Ecole thématique SPM biologie, Sondes Locales 18th Mars 2019, Carry le Rouet, France

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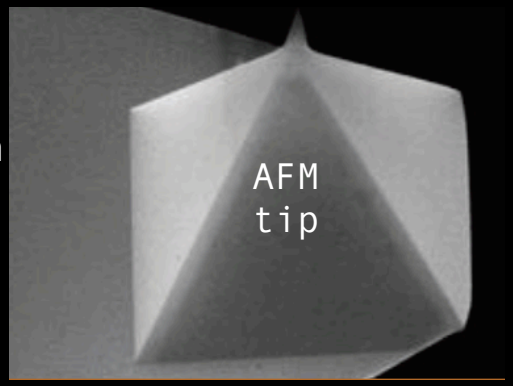
Introduction to High Speed Atomic Force Microscopy

High Speed AFM imaging of molecular dynamics	High Speed AFM mechanical studies	Overview and perspectives

Atomic Force Microscope (AFM)

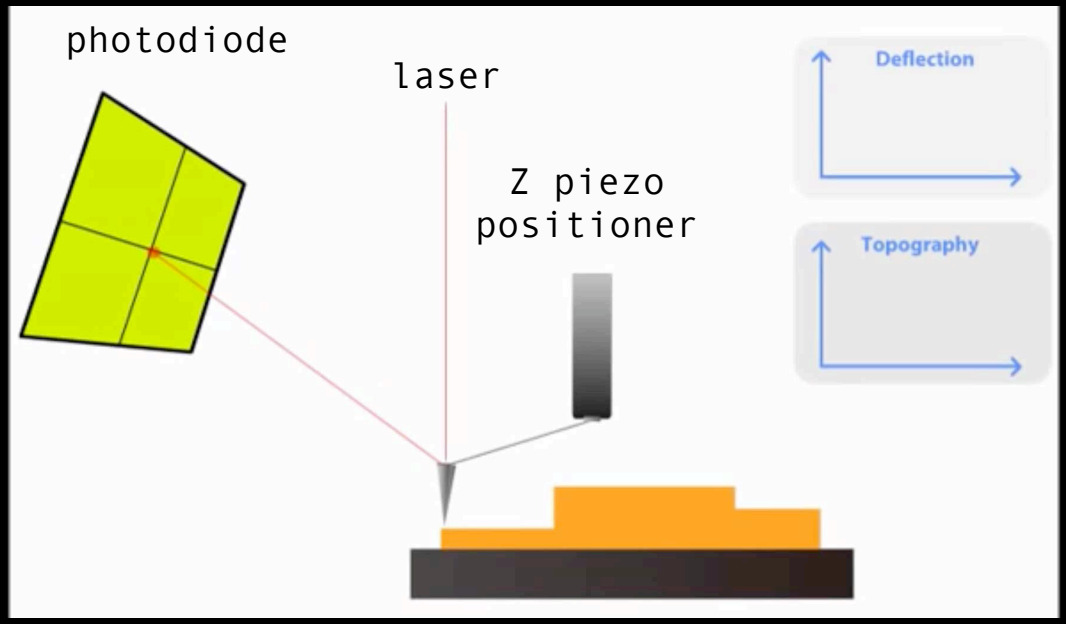
Atomic force microscope

Lever length: ~ µm
Tip radius: ~ nm



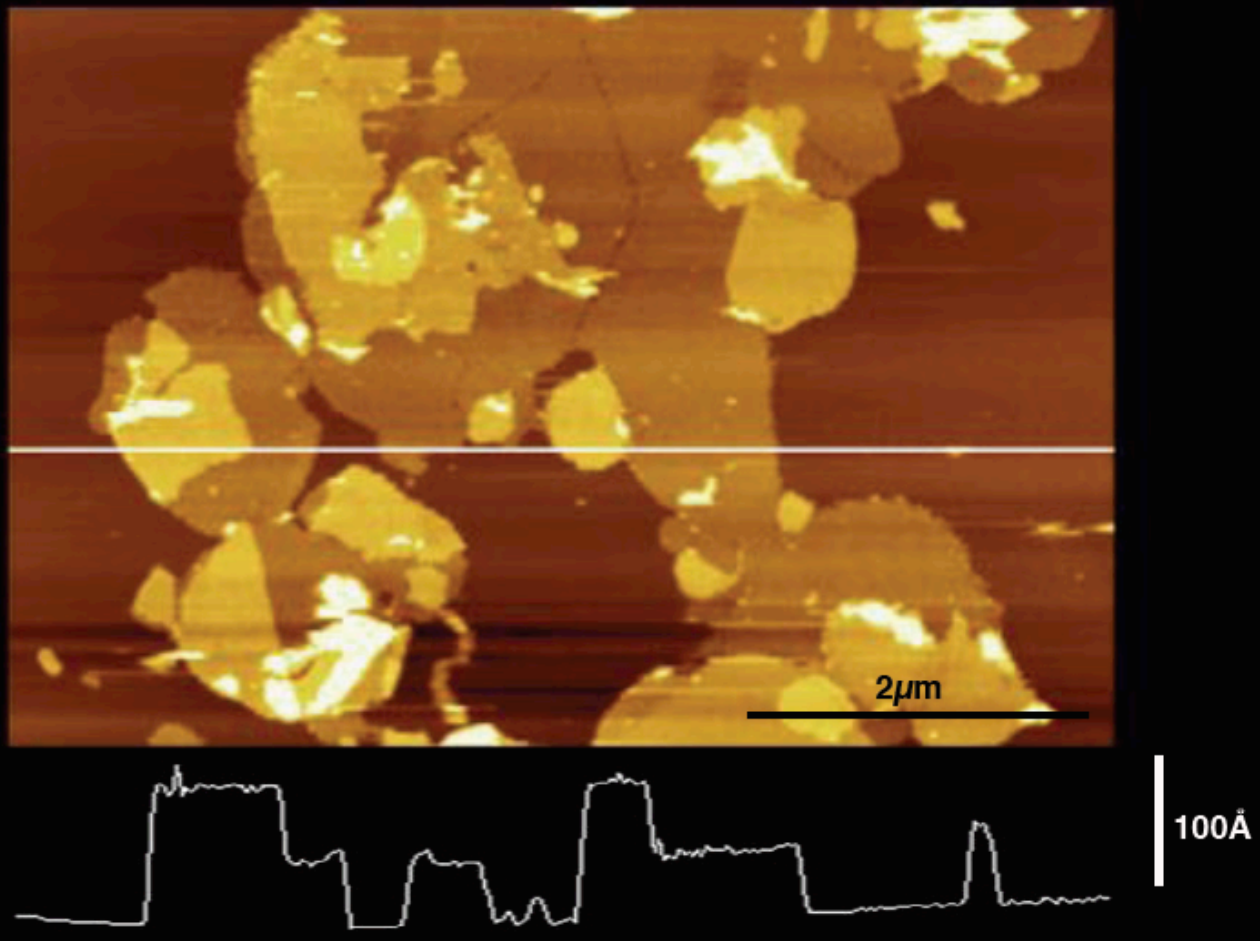
Phonograph

Mounting: ~ mm
Tip radius: ~ µm



Atomic Force Microscope (AFM)

Membranes on mica - The color code

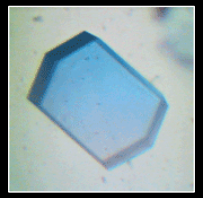


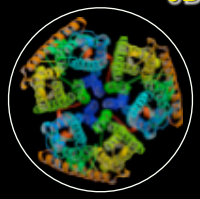
How native are the proteins surroundings by technique

The nativeness of the proteins in structural biology

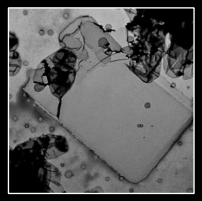
less native

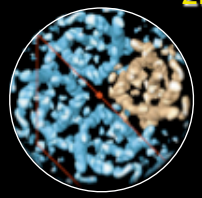
3D-crystal





2D crystal



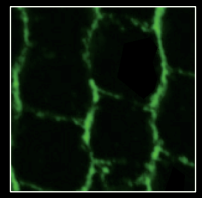


Densely packed reconstitution

Loosely packed reconstition

Native membrane *ex cellula*

Native membrane *in cellula*



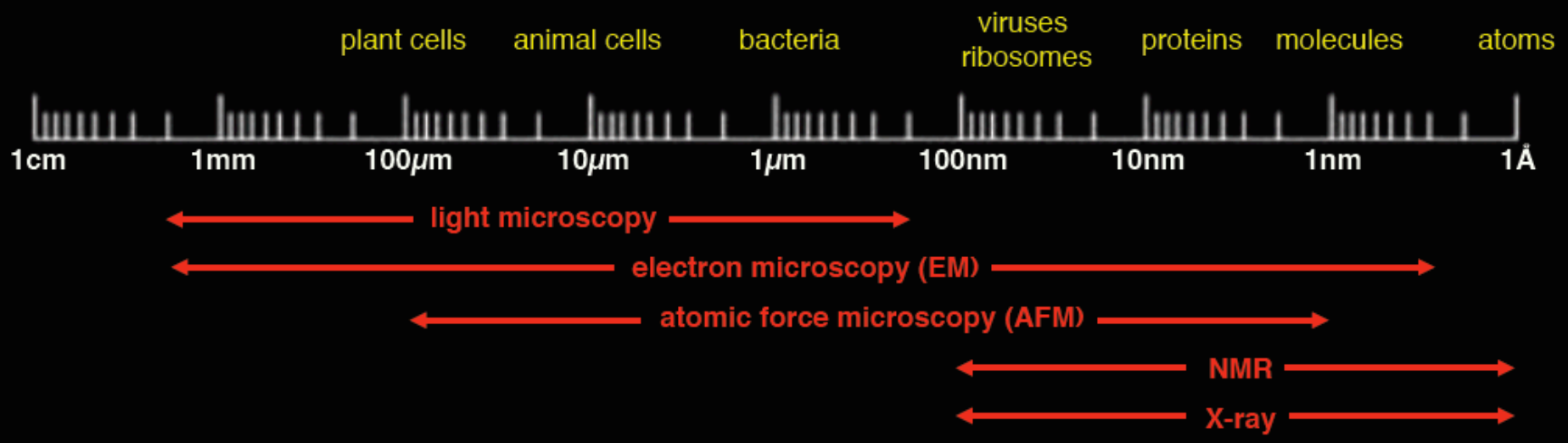
AFM

more native

Atomic Force Microscope
in buffer solution
at ambient pressure
at ambient temperature
and label-free

