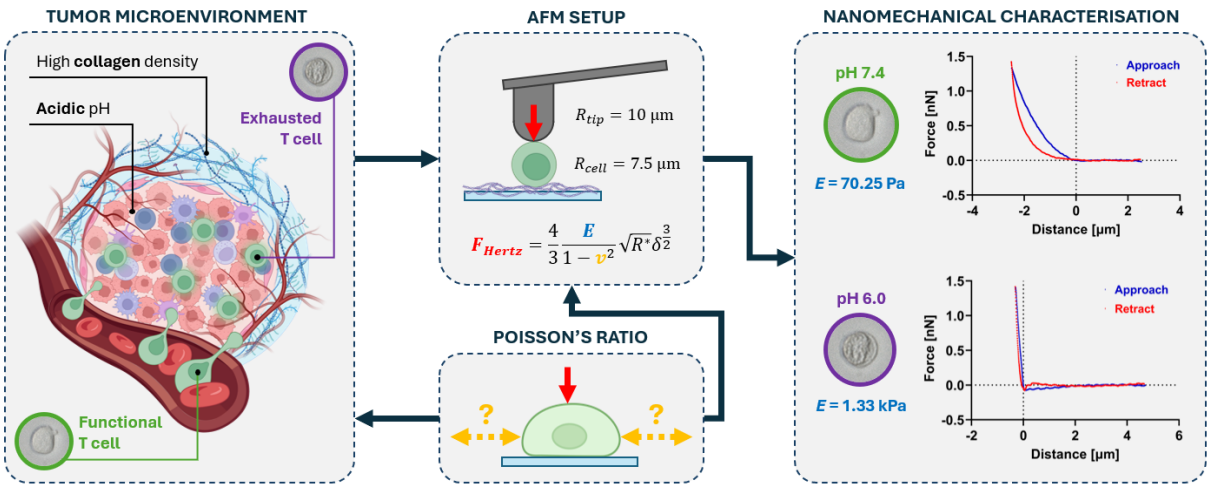
T cells circulate through the bone marrow, thymus, lymphatic system, and bloodstream, undergoing diverse mechanical stimuli. Their cytotoxic function against infected, damaged, or cancerous cells also involves exerting forces and altering mechanical properties [1]. However, our understanding of immunomechanics — the intrinsic mechanical properties of immune cells and their relationship to cellular physiology — remains very limited.

In this proof-of-concept study, we investigate how key tumor microenvironment (TME) conditions [2], such as acidic pH and high collagen concentration, influence T-cell mechanics. Using atomic force microscopy (AFM), fluidic force microscopy (FluidFM) and optical microscopy, we quantified changes in mechanical properties of the cells. Preliminary results indicate that the Young's modulus, rugosity and the stress relaxation response of the T cells, as well as their morphological features, are affected by the altered environmental conditions.

Additionally, since current studies assume T cells to be perfectly incompressible, we aim to experimentally estimate the Poisson's ratio of T cells. This would not only refine Young's modulus measurements obtained through Hertz model fitting but also provide deeper insights into the mechanical implications of T-cell diapedesis — squeezing through endothelial cell junctions — during extravasation, and interstitial migration through the dense TME [3].

Ultimately, this research contributes to deciphering the mechanical fingerprint of T cells in healthy and pathological states, which is crucial for the development and optimisation of immunotherapies. Further research in this direction could enable clinically relevant advances such as the improvement of CAR T cell therapies for solid and brain tumors [4].



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Nanomechanical stimuli and activity of Ca ion channels in HeLa cells

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We have studied the influence of external forces on the activation of ion channels associated with the intracellular Ca^{2+} activity. We have applied three different types of mechanical force: (i) by using compressive forces exerted by an AFM tip; (2) by applying a sustained lateral force and (iii) by applying an oscillatory lateral force. The lateral force was applied by developing a nanostretching device. We observed a significant enhancement of the presence of intracellular Ca^{2+} by applying lateral forces. On the other hand, the application of compressive forces does not produce any observable increase in the fluorescence signal associated with the presence of intracellular Ca^{2+}.

