

Master 2 Biologie Santé EC11 – 16 Novembre 2020

Microscopie à Force Atomique

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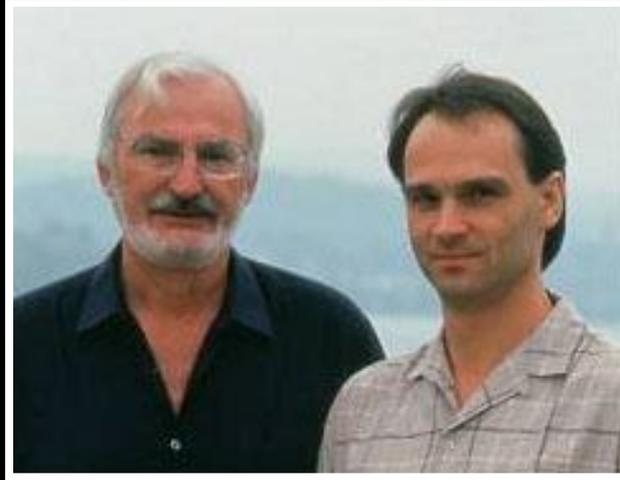
3.1 Propriétés mécaniques

3.2 Manipulation et déplieement

3.3 Détection, localisation et mapping de molécules individuelles

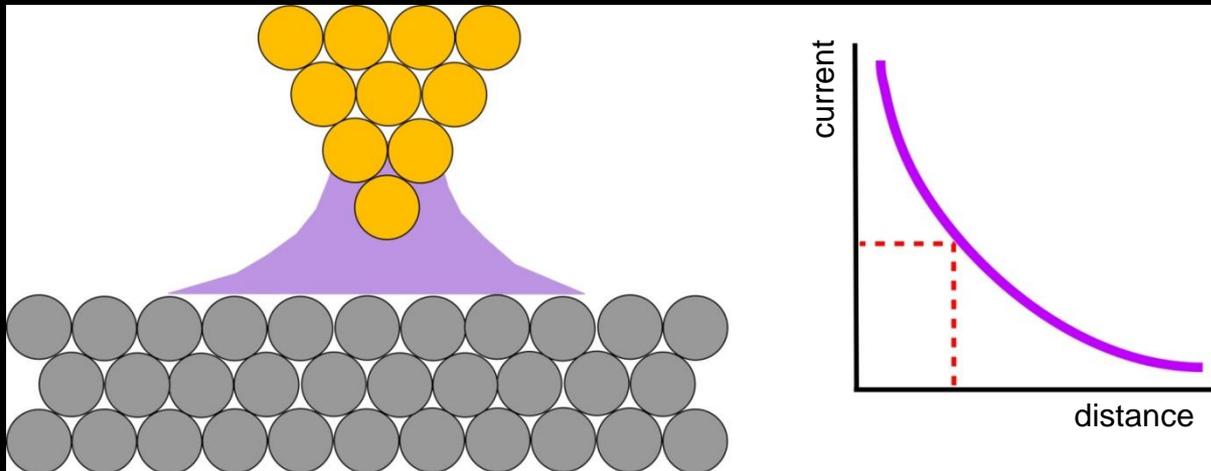
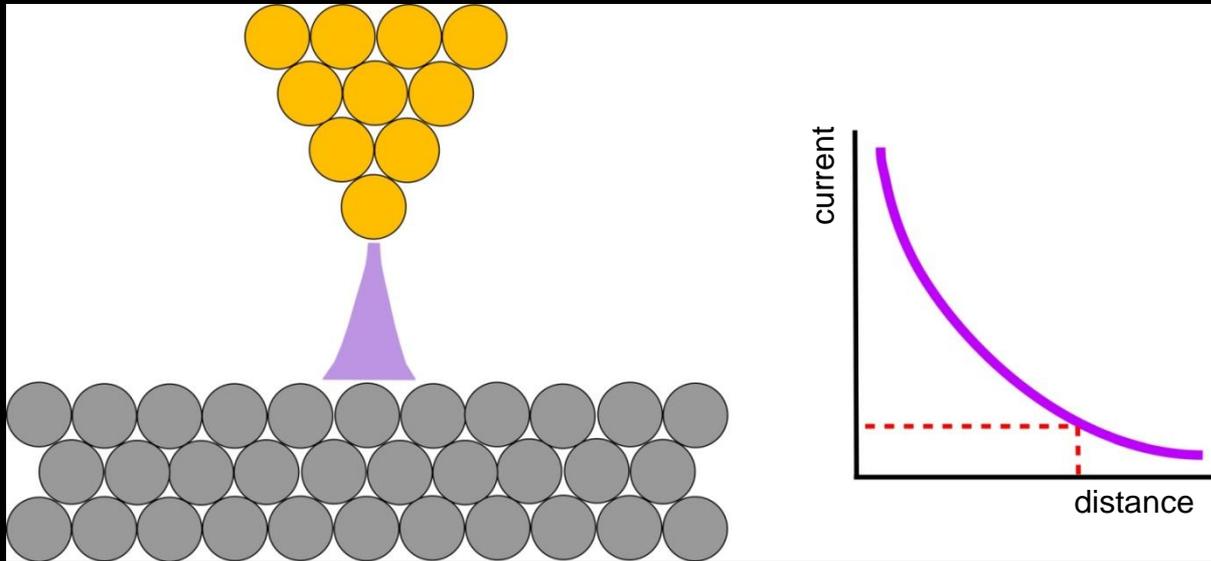
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Scanning Tunneling Microscope (STM)

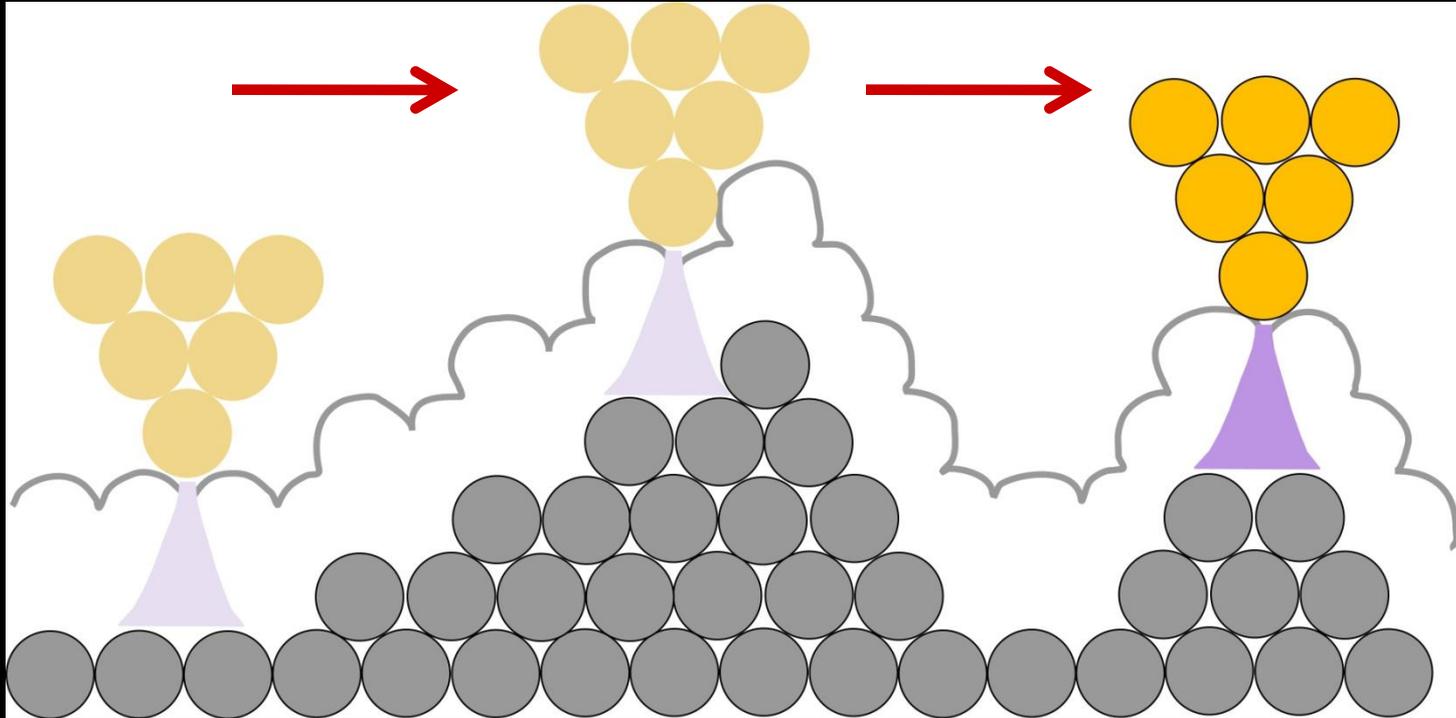


- **1981 : développement du microscope à effet tunnel par Heinrich Rohrer et Gerd Binnig (IBM Zurich)**
→ premières images avec une résolution atomique
- **1986 : prix Nobel de physique**

Scanning Tunneling Microscope : principe



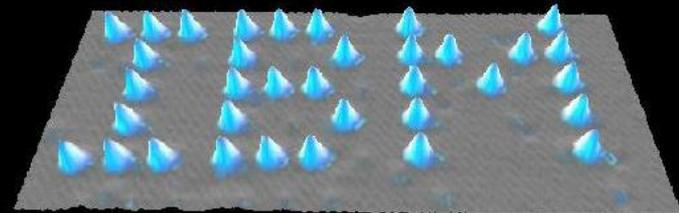
Scanning



- Pointe conductrice amenée à proximité d'une surface conductrice ($z \approx 5 \text{ \AA}$)
- Application d'une tension (10 mV - 1 V)
- Mesure d'un courant tunnel (0,1-10 nA)

Scanning Tunneling Microscope (STM)

- **Avantages**
 - Résolution atomique (résolution latérale : 0.1 nm)
 - Manipulation d'atomes indiv.

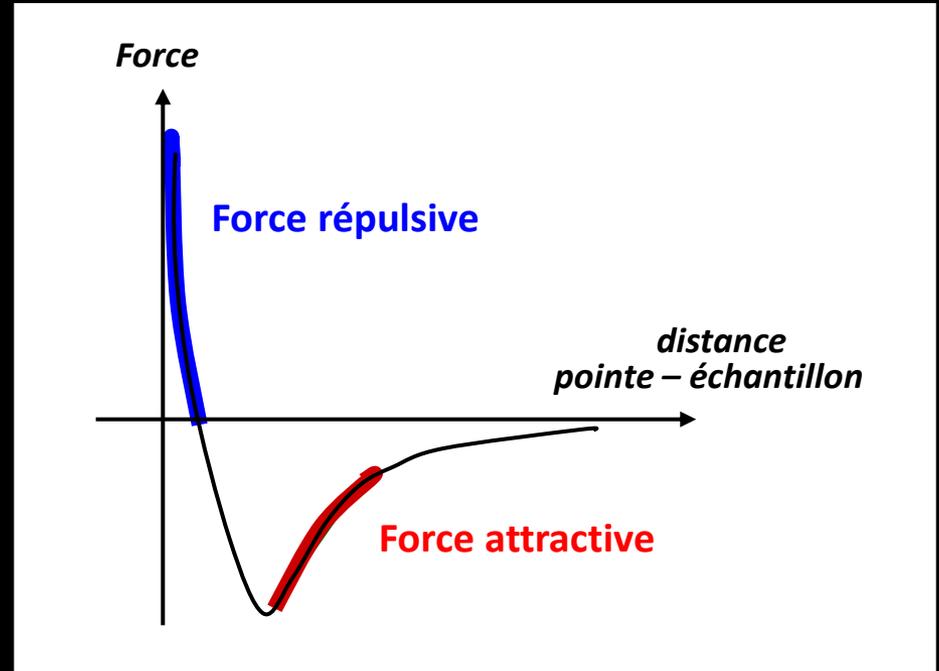
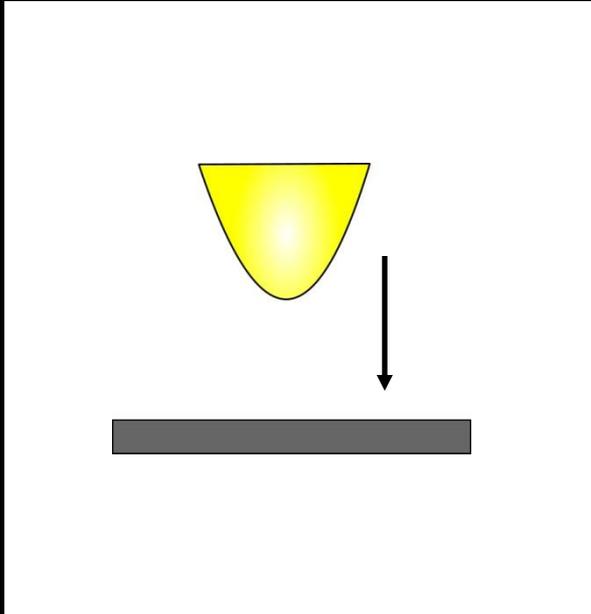


Eigler et al., *Nature*, 344 (1990)

- **Limitations**
 - Matériaux conducteurs
 - Non adapté à des échantillons biologiques !!!

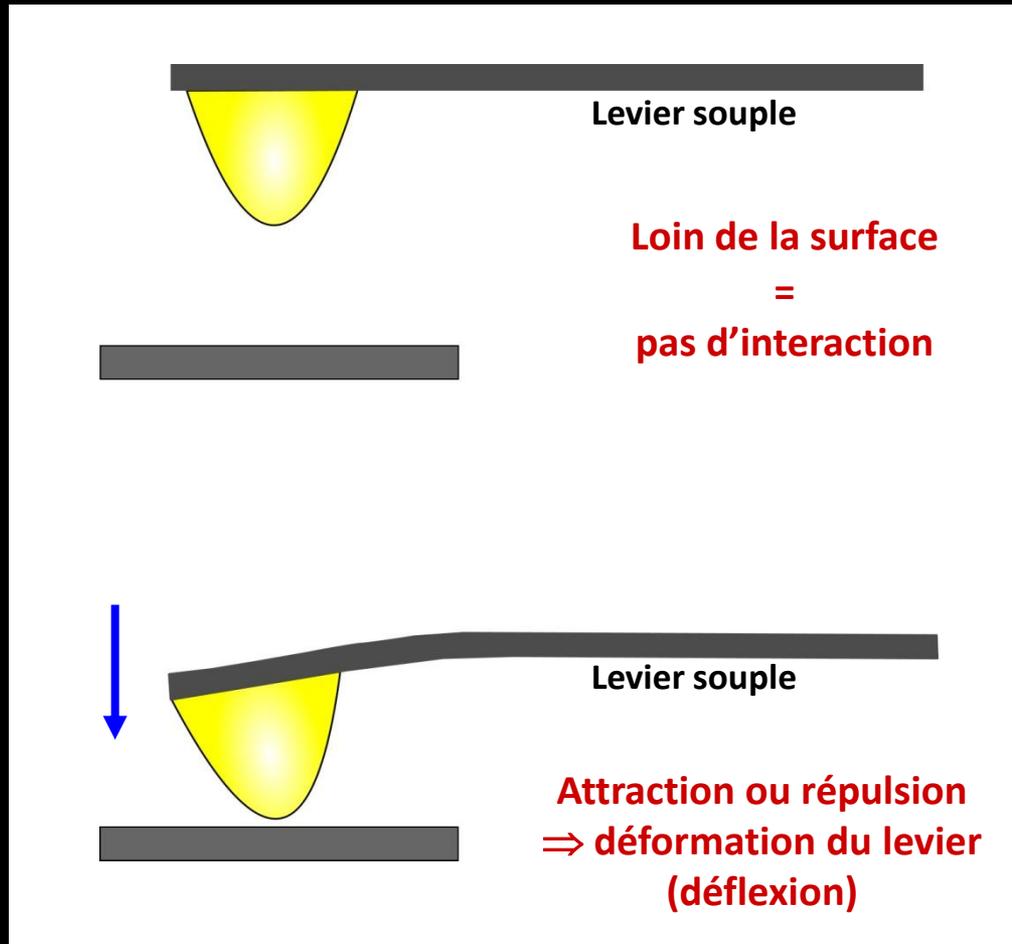
⇒ **Atomic Force Microscope (AFM)**

Microscopie à Force Atomique : principe

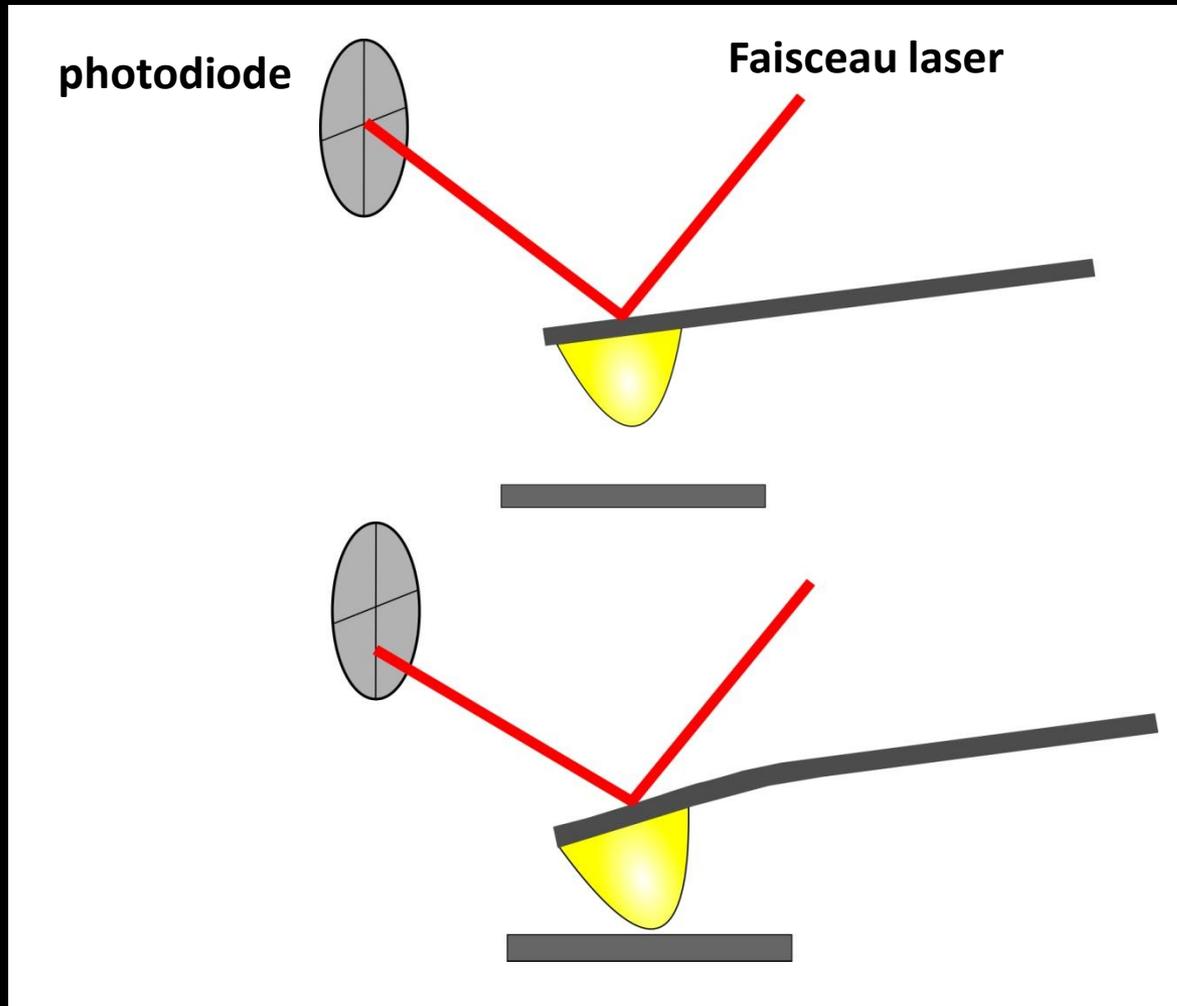


- Interaction de Van der Waals
- Forces électrostatiques
- Forces hydrophobes ...

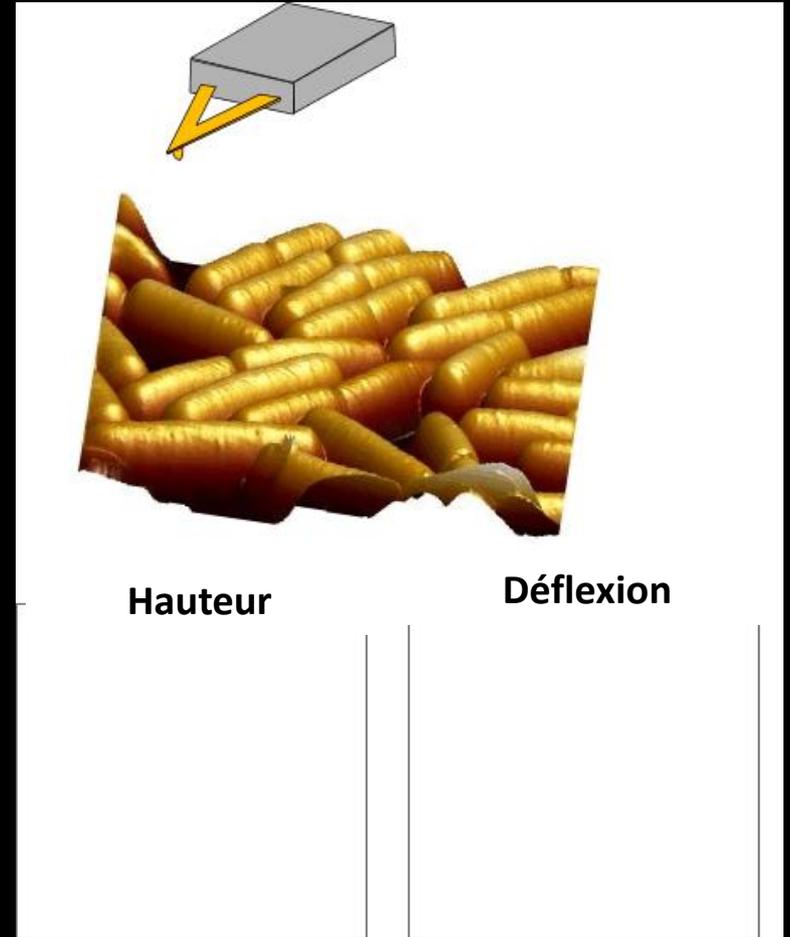
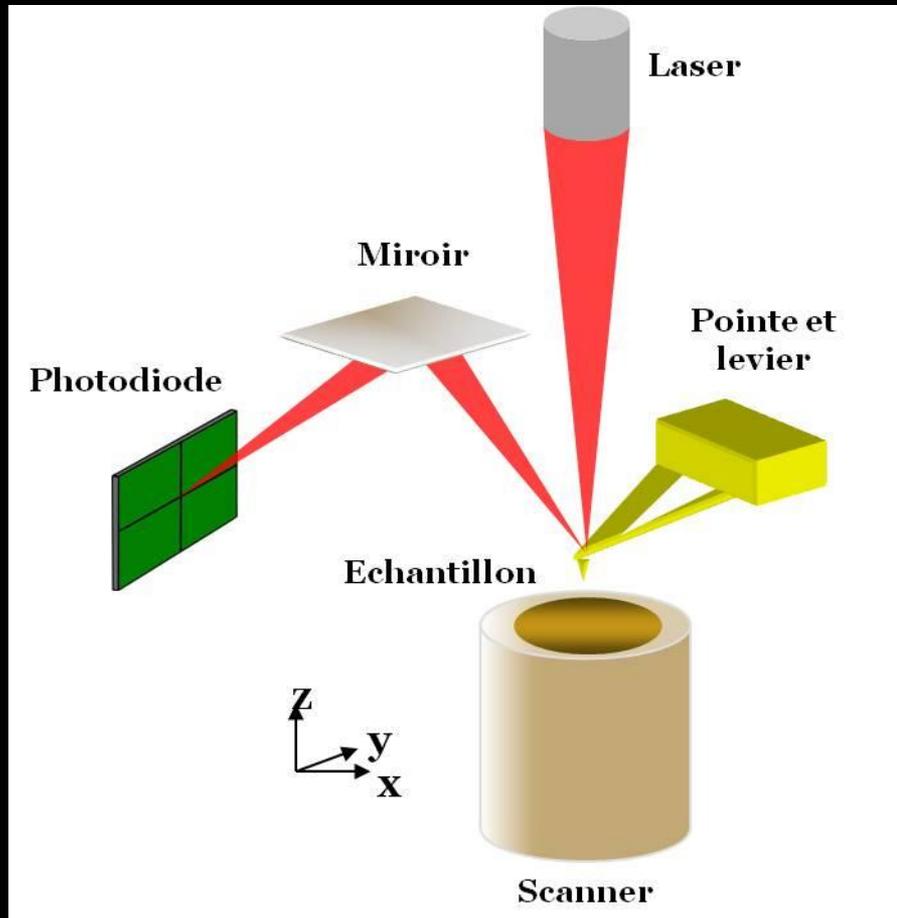
Microscopie à Force Atomique : principe