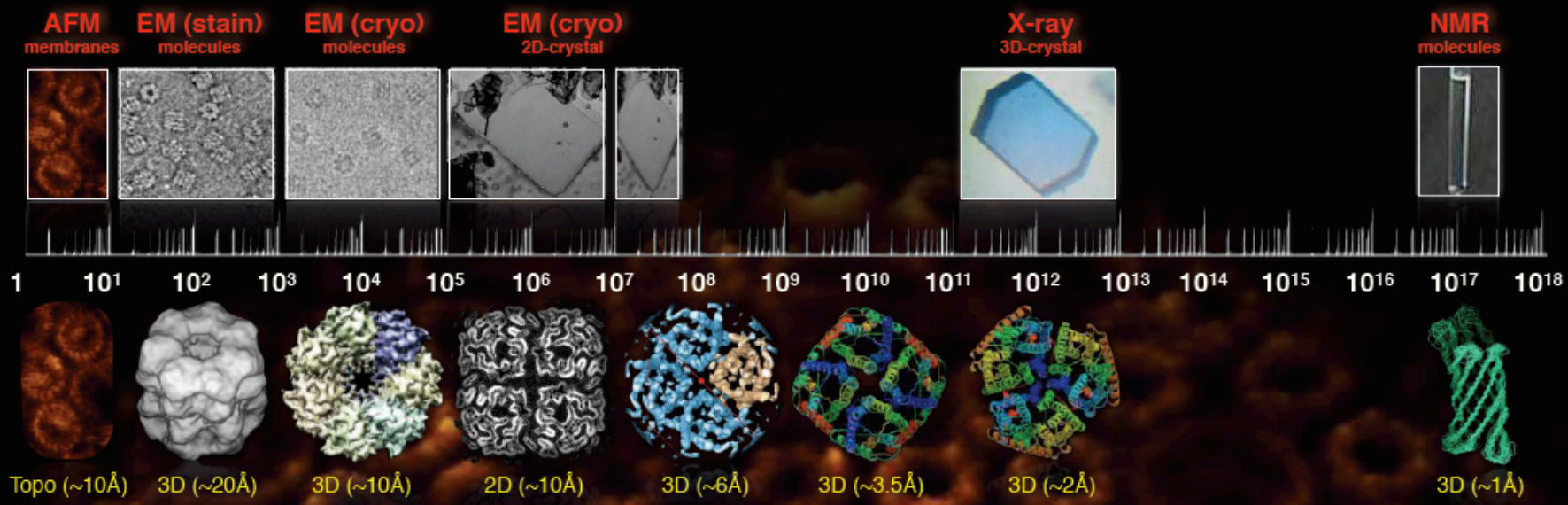
Techniques in structural biology

Dimensions in life sciences - what technique is appropriate



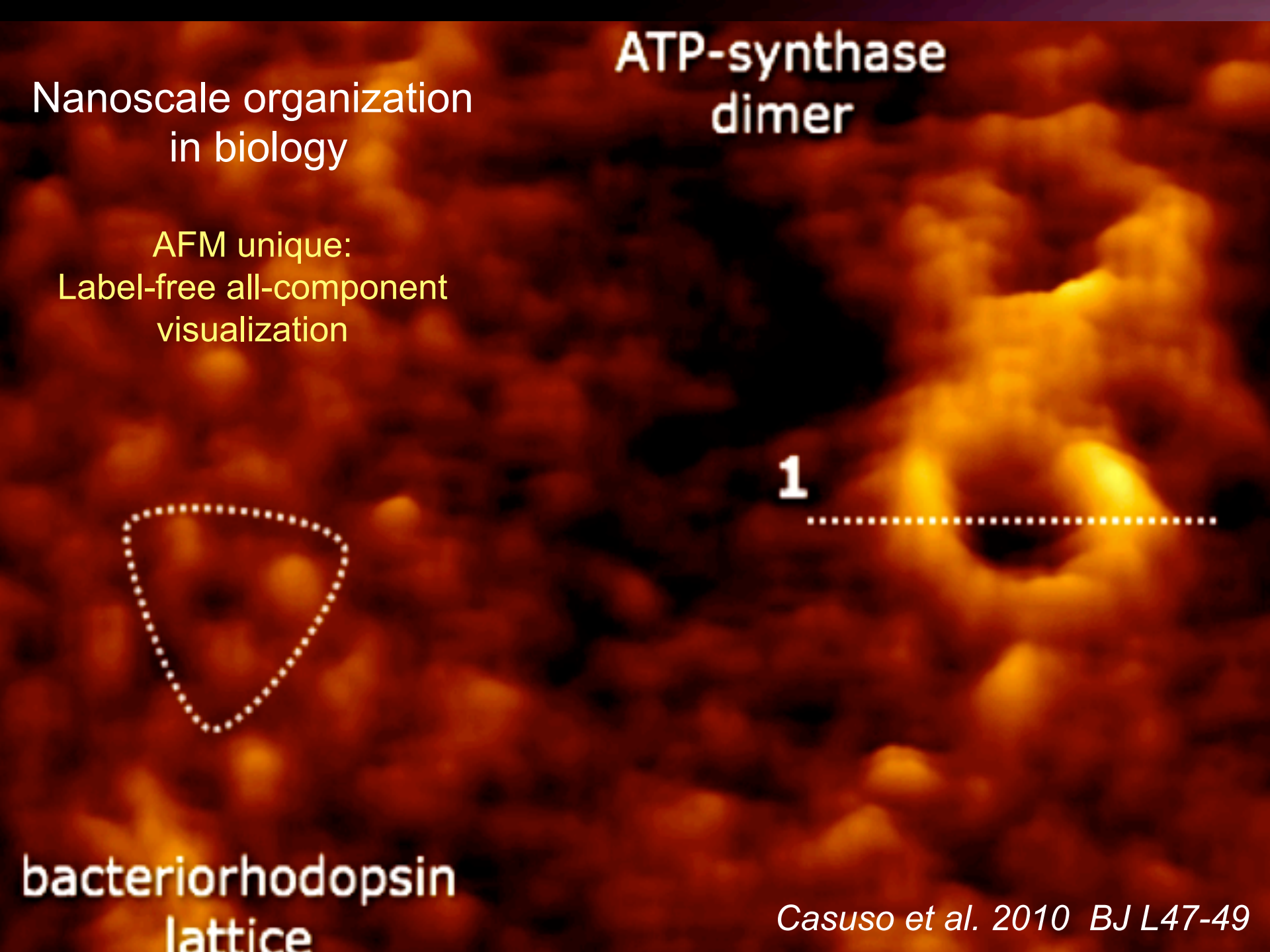
Techniques in structural biology

The signal-to-noise ratio (SNR) vs. The number of molecules to analyze



Nanoscale organization in biology

AFM unique:
Label-free all-component visualization

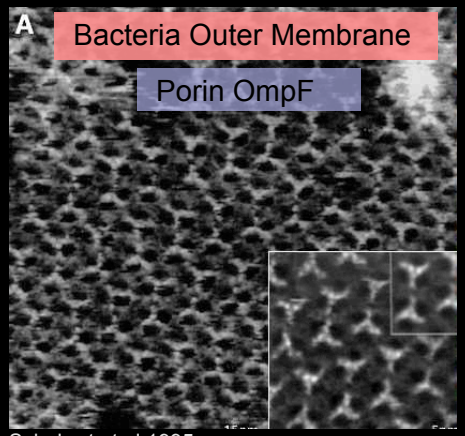


Casuso et al. 2010 BJ L47-49

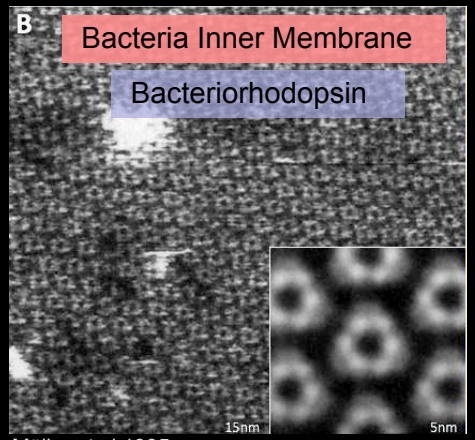
Historical timeline of membranes by AFM

90's

One bacterial protein in crystal



Schabert et al.1995

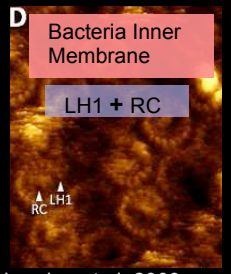


Müller et al.1995

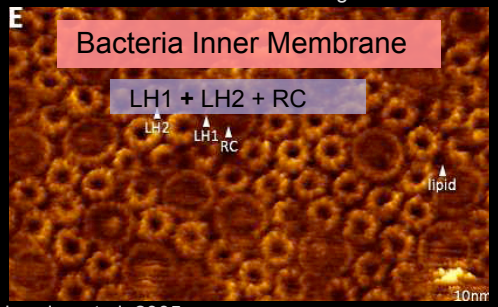
00's

No crystalline / Complex ensembles / Eukaryotes / slow Dynamics

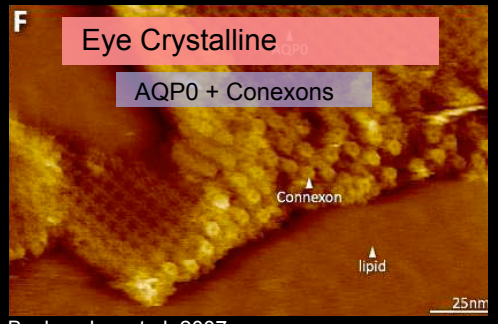
Fotiadis et al. 2003



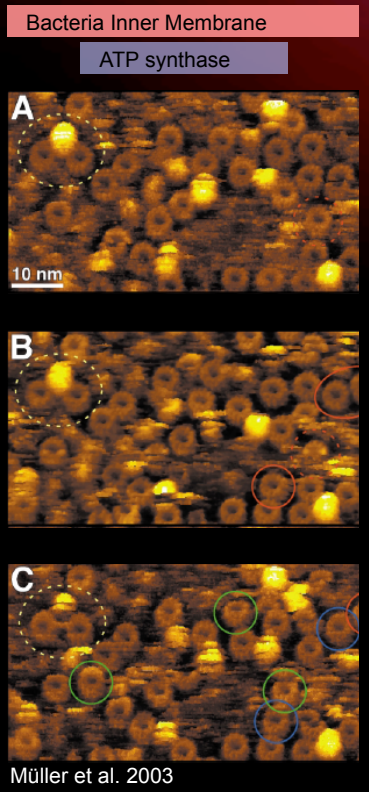
Scheuring et al. 2003



Scheuring et al. 2005



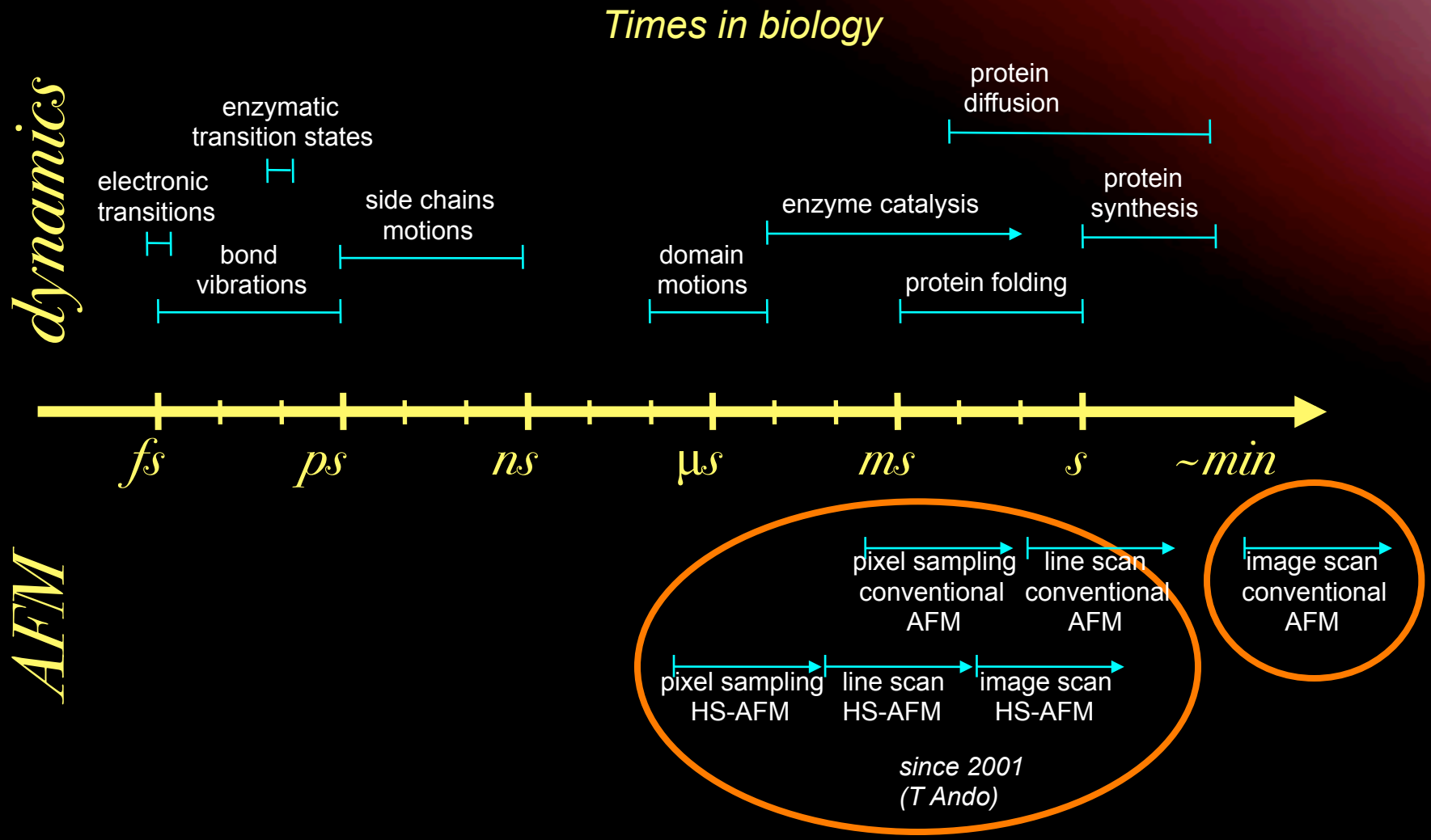
Buzhynskyy et al. 2007



Müller et al. 2003

Physiological Conditions

Conventional versus High Speed AFM

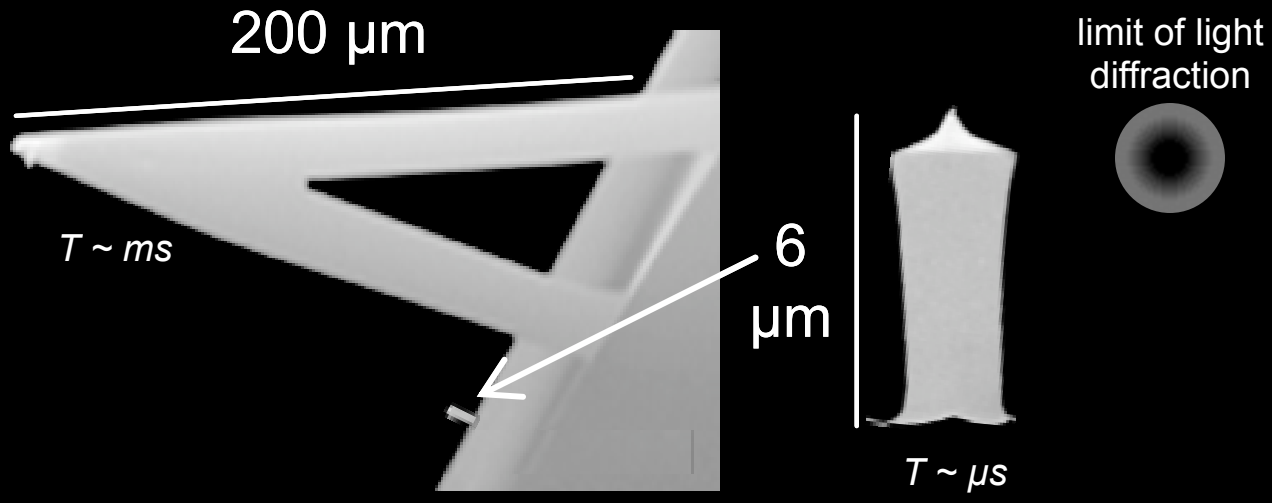


the High Speed AFM. Fundamentals

minimization of moving components to the limit of light difraction

the HS-AFM is x1000 times faster than conventional AFM

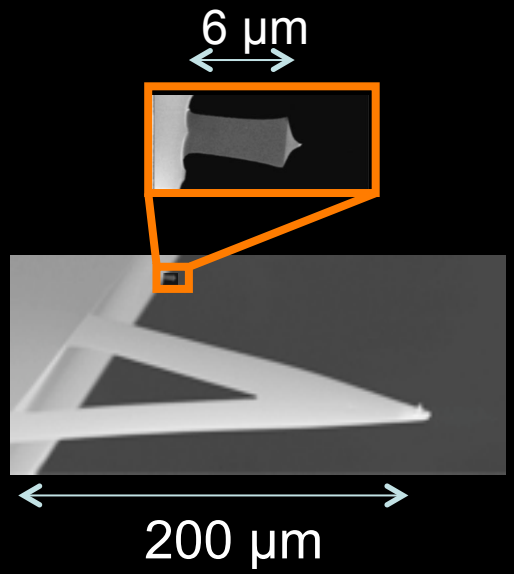
Speed Reaction ~ 1/√ mass

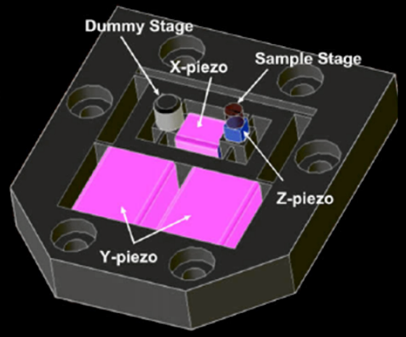


Fundamentals of HS-AFM

Minimization of moving components

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





HighSpeed Cantilever

- Spring constant ~ 0.1 N/m
- Resonance frequency in water ~ 600 kHz
- Q ~ 2
- Typically Amplitude Modulation

Conventional Cantilever

- Spring constant ~ 0.1 N/m
- Resonance frequency in water ~7 kHz
- Q ~ 2
- Typically Contact mode

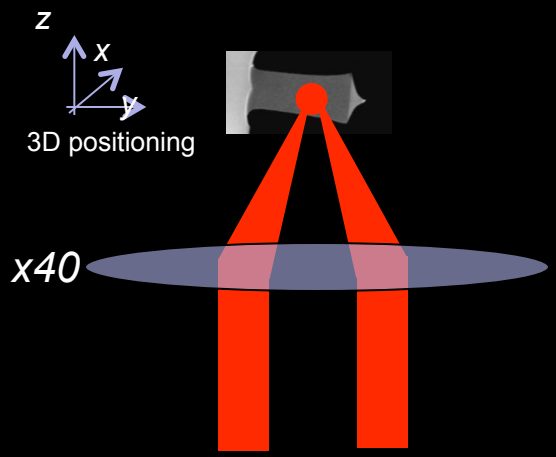
HS-AFM Scanner

- Scan area ~ 1µm^2
- Resonance frequency ~300 kHz

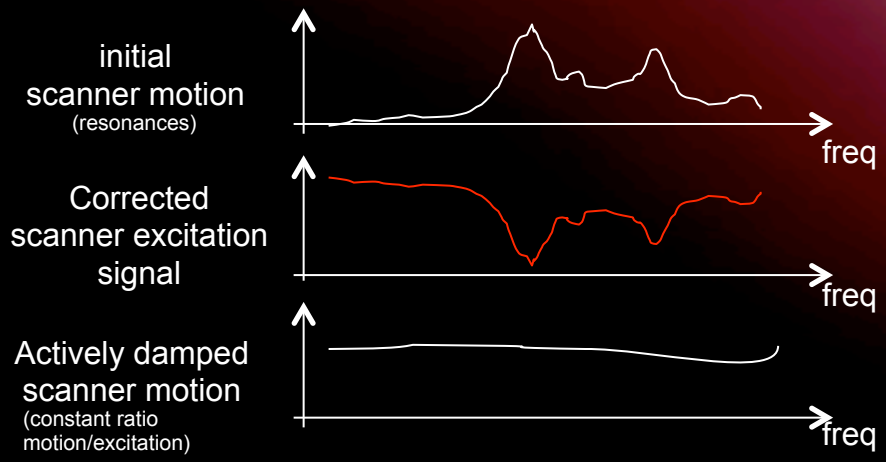
Details of HS-AFM

T. Ando et al. 2001

Laser focusing

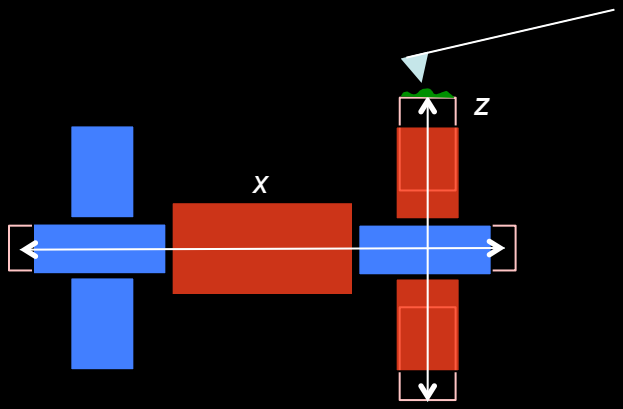


Active damping scanner vibrations

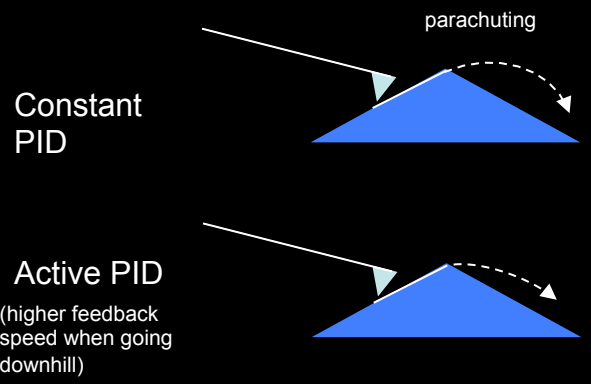


Scanner counterbalance

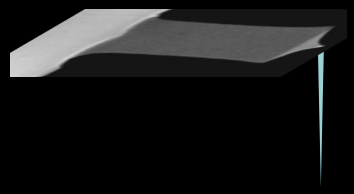
back to tripod



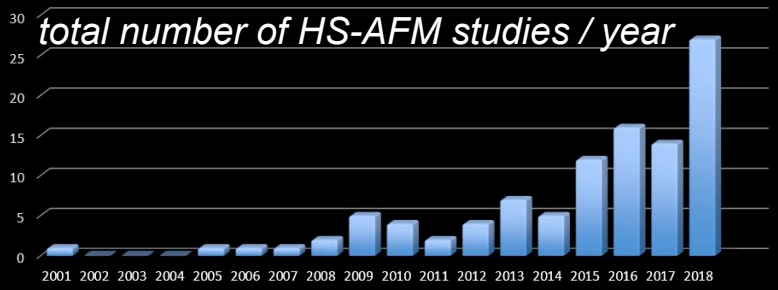
Active feedback

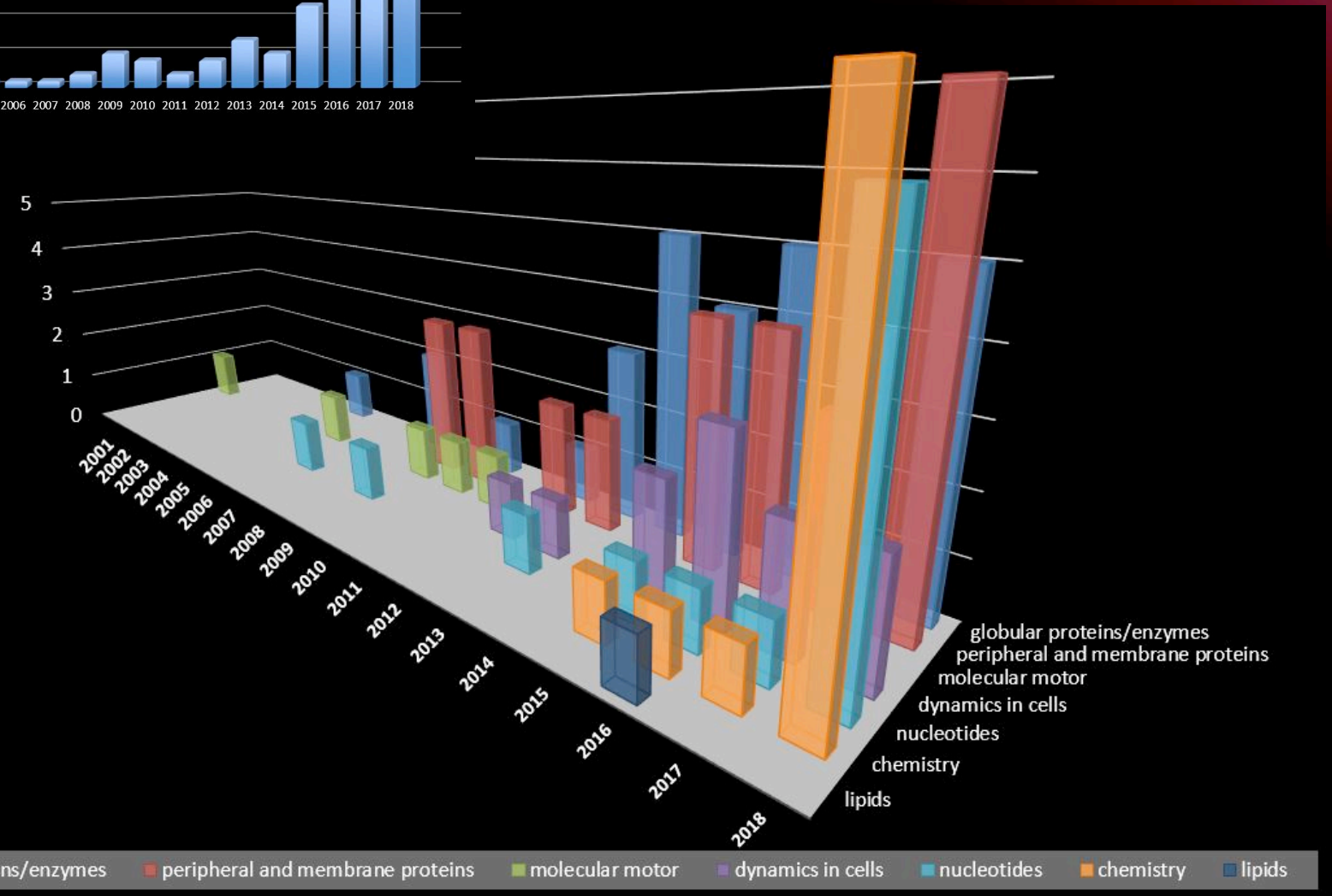


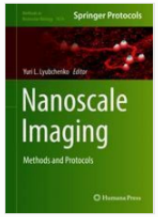
Electron Beam Deposited tip



History line of HS-AFM, 102 studies until Dec 2018







Nanoscale Imaging pp 181-200 | Cite as

High-Resolution and High-Speed Atomic Force Microscope Imaging

Authors | Authors and affiliations

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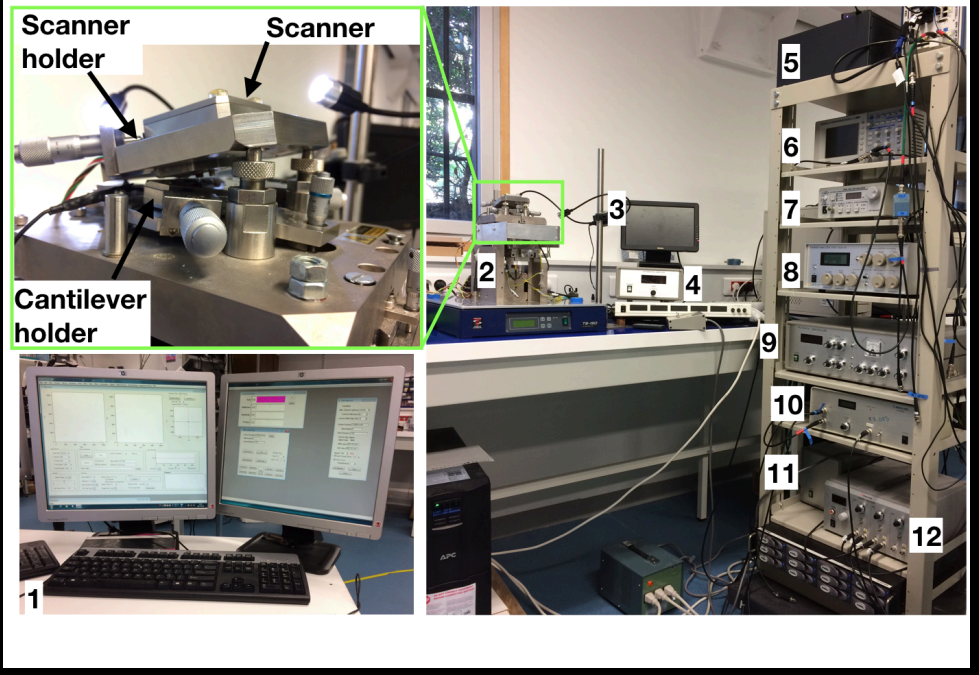
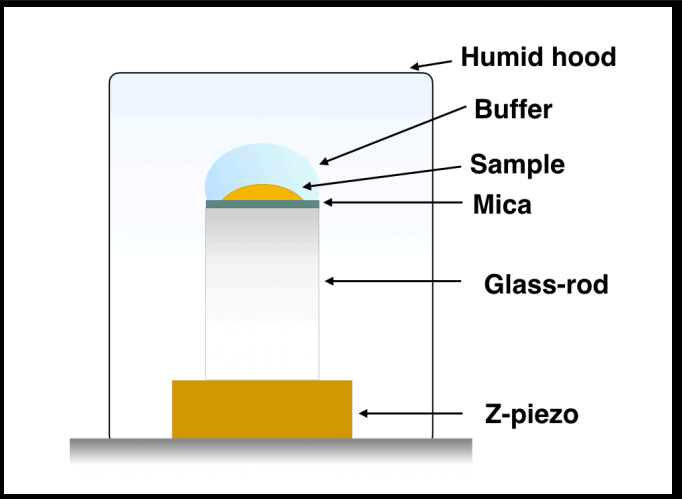
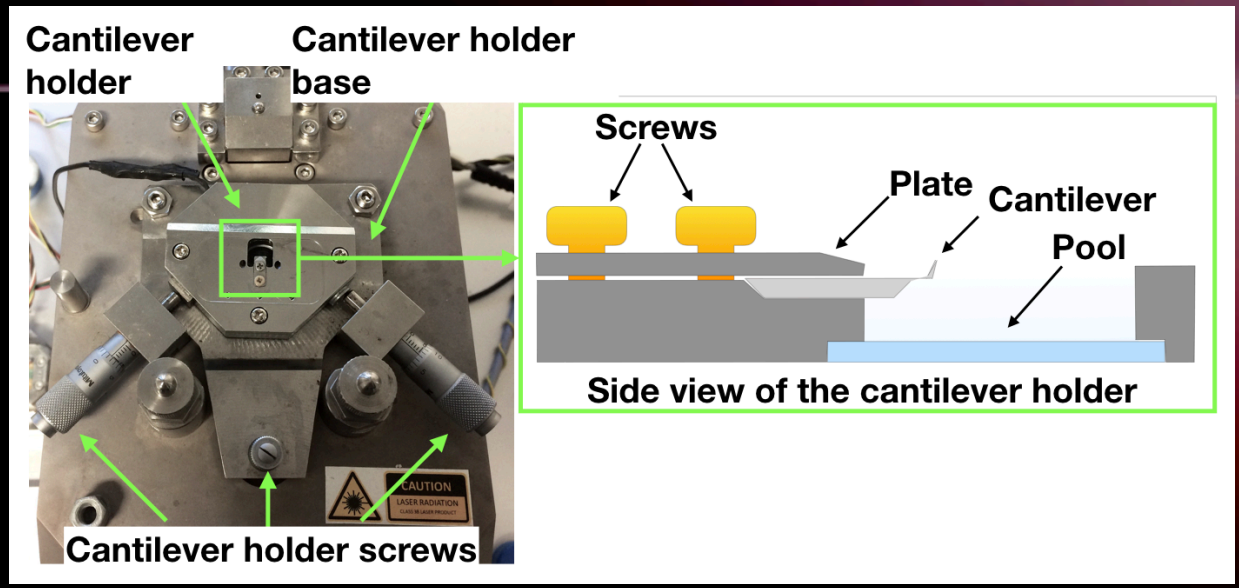
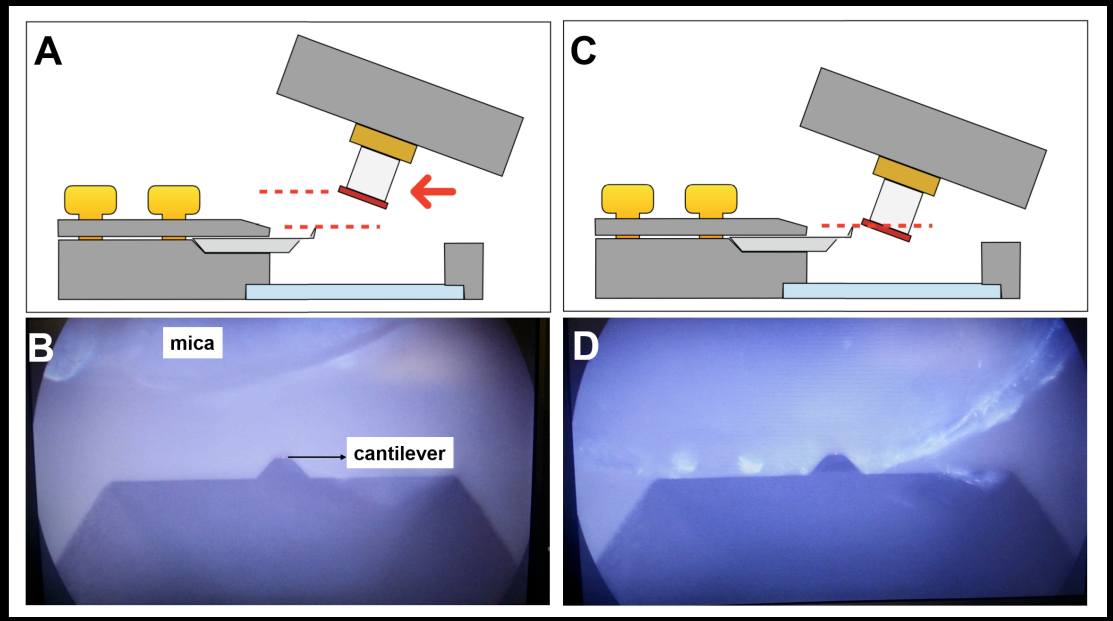
Protocol
First Online: 29 June 2018