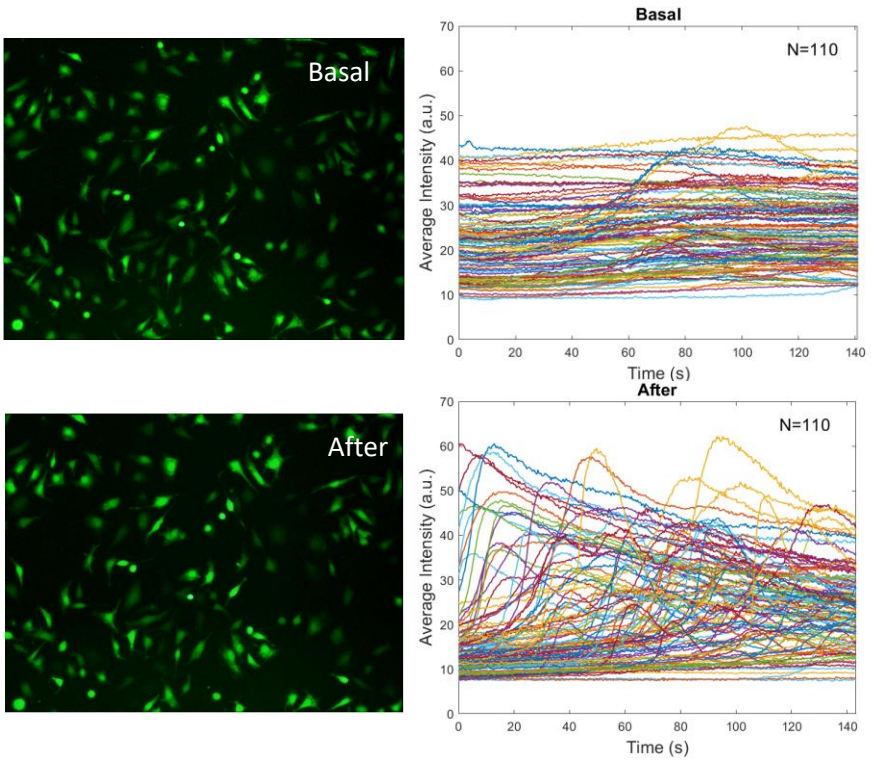

Figure. (a) Fluorescence images of the intracellular Ca^{+2} activity, before and after mechanical stimulation. (b) Average fluorescence signals of the cells

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 [1], [2]. 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 [1]. The present contribution goes beyond these simple approaches, and presents 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 considering information from its surroundings resulting in a better contextualization of each point within the image.

The proposed neural network, trained using data derived from calculations of experimental data, 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 to achieve accurate predictions. This reduces even further the amount of data that is needed and thus the total execution time. The application of this methodology in eukaryotic and bacterial cells is demonstrated, showing the potential of this approach for high throughput nanoscale dielectric imaging of biological samples and for its overall contribution to the development of multiparametric AFM methods.

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[1] M. Checa, R. Millan-Solsona, A. G. Mares, S. Pujals, and G. Gomila. Small Methods, vol. 5, no. 7, p. 2100279 (2021), “Fast Label-Free Nanoscale Composition Mapping of Eukaryotic Cells Via Scanning Dielectric Force Volume Microscopy and Machine Learning,”

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Impaired Nuclear Viscoelasticity upon MG-induced glycation

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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 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 scaling modulus and fluidity coefficient 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. Complementary confocal microscopy reveals a structurally compromised nuclear lamina in MG-treated nuclei, presenting features such as blebbing and nuclear envelope invaginations (Fig. 1D). By connecting MG-induced biochemical changes to nuclear mechanics, this work highlights a potential mechanism for diabetes-associated tissue stiffening and pathogenesis.

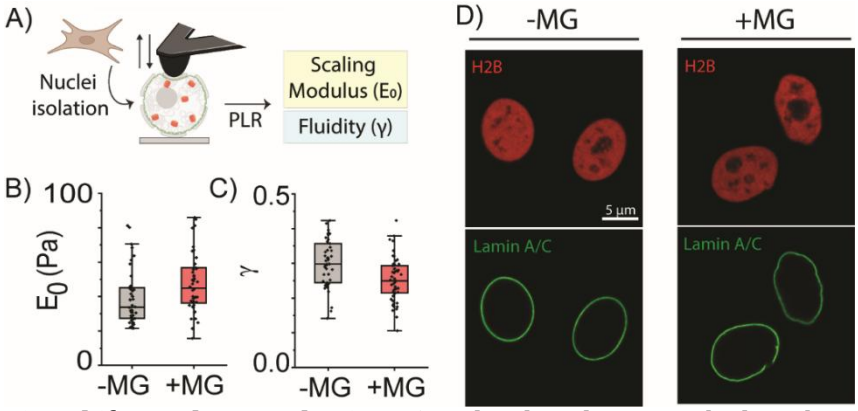

Figure 1. Glycation shifts nuclear mechanics. A) Isolated nuclei are probed to obtain its viscoelastic properties by applying Power Law Rheology (PRL) model. B-C) E0 and γ of the isolated nuclei before (-MG) and after 6 h incubation with methylglyoxal 50 mM (+MG). Each dot represents the mean of three single force-distance curve performed on a nucleus. The box represents the IQ 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.