Microscopie à Force Atomique : principe

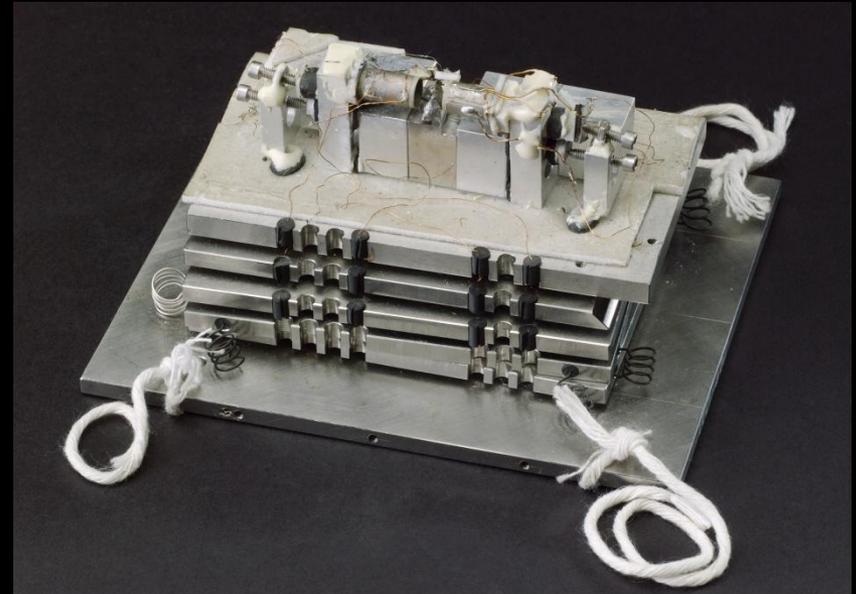
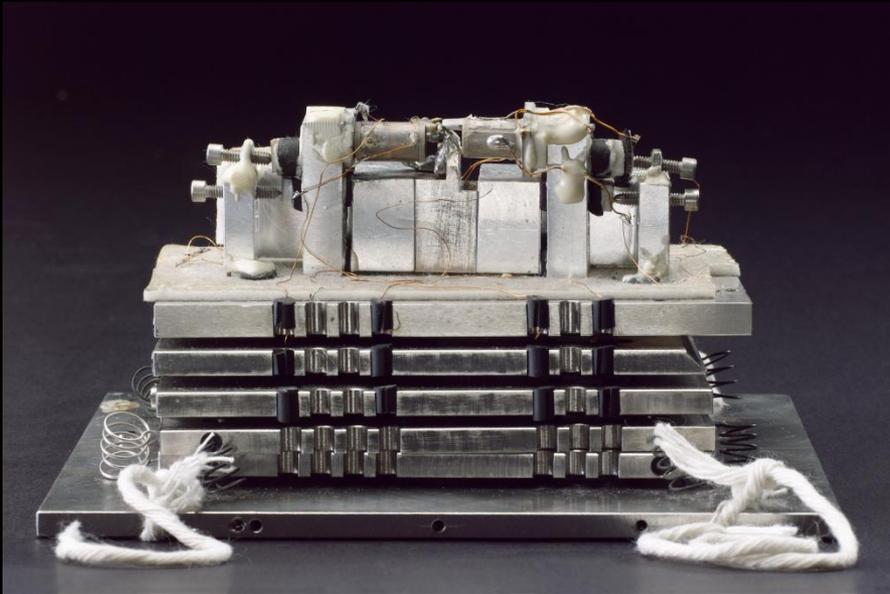


Microscopie à Force Atomique : principe



Microscopie à Force Atomique : principe

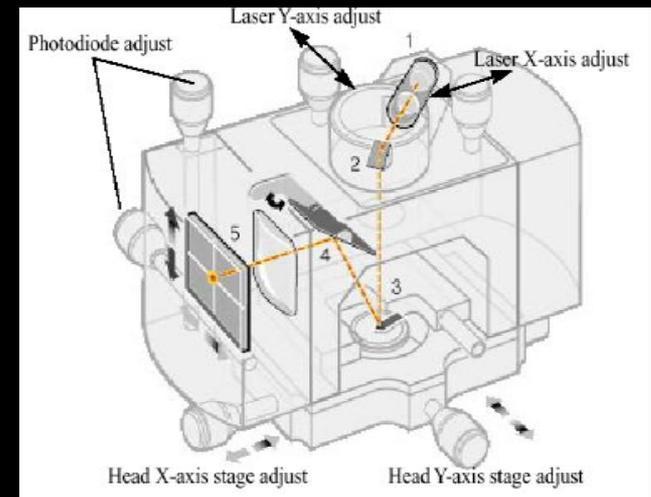
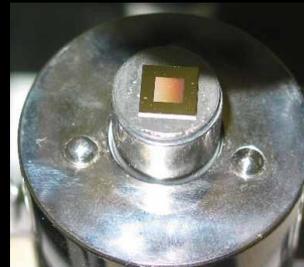
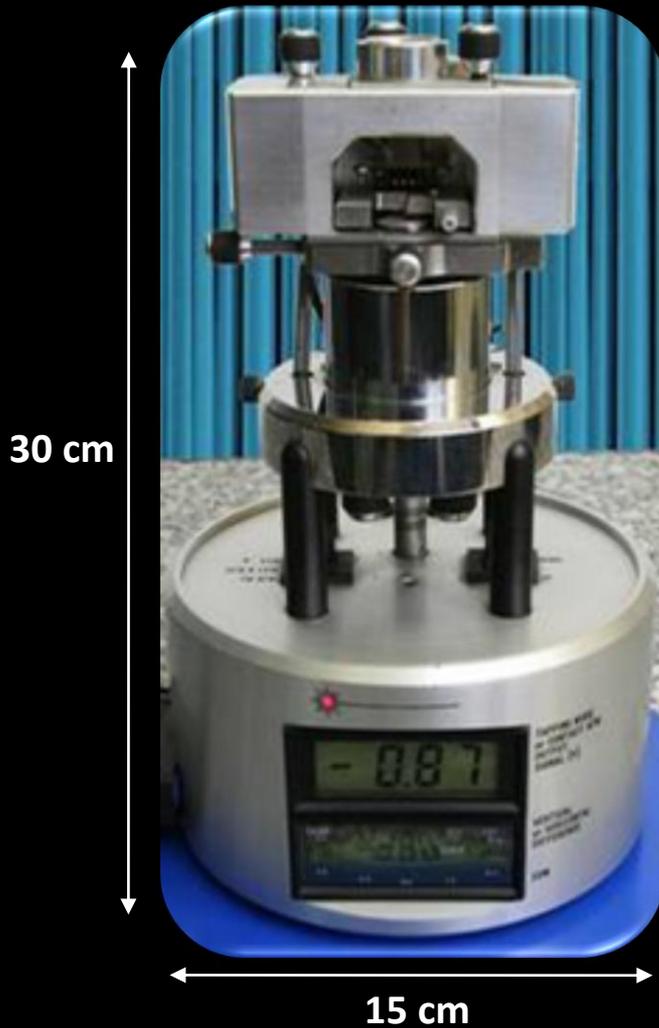
Le « premier » AFM



Binnig, Quate & Gerber, 1985

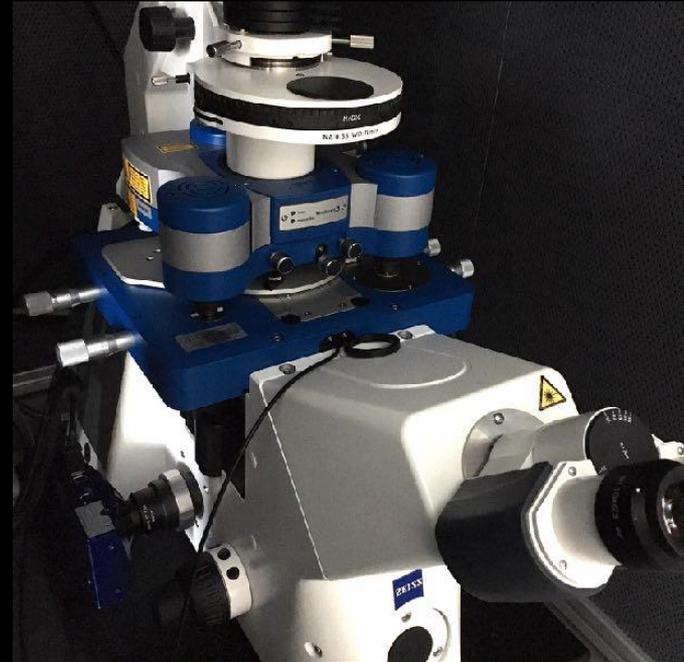
(from Science Museum, London)

Microscopie à Force Atomique : principe



JPK NanoWizard III AFM

→ Différents modèles pour différentes applications



AFM scanning head (xyz) and stage (z)

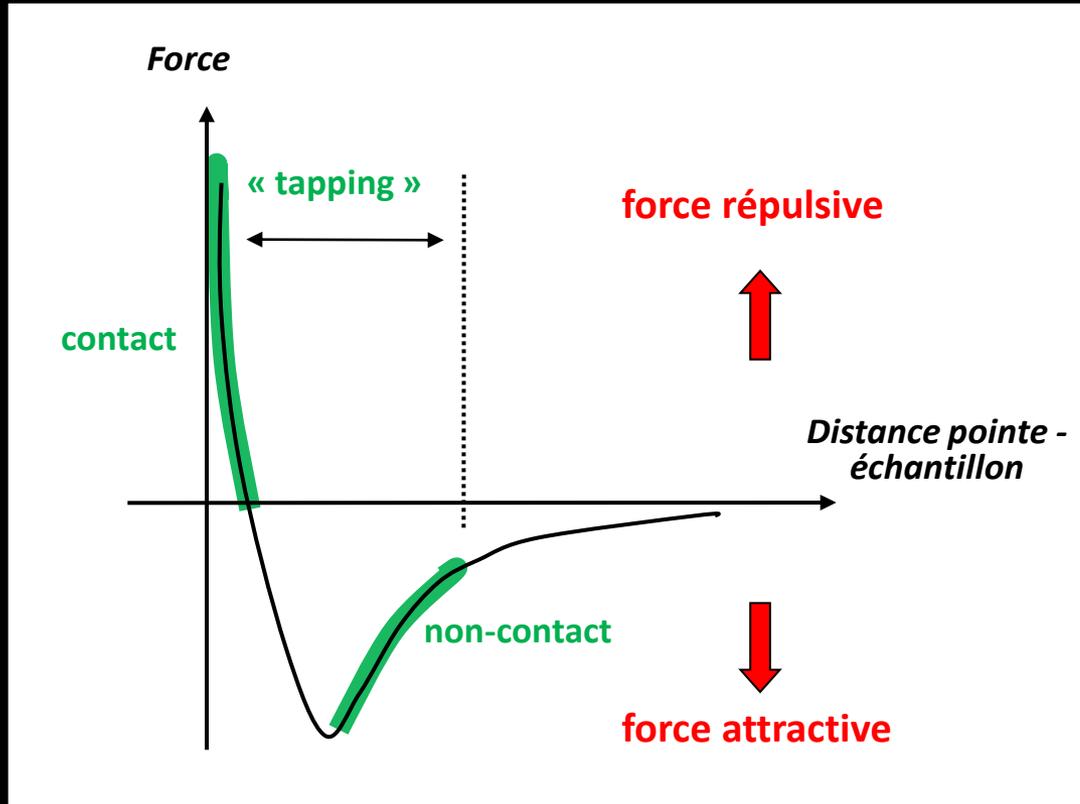


Petri dish heater



Cantilever holder

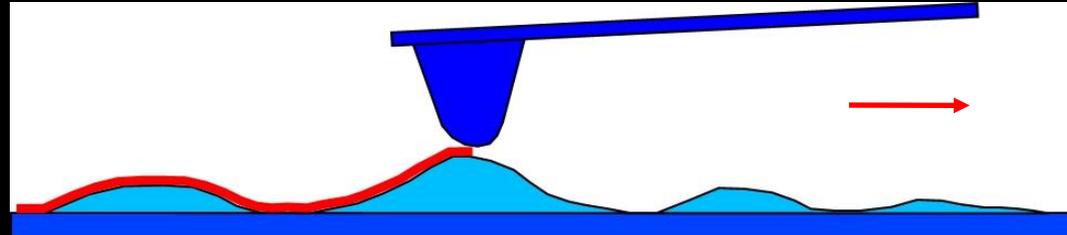
Les différents modes d'imagerie



Les différents modes d'imagerie

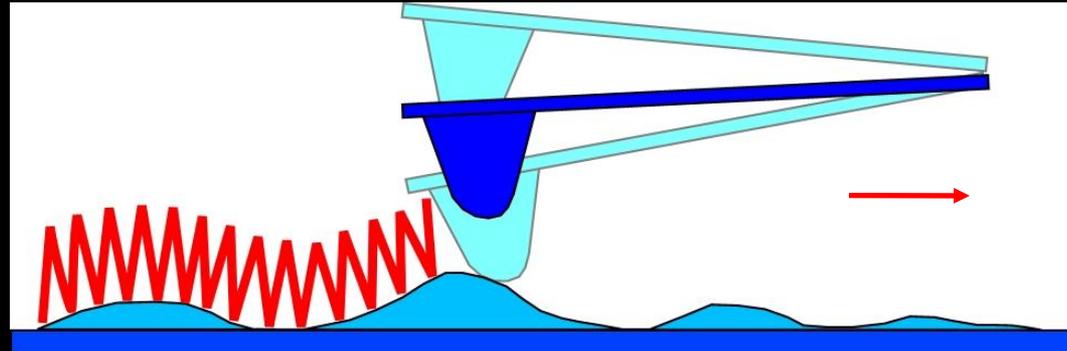
contact

$d < 5\text{\AA}$



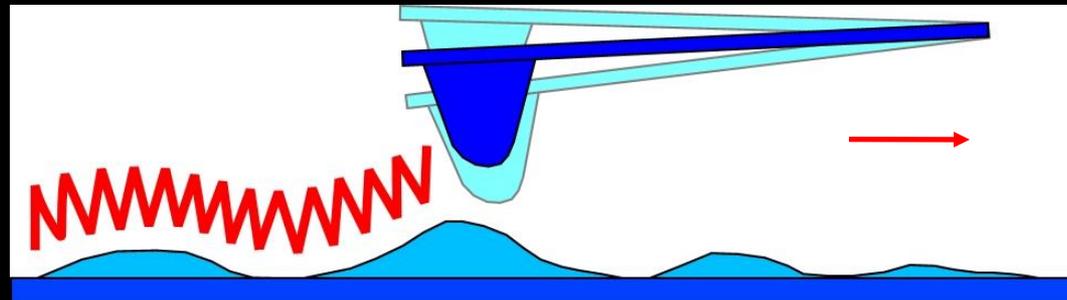
tapping

$d = 5 \text{ to } 20\text{\AA}$

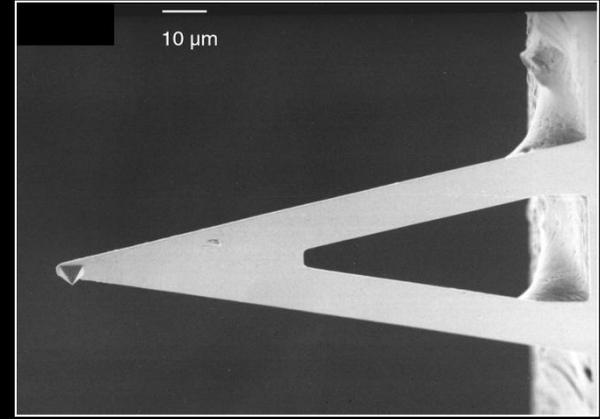
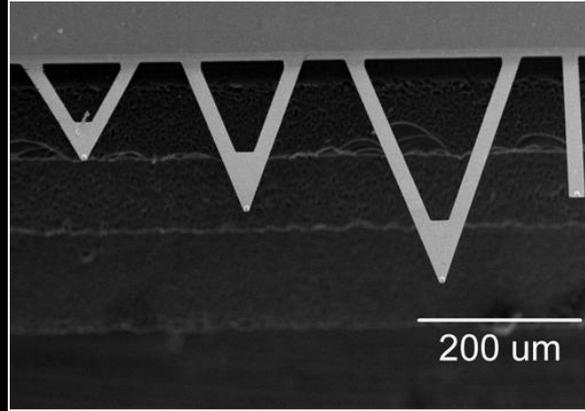
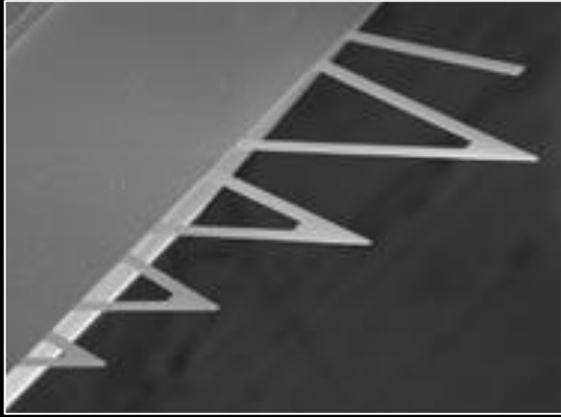


non-contact

$d = 10 \text{ to } 100\text{\AA}$



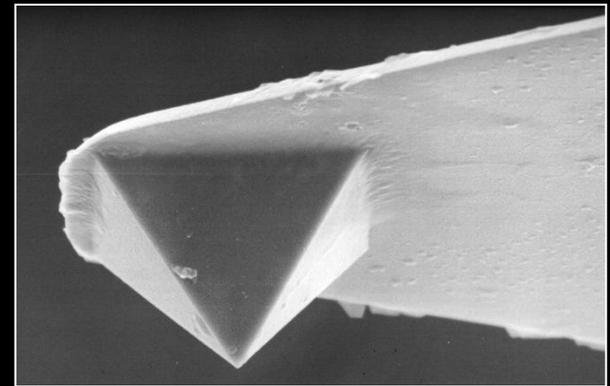
Leviers et pointes



1^{er} levier : $k = 0,01 \text{ N/m}$ $L = 320 \mu\text{m}$

2^e levier : $k = 0,03 \text{ N/m}$ $L = 220 \mu\text{m}$

3^e levier : $k = 0,1 \text{ N/m}$ $L = 140 \mu\text{m}$



⇒ Leviers souples afin de minimiser les interactions avec l'échantillon

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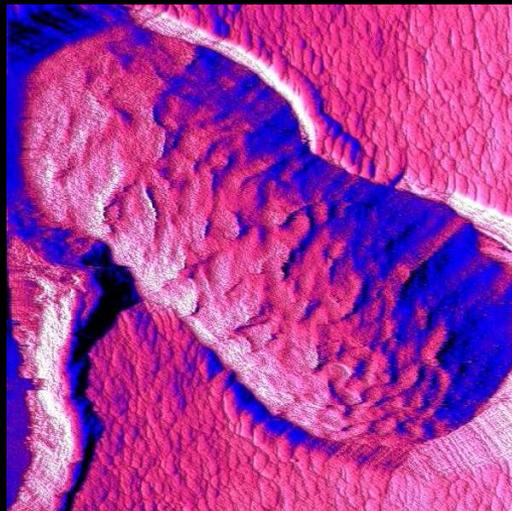
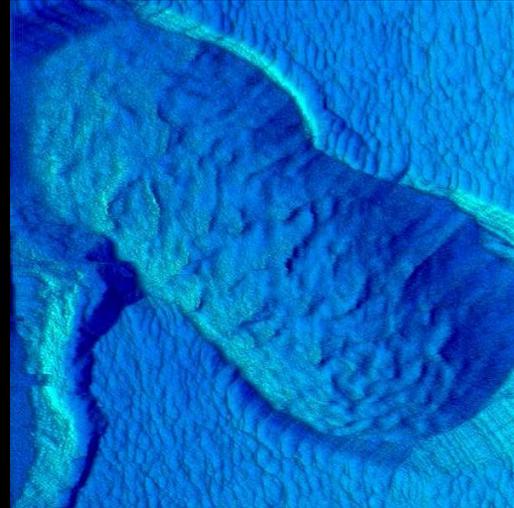
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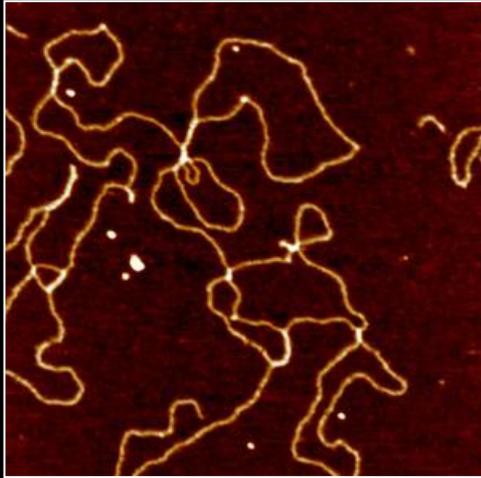
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3. Conclusions et perspectives

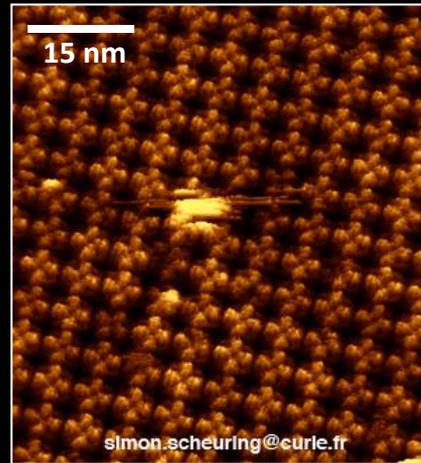
Des images en couleurs mais...



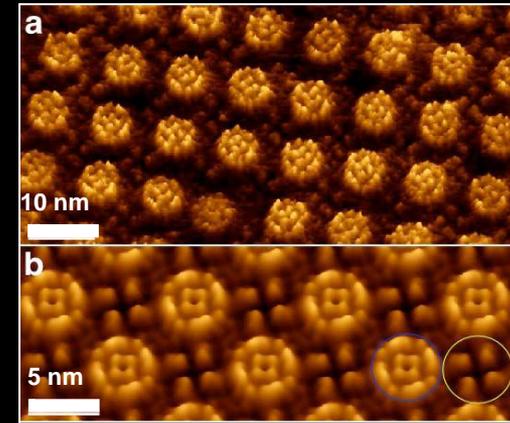
ADN et protéines



ADN adsorbé sur mica
 $1\ \mu\text{m} * 1\ \mu\text{m}$ (2 nm)



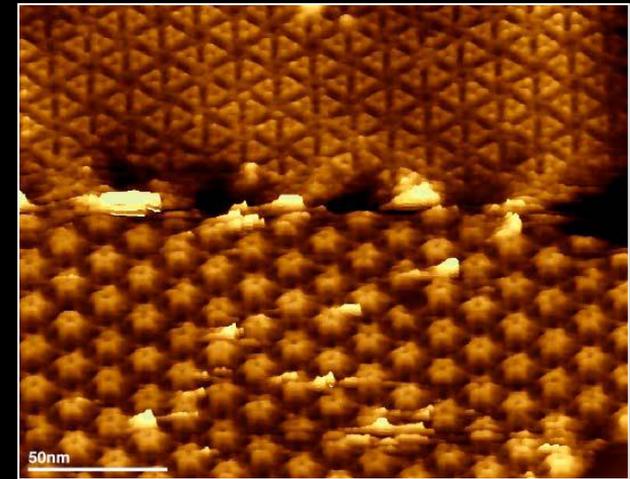
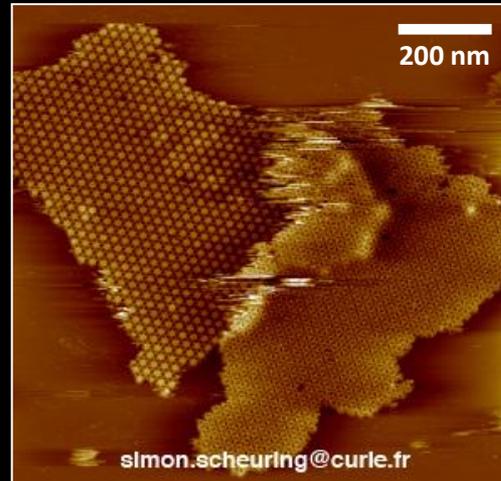
High-resolution image of the
extracellular surface of Aqp2.