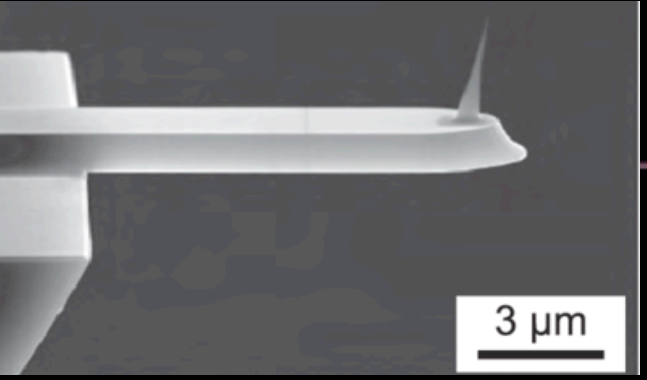
https://hal.archives-ouvertes.fr/hal-01871339/document




Humid hood
Buffer
Sample
Mica
Glass-rod
Z-piezo

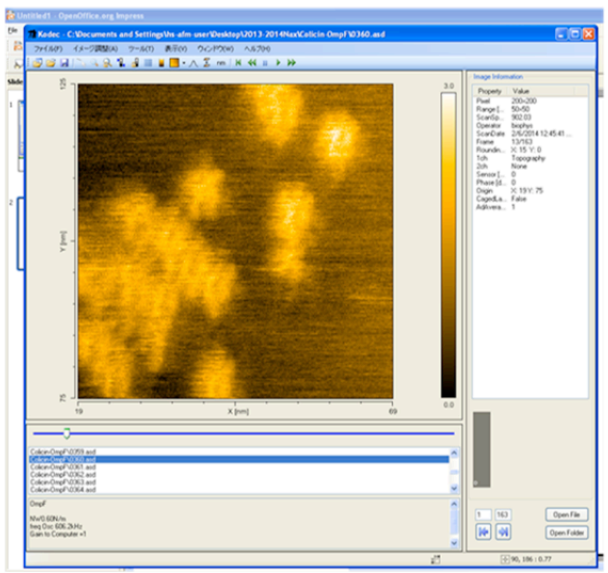

Cantilever holder
Cantilever holder base
Cantilever holder screws
Screws
Plate
Cantilever
Pool
Side view of the cantilever holder


A
C
B
mica
cantilever
D

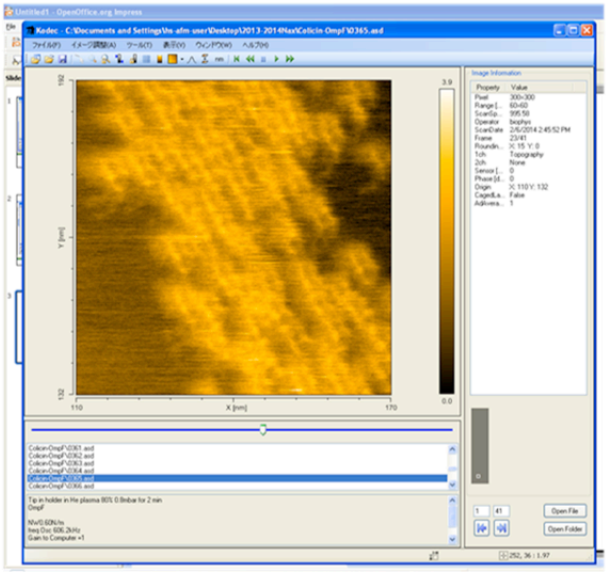


Amorphous carbon Electron Beam Deposition tips used in HS-AFM can be sharpened by plasma attack

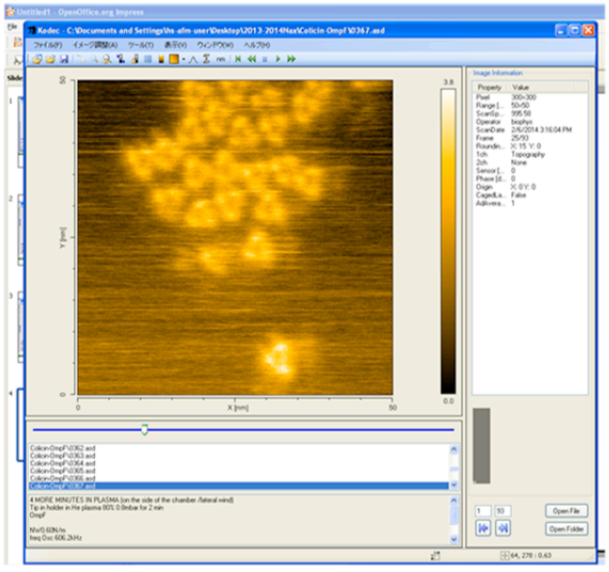
Out of the box EBD tip



+ 2 min He_2(g) plasma



+ 4min He_2(g) plasma



Atomic Force Microscope (AFM)

Invention of the STM and AFM

The Nobel Prize in Physics 1986

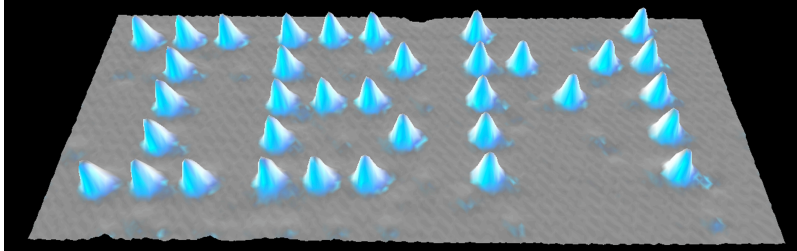
for his fundamental work in electron optics, and for the design of the first electron microscope"

"for their design of the scanning tunneling microscope"

Ernst Ruska
Prize share: 1/2

Gerd Binnig
Prize share: 1/4

Heinrich Rohrer
Prize share: 1/4



Scanning tunneling microscopy – from birth to adolescence
Nobel lecture, December 8 1986

Atomic force microscope
Phys. Rev. Lett. 1986 Mar 3;56(9):930-933

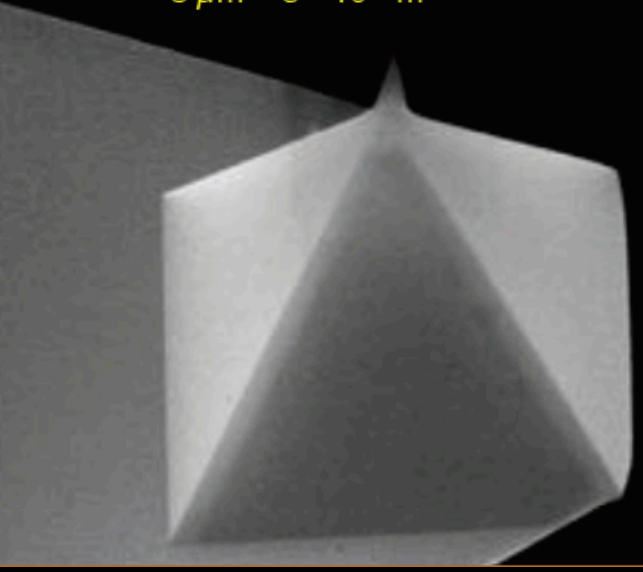
Atomic Force Microscope (AFM)

The tip and the protein

Protein
~ 3 nm = 3 * 10^{-9} m

Basket-Ball
~ 3 dm = 3 * 10^{-1} m

AFM-Tip
~ 3 μm = 3 * 10^{-6} m



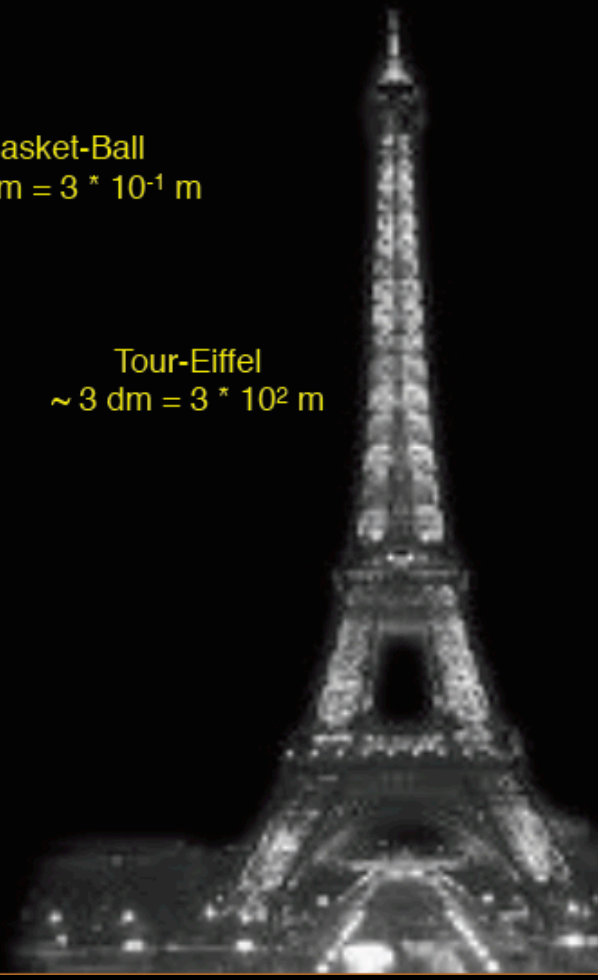
AFM-Tip / Protein

=

Tour-Eiffel / Basket-Ball

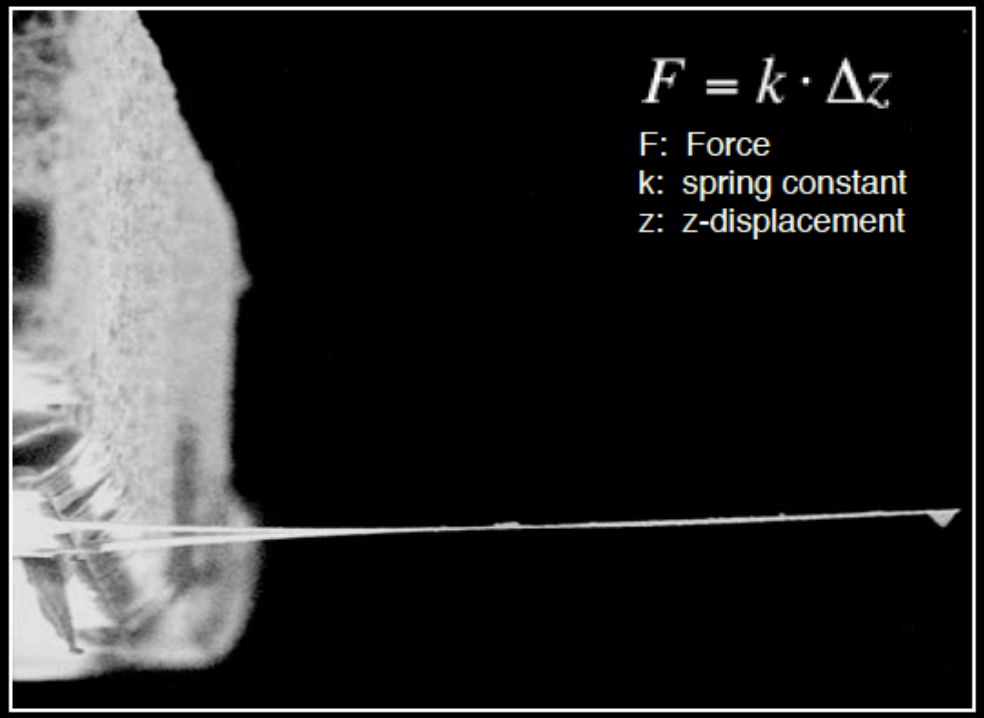
~ 1000

Tour-Eiffel
~ 3 dm = 3 * 10^{2} m



High Resolution AFM imaging

The force measured by the cantilever / The spring constant *(k)* of a cantilever



Geometrical consideration

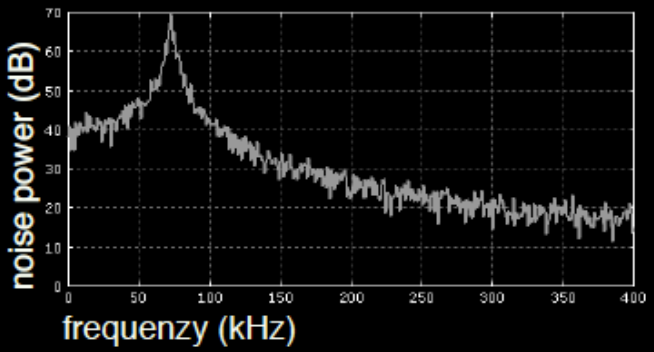
$$k \approx \frac{E \cdot w \cdot t^3}{l^3}$$

E: Young's modulus
w: cantilever width
t: cantilever thickness
l: cantilever thickness

Thermal noise analysis

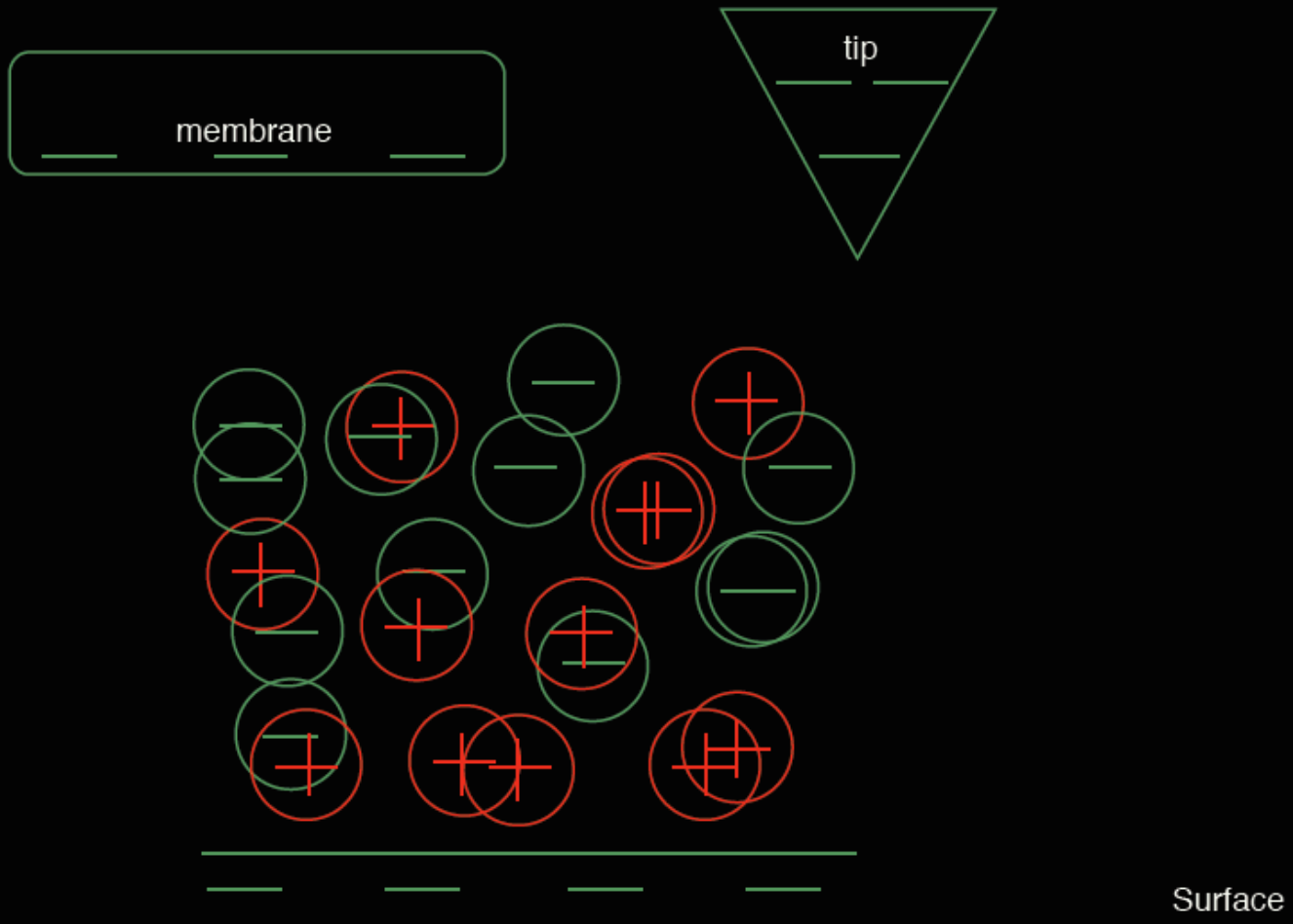
$$k = \frac{k_B \cdot T}{\langle x^2 \rangle}$$

k_B: Boltzmann constant
T: temperature
x^2: mean-squared amplitude of cantilever motion



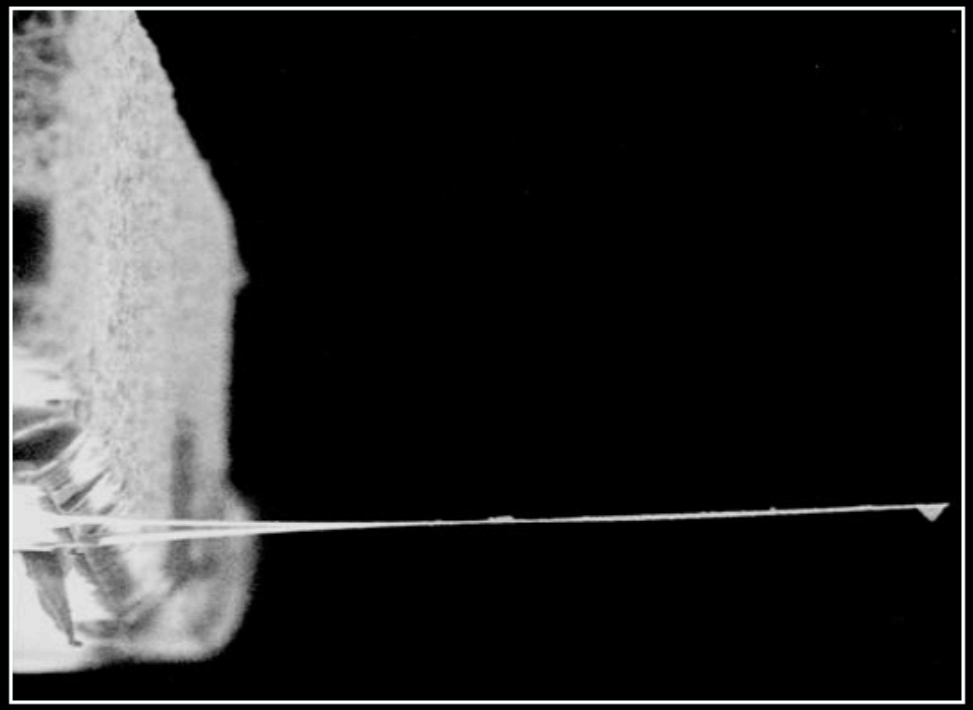
High Resolution AFM imaging

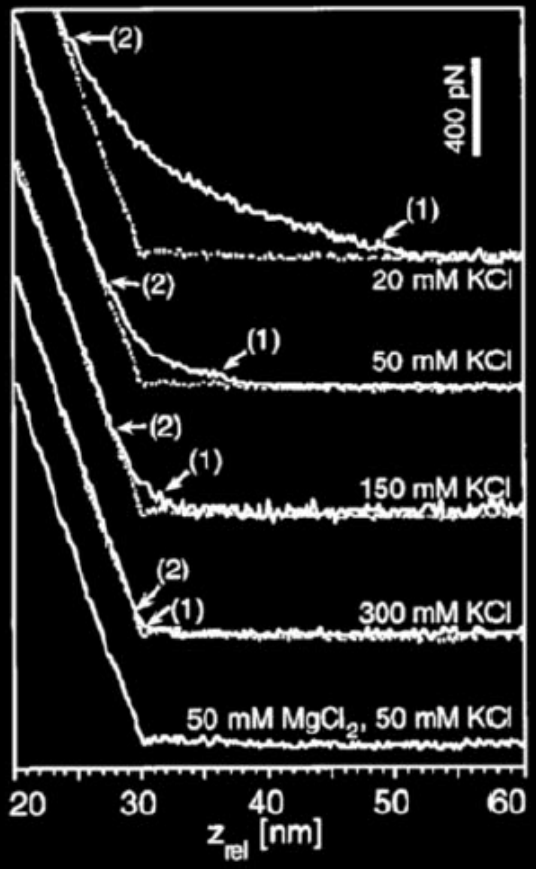
Ions are important



High Resolution AFM imaging

Tip - Sample : Repulsive forces





Daniel J Müller, Dimitrios Fotiadis, Simon Scheuring, Shirley A Müller & Andreas Engel*
Electrostatically balanced subnanometer imaging of biological specimens by atomic force microscopy
Biophys J, 1999, 76 (2): 1101-1111

Atomic Force Microscope (AFM)

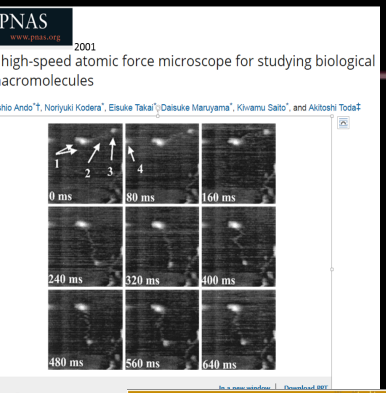
Imaging modes

Contact mode
- Deflection / Force
  (deflection when in contact)

