Optical and atomic force microscopy: from single molecules to living cells

Frédéric EGHIAIAN, Post-doctoral researcher

(INSERM U1006, Director Simon Scheuring, LABEX InFOrM)

My career…

Master/Ph.D (2001-2005, Paris)

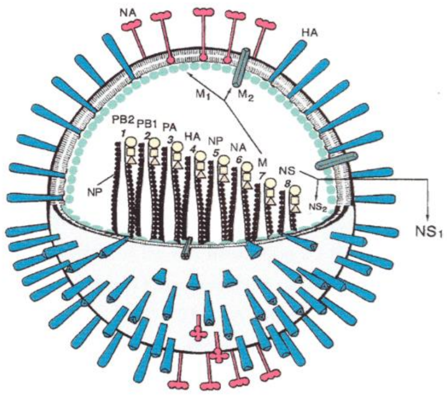
Structural and biochemical studies of the sheep prion protein

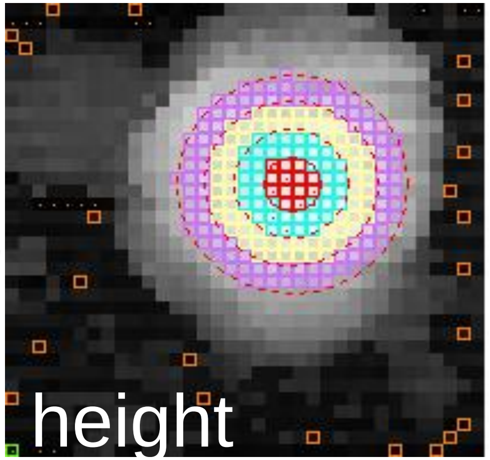
Eghiaian et al, PNAS 2004

Eghiaian et al, PNAS 2007

Post-doc (2005-2009, London, 2009-2012 Göttingen):

Single molecule studies of Influenza and ribosomes





Li et al, BJ 2011

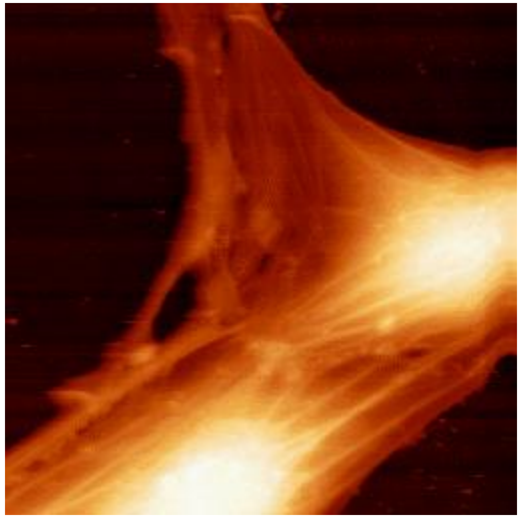
Eghiaian et al. JBC, 2012

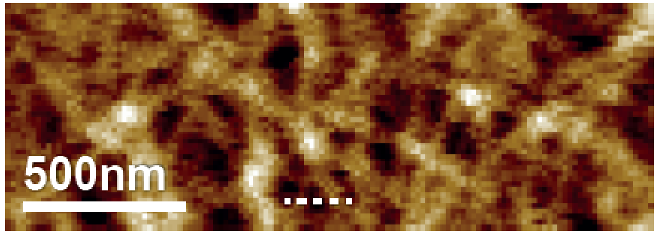
Li et al., BJ 2014

Eghiaian et al, in preparation

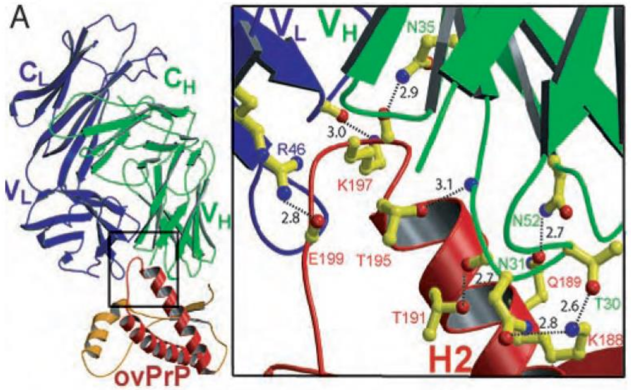
Post-doc and…(2013-…):

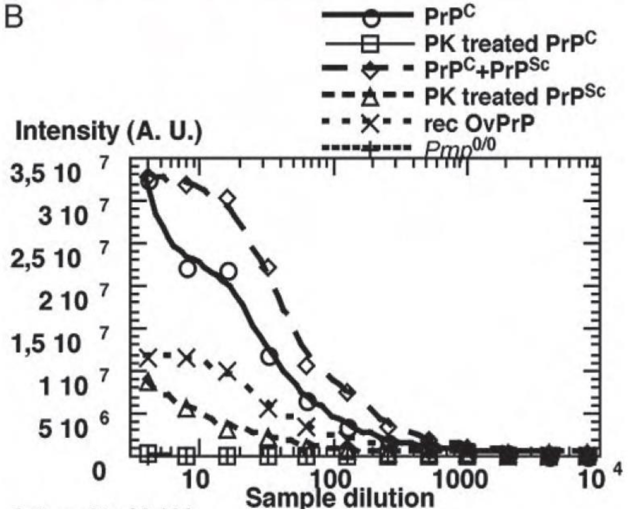
AFM of actin dynamics in live cells

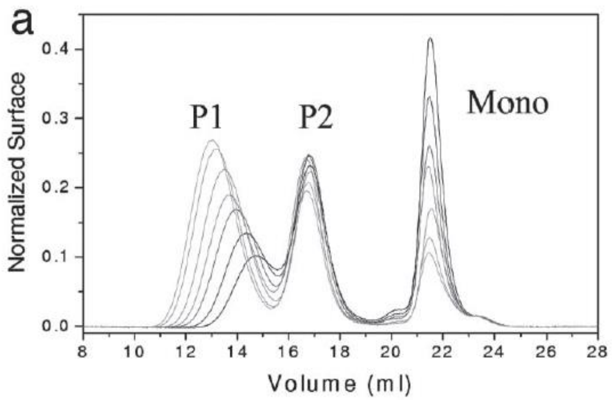


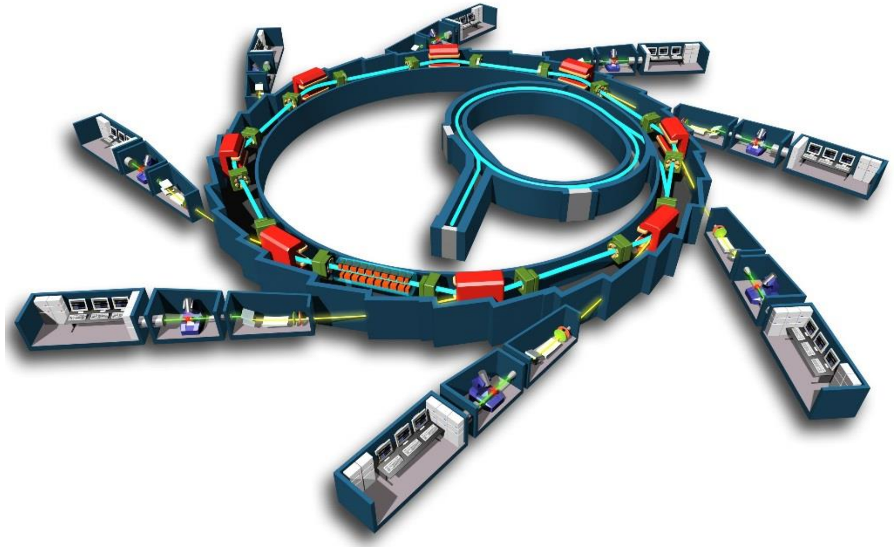


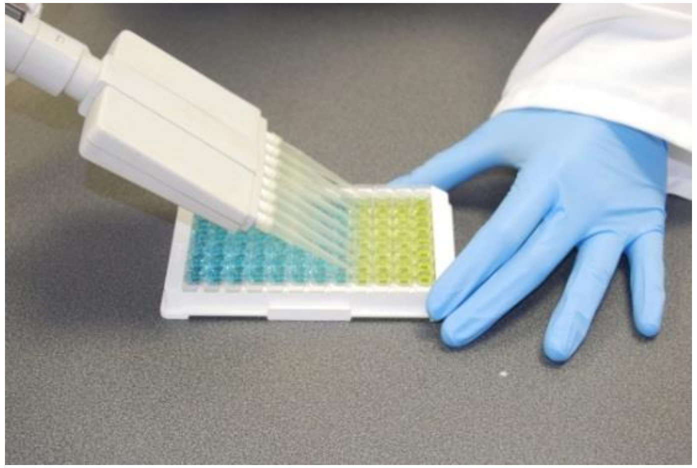
Eghiaian et al, submitted to Mol Cell

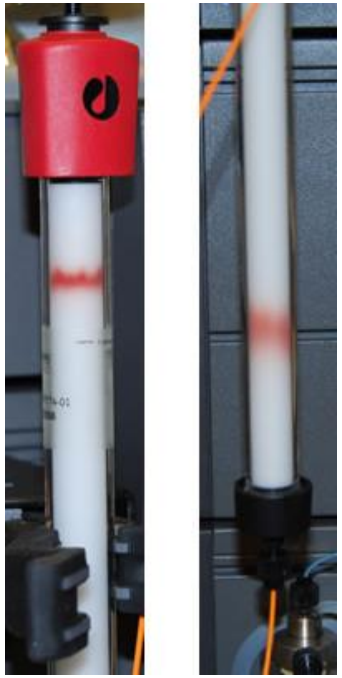












Eghiaian et al, PNAS 2004

Eghiaian et al, PNAS 2007

How can one see dynamics at the single molecule level ?

Single molecule
fluorescence ???

Challenges of single molecule fluorescence

- Contrast: A single fluorophore is not very bright and bleaches fast…
- Resolution: Lateral and Axial resolutions in microscopy are limited !

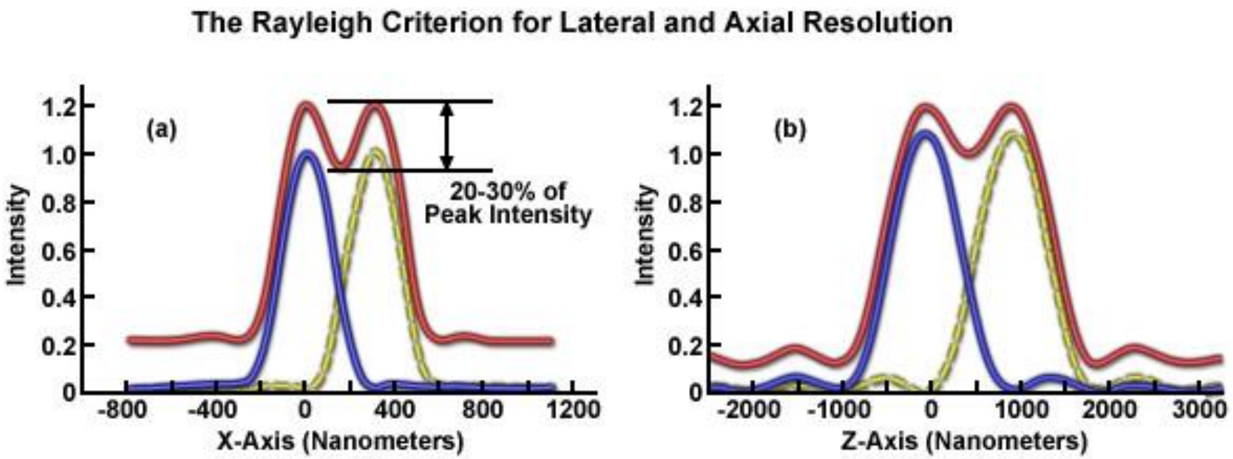


Figure 2

Abbe Resolution$_{x,y}$ = λ/2NA

Abbe Resolution$_{z}$ = 2λ/NA^2

Rayleigh Resolution$_{x,y}$ = 0.61λ/NA

Sparrow Resolution$_{x,y}$ = 0.47λ/NA

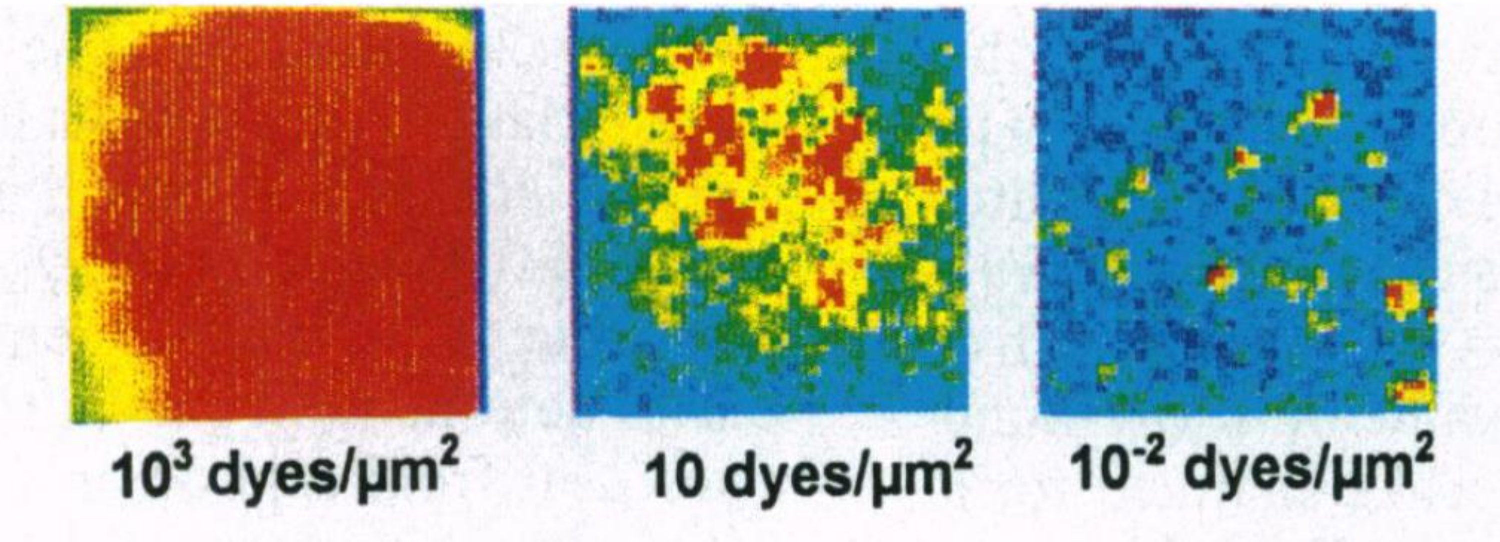
http://www.microscopyu.com/articles/superresolution/diffractionbarrier.html

Detecting single molecules by fluorescence microscopy

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 2926–2929, April 1996
Biophysics

Imaging of single molecule diffusion

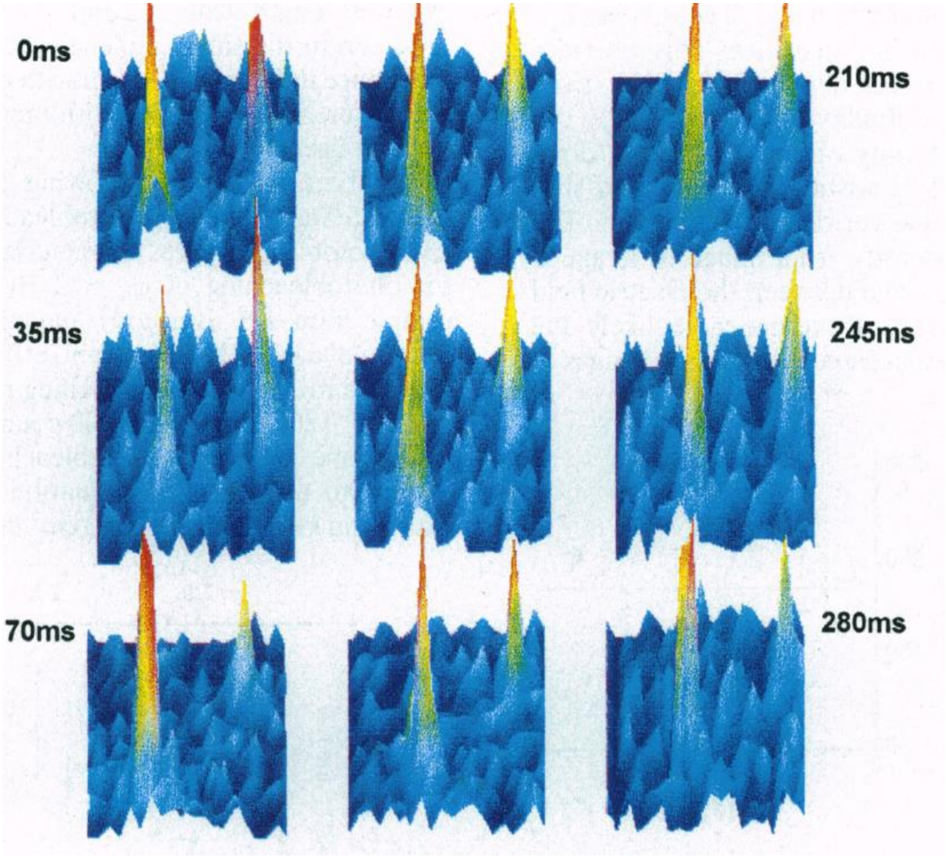
TH. SCHMIDT, G. J. SCHÜTZ, W. BAUMGARTNER, H. J. GRUBER, AND H. SCHINDLER

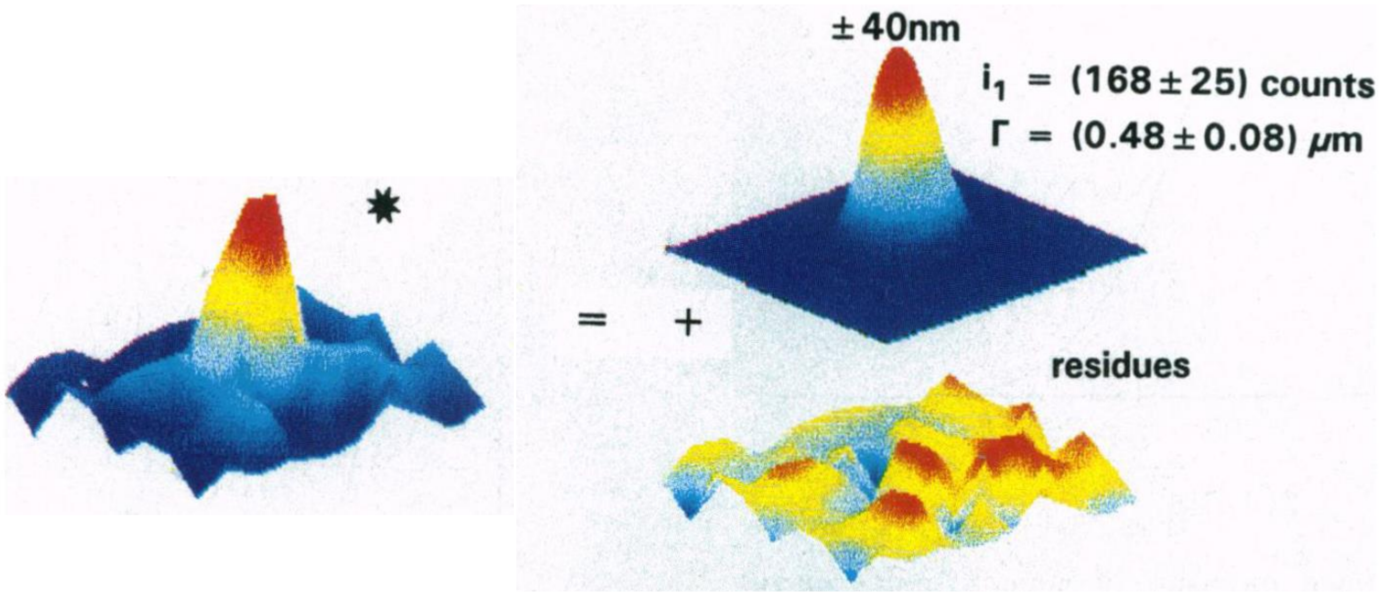


Reduce molecular density of fluorophores: single fluorophores resolved

High illumination power -> High Signal-to-noise ratio

Detecting single molecules by fluorescence microscopy





Schmidt et al, PNAS 1996

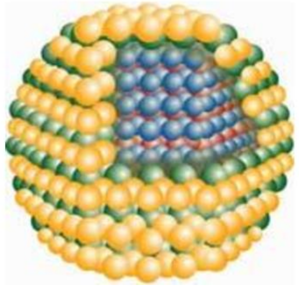
How to increase signal-to-noise ?

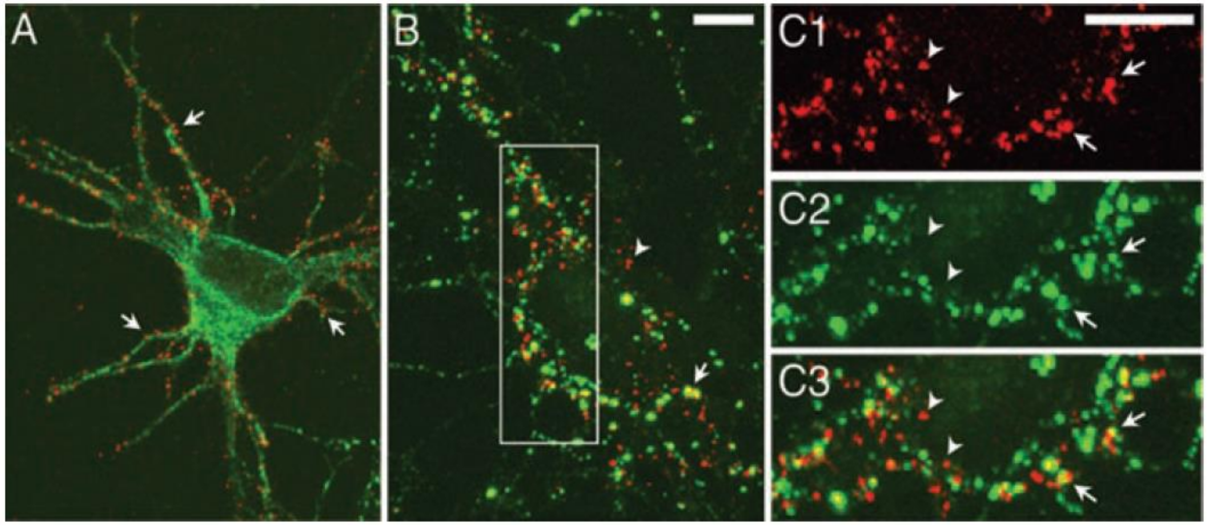
- Decrease background fluorescence

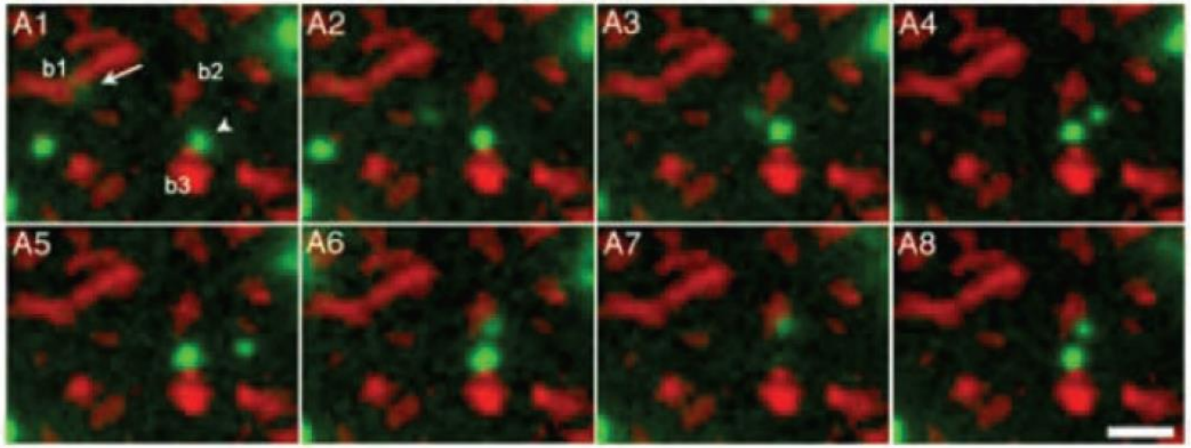
- Increase brightness of fluorophores
  - Inorganic nanoclusters
  - Quantum dots

(Not-so-) New dyes: Quantum dots, inorganic nanoclusters…

Diffusion Dynamics of Glycine Receptors Revealed by Single-Quantum Dot Tracking
Maxime Dahan *et al.*
Science **302**, 442 (2003);
DOI: 10.1126/science.1088525







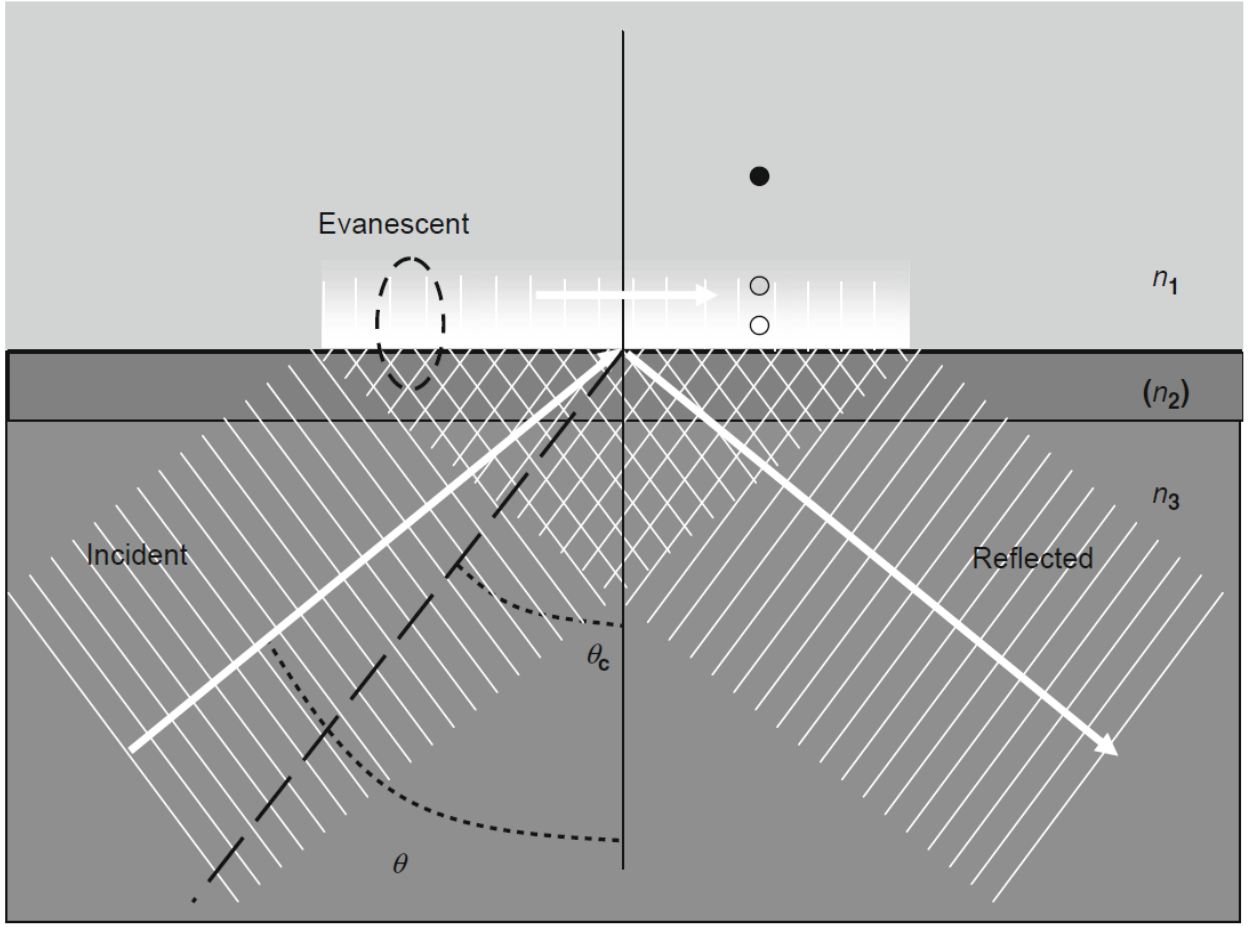
BLINKING !!!