High-resolution image of an AqpZ
2D crystal

Meilleure résolution
(protéines membranaires) :

- latérale : $\sim 0.5\text{-}0.7\ \text{nm}$
- verticale : $\sim 0.1\ \text{nm}$



S layer (*Corynebacterium glutamicum* sur mica)

Pointe et protéine (Simon Scheuring – NY)

Protéine

~ 3nm = $3 \cdot 10^{-9}$ m

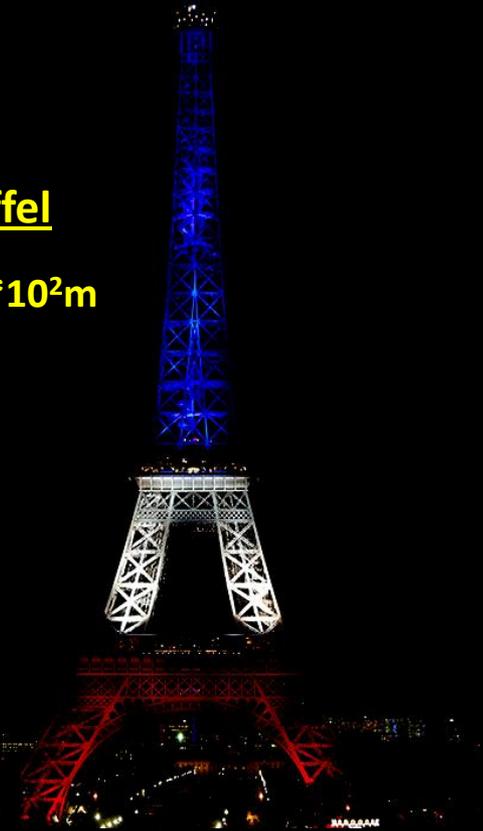


Basket-ball

~ 3dm = $3 \cdot 10^{-1}$ m

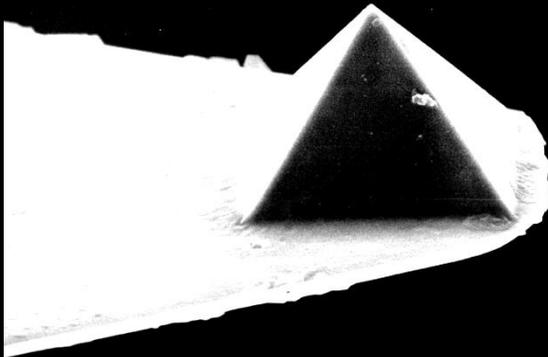
Tour Eiffel

~ 300m = $3 \cdot 10^2$ m



Pointe AFM

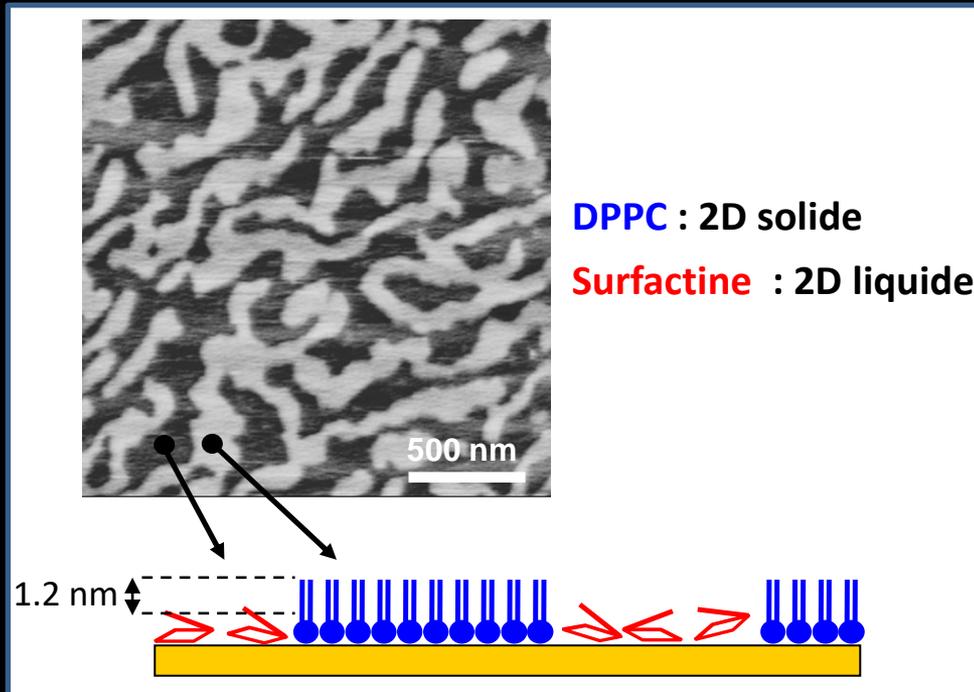
~ 3 μ m = $3 \cdot 10^{-6}$ m



Pointe / protéine
=
Tour Eiffel / basket-ball
~ 1000

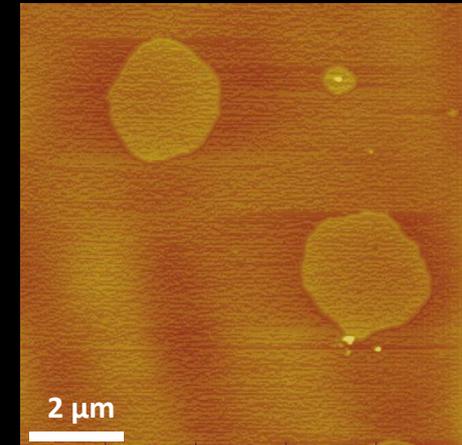
Bicouche lipidique

→ antibiotiques (Fluoroquinolones)

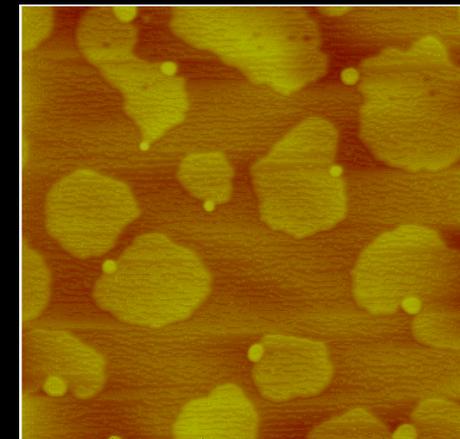


Deleu *et al.*, *Biophys. J.*, **77**, 2304 (1999)

DPPC / DOPC

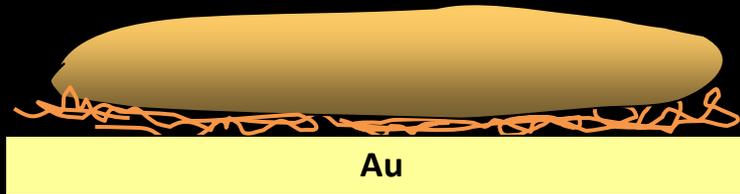


+ fluoroquinolone



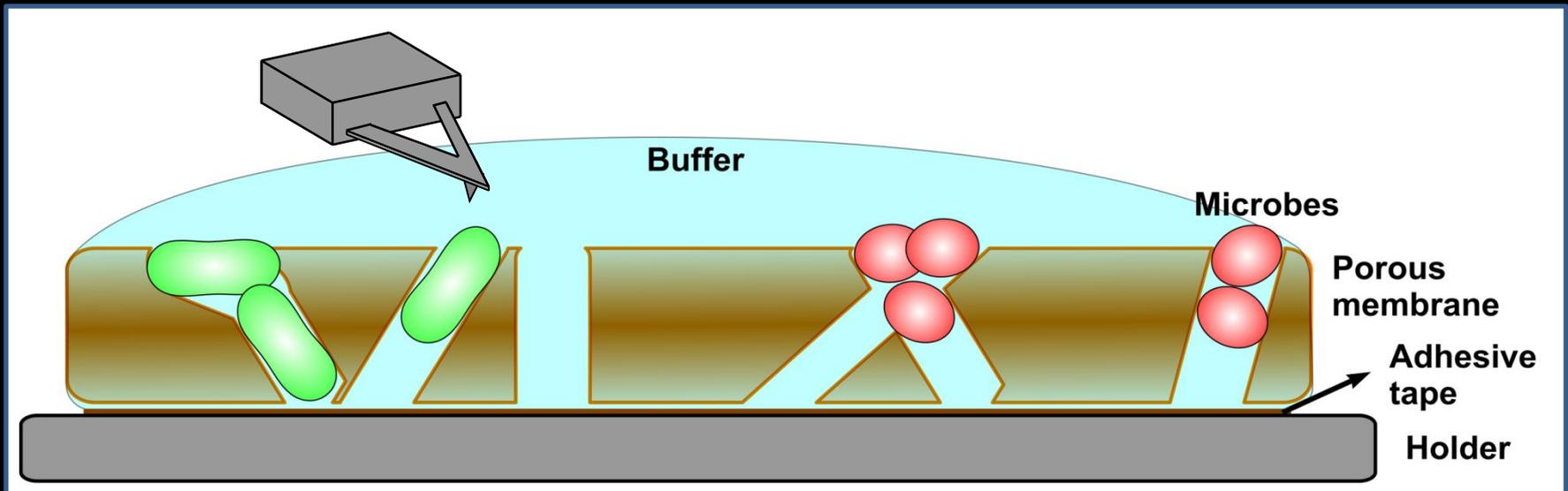
Immobilisation des cellules

→ Fixation chimique



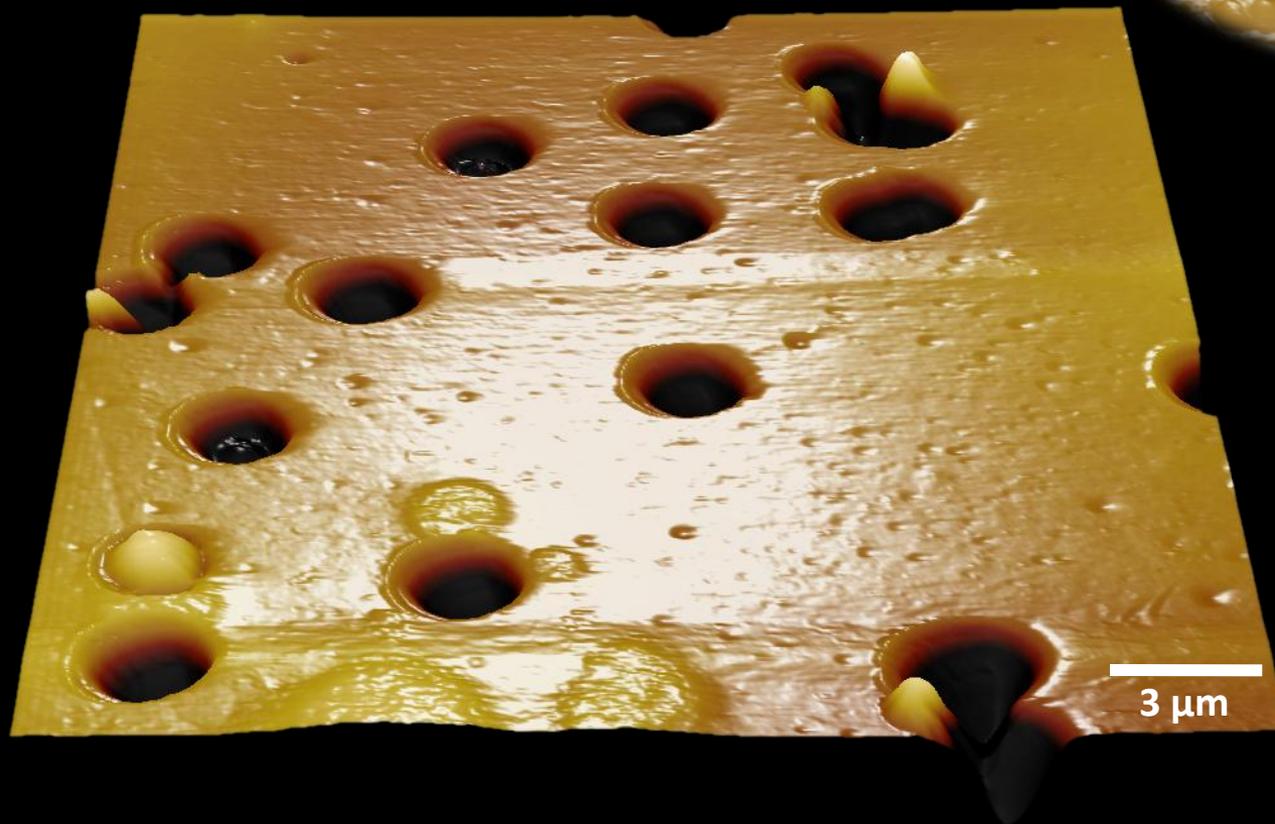
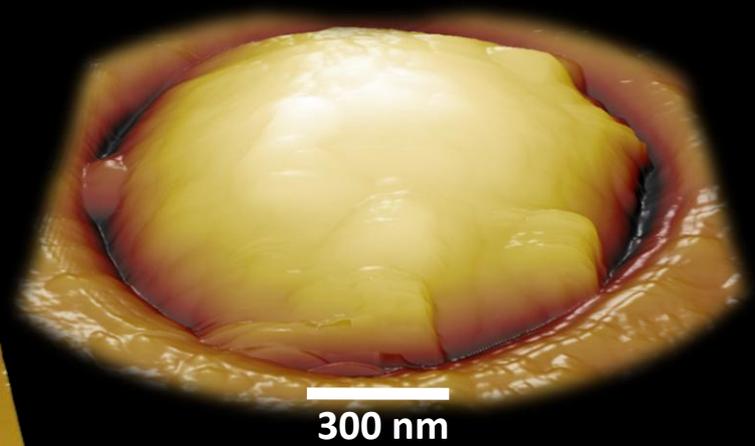
Poly-L-lysine,
Glutaraldéhyde,
Polyéthylène imine (PEI), ...

→ Piégeage mécanique dans des membranes microporeuses



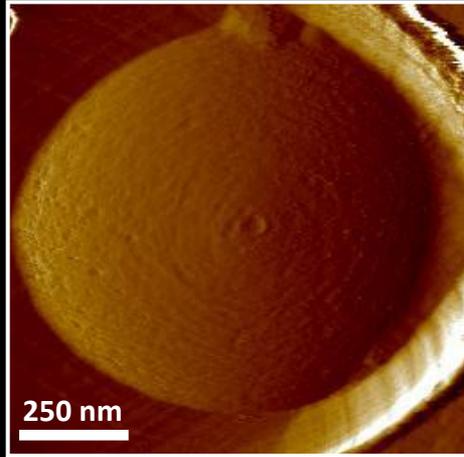
→ Pas de traitement chimique (contamination de la pointe)

→ Différents diamètres (0,8 → 12 μm)

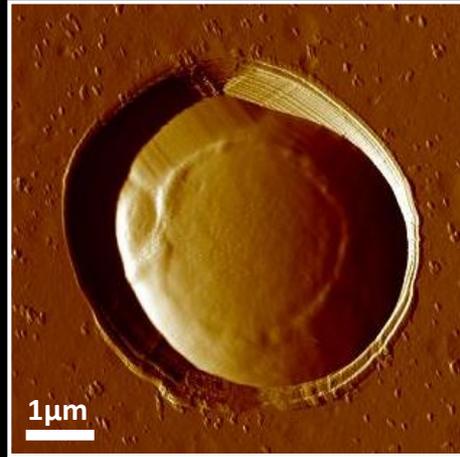


Quelques exemples

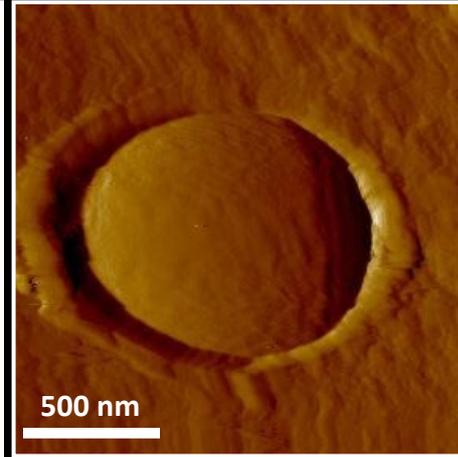
Lactococcus lactis



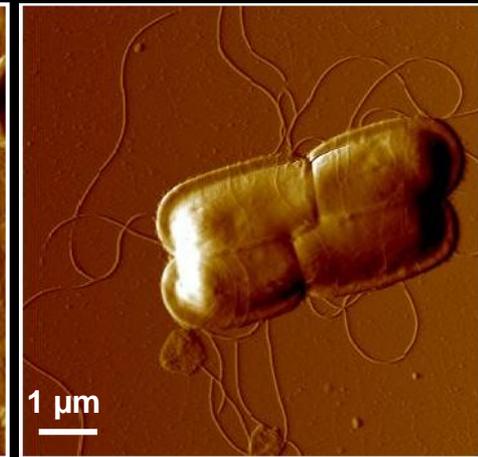
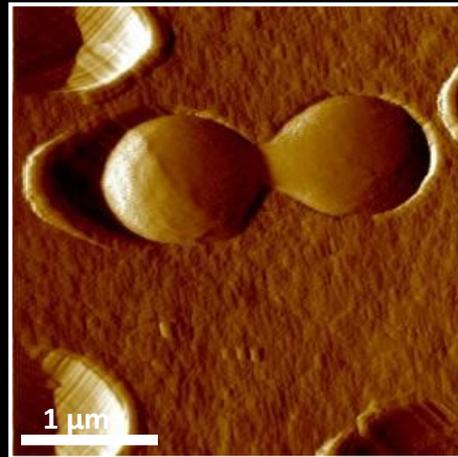
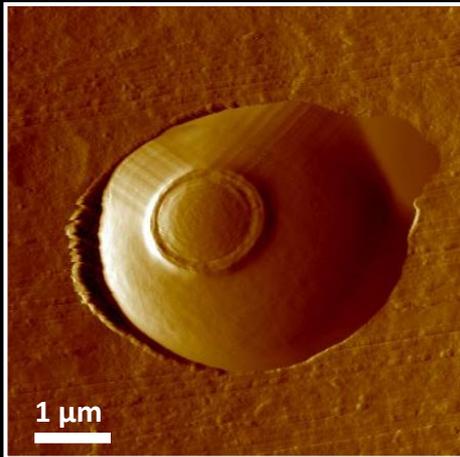
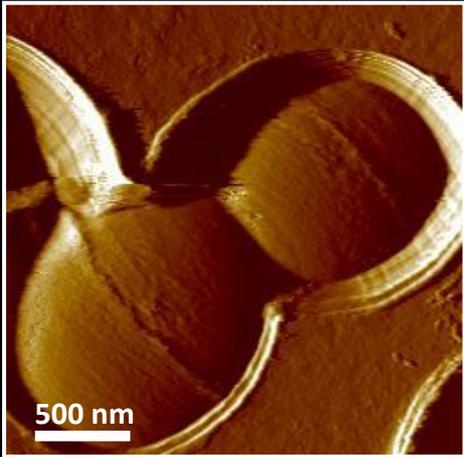
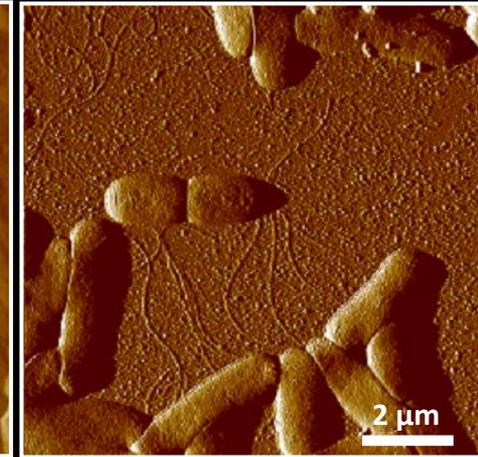
S. cerevisiae



S. aureus



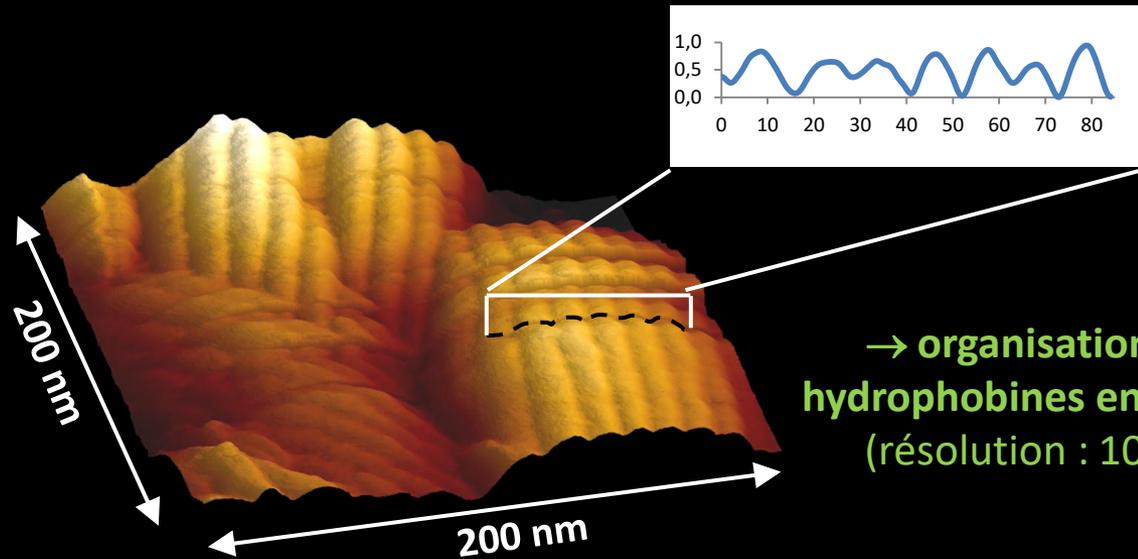
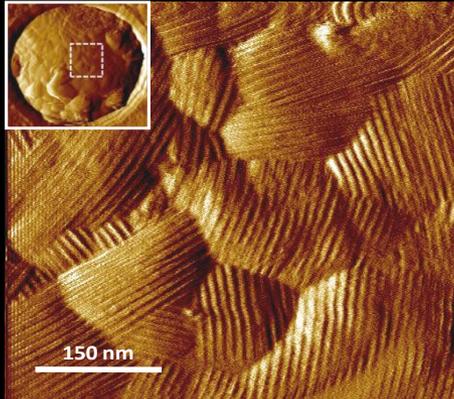
E.Coli (air)



B.thuringiensis (air)

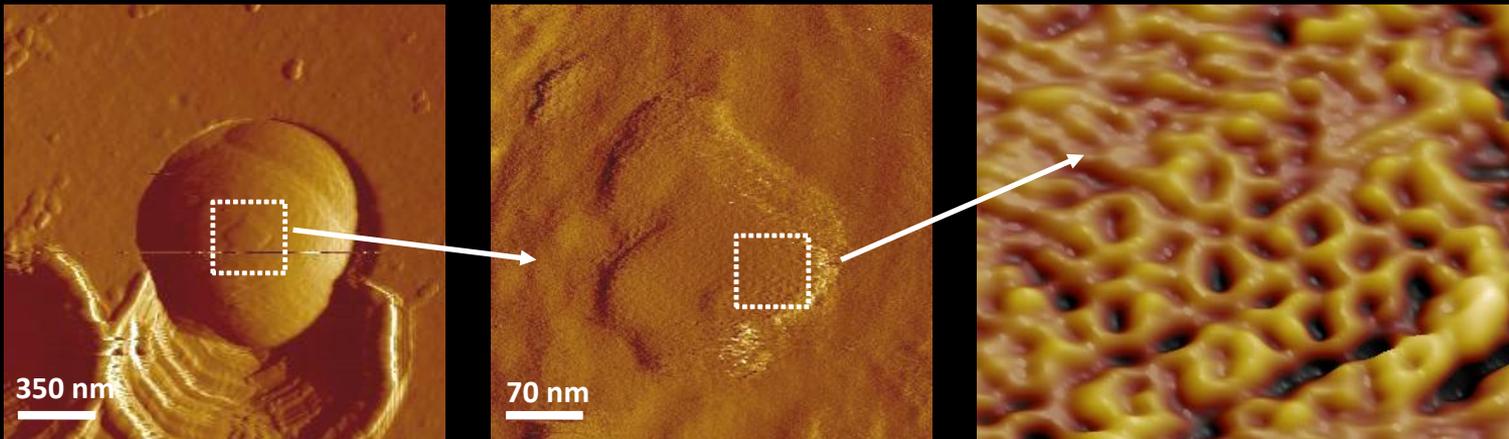
Ultrastructure

Aspergillus fumigatus



→ organisation des hydrophobines en tiges // (résolution : 10 nm)