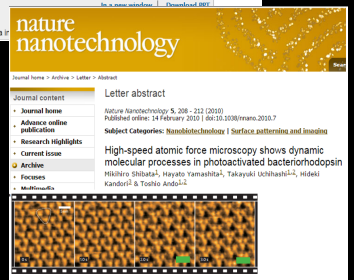
Oscillating mode
- Deflection / Force
- Amplitude
- Phase
  (Amplitude decreases and phase changes when in contact)

The highest impact ten HS-AFM studies

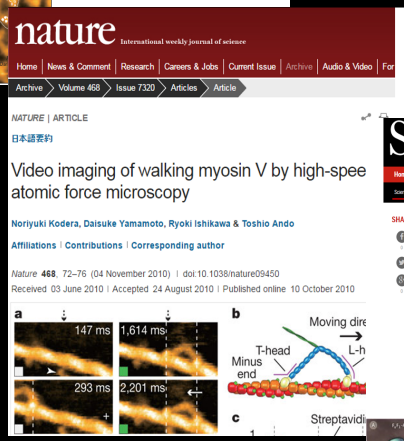
2001 MOLECULAR MOTOR
KANAZAWA



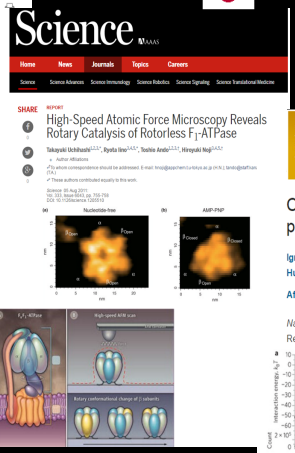
2010 MEMBRANE PROTEIN
KANAZAWA



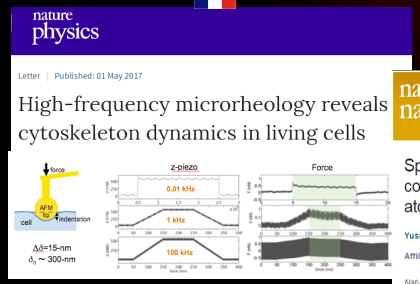
2010 MOLECULAR MOTOR
KANAZAWA



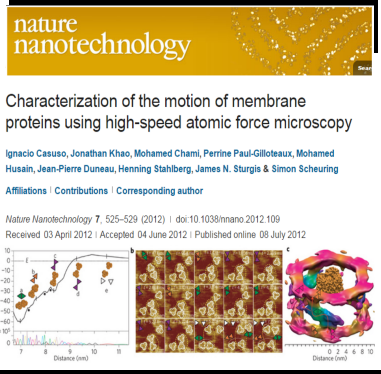
2011 MEMBRANE PROTEIN
KANAZAWA



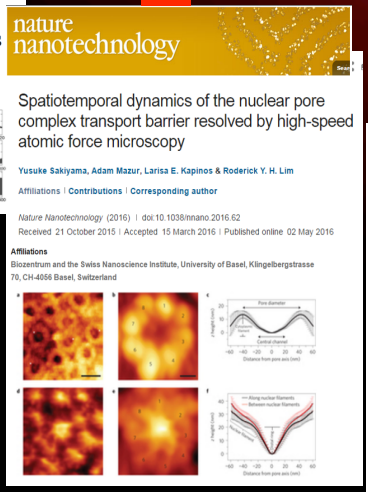
2017 CELLS
MARSEILLE



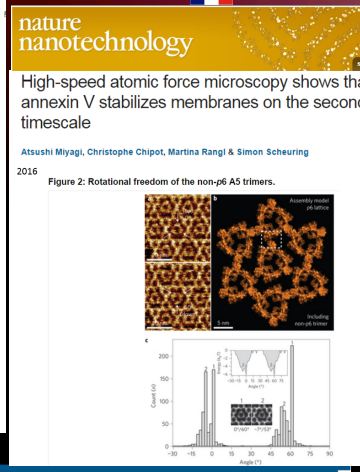
2012 MEMBRANE PROT
MARSEILLE

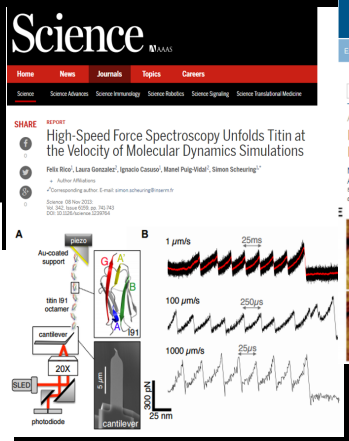


2016 PROTEIN COMPLEX
BASEL



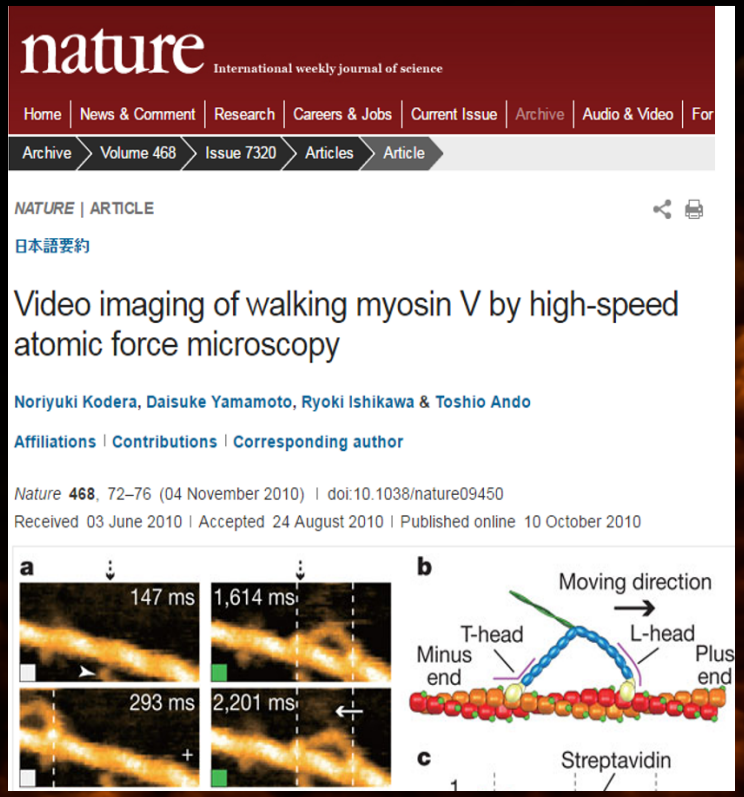
2016 PERIPHERAL
MEMBR PROT
MARSEILLE





2012 GLOBULAR PROT
MARSEILLE

2015 PERIPHERAL
MEMBR PROTEIN
MARSEILLE



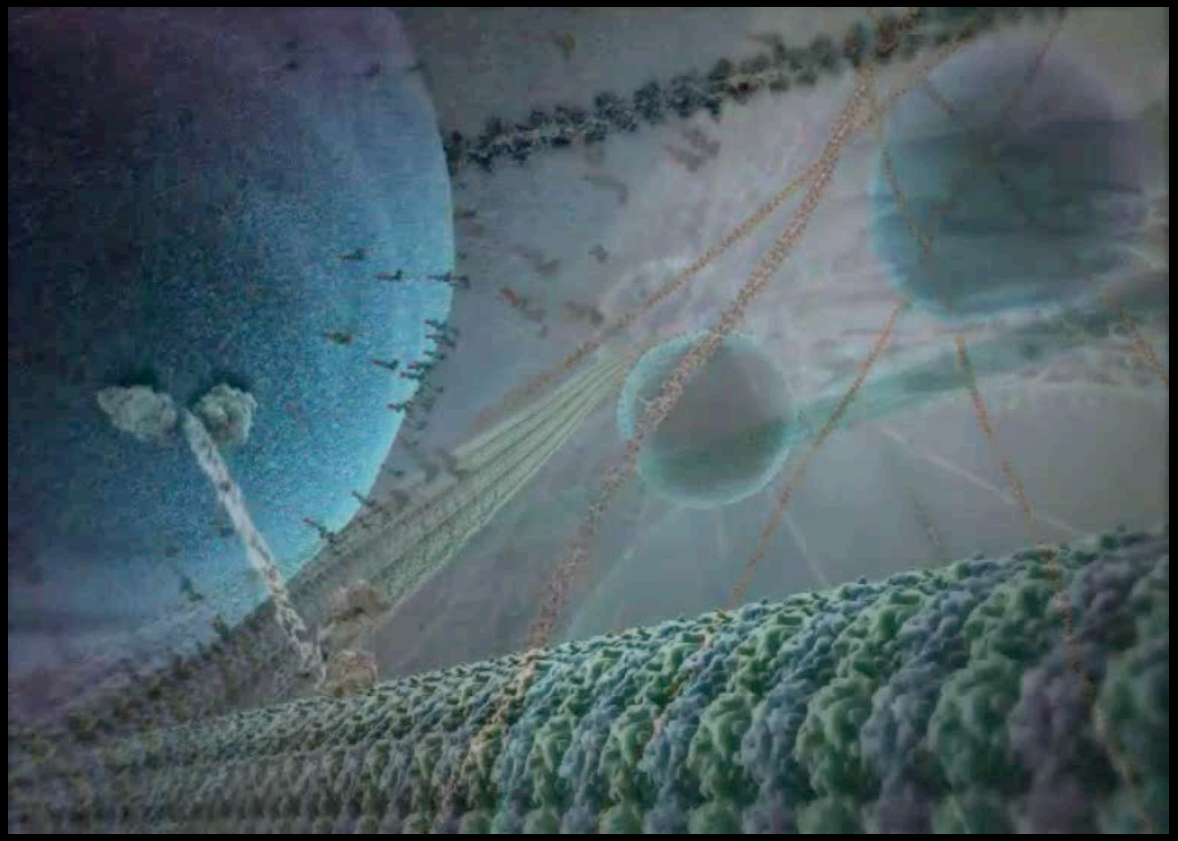
Molecular Motor

Myosin walking Visualized by HS-AFM

Myosin carrying cargo vesicle between organelles

Recreation of the Swinging Lever-Arm Hypothesis

The Inner life of the Cell, 3D computer graphics animation, 2008



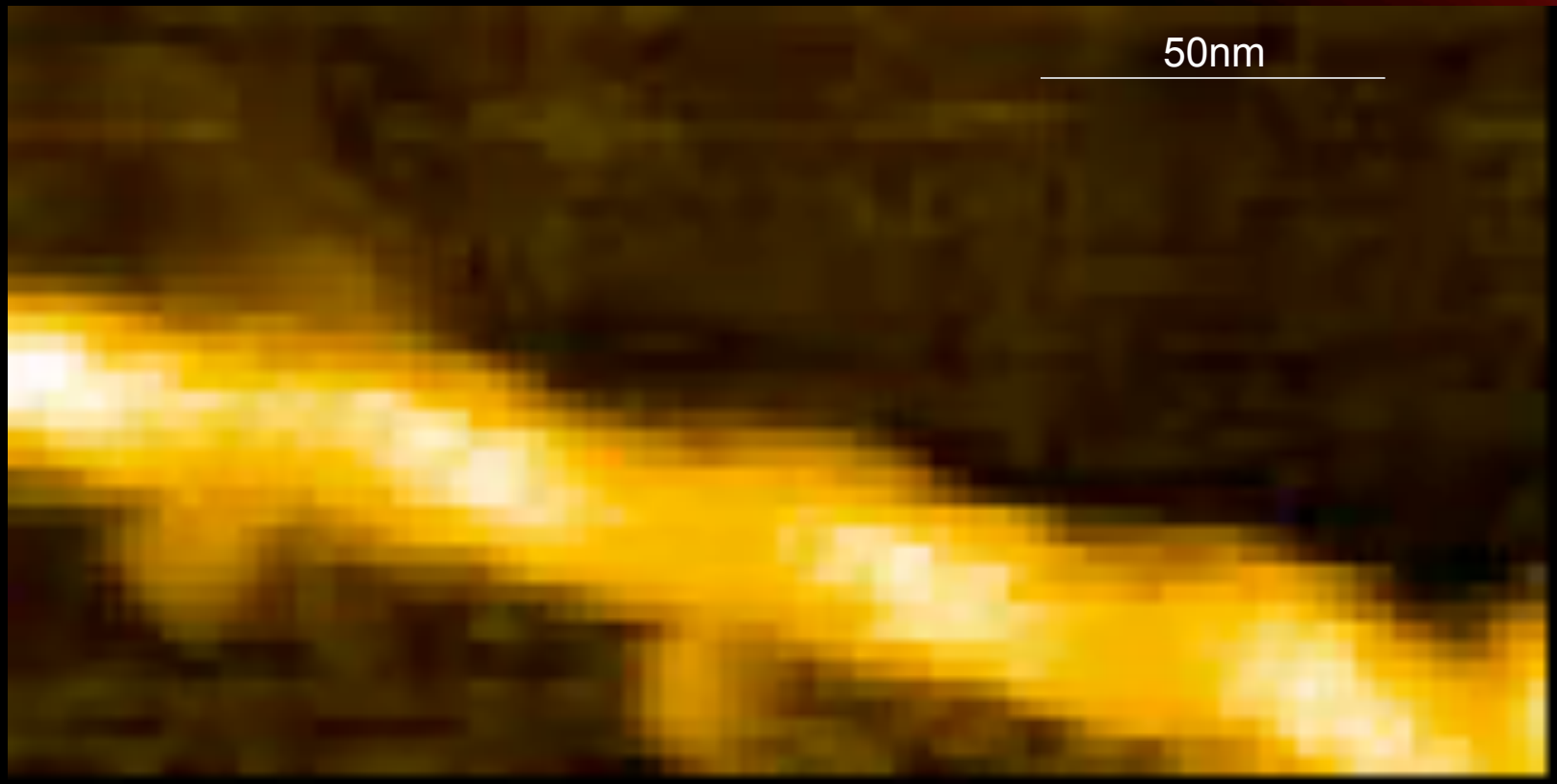
Hypothesis based on data from two sources

(i) Structural data from X-ray and electron microscopy data

(ii) Dynamical data from fluorescence microscopy

Molecular Motor. Myosin observed by HS-AFM

Confirmation of swinging arm hypothesis
Simultaneous structural and dynamical HS-AFM data

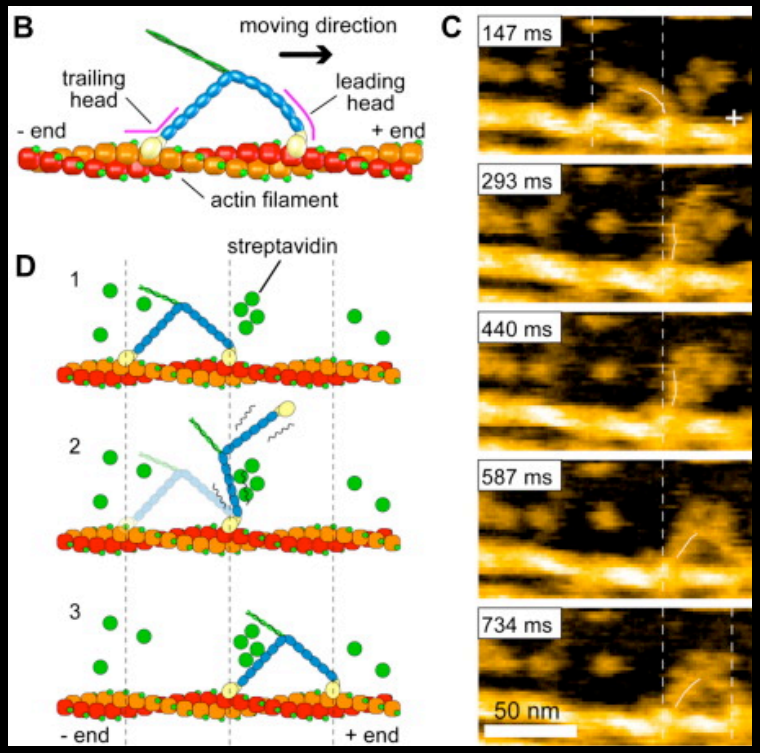


N Kodera *et al*. *Nature* 000, 1-5 (2010) doi:10.1038/nature09450

Model previous to HS-AFM

1 to 1: configuration – chemical state

(info based on static electron microscopy visualizations)

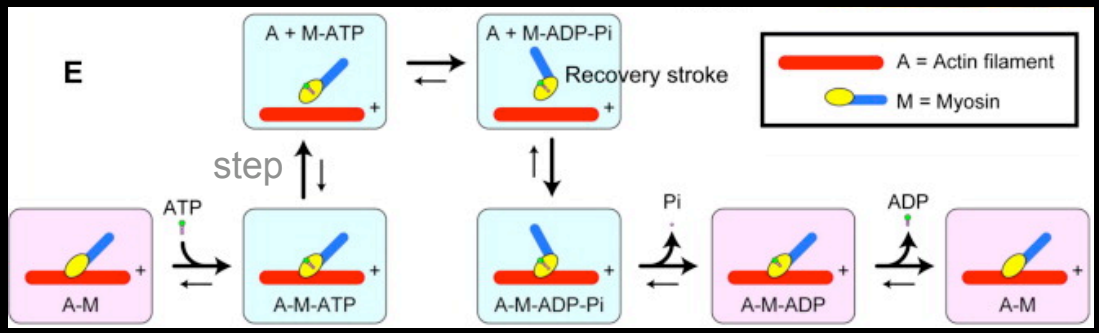


1 μM ATP. 7 fps

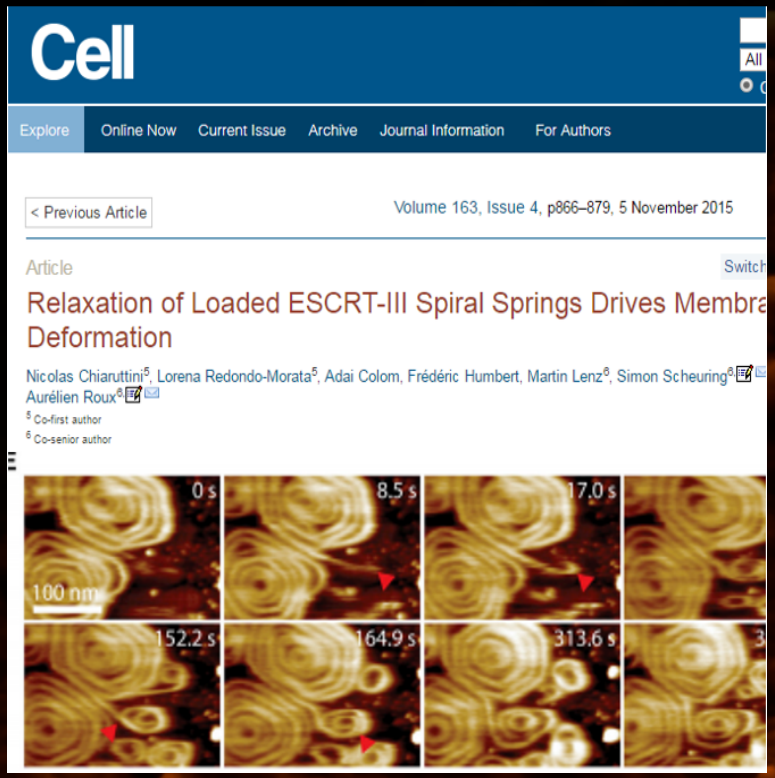
HS-AFM visualization questions the established model

The step forward observed take place without any chemical transition (ATP- ADP- conditions)

New hypothesis chemical energy reaction only plays in the detaching the heads from actin. Change of paradigm

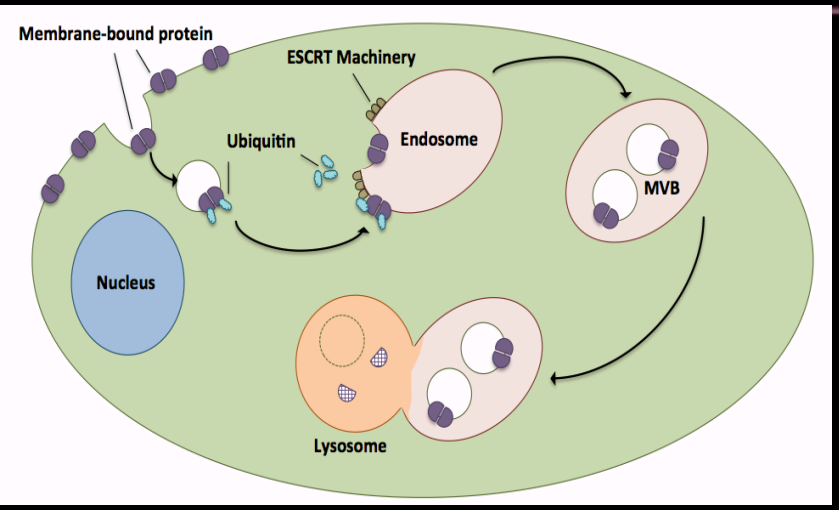


N Kodera *et al*. *Nature* 000, 1-5 (2010) doi:10.1038/nature09450



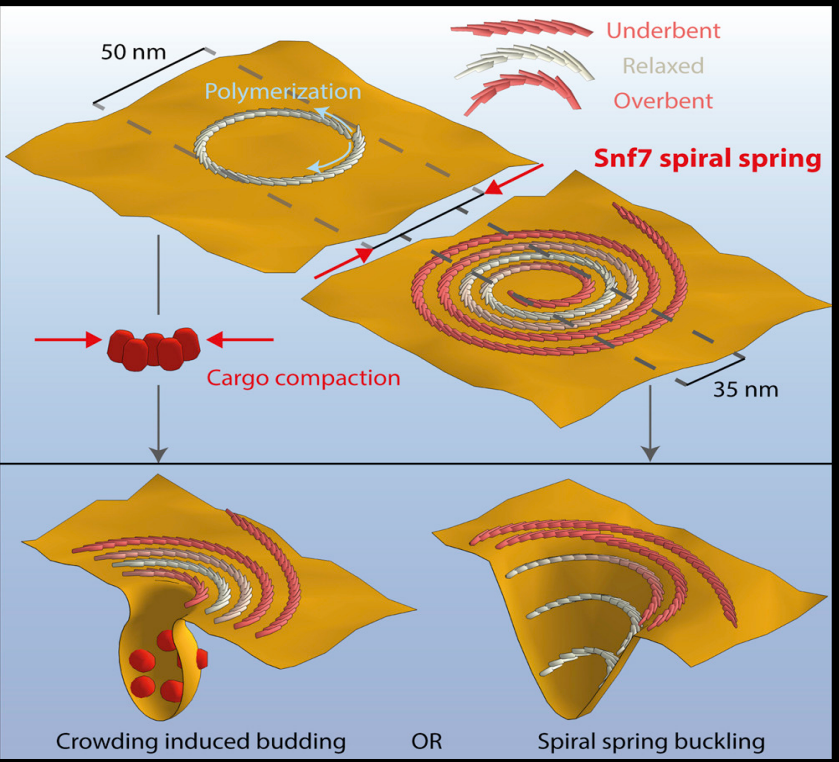
Peripheral Membrane Protein Induced budding process Visualized by HS-AFM

