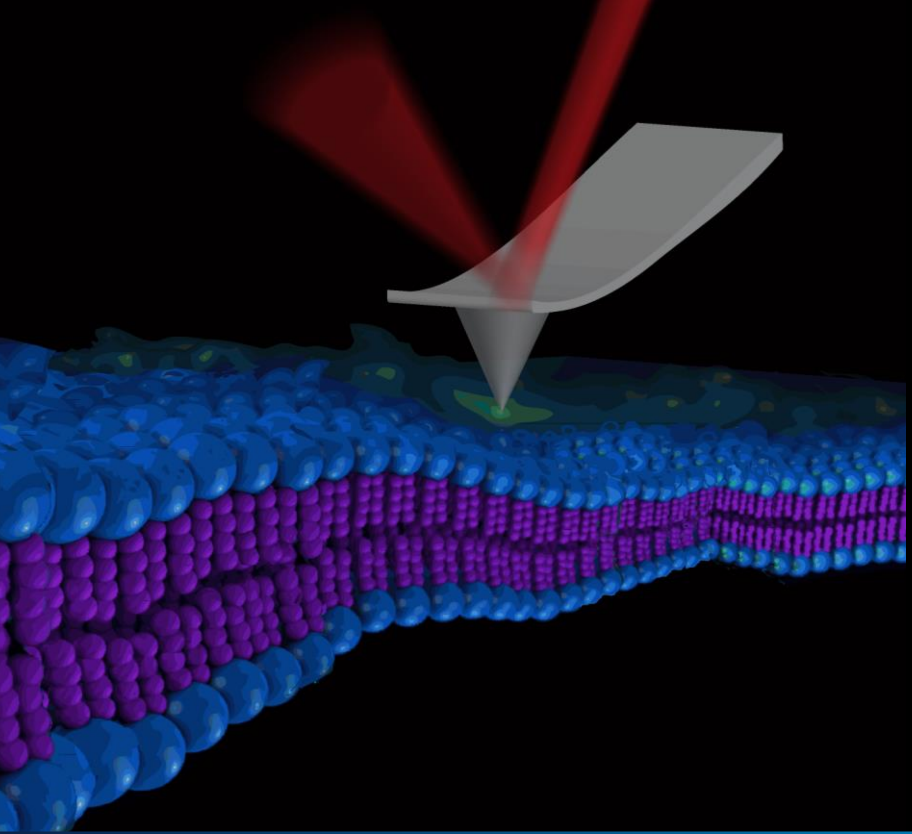
Inserm

La science pour la santé
From science to health

Aix*Marseille université
Socialement engagée



High-Speed Atomic Force Microscopy

A tool for studying dynamic membrane remodeling processes

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26th April 2024

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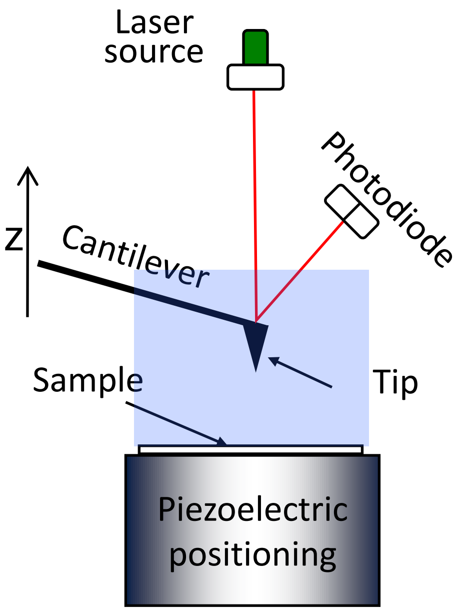
https://sites.google.com/view/fm4b-lab/home

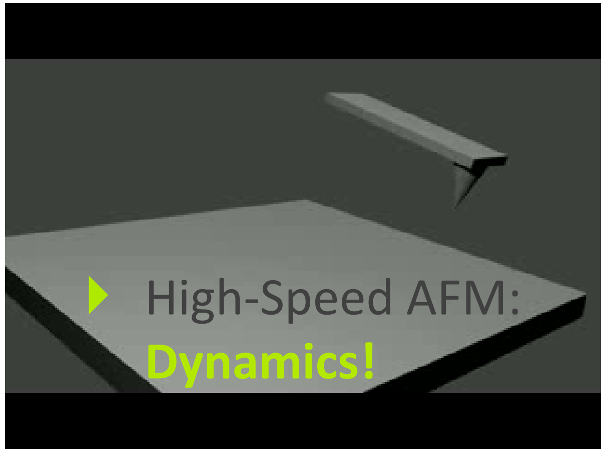
Outline

Atomic Force Microscopy

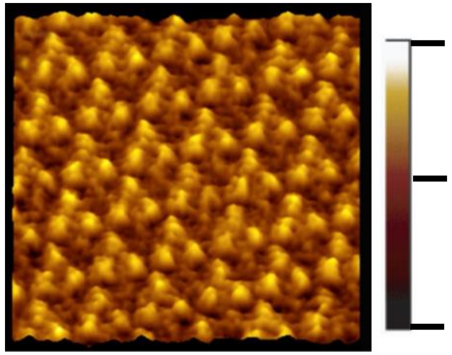


Imaging

- Submolecular resolution
- Liquid environment
- **Structure**



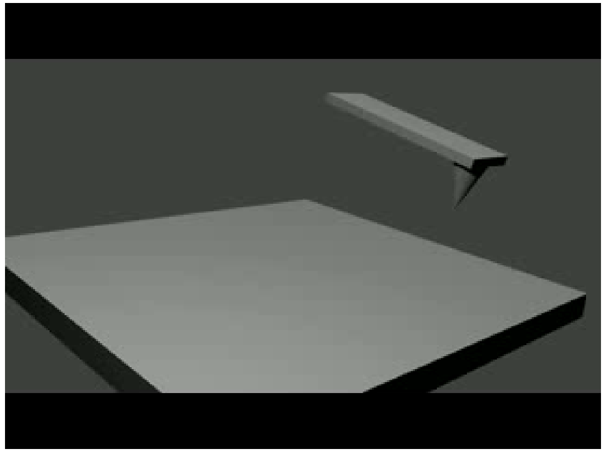
Example: 2D crystal of bacteriorhodopsin

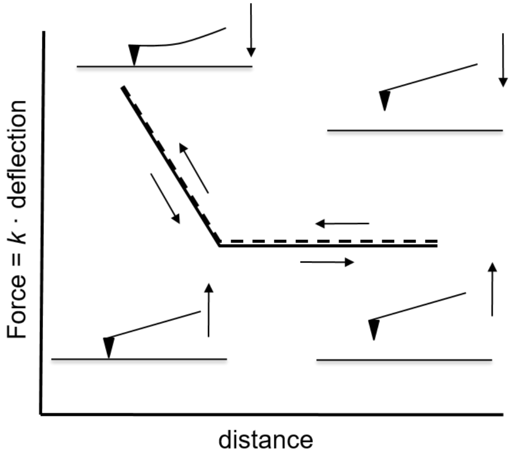


Fotiadis, FEBS Letters 2001

Force Spectroscopy

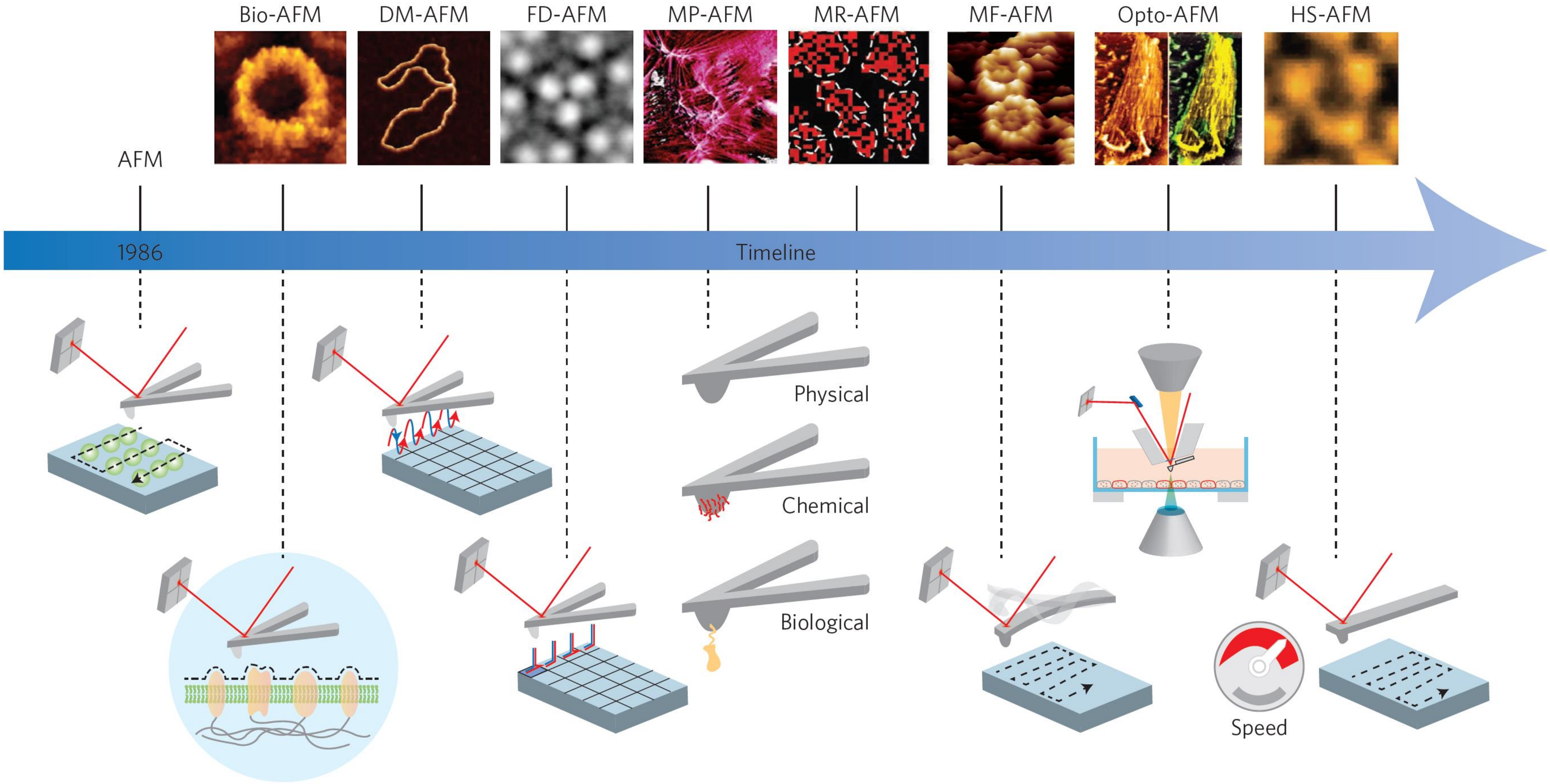
- pN resolution
- Liquid environment
- **Mechanics**





Atomic Force Microscopy

Timeline of key inventions



Dufrene et al. Nat. Nanotech. 2017

Outline

01. Introduction
- Atomic Force Microscopy (AFM)
- Why fast? High-Speed AFM
- High-Speed ‘breakthrough’ examples
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High-Speed AFM

Why fast?

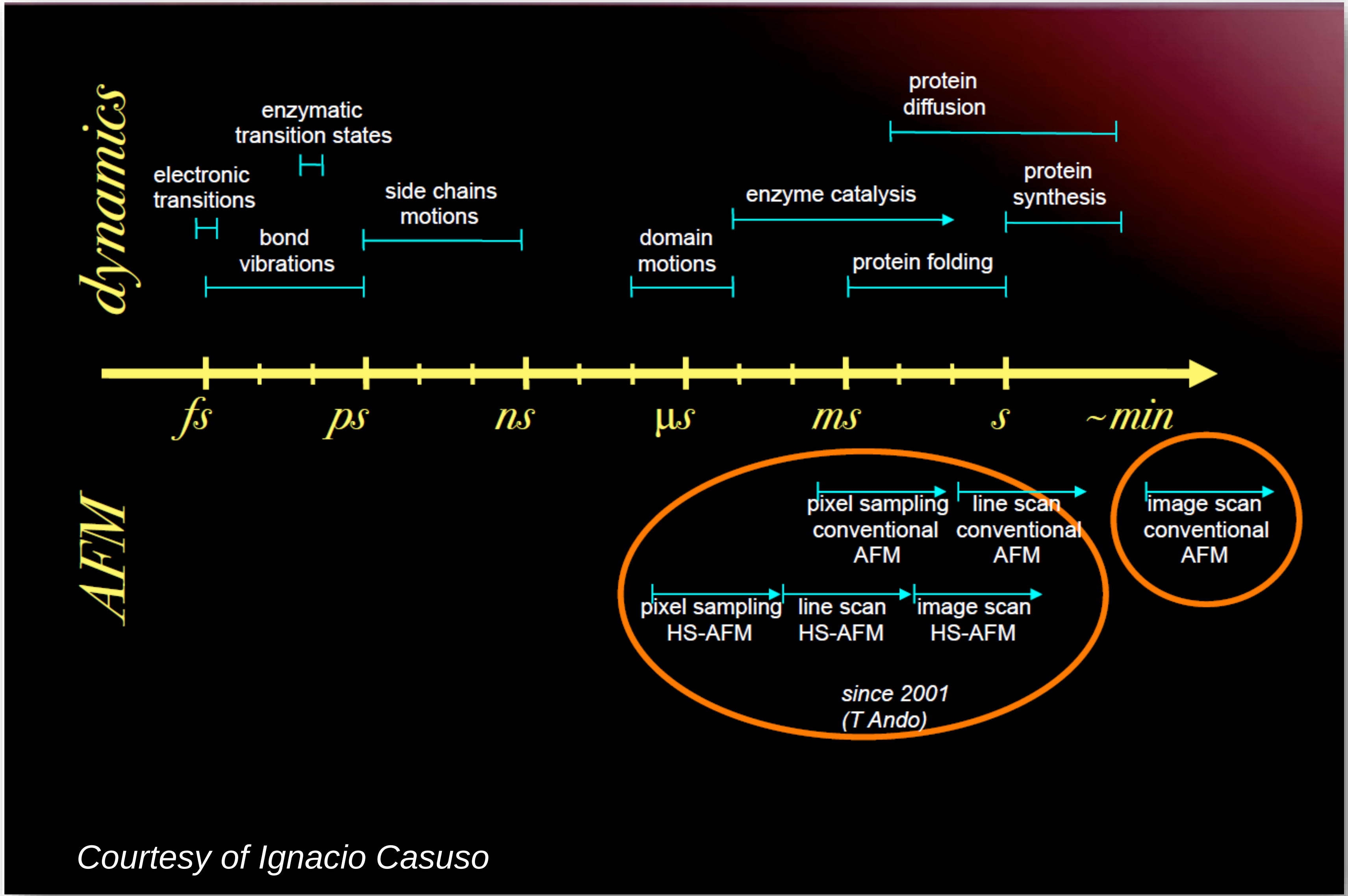
- Most biological phenomena in cells are due to cascades of dynamic molecular processes:

    -Conformational changes
    -Binding and dissociation
    -Assembly and disassembly of proteins
    -...

- We try to understand the dynamics of a small number of molecules.
- Single-molecule approach to monitor individual molecular behaviours.
- Direct method with high spatiotemporal resolution to monitor dynamics!

High-Speed AFM

Why fast?



The born of the High-Speed AFM

- The group of Hansma, 1993 (Viani et al, 2000 Nat Struct Biol 7:644–647)
- Toshio Ando's team: first prototype in 2001
- First results in dynamic imaging of biomolecules by the group of Toshio Ando in 2008 (Ando et al., 2008 Prog Surf Sci 83:337–437)

- Toshio Ando

High-Speed AFM

Operates in tapping mode

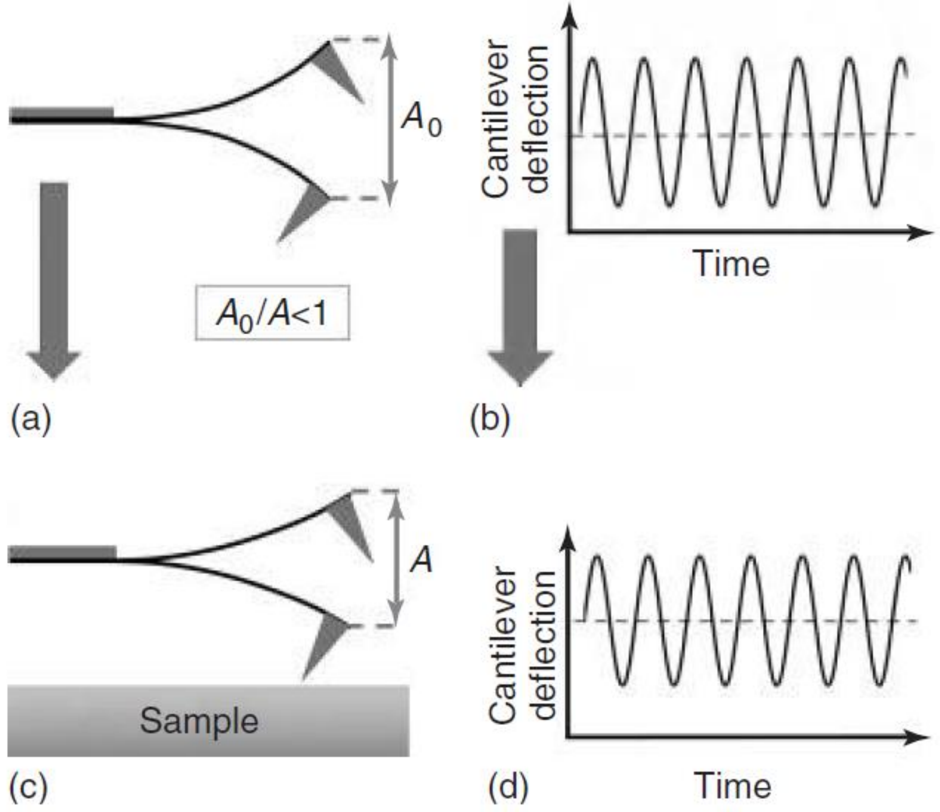
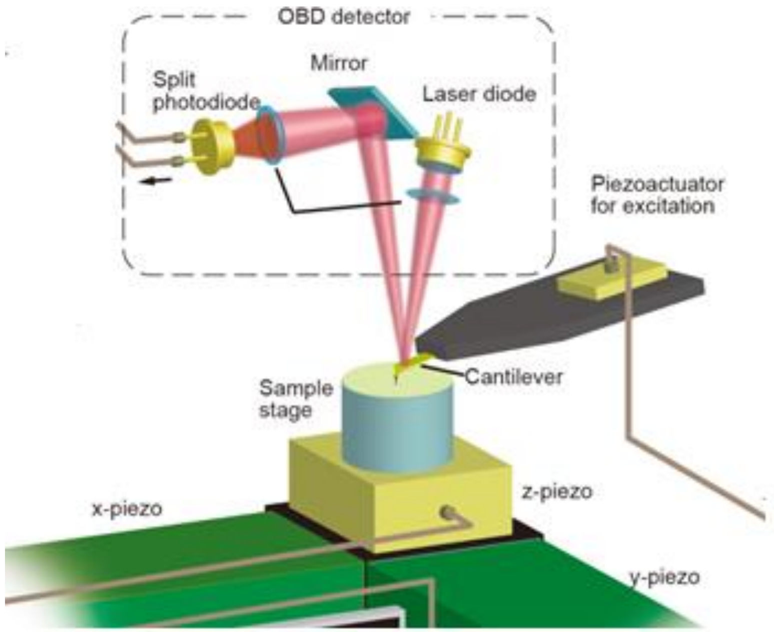


Figure 1.11 Principles of dynamic-mode AFM. (a) Cantilever driven at its free resonance frequency f_0 with amplitude A_0. (b) Rendering of the cantilever deflection as a function of time using, for instance, the beam deflection method. (c) The cantilever is approached to the sample surface and the oscillation amplitude is reduced to A. (d) Same as in (b) but with the cantilever near the surface.

AFM in liquid: biological applications, Ed. Baro & Reifenberger, Wiley 2012

High-Speed AFM



High-Speed AFM:

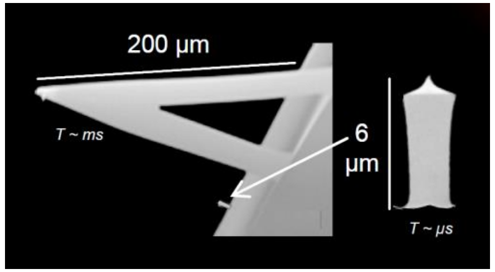
1000 x faster than AFM

Buffer solution

Ambient pressure

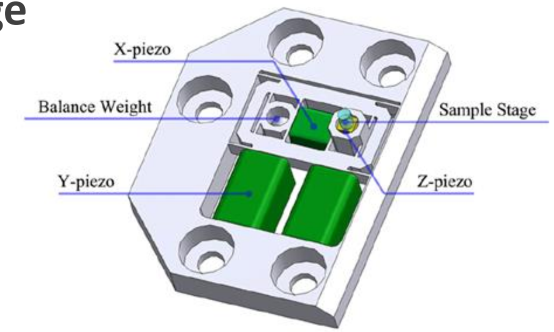
Ambient temperature

01 Small cantilevers



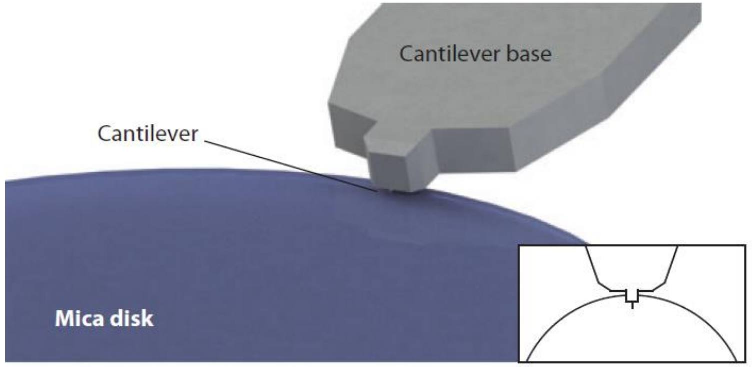
resonance ~1/ $\sqrt{mass}$

02 Small scanners & sample stage



03 Faster electronics

04 Dynamic controller



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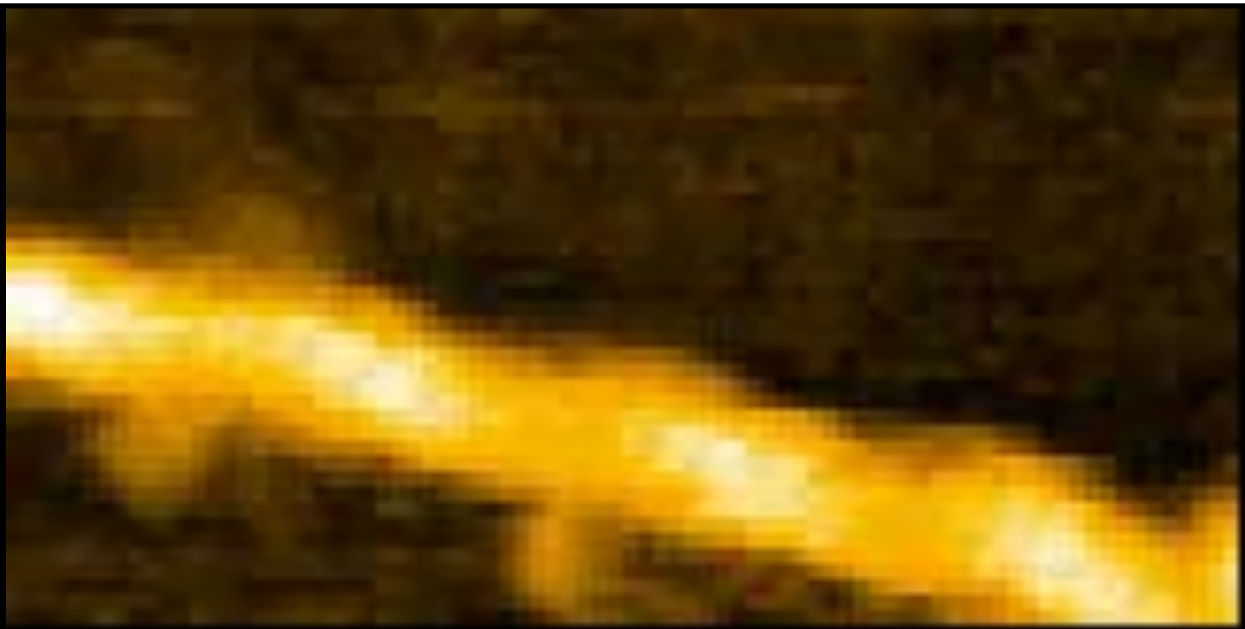
High-Speed imaging: examples

Milestone work of biomolecular imaging showing unprecedented details of myosin V walking and tension



frame rate 140 ms
scan size 130 x 65 nm^2

milestone work of biomolecular imaging showing unprecedented details of myosin V unidirectionally walking and tension generation

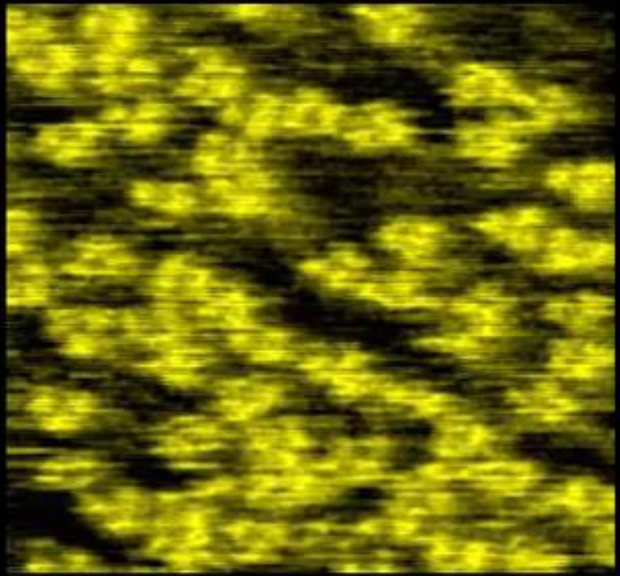
[Kodera et al., Nature, 2010, 468, 72]

High-Speed imaging: examples

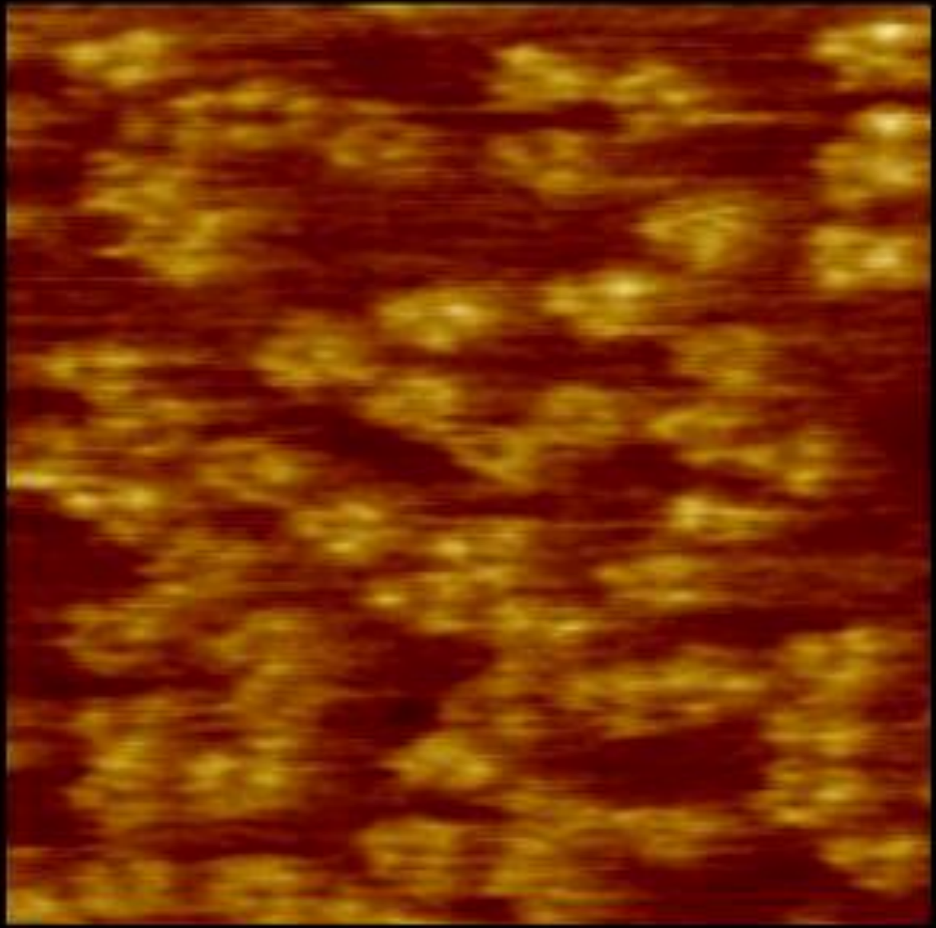
Lateral and rotational diffusion dynamics of OmpF

<u>How are the OmpF molecules distributed in the bacterial membrane ?</u>

Location of Porins in the membrane is critical for Cell Transport



frame rate 477 ms
scan size 75 nm^2

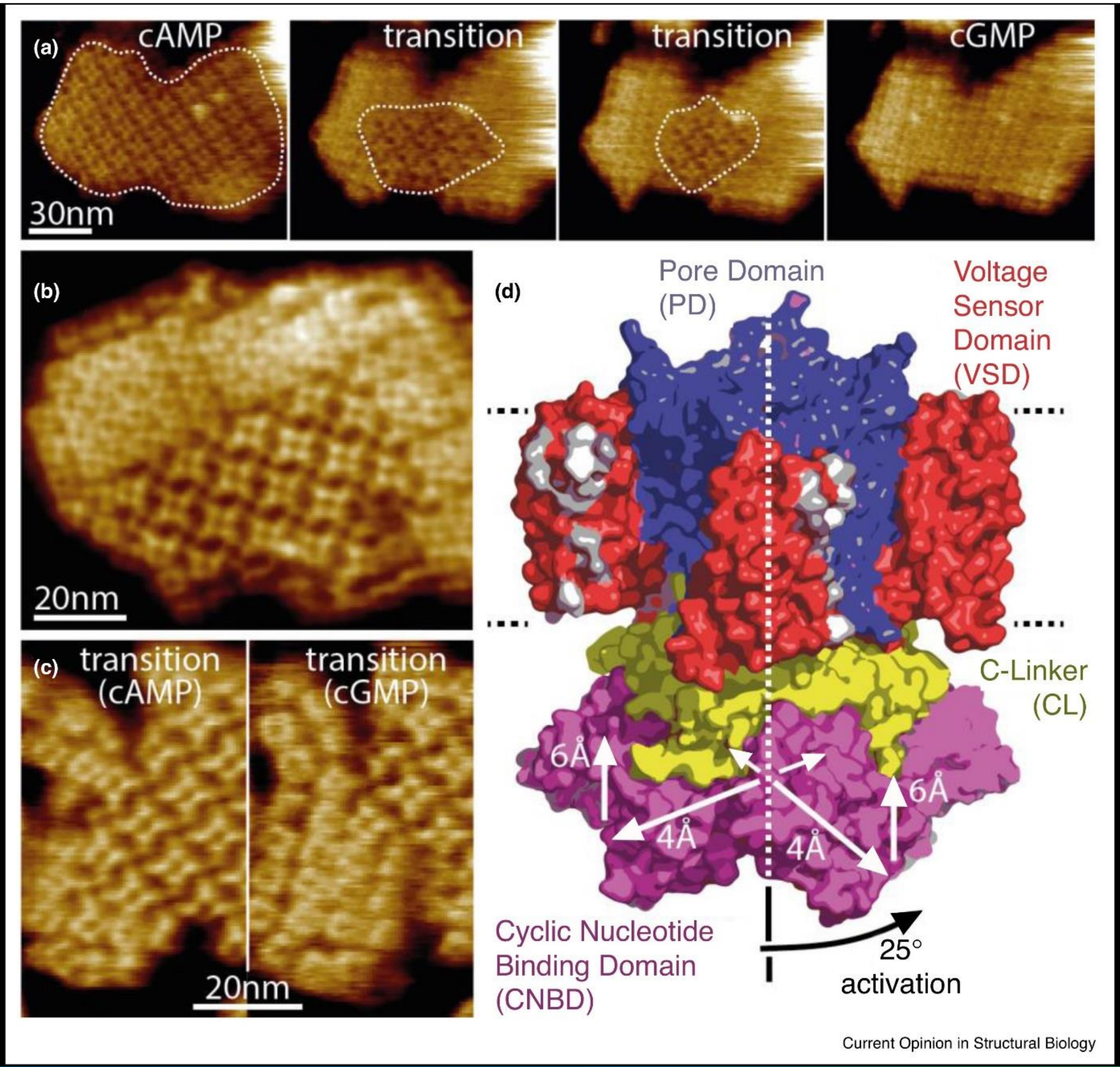


frame rate 204 ms
scan size 75 nm^2

[Casuso et al., Nat. Nanotech., 2012, 7, 525]

High-Speed imaging: examples

Cyclic nucleotide-gated (CNG) ion channels are non-selective cation channels



[Marchesi et al., Nat. Com., 2018, 9, 3978]