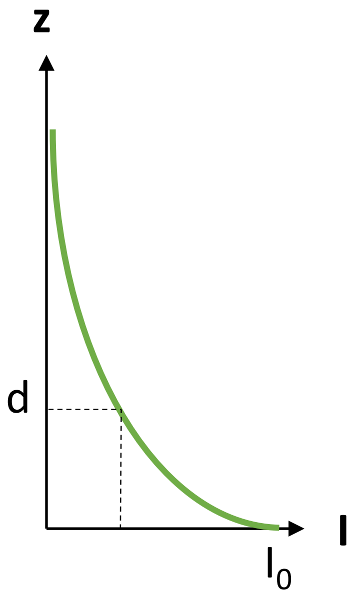
How to increase signal-to-noise ?

- Decrease background fluorescence

- Increase brightness of fluorophores
  - Inorganic nanoclusters
  - Quantum dots

Total Internal Reflection: evanescent wave





$$\theta_c = \sin^{-1}\left(\frac{n_1}{n_3}\right)$$

$$I(z) = I(0)e^{-z/d}$$

$$d = \frac{\lambda_0}{4\pi}(n_3^2\sin^2 - \theta - n_1^2)^{-1/2} = \frac{\lambda_0}{4\pi n_3}(\sin^2\theta - \sin^2\theta_c)^{-1/2}$$

Axelrod et al, Methods Mol Biol 2008

Advantages of TIRF-M

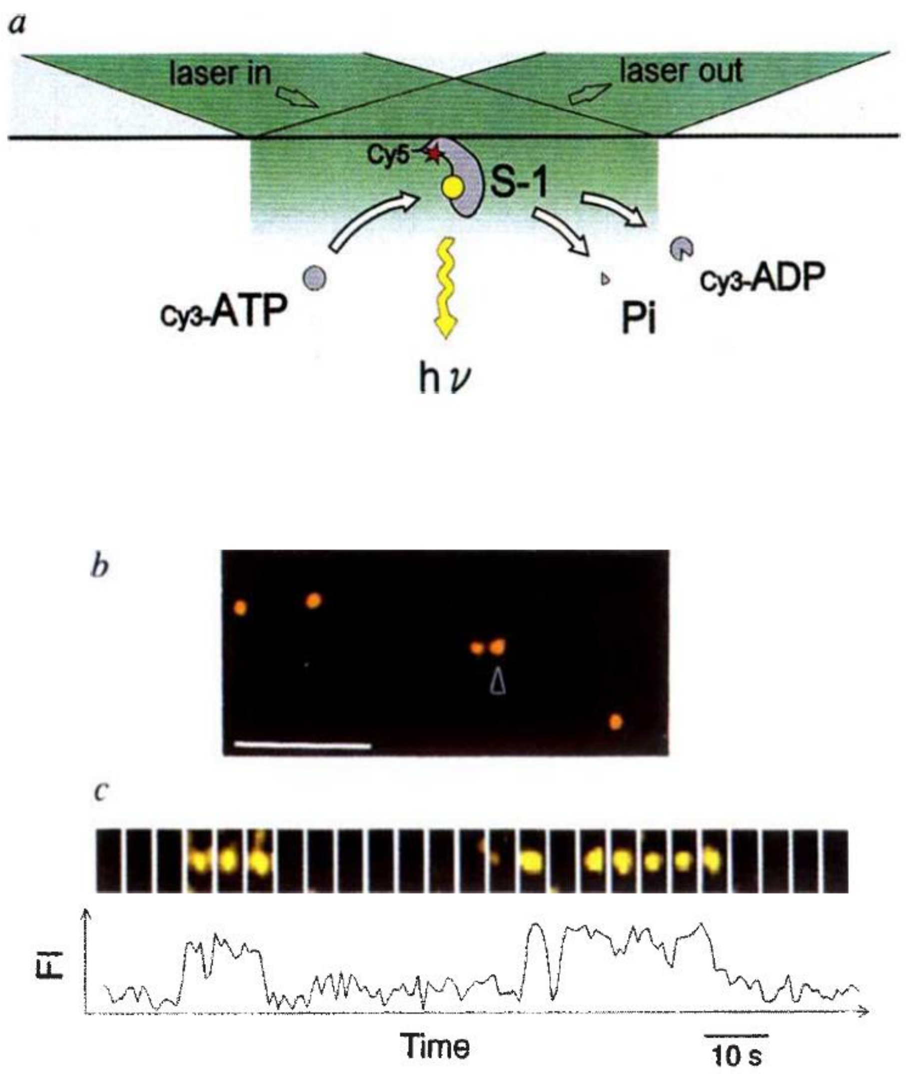
- Restricted excitation volume (close to surface): better signal to noise
- Gain in excitation light intensity
- No loss/gain in resolution

TIRF-M: single molecule imaging

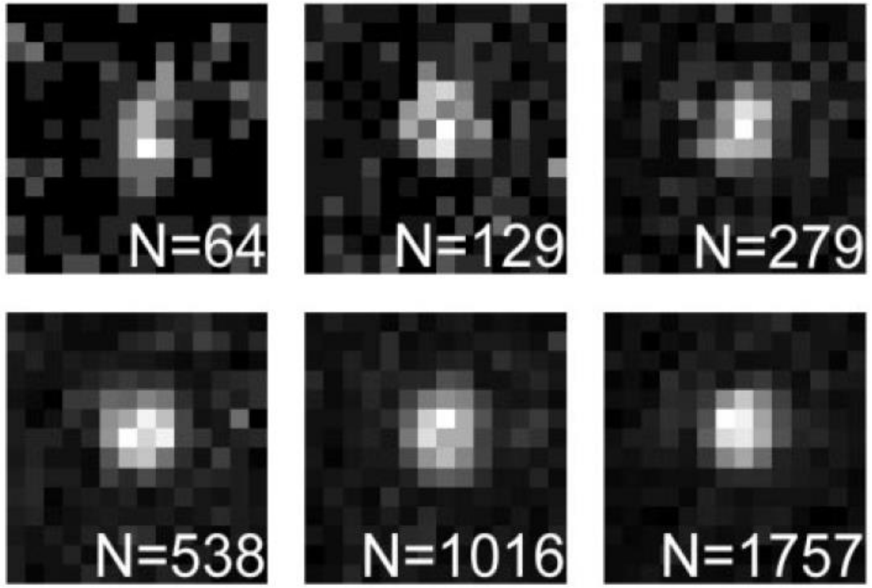
Imaging of single fluorescent molecules and individual ATP turnovers by single myosin molecules in aqueous solution

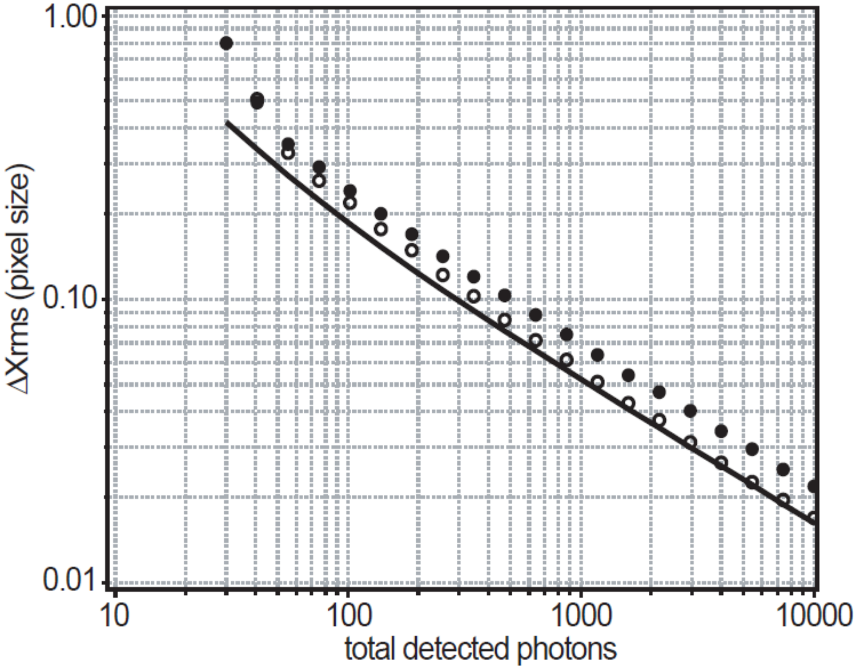
Takashi Funatsu[*], Yoshie Harada[*], Makio Tokunaga[*], Kiwamu Saito[*] & Toshio Yanagida[*†]

NATURE · VOL 374 · 6 APRIL 1995



Increased positional accuracy

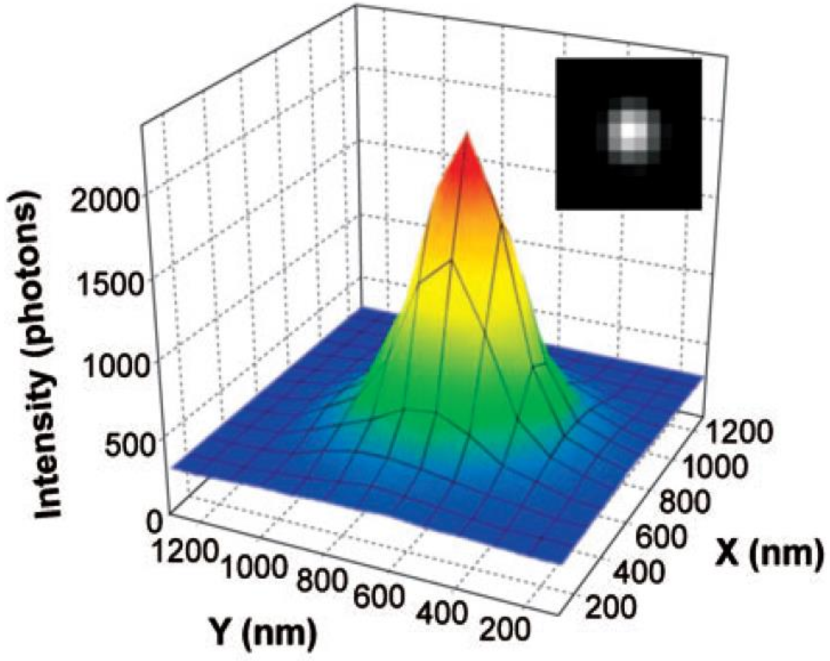




$\langle \Delta x^2 \rangle = s^2/N$

Thompson et al Biophys J 2002

Increased positional accuracy

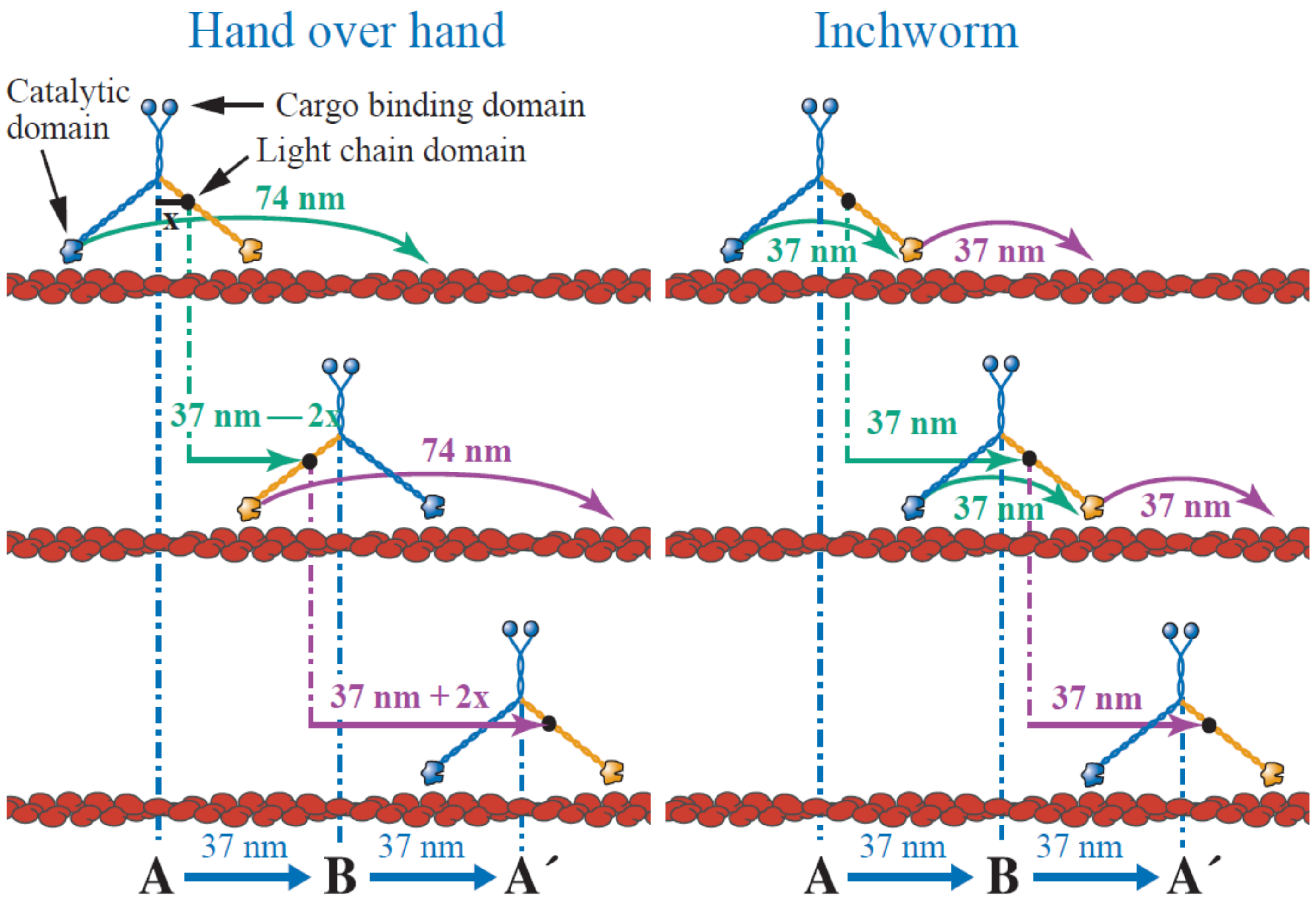


-Gauss fit of point-spread function

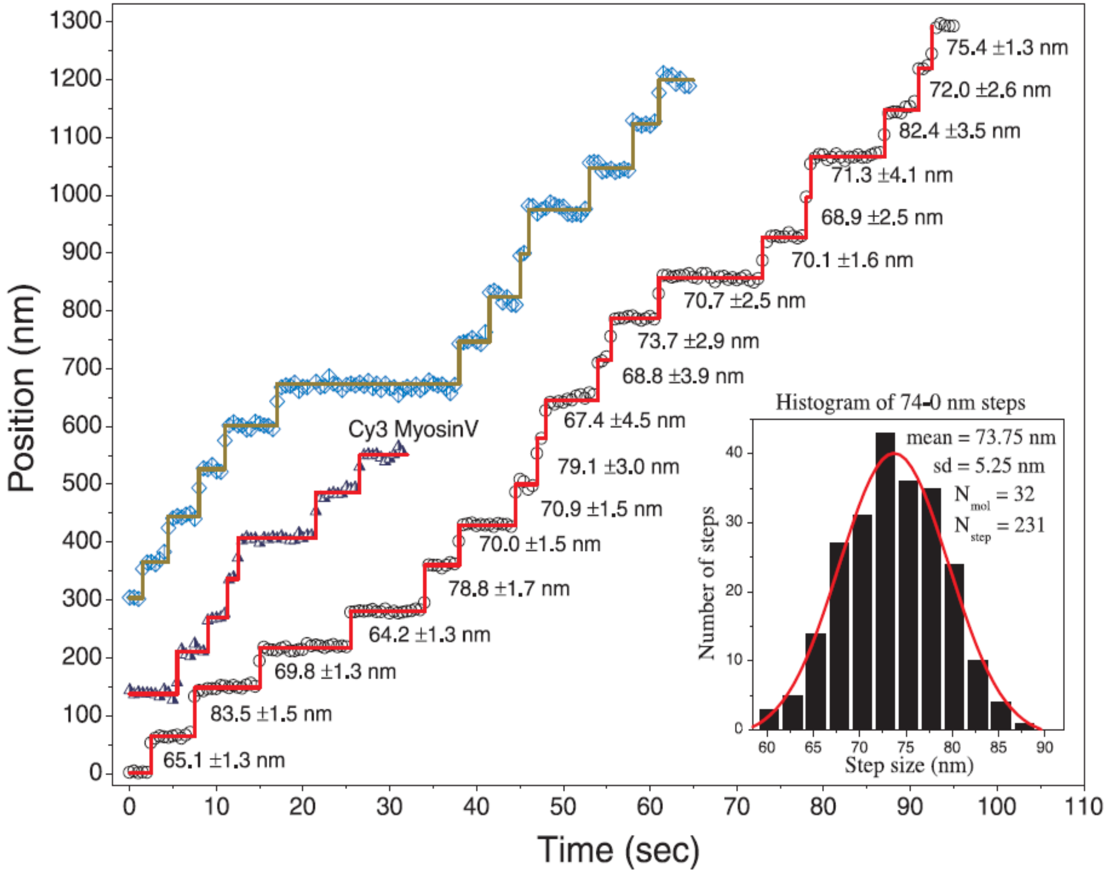
-Standard error of the Gaussian peak=positional accuracy

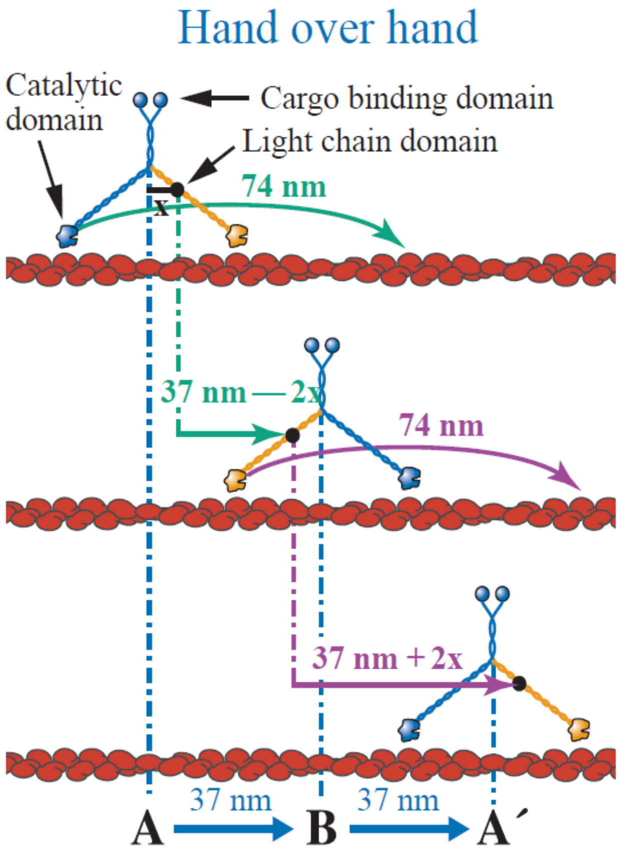
-1nm positional accuracy achieved

What is the step-size of the Myosin V motor



FIONA confirms hand over hand mechanism

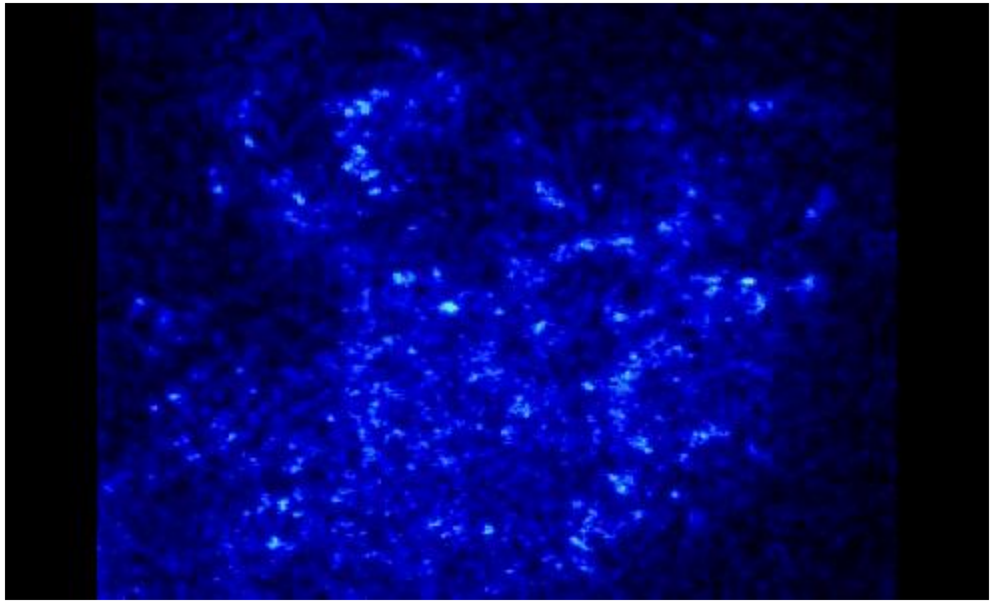




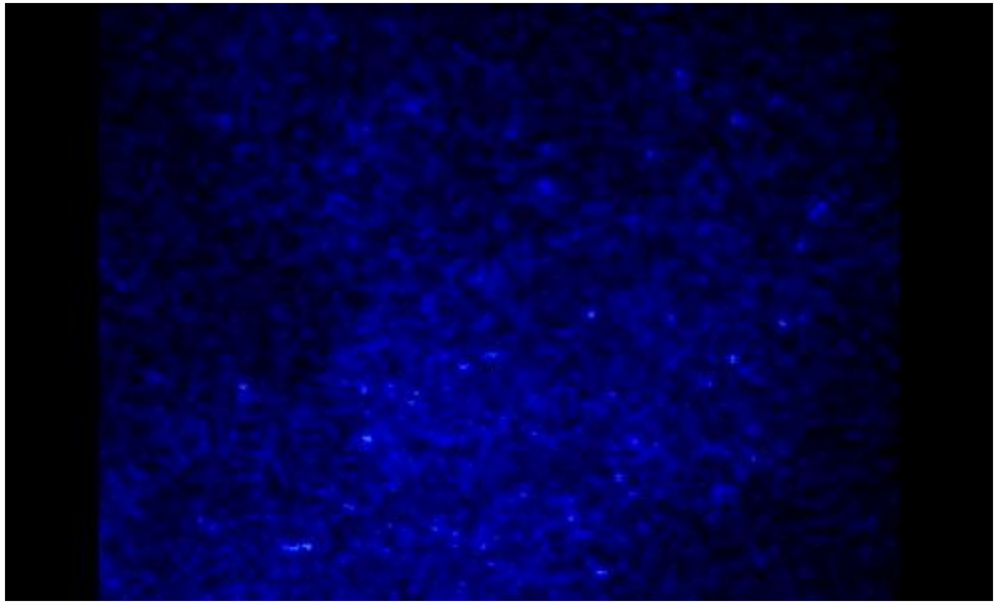
Yildiz et al Science 2003

TIRF-M in live cells

HA-GFP in live HUVECs (low density, 1000 molecules in the image)



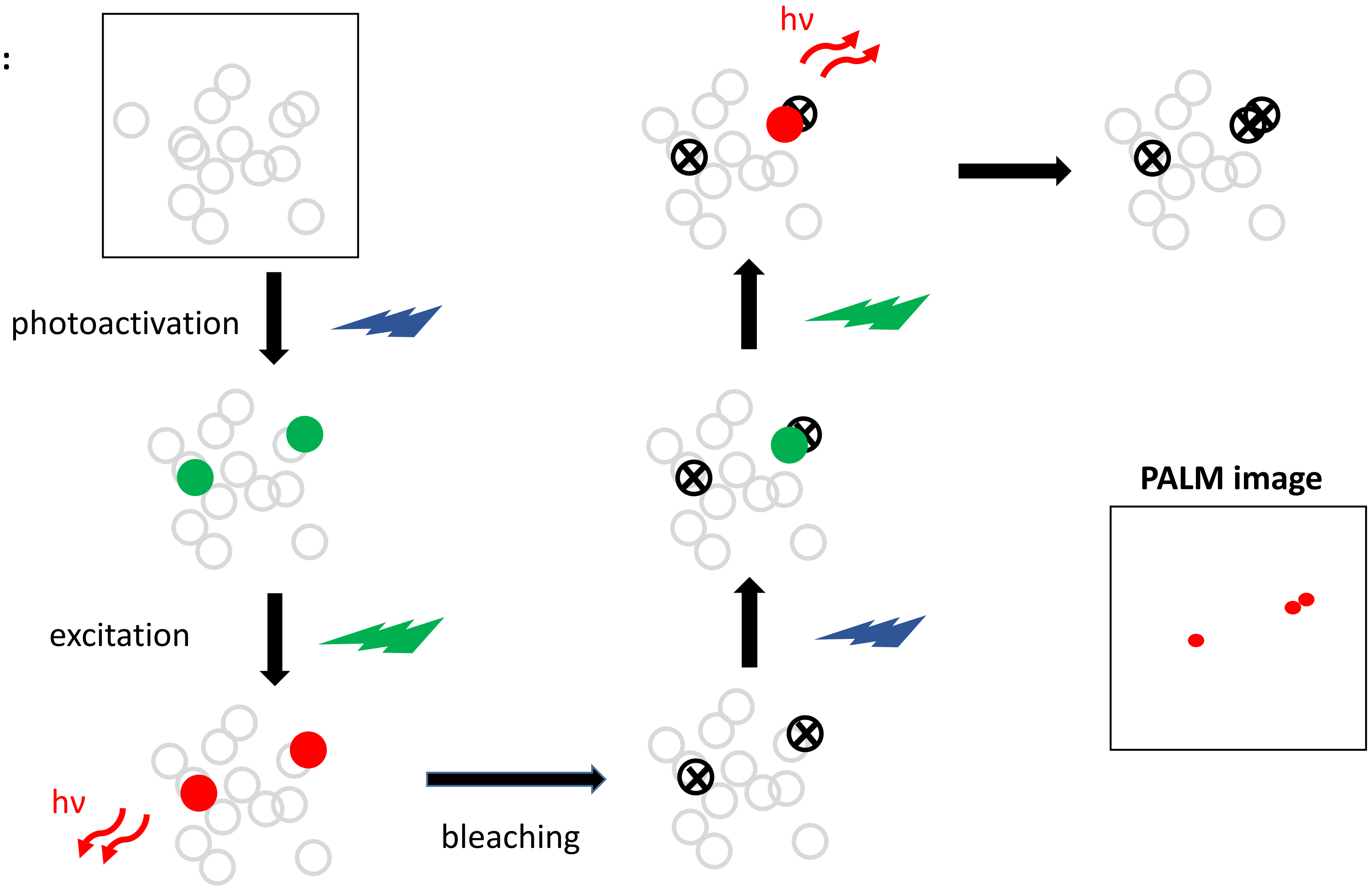
After a few seconds….