Imagerie in vivo d'une S-layer chez *Corynebacterium glutamicum*

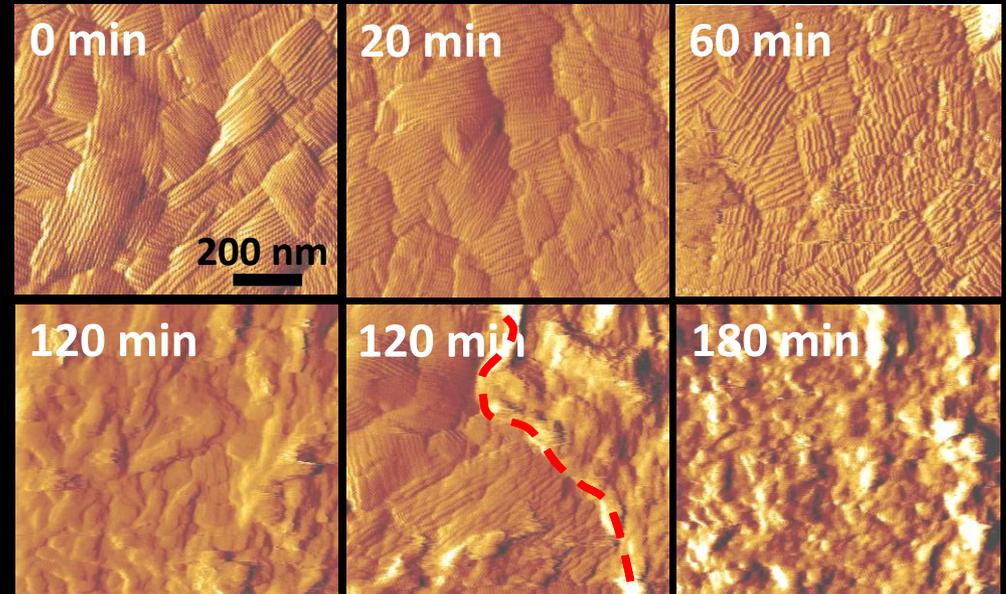
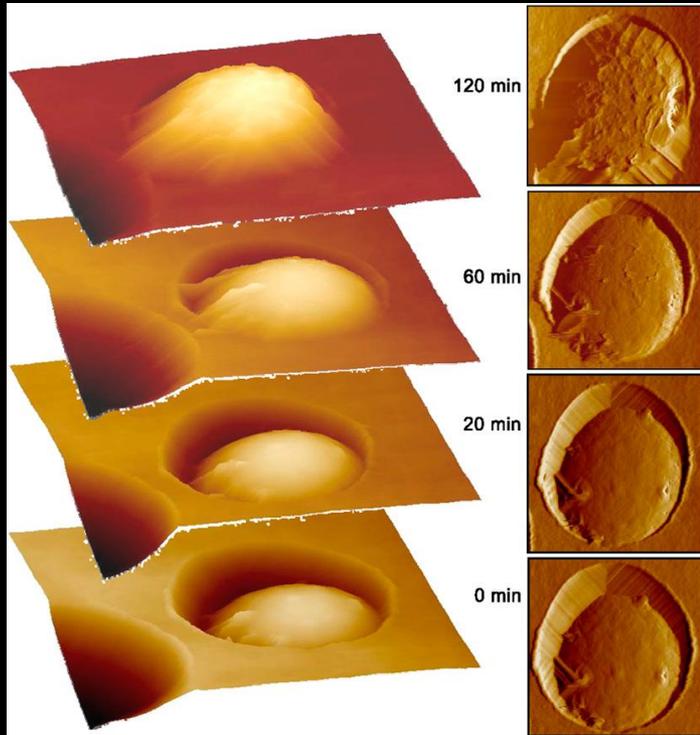


Couche externe: protéines organisées en une S-layer hexagonale (périodicité de 16 nm)

Suivi de la dynamique de la surface cellulaire

Aspergillus fumigatus

Germination en temps réel, 37°C

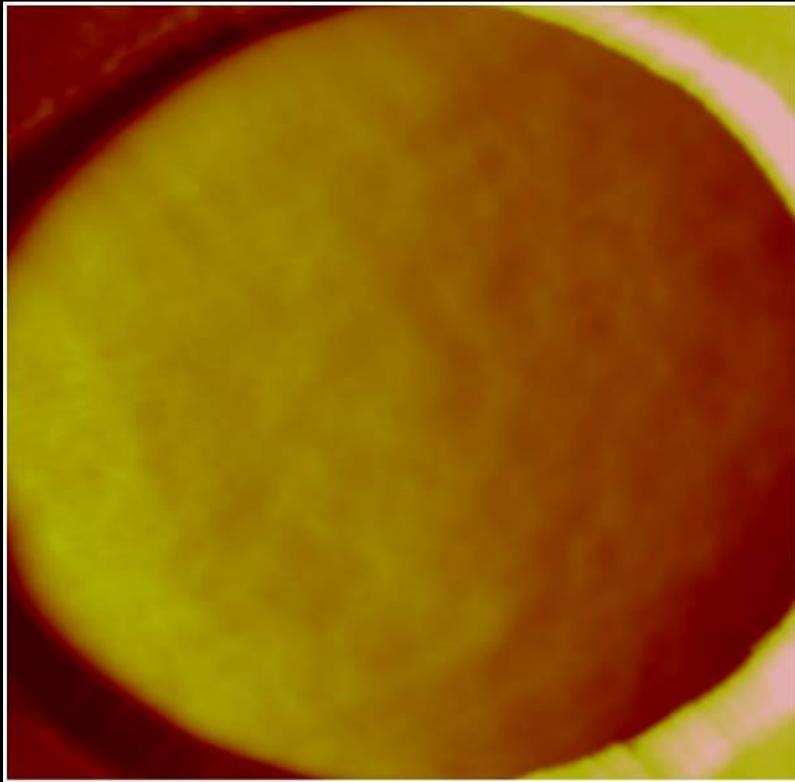


- Gonflement de la spore
- Altérations de l'ultrastructure

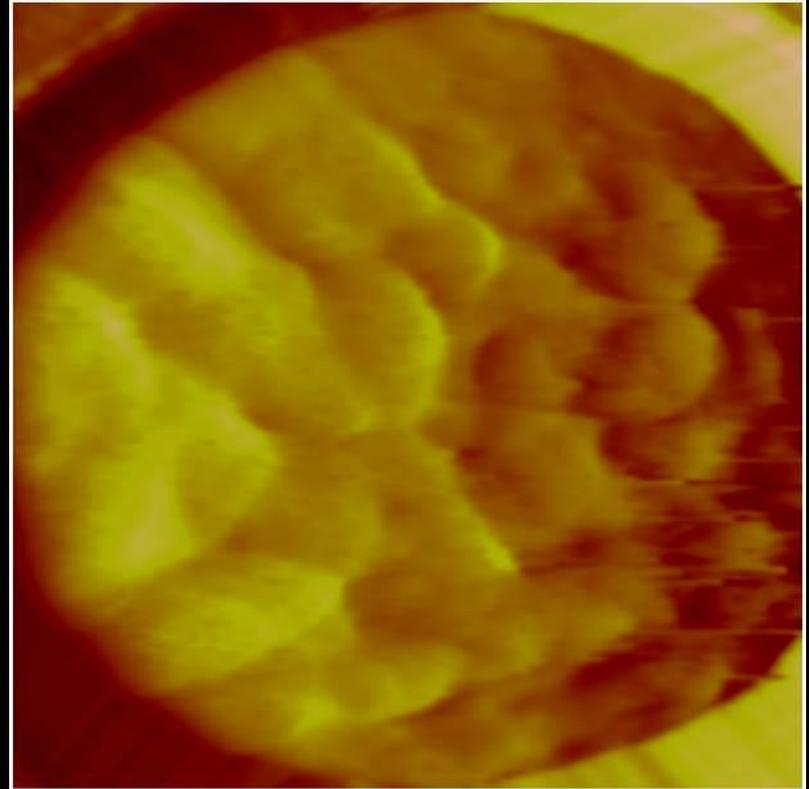
Imagerie *in situ* de la digestion par une protéase

(levure + protéase)

0 min

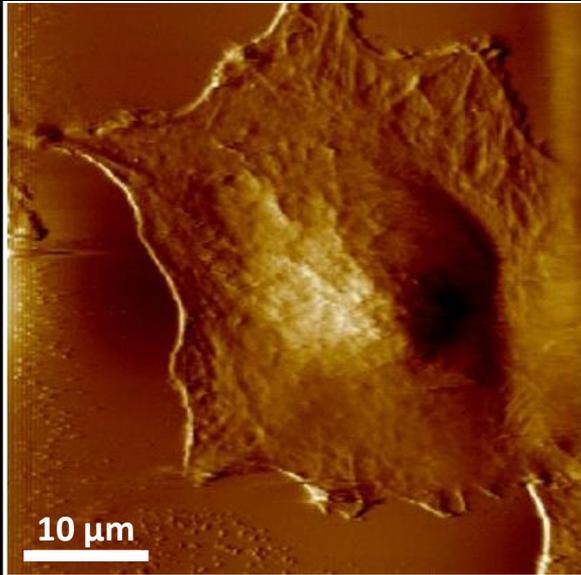


après 60 min

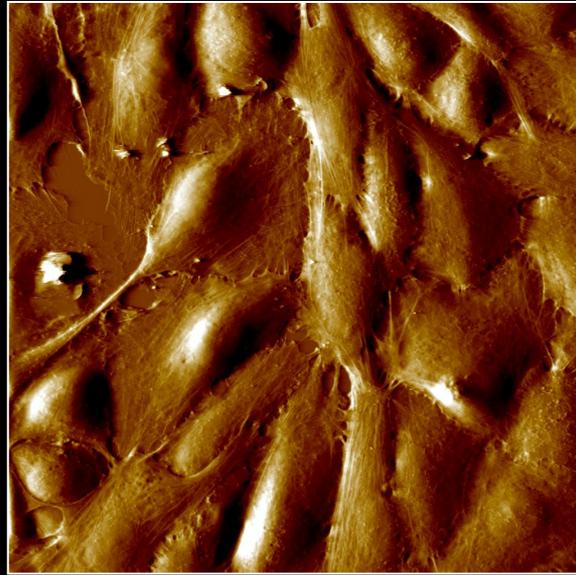


1 μm

Cellules eucaryotes

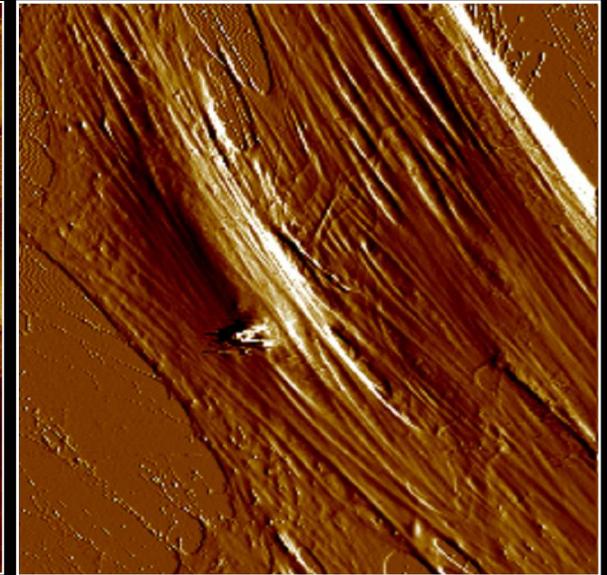


Pneumocytes, A549



Cellules endothéliales

180 * 180 μm²
(Veeco Instruments Inc)



Fibroblastes

120 * 120 μm²

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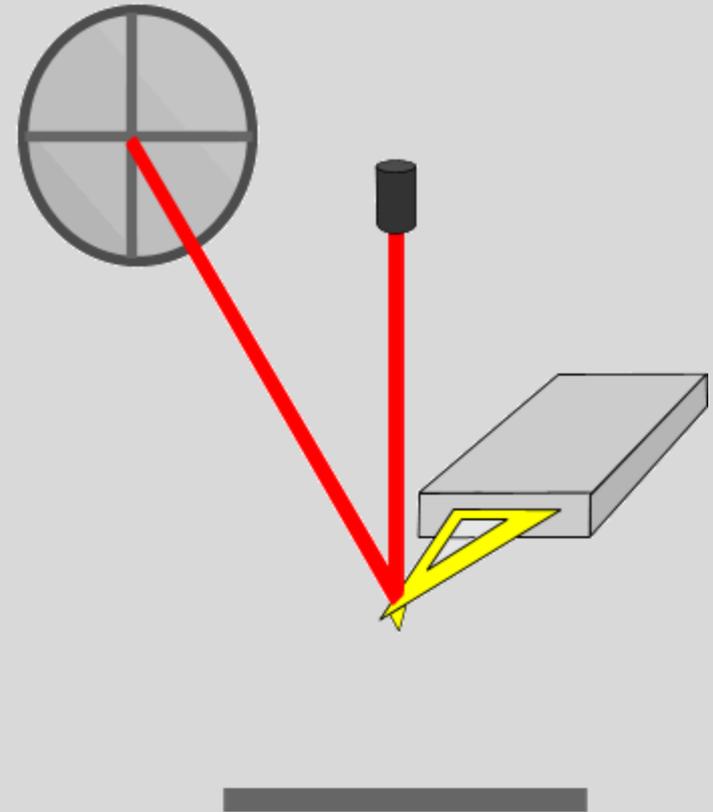
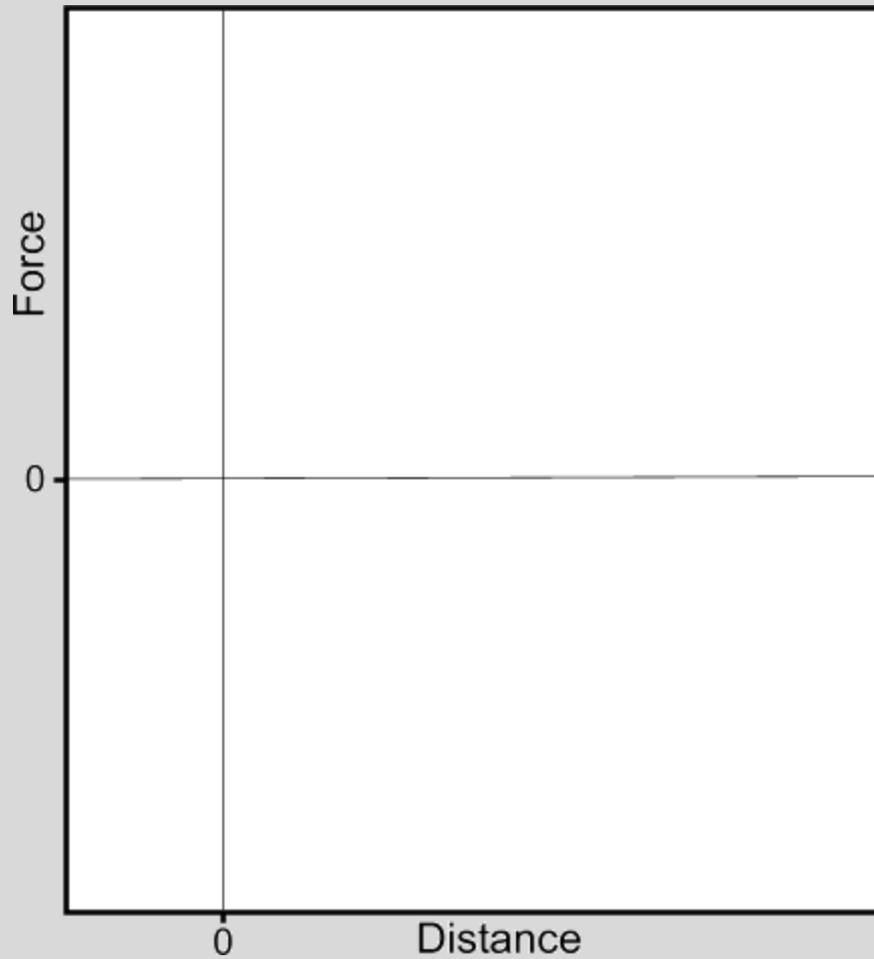
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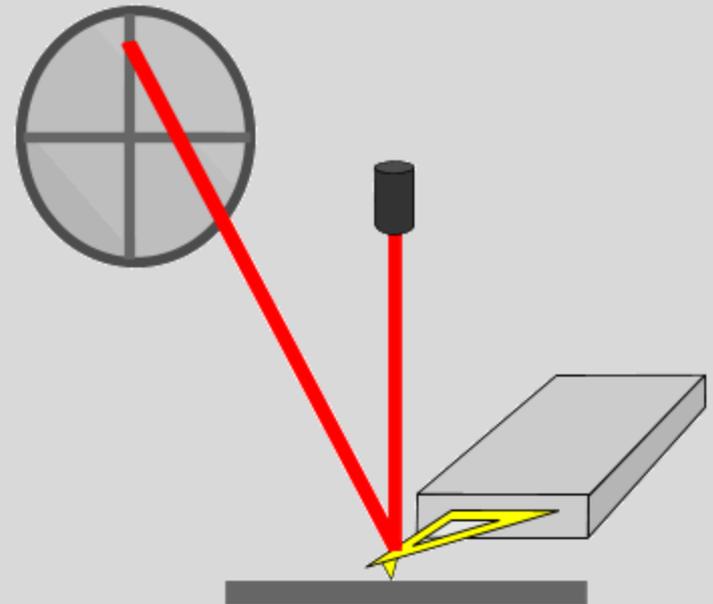
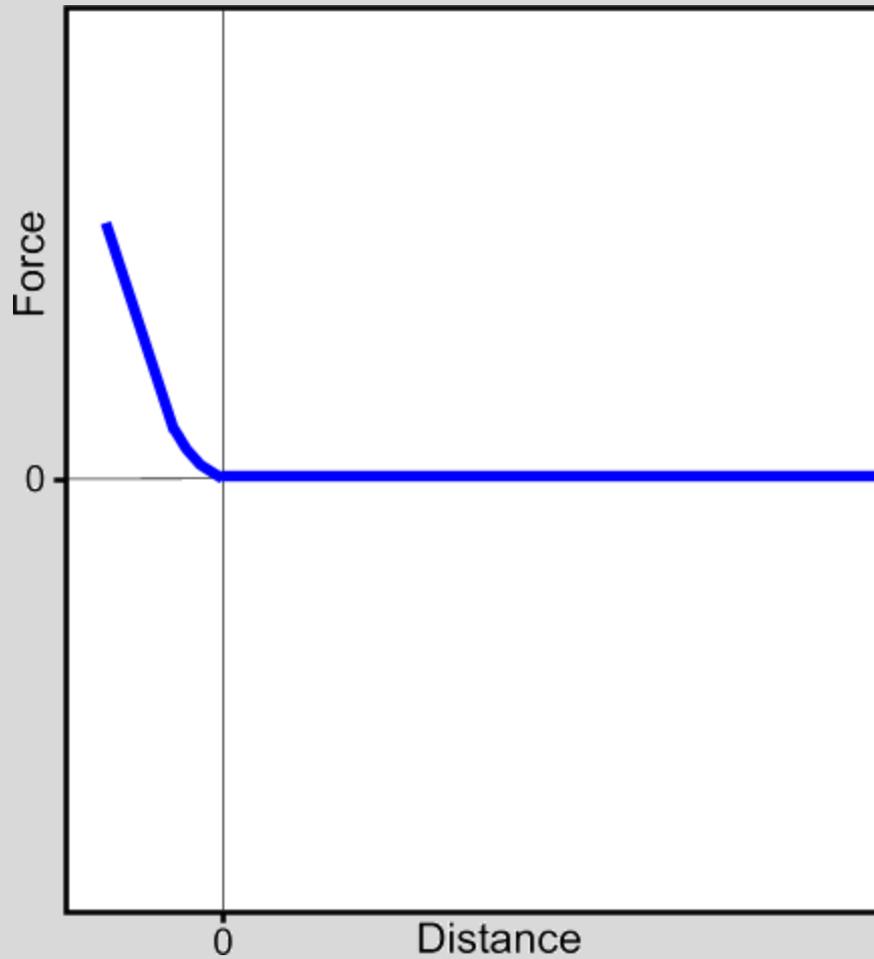
Spectroscopie de force

→ Courbe force-distance (approche)



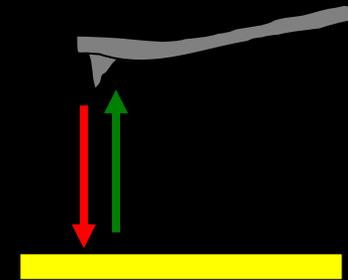
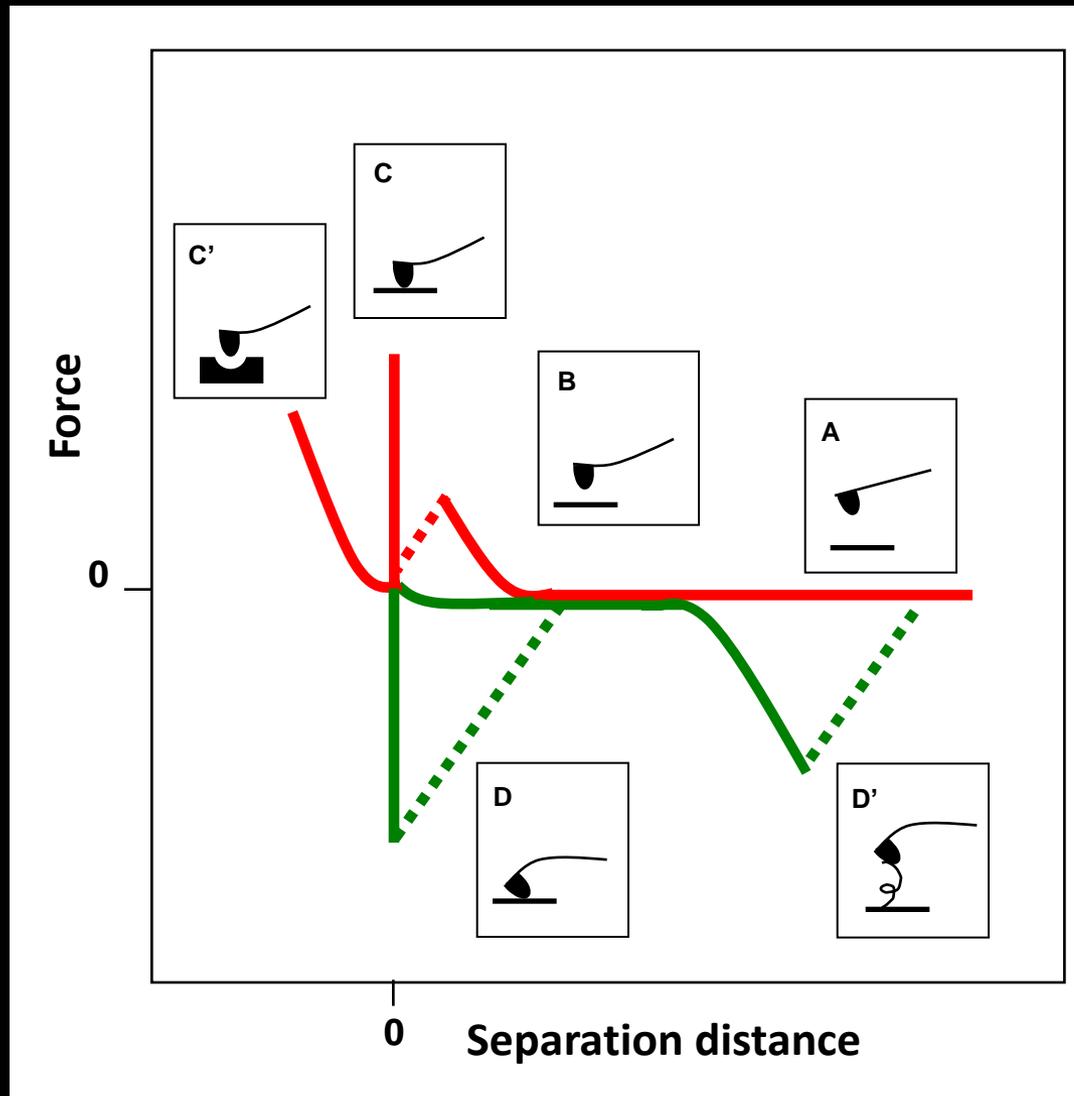
Spectroscopie de force