Eukaryotic endosome cargo sorting



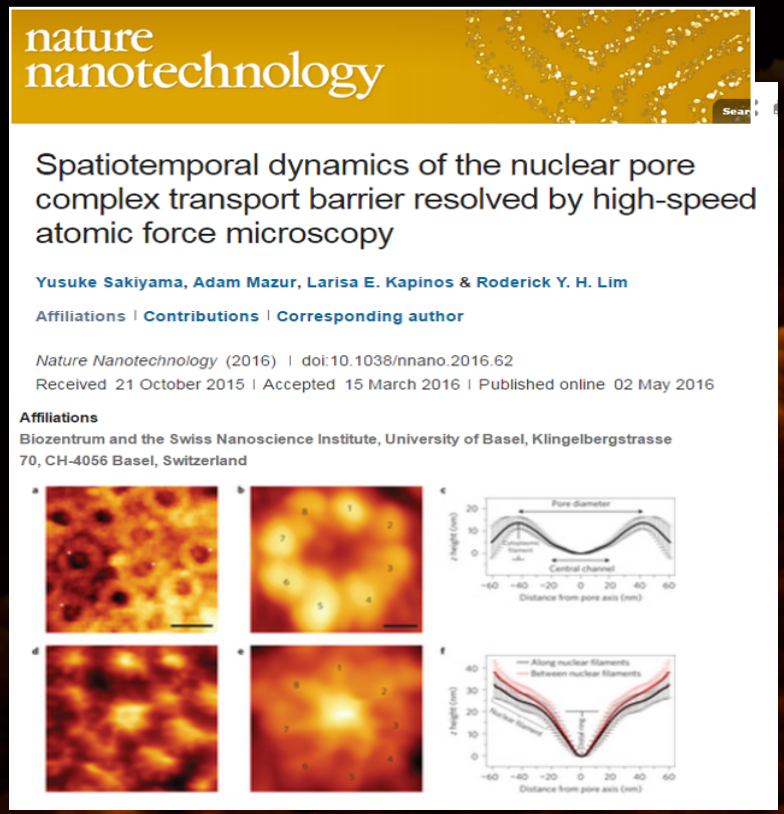
Supplementary video 6

Nucleation of Snf7 disks
High-Speed AFM movie



Findings

- There exist a preferred ring size of the Snf7 rings (Energy minimum)
- The tendency to achieve the preferred ring size drives membrane budding
- Larger Snf7 rings, formed during initial oligomerization, squeze inner rings



nature nanotechnology

Spatiotemporal dynamics of the nuclear pore complex transport barrier resolved by high-speed atomic force microscopy

Yusuke Sakiyama, Adam Mazur, Larisa E. Kapinos & Roderick Y. H. Lim

Affiliations | Contributions | Corresponding author

Nature Nanotechnology (2016) | doi:10.1038/nnano.2016.62

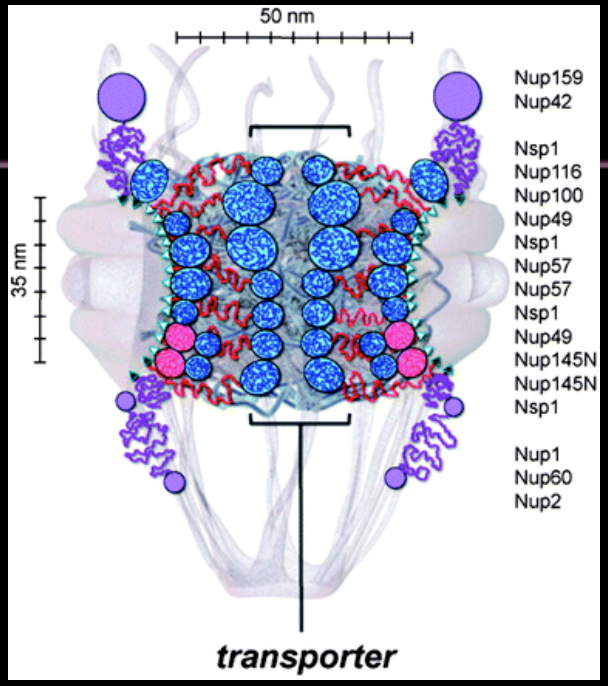
Received 21 October 2015 | Accepted 15 March 2016 | Published online 02 May 2016

Affiliations

Biozentrum and the Swiss Nanoscience Institute, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

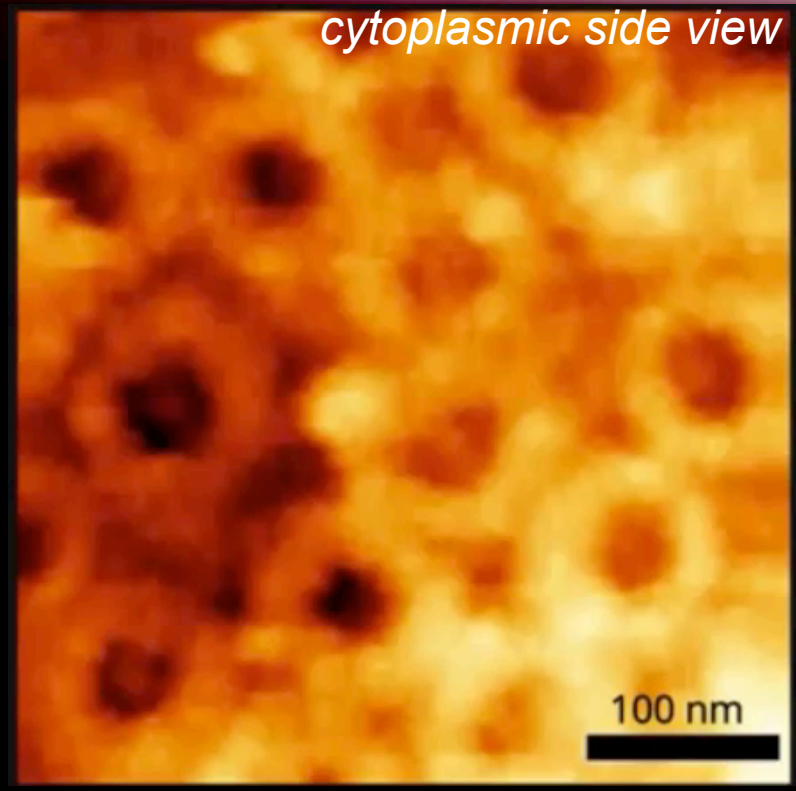
Peripheral Membrane Complex

Nuclear pore complex Visualized by HS-AFM



Y Sakiyama *et al*. *Nature* nano (2016) doi:10.1038/nnano.2016.62

The mechanism by which the NPC selectively allows the transit of import or export complexes, while restricting the passage of inert species is poorly understood.



…highly dynamic FG Nups. Importantly, this brings consensus and clarity to barrier models, which mainly disagree on their static arrangements in the pore…

extended

entangled

radial

nature nanotechnology

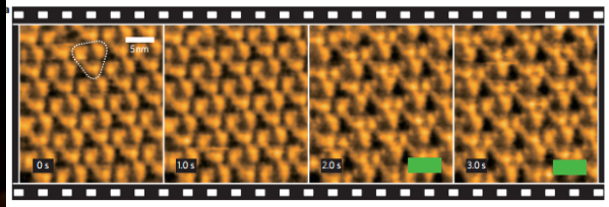
Letter abstract

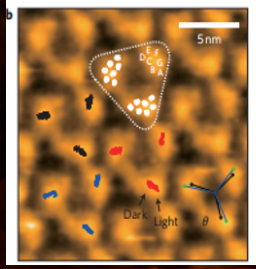
Nature Nanotechnology **5**, 208 - 212 (2010)
Published online: 14 February 2010 | doi:10.1038/nnano.2010.7

Subject Categories: Nanobiotechnology | Surface patterning and imaging

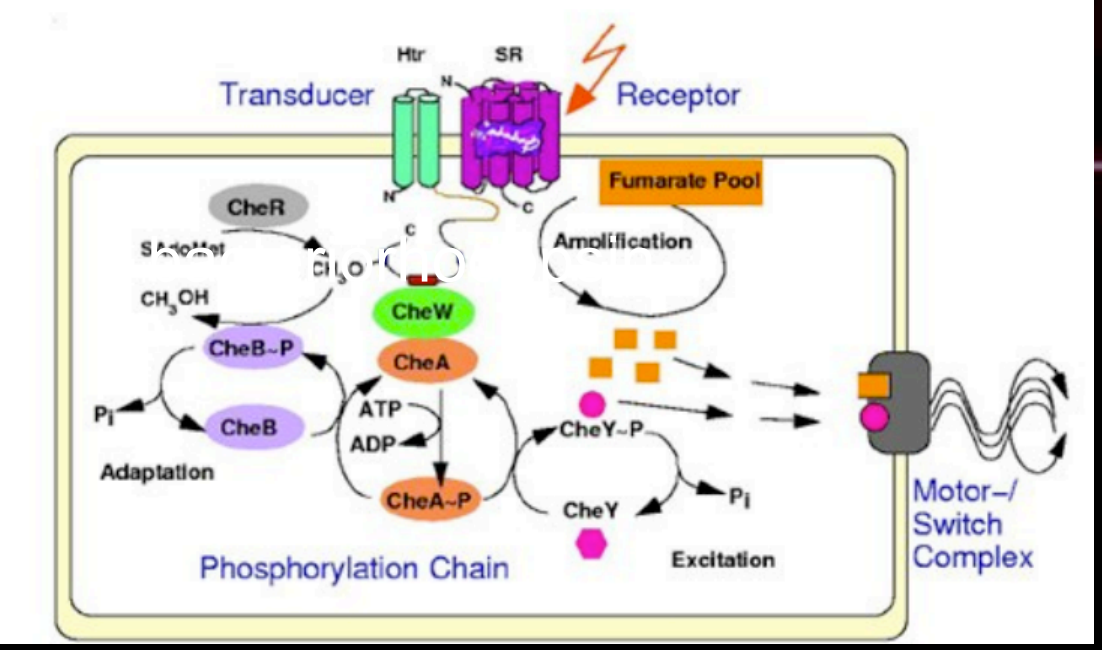
High-speed atomic force microscopy shows dynamic molecular processes in photoactivated bacteriorhodopsin

Mikihiro Shibata[1], Hayato Yamashita[1], Takayuki Uchihashi[1,2], Hideki Kandori[3] & Toshio Ando[1,2]

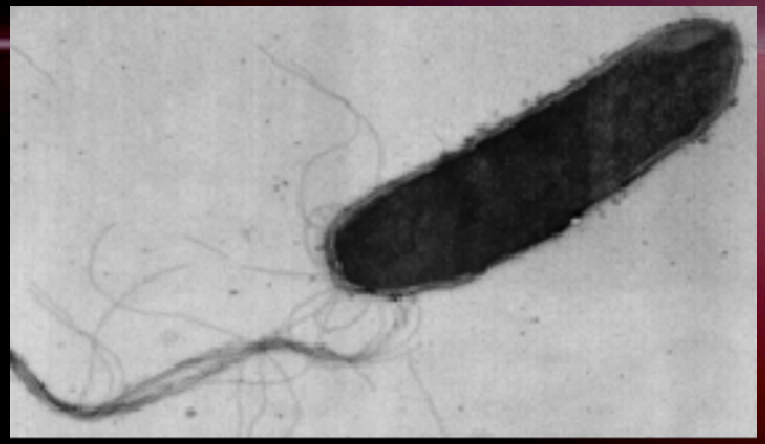




Membrane Protein Configurational change Visualized by HS-AFM

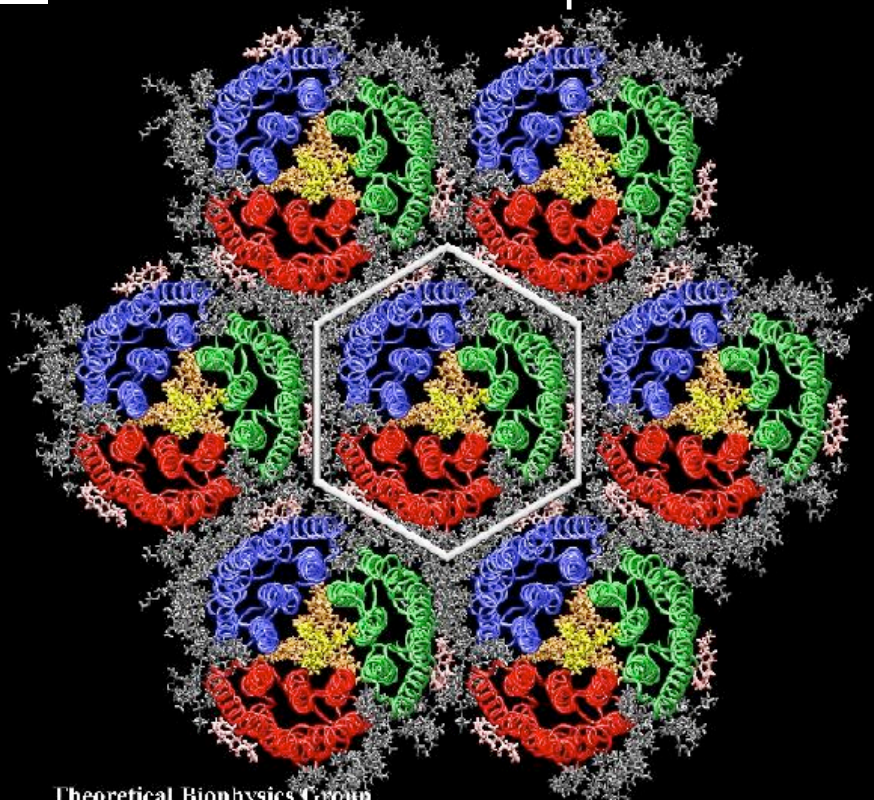


Halobacterium salinarum



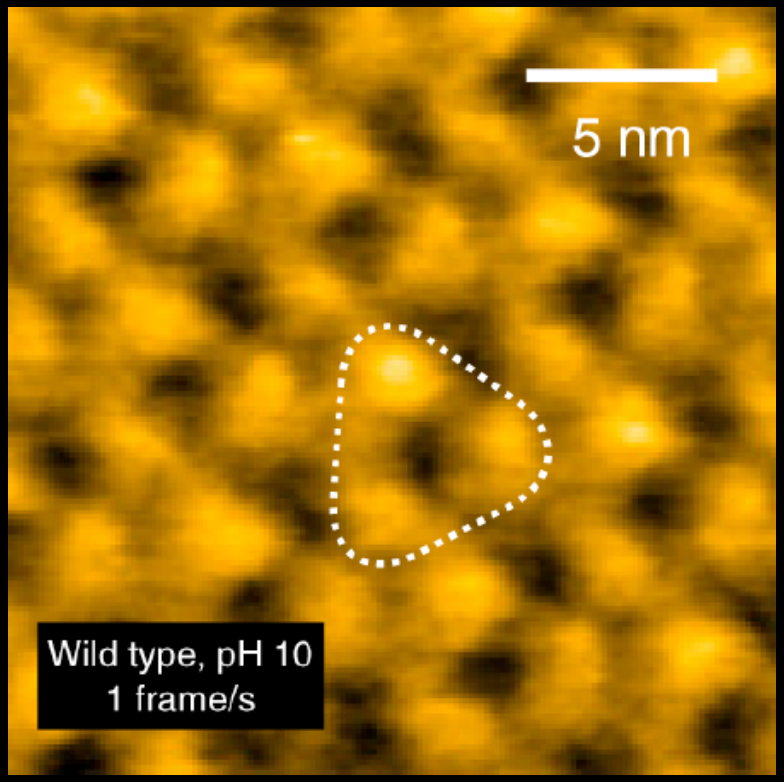
bacteriorhodopsin

- Light driven proton pump
- 7 TM helices (reference for homologue proteins)
- Forms a homotrimer
- Homotrimers aggregate to form the purple membrane
- Stability of trimer by:
  - G113, I117, L48
  - Most stability comes from surrounding lipids

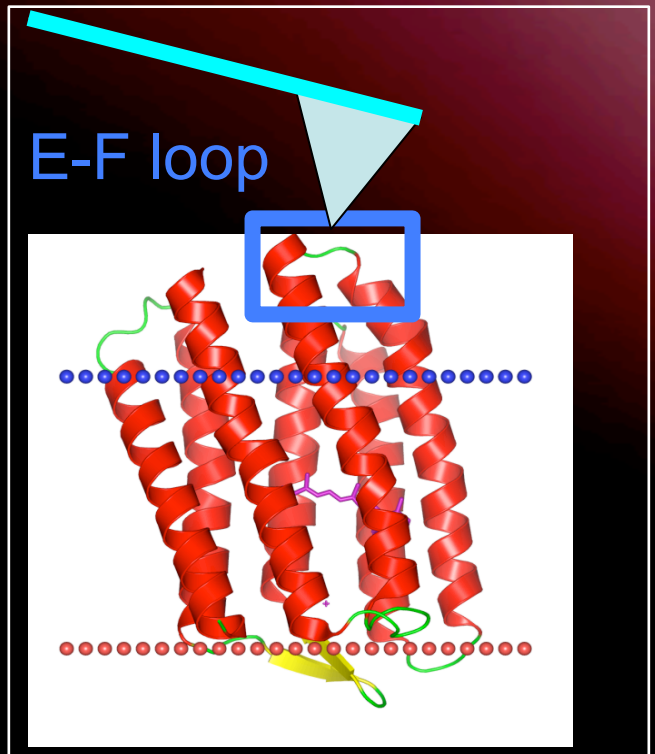


The trefoil assembly alters the decay kinetics of the activated state from individual trimers

bacteriorhodopsin



'trefoil', nearest-neighbour monomers trimer



Latest activated monomers in the trefoil decay time 2.0 s

Early activated monomers decay time lengthens to ~13 s

M Shibata *et al*. *Nature* nano (2010) doi:10.1038/nnano.2010.07

highlights the relevance out of the membrane interactions between neighbor oligomers in protein functioning (E-F loop bending/activation)

nature nanotechnology

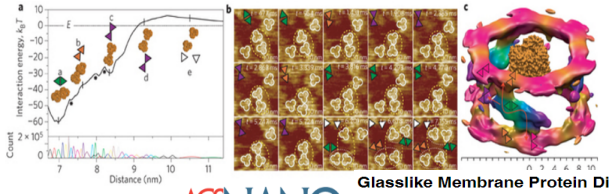
Characterization of the motion of membrane proteins using high-speed atomic force microscopy

Ignacio Casuso, Jonathan Khao, Mohamed Chami, Perrine Paul-Gilloteaux, Mohamed Husain, Jean-Pierre Duneau, Henning Stahlberg, James N. Sturgis & Simon Scheuring

Affiliations | Contributions | Corresponding author

Nature Nanotechnology **7**, 525–529 (2012) | doi:10.1038/nnano.2012.109

Received 03 April 2012 | Accepted 04 June 2012 | Published online 08 July 2012



ACS NANO

Glasslike Membrane Protein Diffusion in a Crowded Membrane

Ignacio Munguira[†], Ignacio Casuso[†], Hirohide Takahashi[†], Felix Rico[†], Atsushi Miyagi[†], Mohamed Chami[‡], and Simon Scheuring[†]

[†] U1006 INSERM, Université Aix-Marseille, Parc Scientifique et Technologique de Luminy, 163 avenue de Luminy, 13009 Marseille, France

[‡] Center for Cellular Imaging and NanoAnalytics, Biozentrum, University of Basel, Mattenstrasse 26, CH-4058 Basel, Switzerland

ACS Nano, **2016**, *10* (2), pp 2584–2590

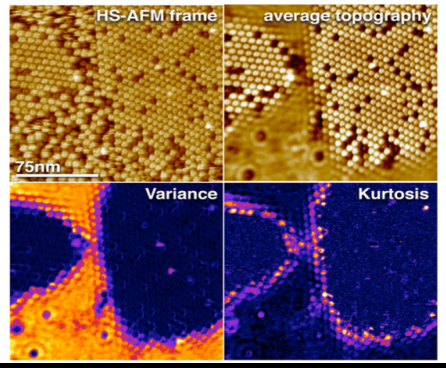
DOI: 10.1021/acsnano.5b07595

Publication Date (Web): February 09, 2016

texte intégral

*E-mail: simon.scheuring@inserm.fr. Tel: ++33-4-91828777. Fax: ++33-4-91828701.