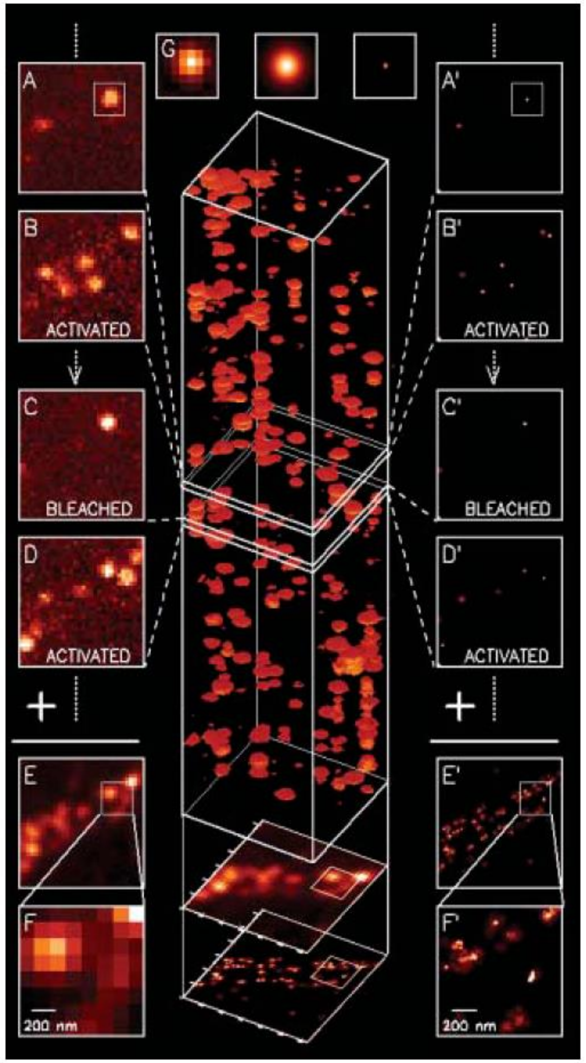
Eghiaian et al, unpublished

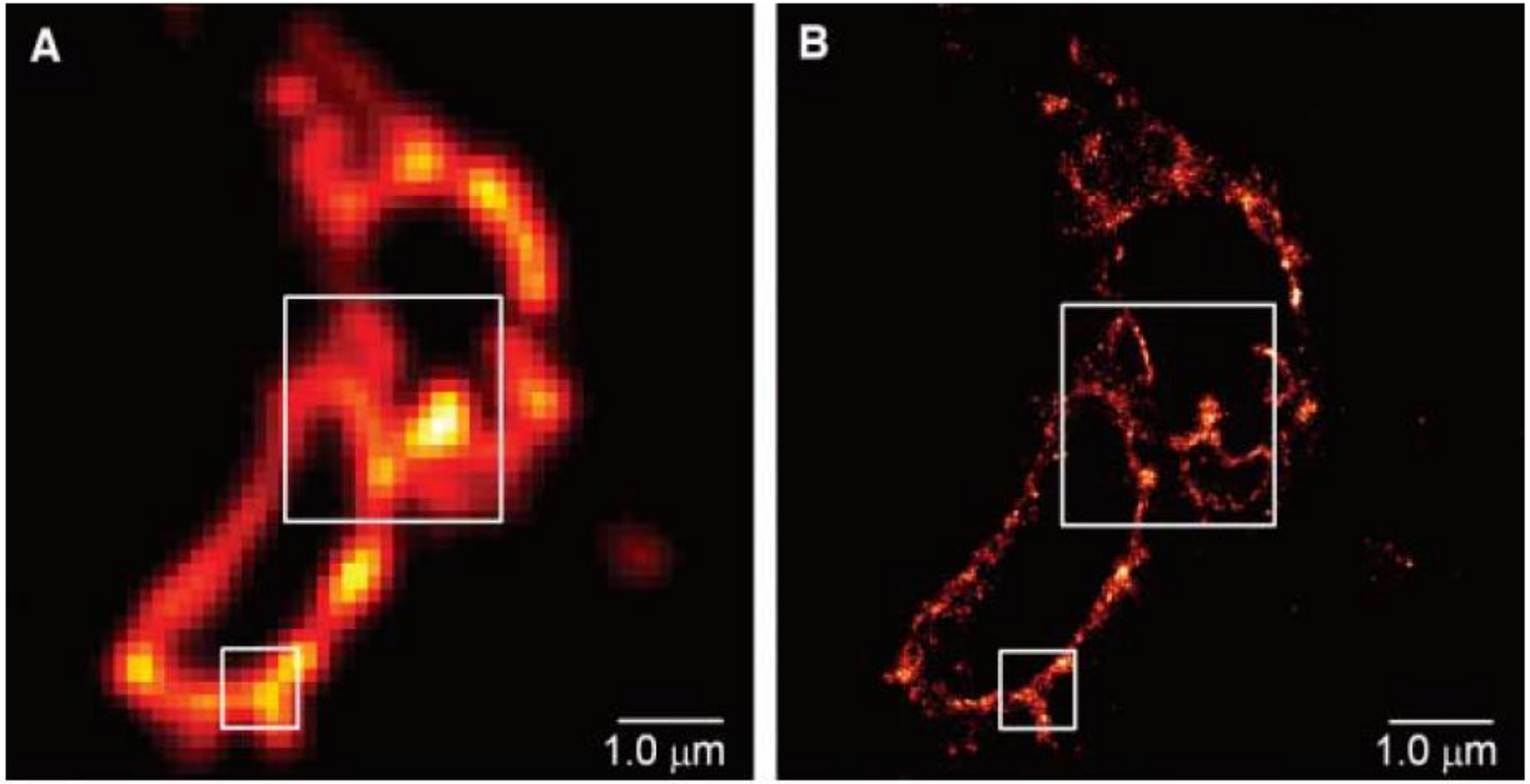
PALM: Photo-Activation Localization Microscopy

Photo-activable protein:
Eos (=GFP)
Dronpa
Kaede
…



PALM: Photo-Activation Localization Microscopy





Betzig et al, Science 2006

Pitfalls of optical microscopy

- **Requires a reporter fluorescent molecule**

  → technical limitation (chemical labelling, molecular biology)

  → potential changes in functionality (misfolding, steric hindrance, loss of a functional site)

- **Limited lateral (10nm) resolution, axial (20nm) and positional accuracy (1nm)**

  → Co-localization ≠ direct interaction

- **One can only see what is labelled**

  → Maximum number of simultaneously detected fluorophores is drastically limited

- **Radiation damage**

  → Fast photobleaching of the fluorophores

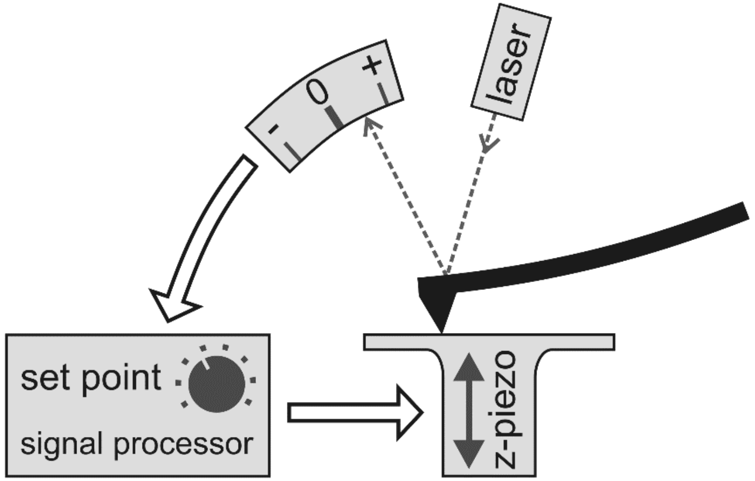
  → High power illumination leads to toxicity

ATOMIC FORCE MICROSCOPY

→ “…The only method that can self-sufficiently perform nanometer-resolution imaging AND nano-manipulation of the sample with pN force resolution (…) in liquid and at room temperature…”

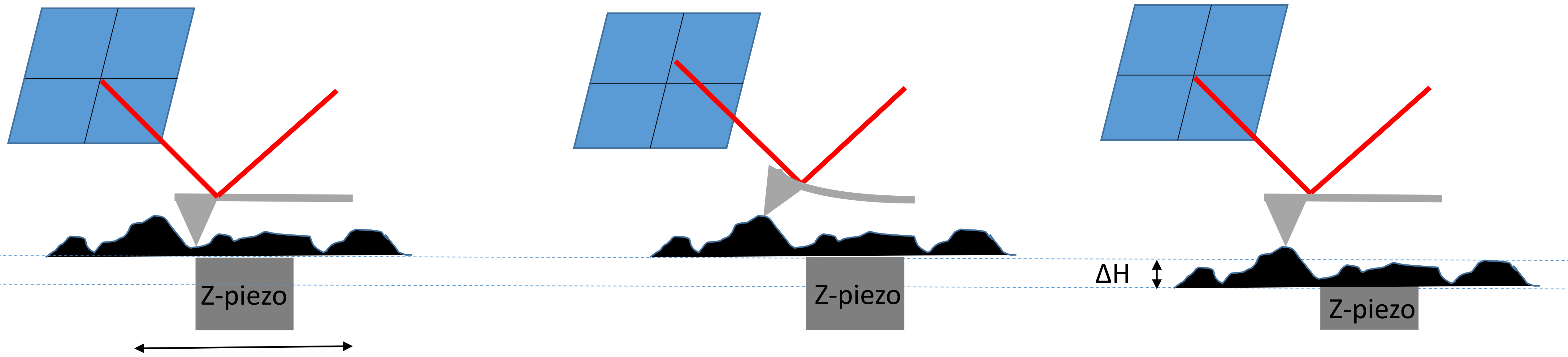
(Eghiaian et al, FEBS Letters review article, to be published)

AFM basics



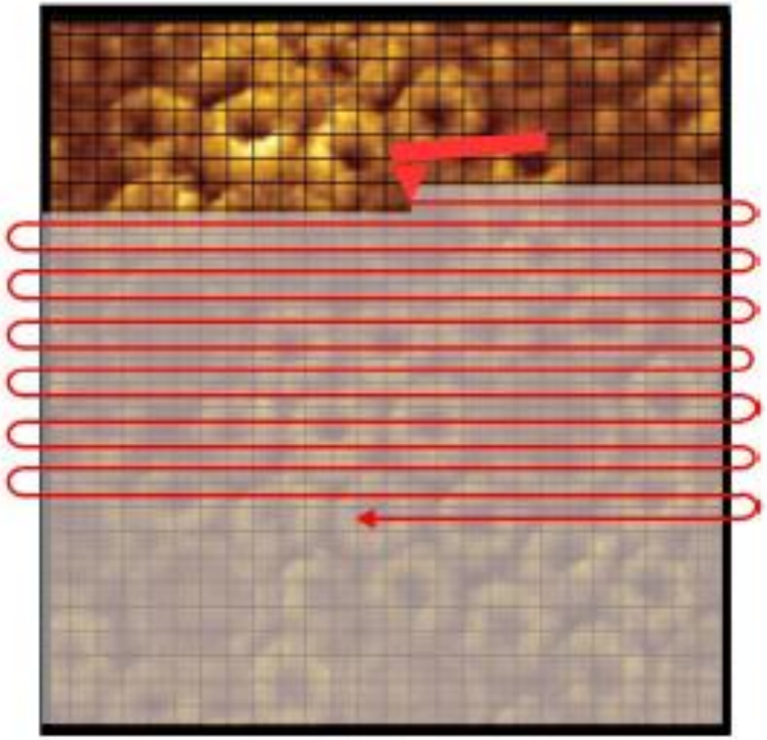
Eghiaian & Schaap, Methods Mol Biol 2011

Basic AFM: contact mode

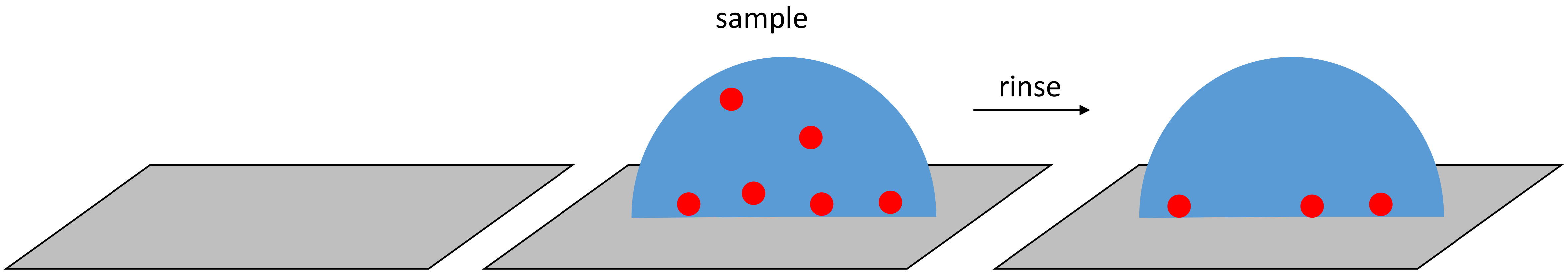


Lever deflection: error signal

Piezo retraction: Height measurement



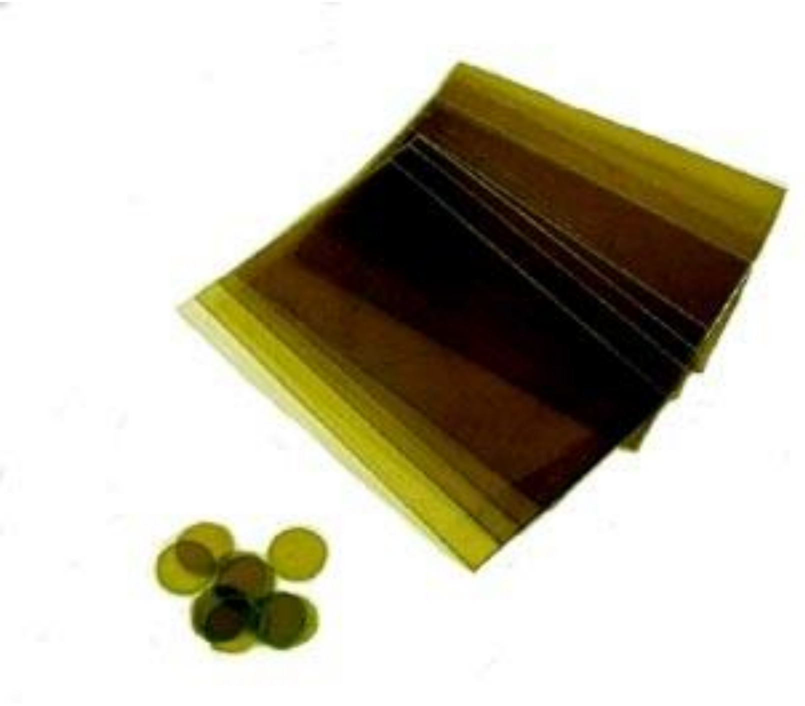
Sample preparation: surface adsorption of a sample



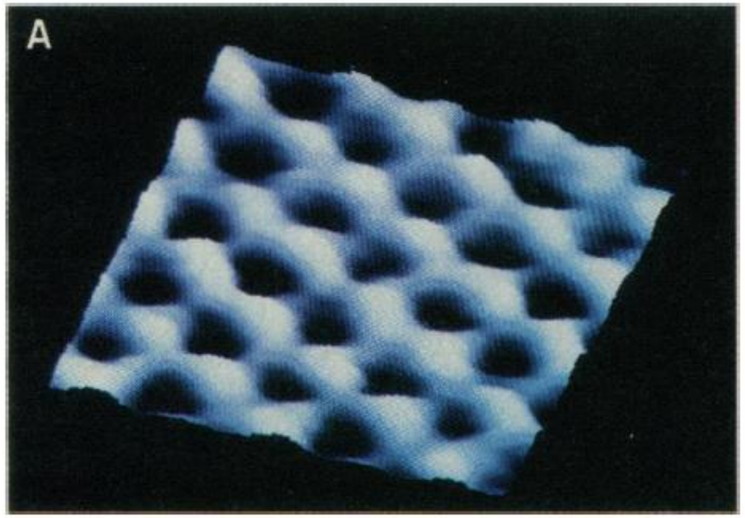
substrate

Unspecific adsorption
-Divalent cations help
-Surface functionalization (silanes, polylysine, patterning, covalent immobilization)
-planar bilayers (membrane proteins, 2D crystals…)

Sample preparation: mica

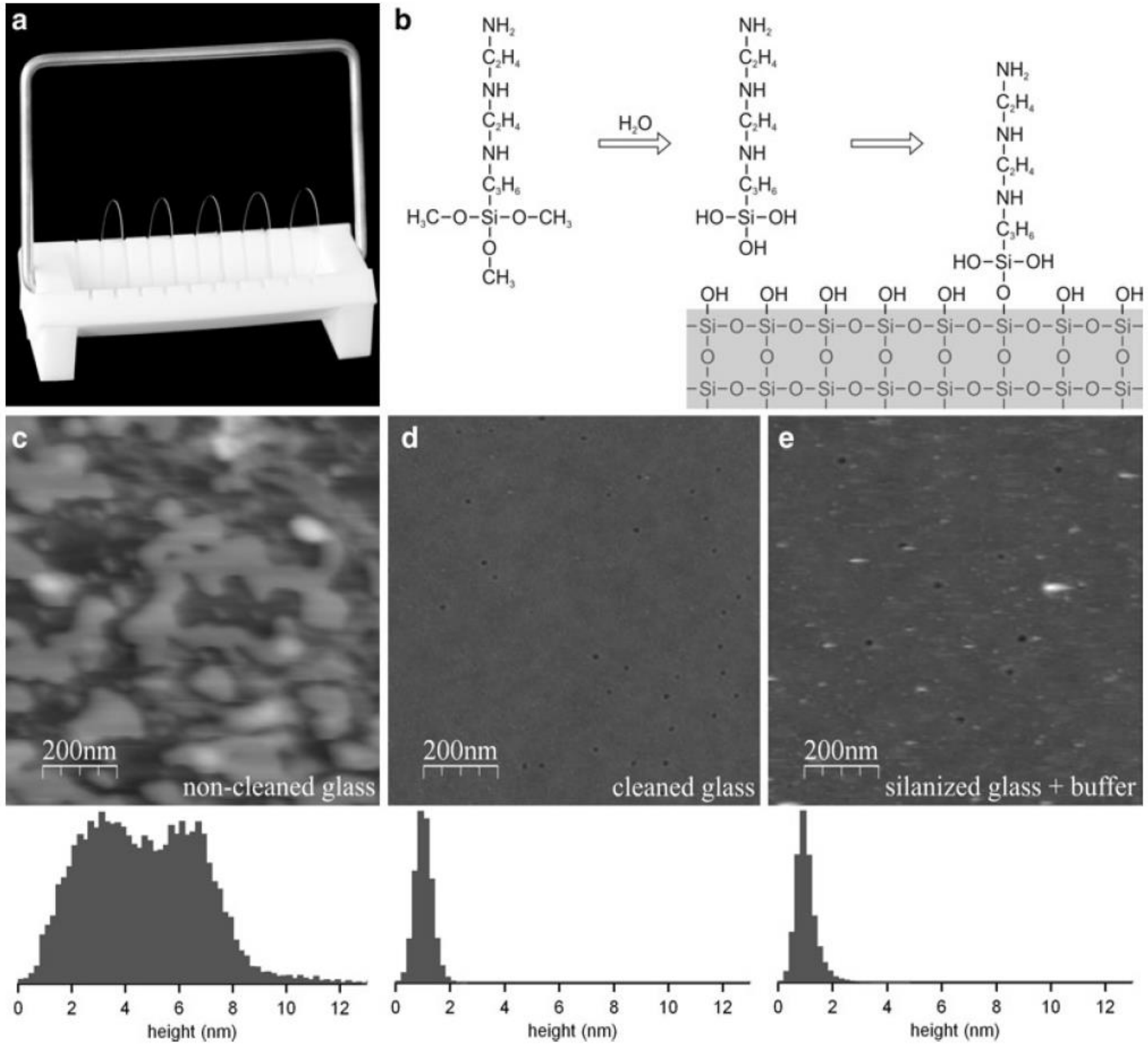


26x26 Å



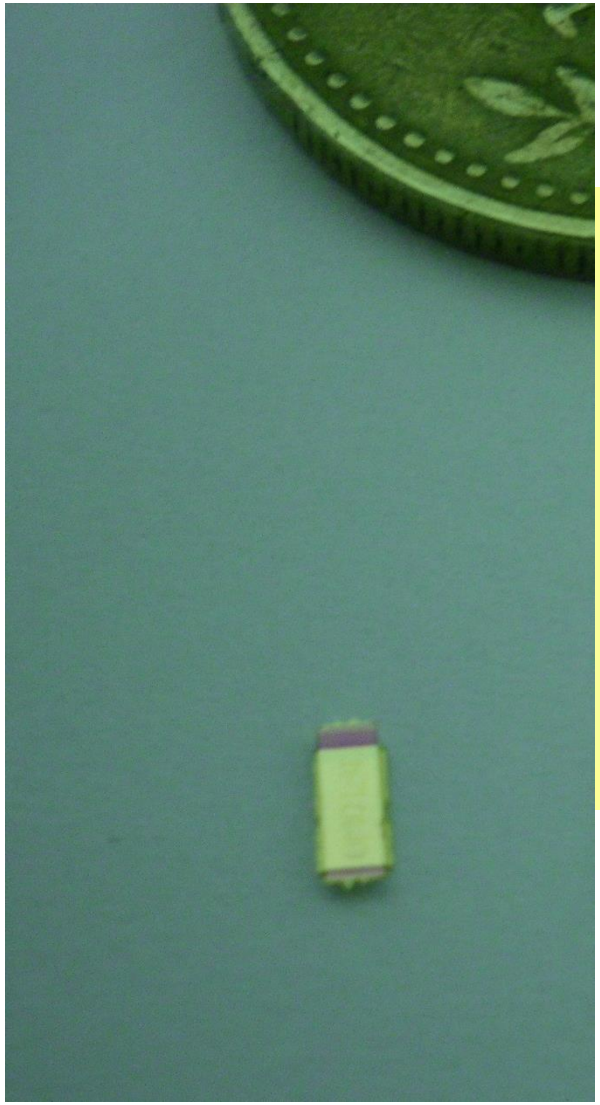
Drake et al, Science 1989

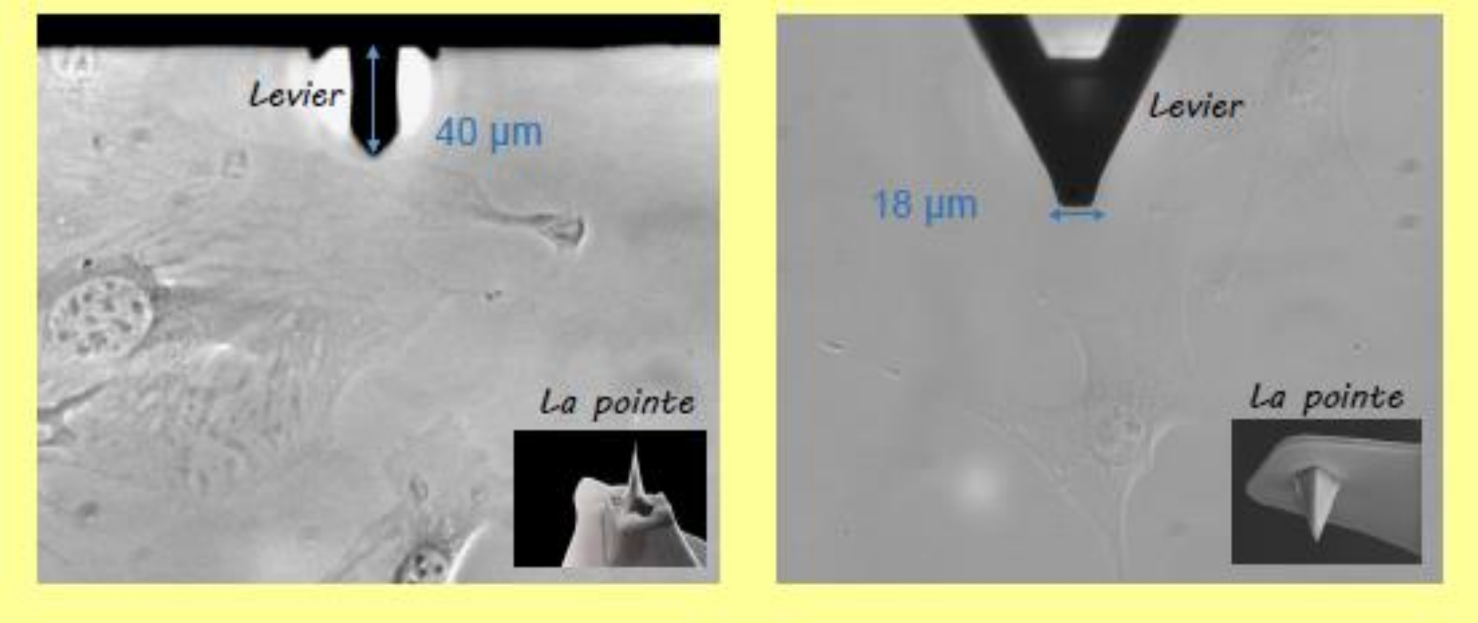
Sample preparation: glass substrate



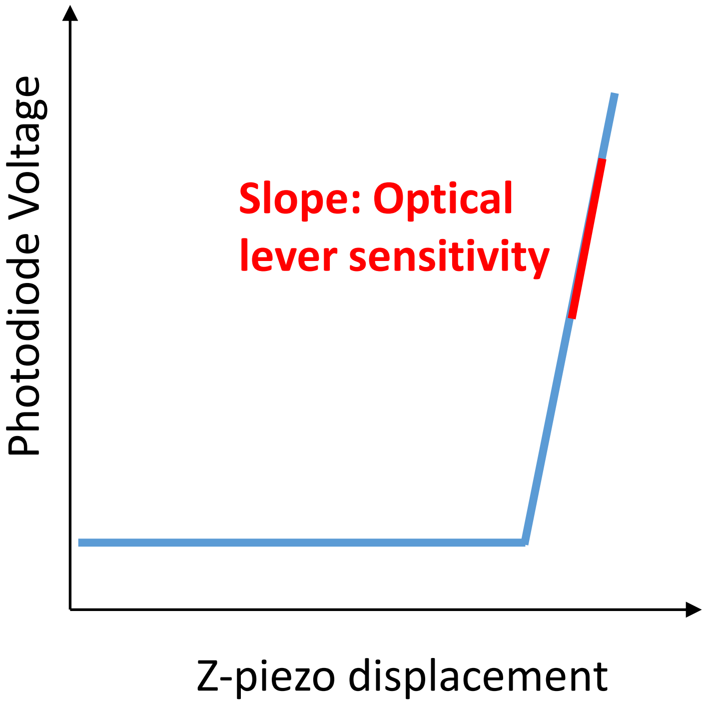
Eghiaian & Schaap, Methods Mol Biol 2011