→ Courbe force-distance (retrait)



Spectroscopie de force

→ Courbe force-distance



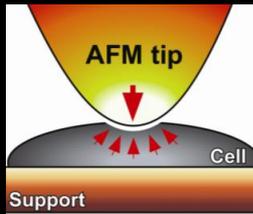
← approche

→ retrait

Spectroscopie de force

Deux courbes :

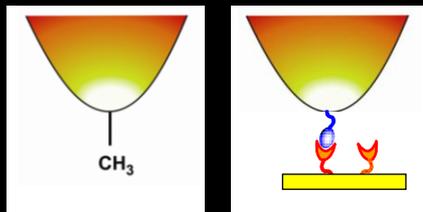
Courbe d'approche
⇒ propriétés mécaniques



Elasticité (module de Young)

Viscosité (viscoélasticité)

Courbe de retrait
⇒ propriétés physico-chimiques



Manipulation et dépliement

Hydrophobicité (CFM)

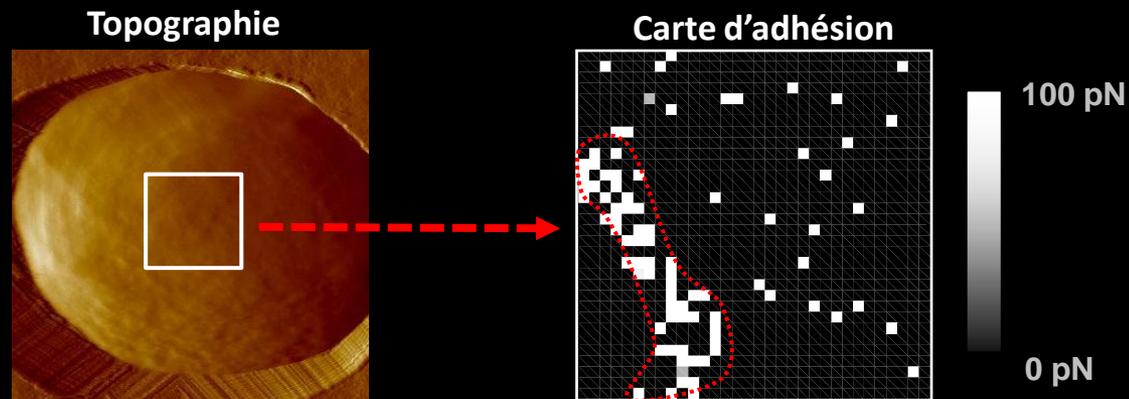
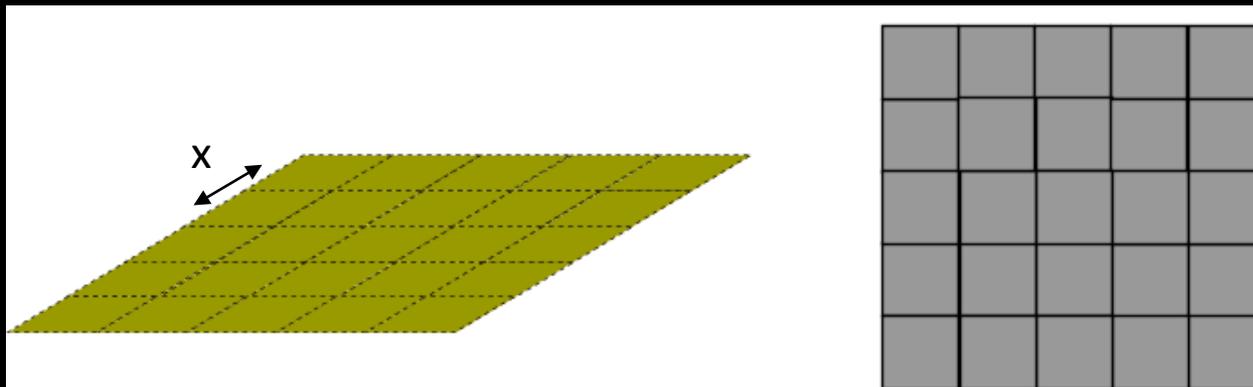
Reconnaissance biomoléculaire
(récepteur-ligand)

Conformation des polymères
(élasticité, longueurs)

Carte d'adhésion

- Nombre de récepteurs
- Distribution

→ Mode Force Volume



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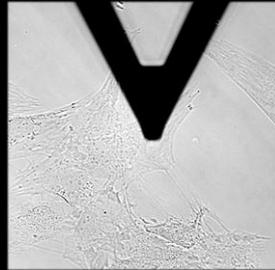
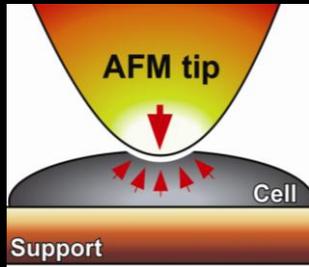
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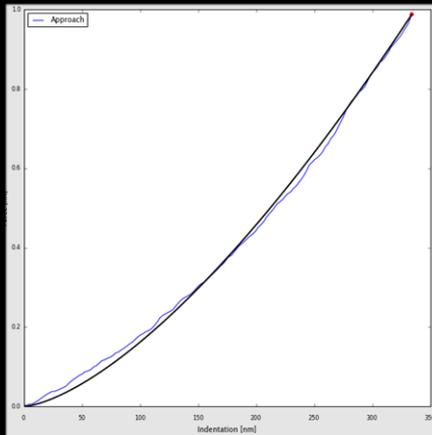
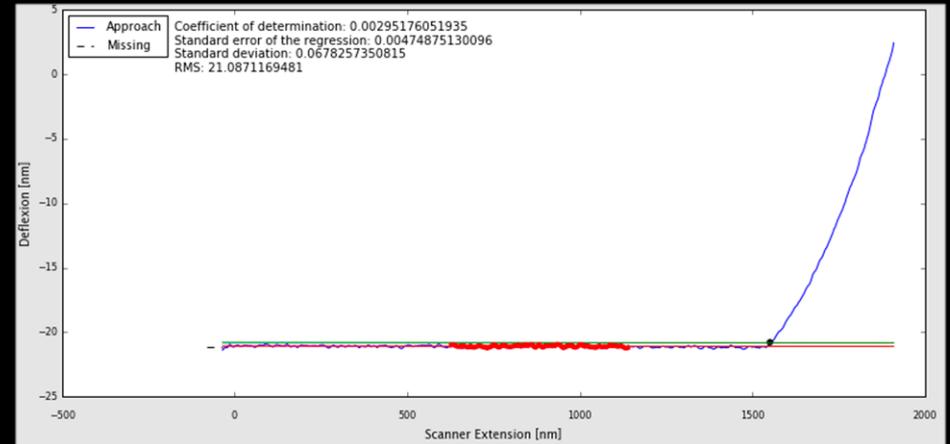
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Propriétés mécaniques : Module d'Young



Courbe de force vs indentation



Hertz model

$$F = \frac{4}{3} \frac{E}{1-\nu^2} \sqrt{R\delta^3}$$

F : force appliquée

R : rayon de courbure

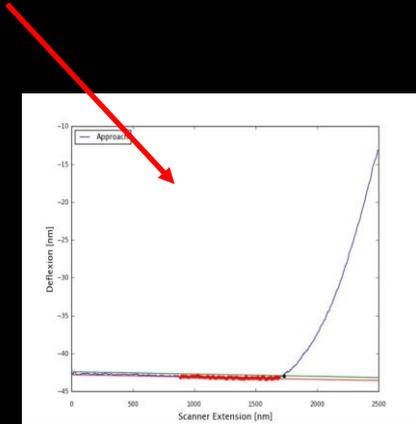
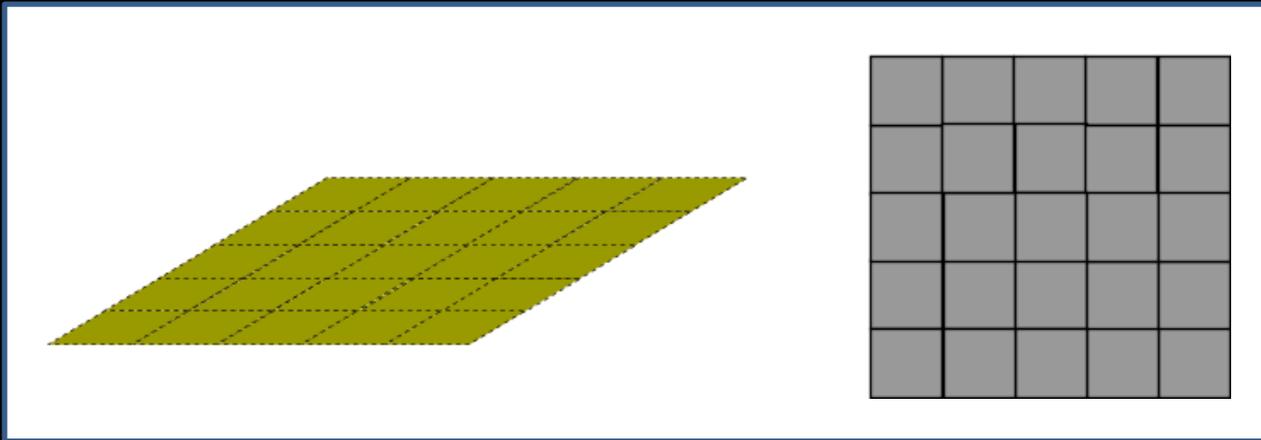
δ : indentation

E : module élastique

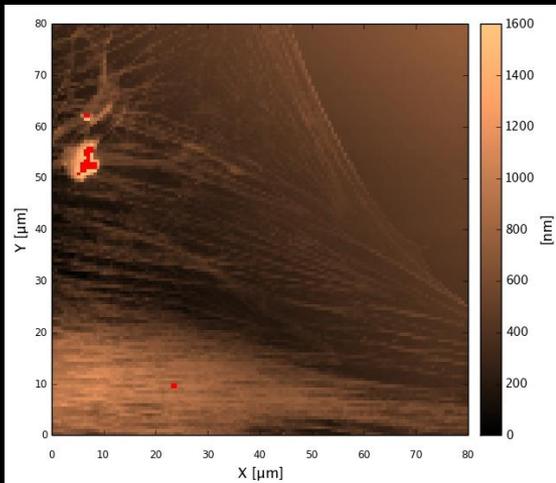
ν : coefficient de Poisson

→ Module élastique E (kPa)

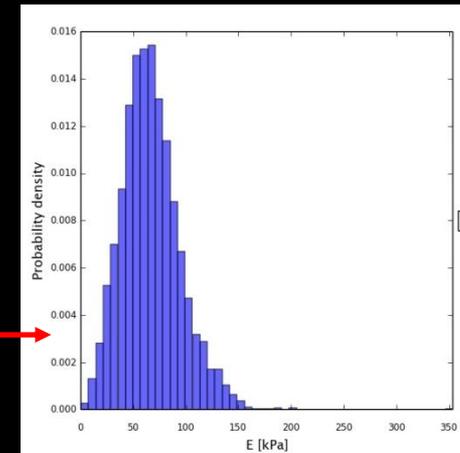
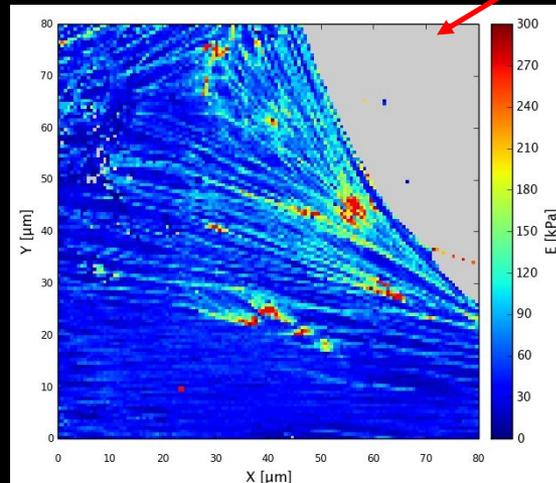
Cartographie des propriétés mécaniques



Topographie

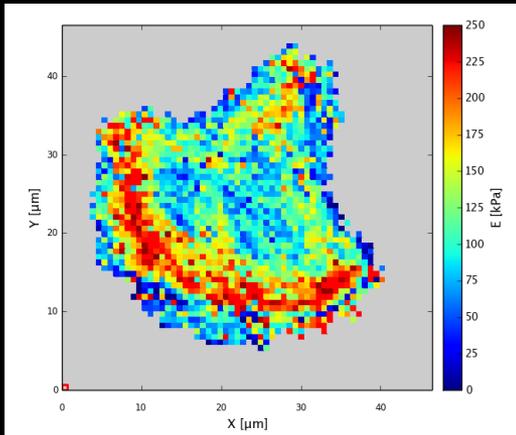


Carte d'élasticité

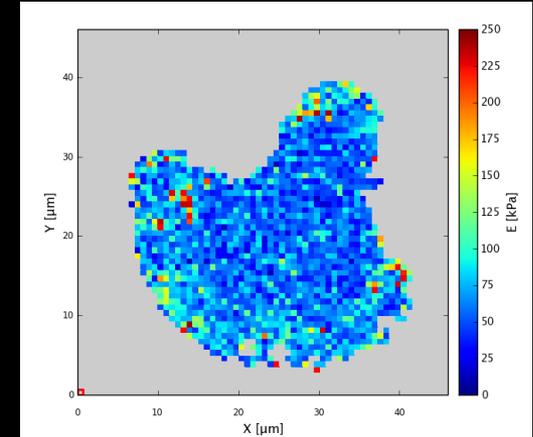
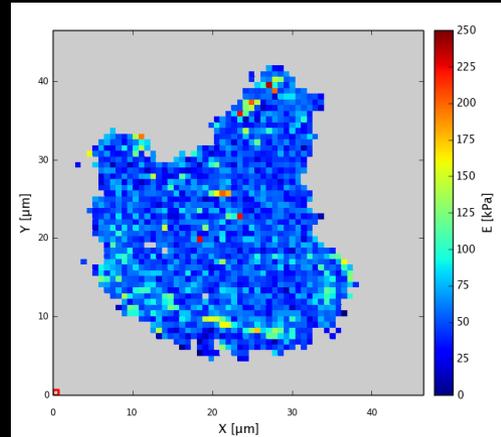


Cartographie des propriétés mécaniques

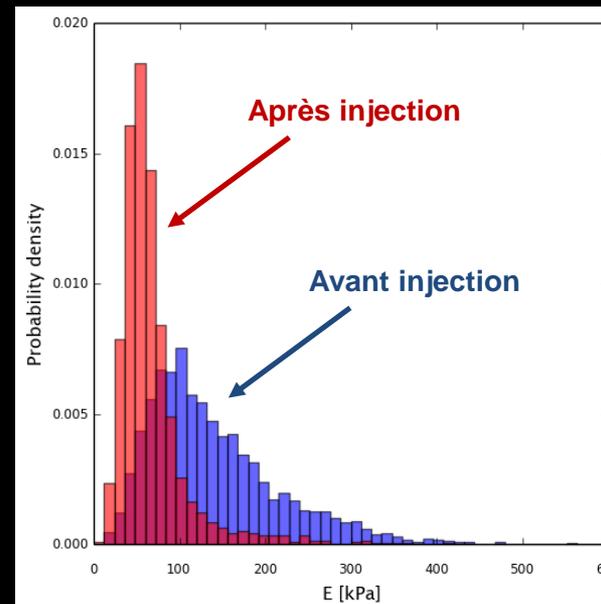
Après injection de latrunculine A (0.2 μ M)



Cellules RPE1



Latrunculine A : toxine qui déstabilise les filaments d'actine dans la cellule

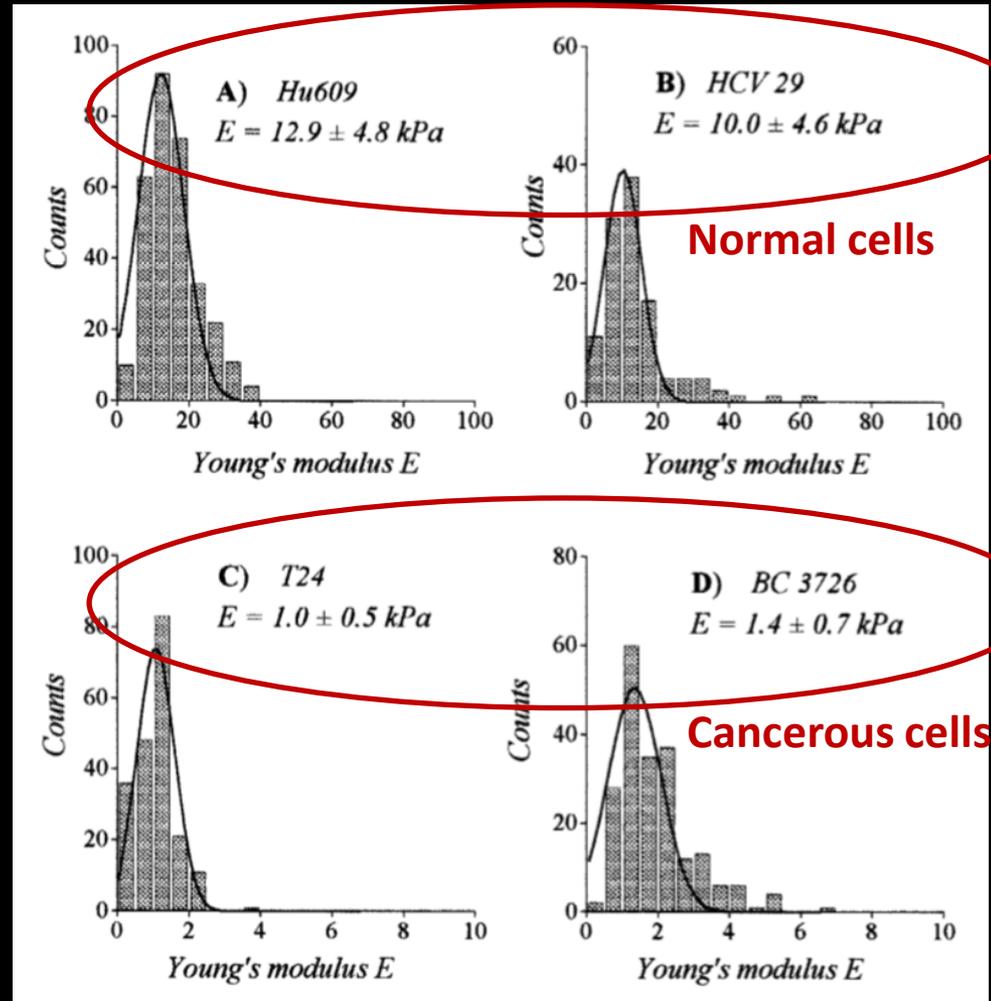
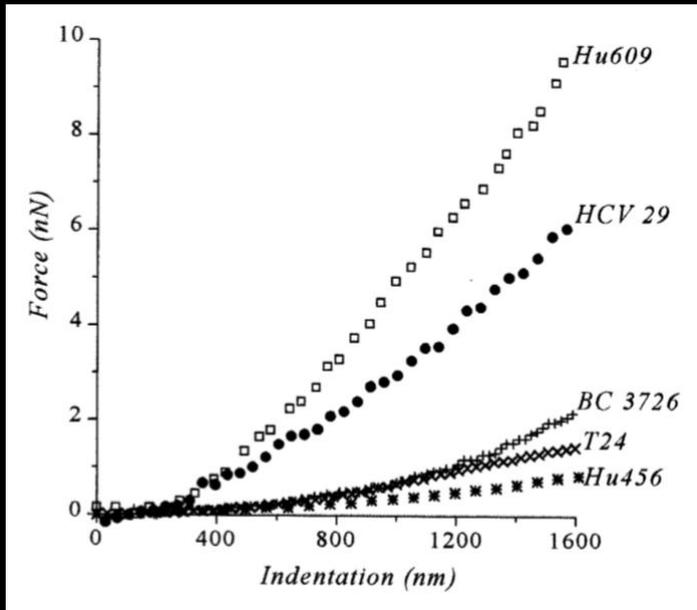


Relevance in Medicine and Physiology

→ Cell stiffness changes during cancer

Human epithelial cells (bladder) :

- Normal cells (Hu609, HCV29)
- Cancerous cells (T24, BC3726)





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Micron

journal homepage: www.elsevier.com/locate/micron



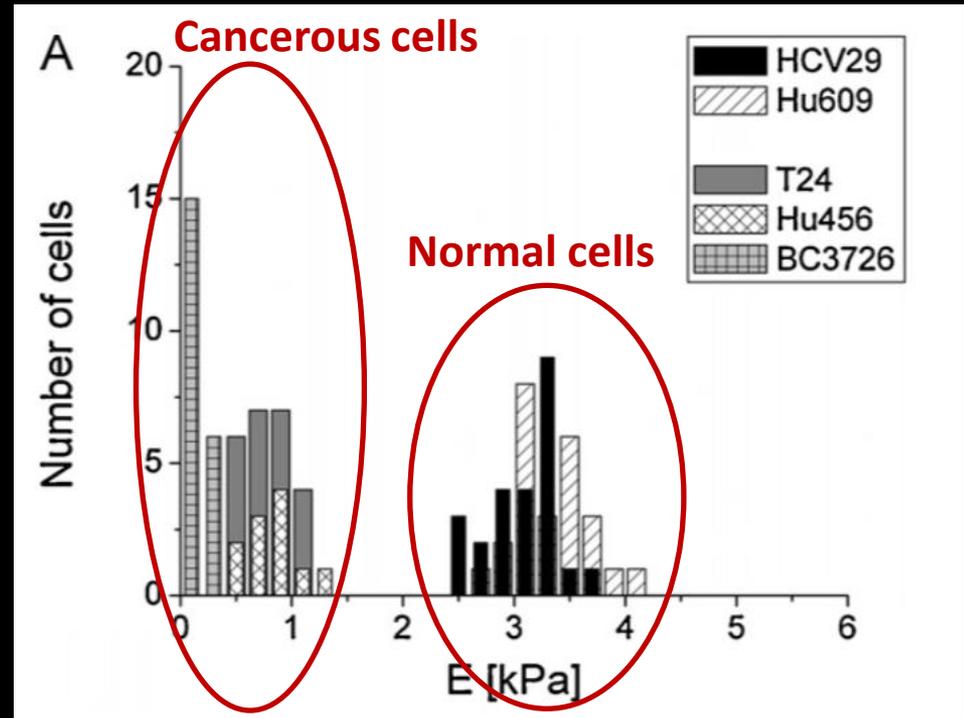
Cancer cell recognition – Mechanical phenotype

Małgorzata Lekka^{a,*}, Katarzyna Pogoda^a, Justyna Gostek^{a,b}, Olesya Klymenko^a,
Szymon Prauzner-Bechcicki^a, Joanna Wiltowska-Zuber^a, Justyna Jaczewska^{a,c},
Janusz Lekki^a, Zbigniew Stachura^a

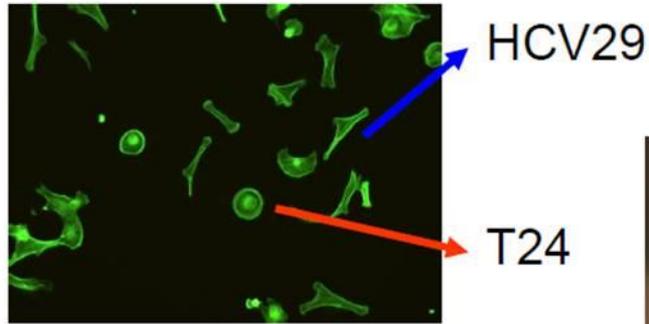
^a The Henryk Niewodniczański Institute of Nuclear Physics, Polish Academy of Sciences, Radzikowskiego 152, 31-342 Kraków, Poland

^b The Marian Smoluchowski Institute of Physics, Jagiellonian University, Reymonta 4, 30-342 Kraków, Poland