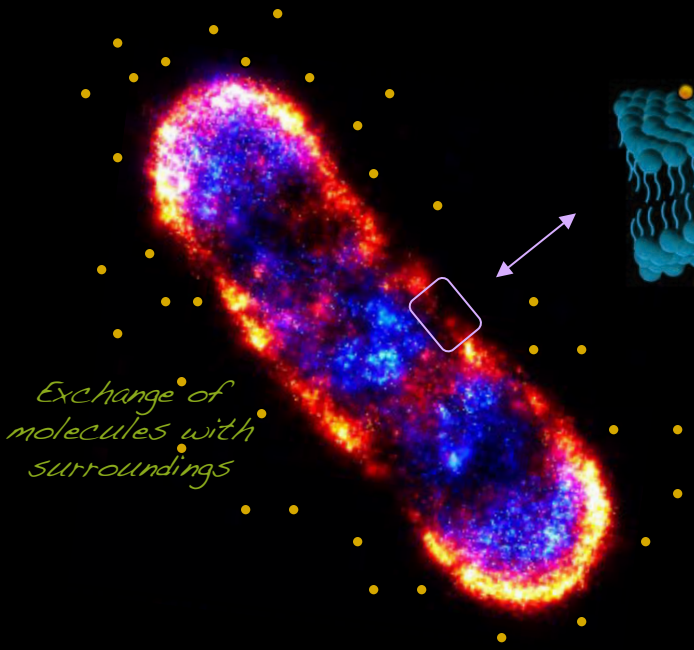
Abstract



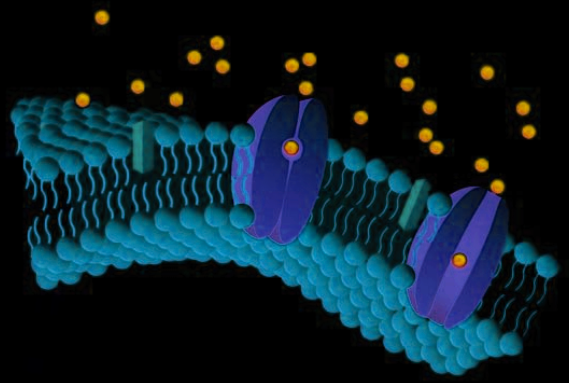
Membrane Protein Membrane mediated protein-protein interaction Visualized by HS-AFM

HS-AFM study of Porin Mediated Cell Membrane Transport

Escherichia Coli

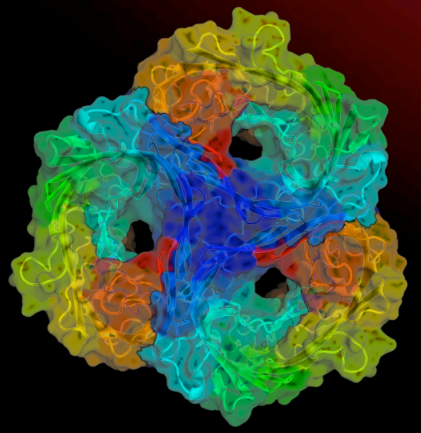


Outer Membrane



Outer Membrane Porin F

OmpF trimer

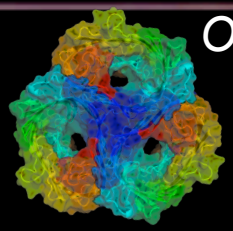


FUNCTION:
Passive diffusion transport of polar nutrients (water, ions sugars) and waste

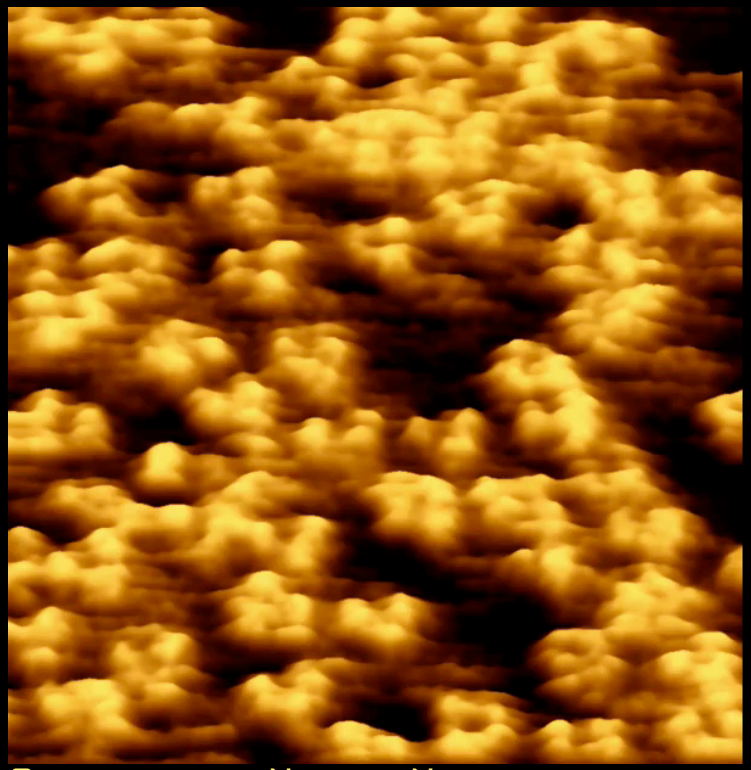
How are the OmpF molecules distributed in the bacterial membrane ?

Location of Porins in the membrane is critical for Cell Transport

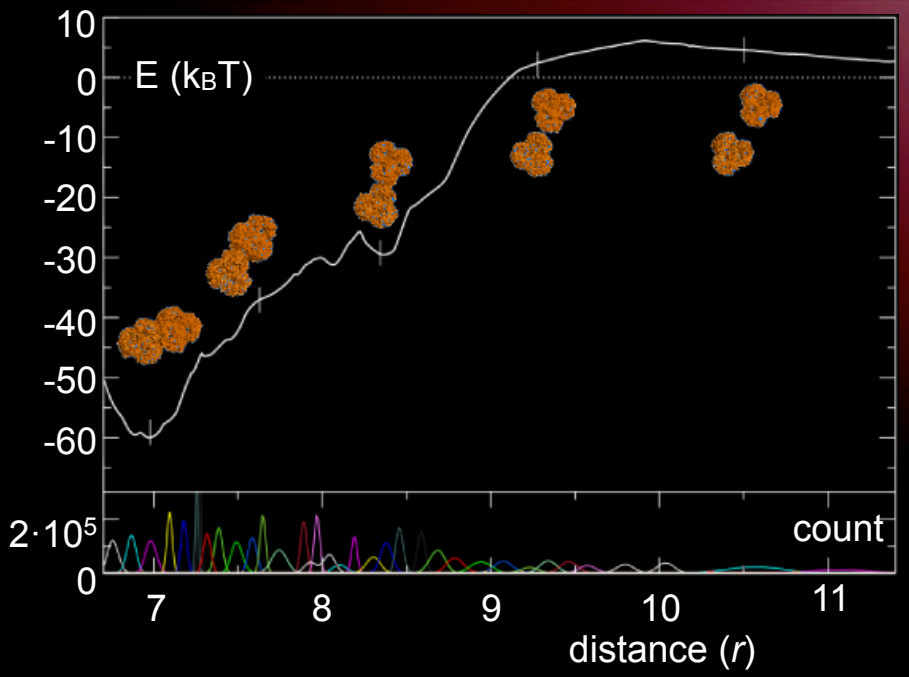
OmpF covers ~40% of the outer membrane surface

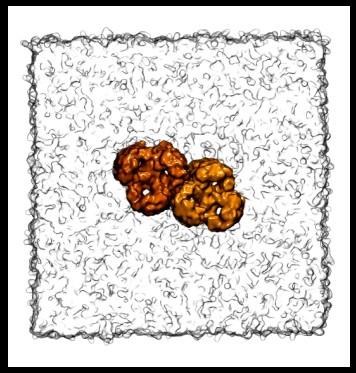


OmpF



Casuso et al; Nature Nanotechnology, 2012, 7 (8): 525-529

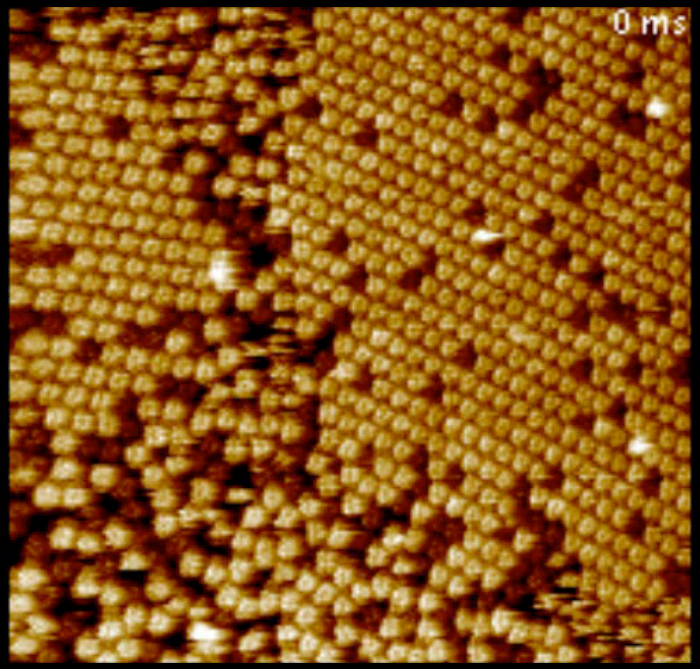


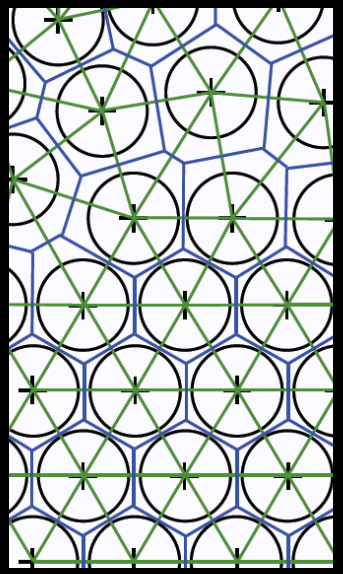


Coarsed grain molecular dynamics simulations

Glassy Behavior in crowded membranes

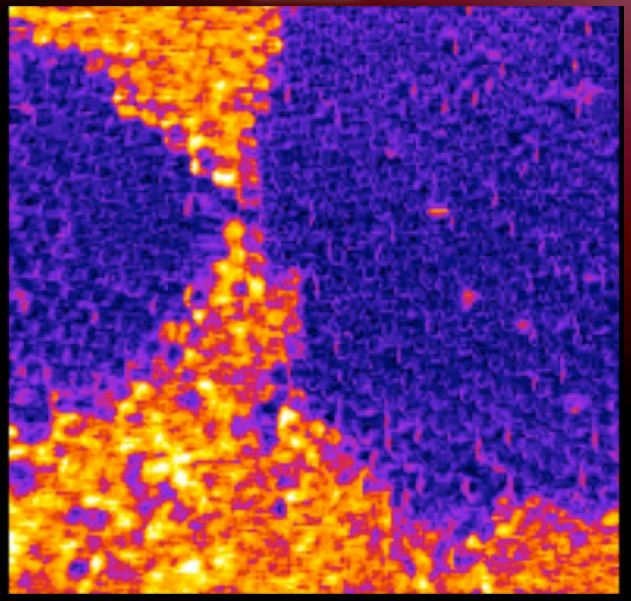
Munguira et al; Nature ACS NANO 2016





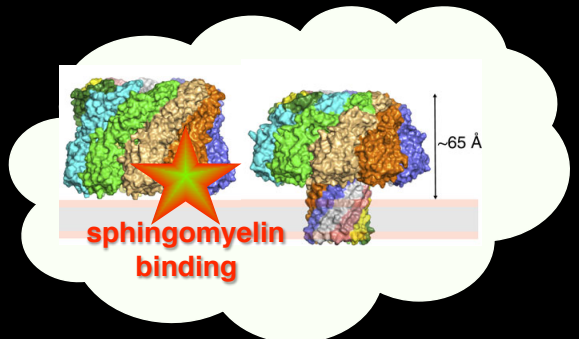
HEAT MAP BY SD

(AT AVERAGE CAGE RESIDENCE TIME)



FLUCTUATION WAVES

DYNAMIC HETEROGENEITY



LYSENIN

PORE FORMING TOXIN

Collective motion is controlled by crowding effects (cages)

In these conditions, the individual molecules diffuse in anomalous-like diffusion trajectories

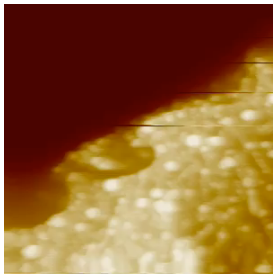
nature COMMUNICATIONS

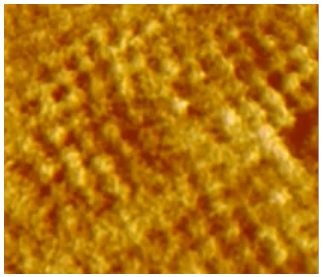
Article | Published: 16 July 2013

A hybrid high-speed atomic force–optical microscope for visualizing single membrane proteins on eukaryotic cells

Adai Colom, Ignacio Casuso, Felix Rico & Simon Scheuring

Nature Communications **4**, Article number: 2155 (2013) | Download Citation

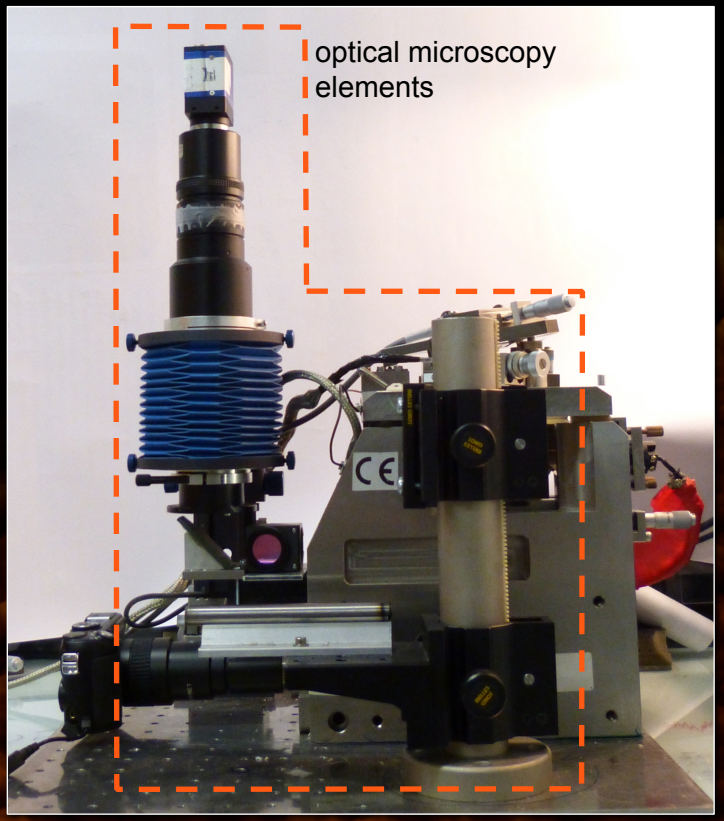


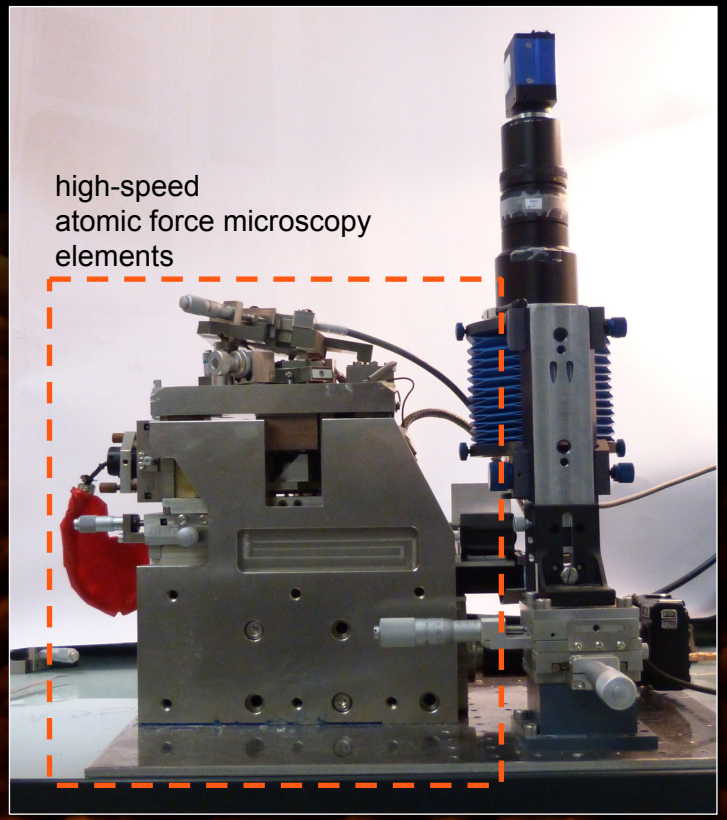


Membrane Protein Imaging directly on Cells HS-AFM Fluorescence Integration

A Hybrid HS-AFM / Optical Microscope Setup

Objective: best HS-AFM performance; epifluorescence OM performance

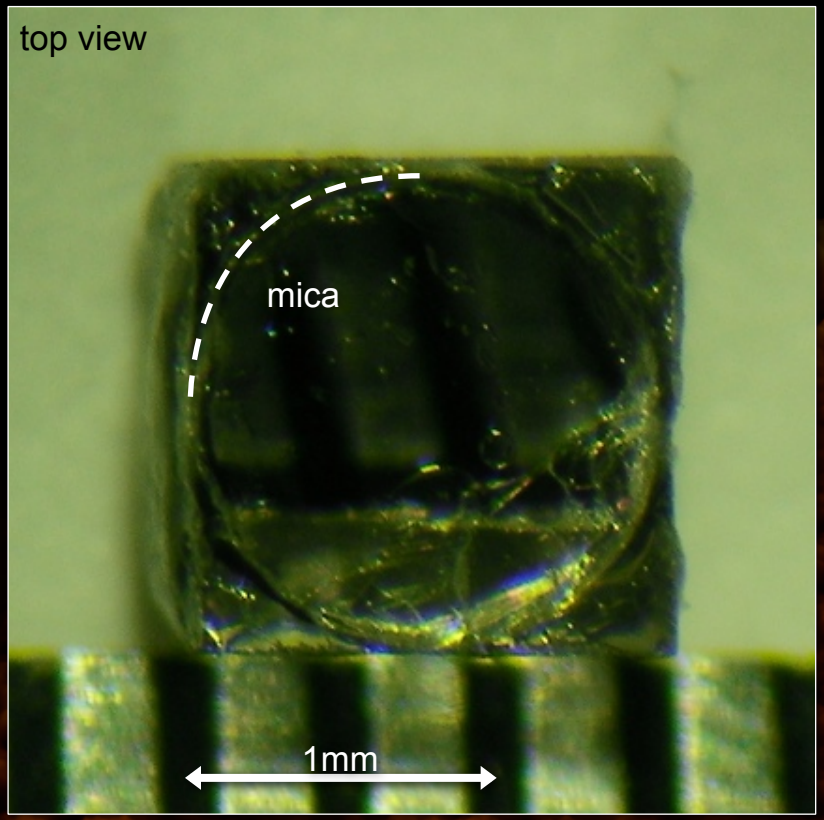


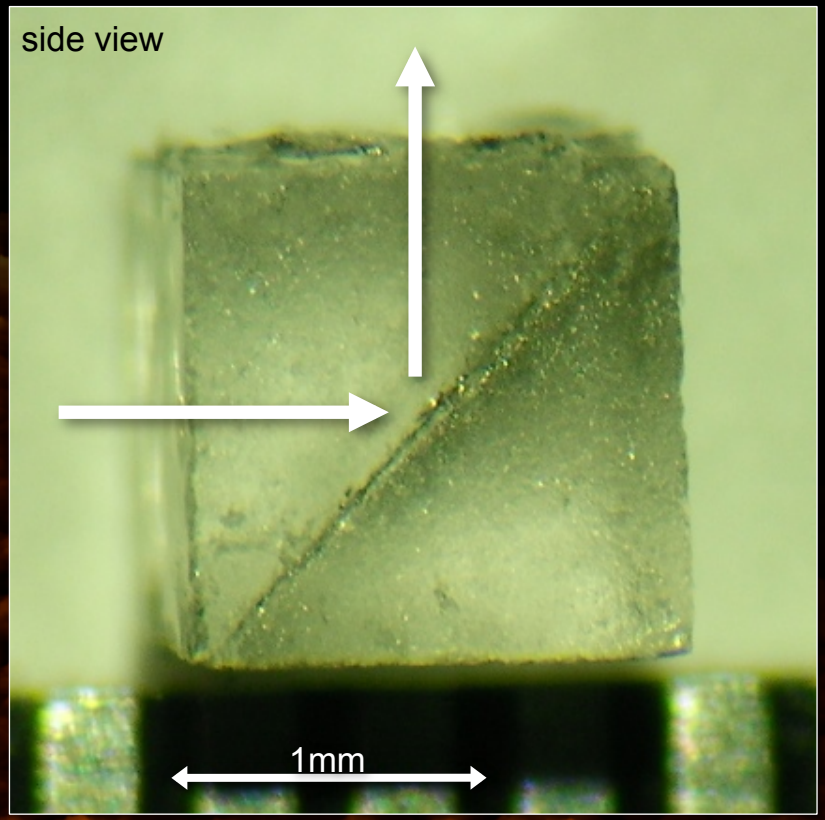


Casuso et al. Nature Communications, 2013, 4 (2155): DOI:10.1038/ncomms3155

A Hybrid HS-AFM / OM Setup

Miniaturized light injection cube for bright field OM illumination





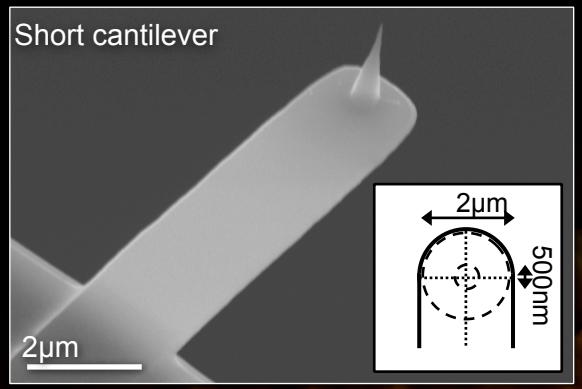
Weight (3.375mm^3, 8.5mg + glue) - original cylinder sample holder (3.534mm^3, 8.9mg)

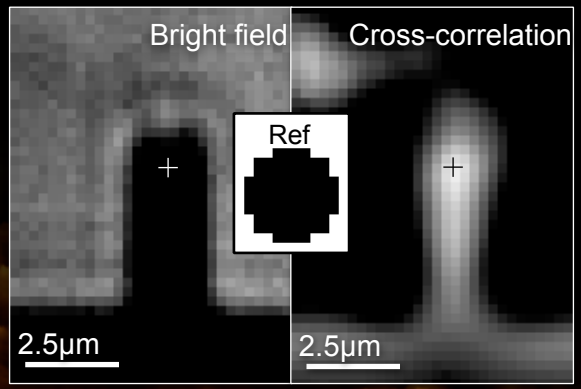
Casuso et al. Nature Communications, 2013, 4 (2155): DOI:10.1038/ncomms3155

A Hybrid HS-AFM / OM Setup

Tip position definition & Tip placement on *E coli* cells

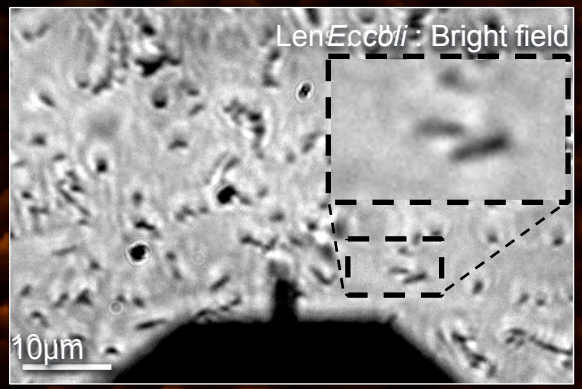
SEM of short cantilever and schematic representation of its geometry (6 x 2 x 0.1 µm)