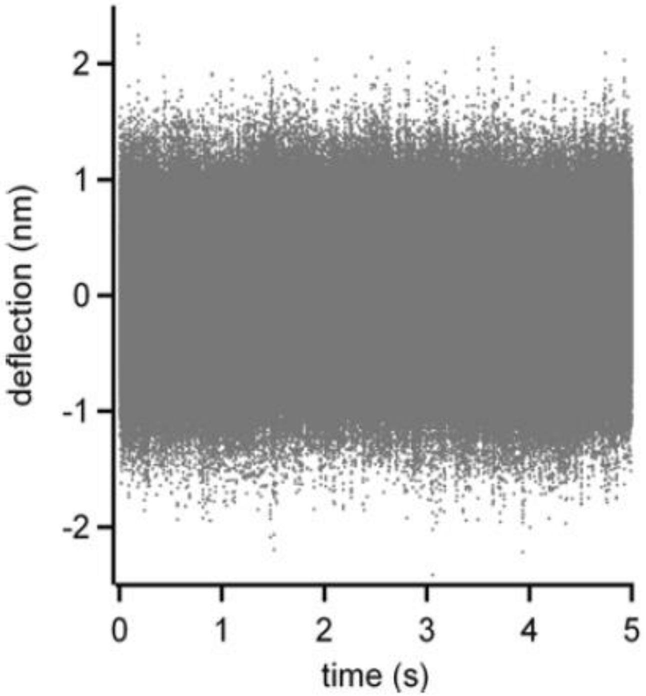
Cantilevers

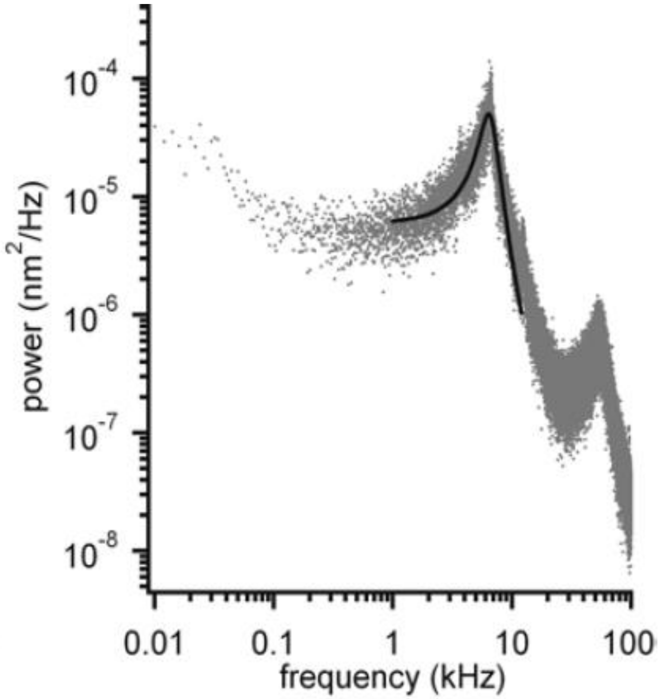




Cantilever calibration





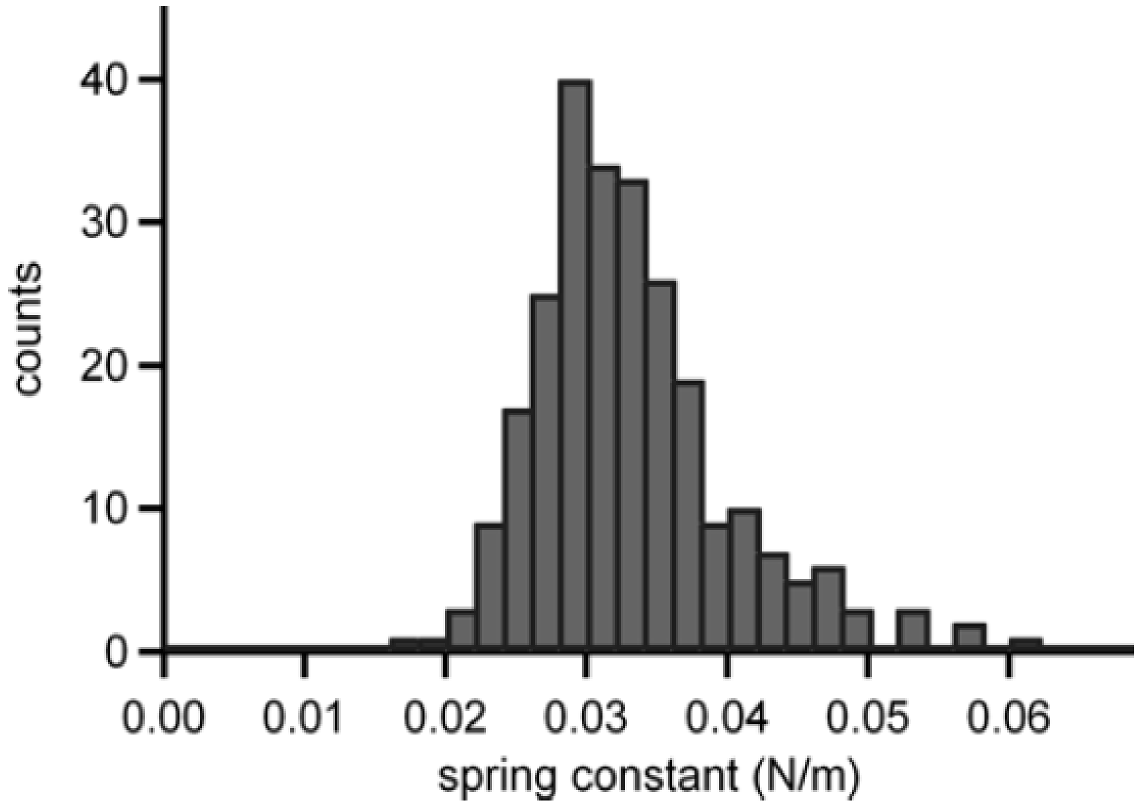


$$S_x(f) = \frac{A}{f} + B + \frac{S_0 f_0^{\,4}}{Q^2 (f_0^{\,2} - f^2)^2 + (f_0 f)^2}$$

$$k = \frac{2 K_B T Q}{\pi S_0 f_0}$$

Eghiaian & Schaap, Methods Mol Biol 2011
Burnham et al, Rev Sci Instr, 2003

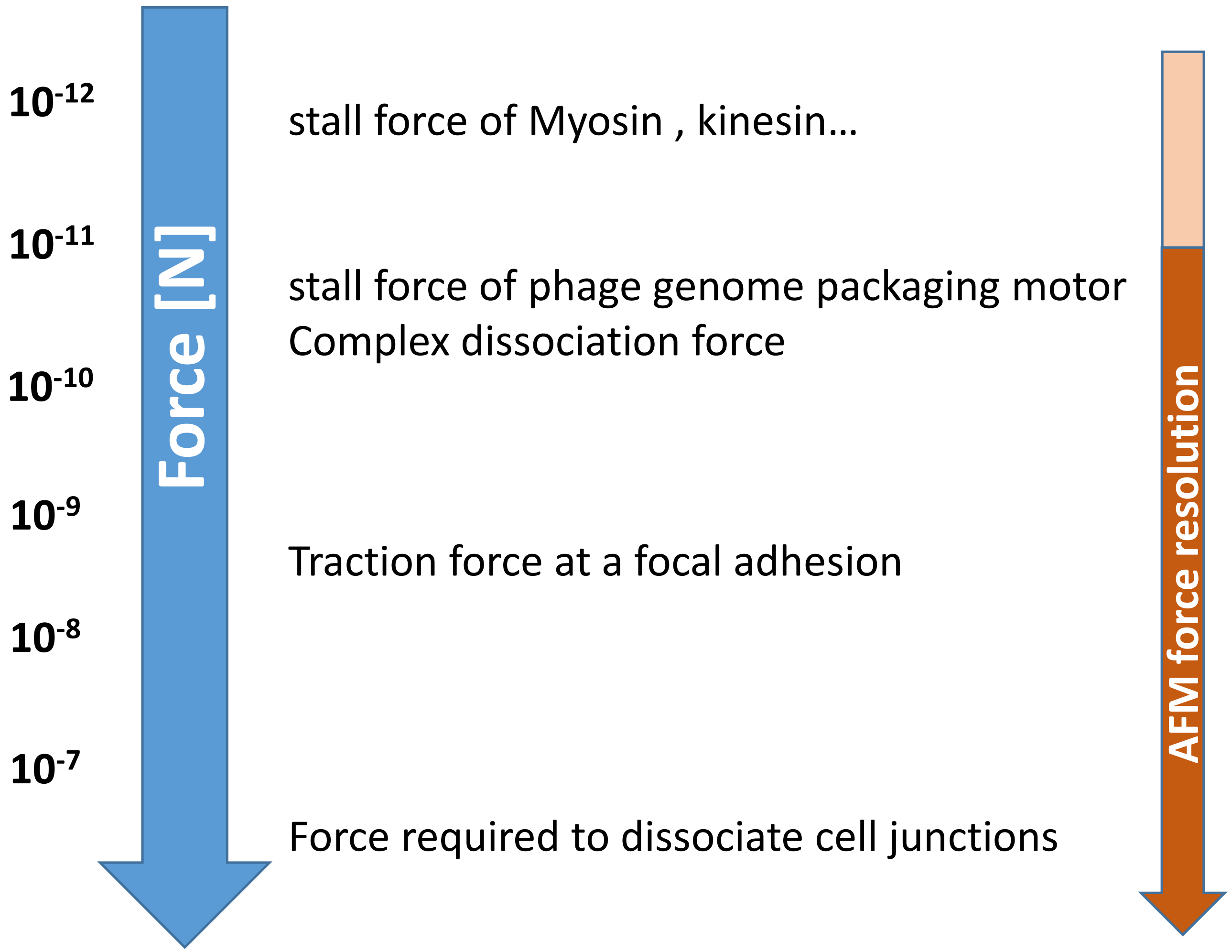
Cantilever calibration



Nominal: 30 pN/nm

Eghiaian & Schaap, Methods Mol Biol 2011

Forces in AFM-Biology

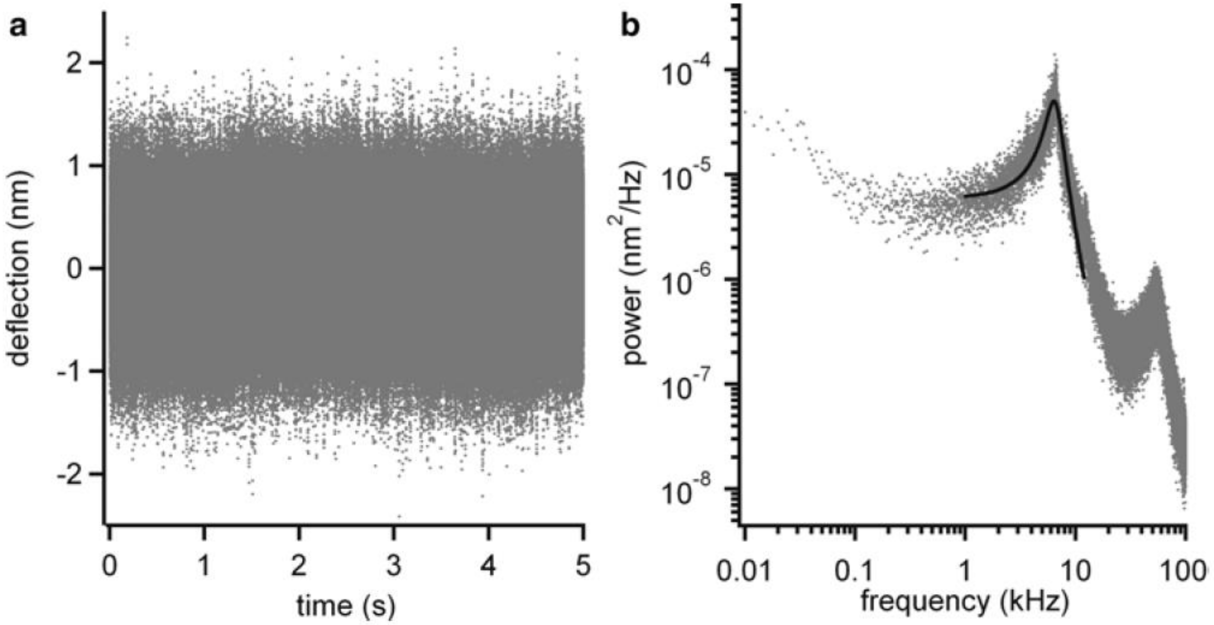


Cantilever theory: force noise

Deflection of a cantilever in liquid

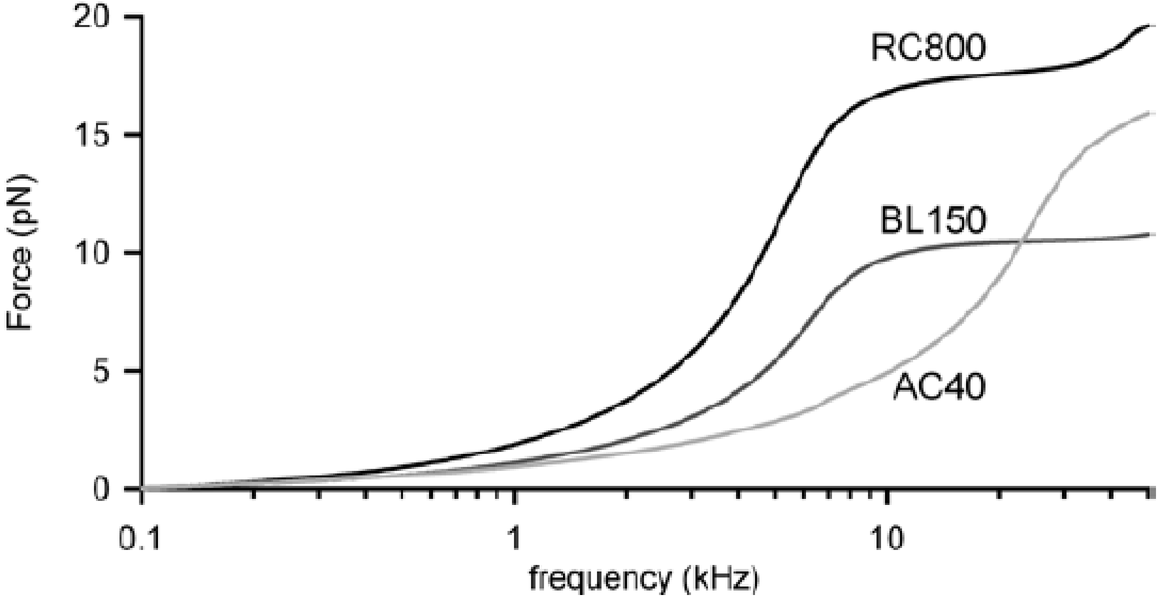
Time-domain

Power Spectrum (PSD)



Force noise of cantilevers in liquid

Integrated PSD * Spring constant



$$\Delta F = \sqrt{4k_B T \Delta f \beta}$$

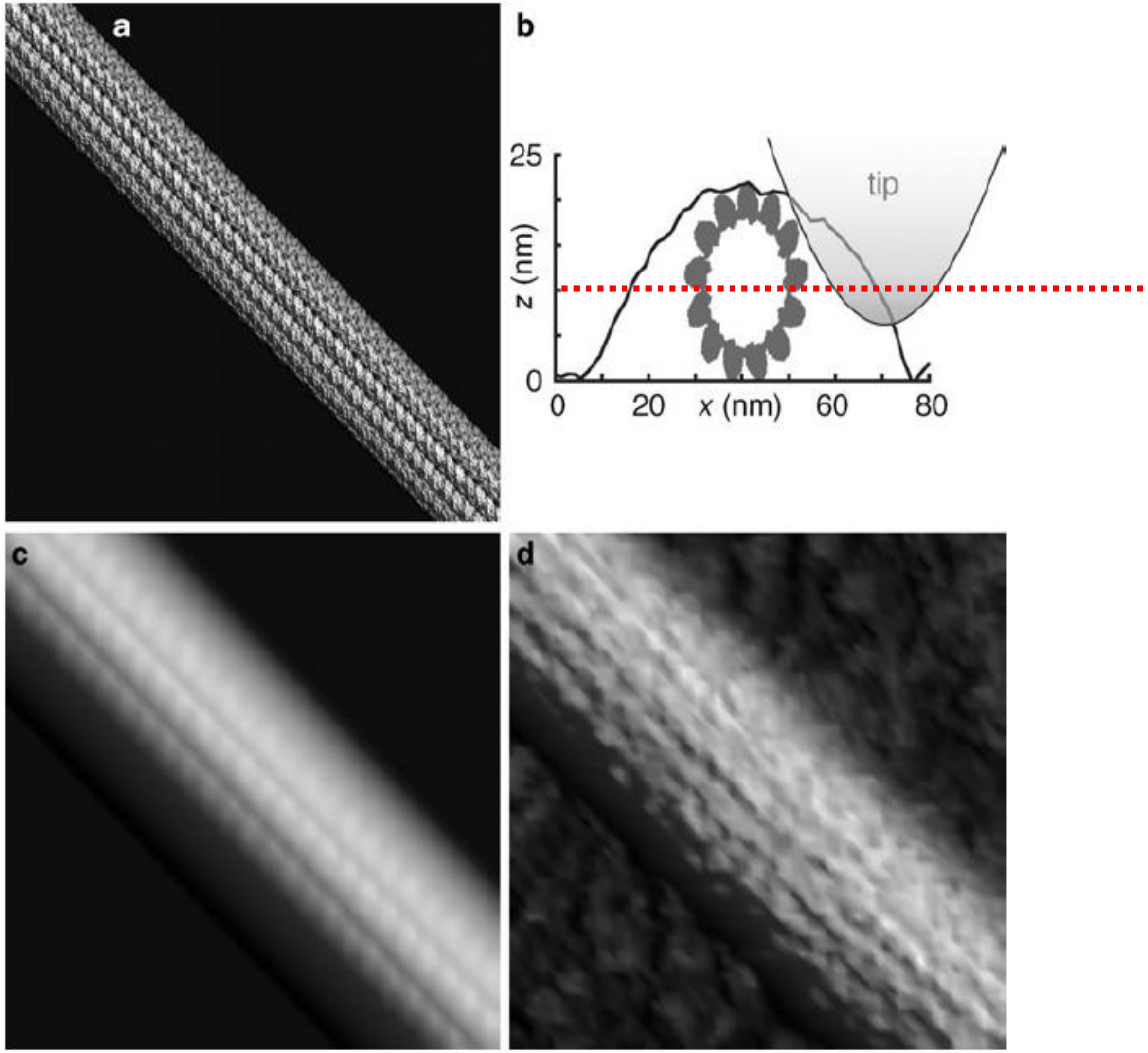
Force limit

Δf : Bandwidth of experiment

β : drag

Eghiaian & Schaap, Methods Mol Biol, 2011

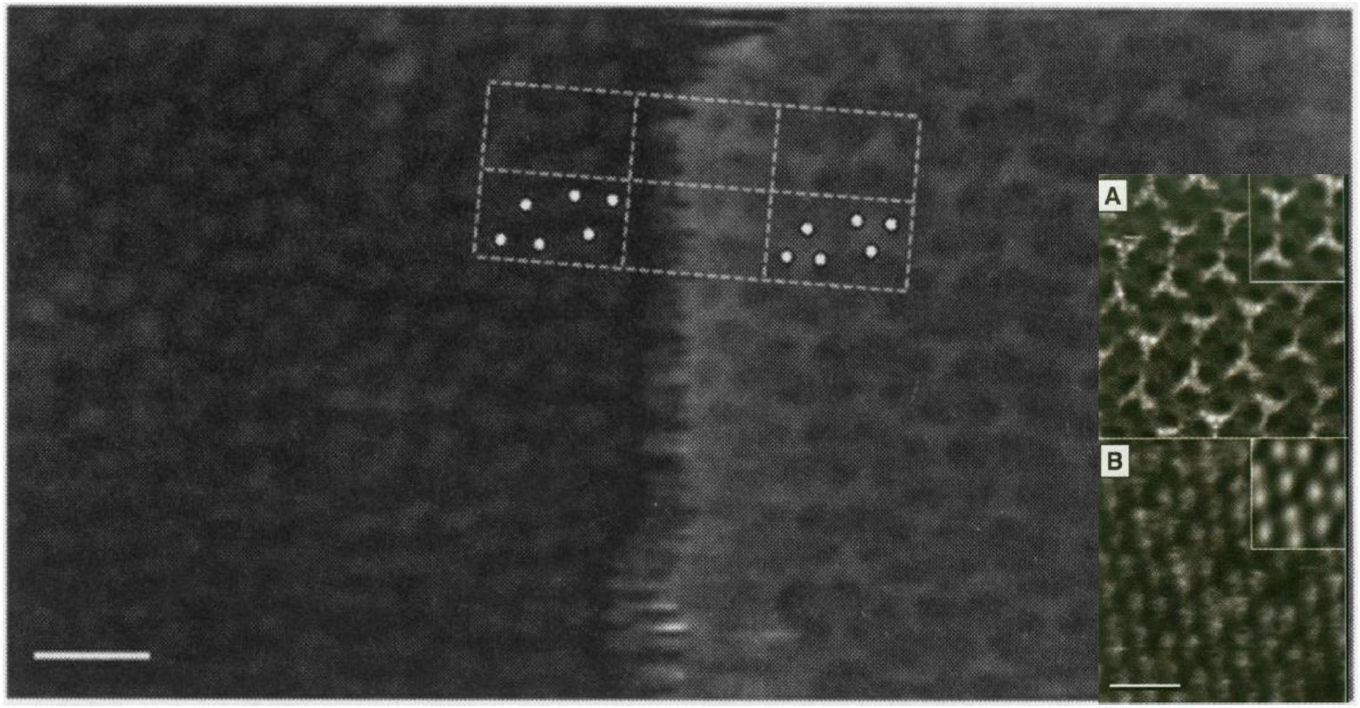
Resolution limit: tip sample dilation



Top half information is more relevant

Eghiaian & Schaap, Methods Mol Biol 2011

Membrane protein imaging by AFM

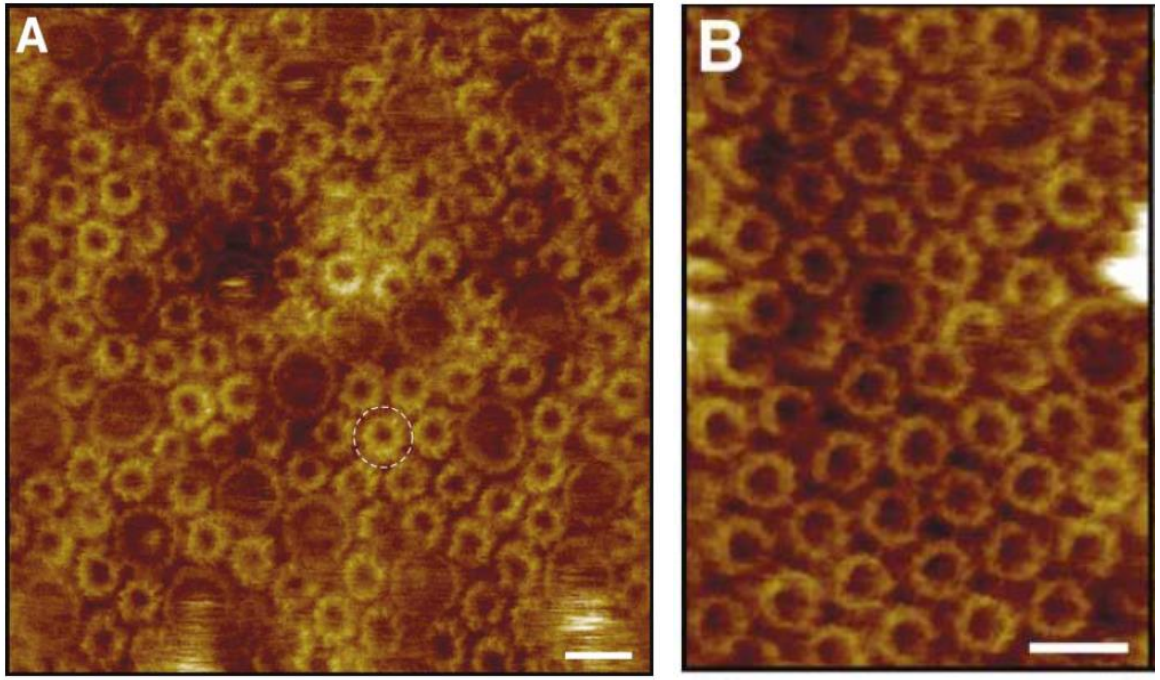


Schabert et al, Science 1994

Membrane protein imaging by AFM

High light conditions

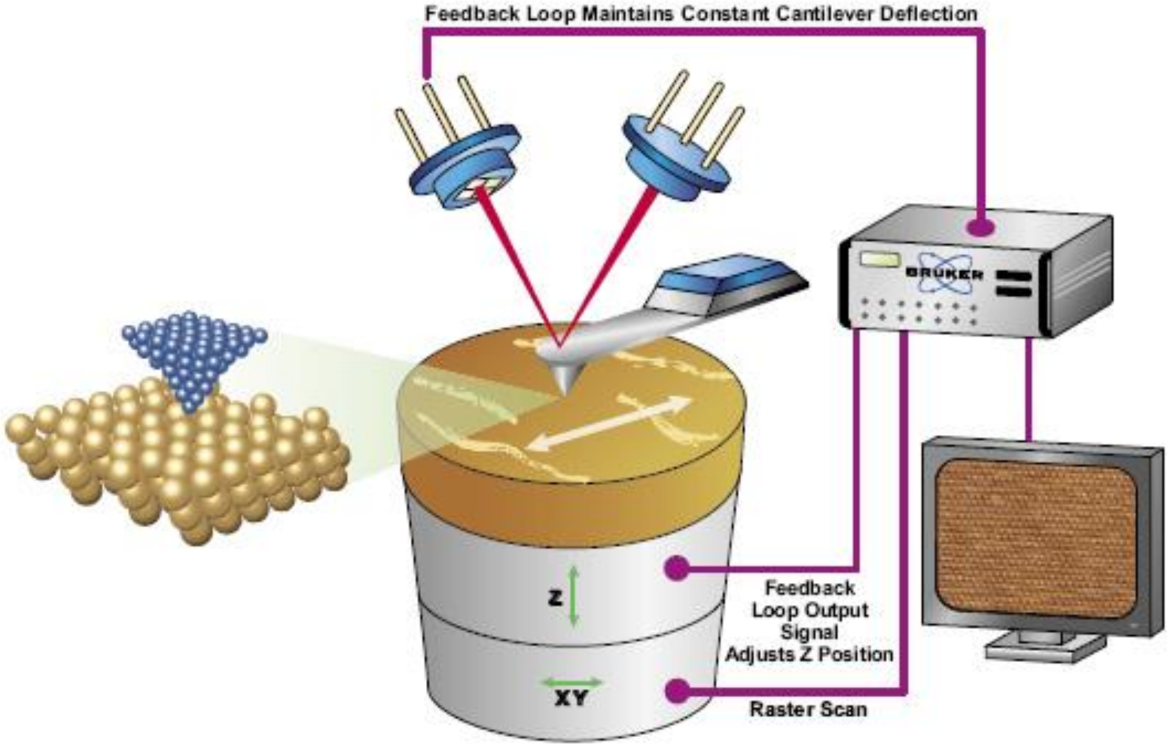
Low light conditions



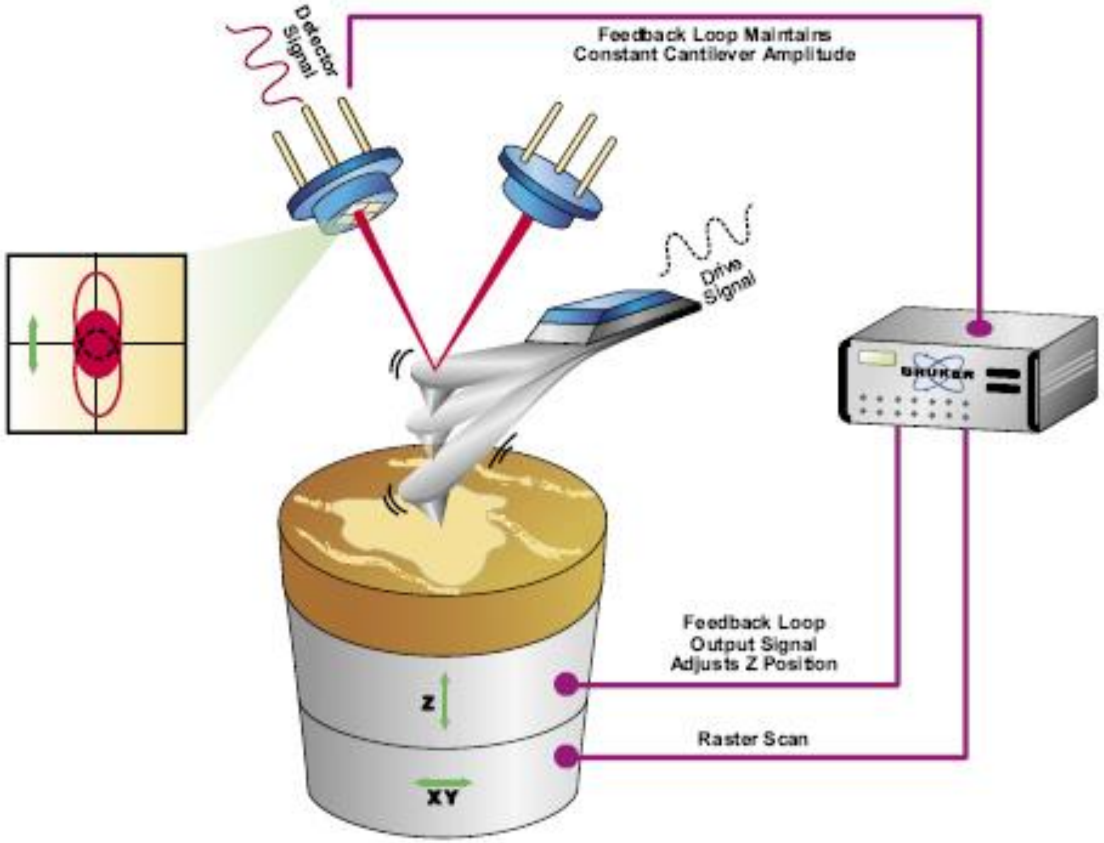
Scheuring et al, Science 2005

Intermittent contact mode

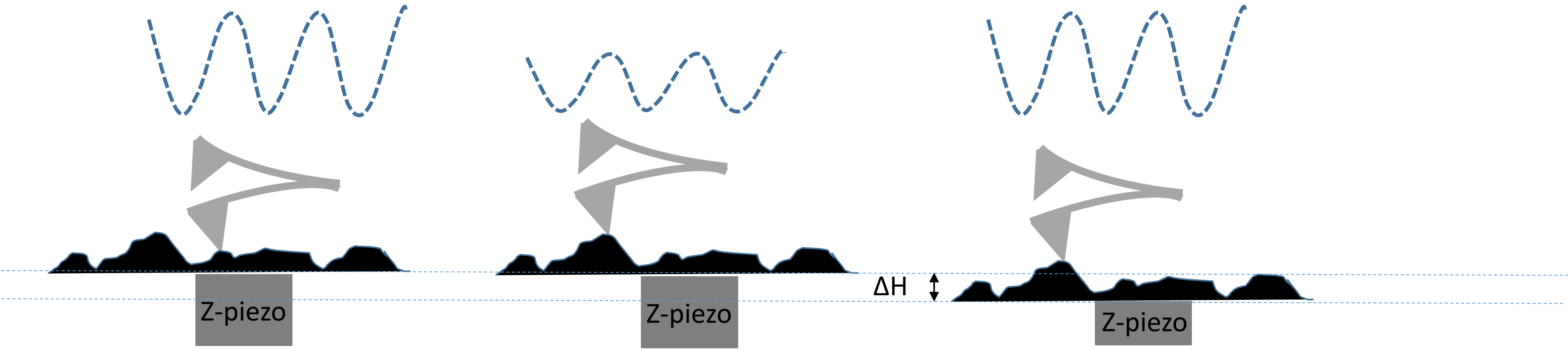
Contact mode (feedback on deflection)