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AFM study of the adaptive mechanisms of blood cells under the influence of physical and chemical factors

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The molecular organization of blood cell membranes is directly related to their functional activity and ability to adapt to conditions such as acidosis, alkalosis, inflammation, genetic modifications, etc. To study these processes in detail, we used atomic force microscopy (AFM), which allowed us to obtain 2D and 3D high-resolution images of cells and assess their stiffness using atomic force spectroscopy.

Our study aimed to develop a comprehensive biophysical approach to analyzing the molecular structure of membranes and studying their adaptive mechanisms.

The study results showed that the membranes of erythrocytes, neutrophils, and macrophages exhibit high plasticity and adaptability [1, 2]. For example, hypoxia improves the properties of erythrocyte membranes, while xenon has a neutral effect [3]. Changes in pH and the presence of iron ions disrupt membrane structure [4]. Neutrophil activation leads to significant alterations in membrane topography associated with the formation of neutrophil extracellular traps (NETs). Macrophages demonstrate different membrane structures depending on their functional state (M1 or M2 phenotype).

AFM enabled a detailed characterization of these processes and the identification of key adaptive mechanisms in blood cell membranes. The obtained results are important for understanding the mechanisms of hemostasis, inflammation, and immune response and highlight AFM as a tool for diagnosing various diseases.

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Morphology of neutrophils under the influence of serum from patients with infectious pathologies

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Neutrophils play a key role in the immune response, particularly in infectious and inflammatory processes. Investigating their functional state under the influence of various biological media, such as blood serum, is of significant interest for understanding the pathogenesis of infections and sepsis. In this study, we examined the effect of serum from healthy donors, patients with localized infection, and patients with sepsis on neutrophils from healthy donors using classical wide-field fluorescent microscopy and atomic force microscopy (AFM). Neutrophils were incubated with serum from three patient groups: (1) healthy donors, (2) patients with localized infection, and (3) patients with sepsis. Fluorescence microscopy was used to assess morphology. AFM was applied to analyze the surface topography of neutrophils.

Fluorescence microscopy did not reveal significant differences in neutrophil morphology between the groups. Data from classical AFM also did not indicate a clear impact of serum on the cells. However, the Peak Force QNM method demonstrated that adhesion was the only parameter that showed group differences. This result suggests subtle changes in neutrophil membrane states that are not detectable by traditional methods. This finding confirms that highly sensitive mechanical property analysis techniques, such as Peak Force QNM, can help study cellular responses to biological factors.