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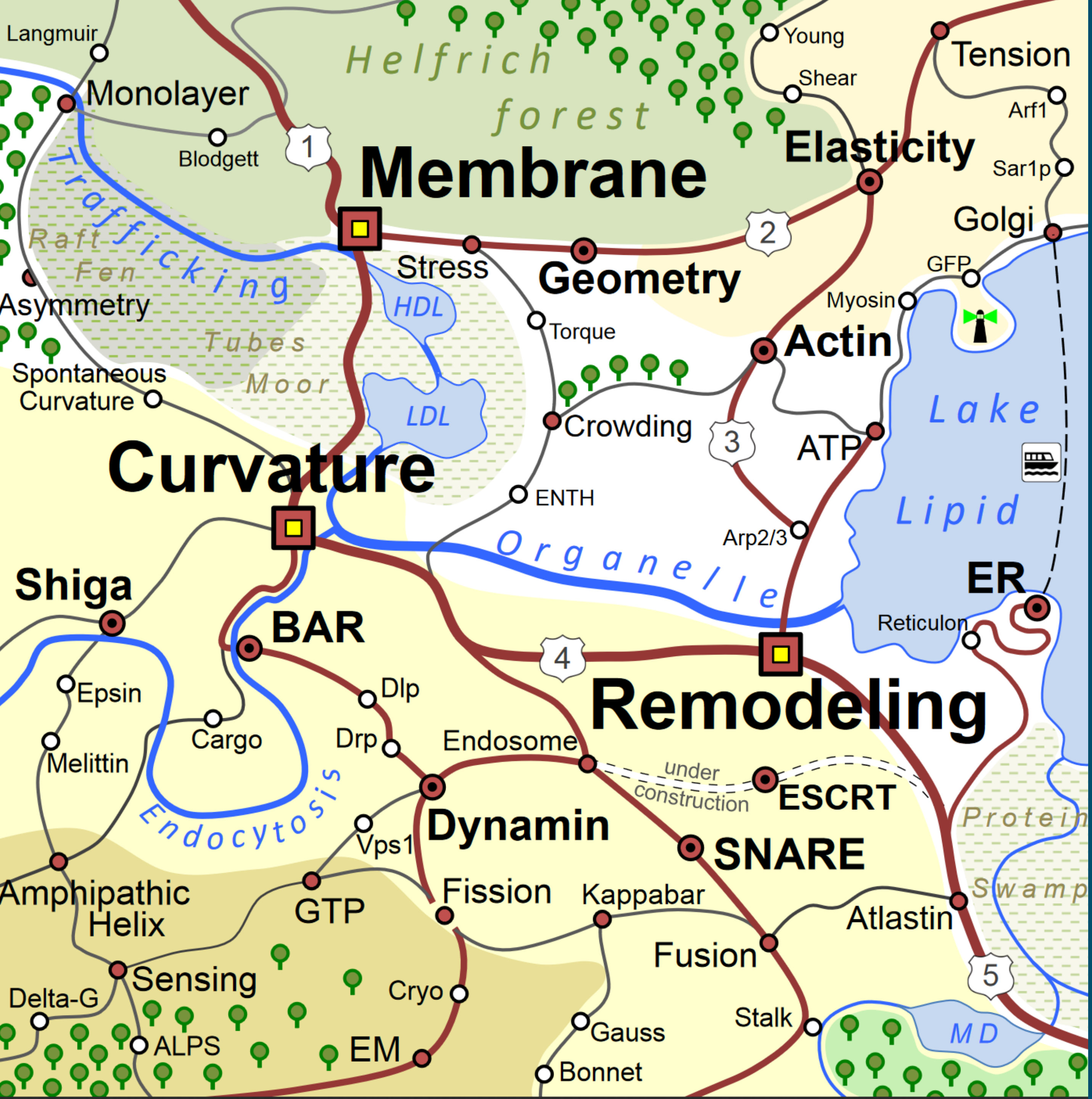


Motivation

Strucure, mechanics and **dynamics** of membrane remodeling

- Deformation
- Fusion and fission
- Cell signaling

The **dynamics** of biological processes (sub-sec to min) is essential to understand their function

Structure, **mechanics** and **dynamics** are only available with advanced microscopies: Atomic Force Microscopy

Image source: M. Deserno in Bassereau *et al.*, J. Phys. D: Appl. Phys. 51, 343001 (2018)

Biological membranes

The current view of the plasma membrane

- **Fluid-mosaic model**

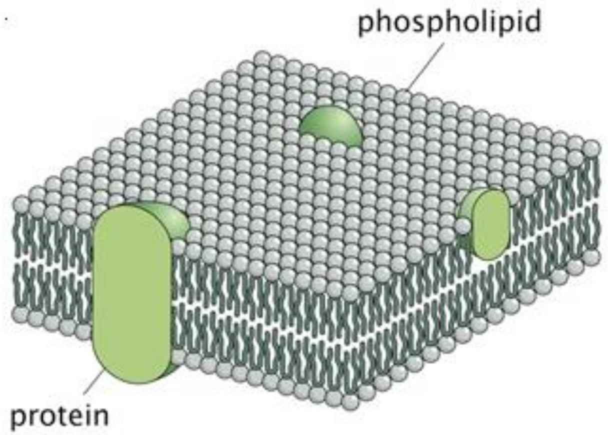
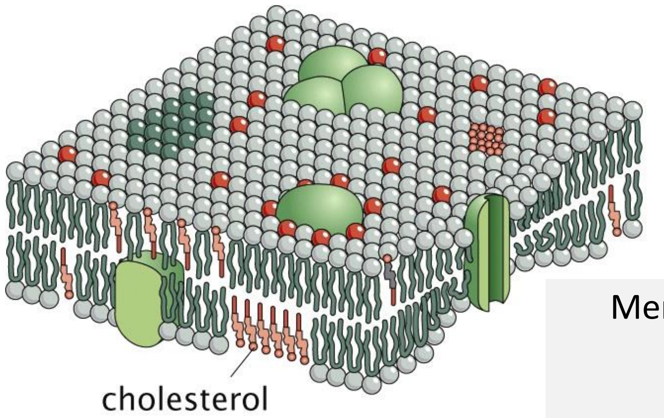


Figure 11.4ab Physical Biology of the Cell, 2ed. (© Garland Science 2013)

- **Updated model!**

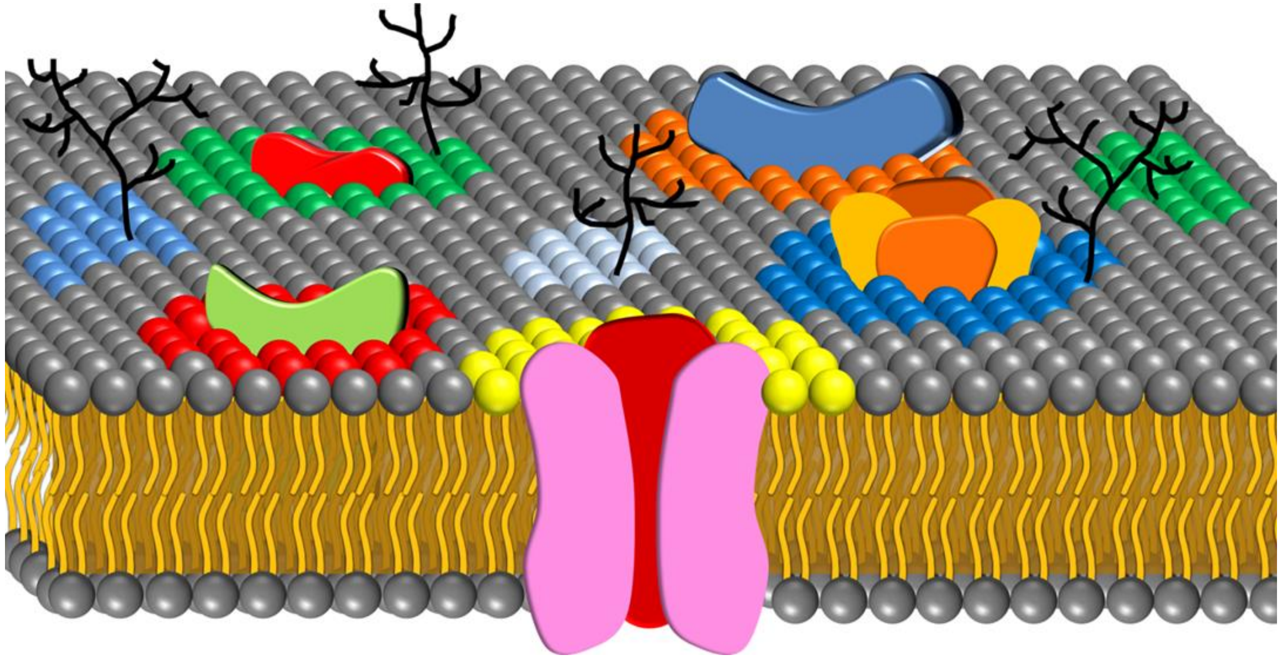


What is the function of this diversity?

- **1997** Lipid rafts as functional lipid-driven domains– **Simons&Ikonen**
- **1973** Detergent resistant membranes– **Yu&Fischman&Steck**
- **1972** Fluid-mosaic model–**Singer&Nicolson**
- **1964** Liposomes: first bilayer–**Bangham**
- **1925** Ultrathin structure of the plasma membrane – **Gorter&Grendel**
- **1891** Quantitative characterization of monolayers–**Agnes Pockels**
- **1889** Membrane concept: a barrier that separates the interior from the exterior – **Charles Ernest Overton**

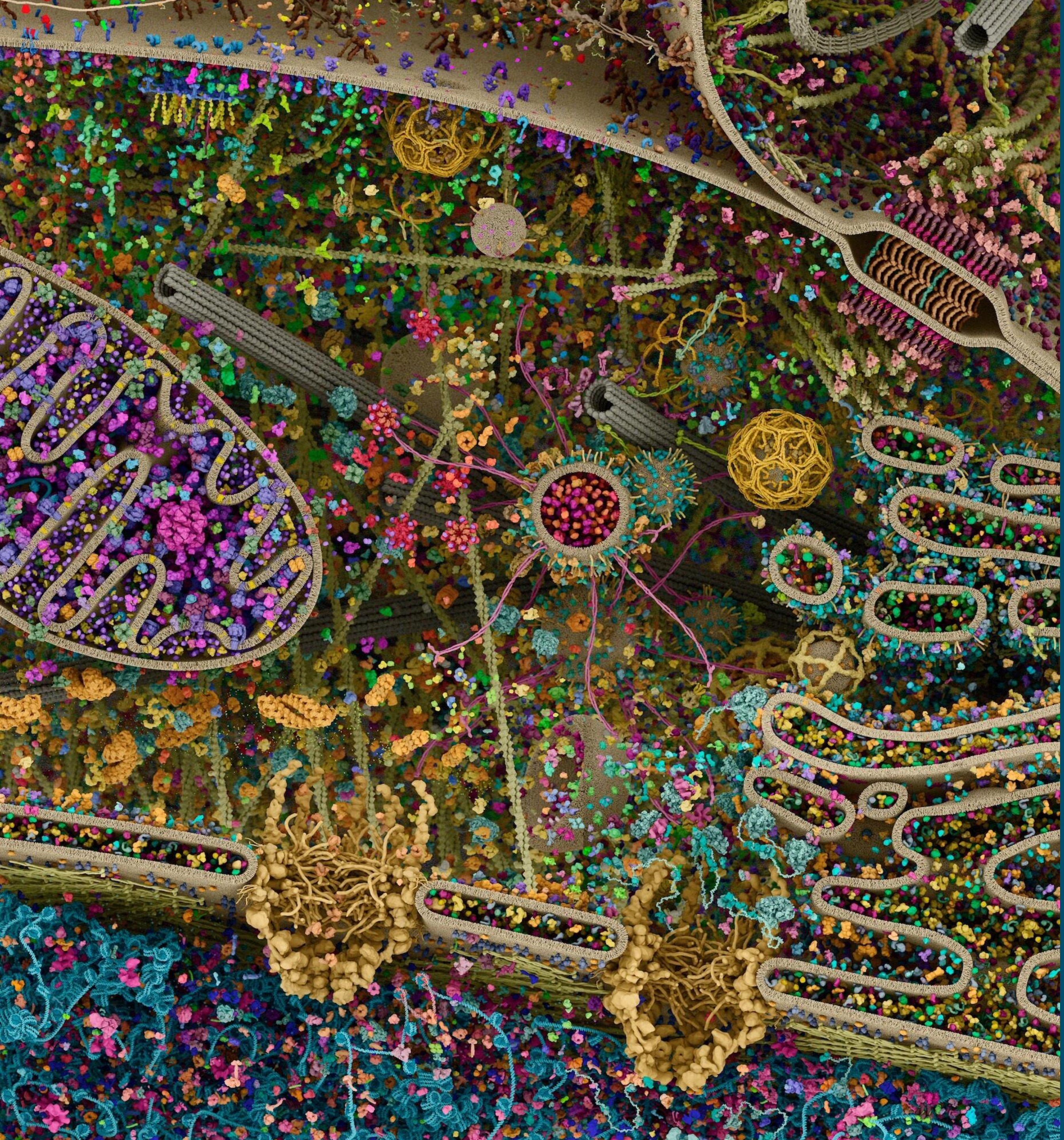
Biological membranes

Updated fluid-mosaic membrane model



Illustation of the modified fluid-mosaic membrane model based on Escribá et al. (2008)

- Better understanding of the high density of transmembrane proteins
- Proteins that bind transiently at the membrane surface
- Existence of phases different from the lamellar phase and their possible physiological relevance
- The curvature of the membrane which depends on the geometry and nanomechanical properties of lipids and proteins
- Lateral heterogeneity of the membranes caused by non-ideal mixing
- Physicochemical properties of the membrane components or deviations from the equilibrium due to transbilayer lipid diffusion which may occur under specific conditions.



Motivation

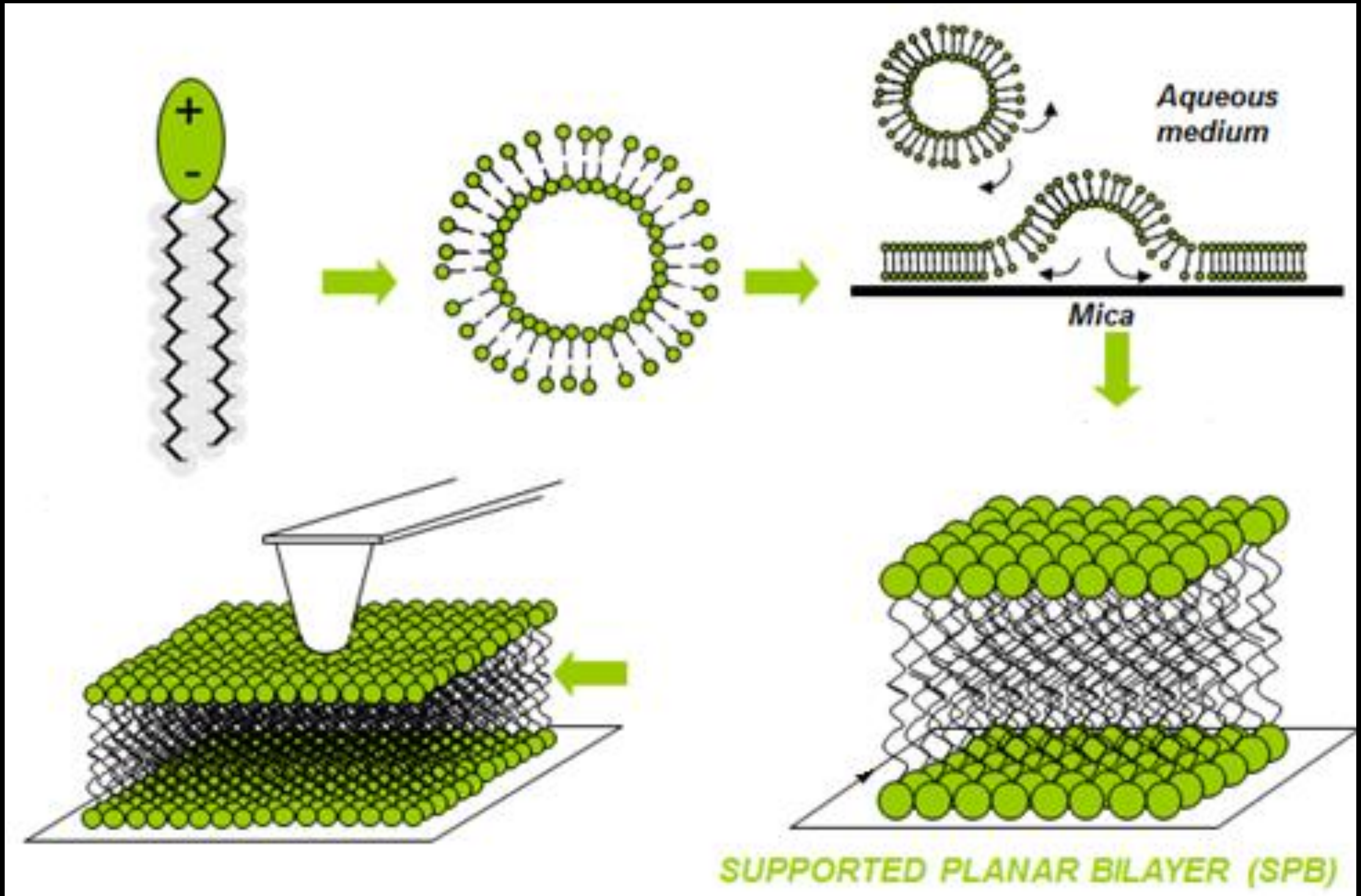
The complexity of cell membranes impedes our investigations to decipher the biophysical principles underlying processes.

Development of model membrane systems that are simpler mimics of the cell membrane with controllable complexity.

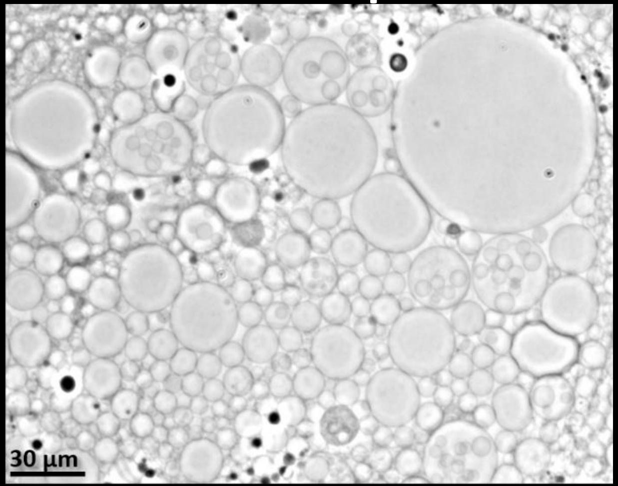
Image source: Evan Ingersoll & Gael McGill
A « simplified » reconstruction of an eukaryotic cell

Lipid bilayer model membranes

Formation of Supported Lipid Bilayers (SLBs)



Vesicles in suspension

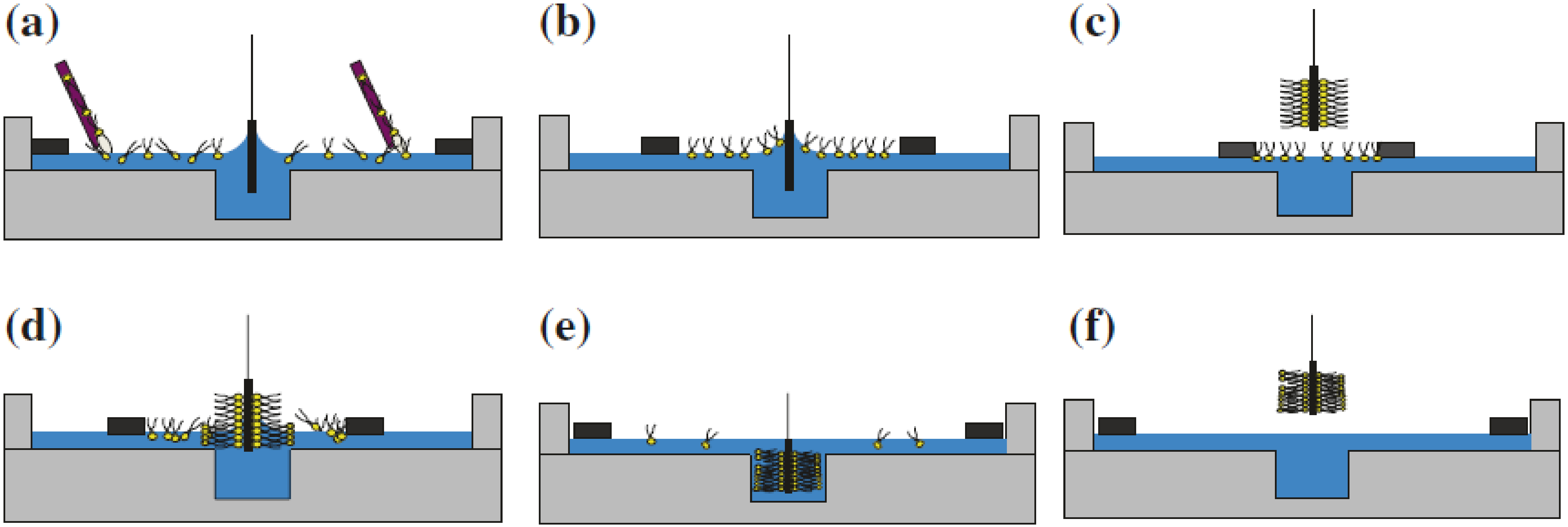


Supported lipid bilayer

800 nm x 800 nm
3 frames per second

Lipid bilayer model membranes

Langmuir-Blodgett films



- Asymmetric bilayers (composition & lateral pressure)
- Leaflet decoupling, dewetting
- Uncompleted coverage
- Defects (holes)

Lateral pressure of biological membranes = 30 mN/m

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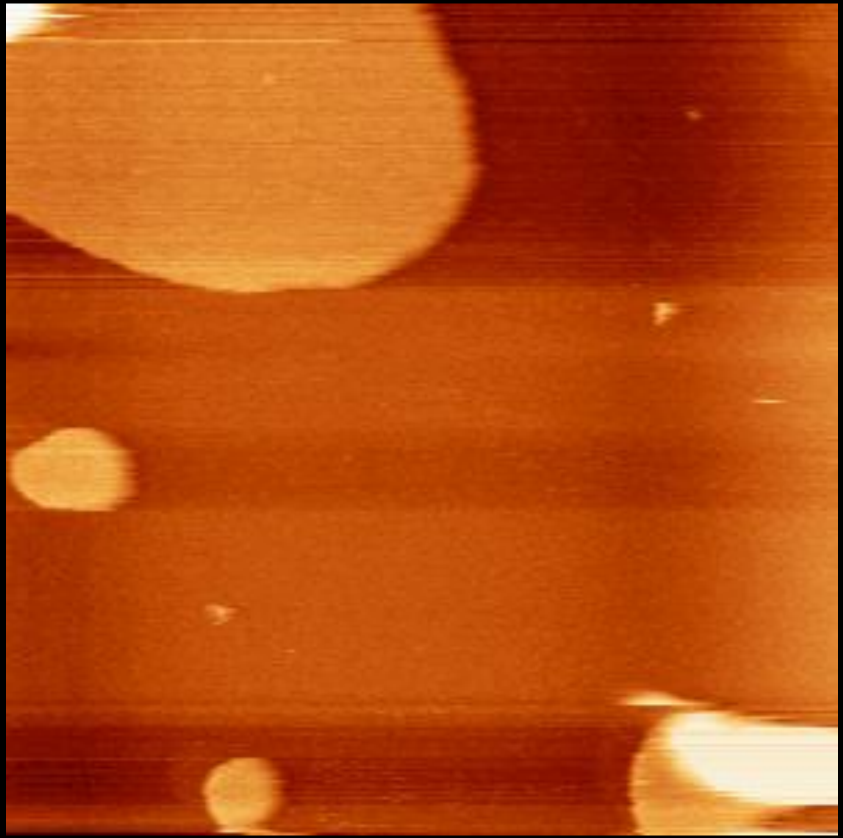
Lipid bilayer model membranes

Formation of Supported Lipid Bilayers (SLBs)

Brain Polar Lipic (BPL): liquid-like phase phospholipid

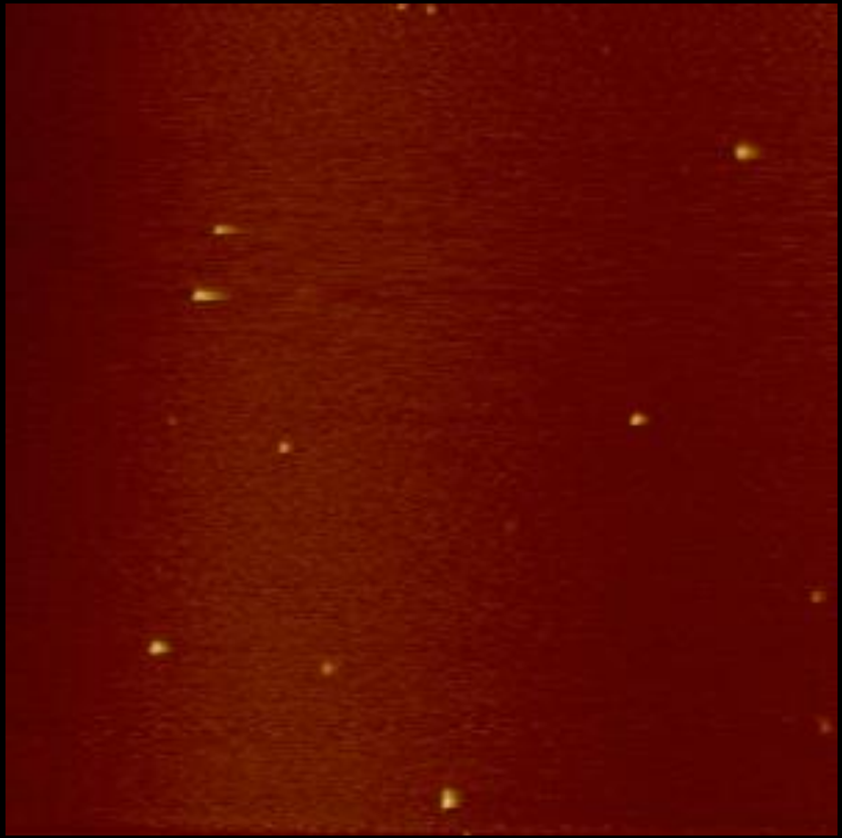
Expansion of a fluid film…



700 nm scan size, 200 x 200 pixels
400 ms per frame

DPPC: solid-like phase phospholipid

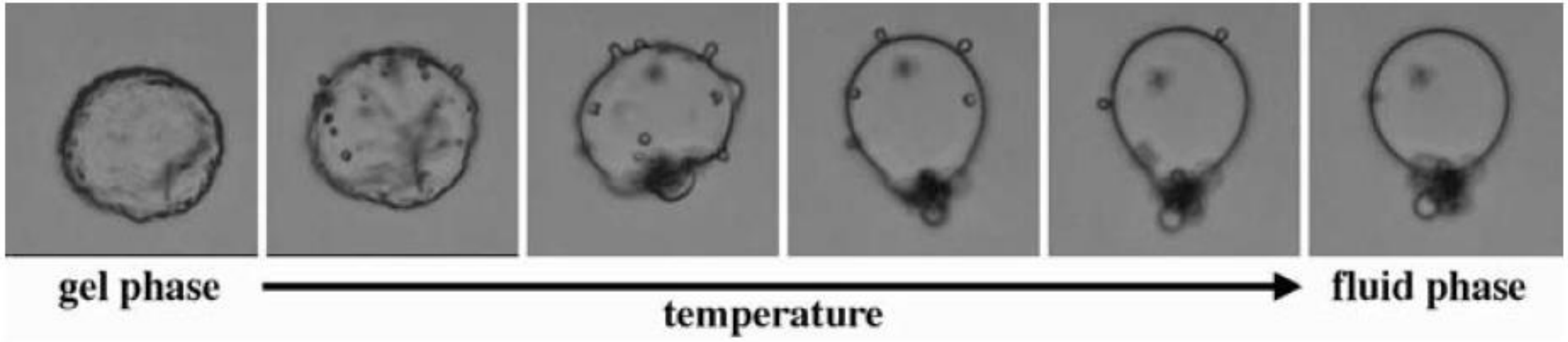
Initial "nucleation" patches that do not grow but fill spaces…



1500 nm scan size, 300 x 300 pixels
1500 ms per frame

Lipid bilayer model membranes

Phase transitions



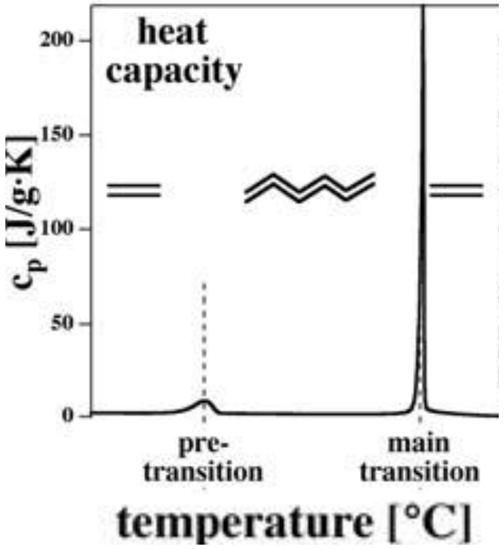
T. Heimburg, Wiley-VCH 2007

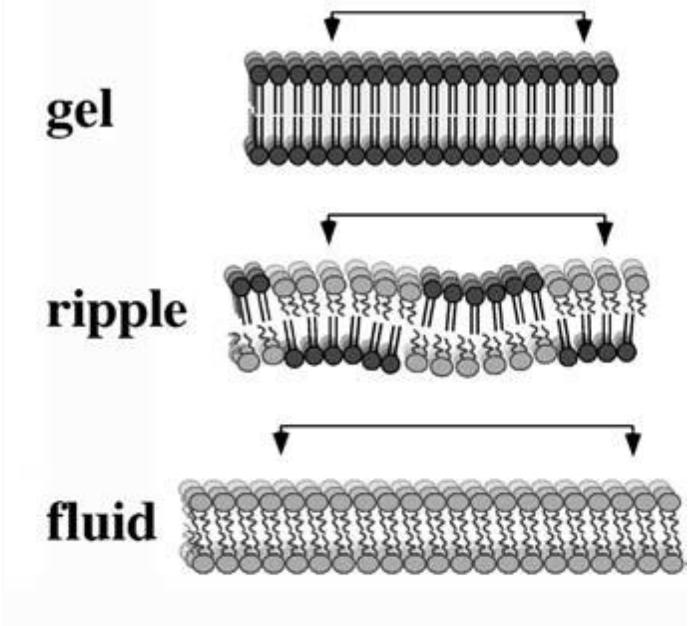
30 to 45 °C
10 s

- Change in vesicular structure of giant DPPC vesicles
- Transient formation of buds resembling events in secretion

Lipid bilayer model membranes

Ripple phase



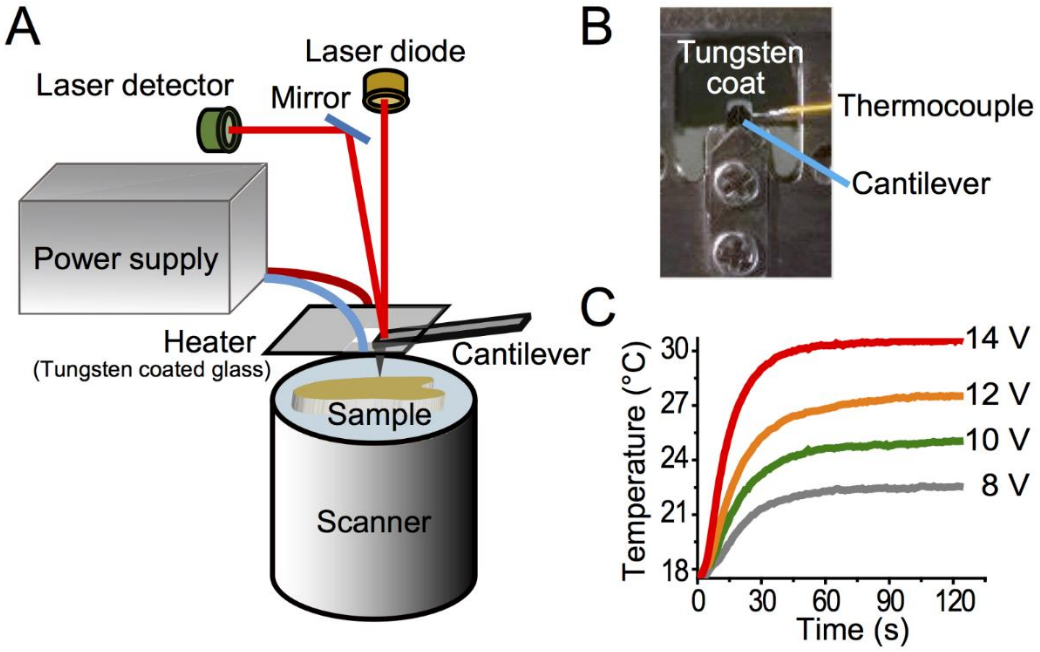


T. Heimburg, Wiley-VCH 2007

- The ripple phase is a structural transition in the vicinity of the main chain melting transition
- Consists in periodic undulations of the membrane

Lipid bilayer model membranes

Ripple phase

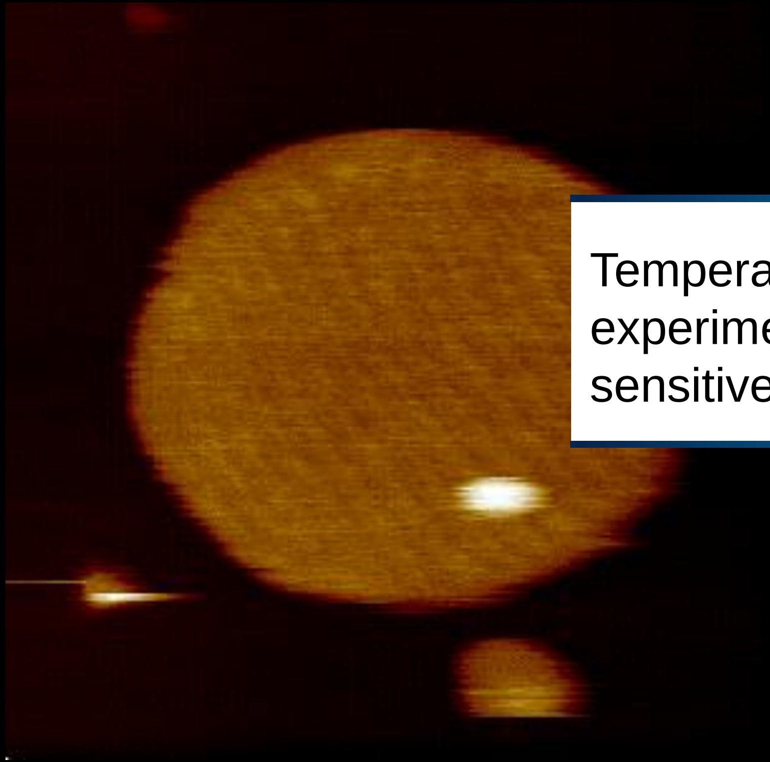


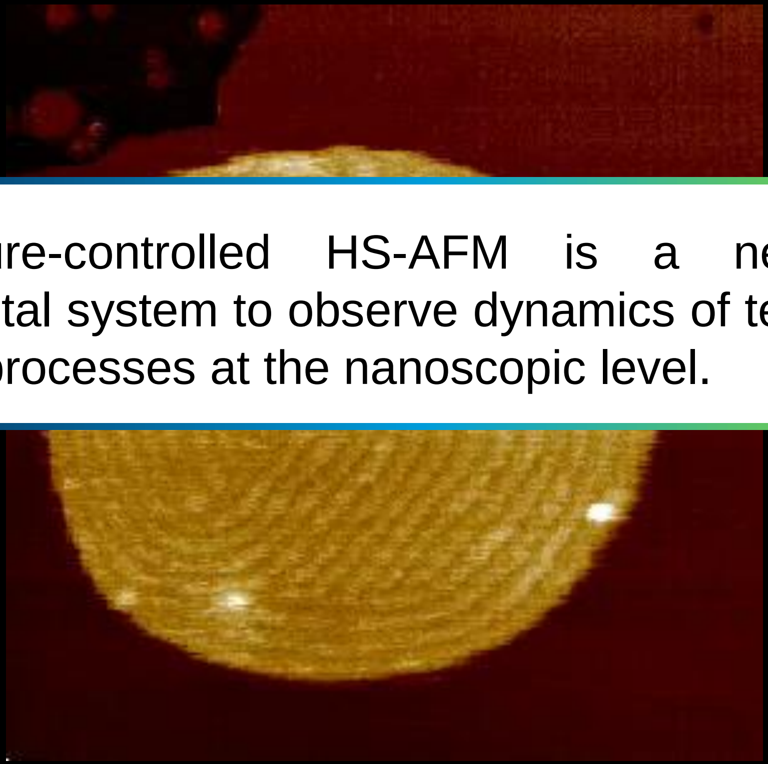
Takahashi et al., *Small 2016; 10.1002/smll.201601549*

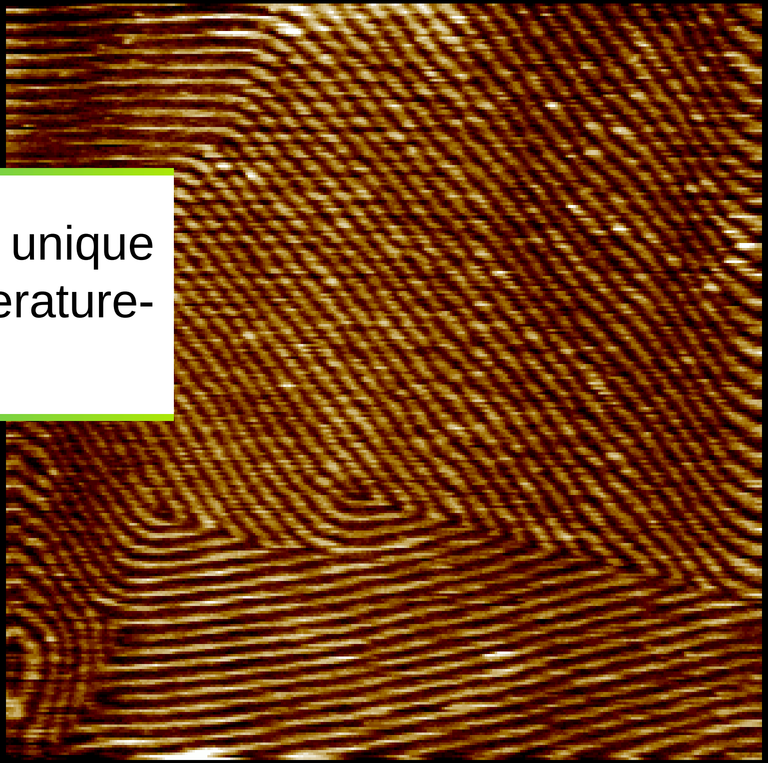
Lipid bilayer model membranes

Real time observation of ripple phase transitions

+ Ca^{2+}







Temperature-controlled HS-AFM is a new unique experimental system to observe dynamics of temperature-sensitive processes at the nanoscopic level.