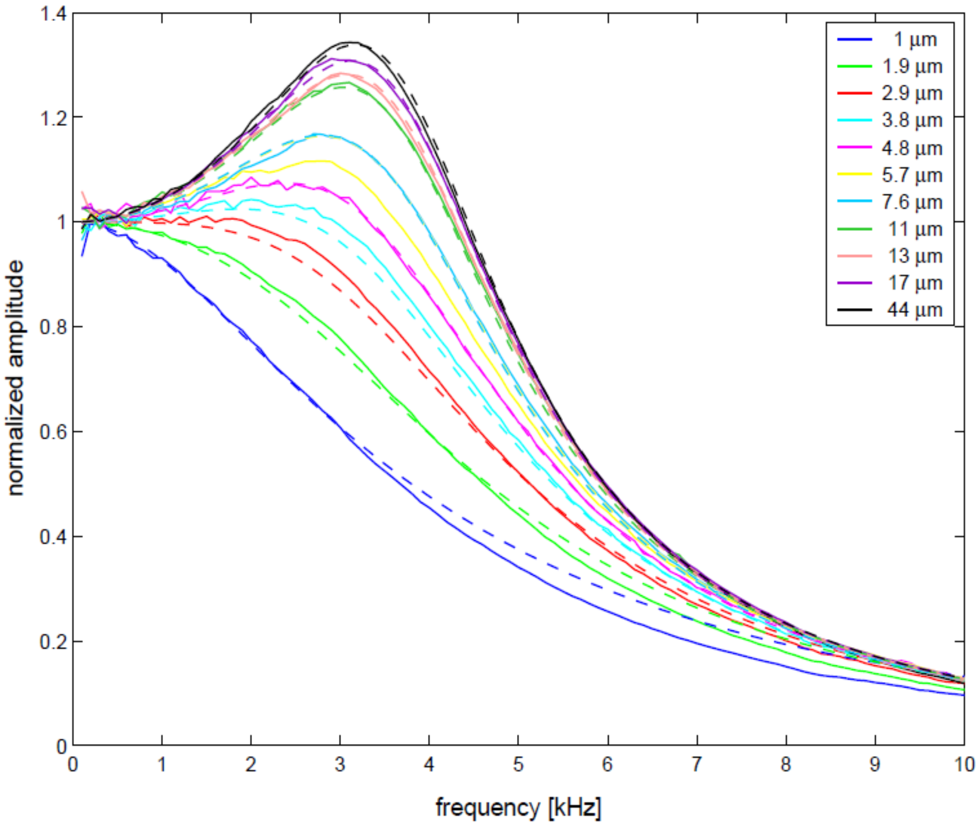
Tapping mode (feedback on amplitude)



Intermittent contact mode



Non-contact mode



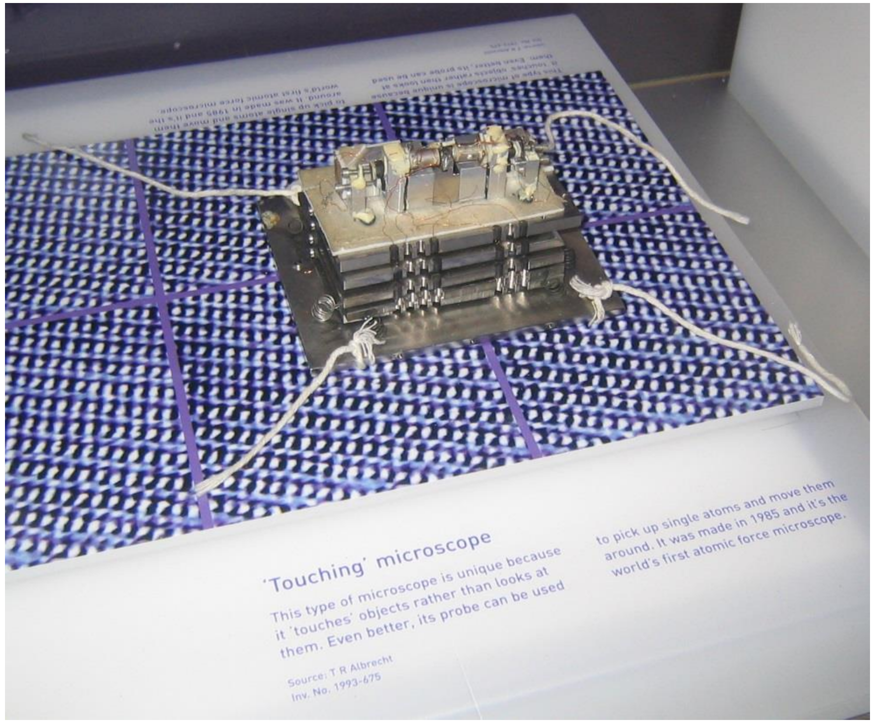
Rankl et al, Ultramicroscopy 2004

Feedback on frequency ???

frequency modulation AFM !
(non-contact mode)

Evolution of the atomic force microscope

1985



AFM prototype !

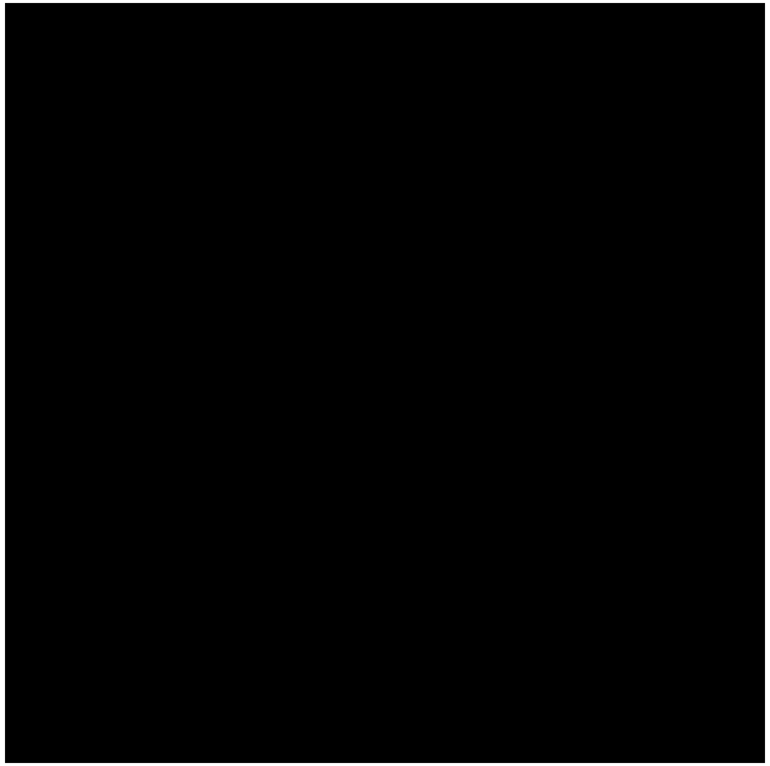
1995

Veeco Nanoscope I (now Bruker)

2005

JPK Nanowizard

Speed limit…



To break the speed limit…

- Increase scanner speed
- Increase cantilever resonance frequency (make smaller)
- Make feedback loop faster

Cantilever theory

Spring constant

$$k = E.t^3.w/4\,L^3$$

E: Young’s modulus
T: thickness
W: width
L: length

Resonance frequency

$$f_0 = \sqrt{(k}/m\Big)$$

m: cantilever mass

THE Breakthrough: High-speed AFM

2010

Pr. Toshio Ando

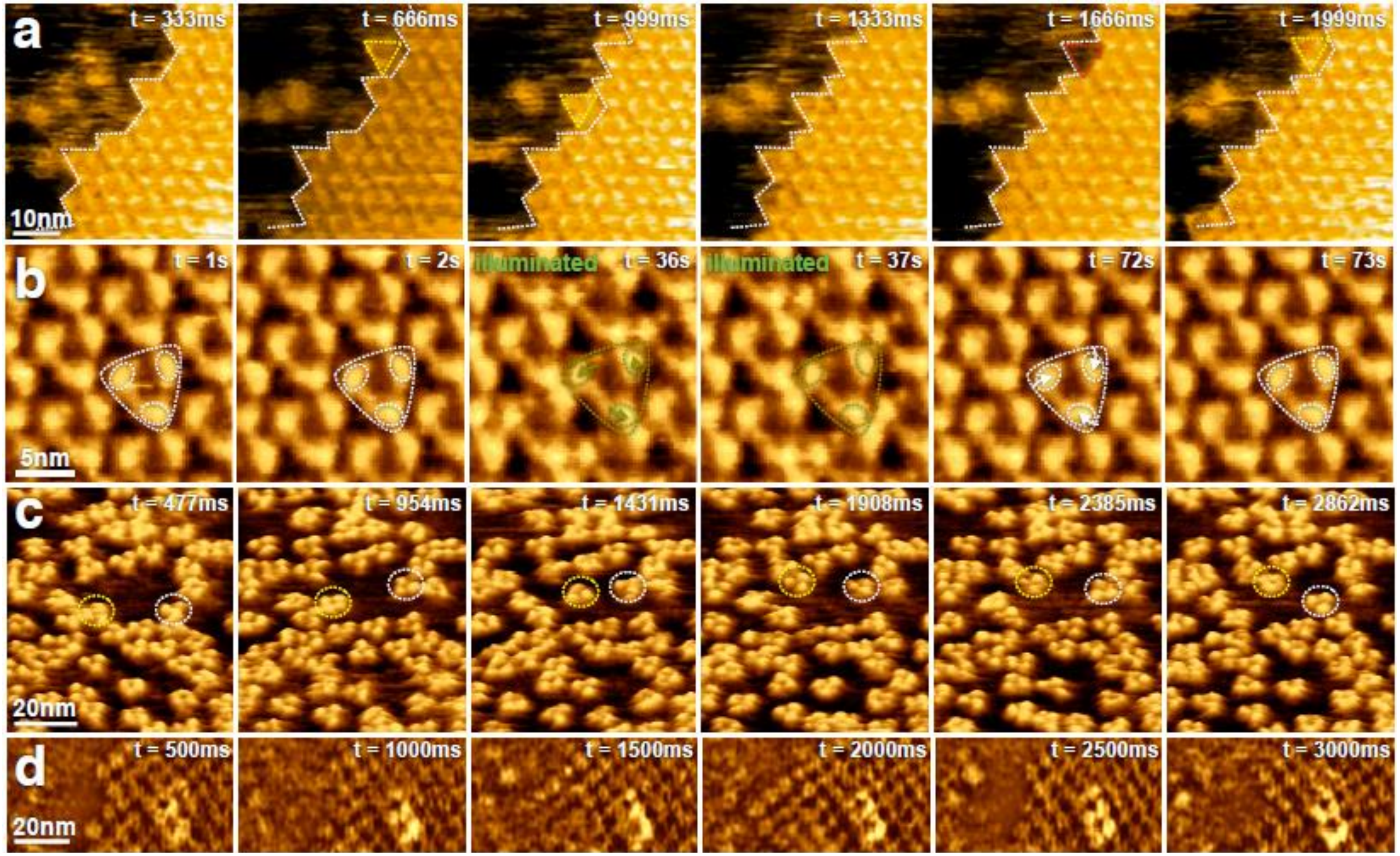
Dynamic imaging of biological membranes by HS-AFM

Purple membrane 2D crystal formation

Bacteriorhodopsin light response

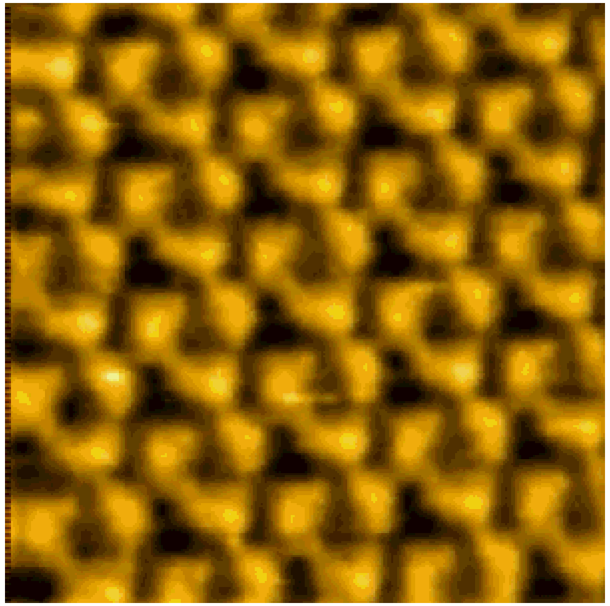
Omp-F protein-protein interactions in native membranes

AQP0 assemblies



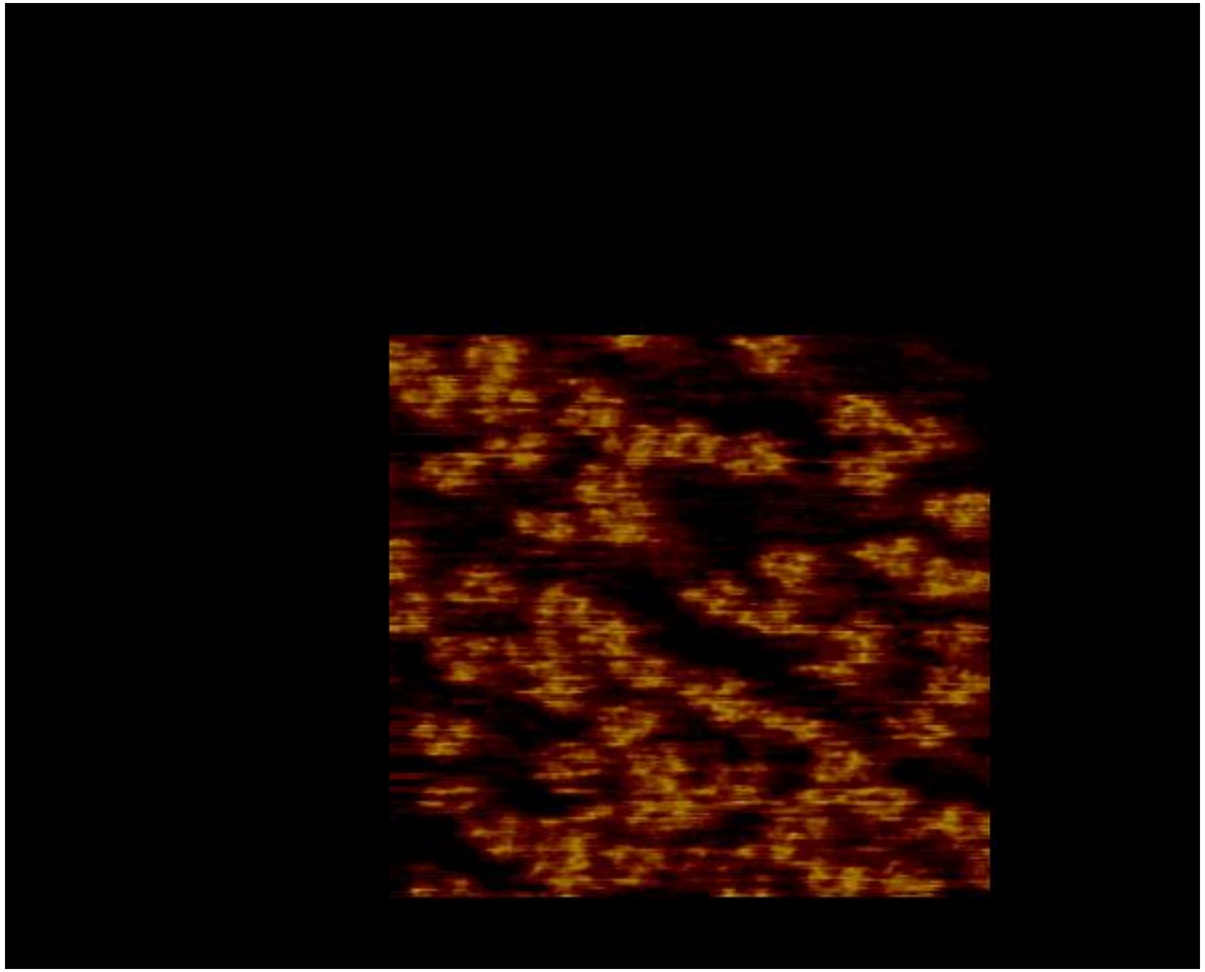
Eghiaian et al, FEBS Letters, in press

Dynamic imaging of biological membranes by HS-AFM



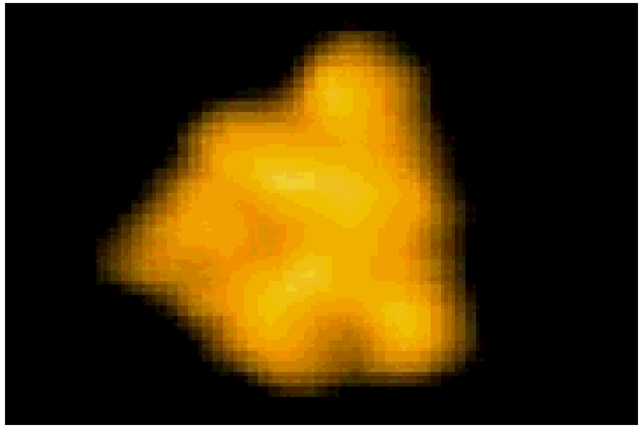
Shibata et al, Nature Nanotechnology 2010

Dynamic imaging of biological membranes by HS-AFM



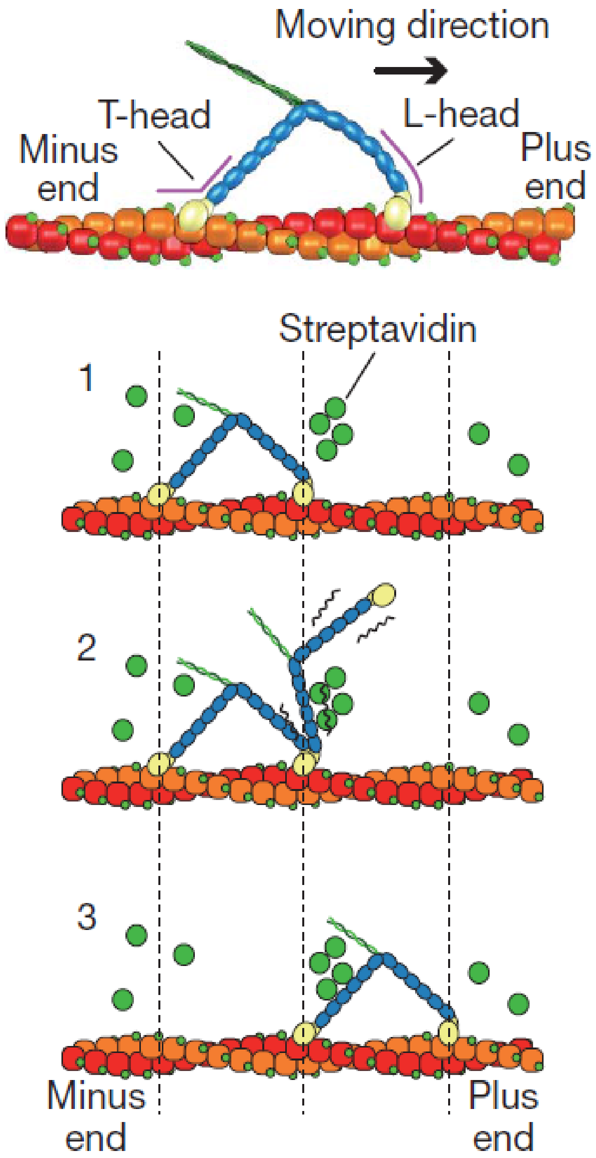
Casuso et al, Nature Nanotechnology 2012

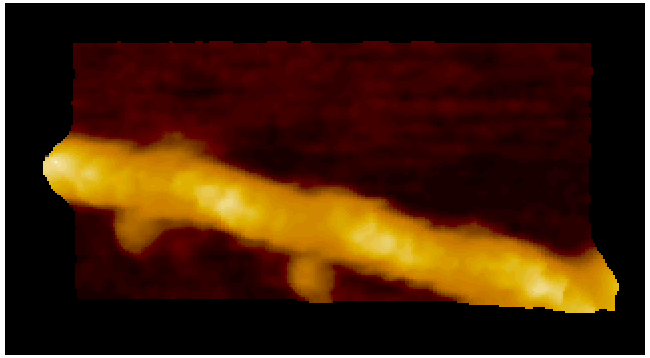
Dynamic imaging of biological systems by HS-AFM