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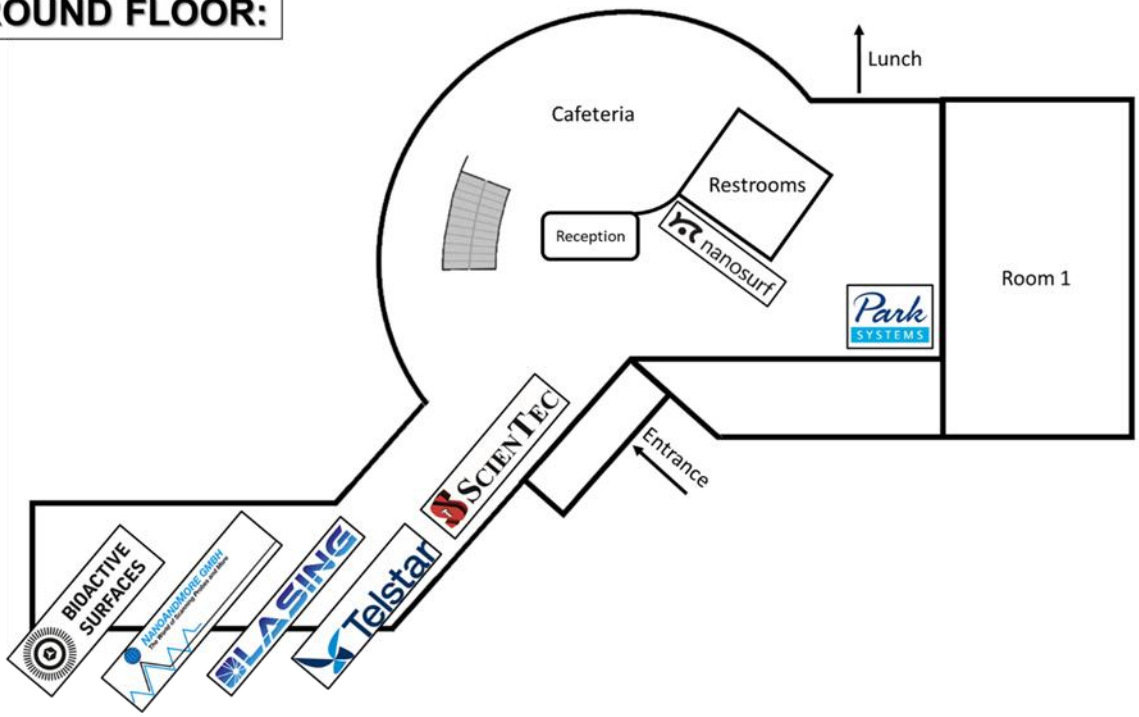
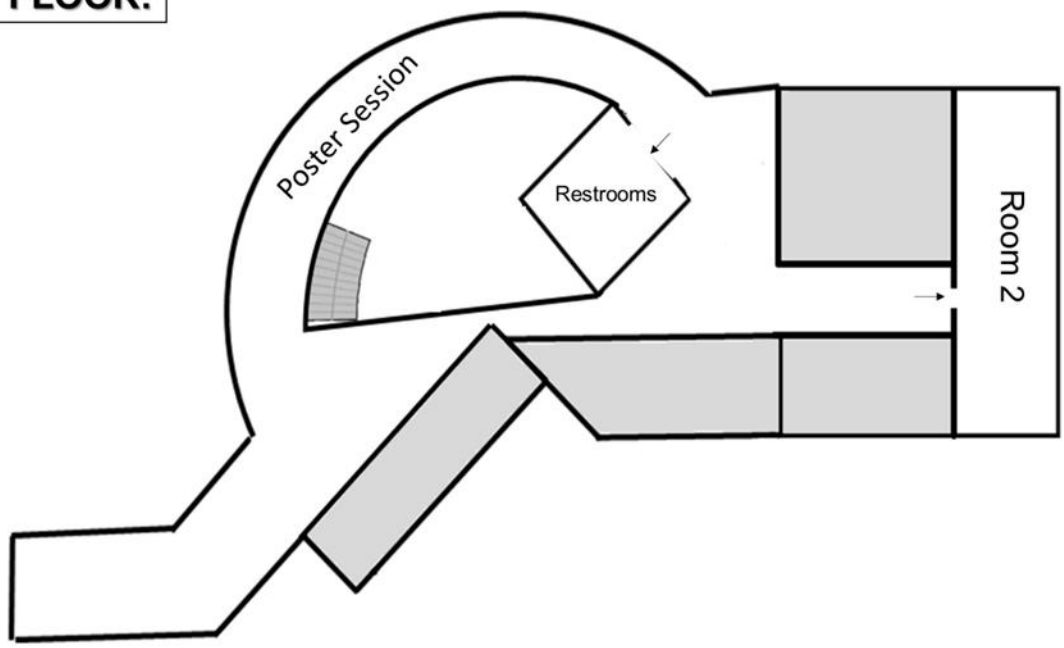
Conference venue and Maps

The venue is the Instituto de Ciencia de Materiales de Madrid (CSIC). The ICMM is located in the Campus of Cantoblanco, c/ Sor Juana Inés de la Cruz 3, 28029 Madrid.

GROUND FLOOR:



FIRST FLOOR:



From Madrid to the Conference venue and back

By train:

In Madrid Chamartin Train Station take the train C4a or C4b in the direction of Alcobendas/San Sebatian de los Reyes or Colmenar Viejo (either of these two work)

Ride 2 stops to Universidad Cantoblanco

Walk to the Institute

By bus:

In Plaza de Castilla Station take the buses 712/713/716 (any of these work).

Ride 8 stops to Ctra M-607 – Hosp Cantoblanco

Walk to the institute.

By train:

Walk to the train Station

Take the train C4a or C4b in the direction of Chamartin

Ride 2 stops to Madrid Chamartin Train Station

By bus:

From the institue, walk to Marie Curie-facultad Informática

Take the bus 714 to Madrid.