400 nm
500 ms per frame

300 nm
500 ms per frame

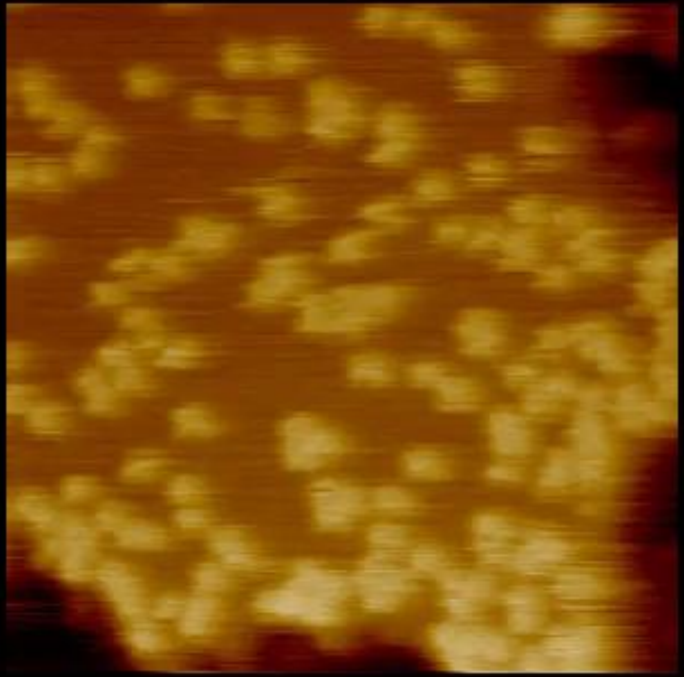
1 µm
1 s per frame

Takahashi et al., Small 2016; 10.1002/smll.201601549

Lipid bilayer model membranes

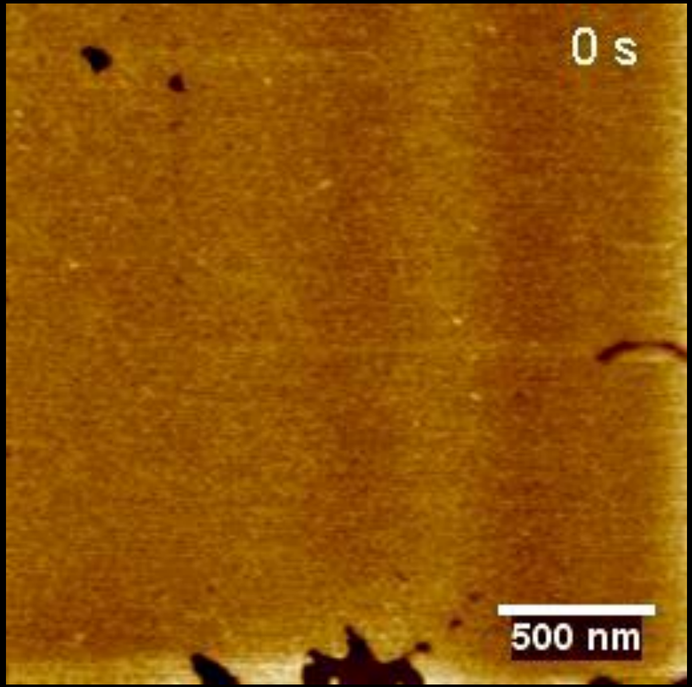
In situ phase composition transition

- Enzymatic degradation of the phospholipid by OmpA



Rangl et al., J Mol Biol, 2017

- Enzymatic conversion from sphingomyeline to ceramide

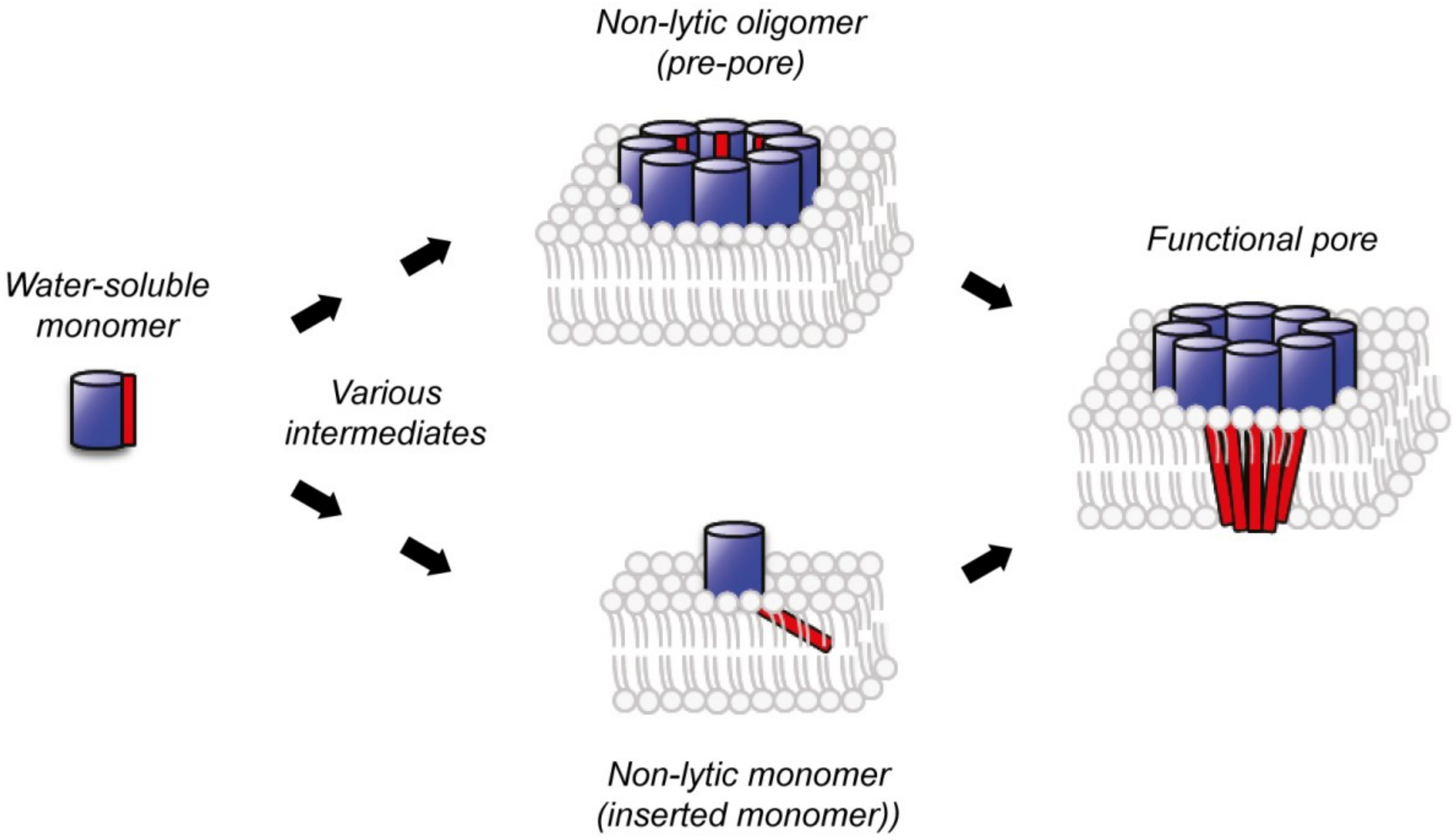


Emilio González (Biofisika, Spain)

Outline

Pore forming toxins

Fragaceatoxin has high affinity for sphingomyelin domains



Morante et al., *J. Biol. Chem.* 2016; 291(37):19210-9

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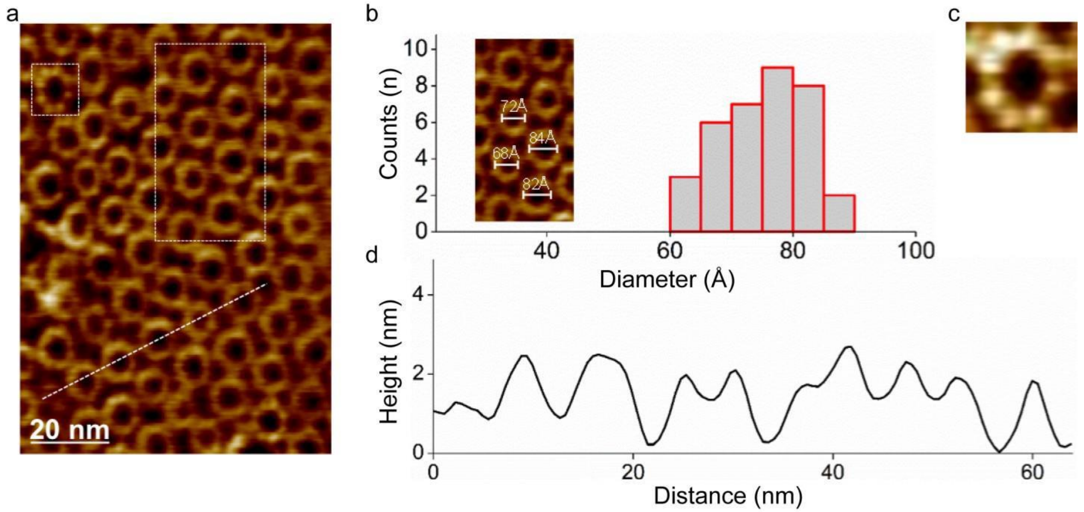

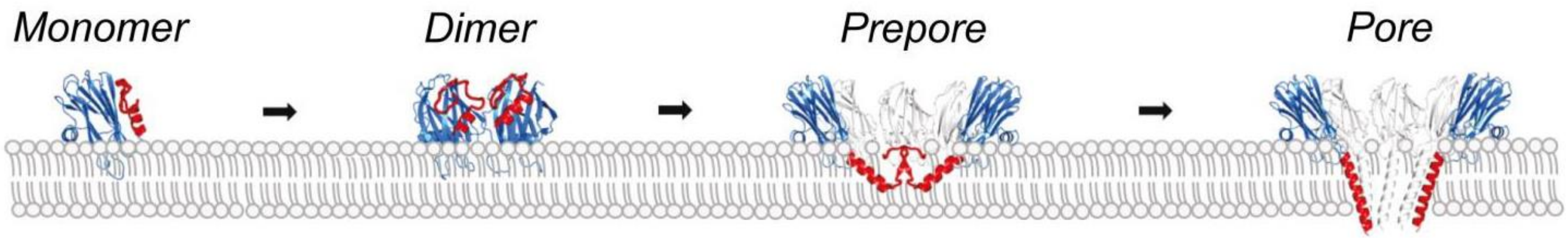

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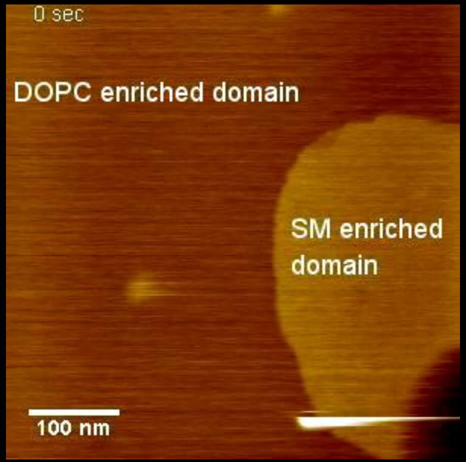

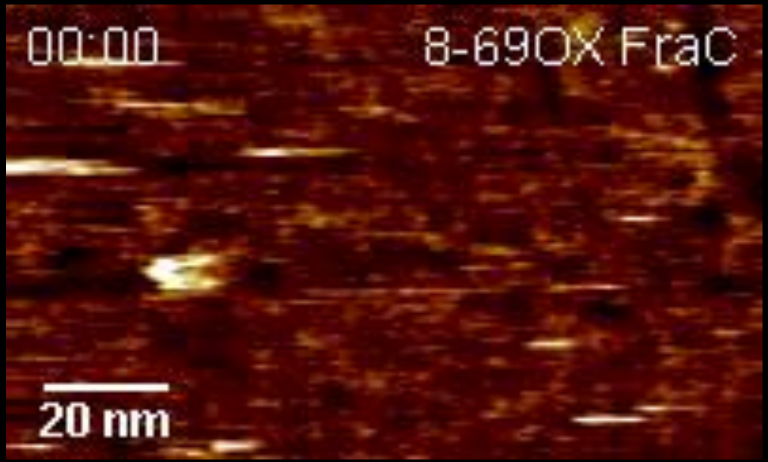

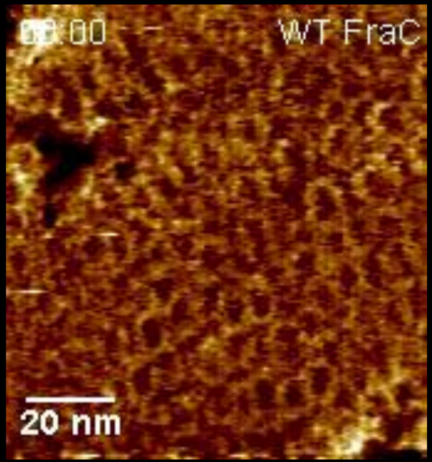

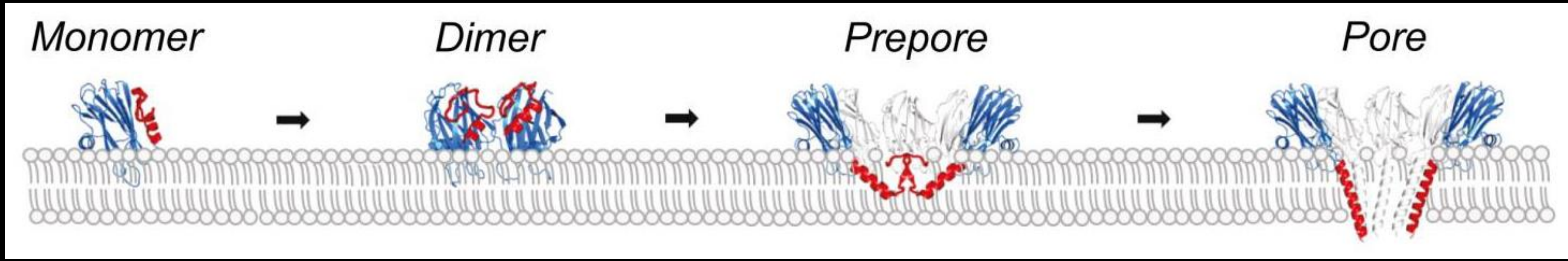

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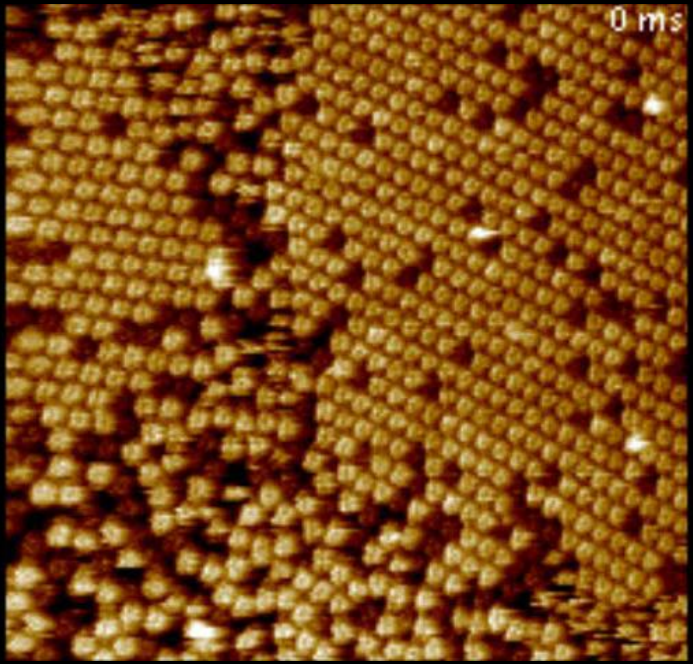
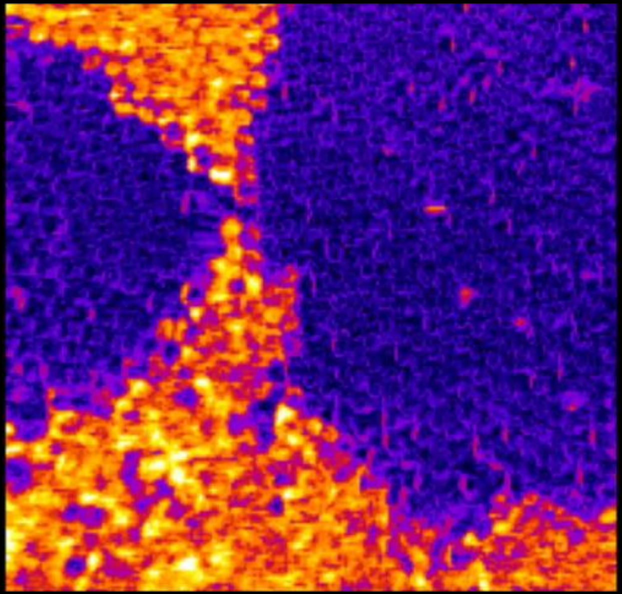
Glassy Behavior in crowded membranes

Anomalous diffusion



HEAT MAP by SD
(at average Cage residence time)



"HS-AFM allows correlating structure with diffusion behavior, and glassy diffusion is only detectable when both movement and environment are simultaneously assessed. Therefore, biologists may have missed glasslike diffusion in crowded membranes due to the technical limitation of only tracking single molecules"

Munguira et al; Nature ACS NANO 2016

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03. High-Speed: how do we do it?
- Short cantilevers
- Moving components

04. Tips on High-Speed imaging membranes
- Sample preparation and imaging

05. Conclusions
- General conclusions
- Recent progresses
- Perspectives

06. Bibliography
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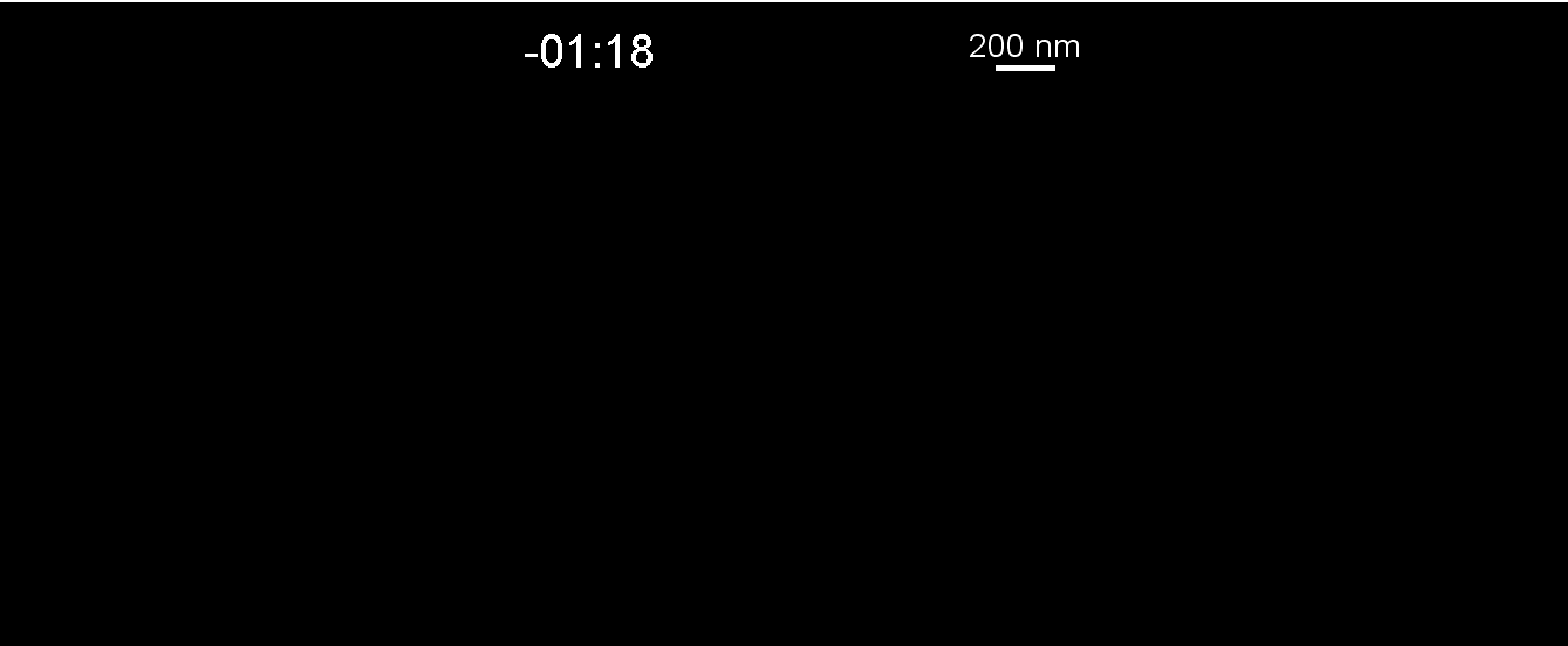
- Biological membranes-related

Kinetics of antibiotics

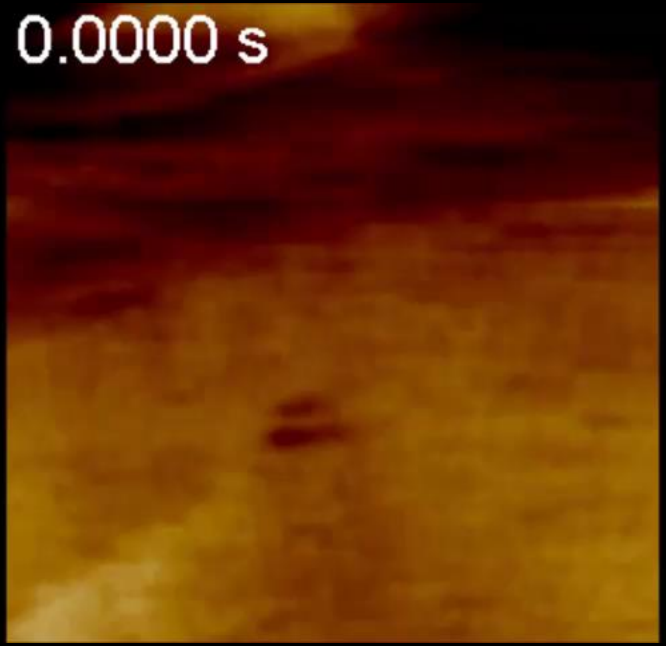
Antimicrobial peptide capsids of de novo design



De Santis et al., Nat. Com. 2017

Kinetics of antibiotics

Molecular mechanism of daptomycin



Zuttion et al., Nat. Com. 2020

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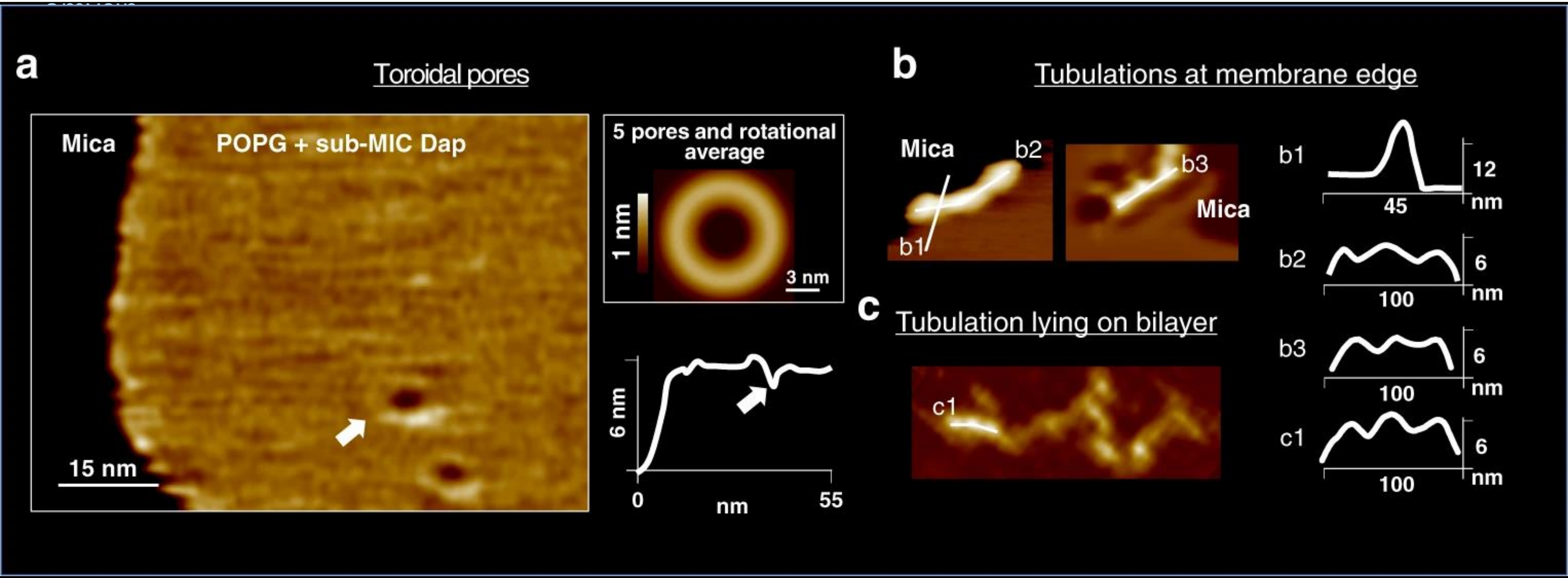

Zuttion et al., Nat. Com. 2020

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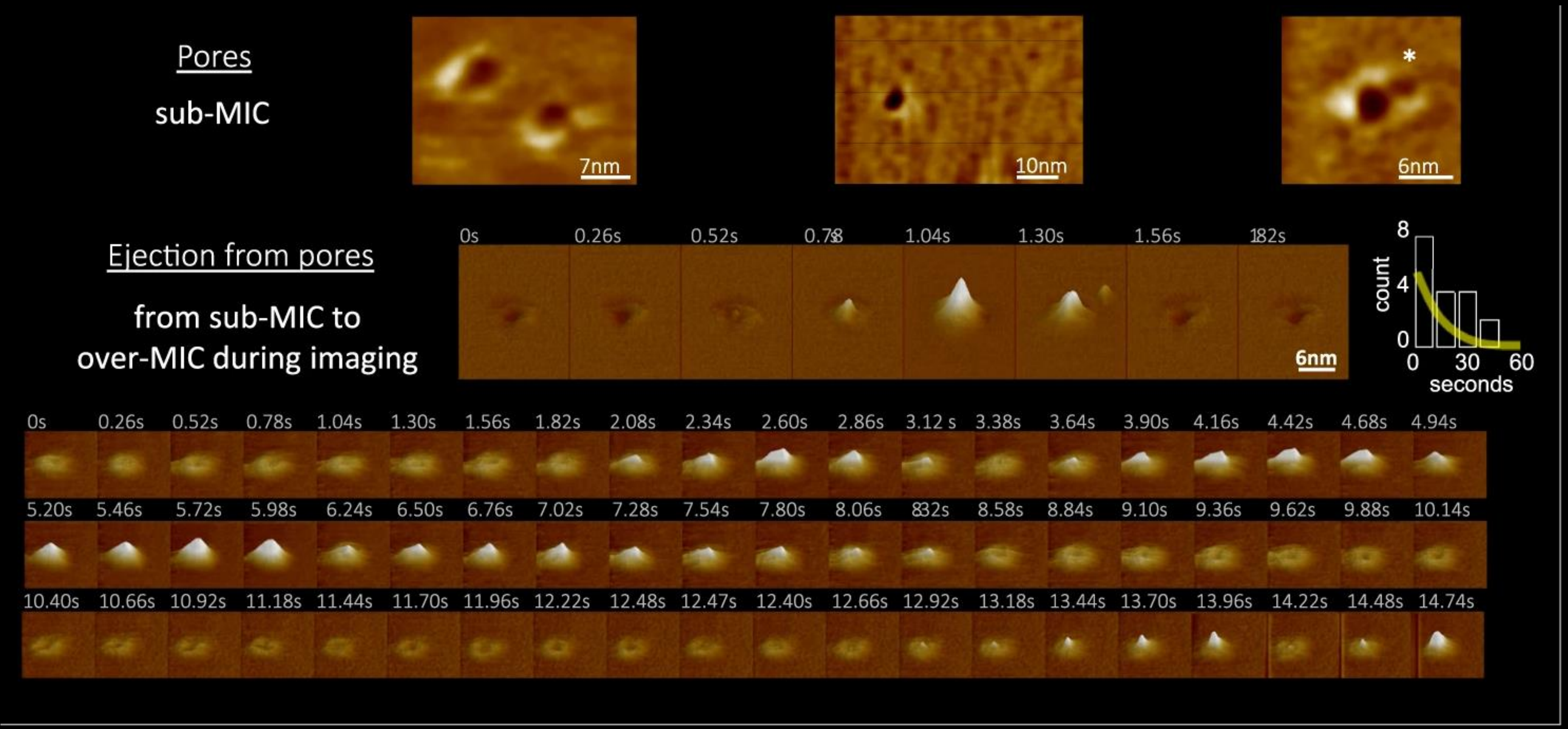
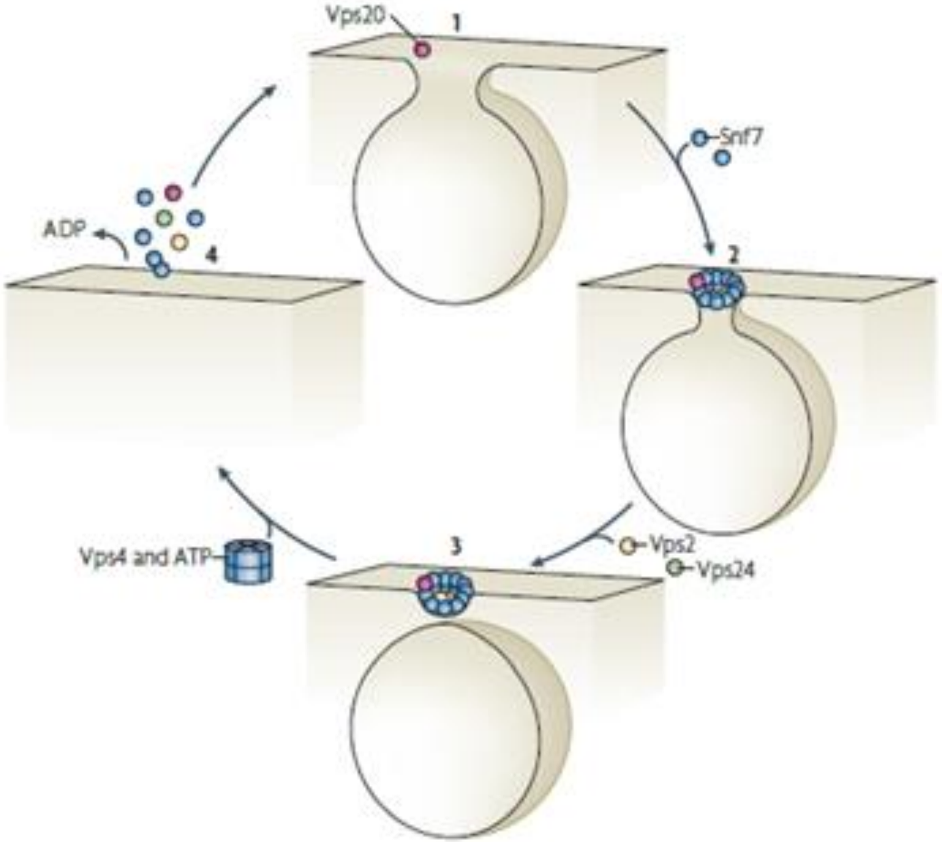


Zuttion et al., Nat. Com. 2020

Outline

What is ESCRT-III?