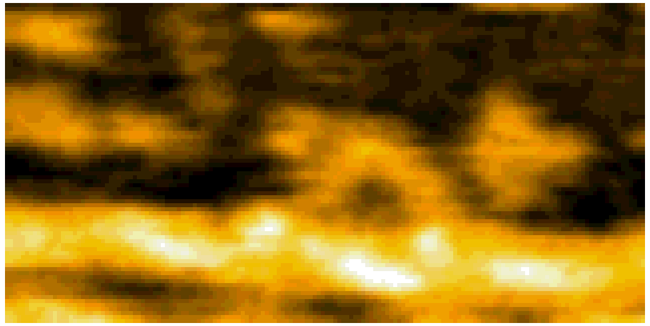


Uchihashi et al, Science 2011

Myosin V walk on actin seen by HS-AFM

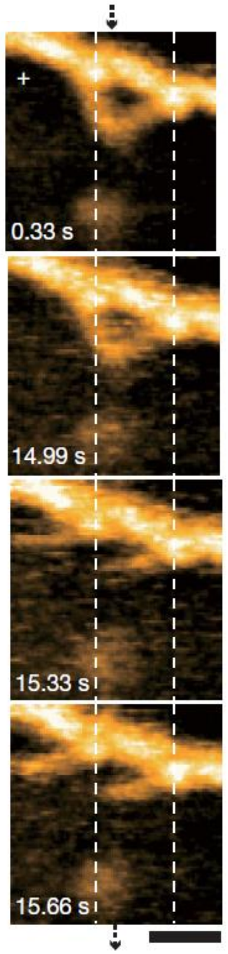


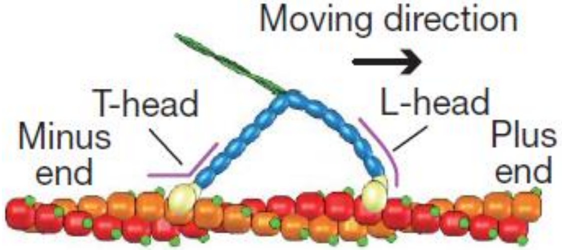


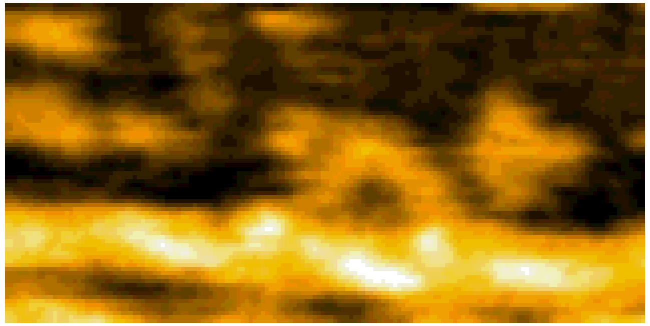


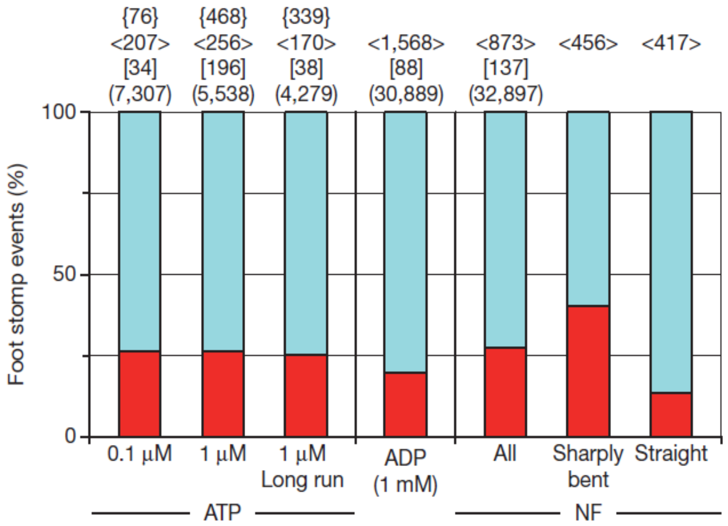
Kodera et al, Nature 2010

Myosin V walk is driven intramolecular tension release



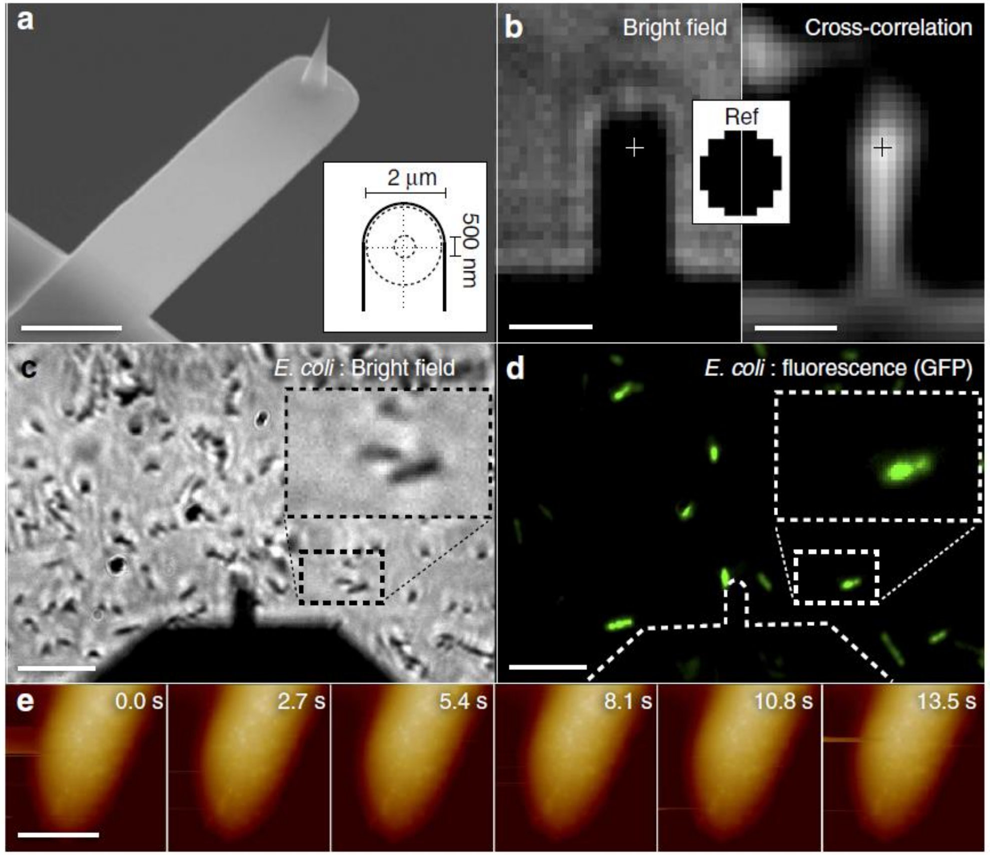


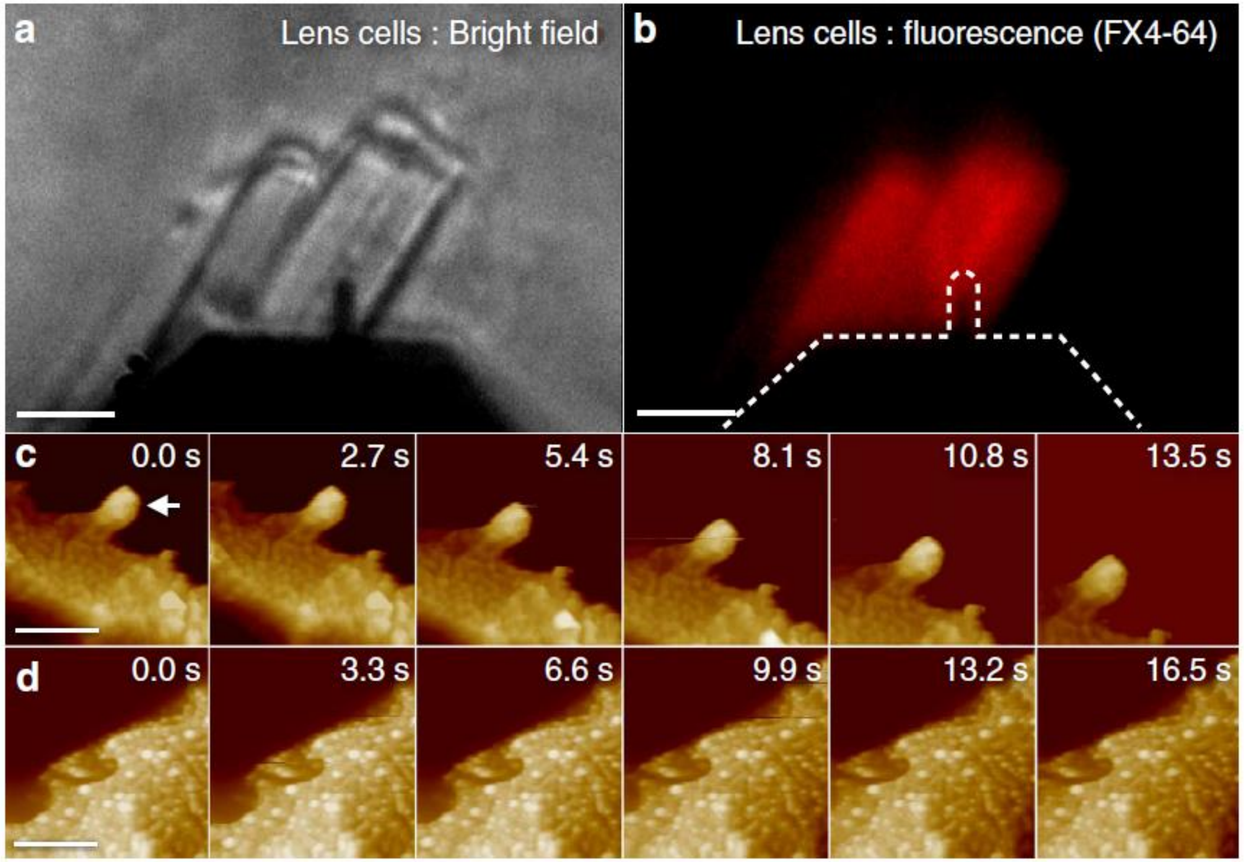




Kodera et al, Nature 2010

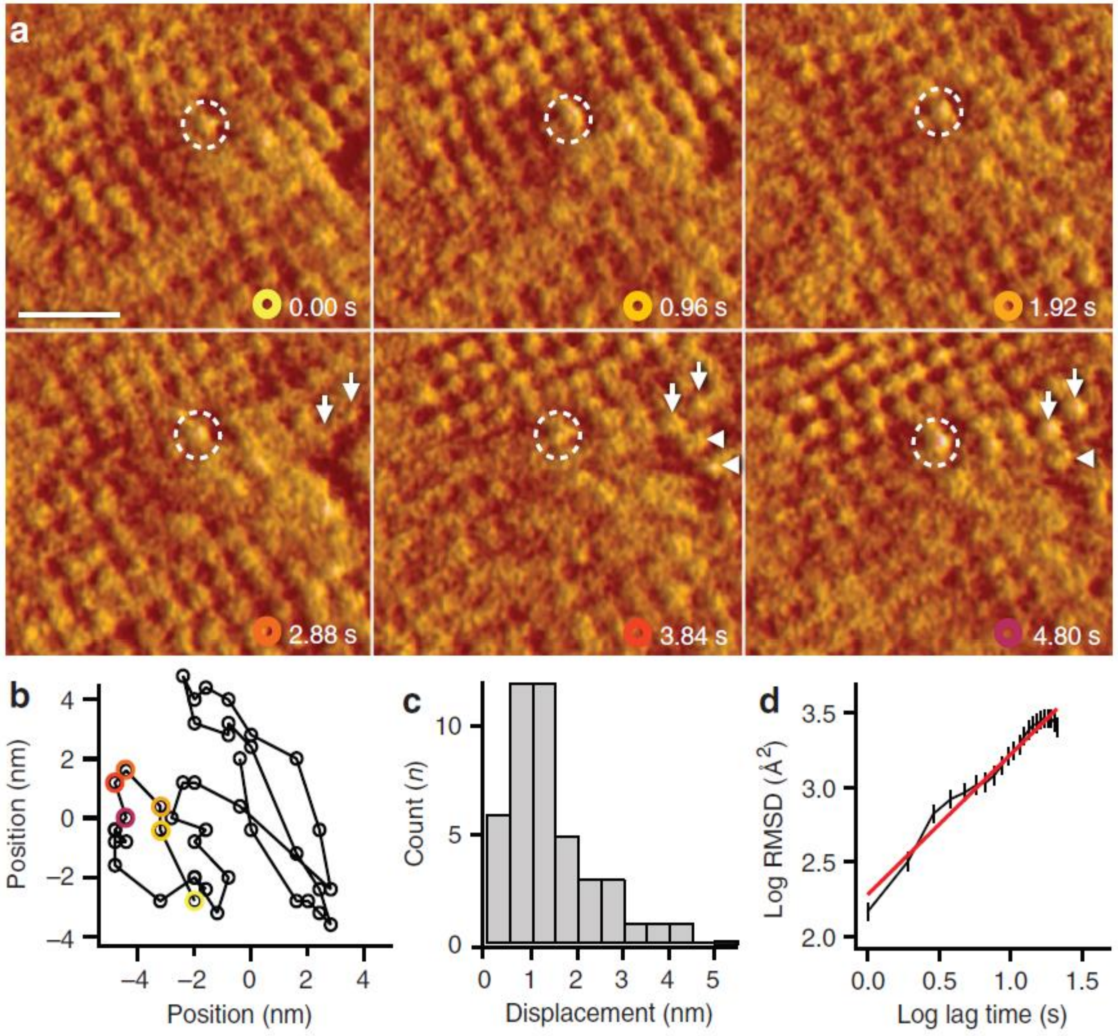
HS-AFM+OM: Imaging of cells





Colom et al Nat. Comms 2013

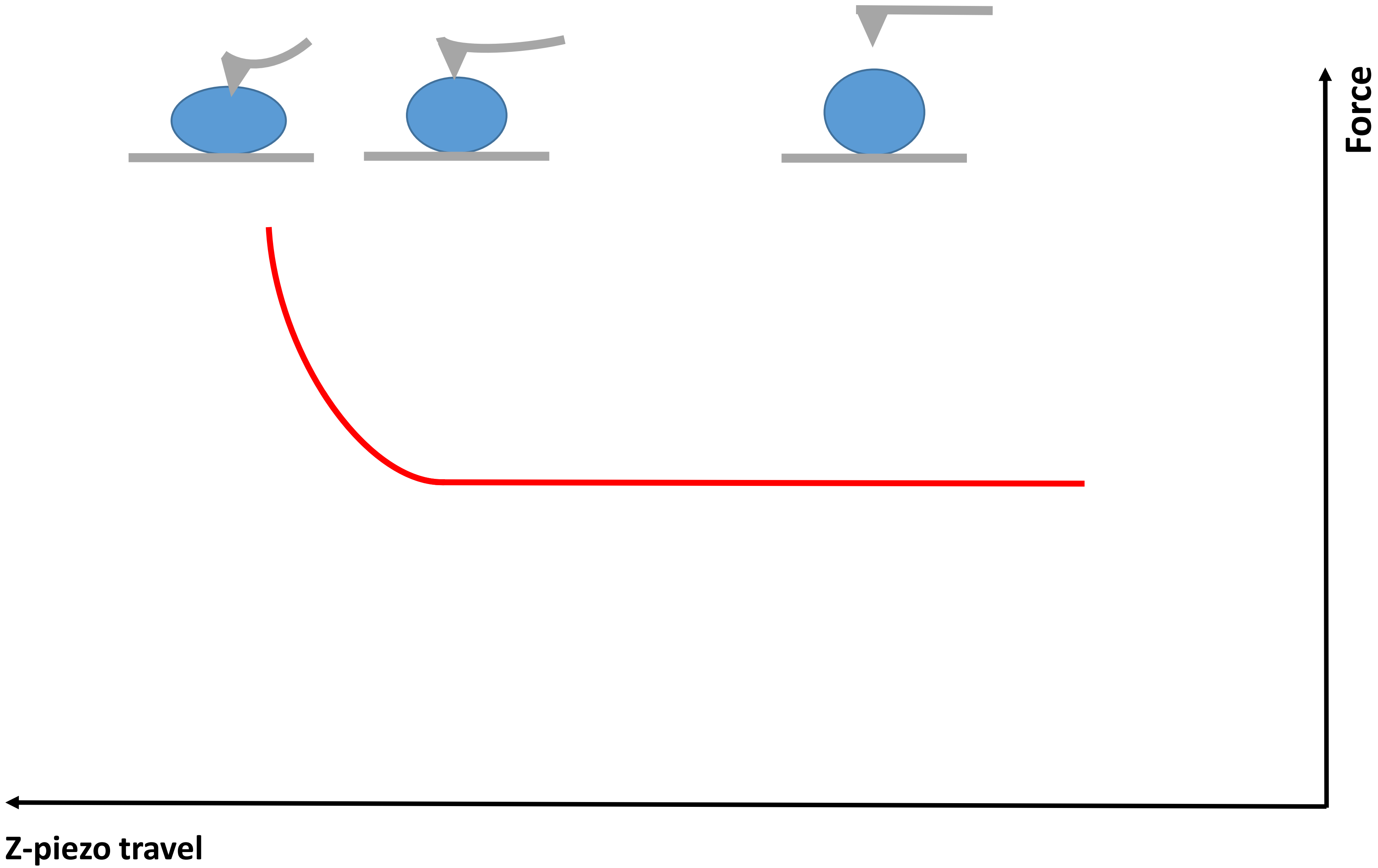
HS-AFM+OM: Characterization of lens cells junctions



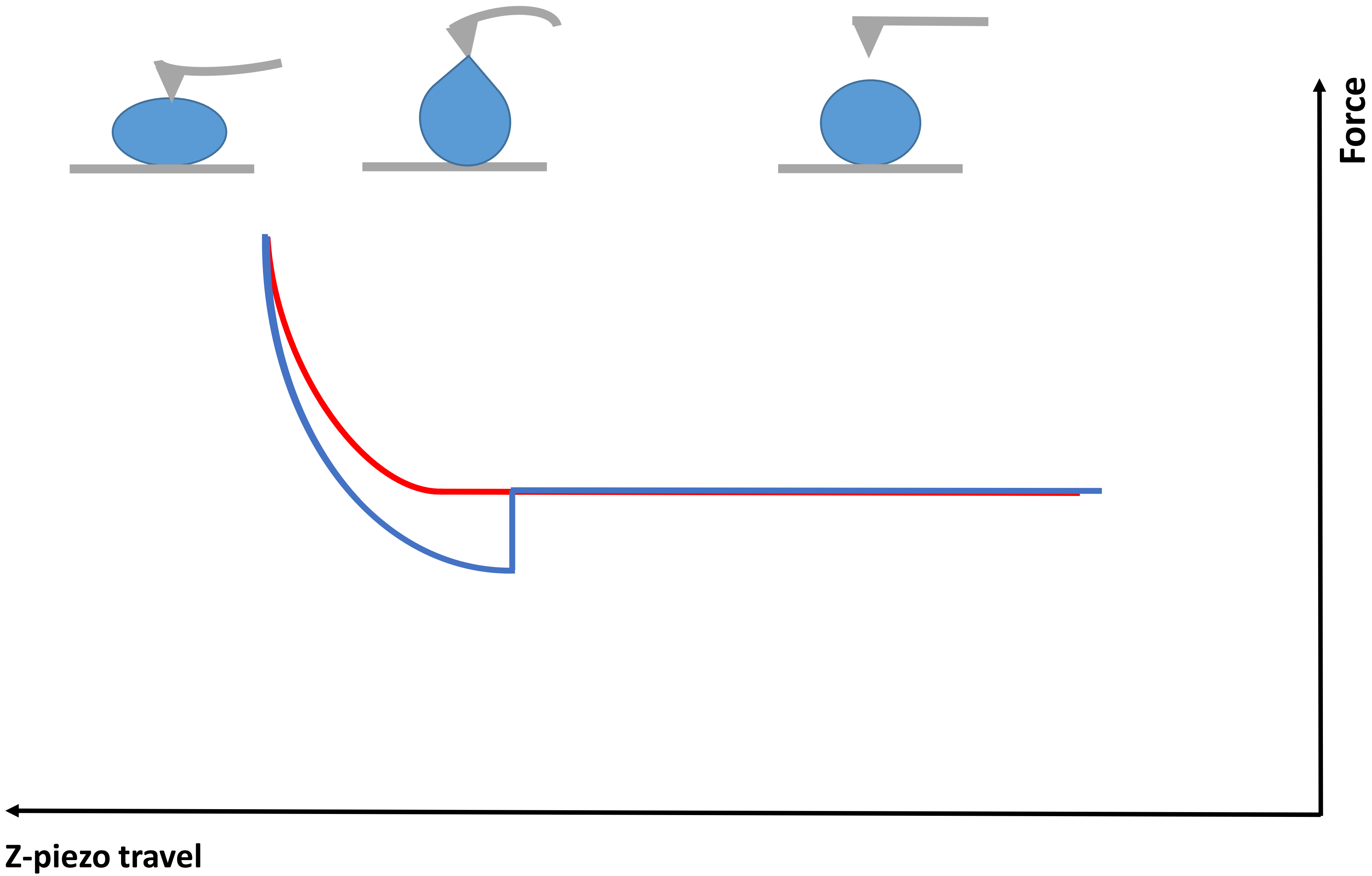
Colom et al Nat. Comms 2013

FORCE MEASUREMENTS BY AFM

Force spectroscopy



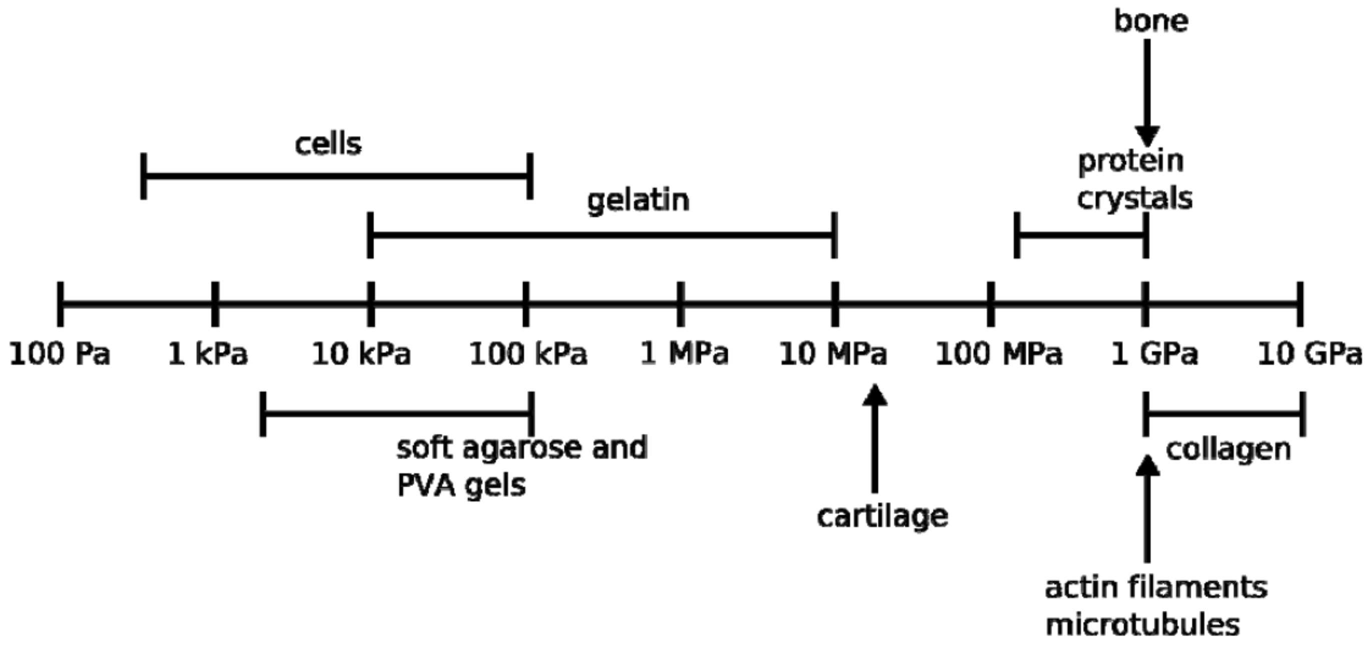
Force spectroscopy



Determining Young's moduli of materials

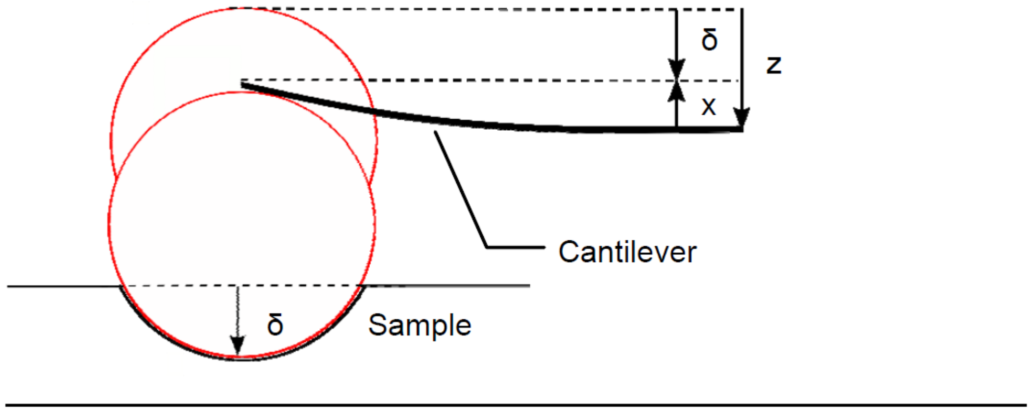
$$E \equiv \frac{\text{tensile stress}}{\text{extensional strain}} = \frac{\sigma}{\varepsilon} = \frac{F/A_0}{\Delta L/L_0} = \frac{F L_0}{A_0 \Delta L}$$

$$F = \left(\frac{E A_0}{L_0}\right) \Delta L = kx$$



usa.**jpk**.com/index.download.5fb2f841667674176fd945e65f073bad

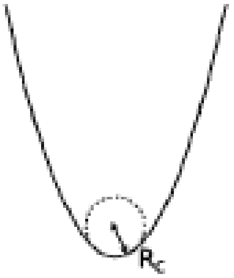
Determining Young's moduli of materials: Hertz model



Parabolic

$$F = \frac{4\sqrt{R_C}}{3}\frac{E}{1-\nu^2}\delta^{3/2} \qquad a = \sqrt{R_C\delta}$$

R_c = radius of tip curvature



Spherical

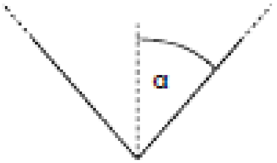
$$F = \frac{E}{1-\nu^2}\left[\frac{a^2+R^2}{2}\ln\frac{R+a}{R-a} - aR\right] \qquad \delta = \frac{a}{2}\ln\frac{R+a}{R-a}$$

R = radius of the sphere

Conical

$$F = \frac{E}{1-\nu^2}\frac{2\tan\alpha}{\pi}\delta^2 \qquad a = \frac{2\tan\alpha}{\pi}\delta$$

α = semi-opening angle of the cone



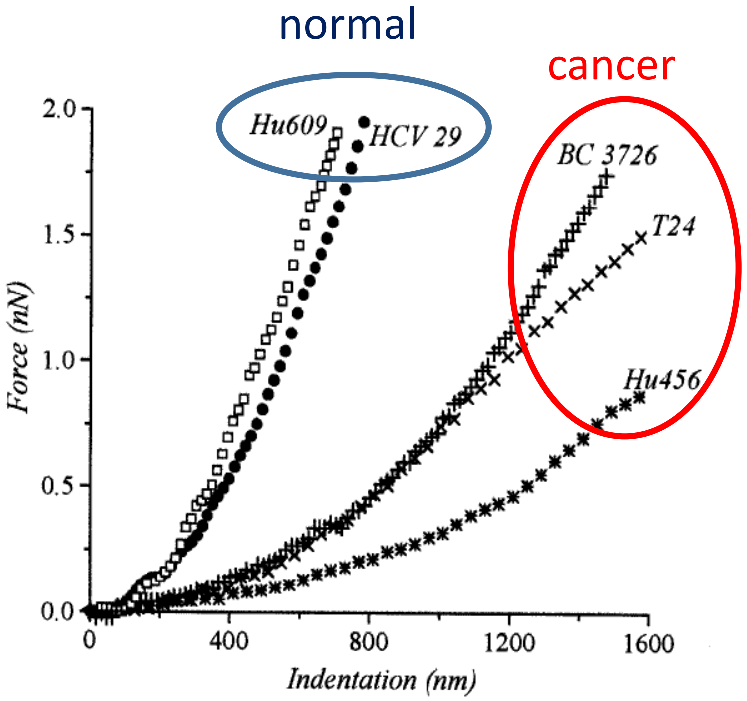
usa.**jpk**.com/index.download.5fb2f841667674176fd945e65f073bad

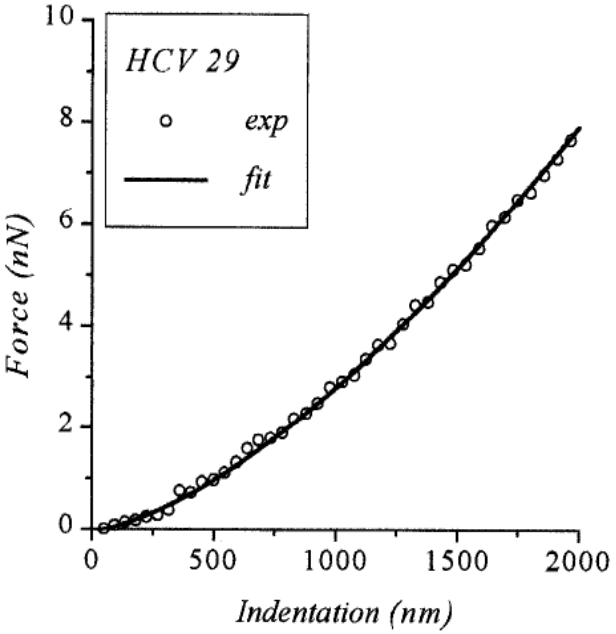
AFM study of cell mechanics

M. Lekka · P. Laidler · D. Gil · J. Lekki · Z. Stachura
A.Z. Hrynkiewicz

Elasticity of normal and cancerous human bladder cells studied by scanning force microscopy

Eur Biophys J (1999) 28: 312–316





Cell line	Young's modulus (kPa) $\mu_{cell}=0$	Young's modulus (kPa) $\mu_{cell}=0.5$	Number of analysed force curves
Hu609	12.9 (±4.8)	9.7 (±3.6)	325
HCV29	10.0 (±4.6)	7.5 (±3.6)	121
BC3726	1.4 (±0.7)	1.0 (±0.6)	214
T24	1.0 (±0.5)	0.8 (±0.4)	201
Hu456	0.4 (±0.3)	0.3 (±0.2)	10

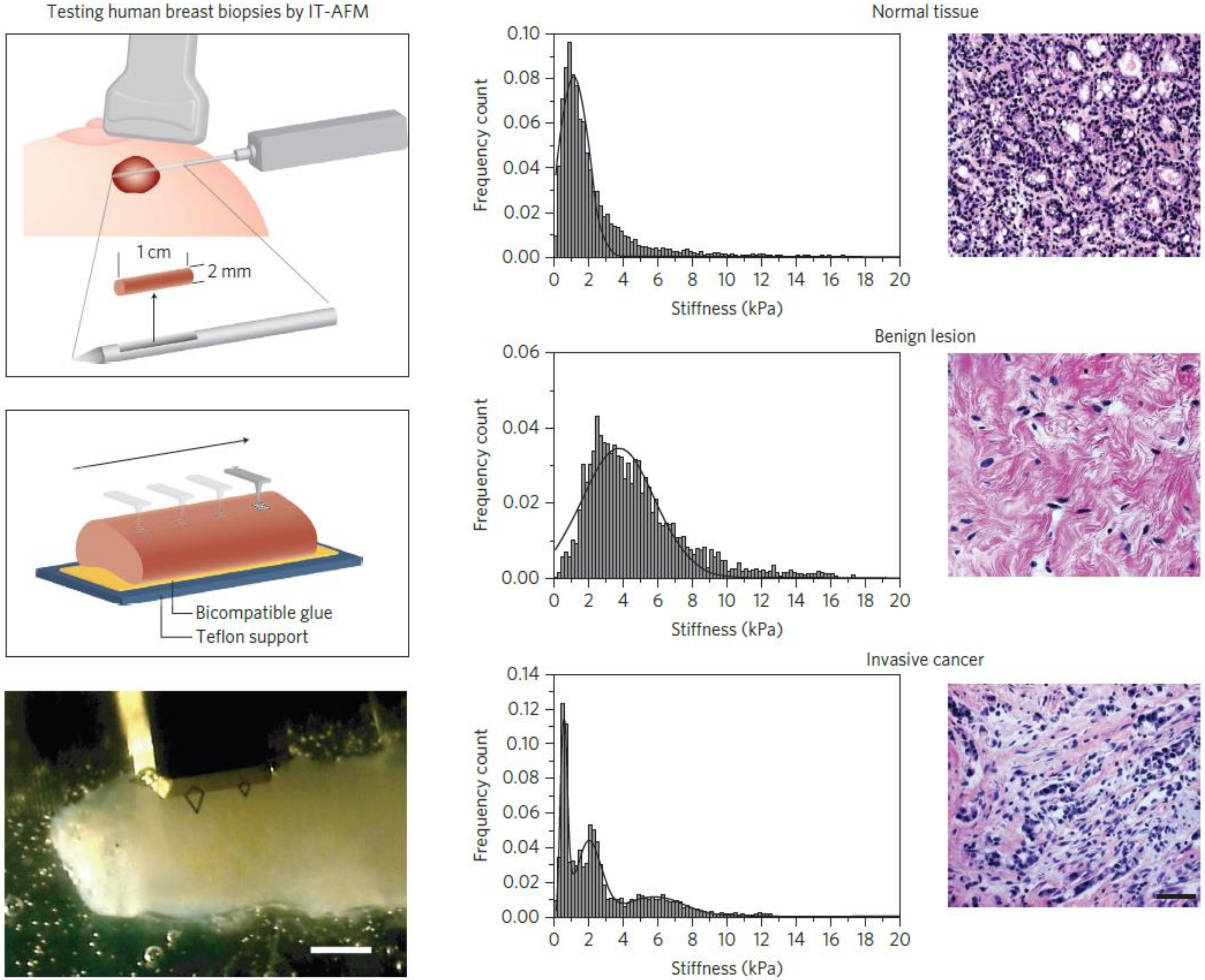
[a] The total number of measured cells is about 20 cells for each line

AFM as a Diagnostic tool ???