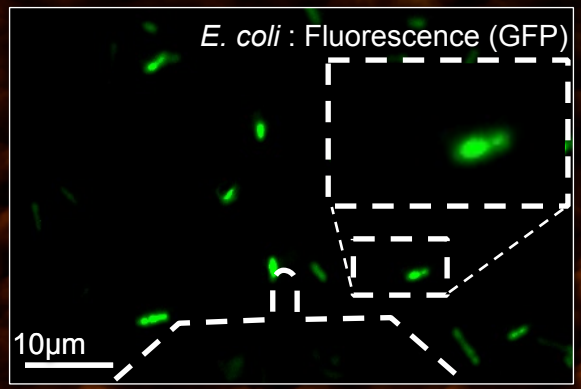




Cross-correlation based tip position definition

Bright field optical microscopy imaging





Fluorescence optical microscopy imaging

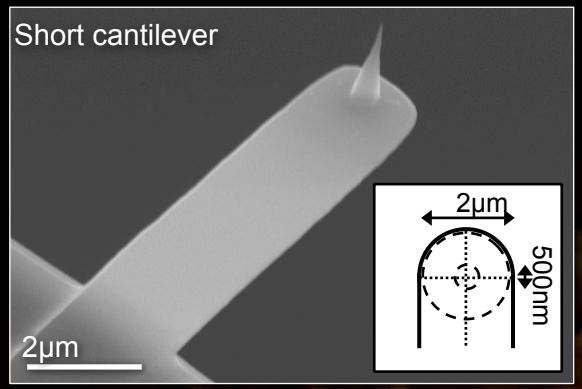
Cantilever placement on molecules / cell / subarea of a cell based on fuorescence microscopy

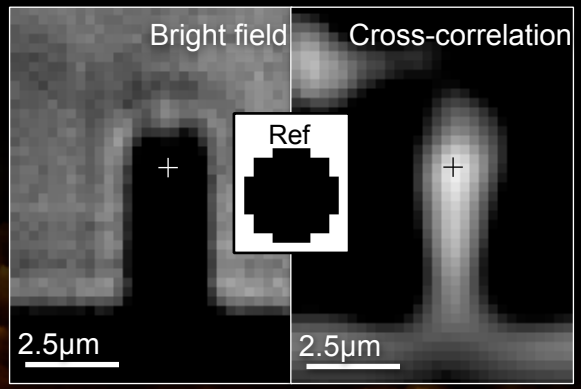
Casuso et al. Nature Communications, 2013, 4 (2155): DOI:10.1038/ncomms3155

A Hybrid HS-AFM / OM Setup

Tip position definition & Tip placement on eye lens cells

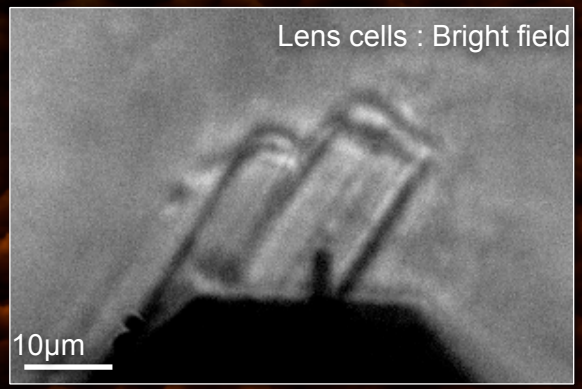
SEM of short cantilever and schematic representation of its geometry (6 x 2 x 0.1 µm)

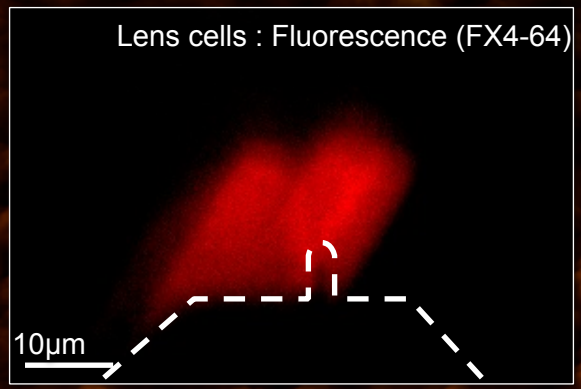




Cross-correlation based tip position definition

Bright field optical microscopy imaging





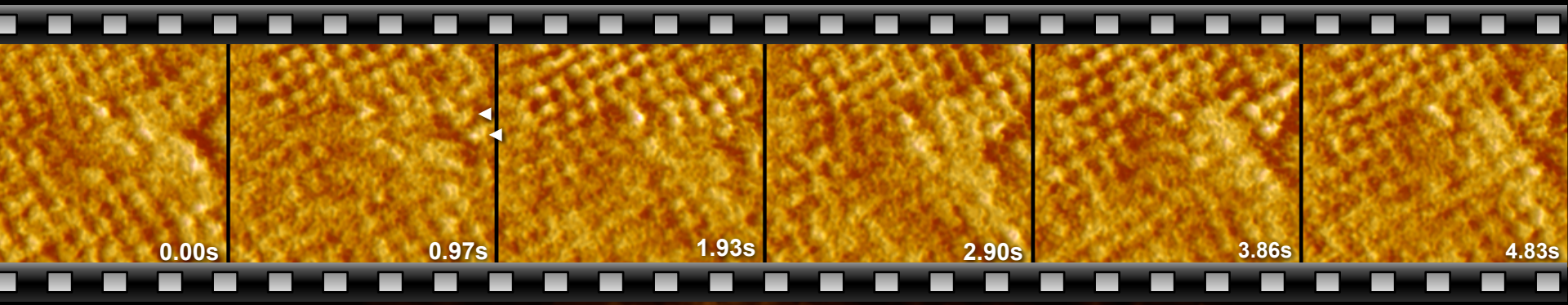
Fluorescence optical microscopy imaging

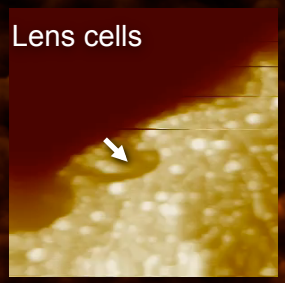
Cantilever placement with <100nm precision on eye lens cells

Casuso et al. Nature Communications, 2013, 4 (2155): DOI:10.1038/ncomms3155

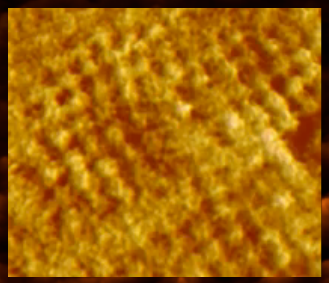
A Hybrid HS-AFM / OM Setup

First HS-AFM movies of unlabeled membrane proteins on a cell

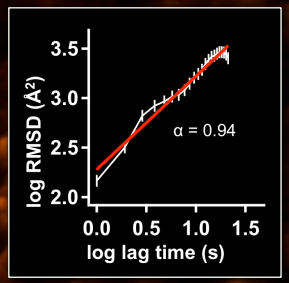




300 x 300 pixels
3.3 s/frame
5 µm frame size



209 x 178 pixels
960 ms/frame
70 nm frame size



slow but free
diffusion of
AQP0 arrays

Visualization of unlabeled AQP0 on lens cells
HS-AFM images acquired in buffer solution and under ambient temperature and pressure

Casuso et al. Nature Communications, 2013, 4 (2155): DOI:10.1038/ncomms3155

Inserm
Institut national de la santé et de la recherche médicale

Agence Nationale de la Recherche ANR

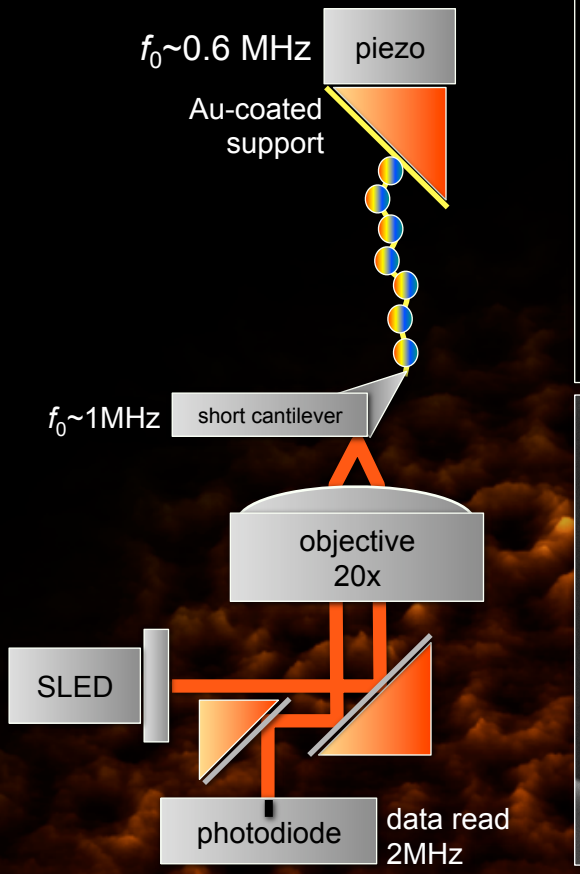
Aix*Marseille université

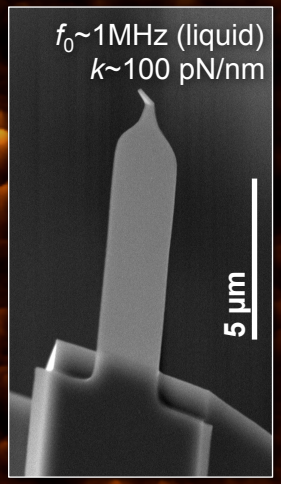
High-Speed Force Spectroscopy (HS-FS)
Titin Unfolding At The Speed Of MD Simulations

Science, 2013, 342 (6159): 741-743

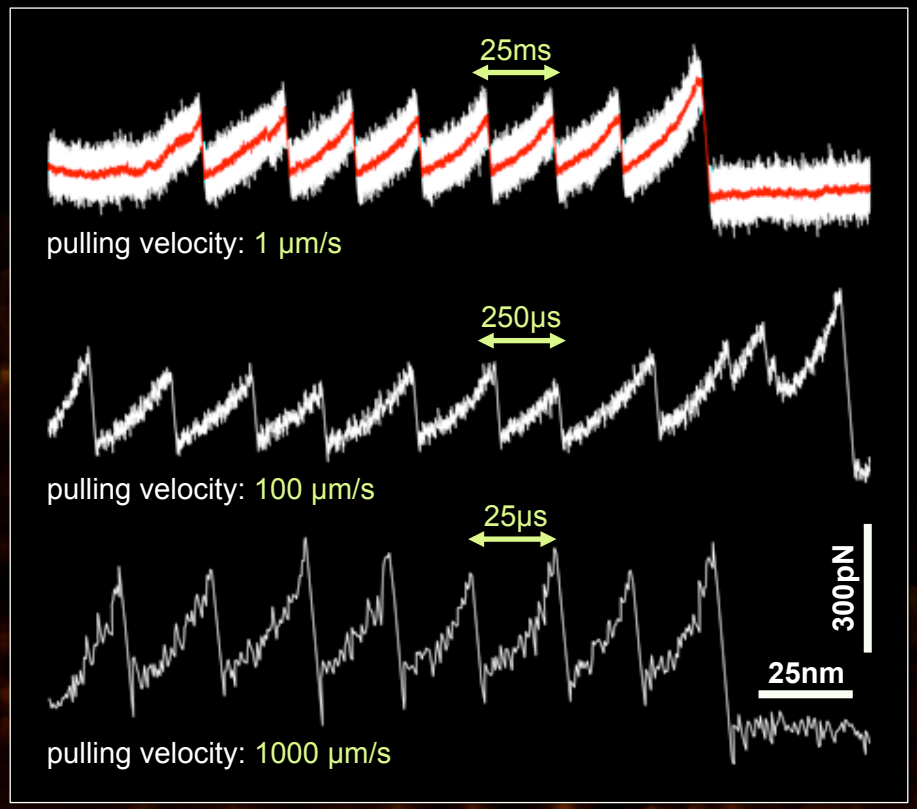
High-Speed Force Spectroscopy (HS-FS)

Experiment and simulation





cantilever: f_0~1MHz ('average' oscillation snap back1µs)
data read: 2MHz (one data point every 0.5µs)

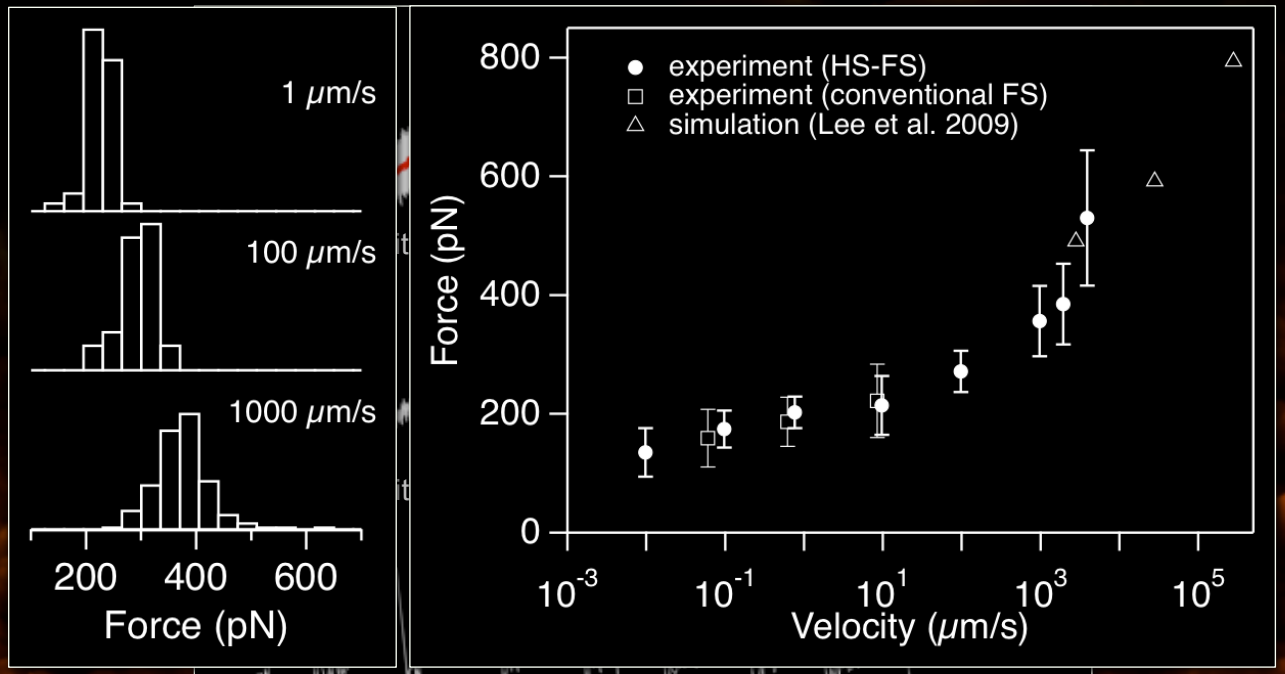


Raw data force curves

Science, 2013, 342 (6159): 741-743

High-Speed Force Spectroscopy (HS-FS)

From force curves to force histograms to the dynamic force spectrum



Unfolding force histograms Dynamic force spectrum

pulling velocity: 1000 μm/s

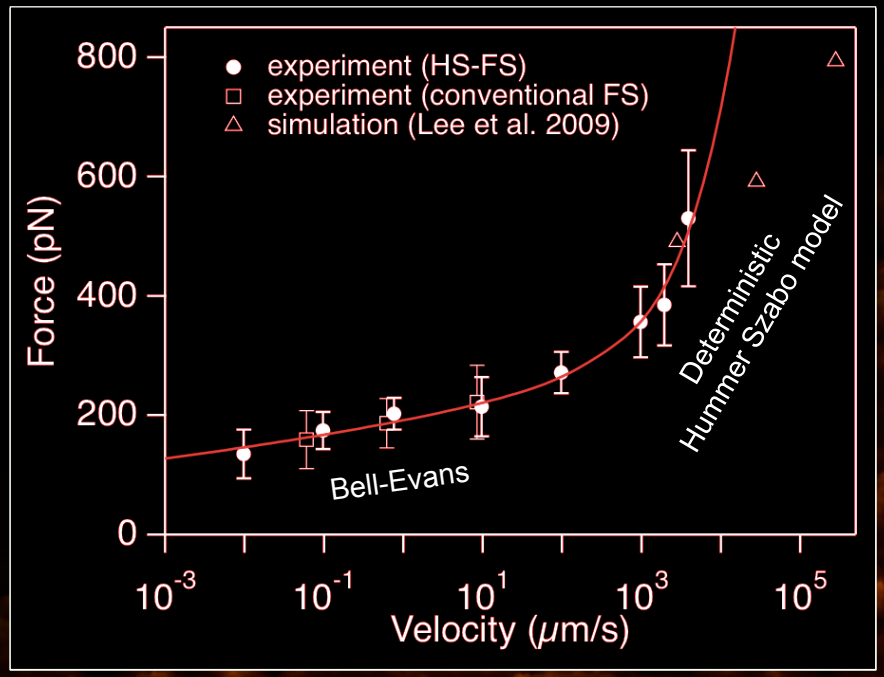
HS-FS bridges conventional FS to molecular dynamic simulations

Raw data force curves

Science, 2013, 342 (6159): 741-743

High-Speed Force Spectroscopy (HS-FS)

The dynamic force spectrum is non-linear
Provides realistic kinetic values and
fitting to the Hummer & Szabo model



Biophysical Journal Volume 85 July 2003 5–15

Kinetics from Nonequilibrium Single-Molecule Pulling Experiments

Gerhard Hummer and Attila Szabo
Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0520 USA

ABSTRACT Mechanical forces exerted by laser tweezers or atomic force microscopes can be used to drive rare transitions in single molecules, such as unfolding of a protein or dissociation of a ligand. The phenomenological description of pulling experiments based on Bell's expression for the force-induced rupture rate is found to be inadequate when tested against computer simulations of a simple microscopic model of the dynamics. We introduce a new approach of comparable complexity to extract more accurate kinetic information about the molecular events from pulling experiments. Our procedure is based on the analysis of a simple stochastic model of pulling with a harmonic spring and encompasses the phenomenological approach, reducing to it in the appropriate limit. Our approach is tested against computer simulations of a multimodule titin model with anharmonic linkers and then an illustrative application is made to the forced unfolding of I27 subunits of the protein titin. Our procedure to extract kinetic information from pulling experiments is simple to implement and should prove useful in the analysis of experiments on a variety of systems.

Spontaneous unfolding:	$k_0 = 2 \times 10^{-10}\ s^{-1}$
Transition barrier:	$x^{\ddagger} = 0.89$ nm
Molecular elasticity:	$k_m = 376$ pN/nm
Unfolding barrier height:	$\Delta G^{\ddagger} = 36.4\ k_B T$
Diffusion coefficient:	$D = 4 \times 10^3$ nm^2/s

Transition barrier according to Bell-Evans model: $x^{\ddagger} = 0.25$ nm

Science, 2013, 342 (6159): 741-743

nature physics

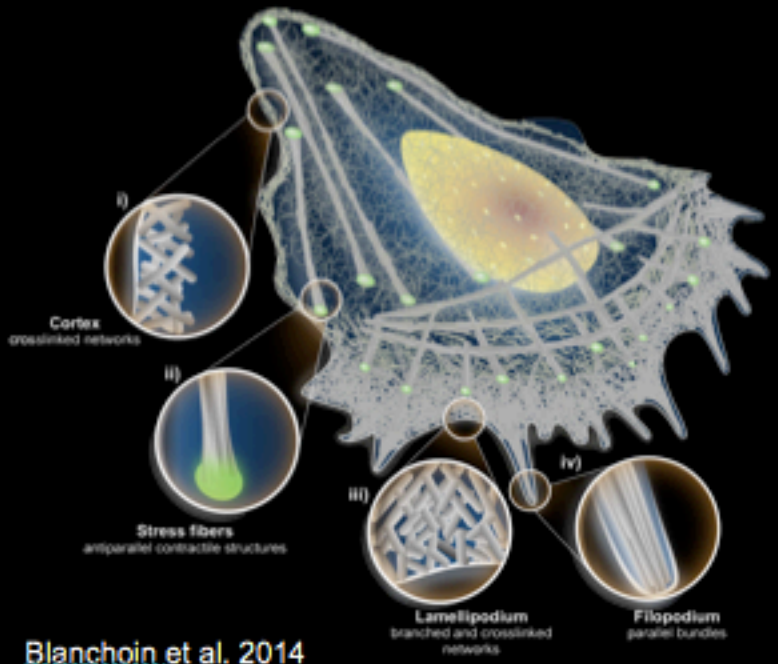
Letter | Published: 01 May 2017

High-frequency microrheology reveals cytoskeleton dynamics in living cells

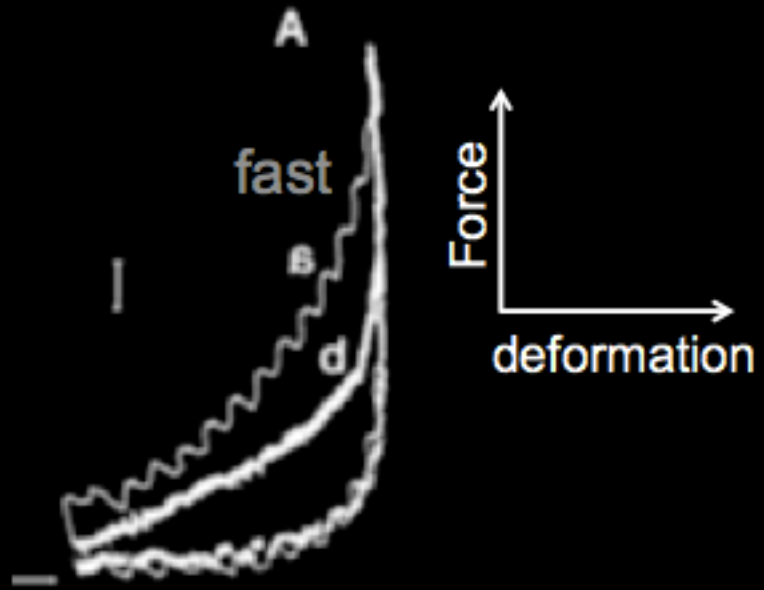
Cells

High speed AFM measures mechanical regimes in cells never measured before

Cells are viscoelastic



Blanchoin et al. 2014



Petersen 1982 PNAS

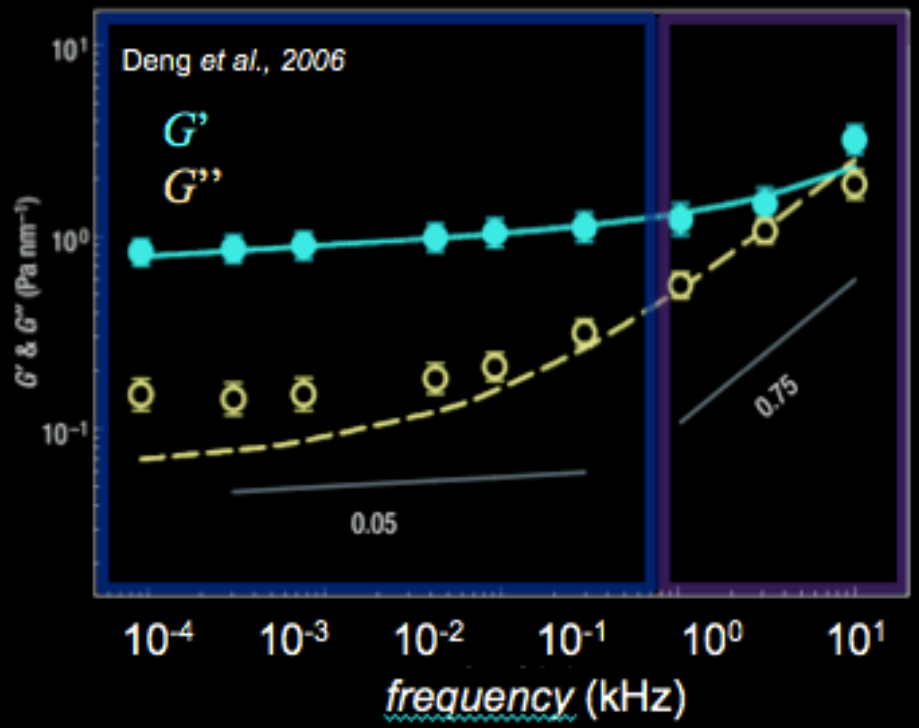
G' G''

Viscoelasticity of cells
Two power law regimes

Phenomenological Soft Glassy Rheol.