Endosomal Sorting Complex Required for Transport



Nature Reviews Mol. Cel. Biol. 2010; 11, 556

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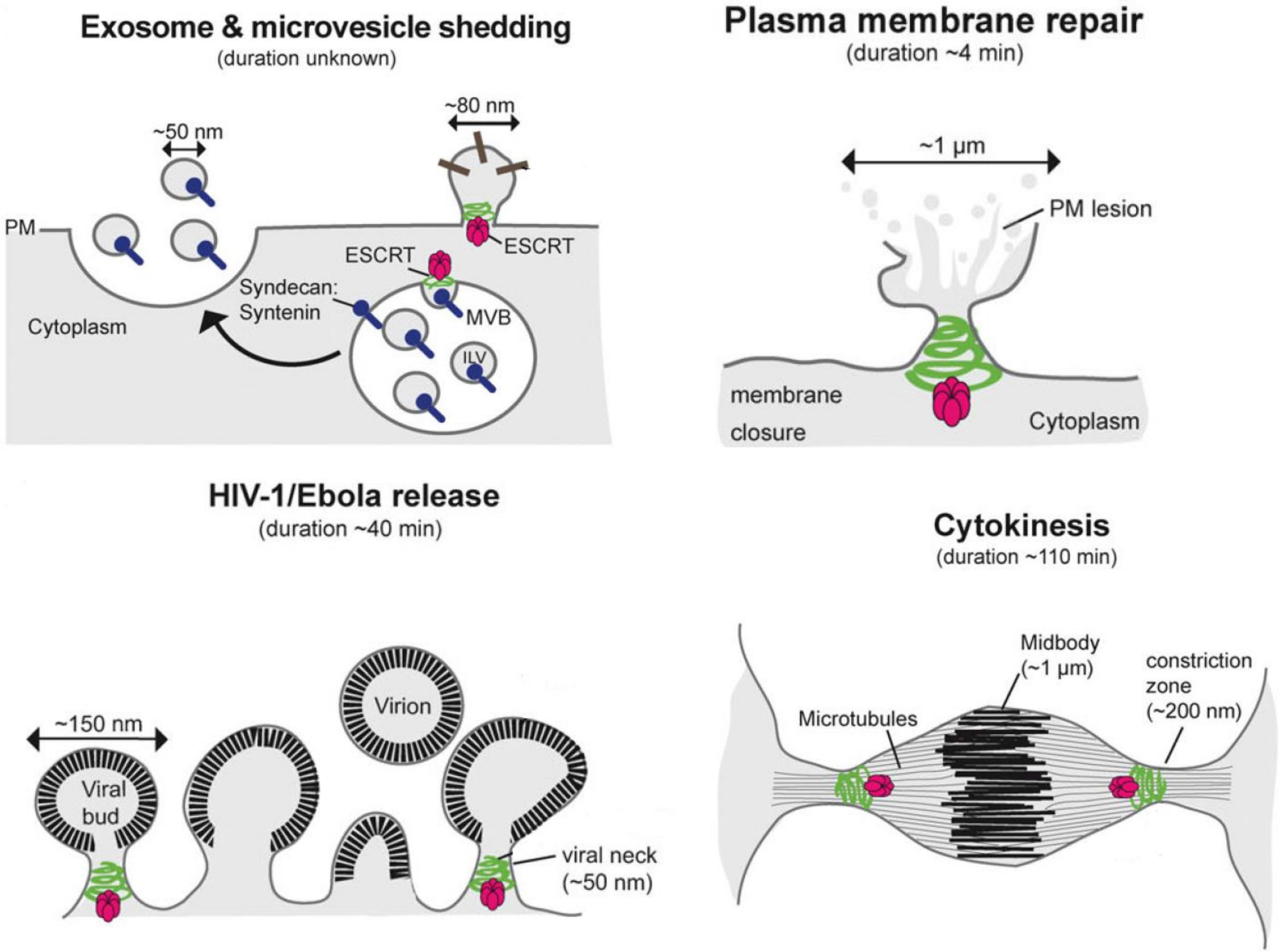
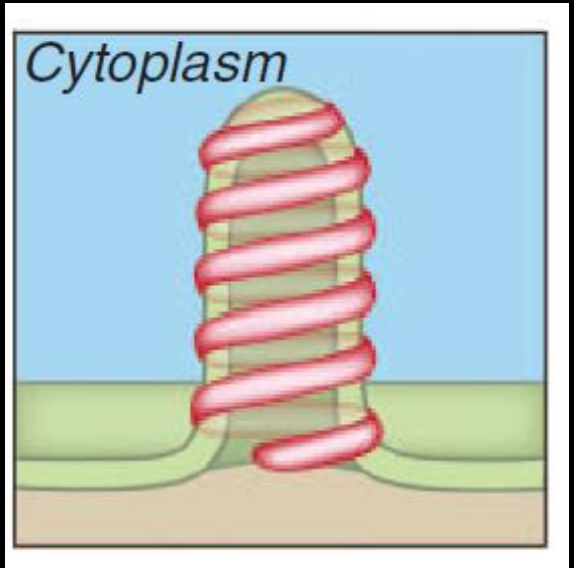


Adapted from Adell et al., *FEBS J.*, 2016

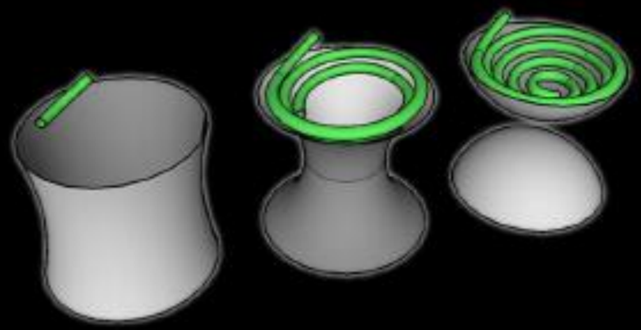
What is ESCRT-III?

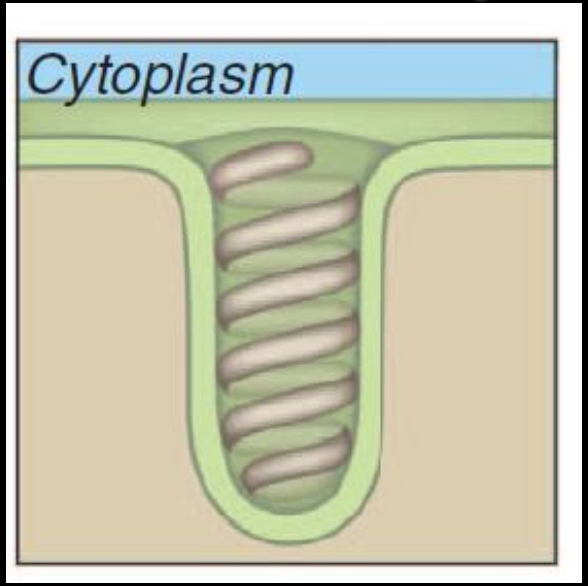
Endosomal Sorting Complex Required for Transport

Dynamin squeezes the membrane neck



ESCRT-III cuts the membrane from the inside

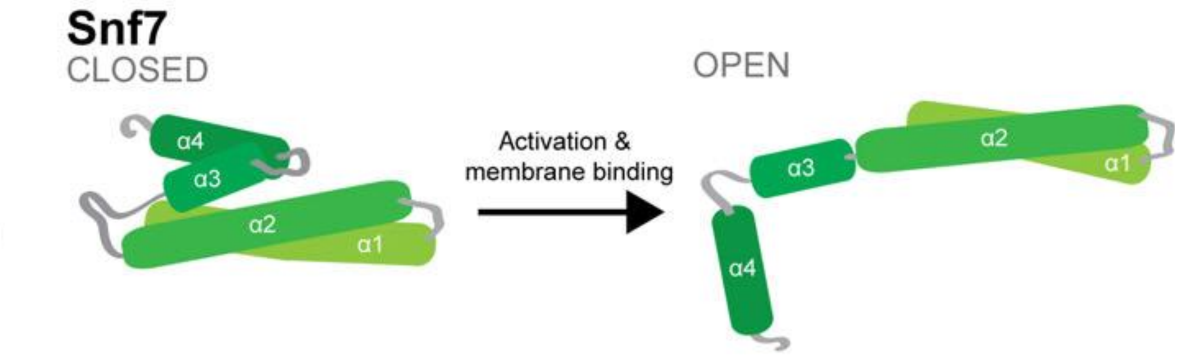


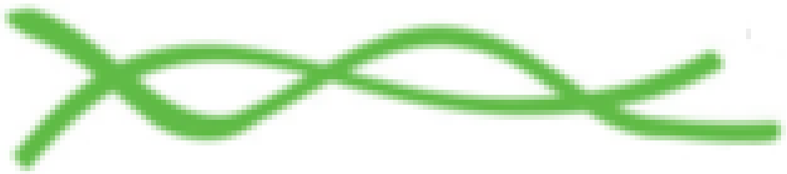


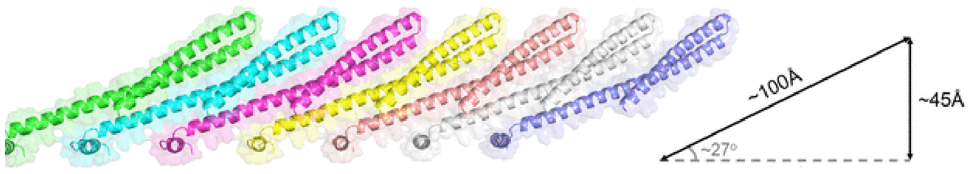
McCullough et al., *Science*, 2016

ESCRT-III polymerization

Snf7 is tha major component in ESCRT-III

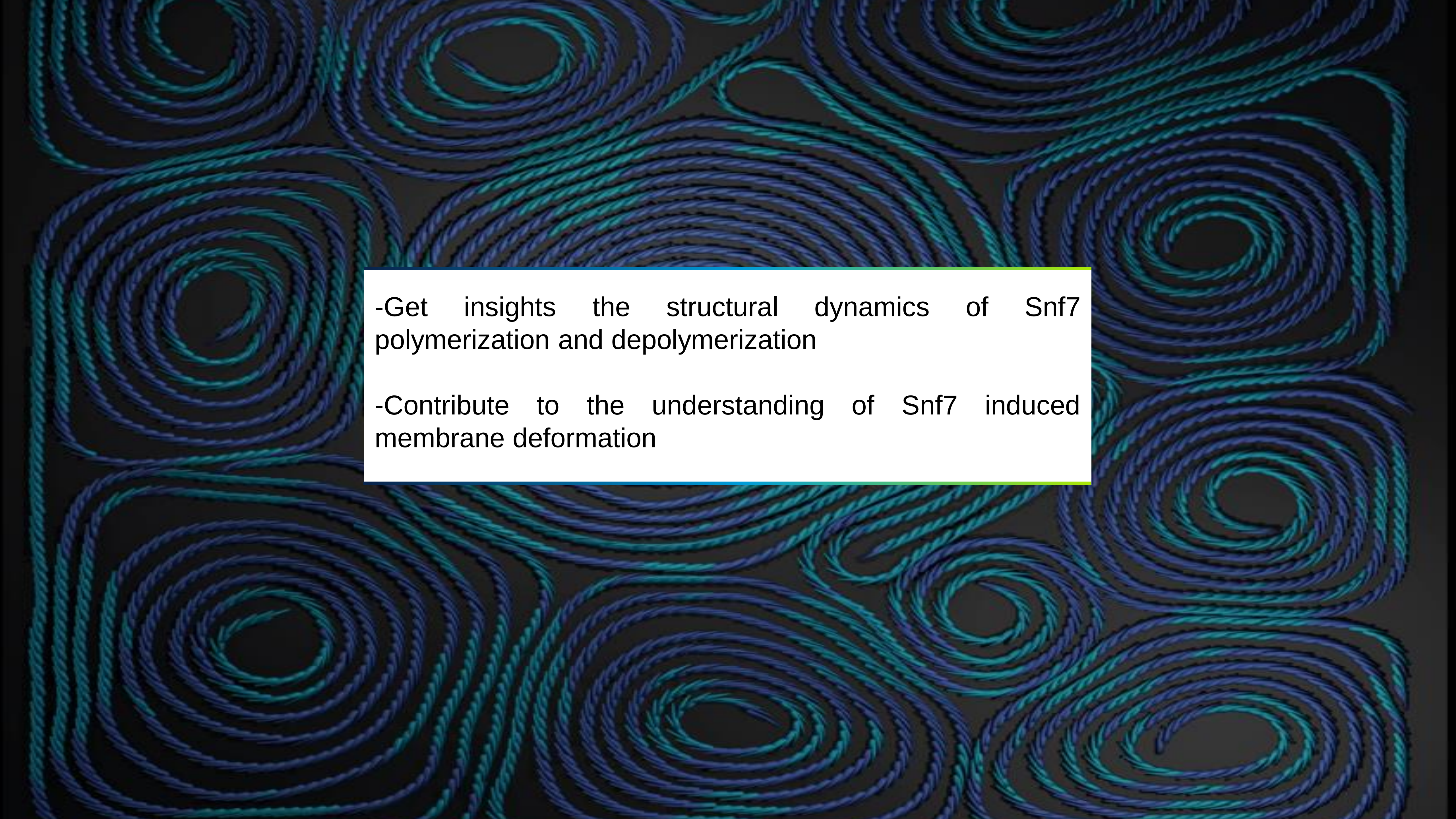






Adapted from Adell et al., *FEBS J.*, 2016

Tang et al. 2015



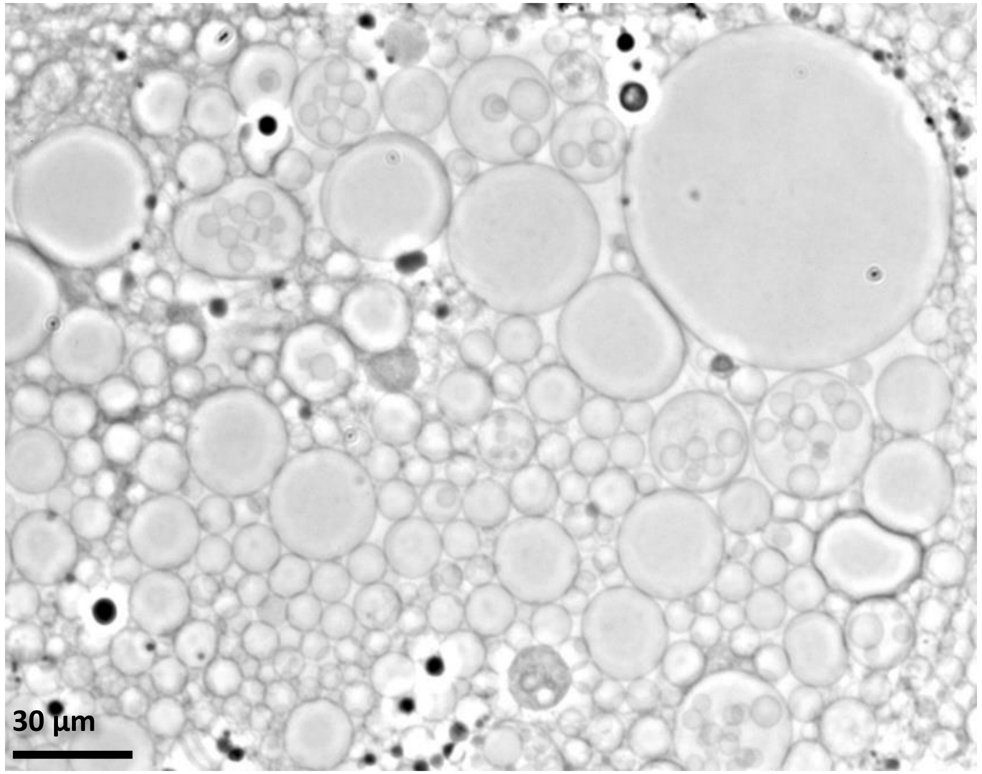
-Get insights the structural dynamics of Snf7 polymerization and depolymerization

-Contribute to the understanding of Snf7 induced membrane deformation

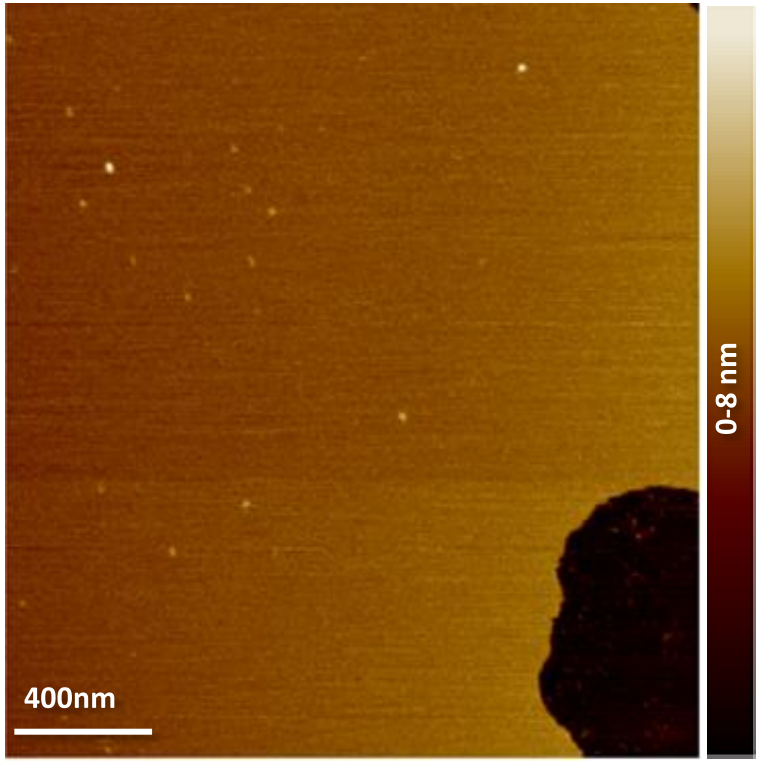
Supported Lipid Bilayer model system

DOPC:DOPS (6:4, mol:mol)

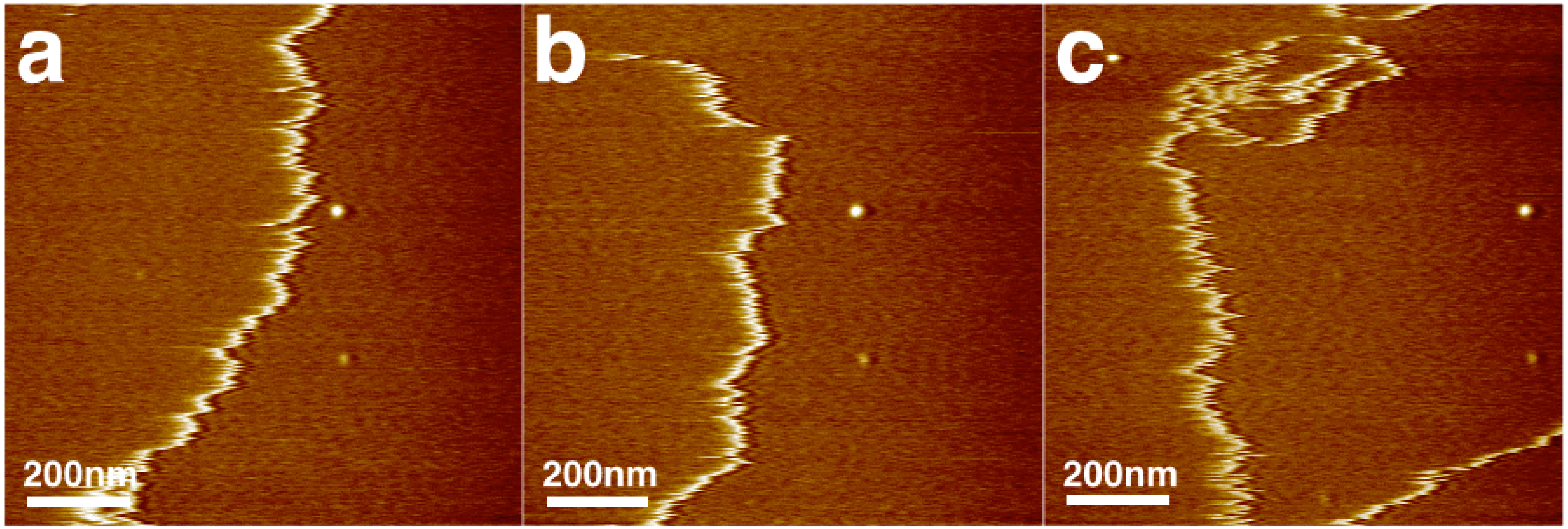
Giant Unilamellar Vesicles



Supported Lipid Bilayer



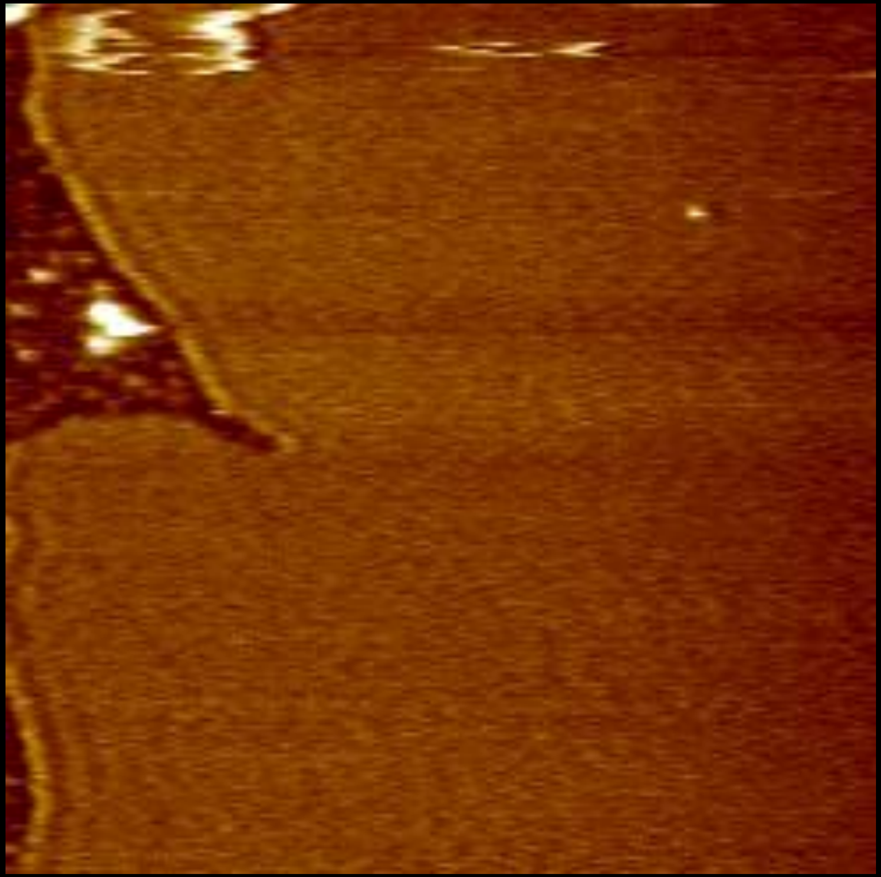
Snf7 filament adsorption



- Filaments are highly diffusive
- Shapes and lengths are variable

Snf7 assembly formation

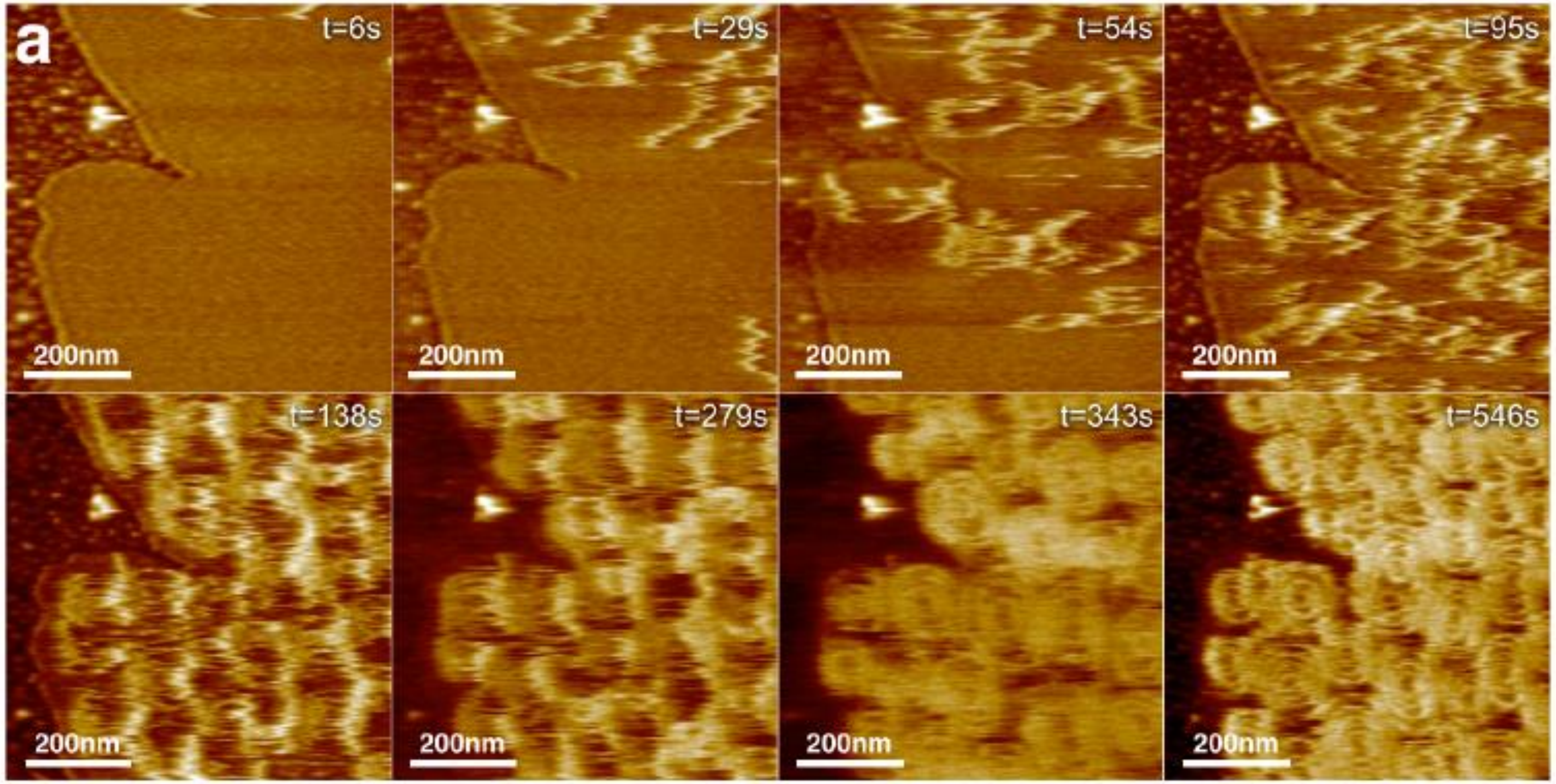
From filaments to initial rings, from rings to mature assemblies

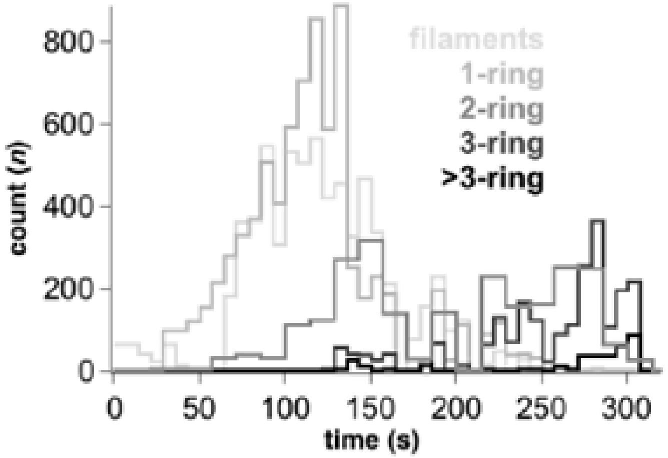


Scan size: 820 nm
Time per frame: 716 ms

Snf7 assembly formation

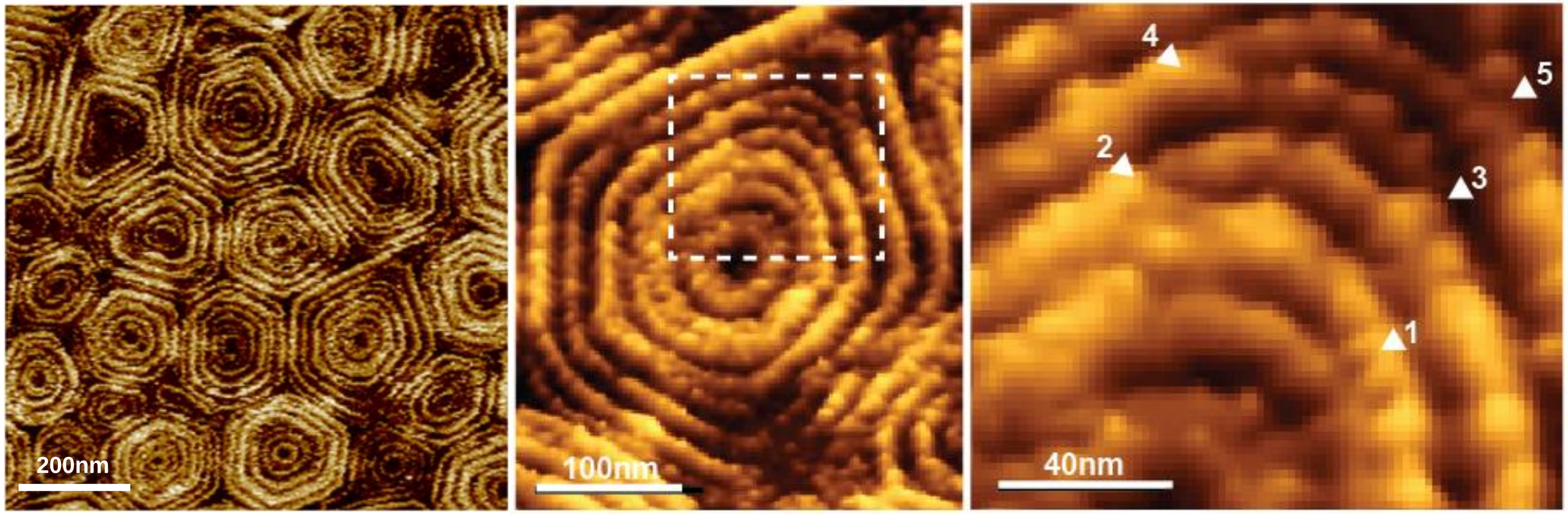
From filaments to initial rings, from rings to mature assemblies