Ride to Plaza de Castilla

From the Residencia to the Conference venue (ICMM, c/ Sor Juana Ines de la Cruz 3)

1 Walk from the Residencia to Nuevos MInisterios train station (about 15 min)

2 Take train either C4a or C4b lines

3 Leave train at Cantoblanco Universidad

4 Walk to the ICMM (10 min)

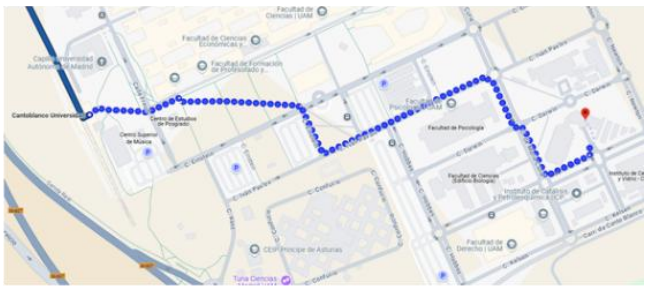
Back, same but reverse the sequence

Directions from the Residencia de Estudiantes to the metro Station:

Entrance to the Residencia de Estudiantes →

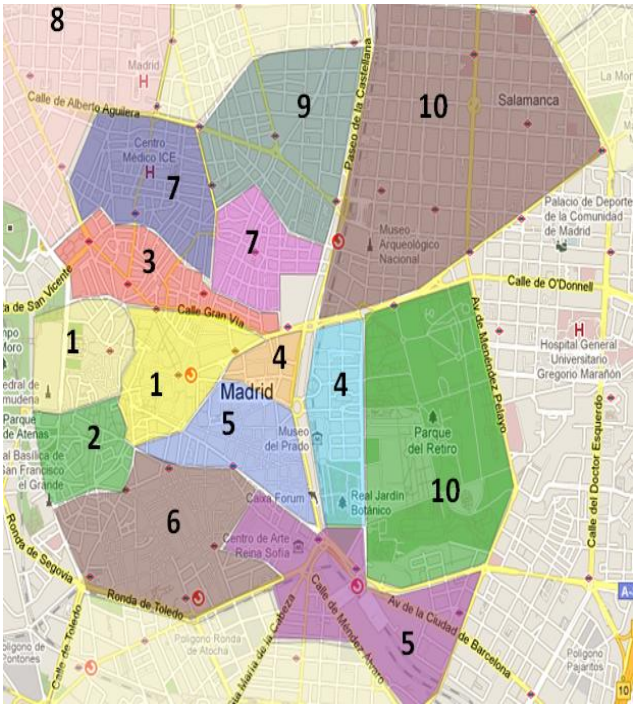
From Hotel EXE Tres Cantos to the Conference venue and back (20-25 min)

1. Walk to the train station Tres Cantos (line C4b)
2. Take the train (in the direction of Parla)
3. Ride 2 stops to Cantoblanco
4. Walk to the institute



1. From the Institute walk to the train station Cantoblanco (line C4b)
2. Take the train (in the direction of Colmenar Viejo)
3. Ride 2 stops to Tres Cantos
4. Walk to the Exe Hotel

DISCOVER MADRID



1.- **Puerta del Sol and Opera:** Madrid's core METRO: Sol (L1, L2 and L3) / Opera (L2, L5 and R)

2.- **Austrias and La Latina:** Old Madrid and popular Madrid. METRO: AUSTRIAS: Sol, Opera (L2) LA LATINA: La Latina (L5)

3.- **Gran Vía street up to Plaza de España:** Callao shopping area. METRO: Gran Vía (L1, L5) / Callao (L3, L5) /Plaza de España (L3, L10, L2)

4.- **Paseo del Prado - Cortes:** Museums area. METRO: Banco de España (L2) / Atocha (L1)

5.- **Atocha - Huertas area:** Literary Madrid. METRO: ATOCHA: Atocha & Atocha station (L1) HUERTAS: Antón Martín (L1), Sevilla (L2)

6**.- Lavapiés and El Rastro fleemarket:** Multicultural Madrid. METRO: Lavapiés (L3)/ Tirso de Molina (L1)/ La Latina (L5) / Antón Martín (L1)

7.- **Chueca and always young Malasaña:** Madrid's Soho. METRO: CHUECA: Chueca (L5) MALASAÑA: Tribunal (L1,L10) /Bilbao (L1, L4) /Noviciado (L2)

8.- **Argüelles and Moncloa: Complutense University area.** METRO: Plaza de España (L3, L10, L2) /Ventura Rodriguez (L3) /Arguelles (L3, L4) / Moncloa (L3, L6)

9.- **Colón, Alonso Martínez and Bilbao:** Residential areas. METRO: Bilbao (L1,L4) /Alonso Martinez (L4,L5,L10) /Colón (L4)

10.- **Salamanca and Retiro: Exclusive shopping area.** METRO: Colón/ Serrano/ Velázquez/ Lista (L4)/ Goya (L3, L5)/ Retiro/Príncipe de Vergara (L2)

You can check touristic alternatives at https://www.esmadrid.com/en (english).

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