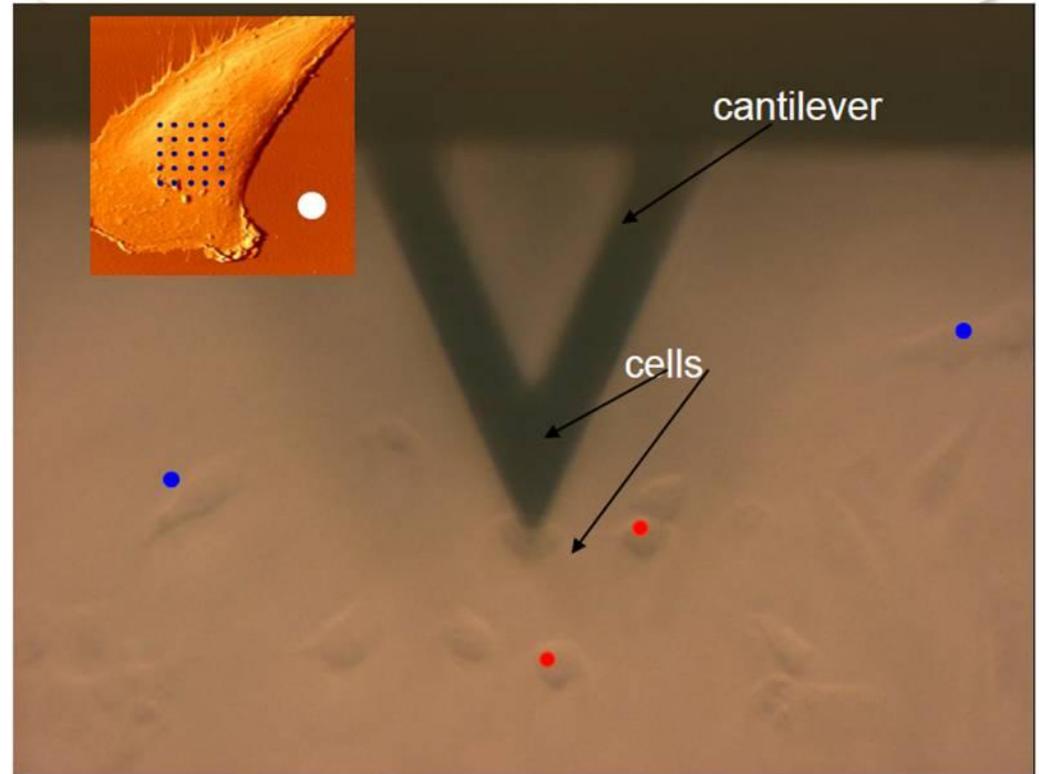
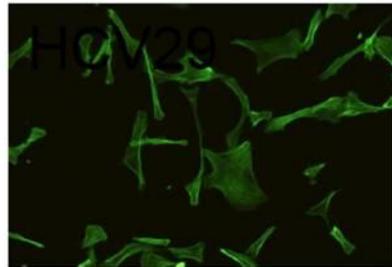
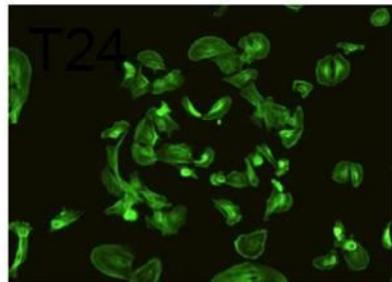
^c Florida Atlantic University, Charles E. Schmidt College of Medicine, 777 Glades Rd., Boca Raton, FL 33431, United States



Cancer cell recognition

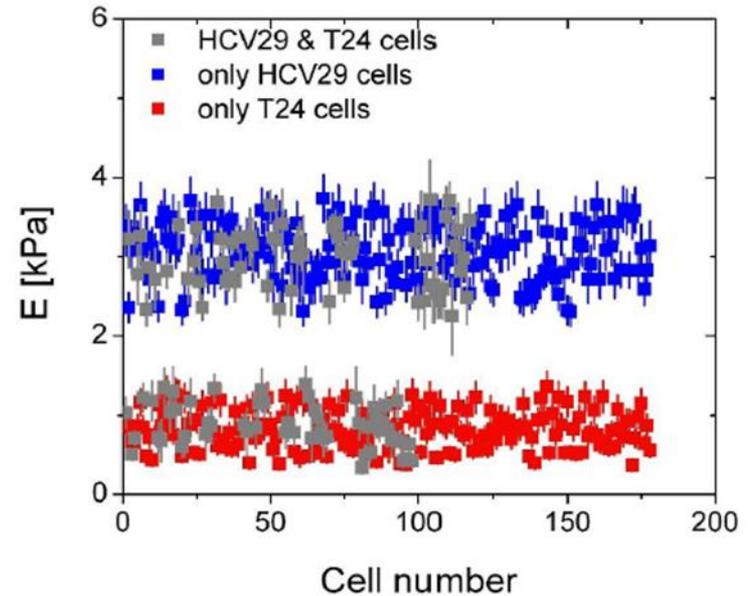
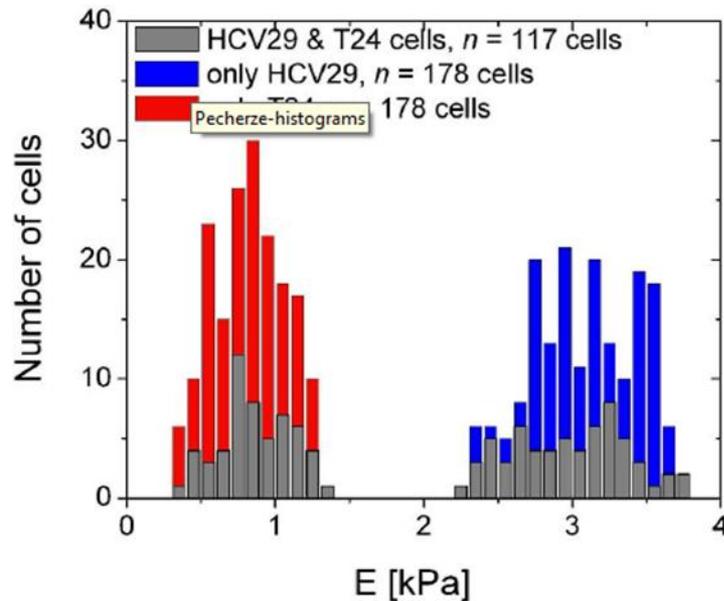


Can we recognized mechanically altered cell in the environment of other cells ?



initial cell number
44000 (T24) and 67000 (HCV29) per ml; 24h of growth

Cancer cell recognition



initial cell number 44000 (T24) and 67000 (HCV29) per ml; ratio 1:0.7; 96h

Same results as for separate cell lines

→ Cell stiffness changes during cancer (tissue)

nature
nanotechnology

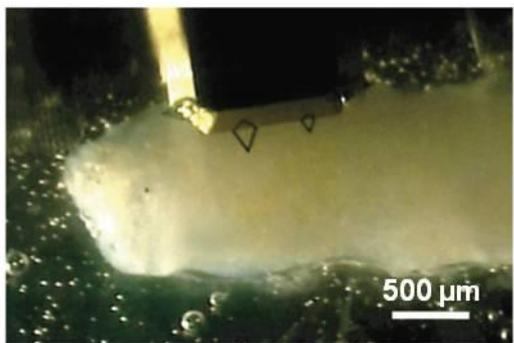
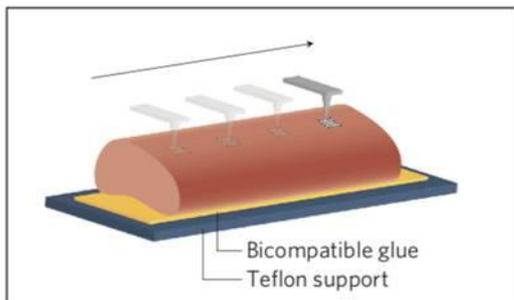
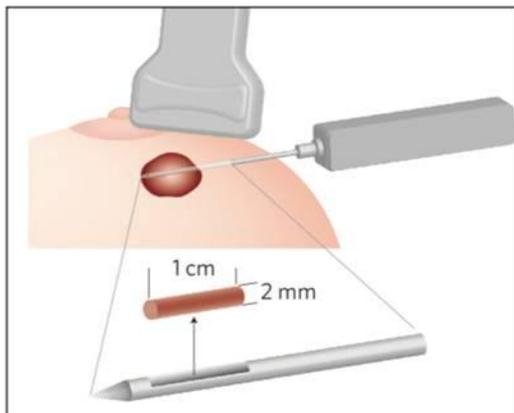
ARTICLES

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

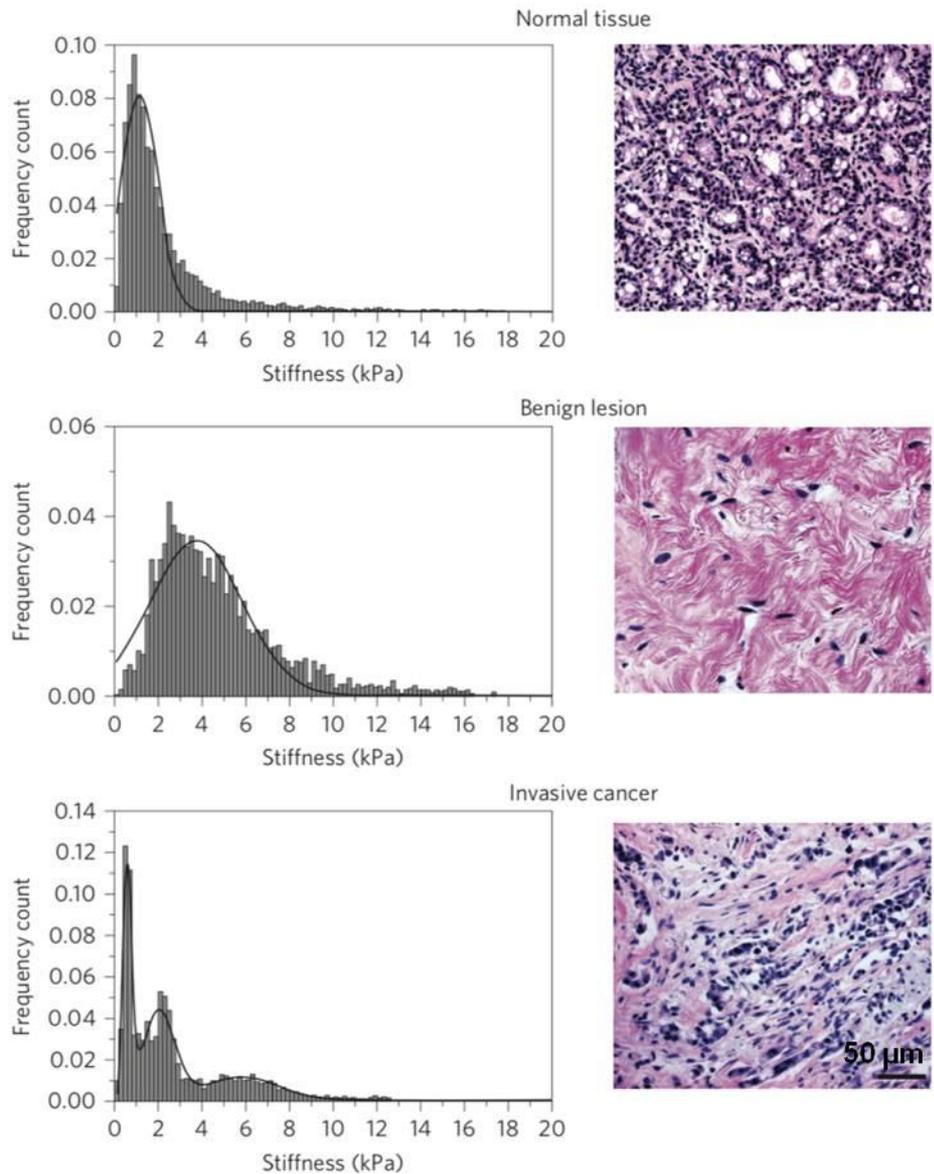
The nanomechanical signature of breast cancer

Marija Plodinec^{1,2}, Marko Loparic^{1,2†}, Christophe A. Monnier^{1†}, Ellen C. Obermann^{3†},
Rosanna Zanetti-Dallenbach^{4†}, Philipp Oertle¹, Janne T. Hyotyla¹, Ueli Aebi², Mohamed Bentires-Alj⁵,
Roderick Y. H. Lim^{1*} and Cora-Ann Schoenenberger²

a Testing human breast biopsies by IT-AFM



b



Sommaire

1. Introduction et principe

2. Imagerie

3. Spectroscopie de force

3.1 Propriétés mécaniques

3.2 Manipulation et dépliage

3.3 Détection, localisation et mapping de molécules individuelles

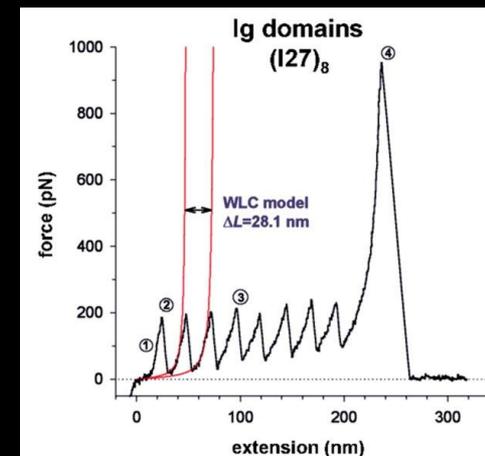
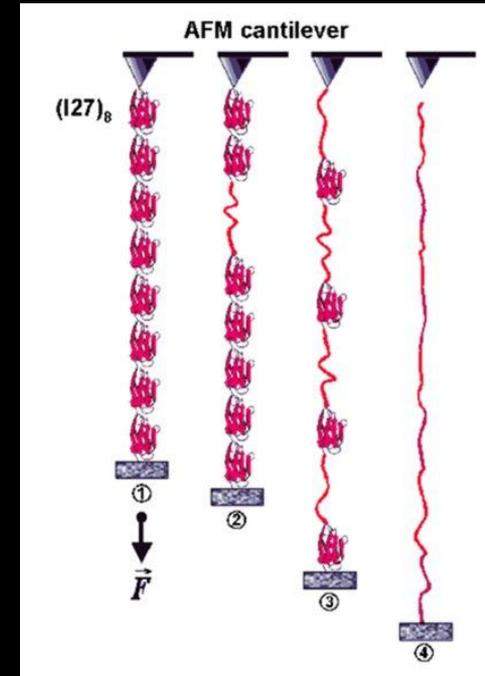
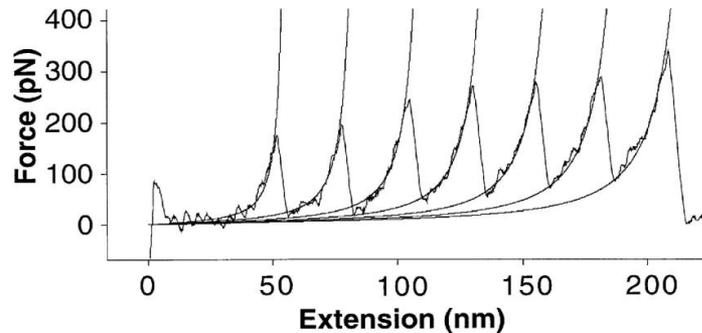
3. Conclusions et perspectives

Manipulation de protéines individuelles

Reversible Unfolding of Individual Titin Immunoglobulin Domains by AFM

Matthias Rief, Mathias Gautel, Filipp Oesterhelt,
Julio M. Fernandez, Hermann E. Gaub*

SCIENCE • VOL. 276 • 16 MAY 1997

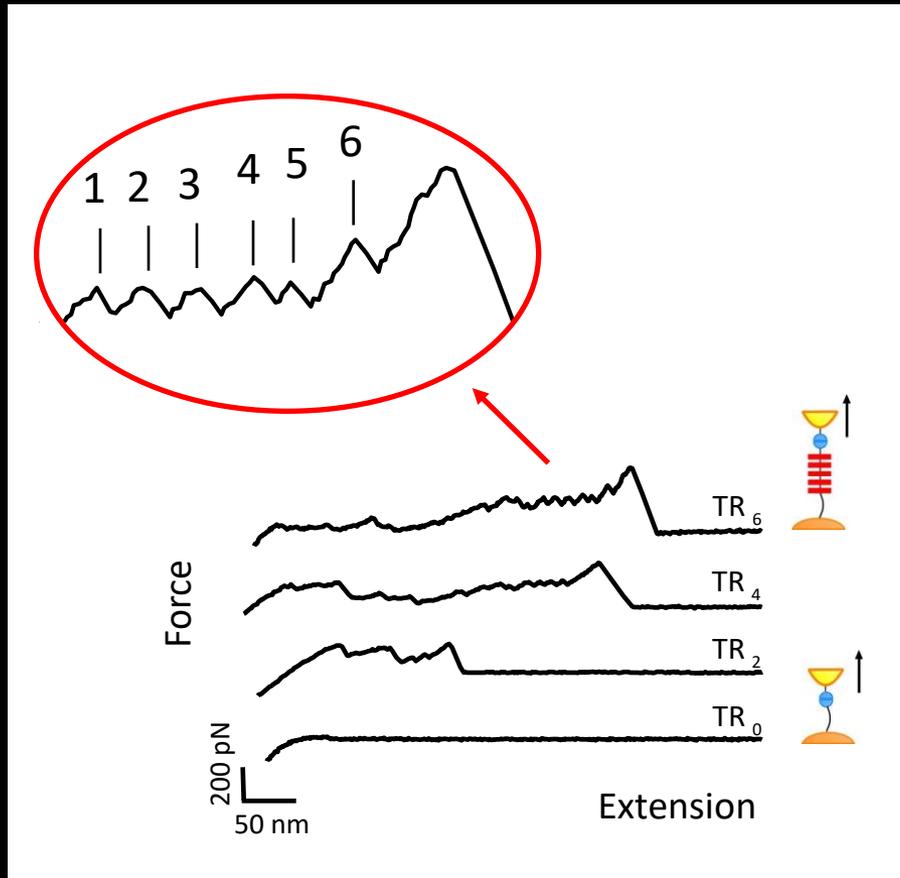


→ Interactions intramoléculaires

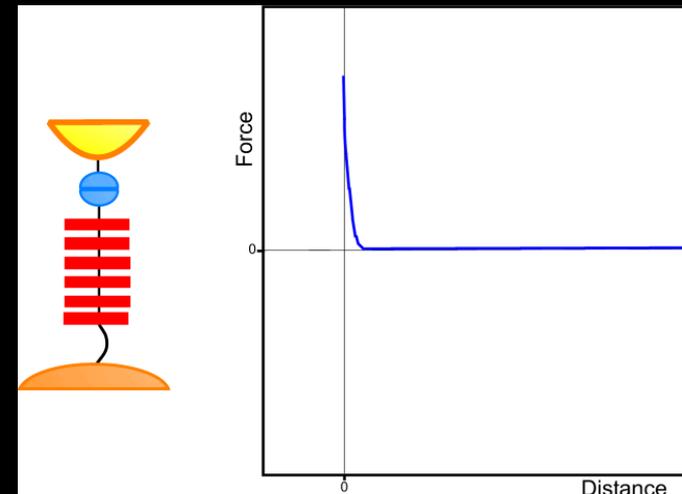
- Propriétés mécaniques
- Conformation de molécules indiv.
- Elasticité

Déplieusement de la protéine Als5p de *Candida albicans*

→ localiser et sonder les propriétés mécaniques des protéines Als5p sur cellule



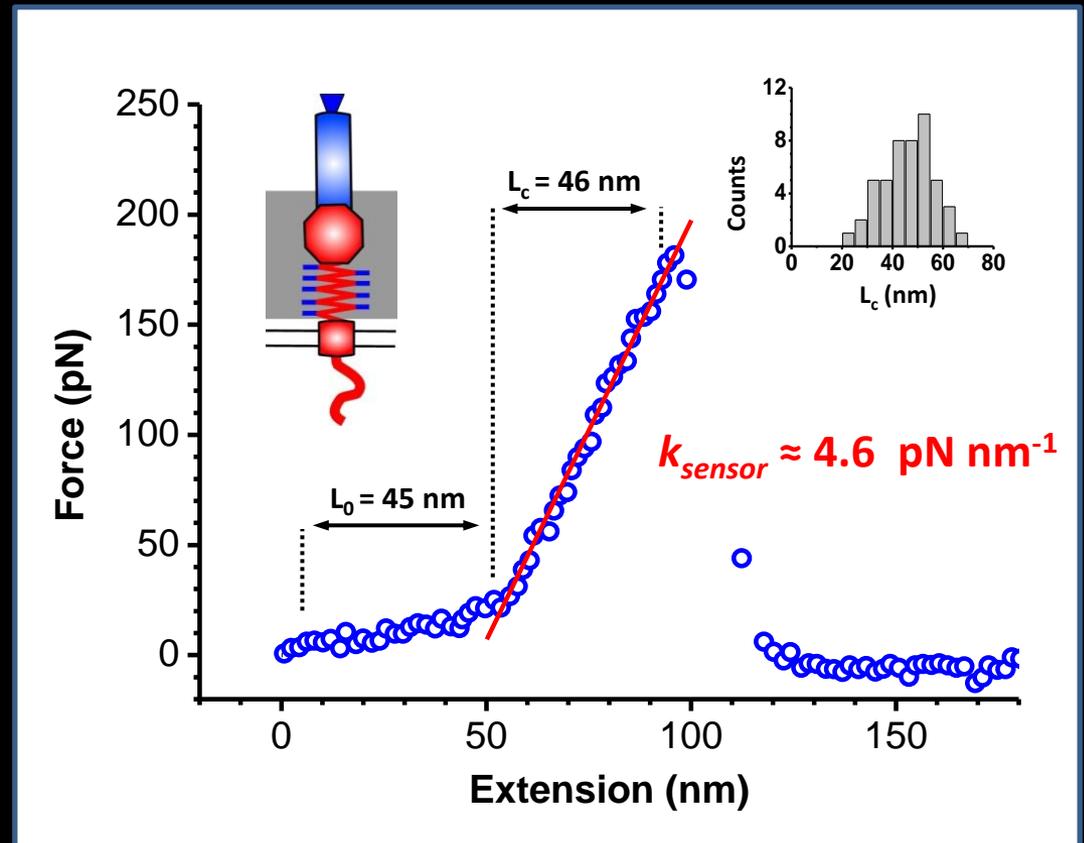
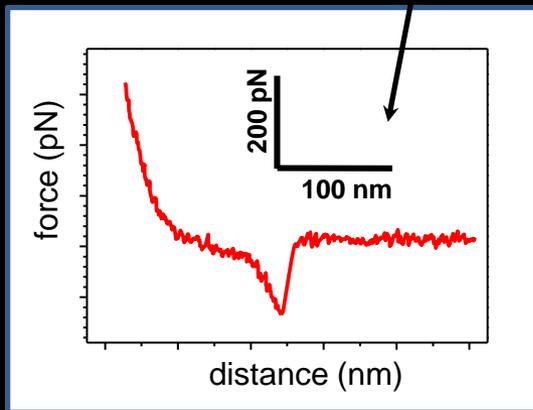
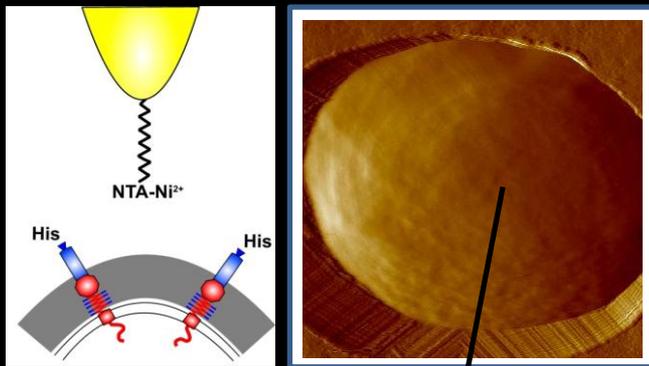
→ cellules exprimant des protéines Als5p avec des nombres variables de TRs



Senseur Wsc1 de *S. cerevisiae*

Force proportionnelle à l'extension = ressort (loi de Hooke)