Snf7 assembly architecture

Rings and spirals

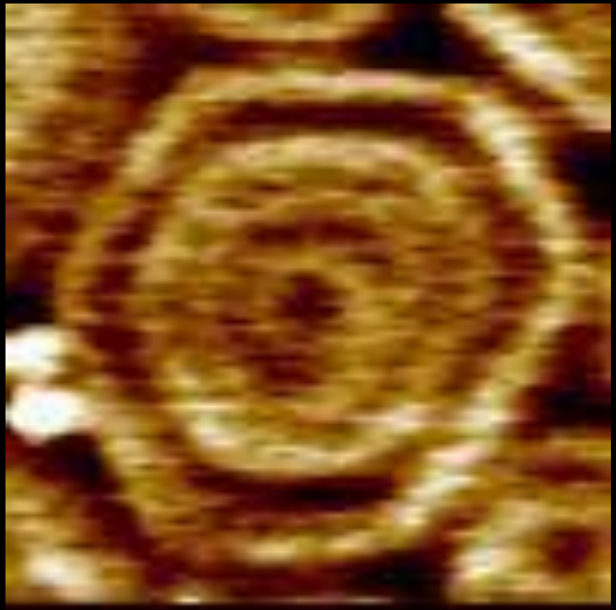


- Assemblies are single filaments that locally engages into double or multiple strands

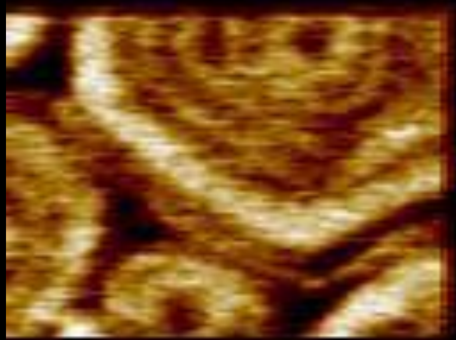
Snf7 assembly architecture

Dynamic equilibrium between ring and spirals

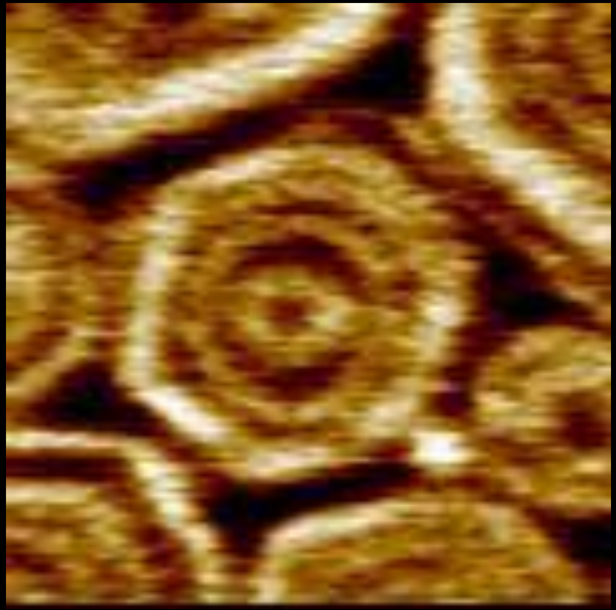
Scan size: 100 nm
Time per frame: 716 ms



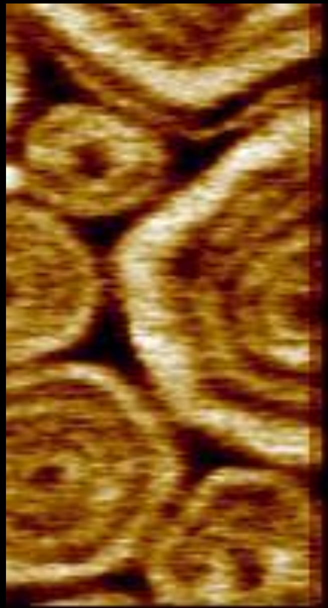
Scan size: 120 nm
Time per frame: 716 ms



Scan size: 170 nm
Time per frame: 716 ms



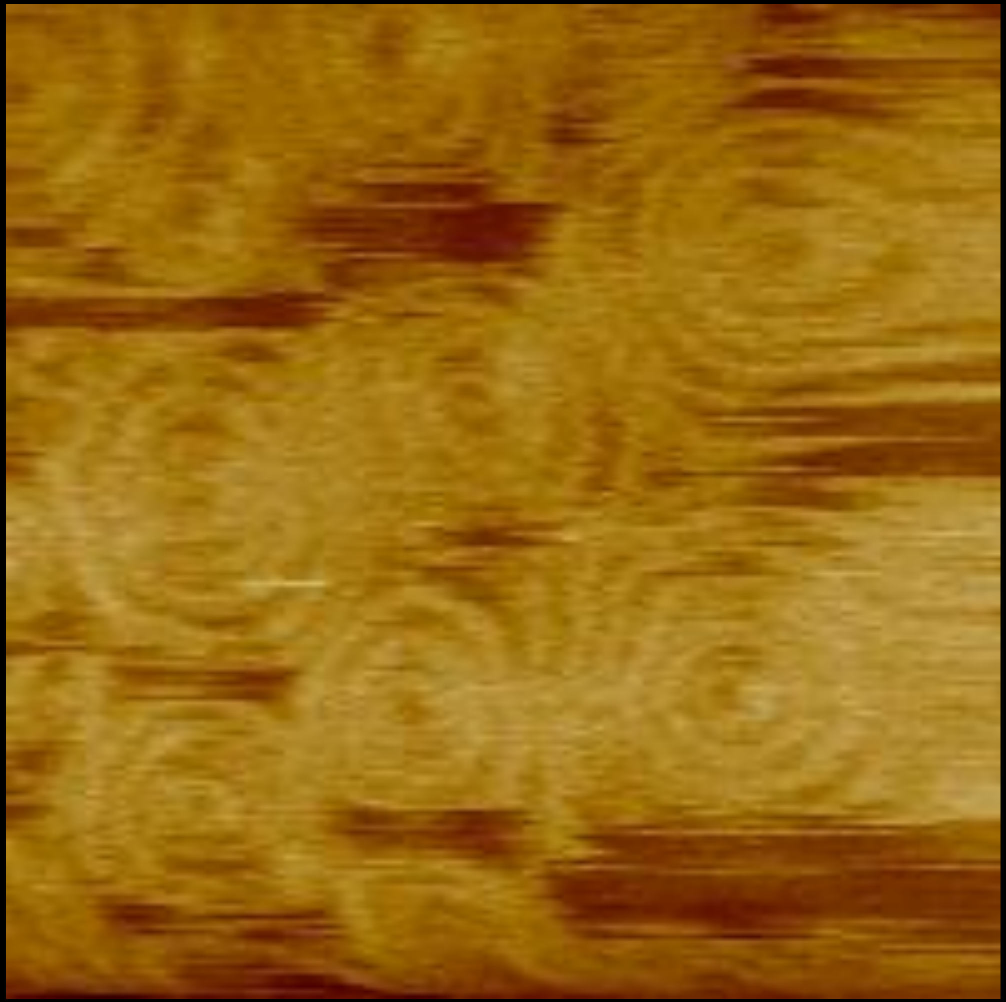
Scan size: 200 nm
Time per frame: 716 ms



- Assemblies are single filaments that locally engages into double or multiple strands

Snf7 under force disruption

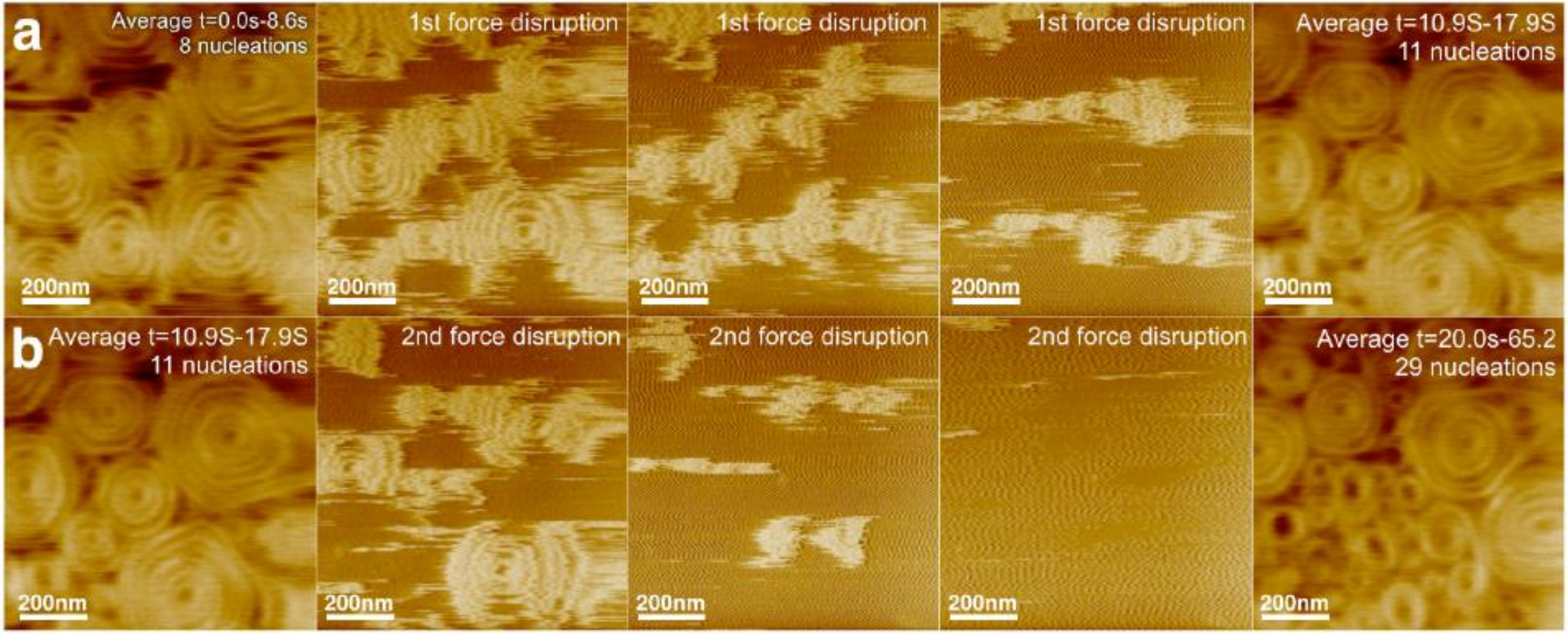
Snf7 reversible assembly - destruction - assembly - destruction - assembly ...



Scan size: 500 nm
Time per frame: 840 ms

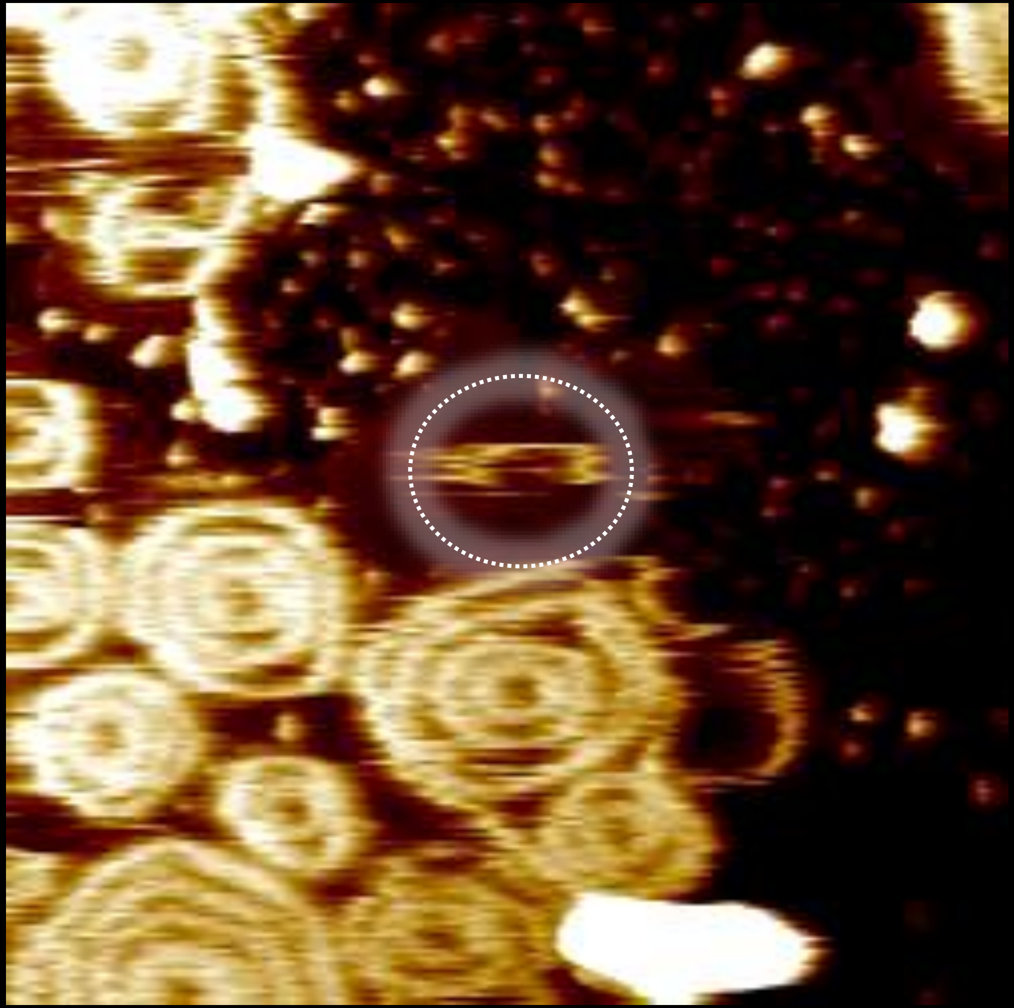
Snf7 under force disruption

Fragmentation of assemblies sets novel nucleations
- seed potential



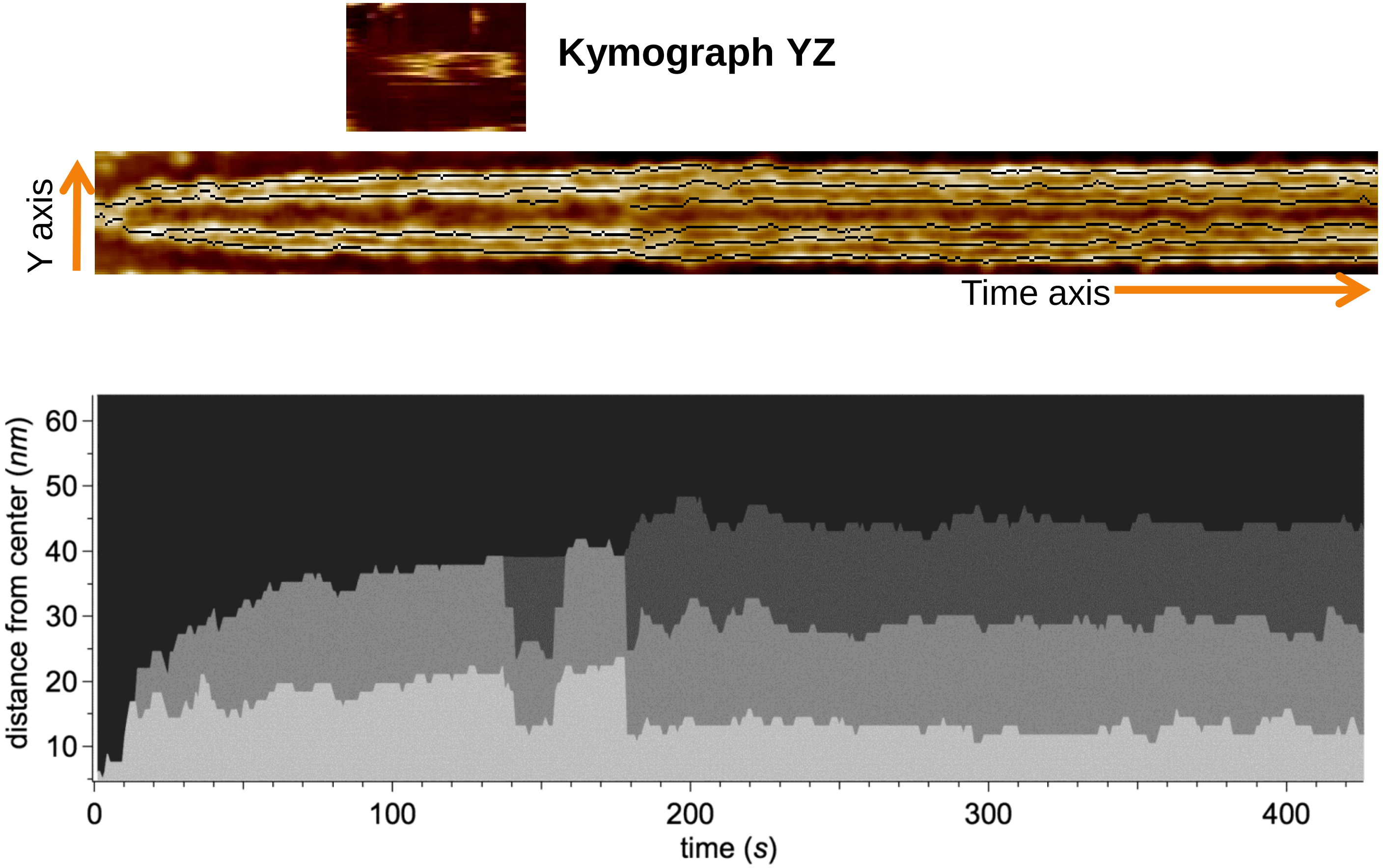
Snf7 assembly formation

Birth of a Snf7 assembly - from filament to concentric assembly

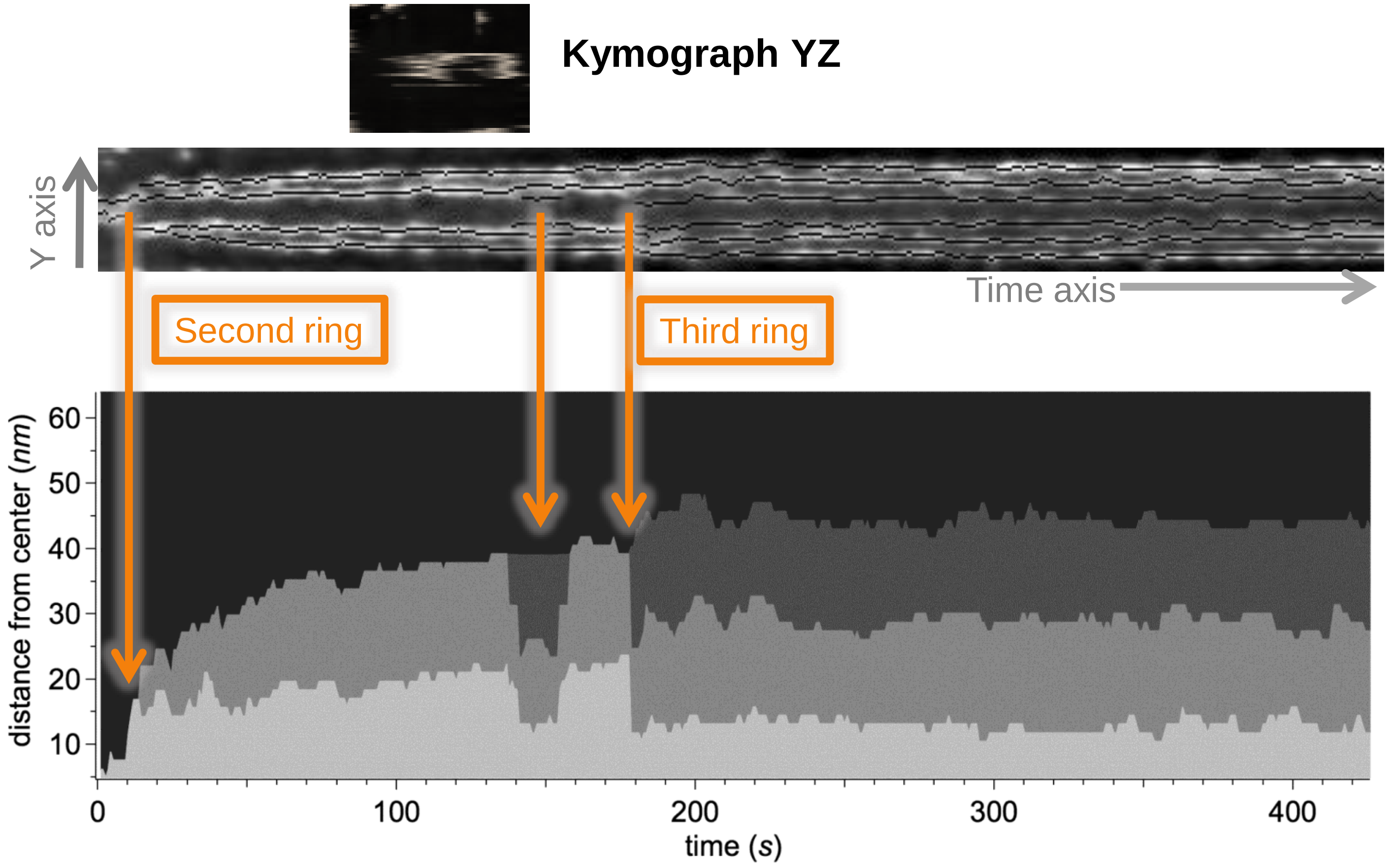


Scan size: 666 nm
Time per frame: 850 ms

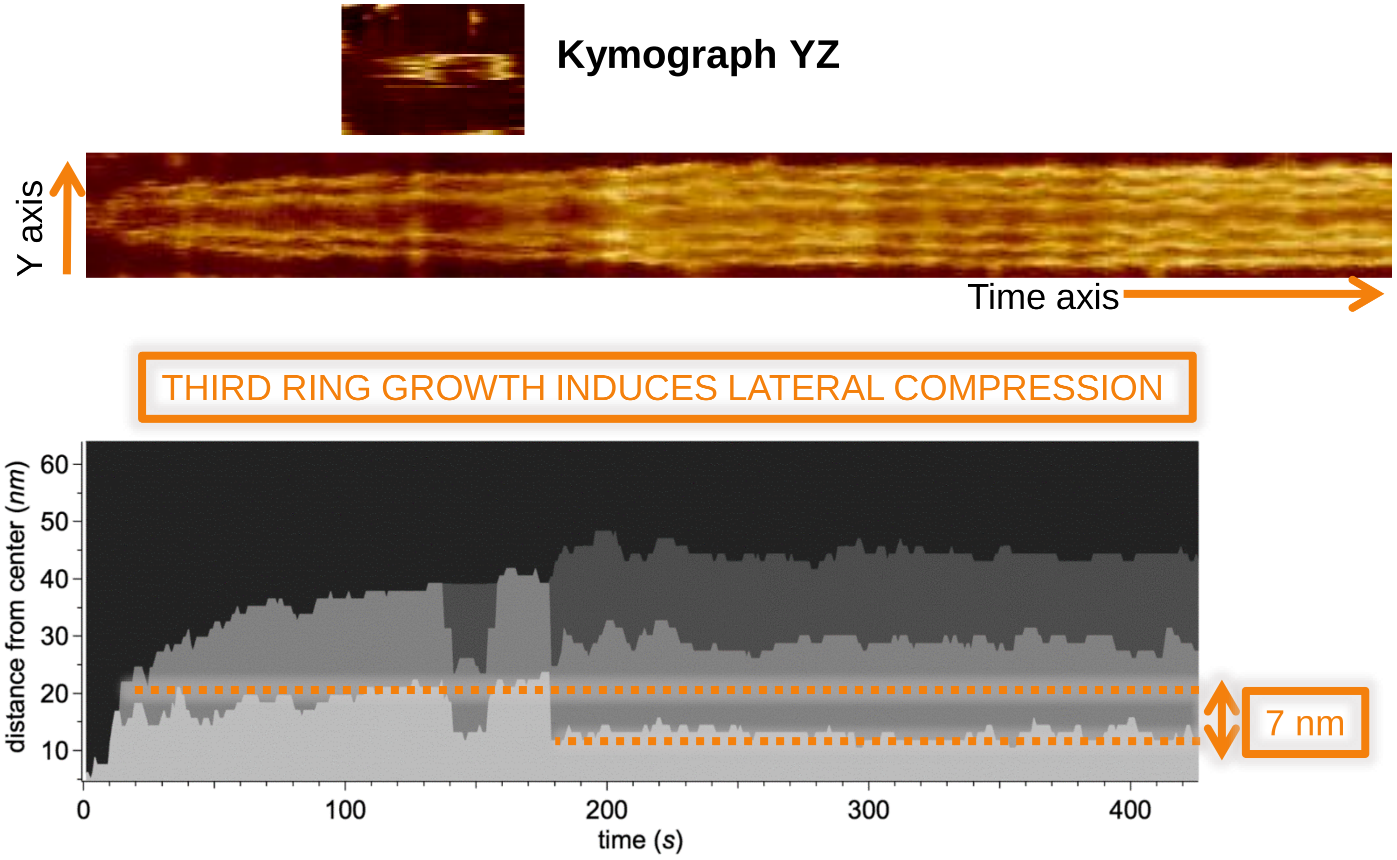
Snf7 assembly formation



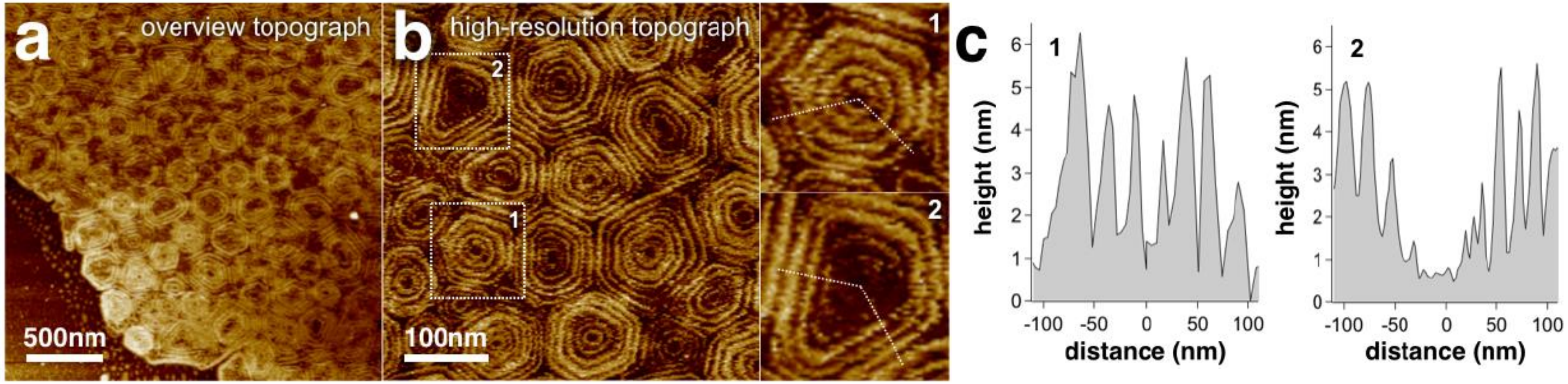
Snf7 assembly formation



Snf7 assembly formation

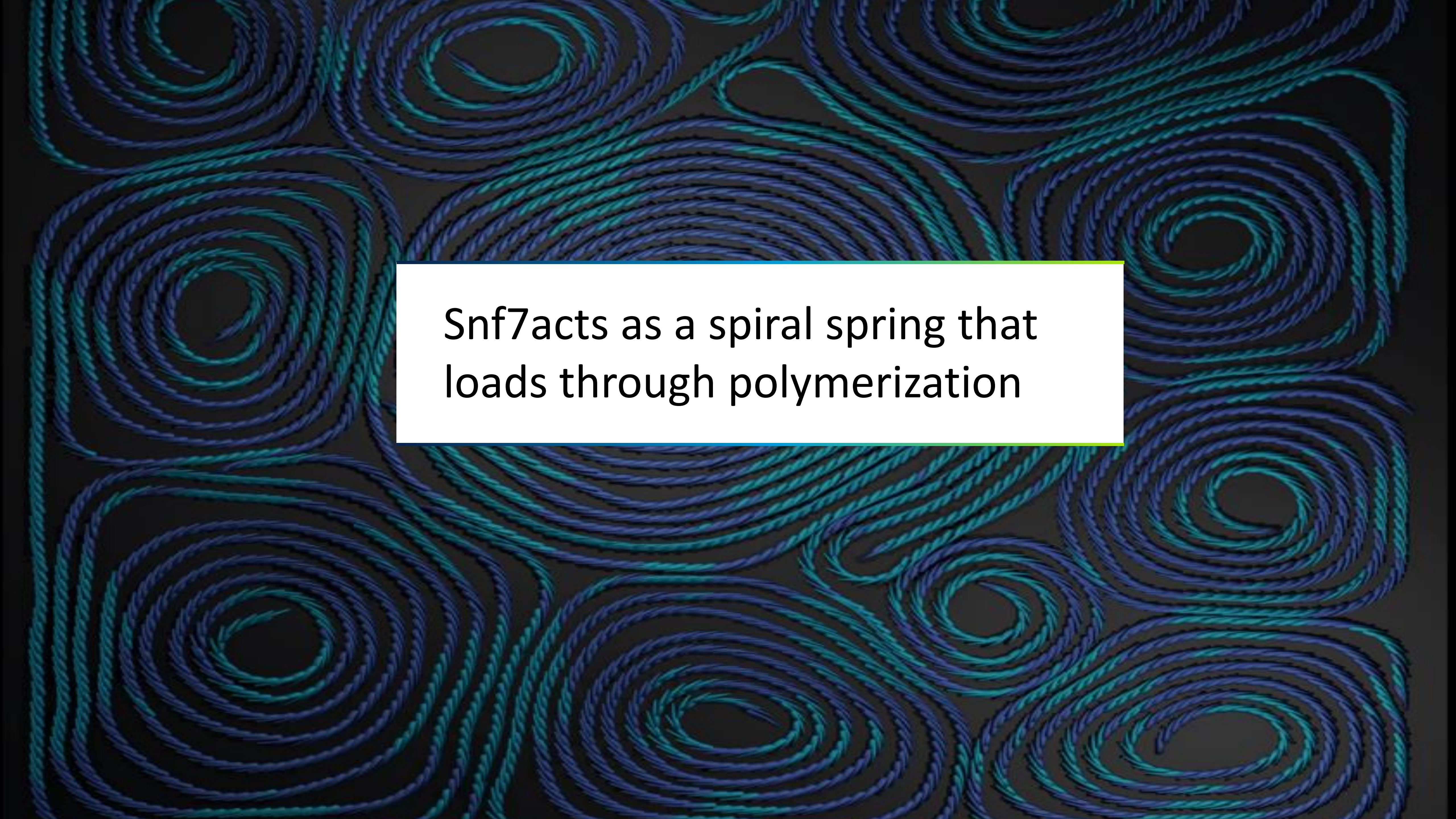


Snf7 assembly formation

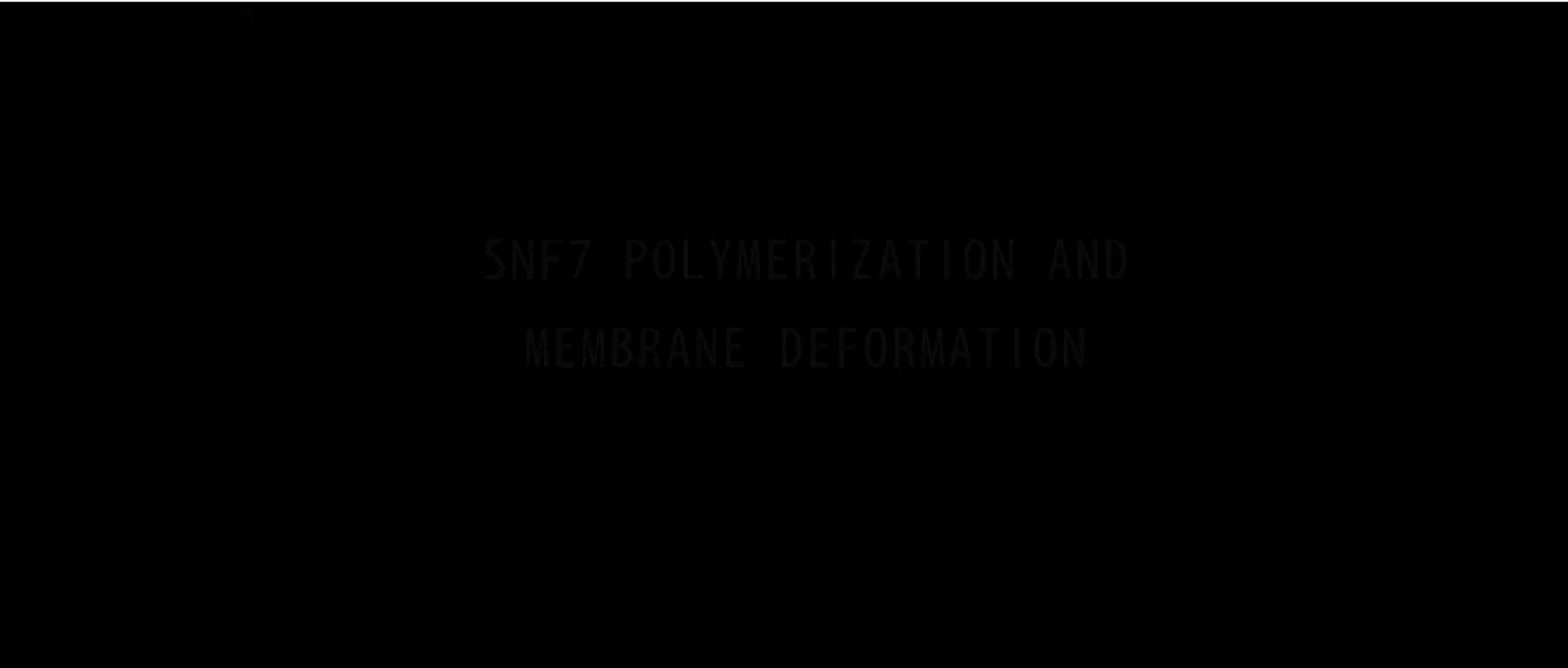


- The assemblies are maturated and under lateral pressure
- Transient formation of buds resembling events in secretion