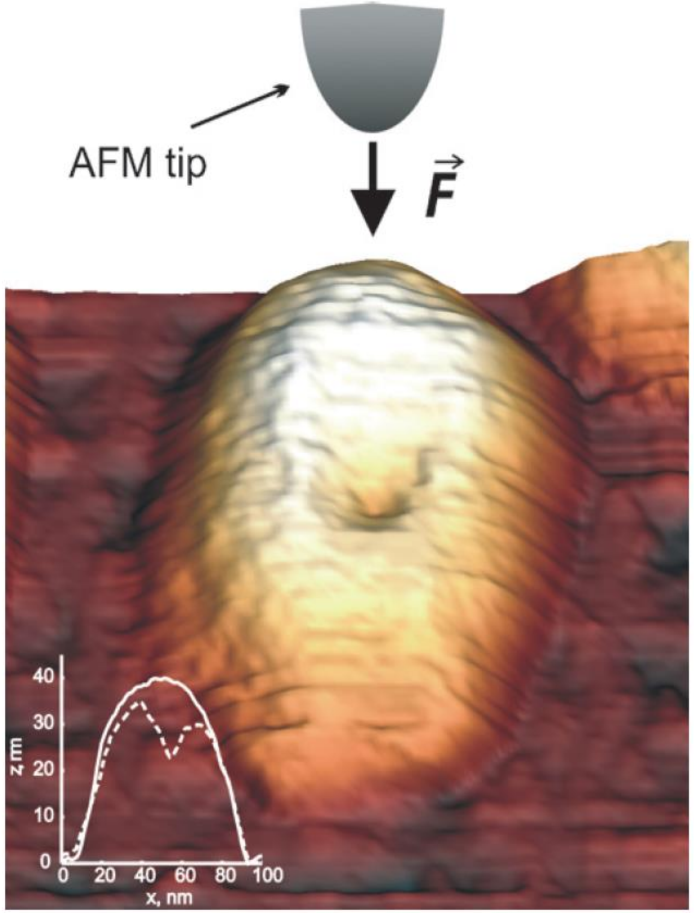
nature nanotechnology | ARTICLES
PUBLISHED ONLINE: 21 OCTOBER 2012 | DOI: 10.1038/NNANO.2012.167

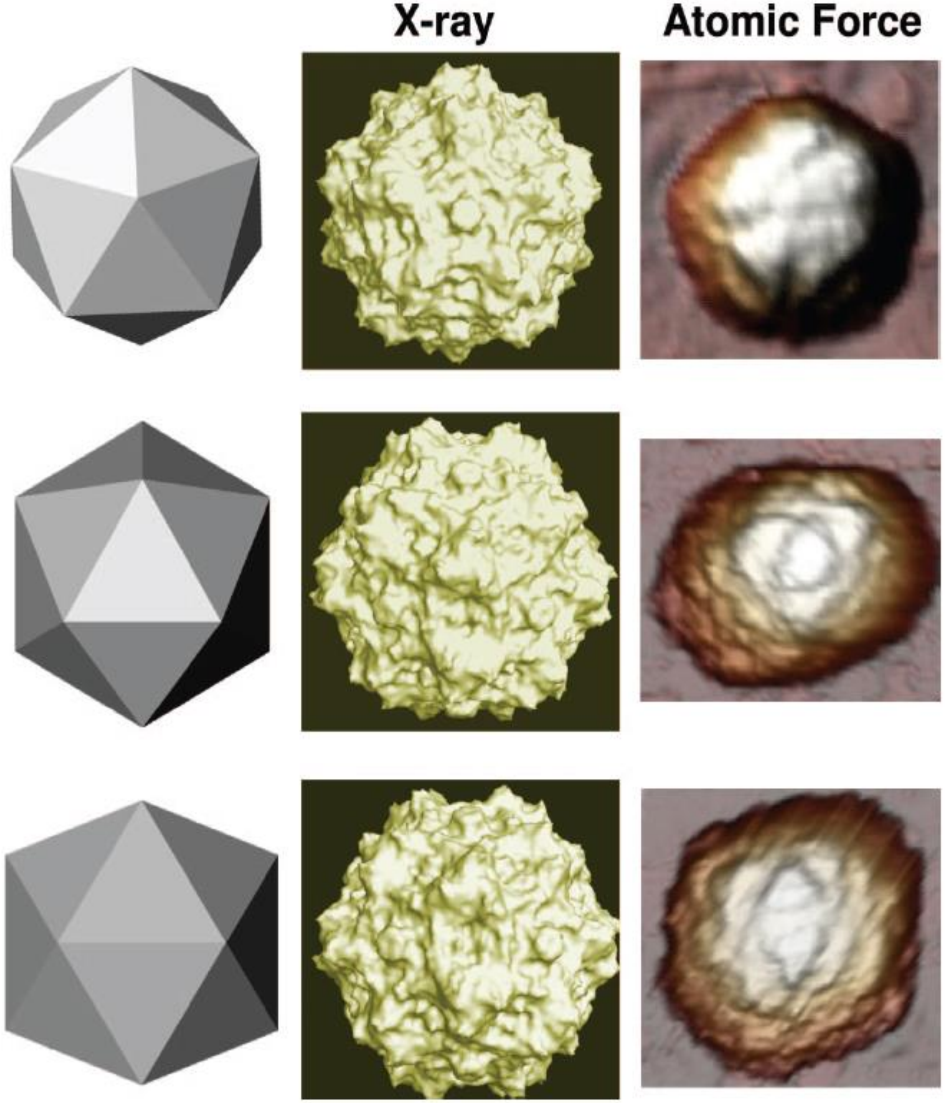
The nanomechanical signature of breast cancer

Marija Plodinec[1,2], Marko Loparic[1,2†], Christophe A. Monnier[1†], Ellen C. Obermann[3†], Rosanna Zanetti-Dallenbach[4†], Philipp Oertle[1], Janne T. Hyotyla[1], Ueli Aebi[2], Mohamed Bentires-Alj[5], Roderick Y. H. Lim[1]* and Cora-Ann Schoenenberger[2]



AFM studies of virus mechanics

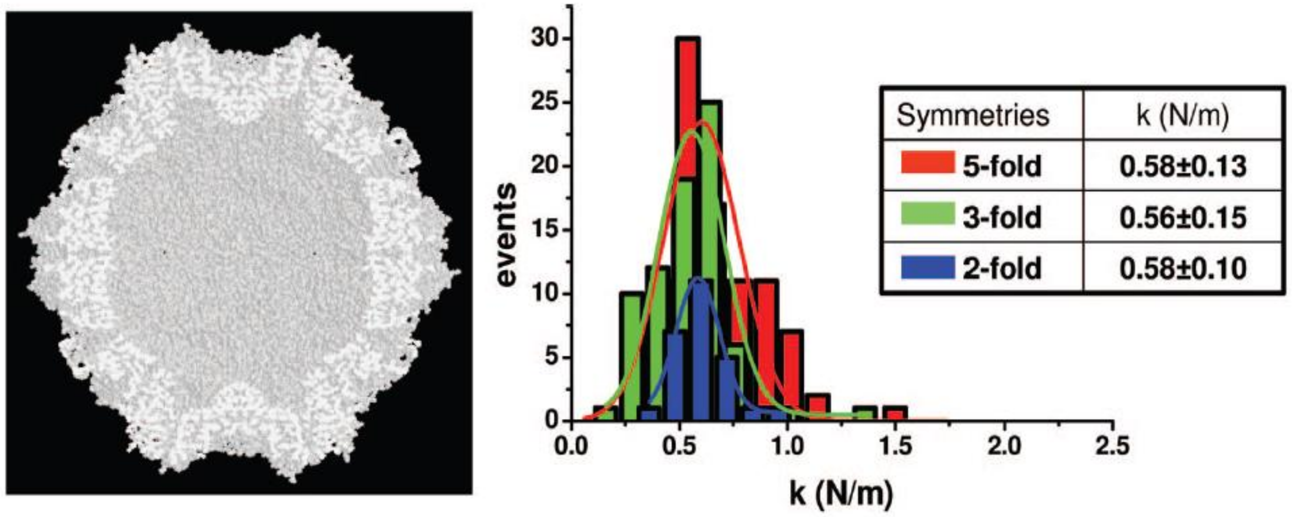




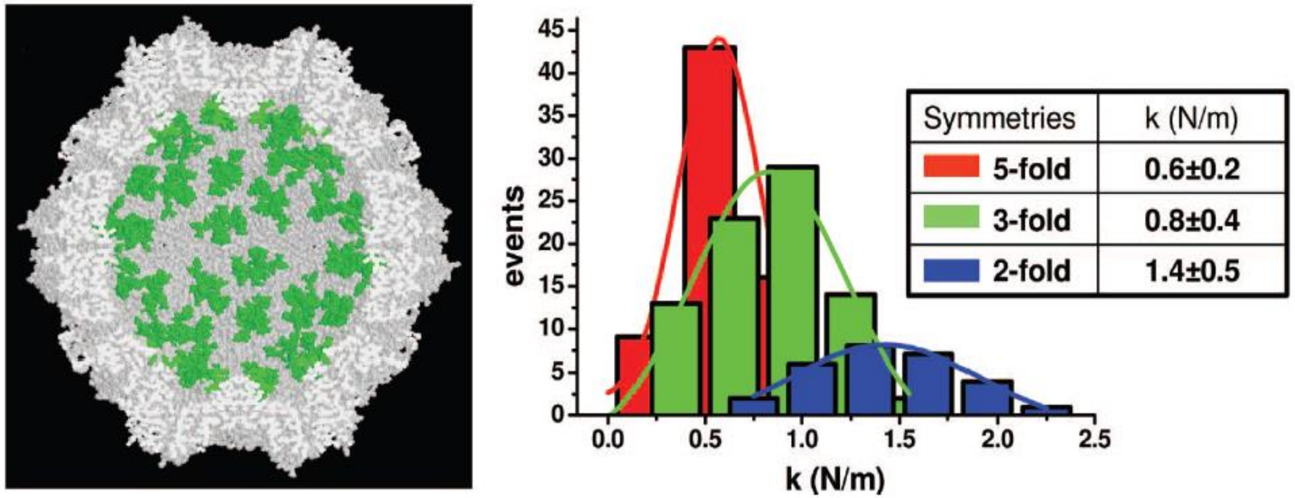
Carrasco et al, PNAS 2006

AFM studies of virus mechanics

Empty capsid



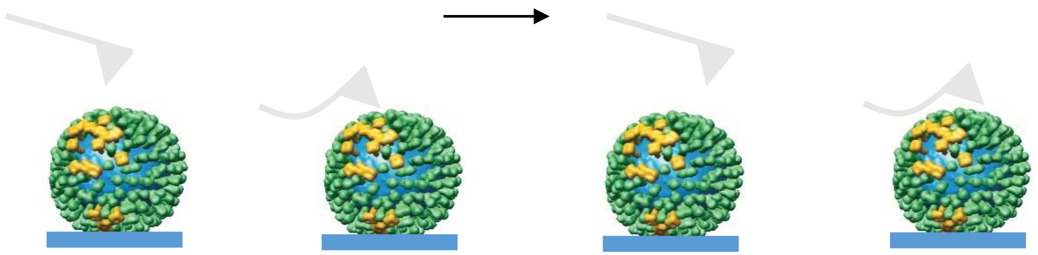
Virion

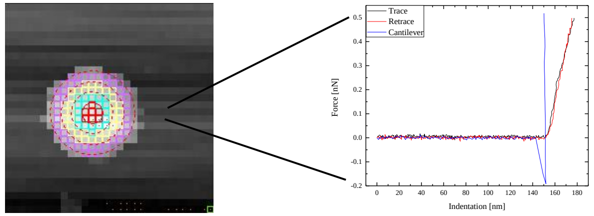


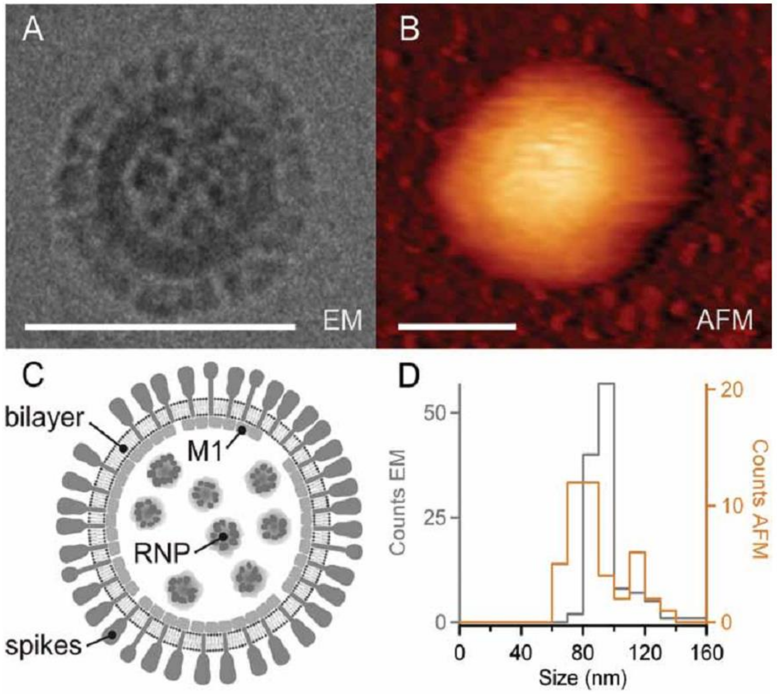
Carrasco et al, PNAS 2006

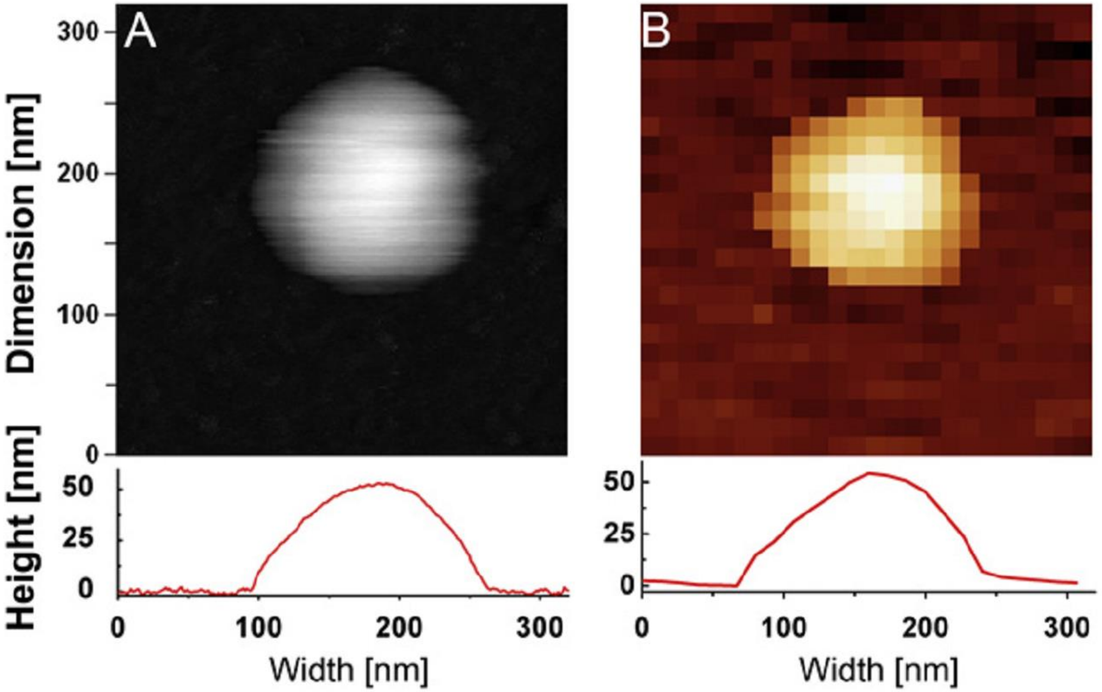
Force mapping of flu virions

Force mapping



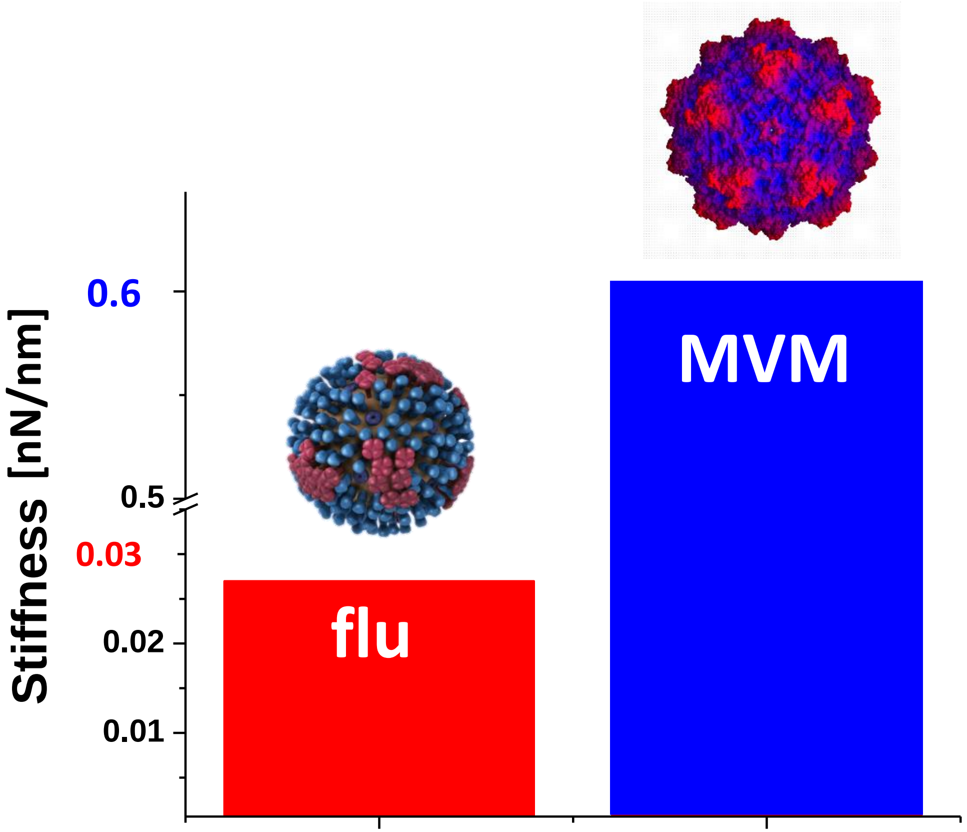


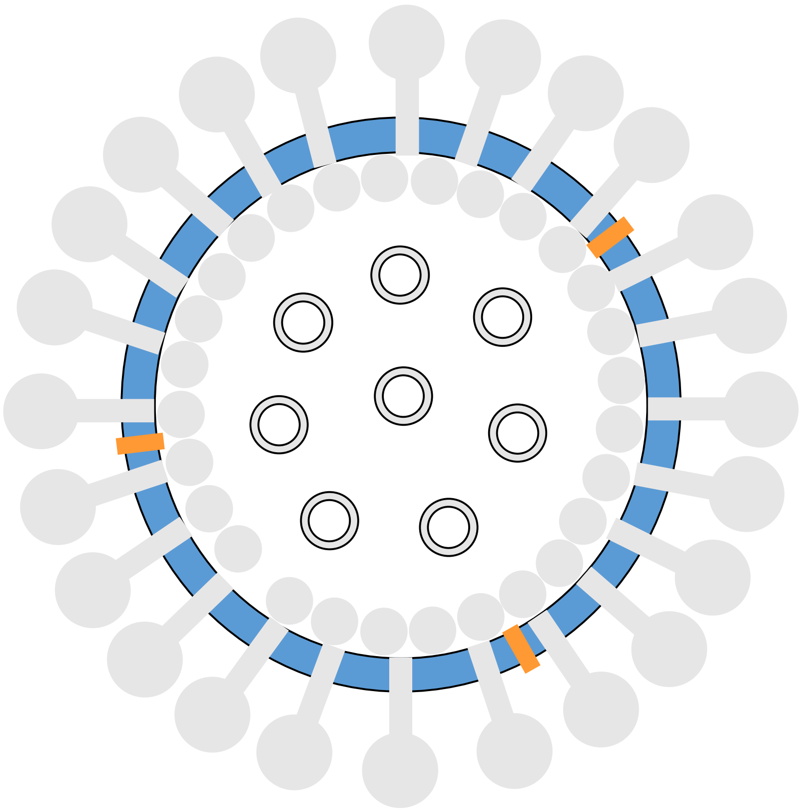




Li et al, Biophys J 2011, Eghiaian et al JBC 2012, Li et al, Biophys J 2014

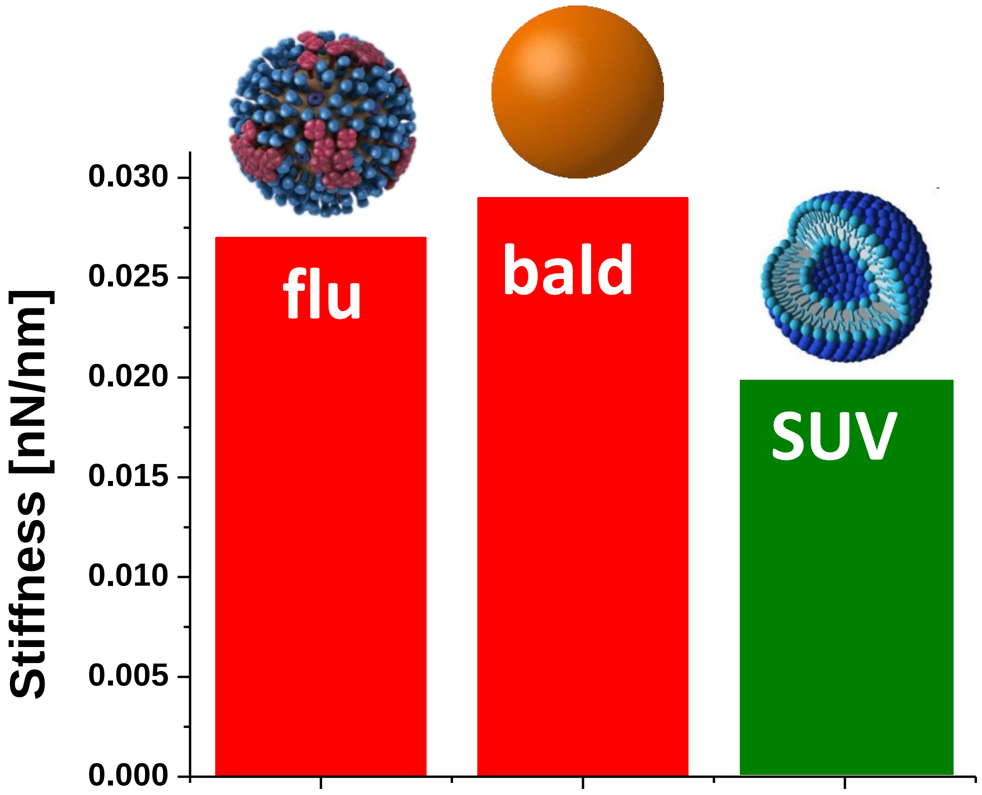
AFM studies of influenza virus mechanics