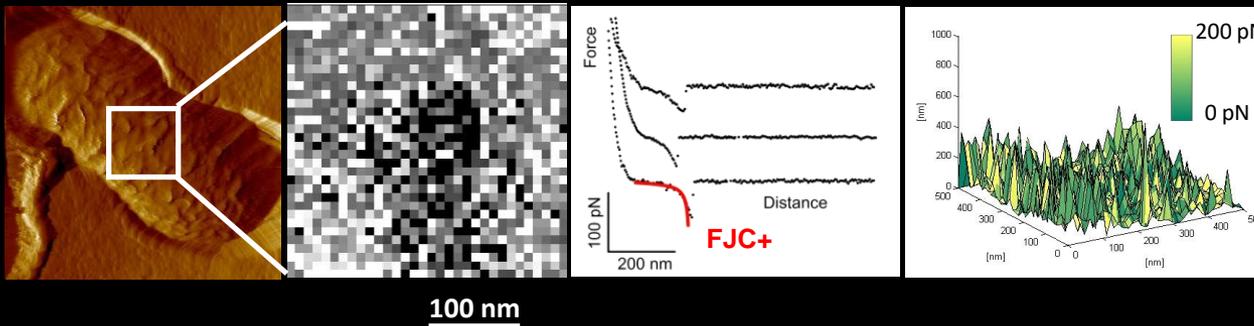
~~(WLC fit)~~



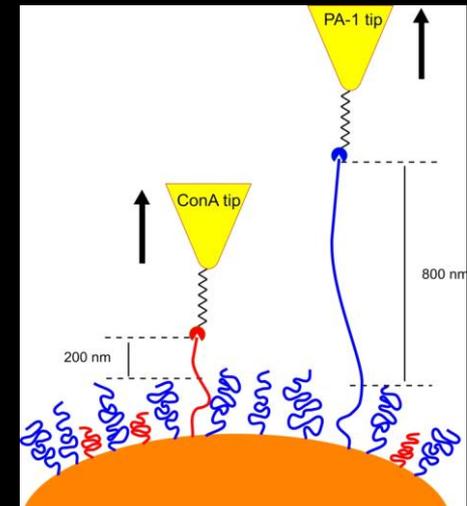
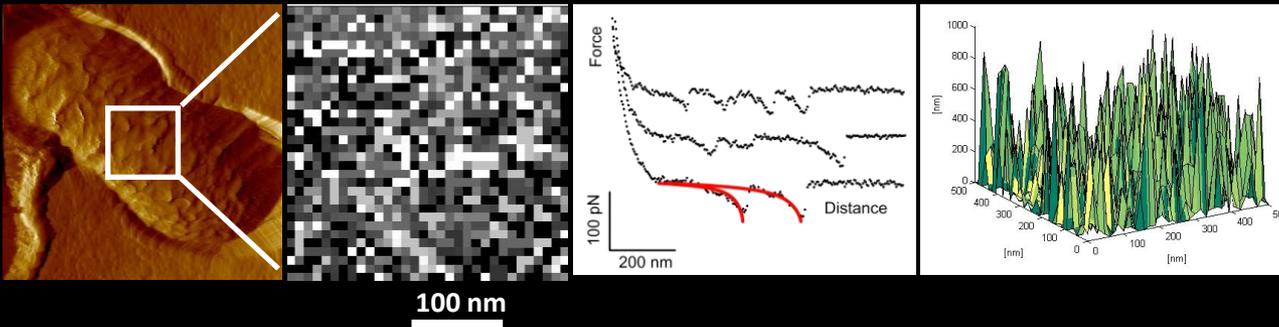
Détection, localisation de polysaccharides

Lactobacillus rhamnosus GG

Pointes ConA → Mannose



Pointes PA1 → Galactose



→ deux types de polysaccharides

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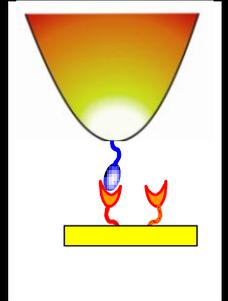
3.1 Propriétés mécaniques

3.2 Manipulation et dépliage

3.3 Détection, localisation et mapping de molécules individuelles

3. Conclusions et perspectives

Préparation de pointes AFM modifiées



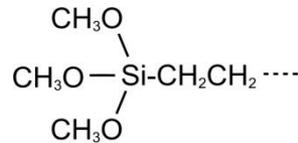
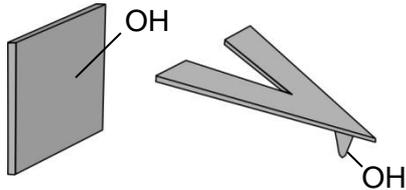
- Des contraintes à respecter :

- (1) La force permettant le greffage doit être supérieure à l'interaction que l'on souhaite mesurer
- (2) Protocole de greffage "doux"
- (3) Densité de couplage faible pour garantir la détection de mol. individuelles
- (4) Mobilité pour interagir librement avec les molécules complémentaires
- (5) Minimiser l'adhésion non spécifique
- (6) Couplage orienté (exposition optimale de la molécule greffée)

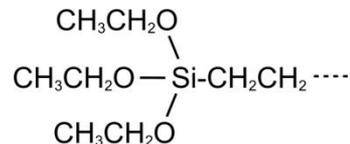
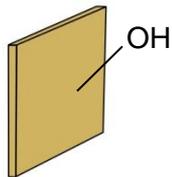
→ Différentes stratégies d'immobilisation

Greffage de biomolécules sur les pointes AFM

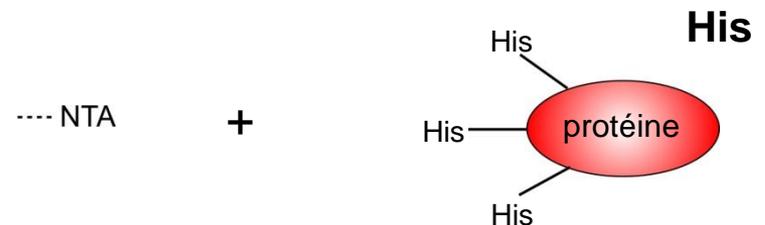
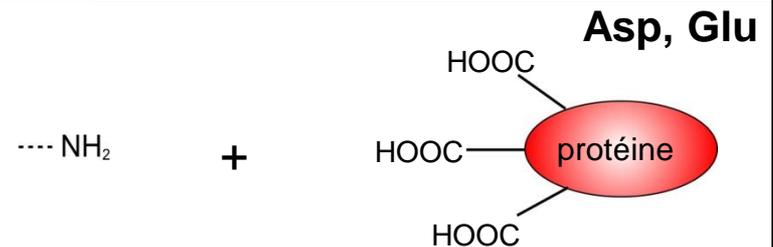
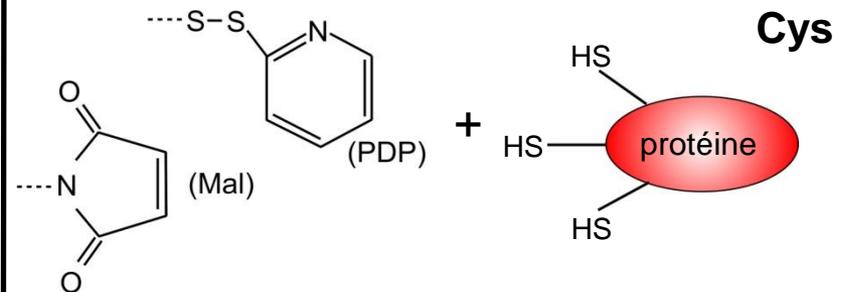
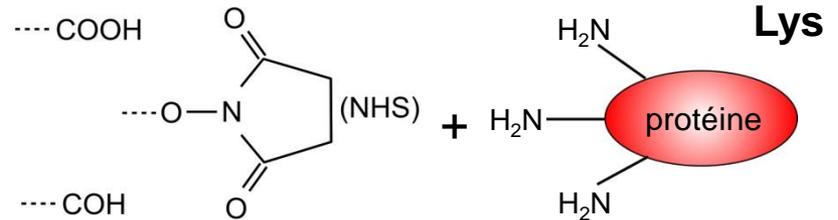
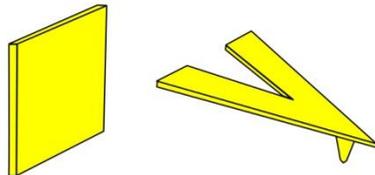
Silicium ou nitrure de silicium



Verre ou mica

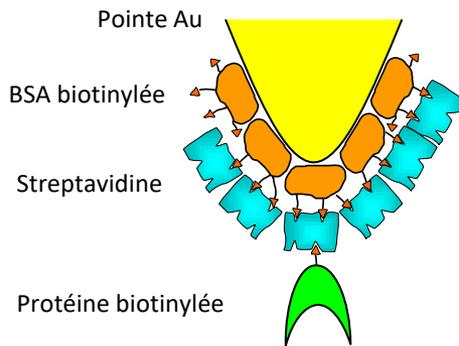


Au

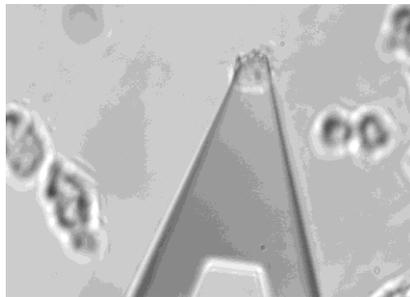


Greffage de biomolécules sur les pointes AFM

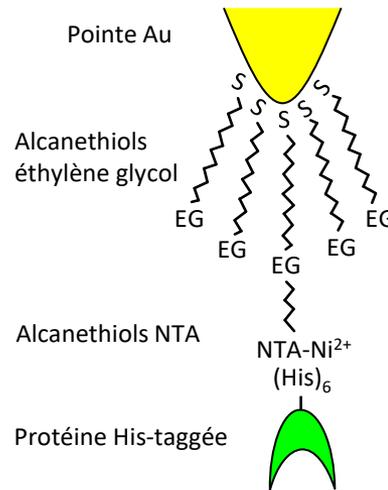
Physisorption de protéines



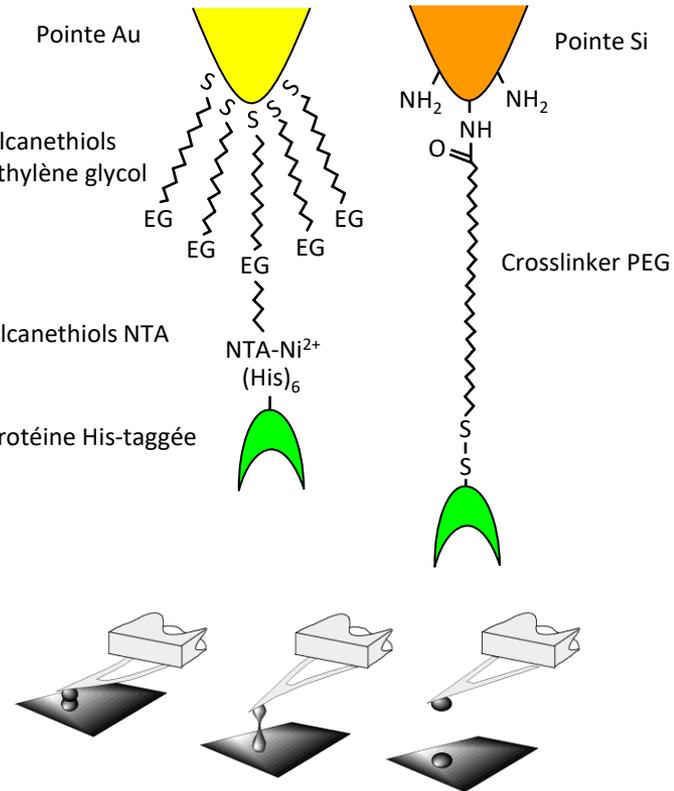
« Cell probes »



Thiols sur or

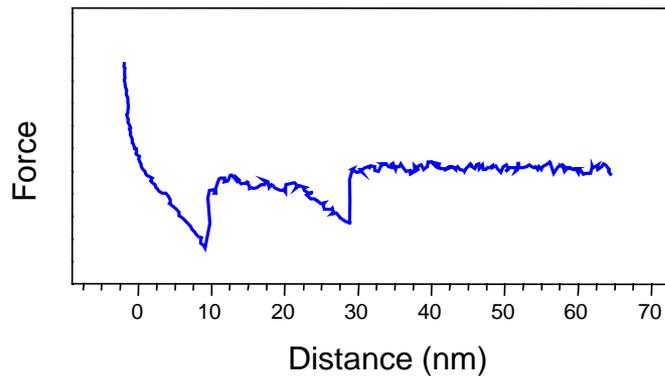
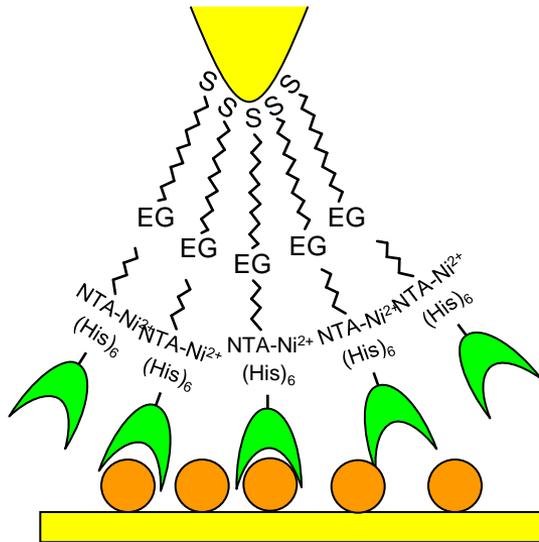


Silanes sur silicium

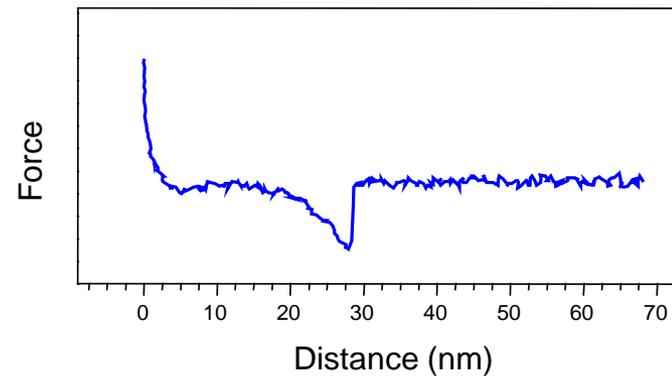
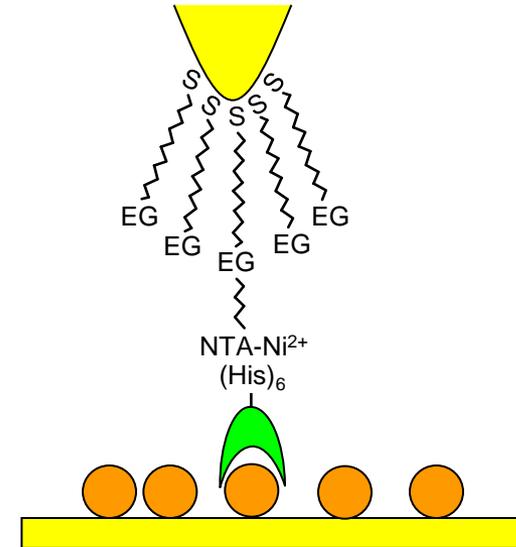


Dilution, pourquoi?

Sans dilution



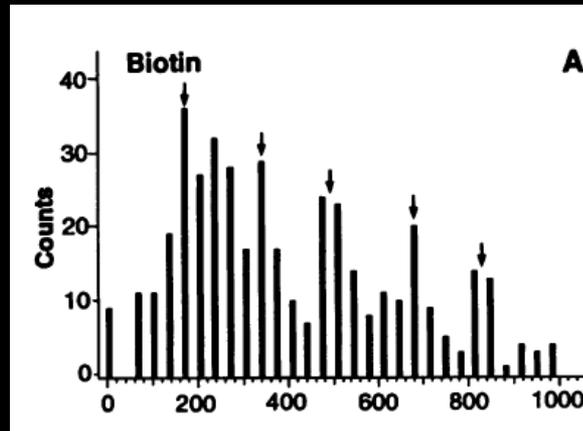
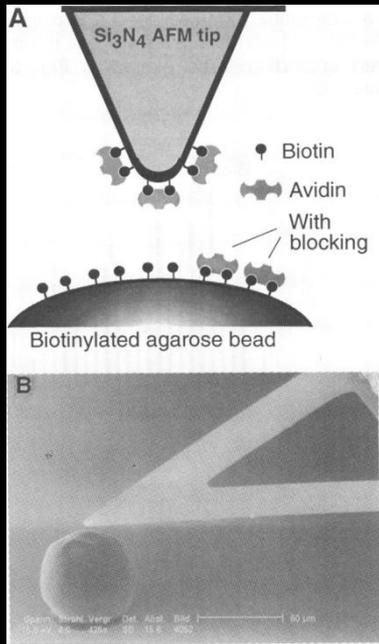
Avec dilution



→ Interaction individuelle entre récepteur et ligand (single molécule)

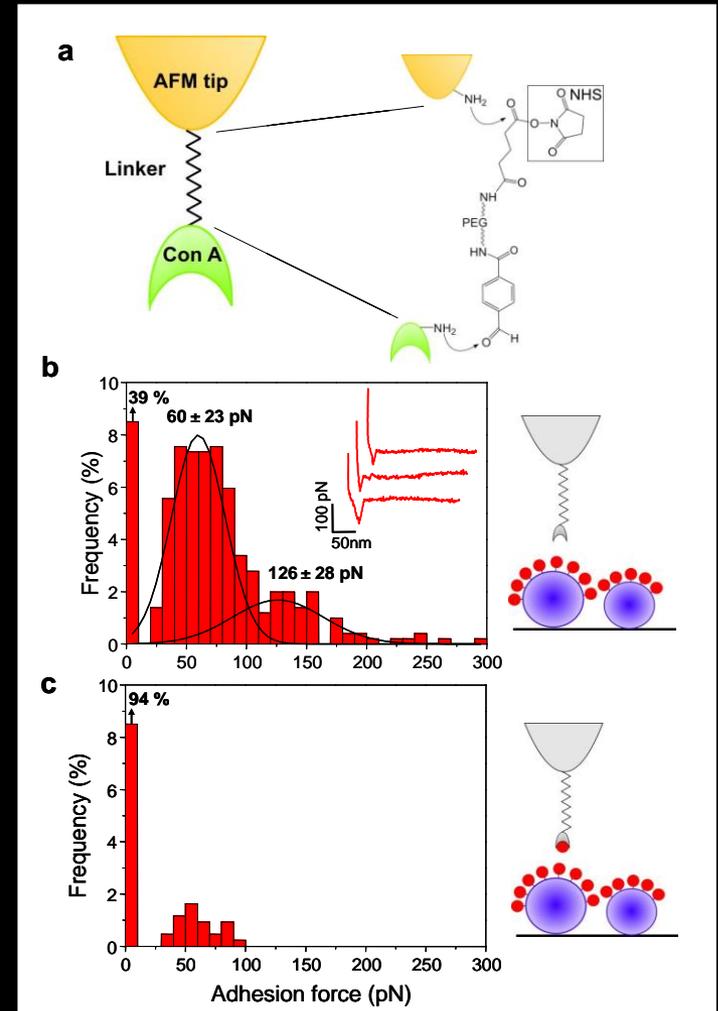
Dilution, pourquoi?

Biotin-avidin



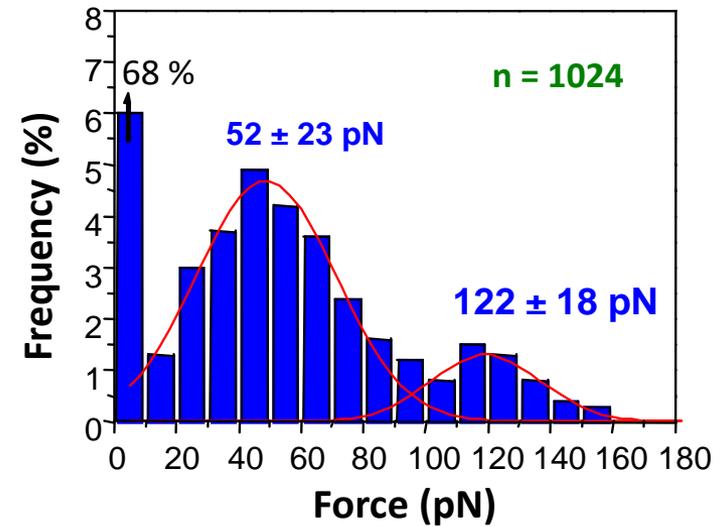
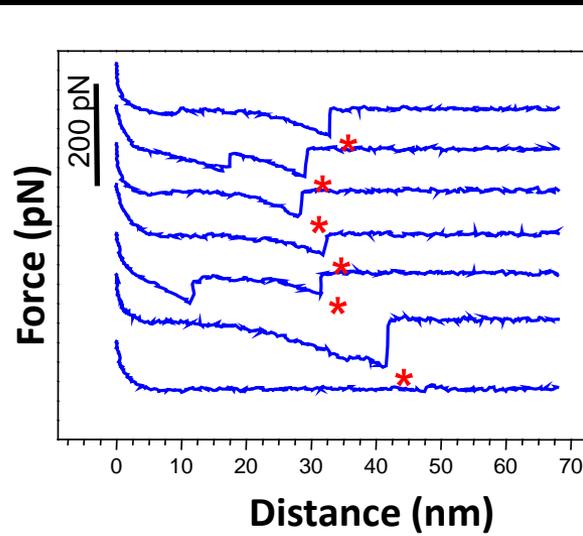
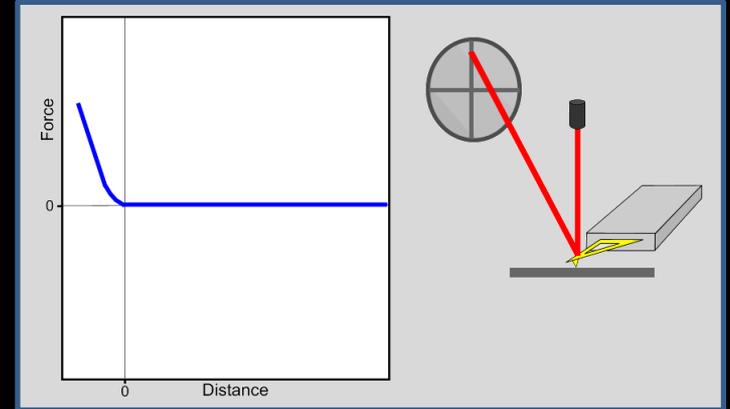
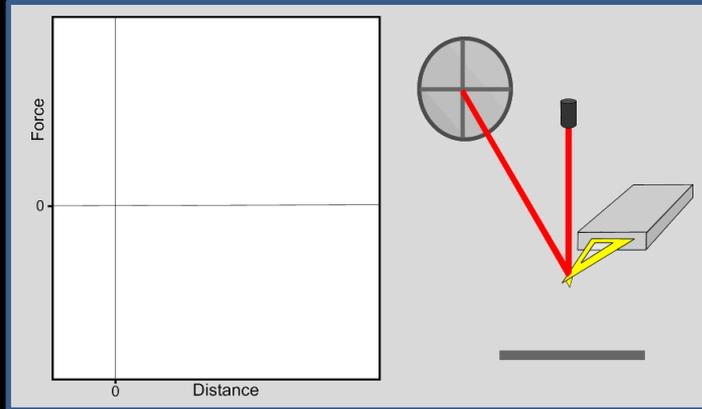
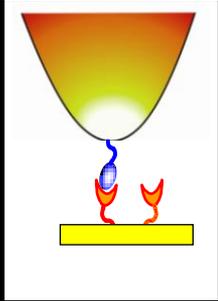
Florin *et al.*, *Science*, 1994

ConcanavalinA-mannose



Alsteens *et al.*, *Nanotechnology*, 2008

Forces d'interaction

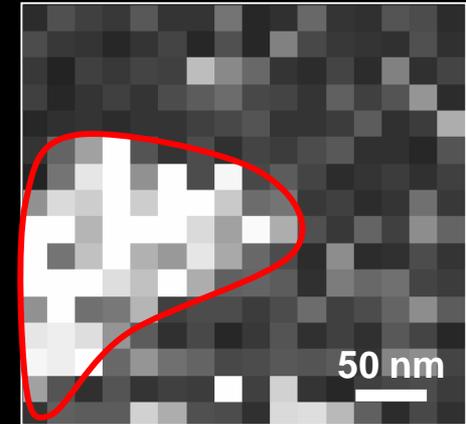
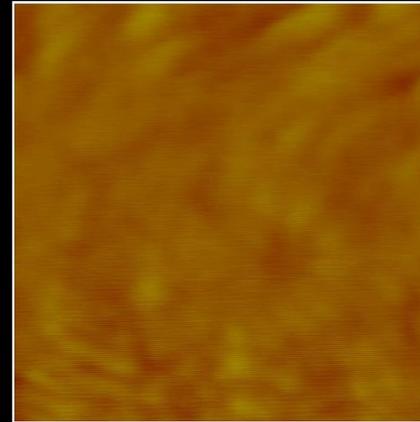
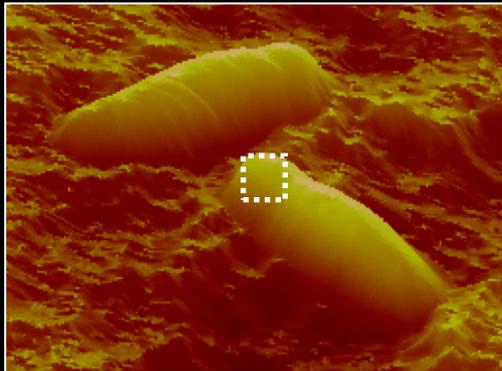