Snf7acts as a spiral spring that loads through polymerization

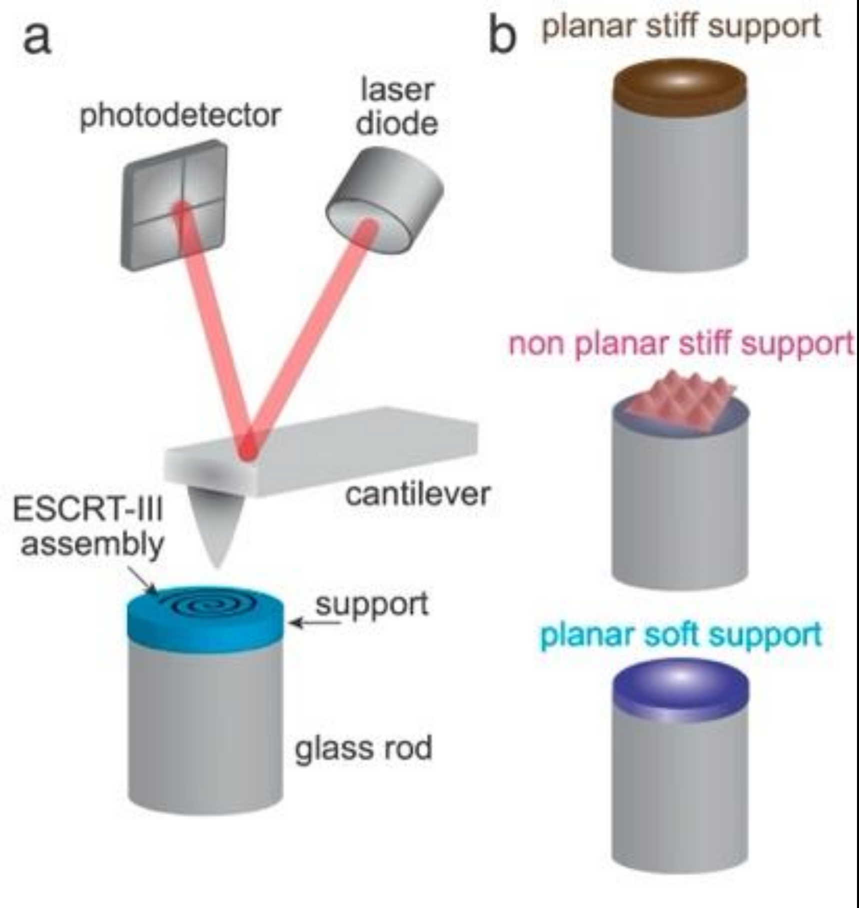


Snf7 induces compression

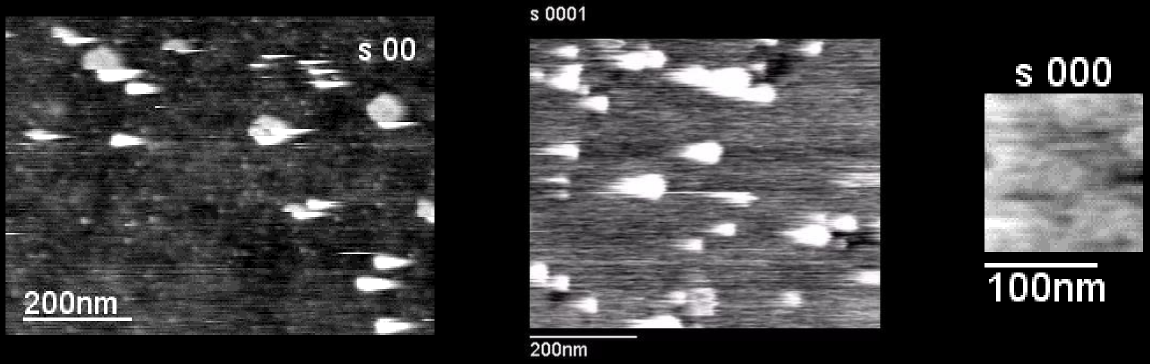


Chiaruttini & Redondo-Morata et al., *Cell 2015; 163, 1-14*

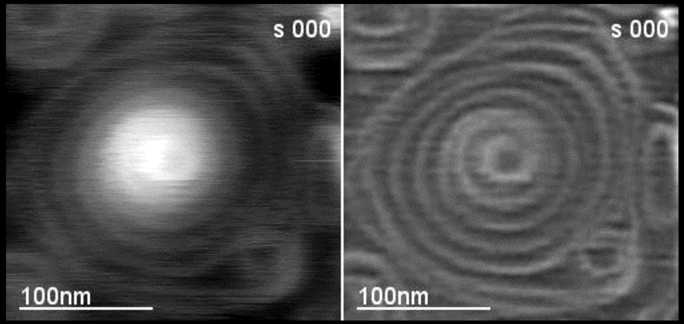
Snf7 sense and alter curvature

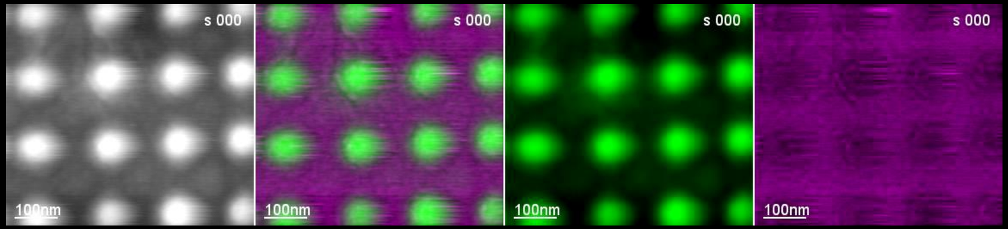


Planar soft support (PDMS)



Nanopatterned rigid support





Jukic et al., Nat. Comm.; 2022, 13 (2174)

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- High-Speed ‘breakthrough’ examples
- Biological membranes

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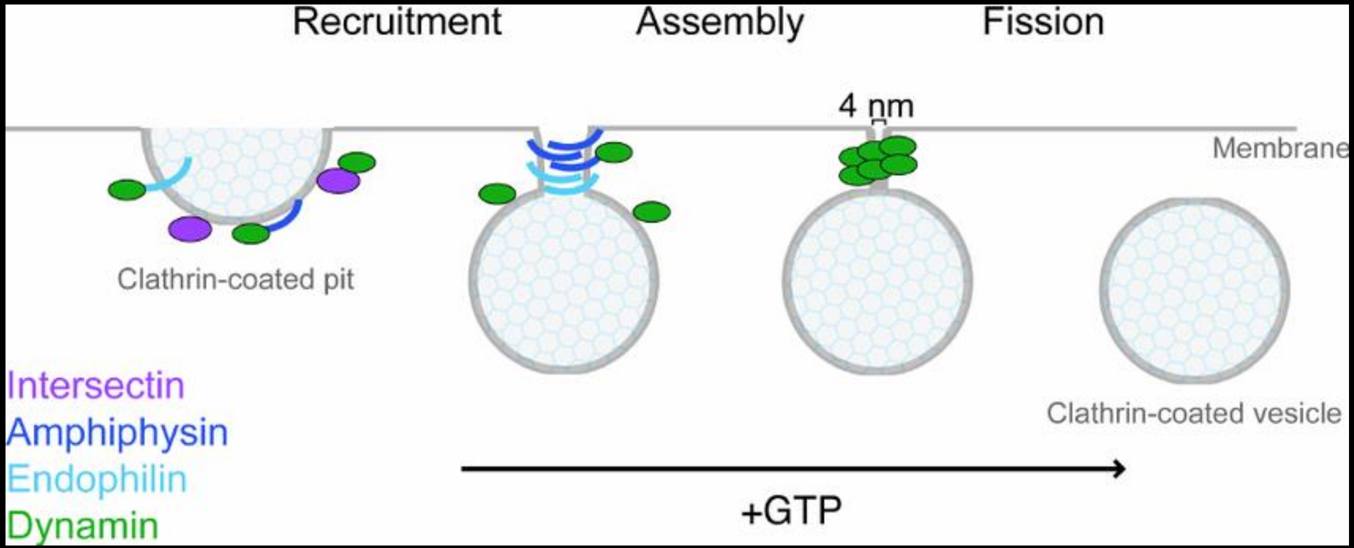
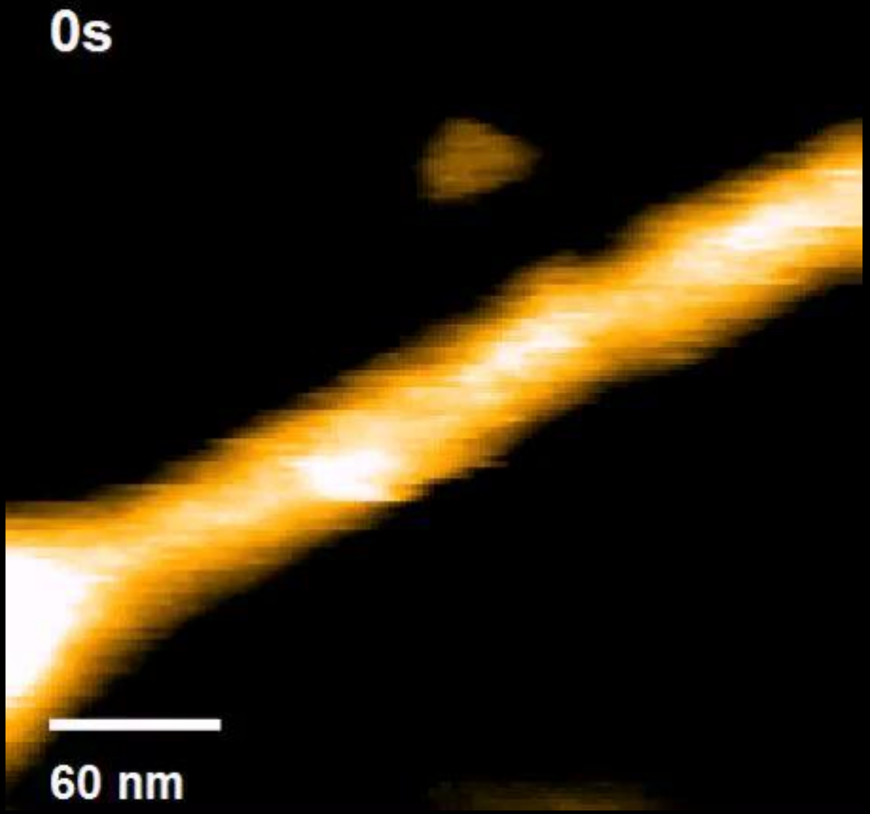
Dynamin-mediated membrane fission

The GTPase dynamin catalyzes membrane fission and is essential in endocytosis and other events such as organelle division



Sundborger & Hinshaw, F1000Prime Reports 2014



Takeda et al., eLife 2018;7:e30246

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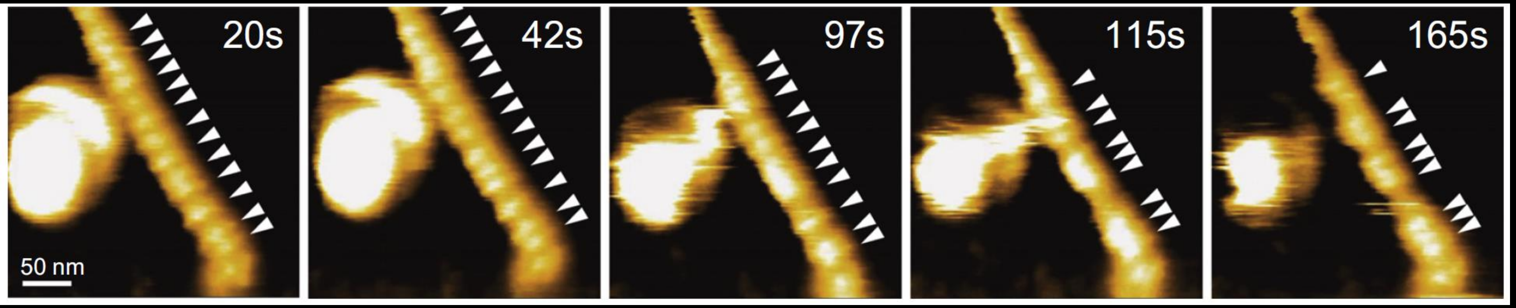

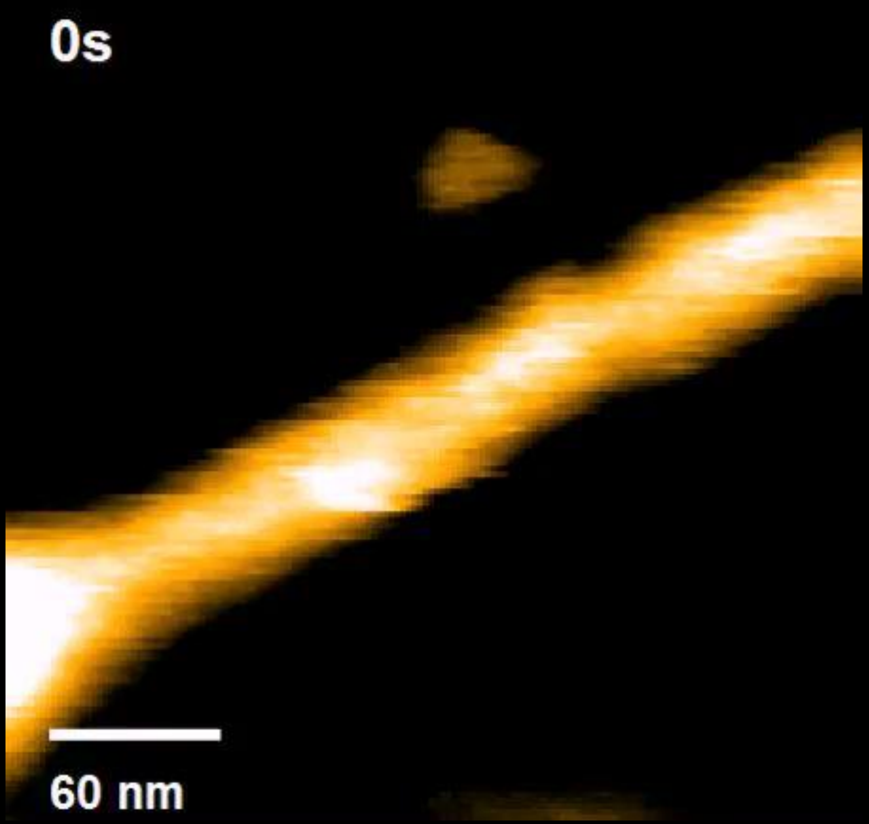

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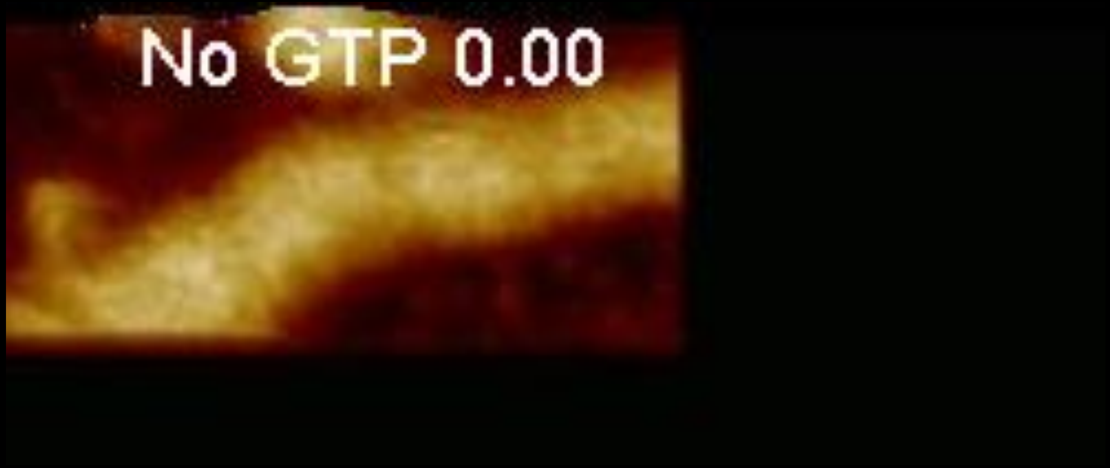
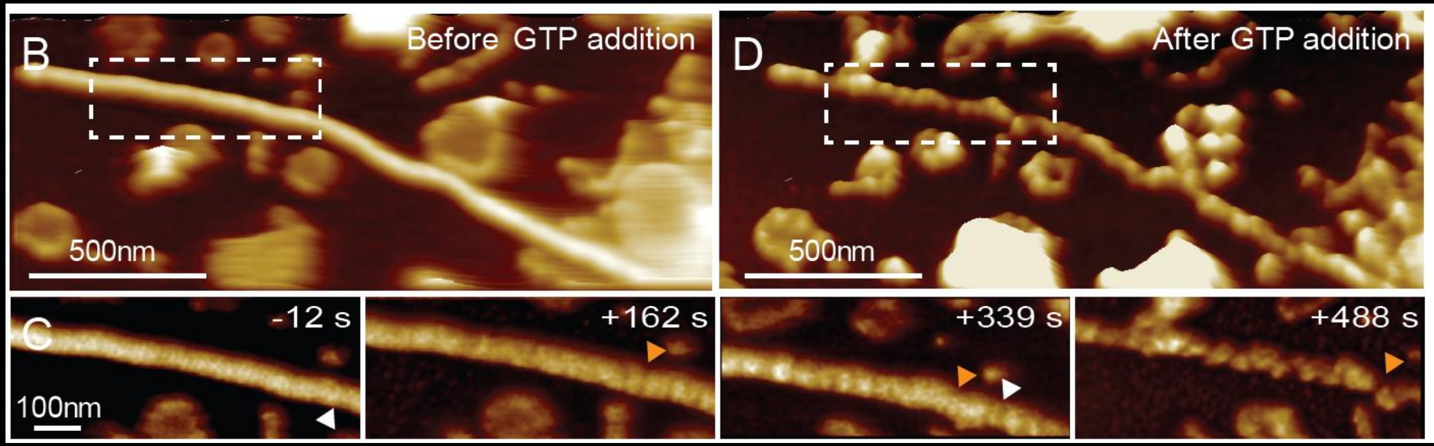


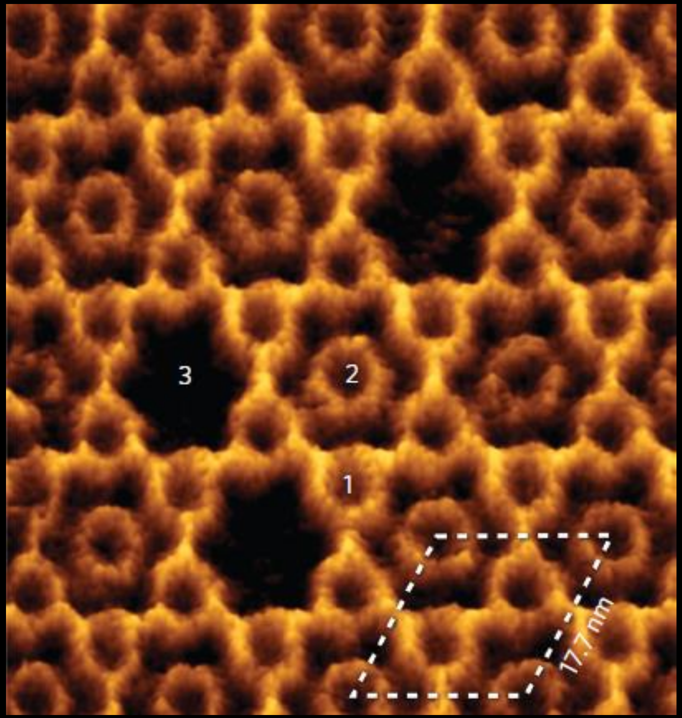


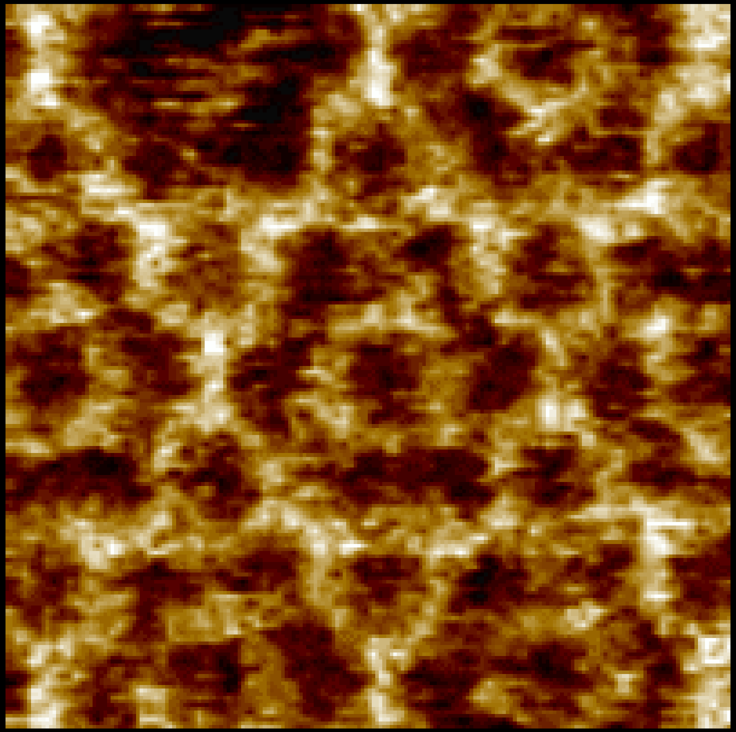
Colom et al., PNAS 2017

Outline

Annexin V and ‘Bioenhanced AFM’

Annexin V displays different effective Ca^{2+} and membrane affinities depending on assembly location

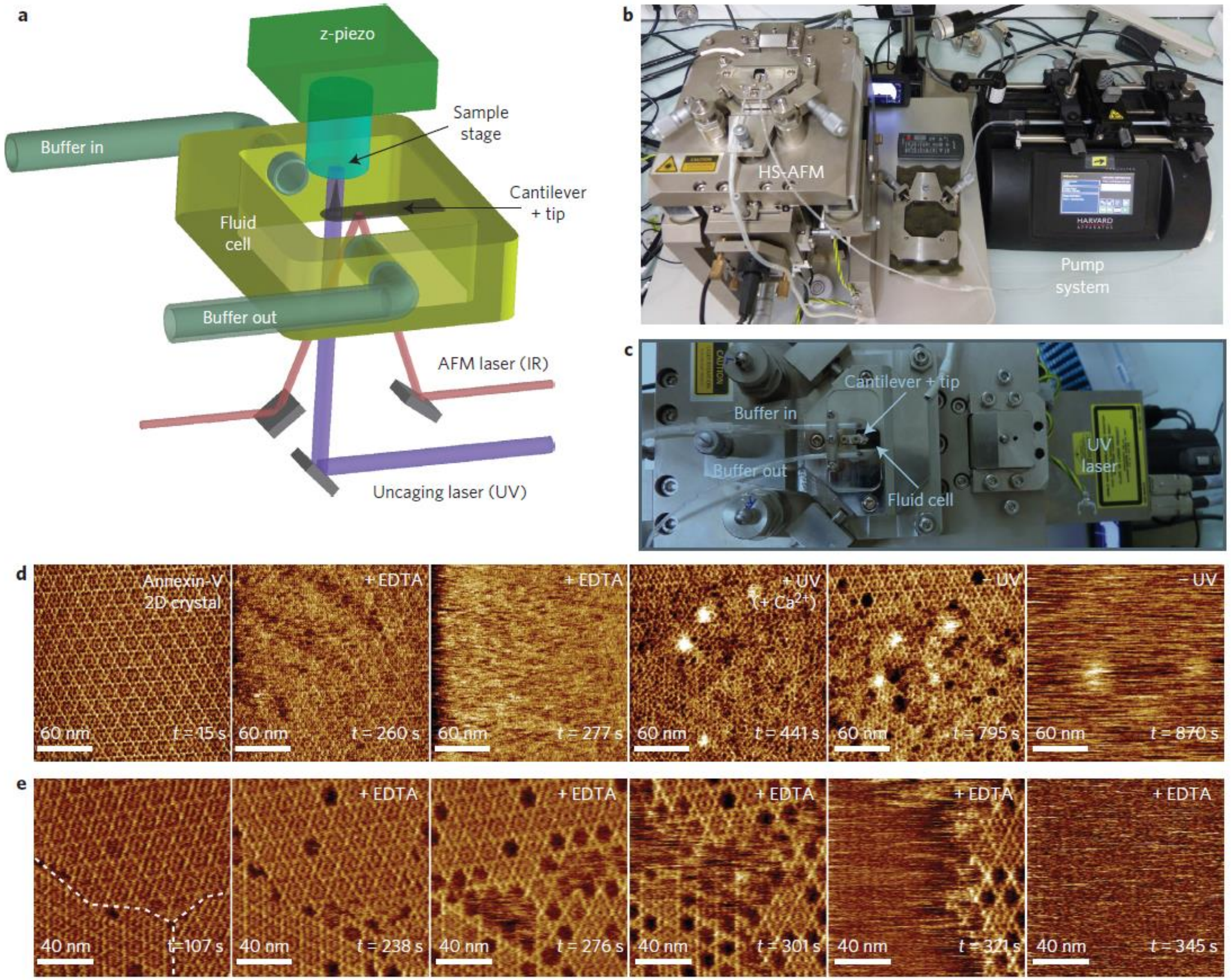




Miyagi et al., *Nat. Nanotec.* 2016; 11, 783-790

Annexin V and ‘Bioenhanced AFM’

Fluid exchange pumping system and an optical pathway for pulsed UV laser uncaging of caged compounds



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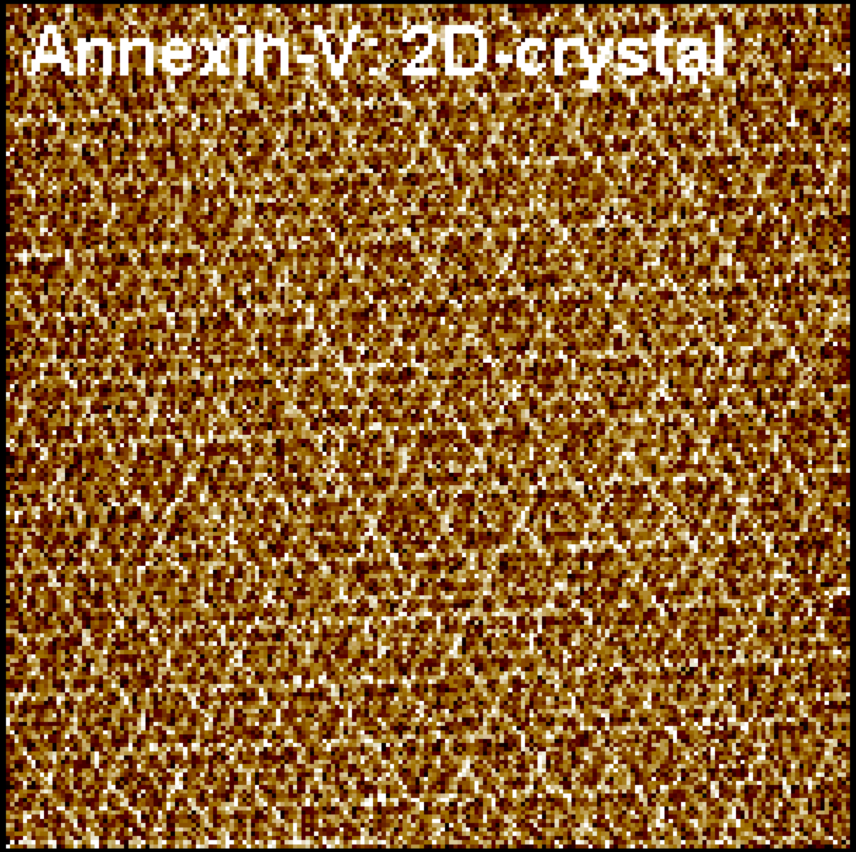

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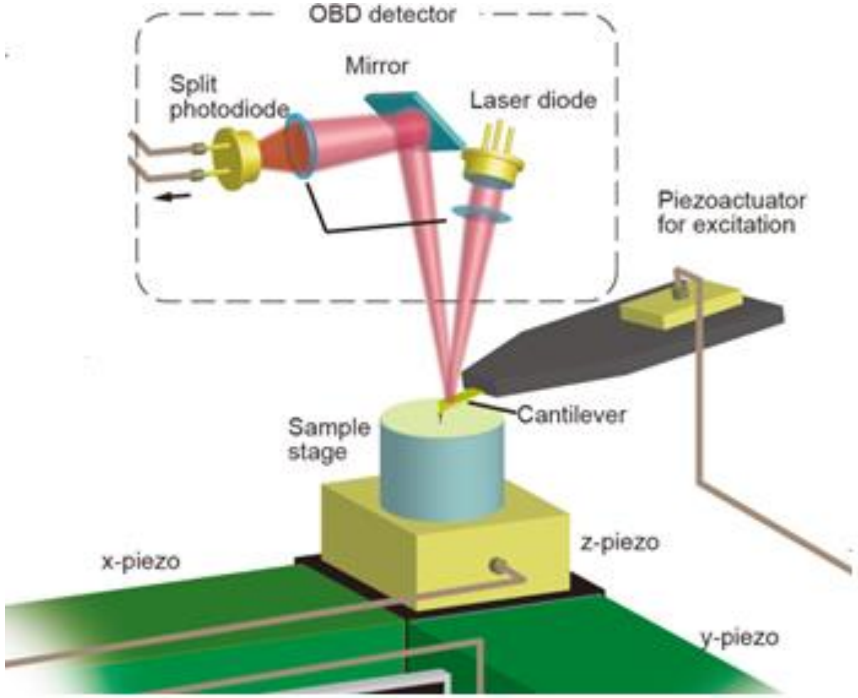
High-Speed AFM



High-Speed AFM:

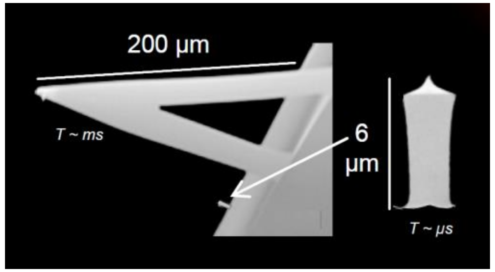
1000 x faster than AFM

Buffer solution

Ambient pressure

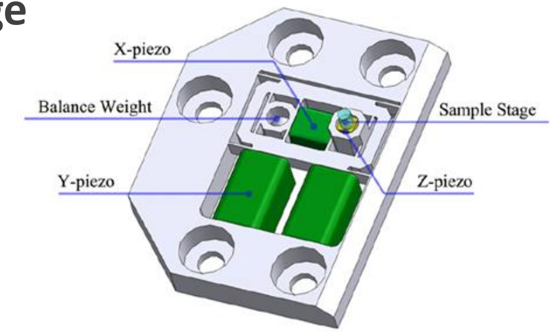
Ambient temperature

01 Small cantilevers



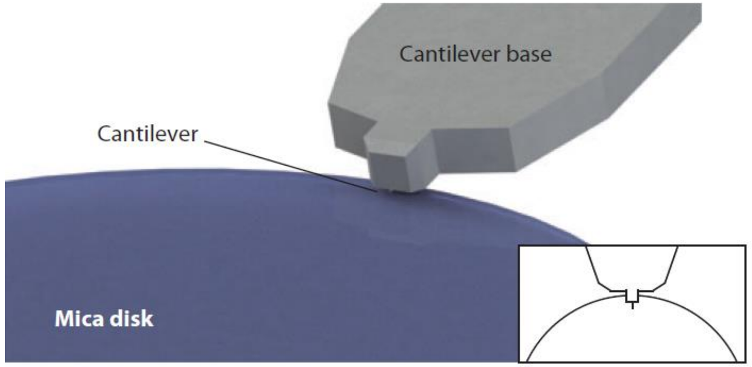
resonance ~1/ $\sqrt{mass}$

02 Small scanners & sample stage



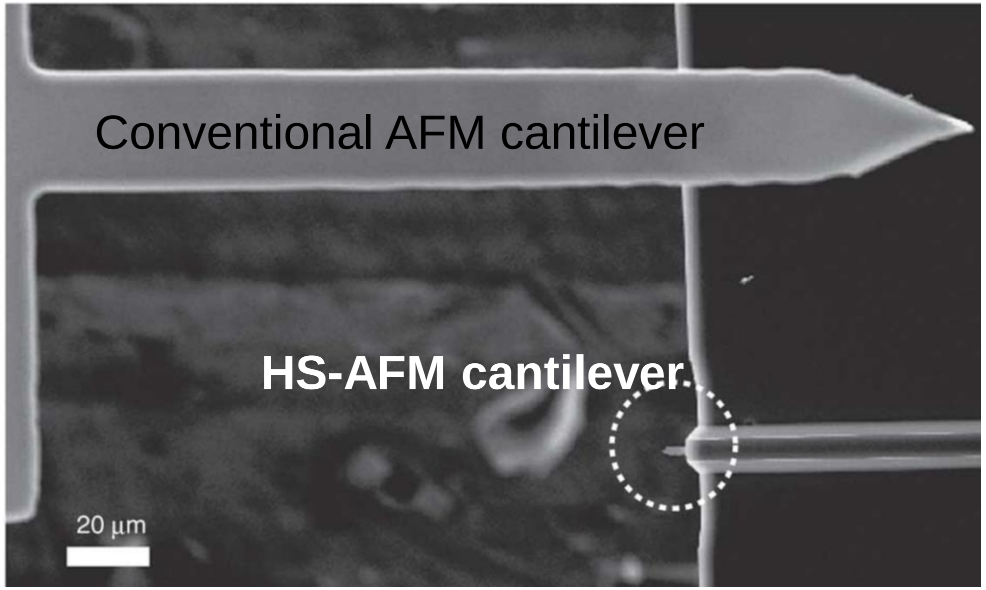
03 Faster electronics

04 Dynamic controller



Ultrashort cantilevers

The cantilever spring constant has to be as low as possible to reduce forces on the sample