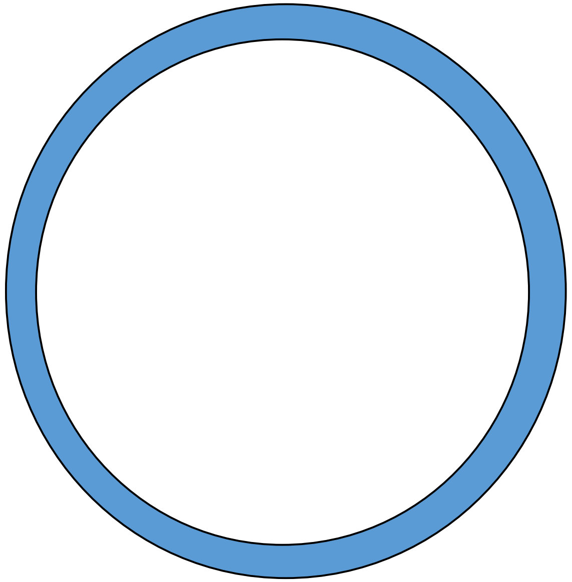




Schaap, Eghiaian et al,JBC 2012

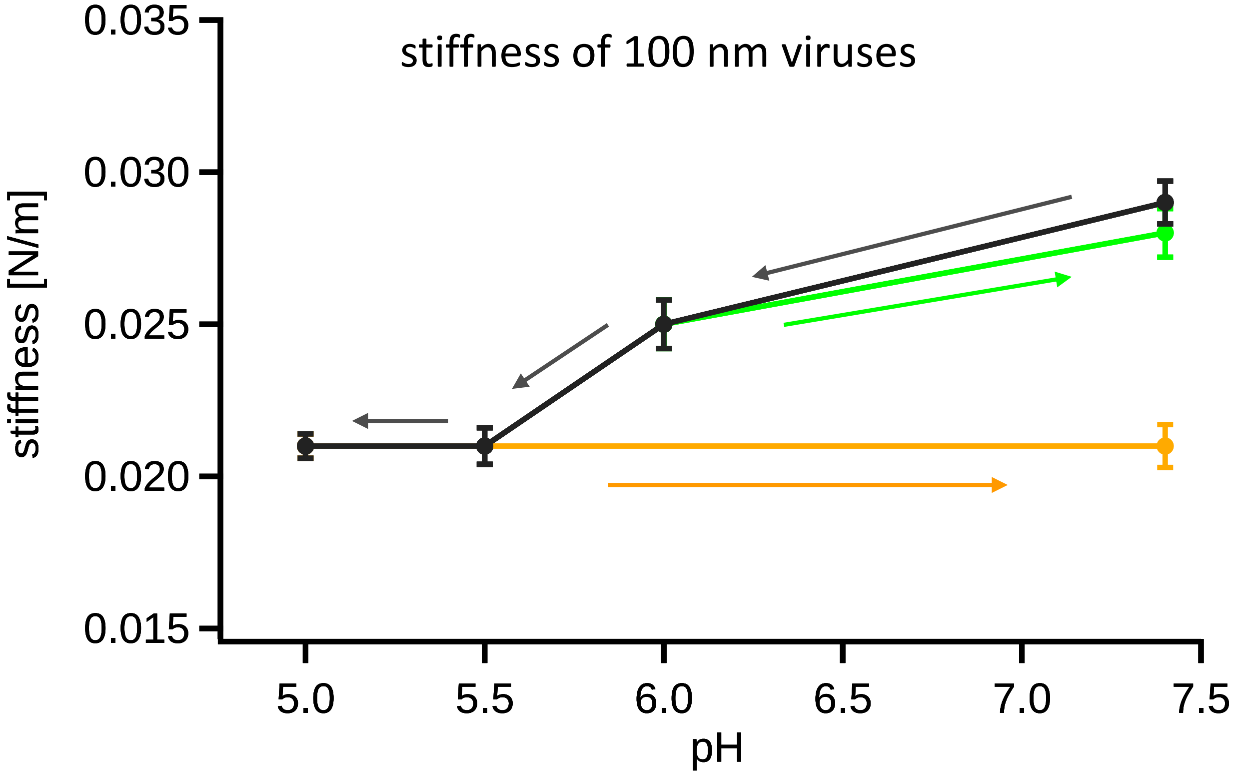
Lipids define mechanical response





Schaap, Eghiaian et al,JBC 2012, Li et al Biophys J 2011

pH-dependent viral stiffness

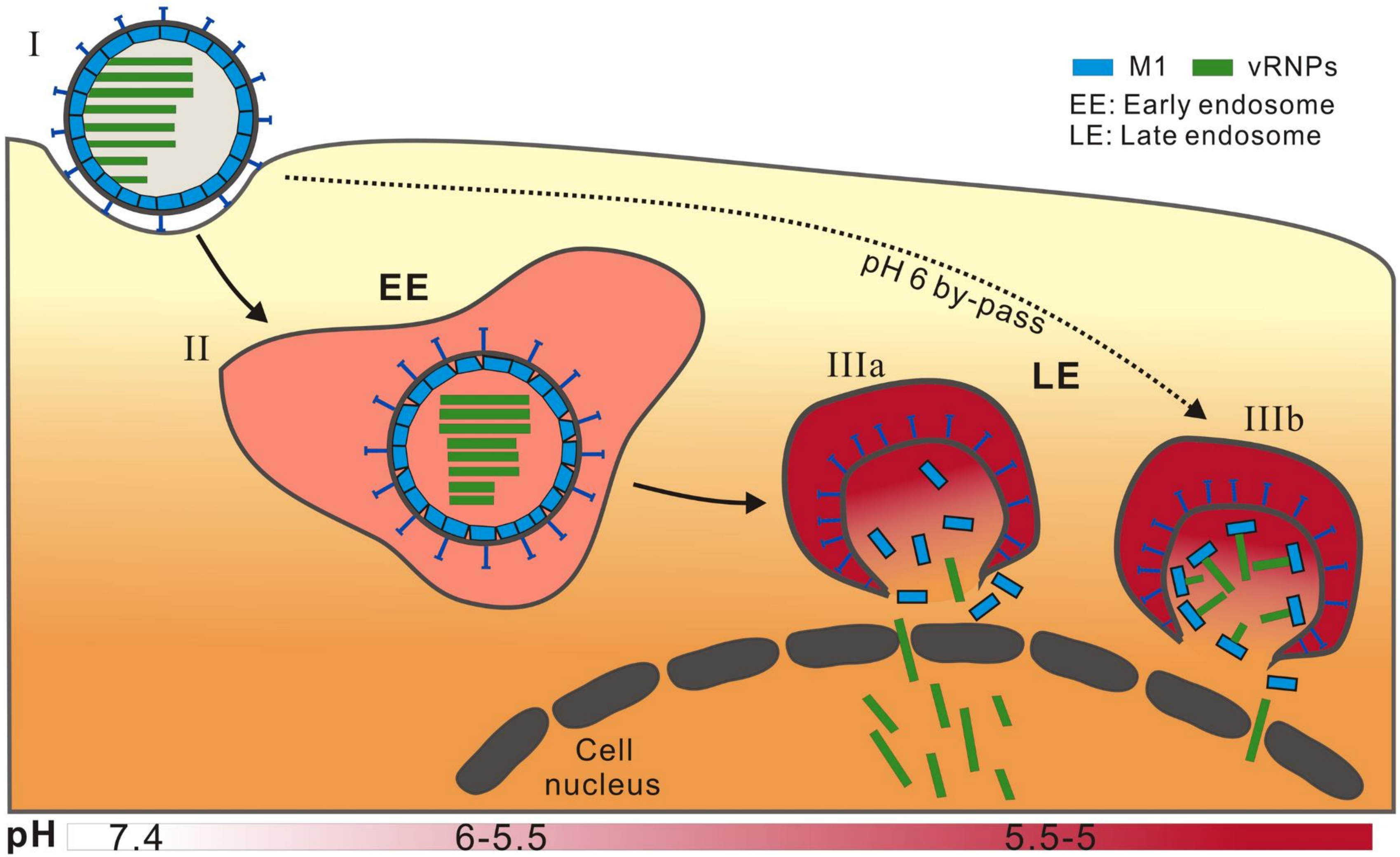


protein capsid disassembly reduced stiffness only by 30 %

irreversible below pH 6

what determines the remaining 70 % of stiffness?

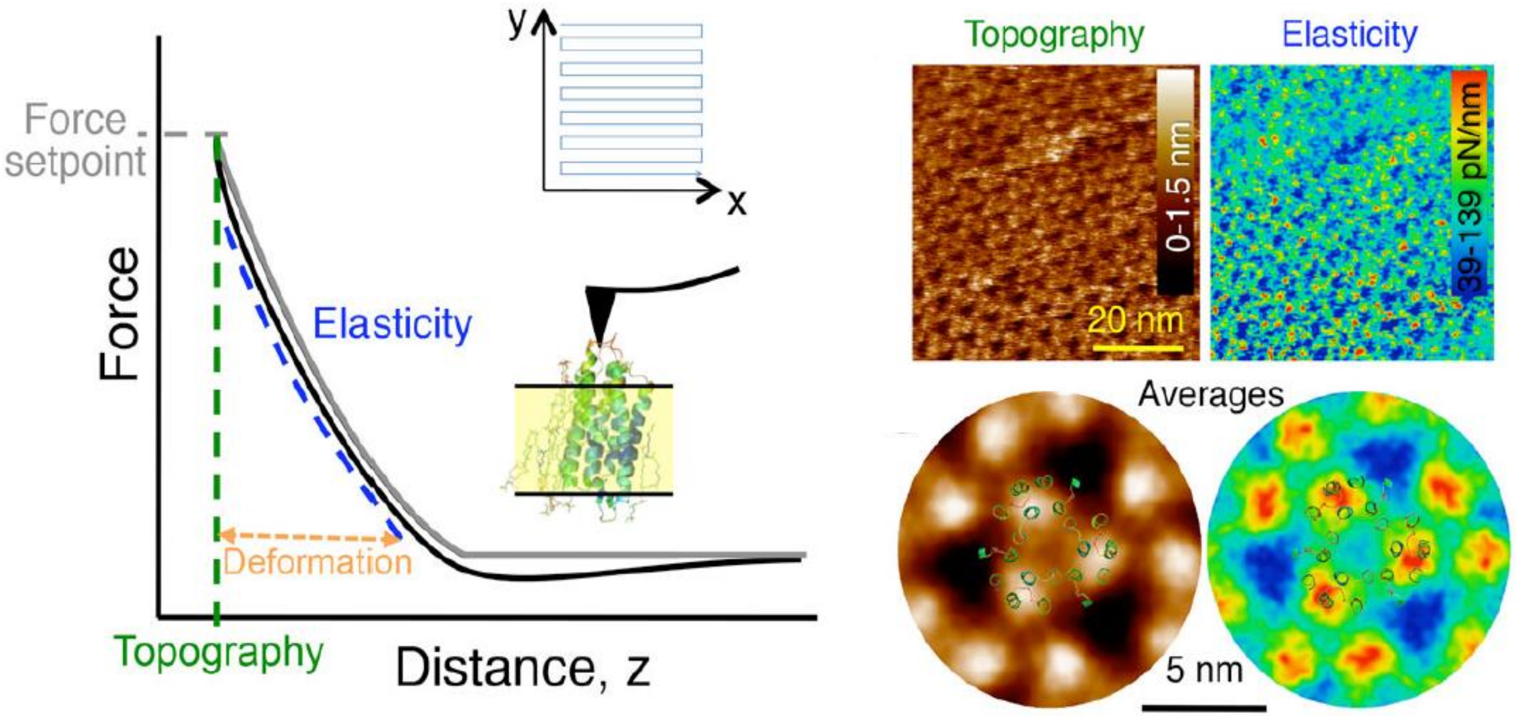
Li et al, Biophys J 2014



Li et al, Biophys J 2014

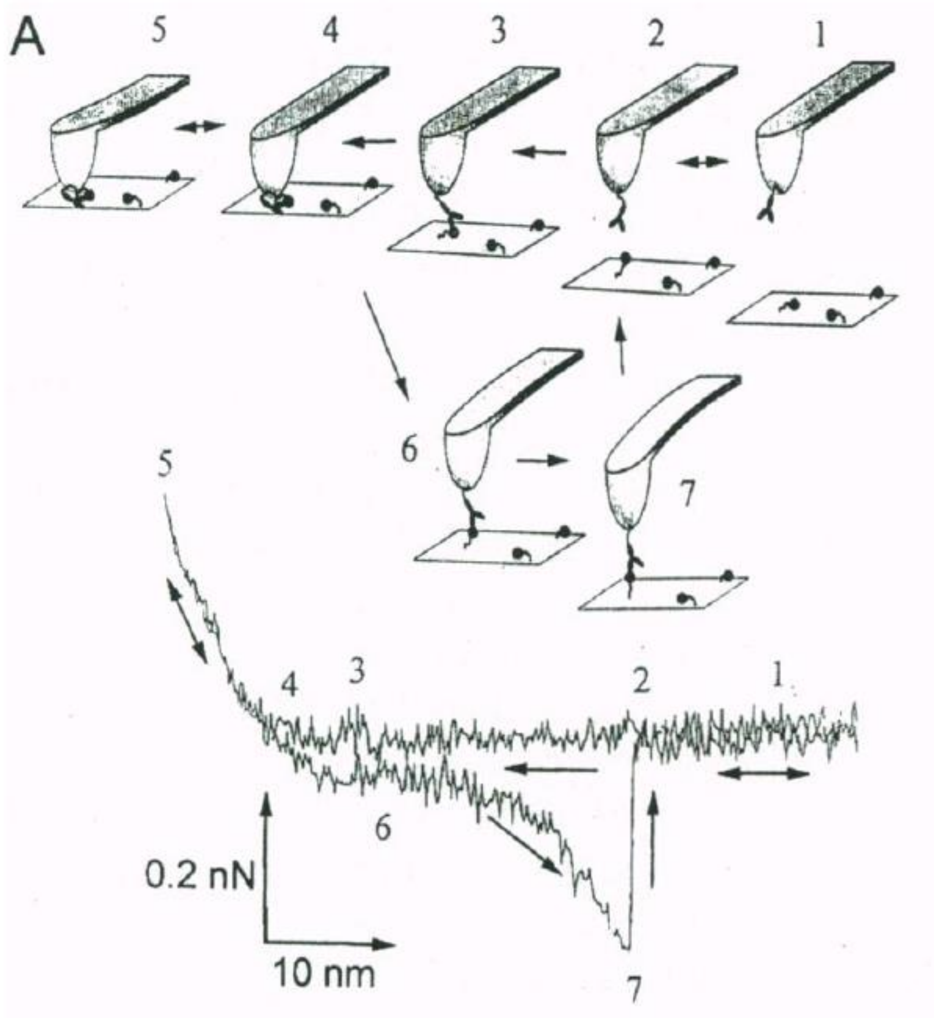
Peak force tapping™

Mechanical measurements on single proteins



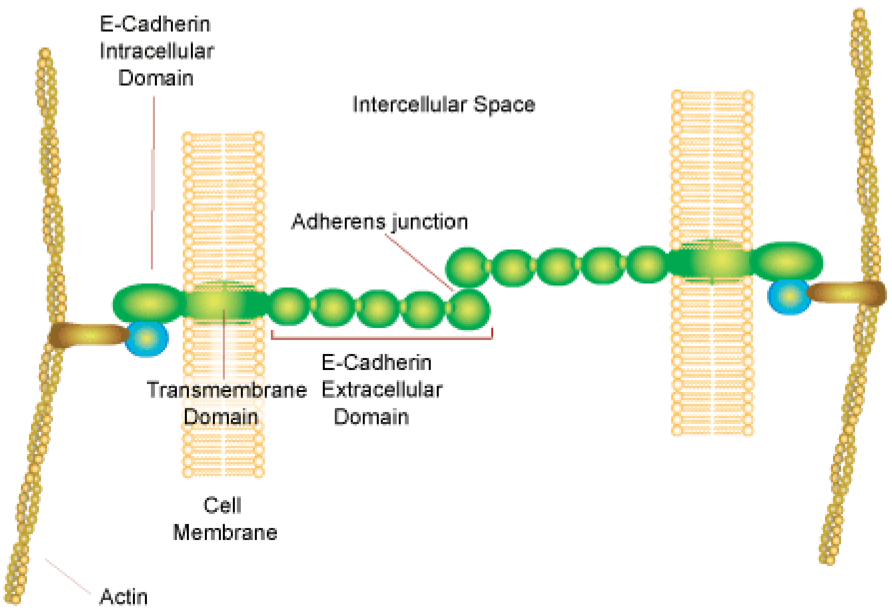
Rico et al, Nano Lett. 2011

Single pair interaction

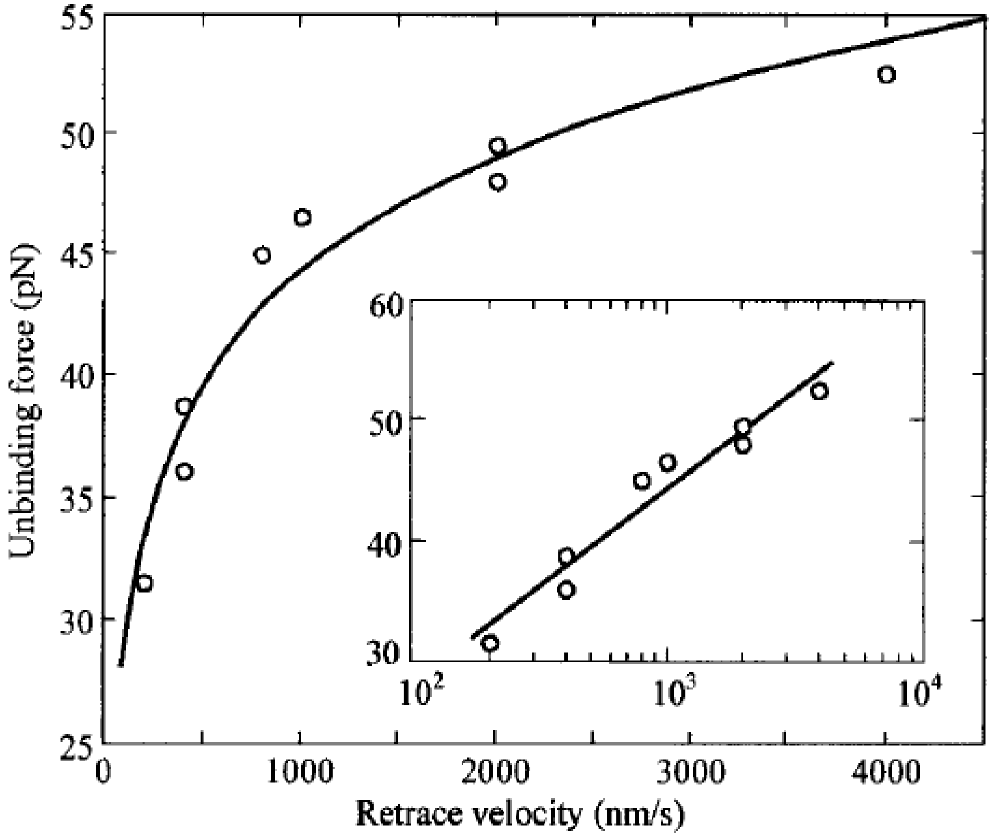


Hinterdorfer et al, PNAS 1996

Single pair interaction



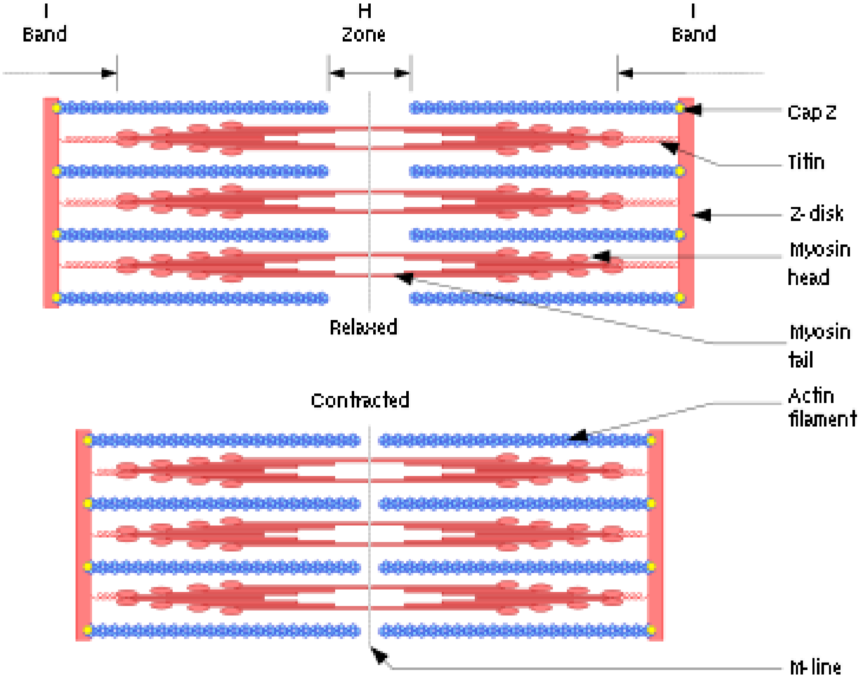
Bell-evans

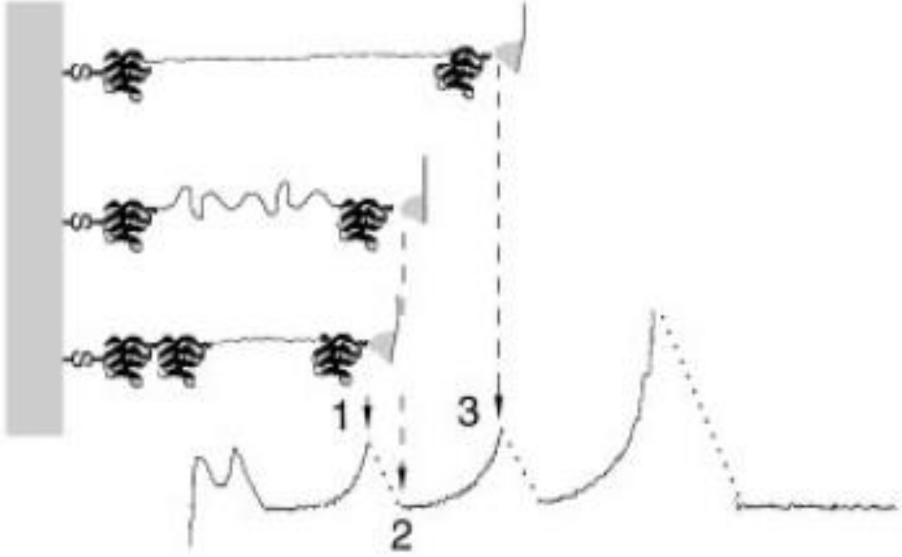


$$\tau(f_u) = \tau_0 \exp(-l_r f_u / k_B T)$$

Baumgartner et al, PNAS 2000
Bell, Science 1977
Evans & Ritchie, BJ 1997

Mechanical characterization of muscle Titin



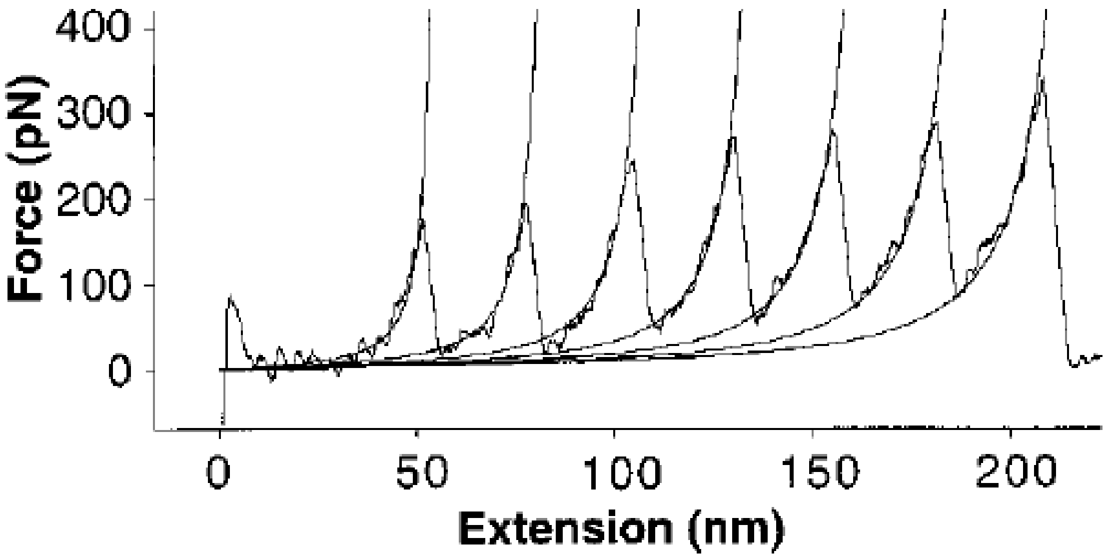


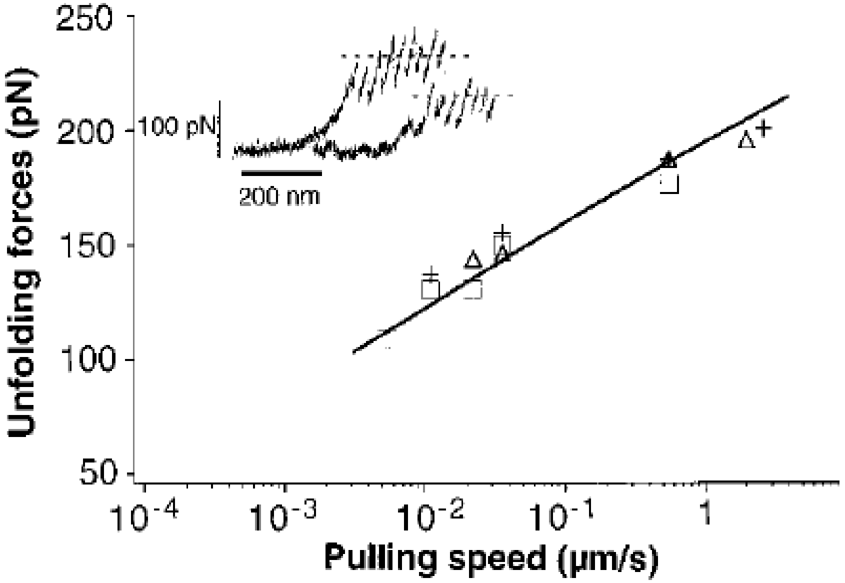
http://en.wikipedia.org/wiki/Titin

Rief et al Science 1997

Single molecule unfolding

Reversible Unfolding of Individual Titin Immunoglobulin Domains by AFM
Matthias Rief *et al.*
Science **276**, 1109 (1997);





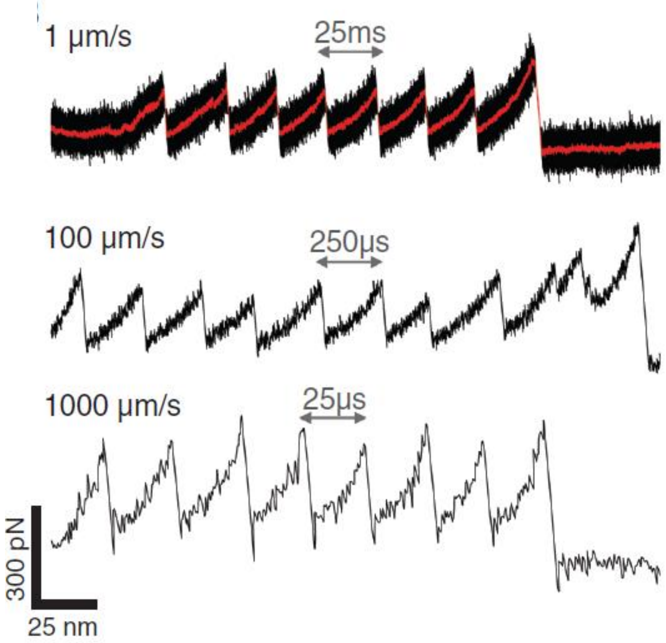
$$F(x) = (kT/b)\,[0.25(1 - x/L)^{-2} - 0.25 + x/L]$$

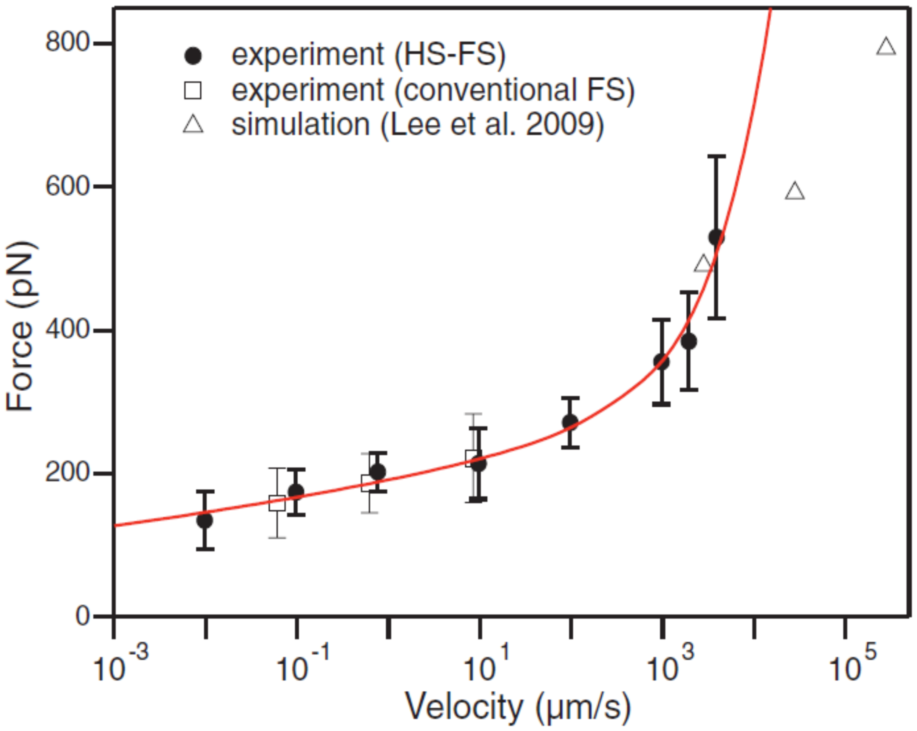
$$\alpha = \alpha_0 \exp(F\Delta x/kT)$$

L: contour length of the polymer chain

Rief et al Science 1997

High-speed single molecule unfolding





Rico et al, Science 2013

Credits

U1006 Marseille
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Christian SIEBEN
Kai LUDWIG
Chris Höfer
Salvatore Chianta

Universität Göttingen
Iwan SCHAAP
Sai LI

Columbia (NYC, J Frank's lab)
Amédée des Georges

NIMR Mill Hill (UK)
Claudia VEIGEL

CNRS Gif-sur-Yvette/INRA Jouy en Josas
Marcel KNOSSOW (thesis director)
Human REZAEI (unofficial co-director)

Institut Pourquier
Stephanie LESCEU

Recommended readings

- Mechanics of Motor Proteins and the Cytoskeleton (J Howard)

- Ando et al, Chem Rev 2014, Filming biomolecular processes by high-speed atomic force microscopy

- Mechanics of the Cell (David Boal)