Adhésine HBHA sur mycobactérie

Mycobacterium bovis BCG

Topographie

Carte d'adhésion

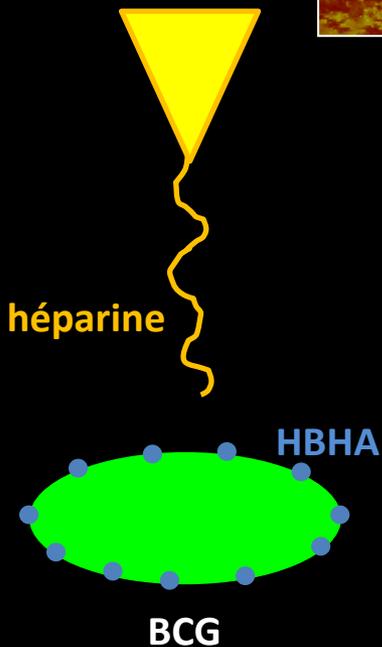


100 pN

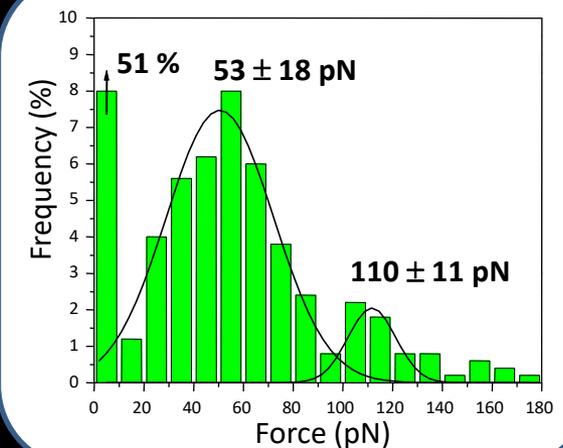
0 pN

50 nm

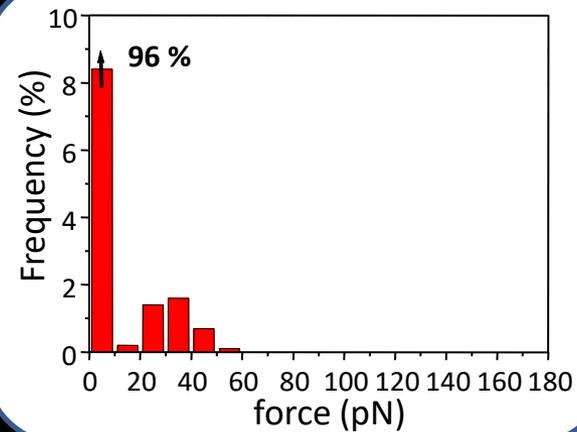
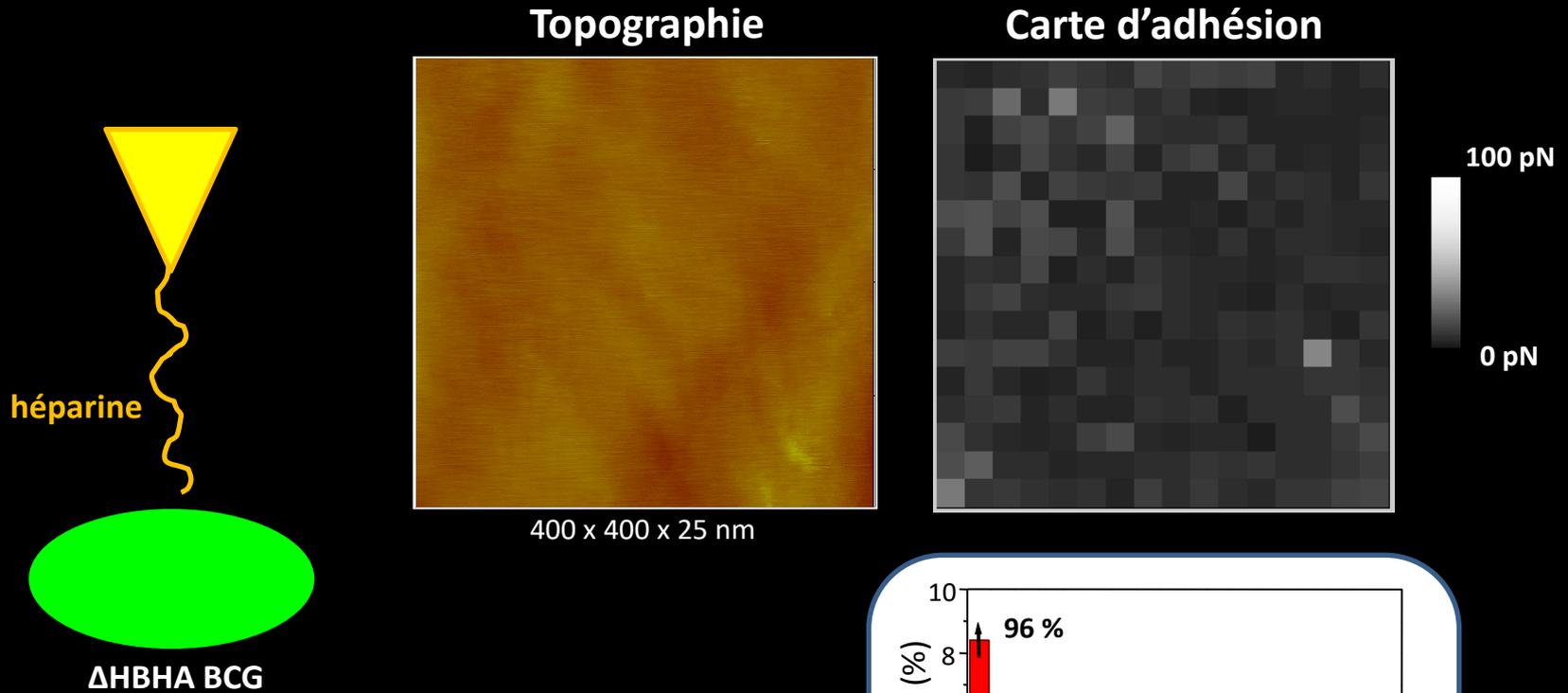
300 x 300 x 25 nm



- Adhésines individuelles détectées
- Nanodomains
- Cibles potentielles

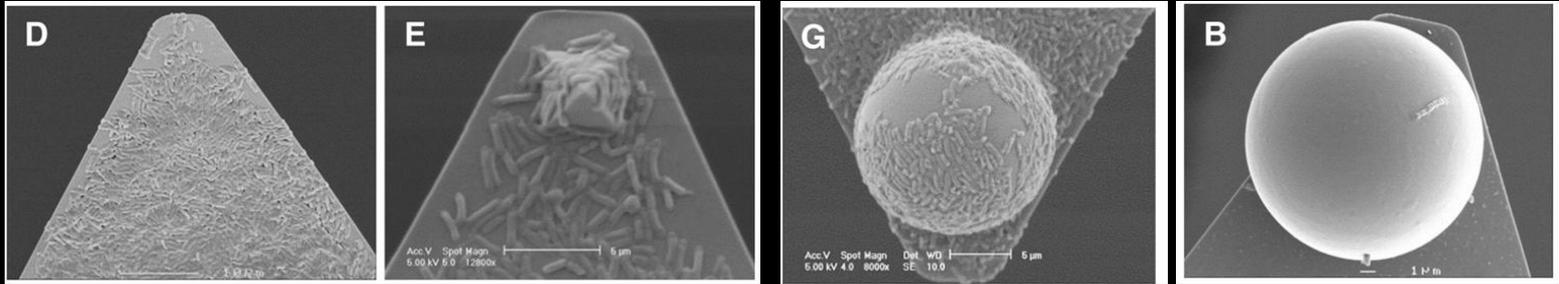


Contrôle : souche mutante sans adhésine



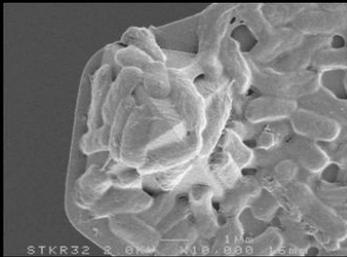
Cell probes

Acidithiobacillus thiooxidans

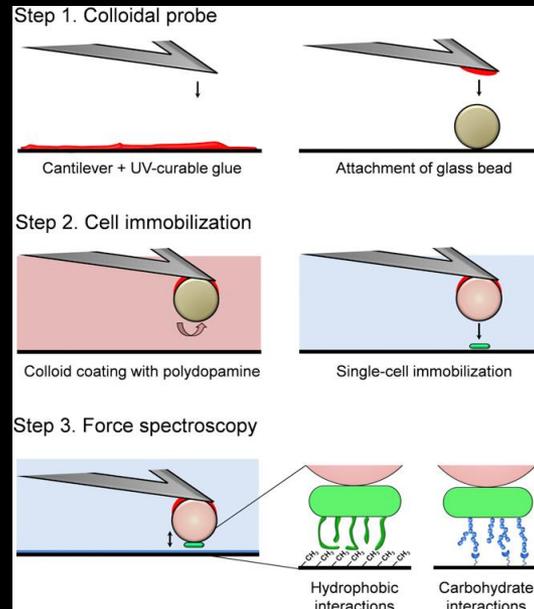


Diao et al., *J. Microbiol. Methods*, 2014

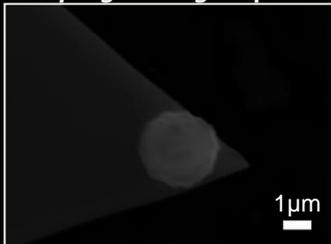
Klebsiella terrigena



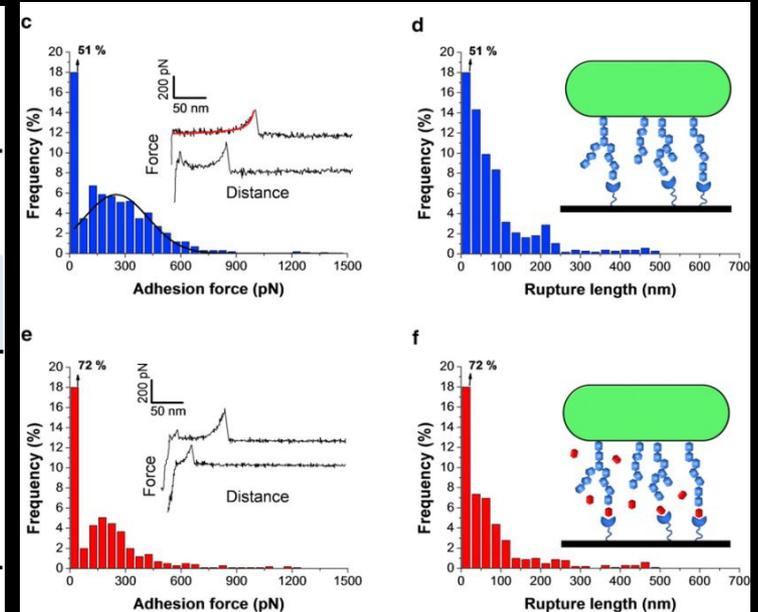
Vadillo-Rodriguez et al., *AEM*, 2004



Aspergillus niger spore



Wargenau et al., *Langmuir*, 2010



Beaussart et al., *BiophysJ*, 104, 1886 (2013)

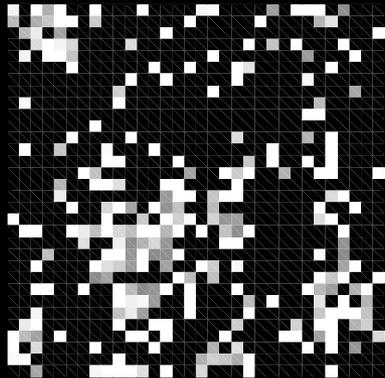
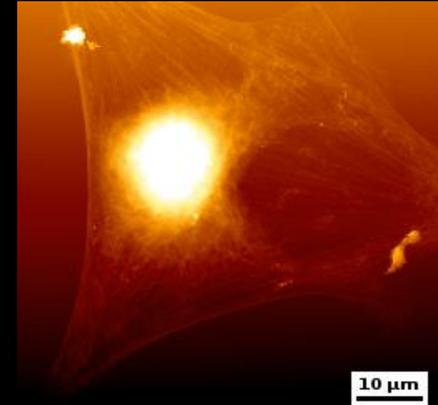
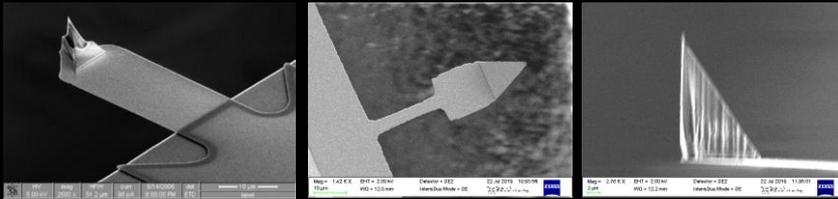
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 - 3.2 Manipulation et déplieement**
 - 3.3 Détection, localisation et mapping de molécules individuelles**
- 3. Conclusions et perspectives**

Perspectives : resolution

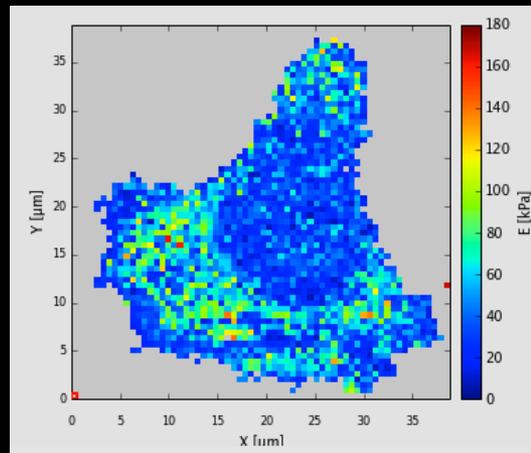
Recent AFM developments :

- new probes
- new modes (FV → QI, PFT)



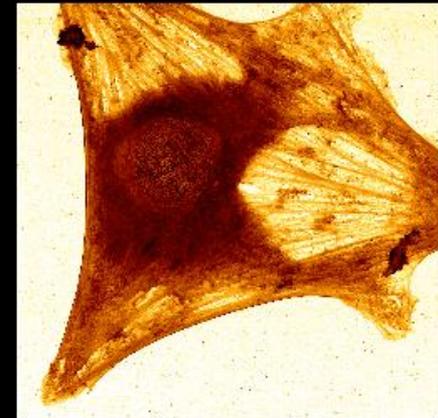
FV 32*32

Acquisition : 30 à 45min
Treatment : ≈ 3-4 heures



QI 64*64

Acquisition : ≈ 5 min
Treatment : ≈ qq minutes

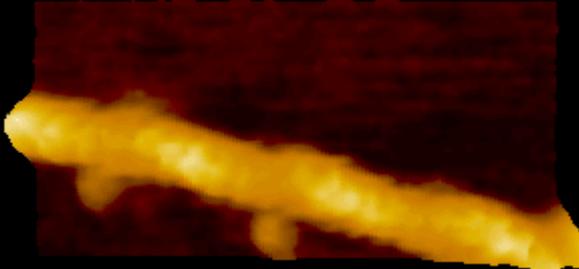


QI 256*256

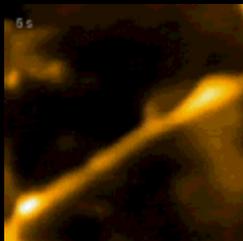
Acquisition : ≈ 10 min
Treatment : ≈ qq minutes

High-speed AFM

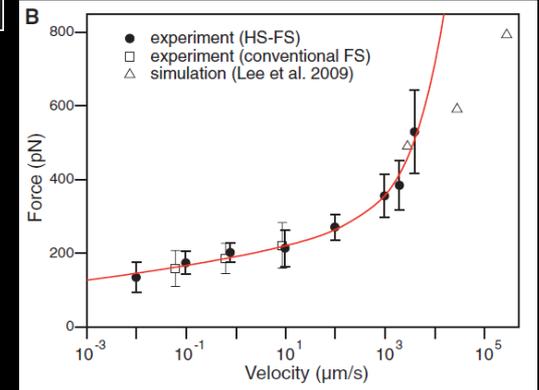
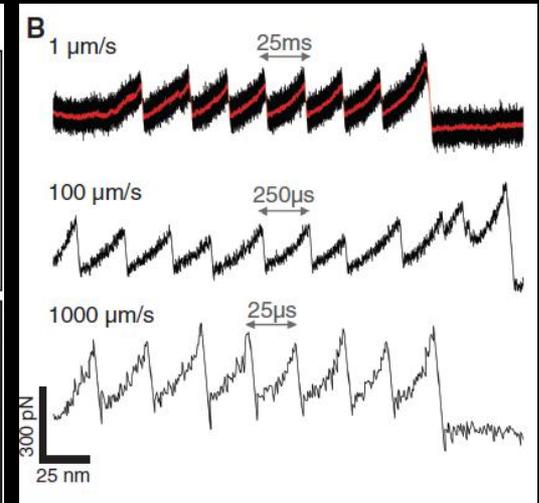
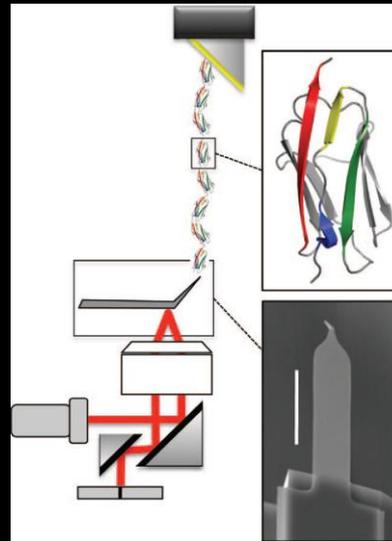
High-speed force spectroscopy unfolds Titin



walking myosin V (realtime)



Dendrite growth in Neuron
5 μm x 5 μm

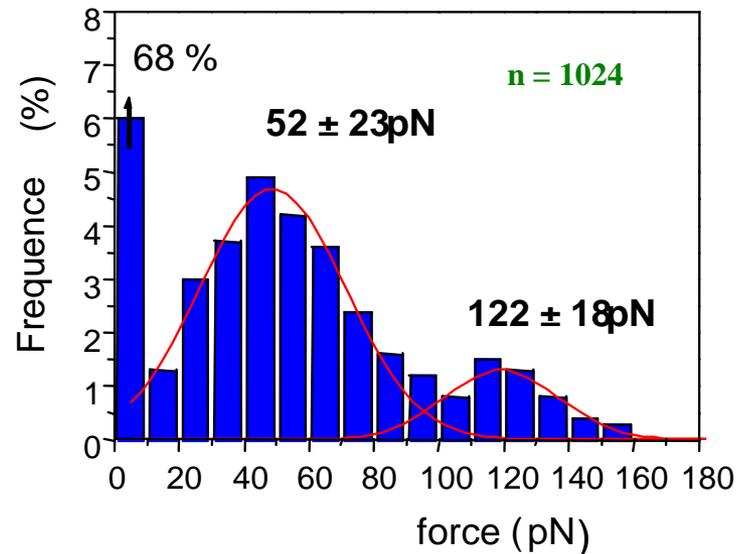
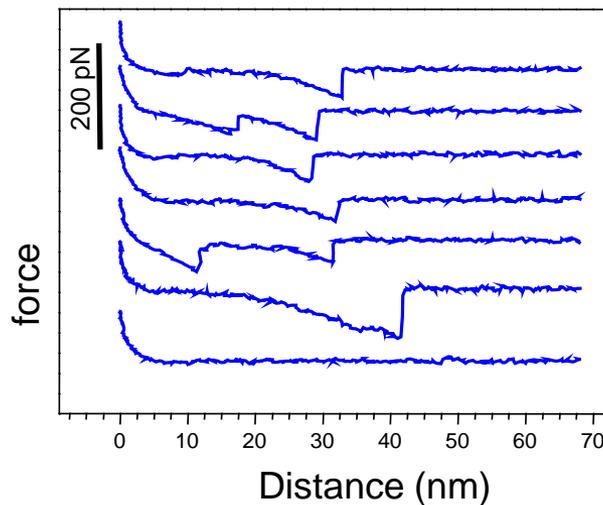


High-throughput force measurement

Expériences de spectroscopie de force :

- Nécessitent de multiplier les expériences (statistiques)
- Expériences sur molécules uniques : faible proportion de courbes de force pouvant présenter une interaction spécifique (10-20%)

→ collection d'une grande quantité de données



Spectroscopie de force : répétitions de mesures, éventuellement en changeant de position ou quelques paramètres (vitesse de retrait, temps de pause...)

→ **Intervention limitée de la part de l'expérimentateur**

2009 : lancement par la firme JPK d'un modèle capable d'effectuer de façon automatique des milliers de mesures et de les analyser (courbes force – distance)

→ **JPK ForceRobot 300**

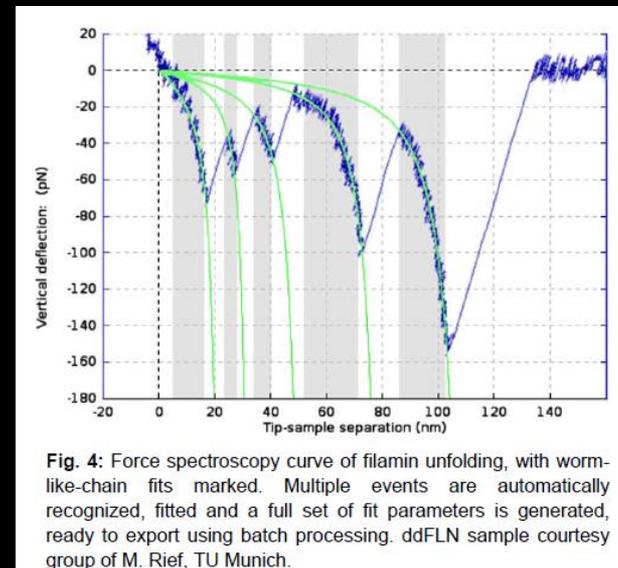


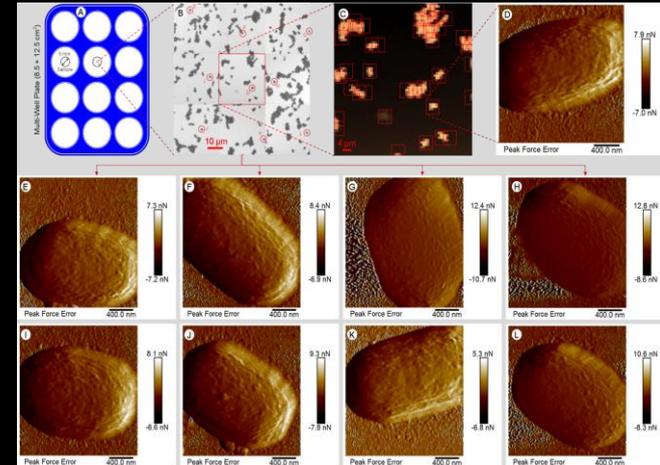
Fig. 4: Force spectroscopy curve of filamin unfolding, with worm-like-chain fits marked. Multiple events are automatically recognized, fitted and a full set of fit parameters is generated, ready to export using batch processing. ddFLN sample courtesy group of M. Rief, TU Munich.

→ **ne concerne que la spectroscopie de force (molécules individuelles)**

→ **quelle solution pour les cellules?**

→ automatisation AFM

→ cellules procaryotes (microbes)



Dujardin *et al.*, PloS One (2019)

→ Cellules eucaryotes et tissu



Conclusion

- **Forces**

- **Imagerie (préparation, liquide, résolution)**
(cellules procaryotes, eucaryotes)
- **Propriétés physiques (adhésion, élasticité, hydrophobicité)**
- **Mesures d'interaction (pointes fonctionnalisées)**
→ détection, cartographie

- **Faiblesses**

- **Troughput**
→ HS-AFM, automatisation, nouveaux modes...
- **Standardisation**
- **Technique limitée à la surface de l'échantillon**
→ couplages...