Mesoscopic/structural elements

$G \sim f^{\alpha}$, $\alpha \sim 0.05\text{-}0.20$, $G'' < G'$



Universal?

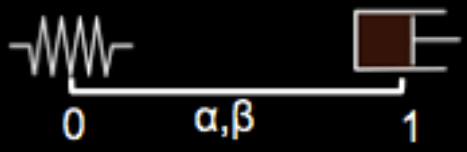
Mechanistic

Single filaments dynamics

$G \sim f^{\beta}$, $\beta \sim 3/4$, $G'' > G'$

Transverse fluctuations $f^{3/4}$ (<1kHz)

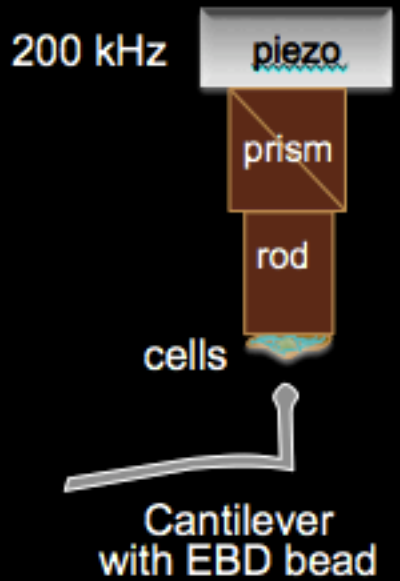
$$G^*(f) = A(if/f_0)^{\alpha} + B(if/f_0)^{\beta}$$



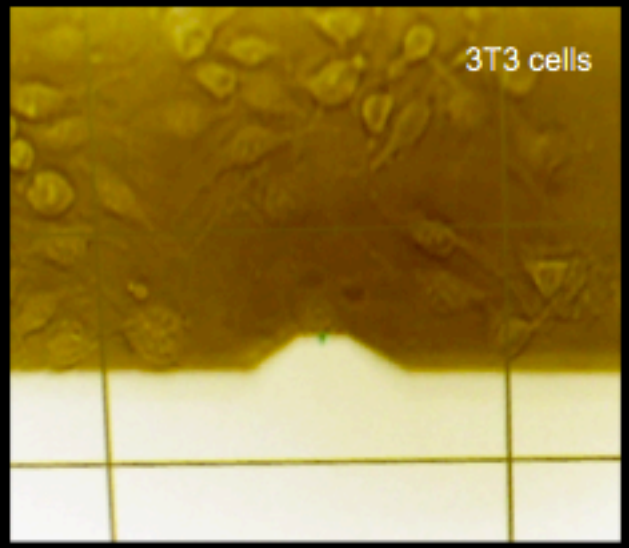
Aim: to probe viscoelastic response of living cells at high frequencies

High frequency microrheology of living cells

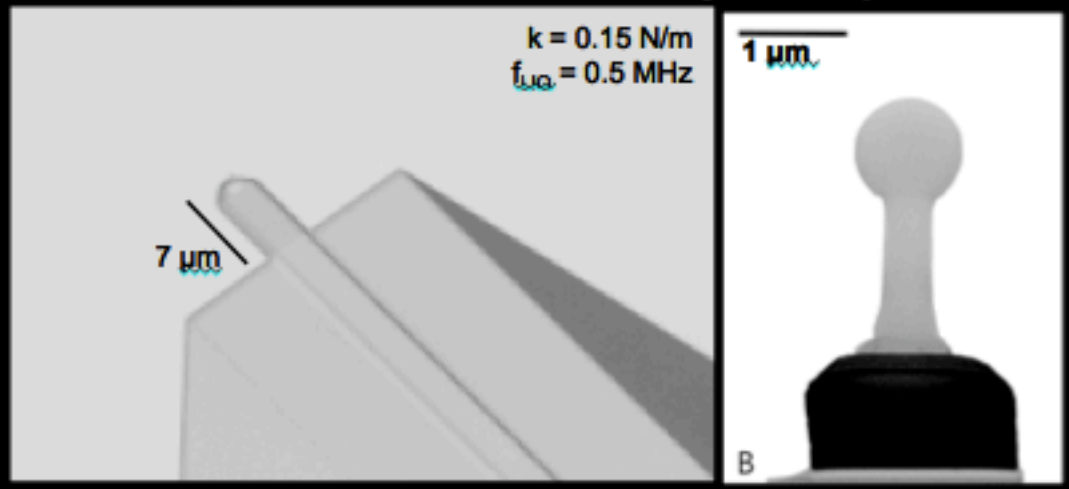
HS-AFM setup



Prism: bright field image of cells



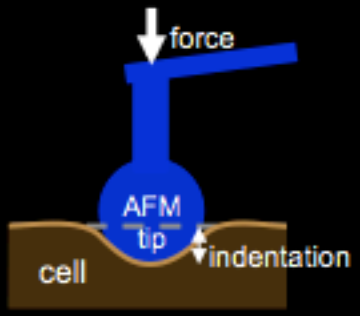
EBD sphere: defined contact geometry



Calibration
Spring constant (Sader in air)
Sensitivity (thermal in liquid)

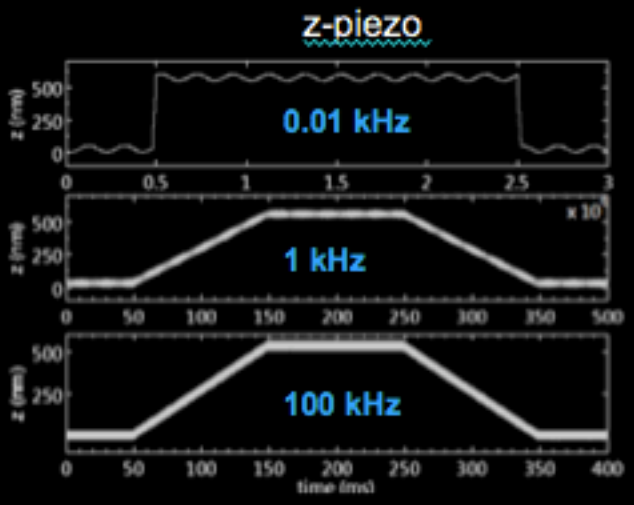
Rigato *et al.* 2017 *Nat Physics*

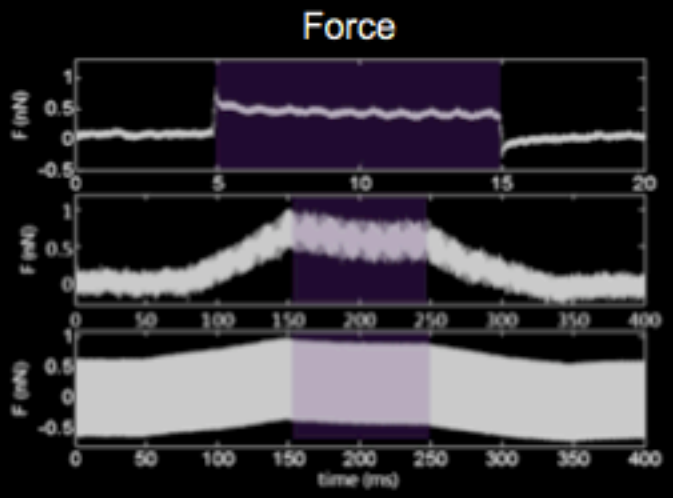
High frequency microrheology of living cells



$\Delta\delta$=15-nm

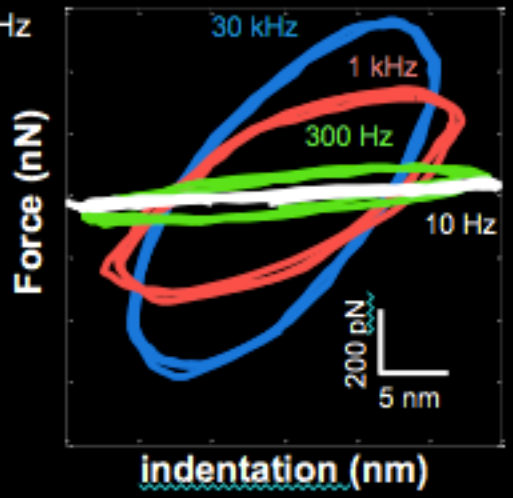
δ_0 ~ 300-nm





Force-indentation loops

Freq< 500 kHz



Frequency response

higher slope → increased elasticity $G'(f)$

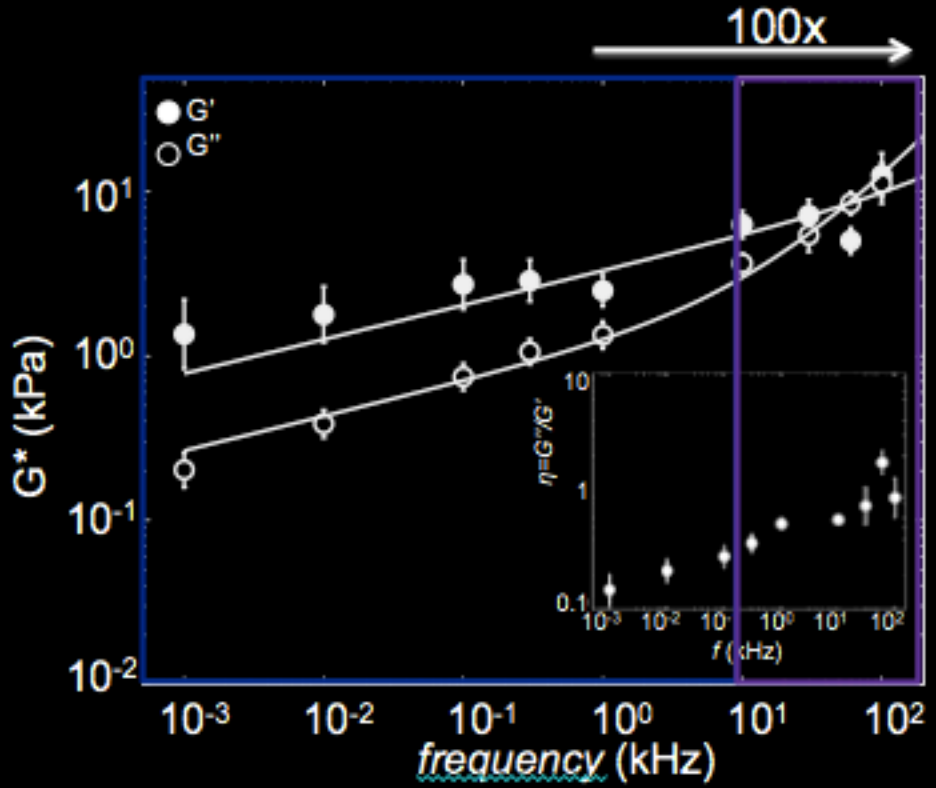
higher hysteresis → increased viscosity $G''(f)$

$$H_{tot}(f)=F(f)/\delta(f)e^{-i\varphi}$$

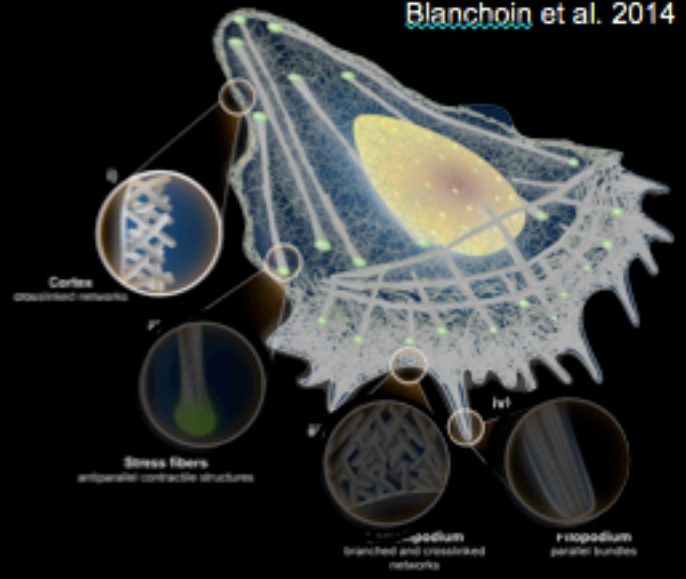
$$G\uparrow*(f)=H\downarrow s(f)\cdot 1-\nu/4\cdot\sqrt{R}\cdot\delta\downarrow 0$$

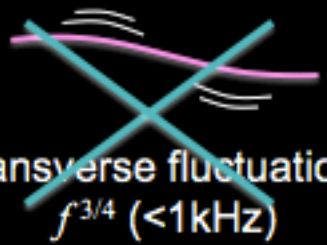
Rigato *et al.* 2017 *Nat Physics*

High frequency microrheology of living fibroblasts

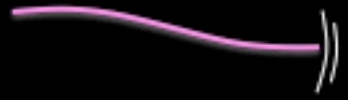


Blanchoin et al. 2014





Transverse fluctuations
$f^{3/4}$ (<1kHz)



Longitudinal fluctuations
$f^{7/8}$

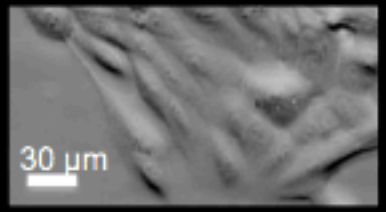
$$G^*(f) = A(if/f_0)^{\alpha} + B(if/f_0)^{\beta}$$

Condition	A (kPa)	α (low freq.)	B (kPa)	β (high freq.)
Untreated	3.48 ± 0.17	0.21 ± 0.01	0.15 ± 0.03	0.92 ± 0.03

Rigato *et al.* 2017 *Nat Physics*

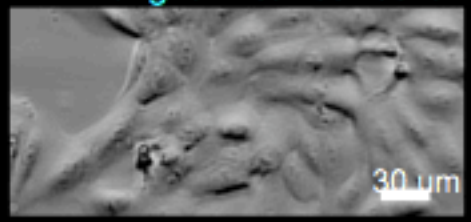
High frequency microrheology of cancer cells

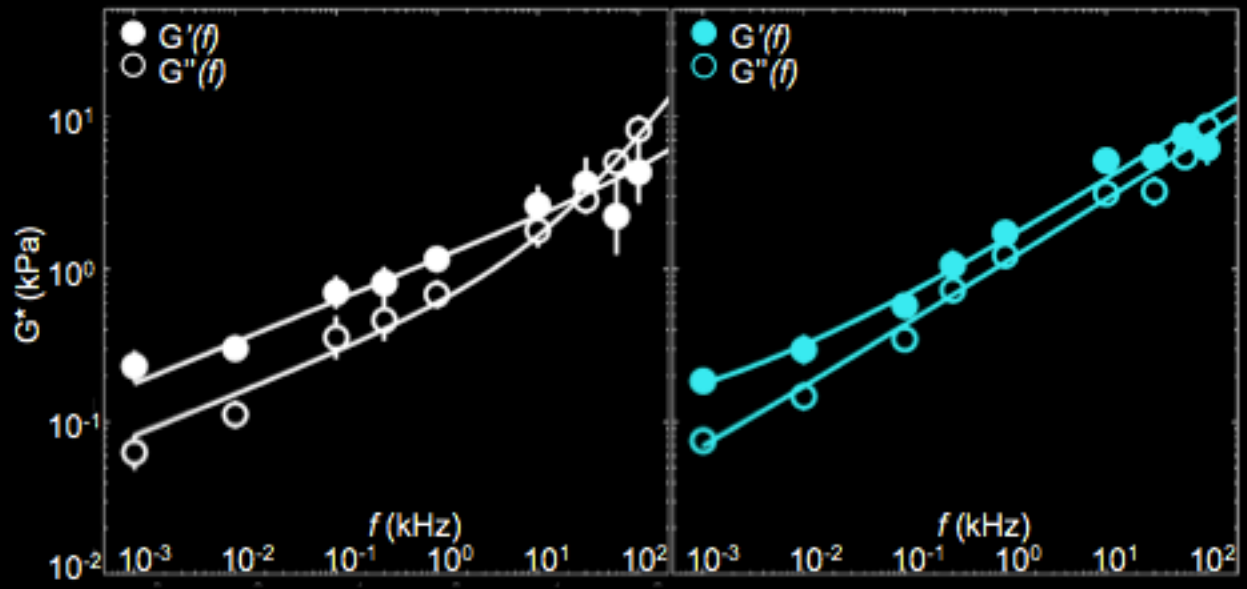
MCF-10A
benign cancer cells

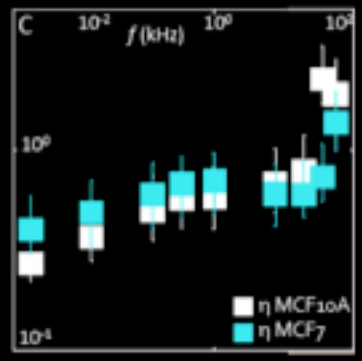


Physical features
- MCF-7 softer (lower *G'*)
- MCF-7 generate higher intracellular forces

MCF-7
malignant cancer cells

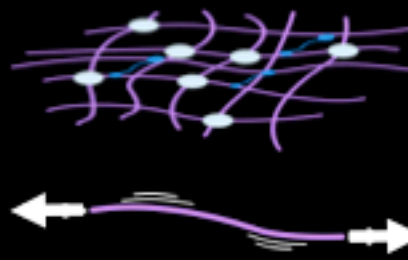


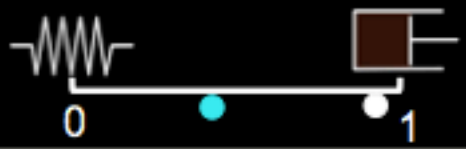




$$G^*(f) = A(if/f_0)^{\alpha} + B(if/f_0)^{\beta}$$

Cell type	A (kPa)	α (low freq.)	B (kPa)	β (high freq.)
MCF-10A	1.27 ± 0.26	0.27 ± 0.05	0.08 ± 0.28	0.93 ± 0.24
MCF-7	0.14 ± 0.03	0.05 ± 0.02	1.77 ± 0.11	0.41 ± 0.01





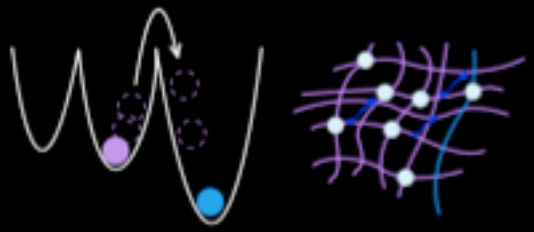
Rigato *et al.* 2017 *Nat Physics*

Active **high-frequency microrheology** >5 frequency decades (>100X faster)

Cell viscoelasticity: $G^*(f) = A(if/f_0)^{\alpha} + B(if/f_0)^{\beta}$

Low frequency → Mesoscale dynamics

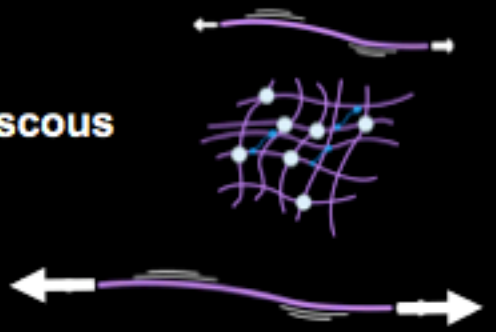
α ~ 0.1 – 0.3 **Soft glassy rheology**



High frequency → Deterministic, single filament dynamics

β ~ 0.75-1 **Unstressed single filament dynamics, purely viscous**

β ~ 1/2 **Stressed single filament dynamics**



Rich viscoelastic response at high frequencies

Univocal mechanical phenotype (β): biomarker (diagnosis?)

Thank You !

Force microscopy division @ LAI
https://sites.google.com/view/fm4b-lab

Toshio Ando visiting

Ignacio Munguira
Now with Wouter H. Roos

Francesca Zuttion at work

Laboratoire d'Adhesion et Inflammation
https://labadhesioninflammation.org/