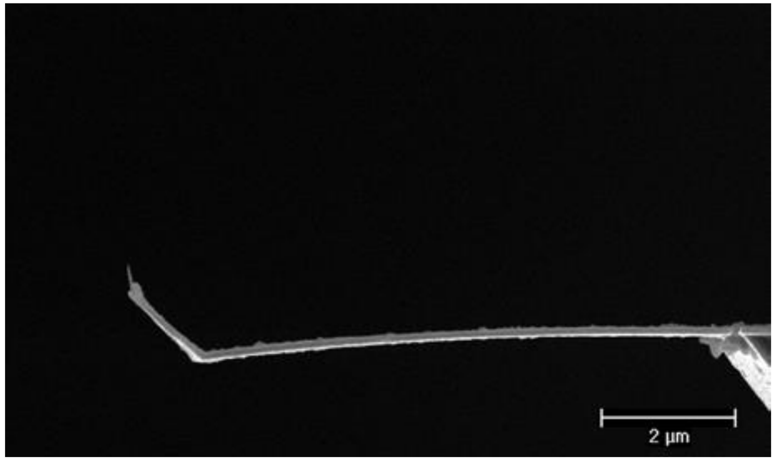


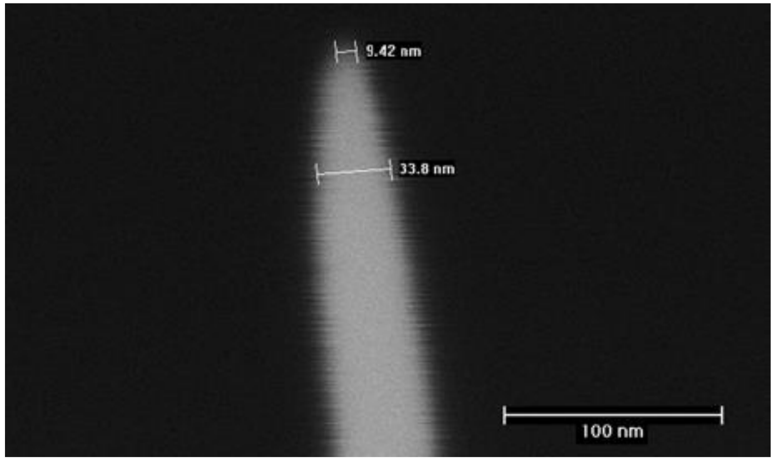
- High frequency f_c and low spring constants k_c are achieved with cantilever of small dimension

$$resonance \sim 1/\sqrt{mass}$$

Ultrashort cantilevers

Electrom Beam deposit (EBD) growth tips





Sakiyama et al. Nat Nanotech. 11:719–723

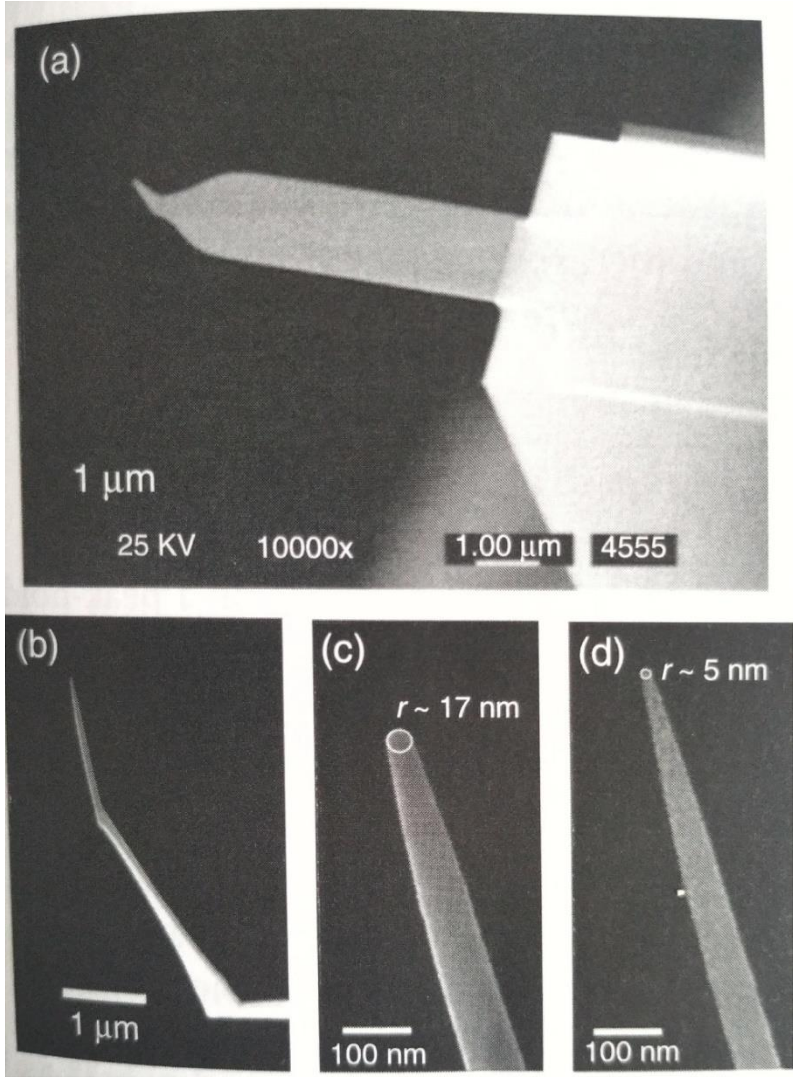
Ultrashort cantilevers

Plasma etching the carbon tips to increase the aspect ratio

- Monoatomic plasma: oxygen, nitrogen…
- In general, we prefer the use of Helium or Argon

Ultrashort cantilevers

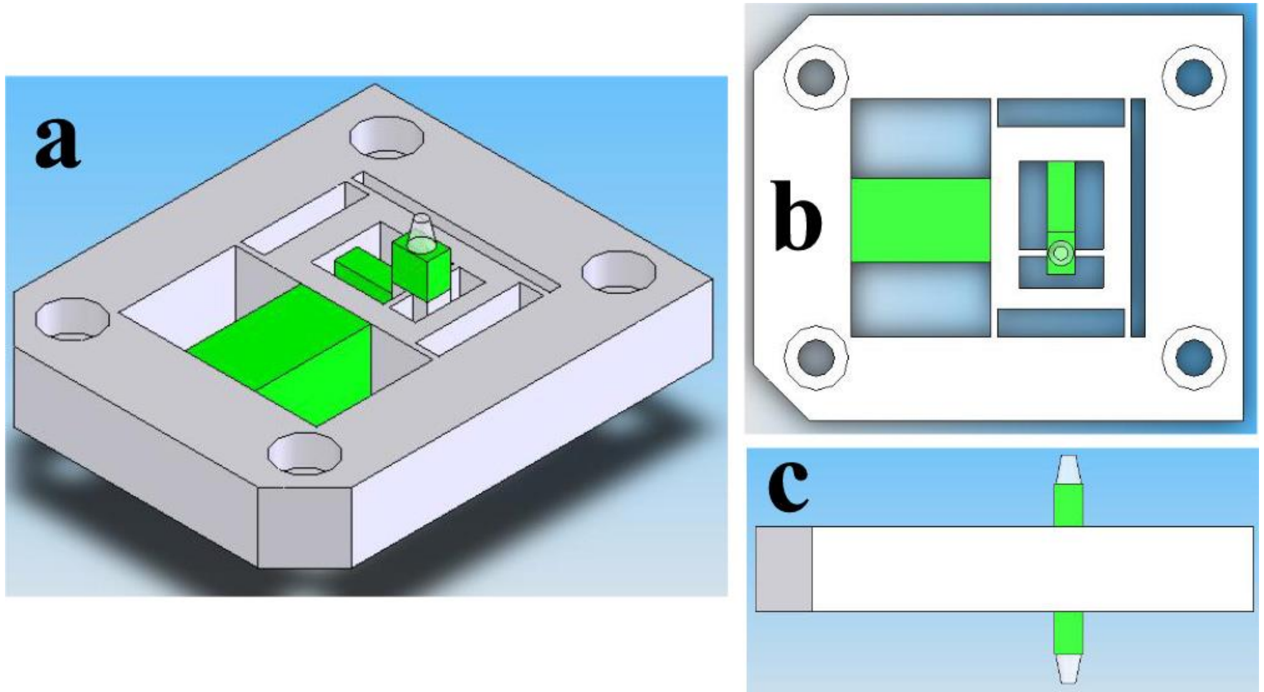
Electrom Beam deposit (EBD) growth tips



Ando et al. in "Atomic force microscopy in liquid", Arturo M. Baró & Donald Refenberger, Wiley-VCH Verlag GmbH (2011/12/15) ISBN-10: 3527327584, ISBN-12: 978-3527327584.

Moving components

HS scanner is constructed using stack piezoactuators and flexures monolithically fabricated within a metal base.



Surf. Sci. Nanotech. Vol. 3 (2005) 384-392

- Sketches of the high-speed scanner. (a) 3D view, (b) top view, and (c) side view. The green blocks are piezo actuators. A sample stage is attached on the top of the upper *z*-piezo actuator, and a dummy stage is attached on the top of the lower *z*-piezo actuator used for counterbalancing. The dimensions (*W* × *L* × *H*) of the *x*-, *y*-, and *z*-actuators are 2 × 3 × 5, 5 × 5 × 10, and 2 × 3 ×3 mm3, respectively.

Moving components

We need to damp the frequencies of the piezo elements

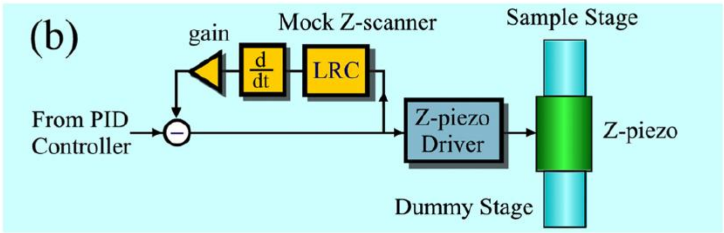
- **1. Passive damping:**

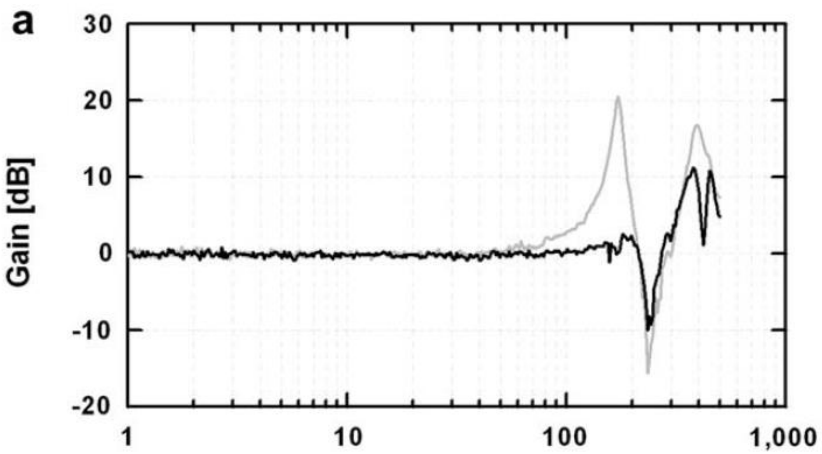
 the X and Y piezos are embeedded in a silicon elastomer
The Z piezo has a counterbalance (dummy piezo)

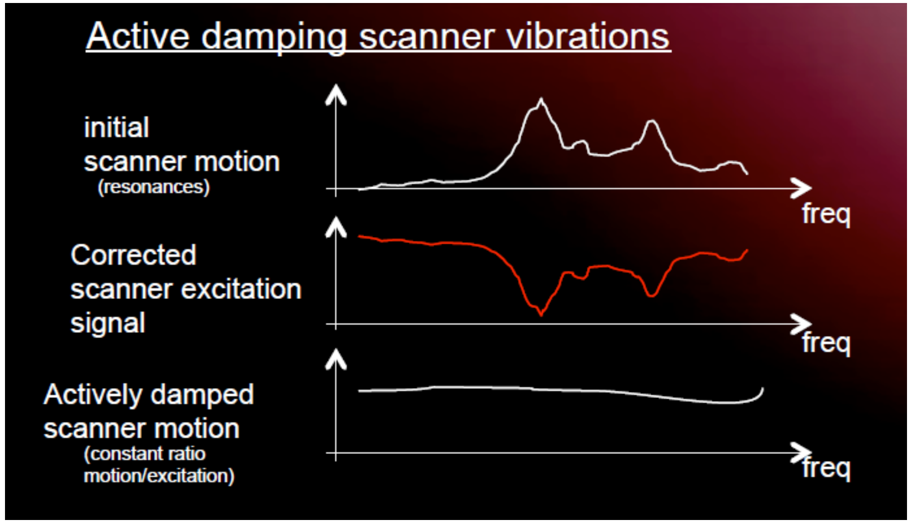
- **2. Active damping: « Q-control »**

Moving components

We need to damp the frequencies of the piezo elements



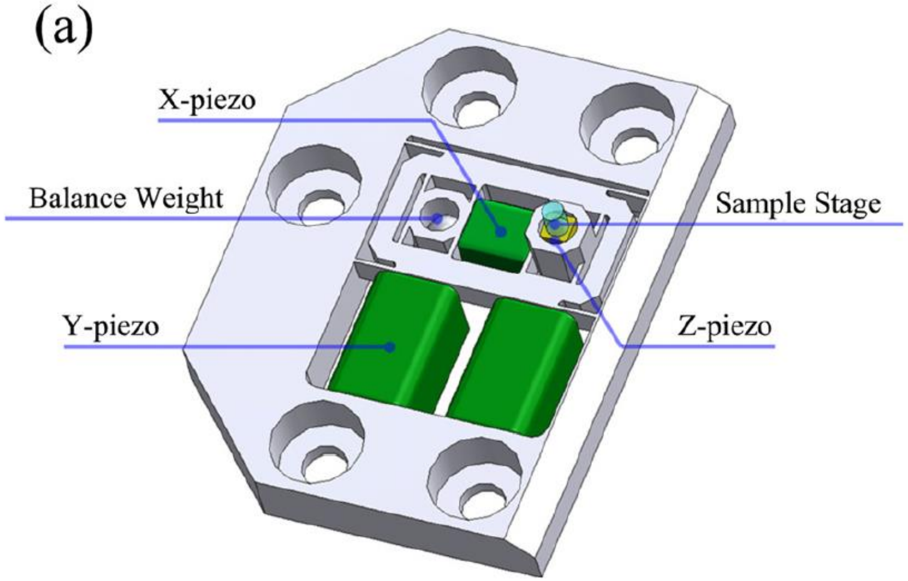


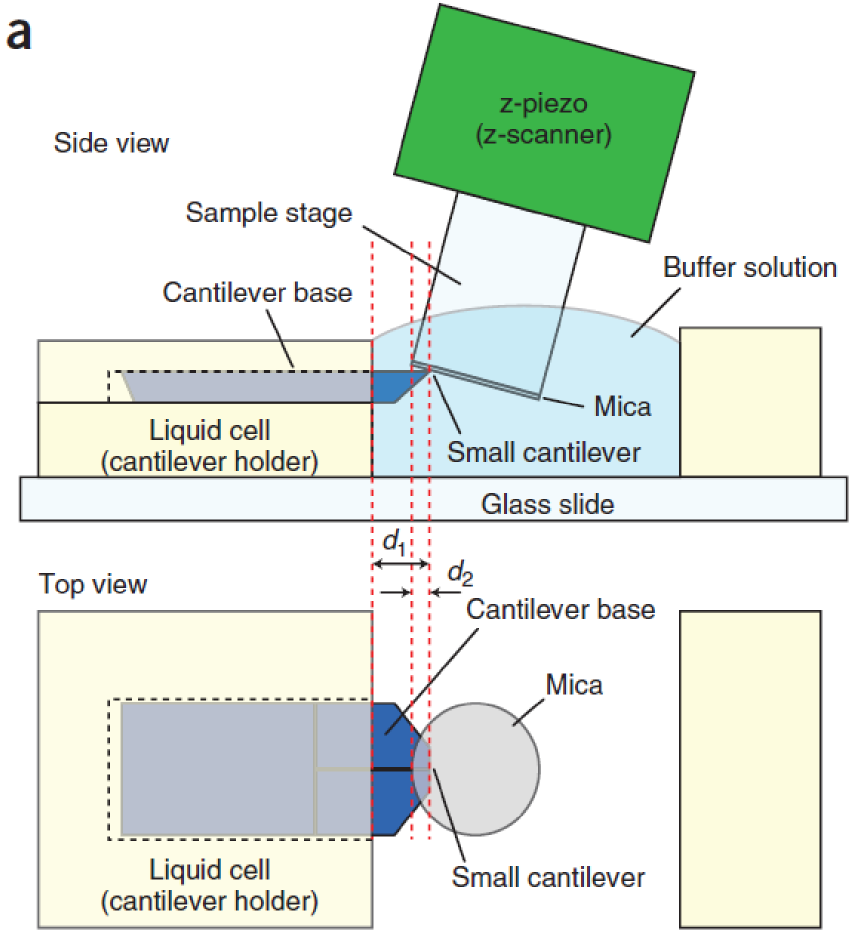


- A schematic of the active damping method. The feedback signal (output from the PID controller) is fed to the active Q-control circuit. The transfer function of the LCR circuits (mock z-scanners) is adjusted so as to become very similar to the z-scanner. The mock z-scanners can contain a number of LCR circuits corresponding to the multiple resonant components of the z-scanner.

Moving components

Small sample stage (2mm diameter) to avoid hydrodynamic pressure

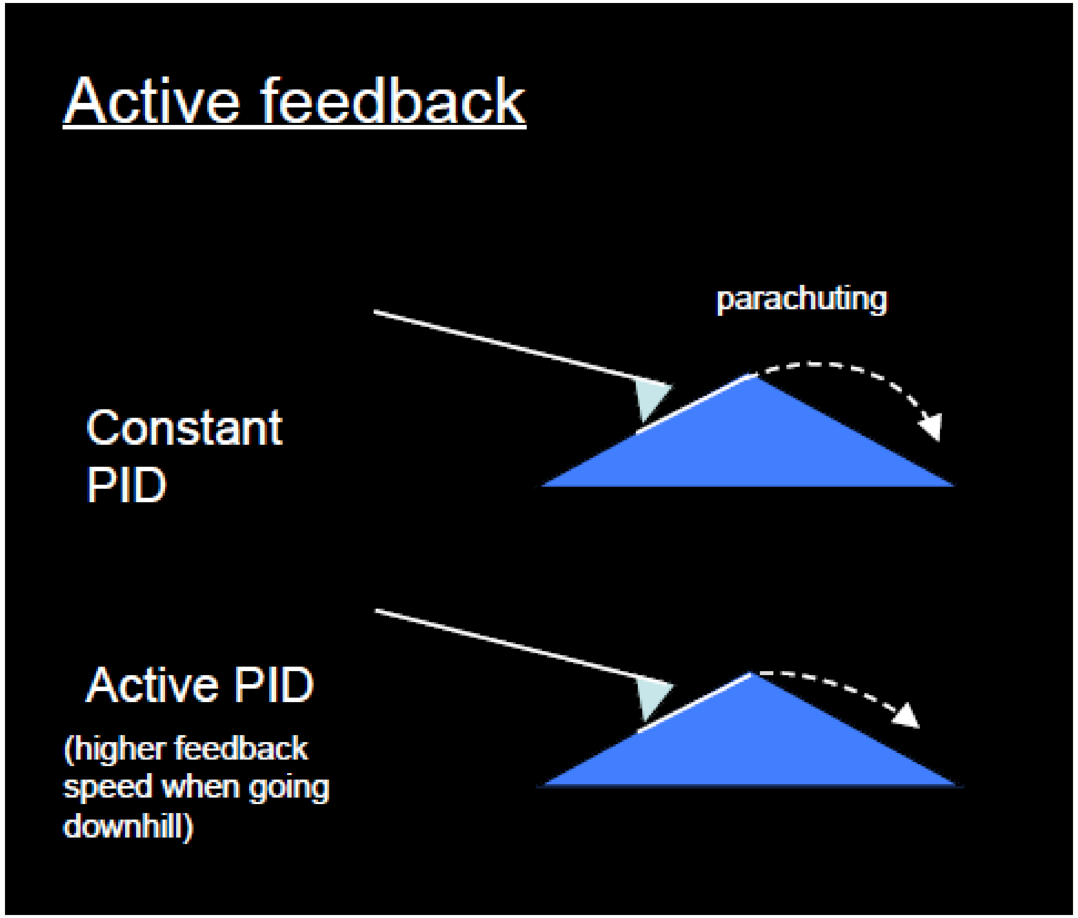


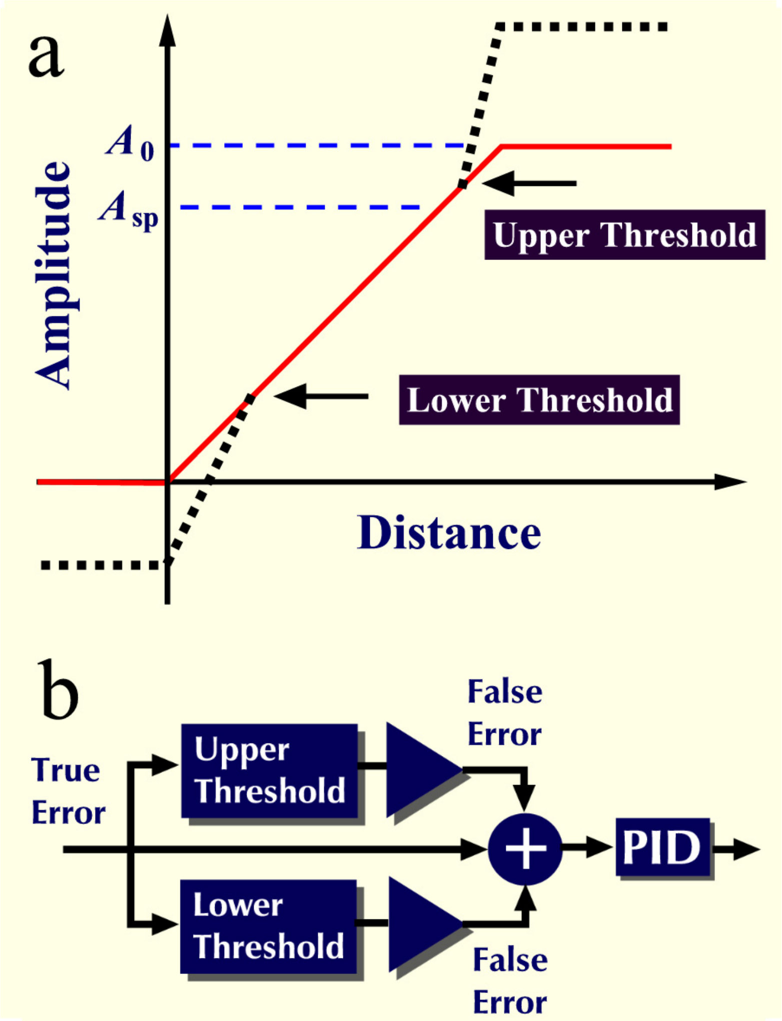


Ando et al. in "Atomic force microscopy in liquid", Arturo M. Baró & Donald Refenberger, Wiley-VCH Verlag GmbH (2011/12/15) ISBN-10: 3527327584, ISBN-12: 978-3527327584.

Dynamic PID

Small sample stage (2mm diameter) to avoid hydrodynamic pressure



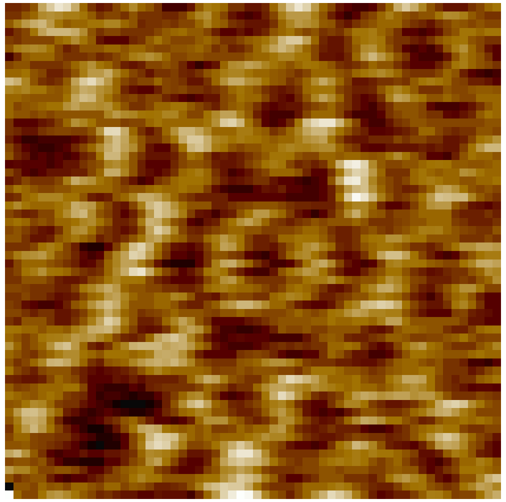


Surf. Sci. Nanotech. Vol. 3 (2005) 384-392

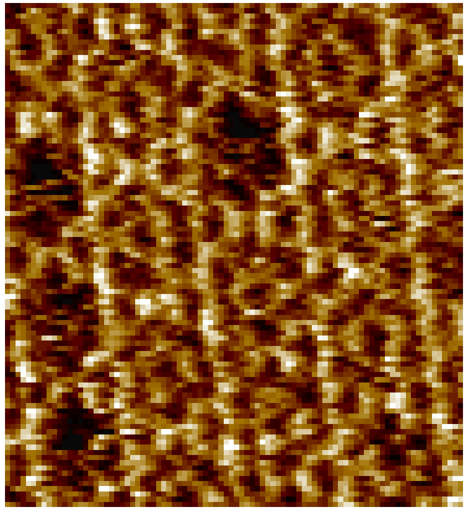
Tip-sample interaction

We need to damp the frequencies of the piezo elements

- "Disappointingly, analysing movies at 650 ms or much faster, at 100 ms and 20 ms frame rates resulted in very different lifetimes depending on the image acquisition rate, meaning that despite our efforts to use a minimal cantilever- drive amplitude and minimal amplitude damping, the energy that the cantilever adds to the system increased the rotation of the molecules"



20 ms per frame
30 nm

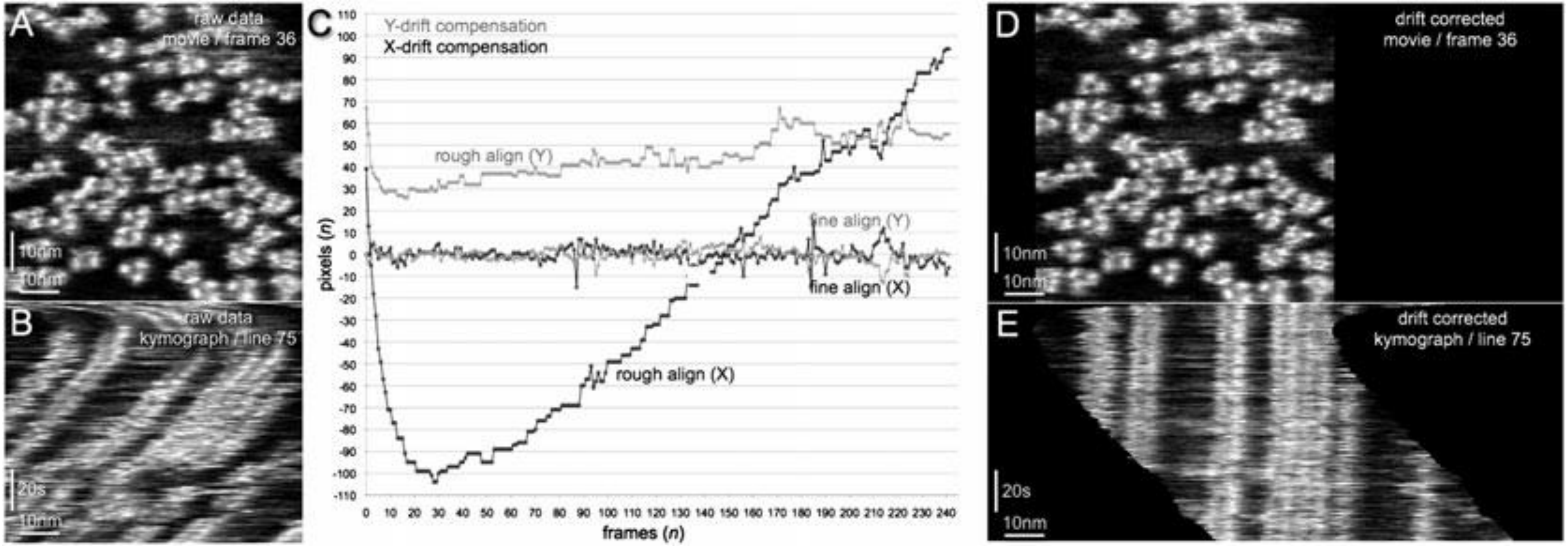


100 ms per frame
66 nm

Miyagi et al., Nat. Nanotech. 11, 783 (2016).

Image analysis

Drift correction or image registration



Husain et al., Journal of Molecular Recognition 25(5):292-8 · May 2012

Conclusions

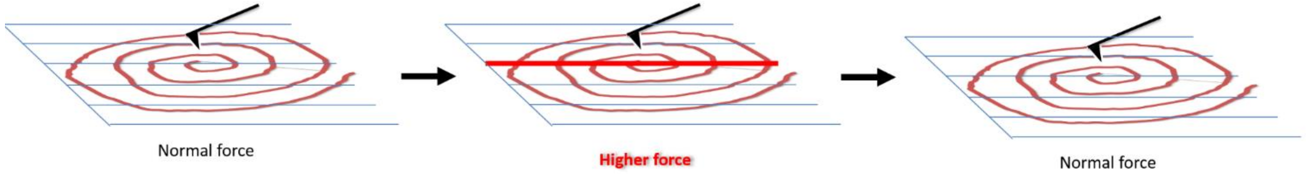
01 The molecular movies obtained by High Speed-AFM provide insights otherwise not accessible by other means to date

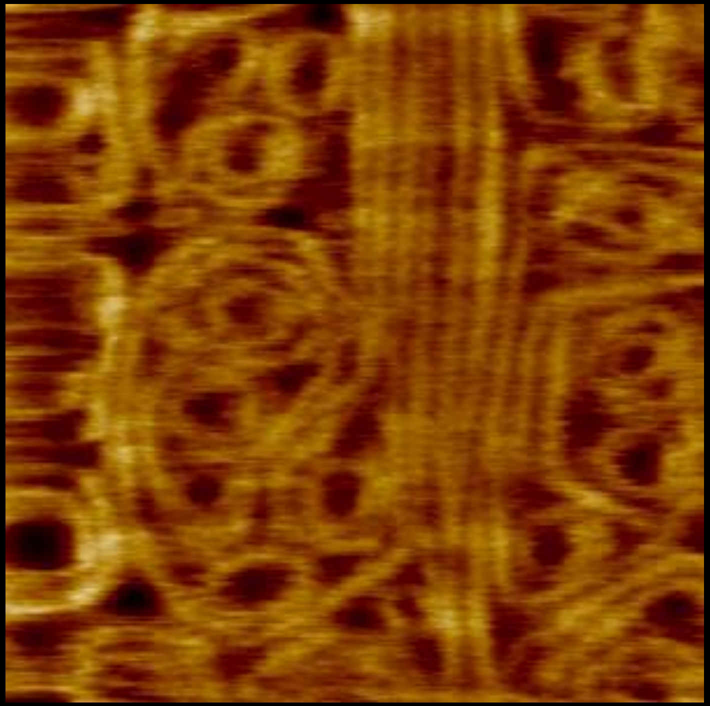
02 The technique itself, alone or combined with other techniques is in continuous development and its relevance is foreseen to expand in the future

03 High-impact in biophysics

Recent progresses

Interactive mode

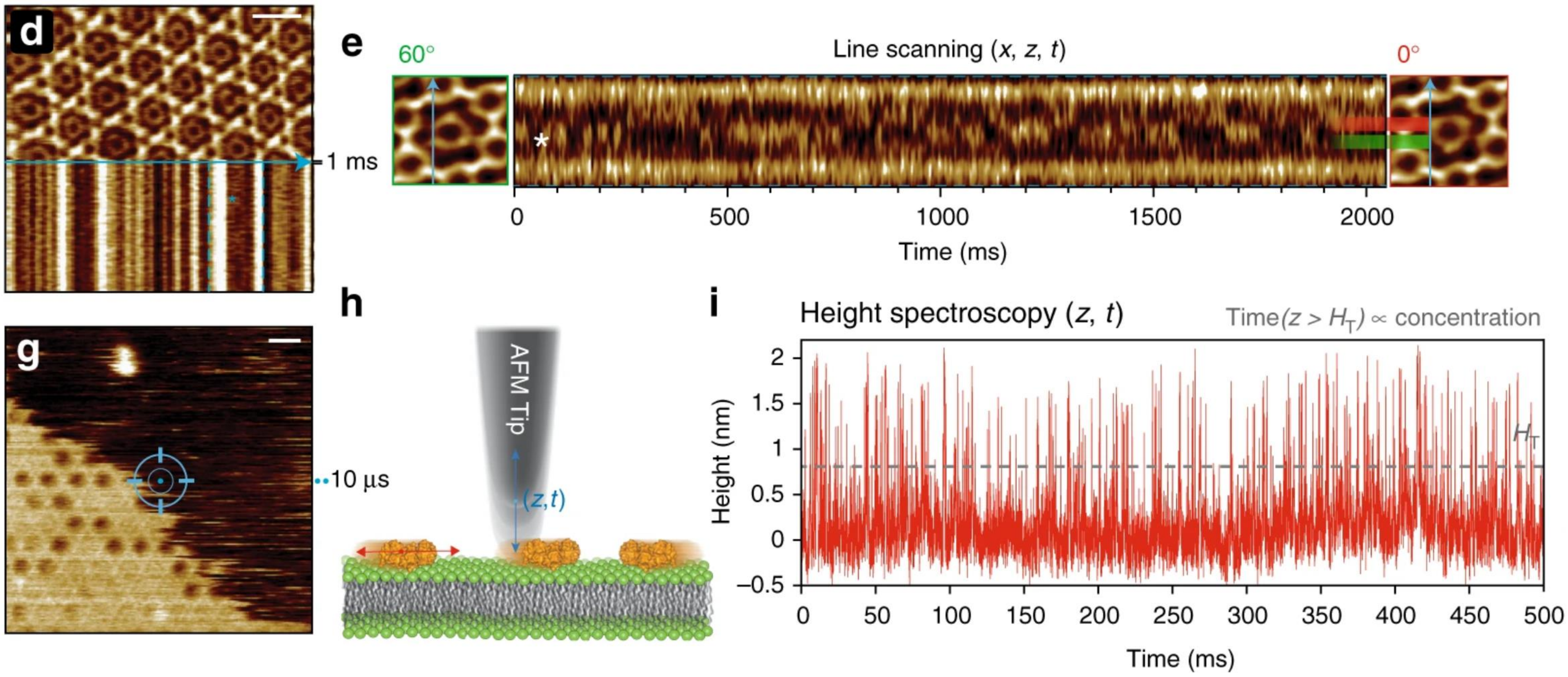




Scan size: 600 nm
Time per frame: 800 ms

Recent progresses

Reduce temporal resolution by reducing dimensionality of data acquisition

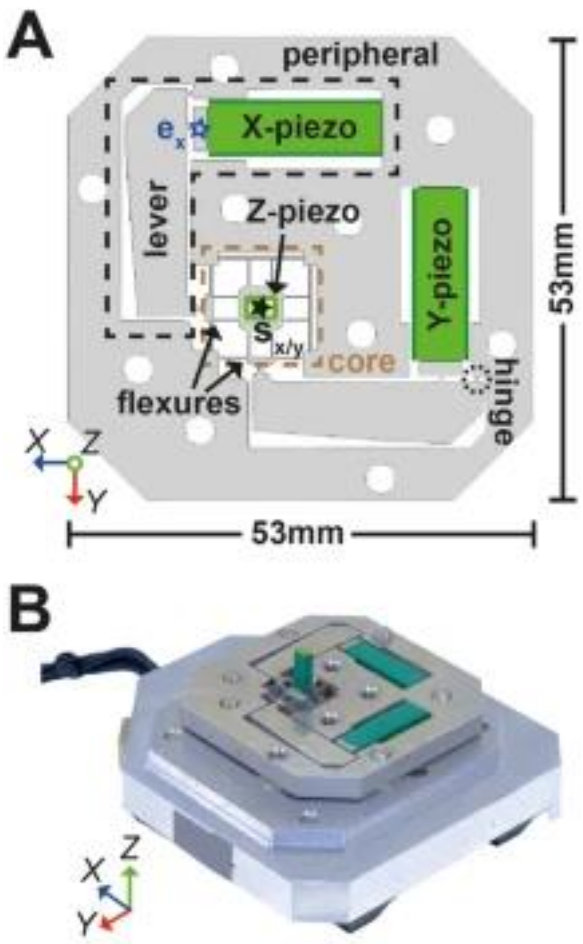


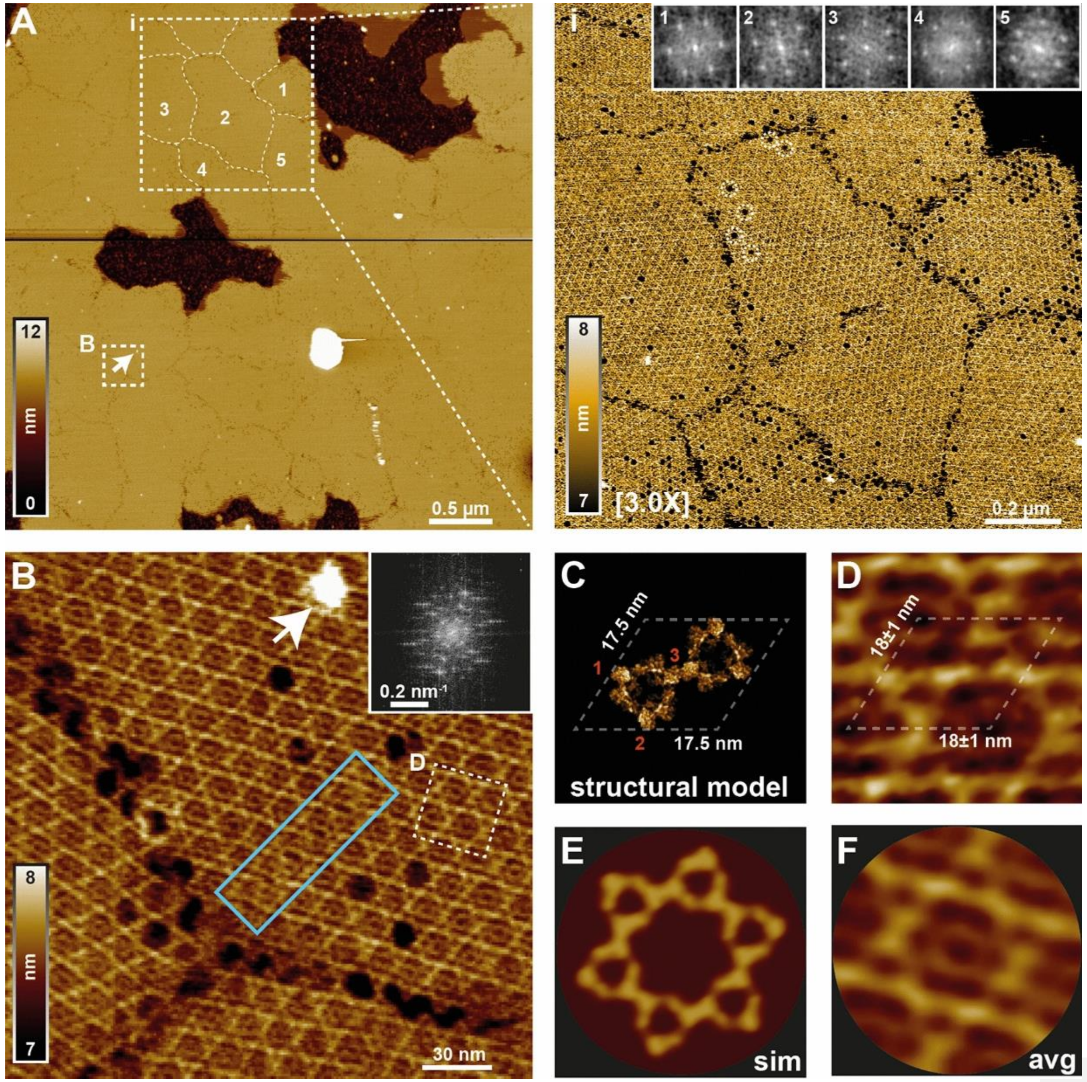
Heath & Scheuring, Nat. Com. (2018)

Recent progresses

An ultra-wide scanner for large-area high-speed atomic force microscopy with megapixel resolution

- The improved design extends the scanner’s acquisition bandwidth and permits high-fidelity, low-noise imaging at 0.5 fps (100 Hz line rate) over a 36 × 36 µm2 area, corresponding to a high scan speed of 7.2 mm/s.



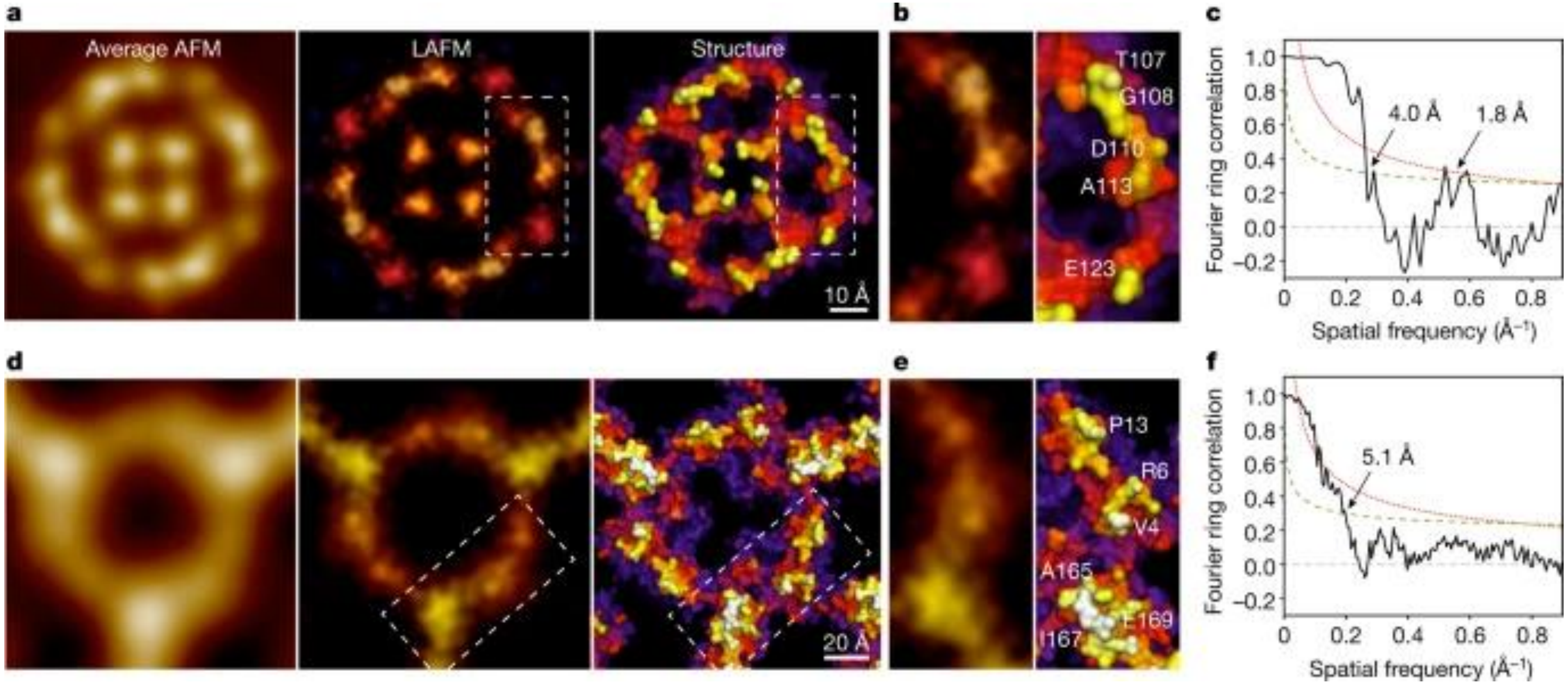


Marchesi et al., *Scientific Reports* vol. 11, 13003 (2021)

Recent progresses

Localization atomic force microscopy (LAFM)

- to resolve time- or environment-dependent conformational changes
- to provide high-resolution information of single molecules or of non-ordered supramolecular assemblies



Heath et al., *Nature, 594, 385–390 (2021)*

Recent progresses

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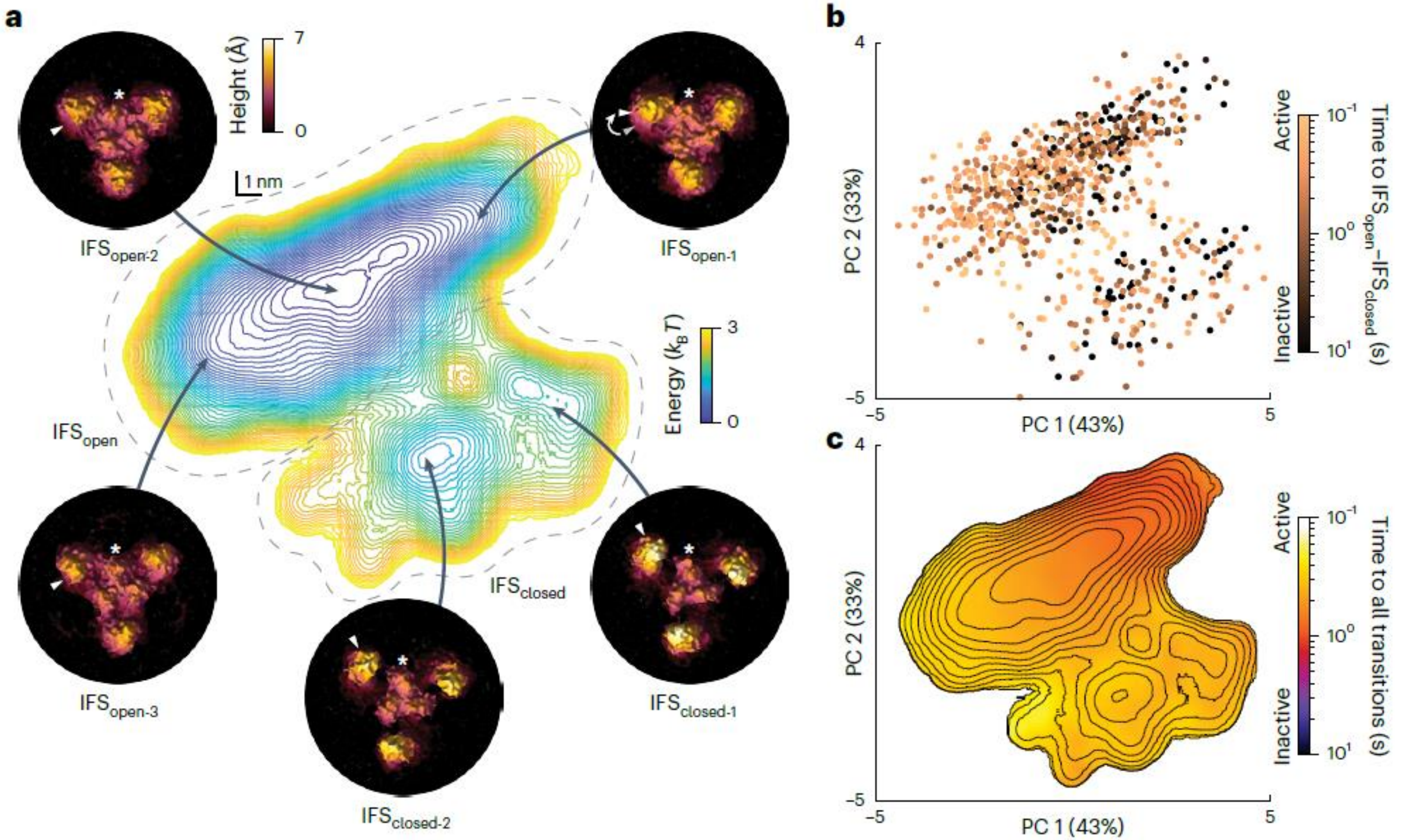
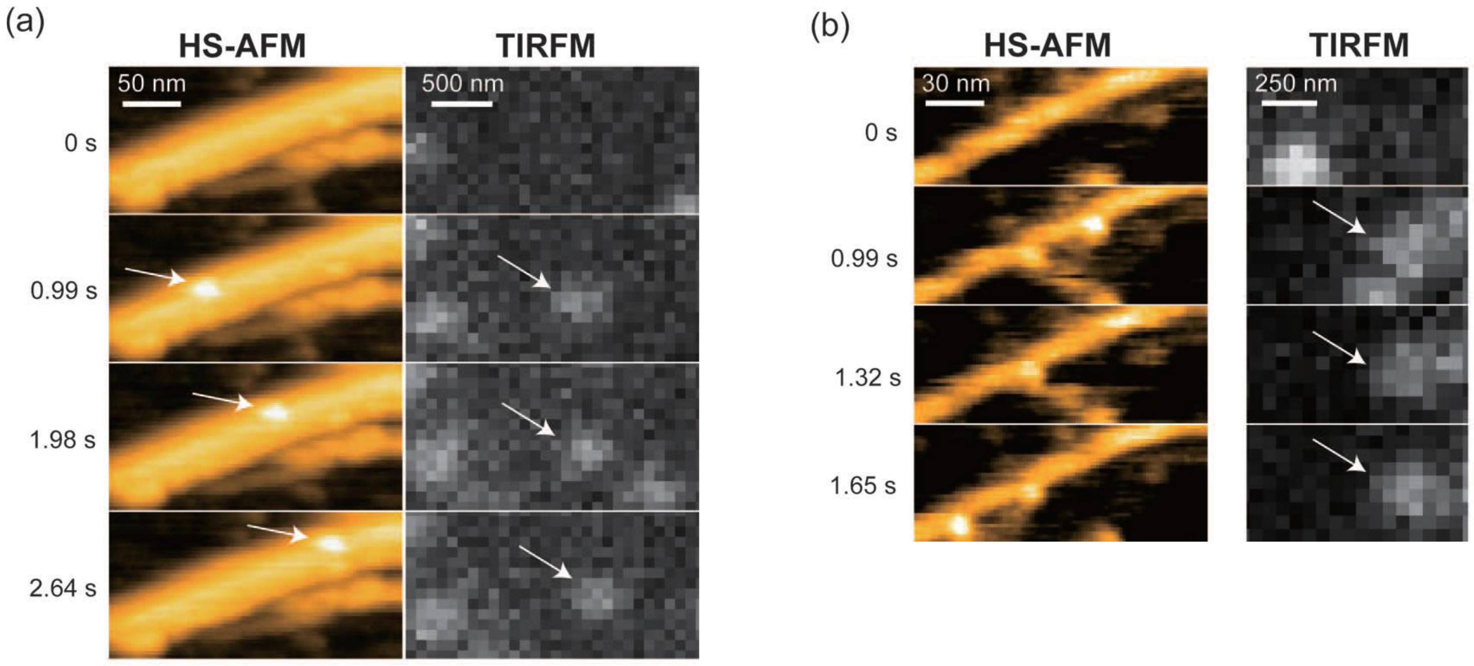


Jiang et al., *Nat. Str. Mol. Biol., 2024*

Perspectives

High-Speed AFM + Optical Microscopy

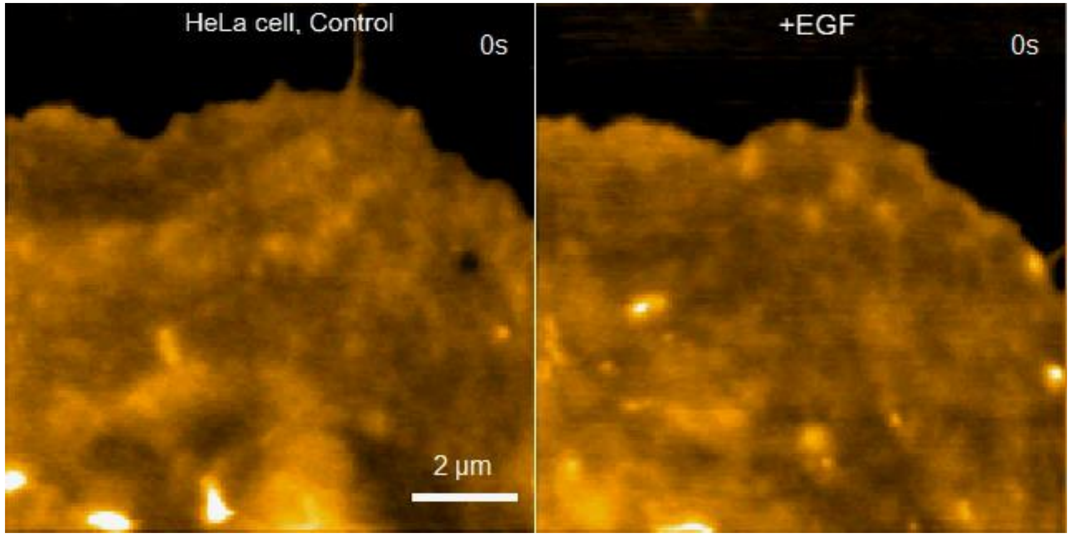


Fukuda et al., Review of Scientific Instruments 84, 073706 (2013)

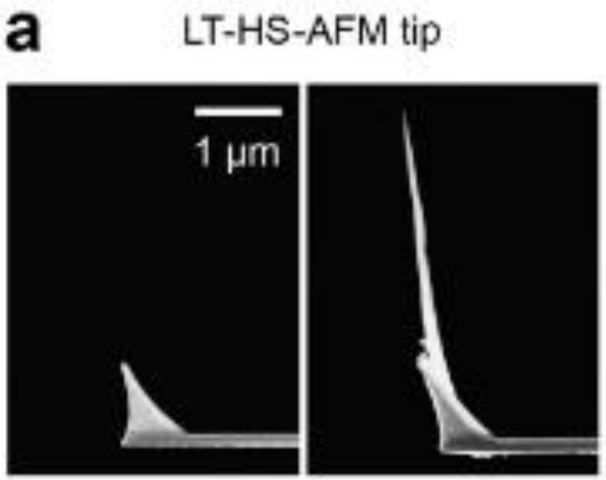
Perspectives

Wide area scanners

Attaching an extremely long (~3 µm) and thin (~5 nm) tip by amorphous carbon to the cantilever allows us to image the surface structure of live cells with the spatiotemporal resolution of nanometers and seconds

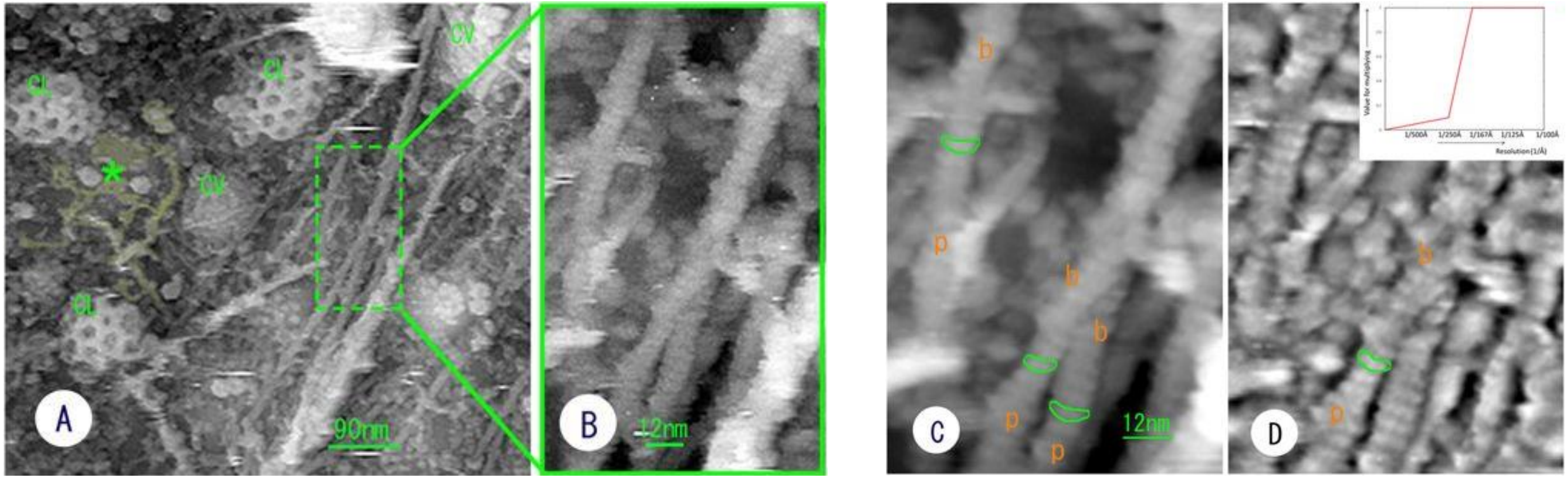


Shibata et al., Sci Rep. 2015; 5: 8724.



Perspectives

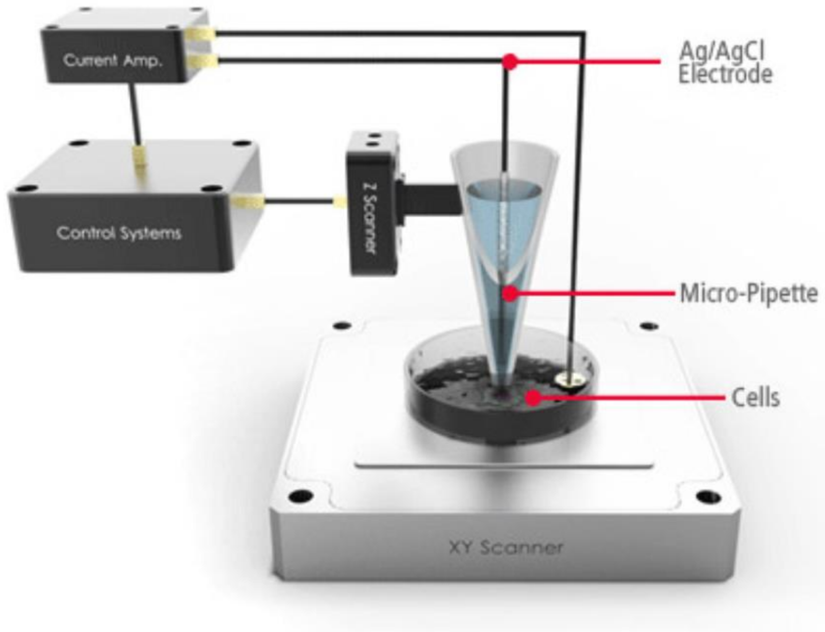
Interior of de-roofed cells



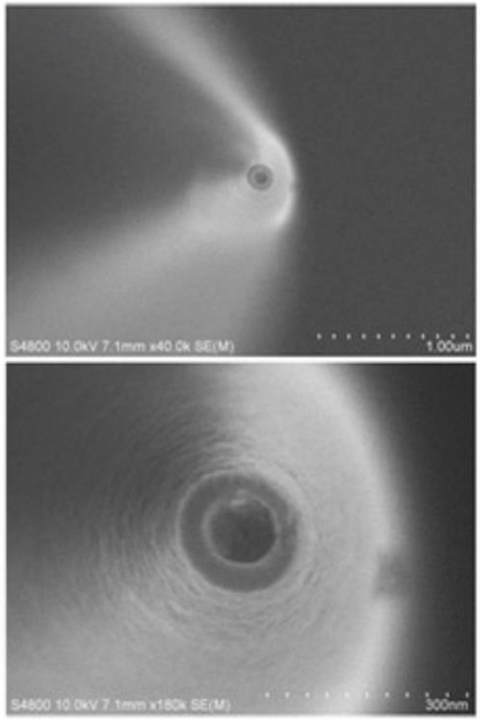
Usukura et al., *Scientific Reports* **volume 6**, Article number: 27472 (2016)

Perspectives

Scanning Ion Conductance Microscopy



Configuration of SICM

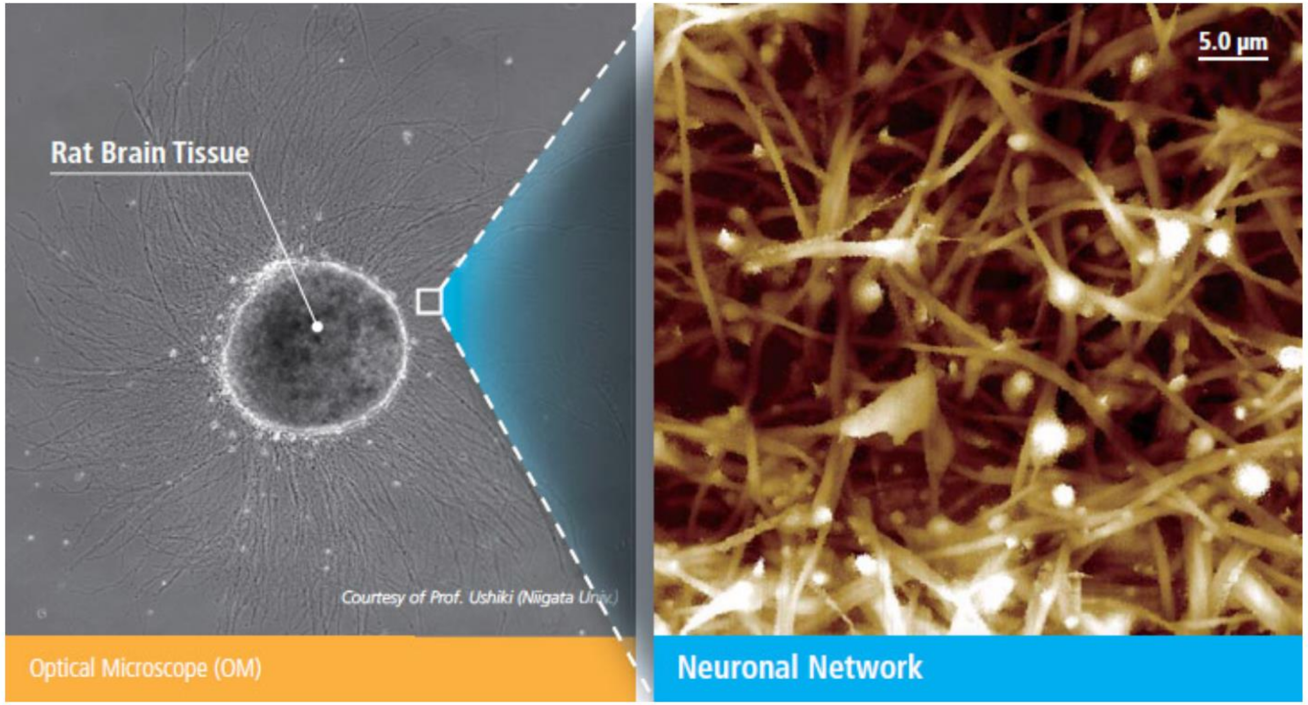


Nano-pipette End Opening (SEM image)

Images from Park Systems [www.parkafm.com]

Perspectives

Scanning Ion Conductance Microscopy



Scanning Ion Conductance Microscope

Neuronal Netwrok, measured in fluid

Images from Park Systems [www.parkafm.com]

Outline

01. Introduction
- Atomic Force Microscopy (AFM)
- Why fast? High-Speed AFM
- High-Speed 'breakthrough' examples
- Biological membranes

02. AFM applied to in vitro membrane reconstituted systems
- Lipid bilayers and phase transitions
- Pore-Forming Toxins: identification of pre-pore species
- Pore-Forming Toxins: Anomalous diffusion
- Kinetics of antimicrobial peptides
- ESCRT assembly and disassembly
- Membrane fission driven by dynamin
- Annexin and Bio-enhanced HS-AFM

03. High-Speed: how do we do it?
- Short cantilevers
- Moving components

04. Tips on High-Speed imaging membranes
- Sample preparation and imaging

05. Conclusions
- General conclusions
- Recent progresses
- Perspectives

06. Bibliography
- HS-AFM-related
- Biological membranes-related

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High-Speed Atomic Force Microscopy related

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  T. Ando, T. Uchihashi, N. Kodera, M. Shibata, D. Yamamoto, H.Yamashita
  Chapter 7 (pp.189-210), "High-speed AFM for observing dynamic processes in liquid"
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- Dufrêne Y., Ando T., Garcia R., Alsteens D., Martinez-Martin D., Engel A., Gerber Ch., Müller D.; "Imaging modes of atomic force microscopy for application of molecular and cell biology"; Nat. Nanotechnol. 12 (2017) p.295-307 DOI: 10.1038/NNANO.2017.45
- Ando T., Uchihashi T., Scheuring S.; "Filming biomoleculear processes by high-speed atomic force microscopy"; Chem. Rev. 114 (2014) p.3120-3188 doi:0.1021/cr4003837
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- **List of papers on High-Speed AFM : http://www.highspeedscanning.com/hs-afm-references.html**

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- Physical Biology of the cell, R. Phillips et al.
- Methods in Membrane Lipids, D. M. Owen
- Atomic force microscopy in liquid: biological applications, Eds. A. Baro & R. Reifenberger

- **Protocol:**

Zuttion F., Redondo-Morata L., Marchesi A., Casuso I. (2018) High-Resolution and High-Speed Atomic Force Microscope Imaging. In: Lyubchenko Y. (eds) Nanoscale Imaging. Methods in Molecular Biology, vol 1814. Humana Press, New York, NY

Acknowledgements

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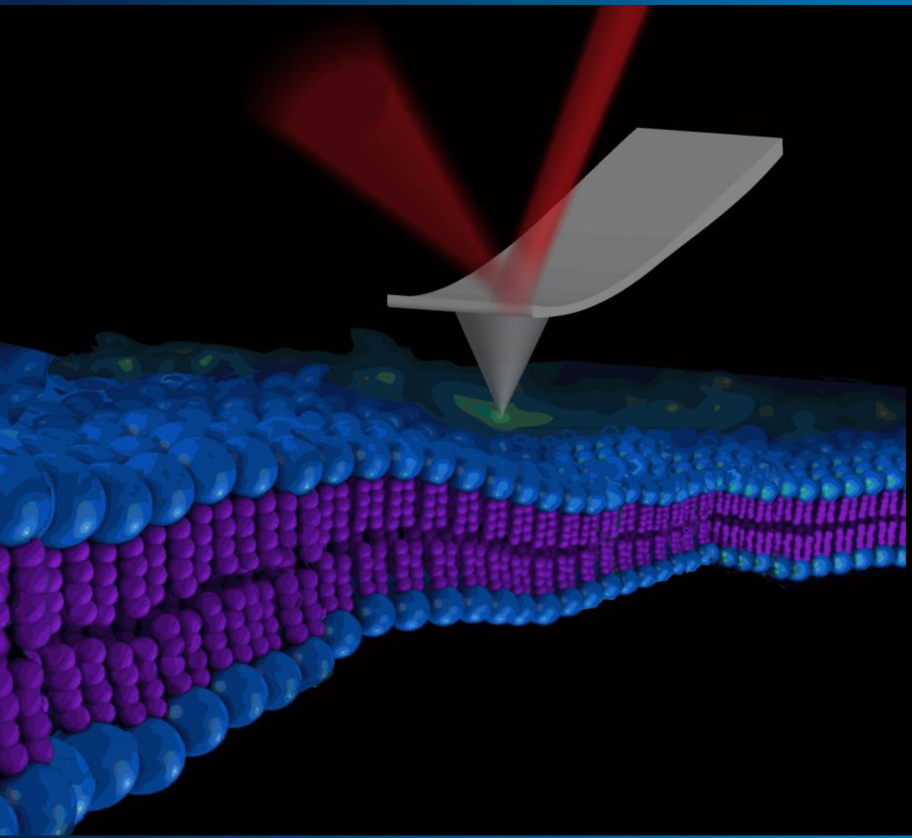

High-Speed Atomic Force Microscopy

A tool for studying dynamic membrane remodeling processes

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Forum Sonde Locale

Valpré 26th April 2024

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https://sites.google.com/view/fm4b-lab/home