

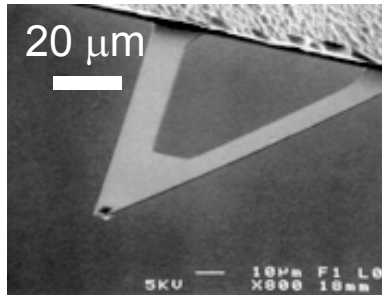
Atomic Force Microscopy in Biology



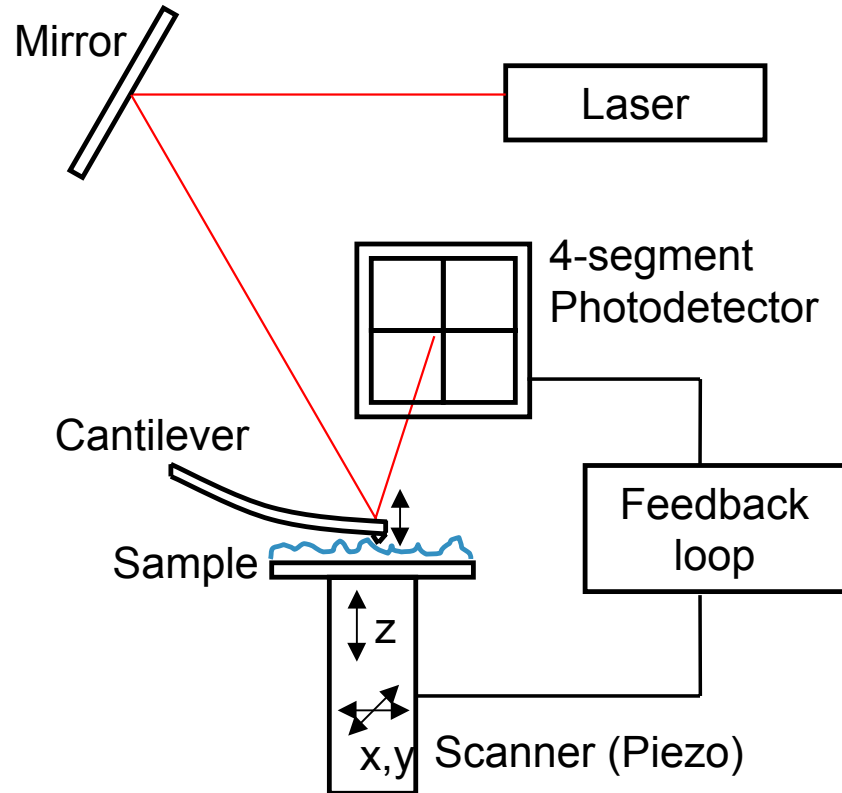
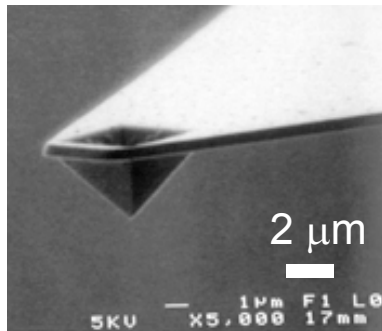
Alain Brisson - Imagerie Moléculaire et Nano-Bio-Technologie, IECB
UMR-CNRS 5471 Biophysique Structurale
Université de Bordeaux 1 - Talence, France
a.brisson@iecb.u-bordeaux.fr

AFM principle

Cantilever



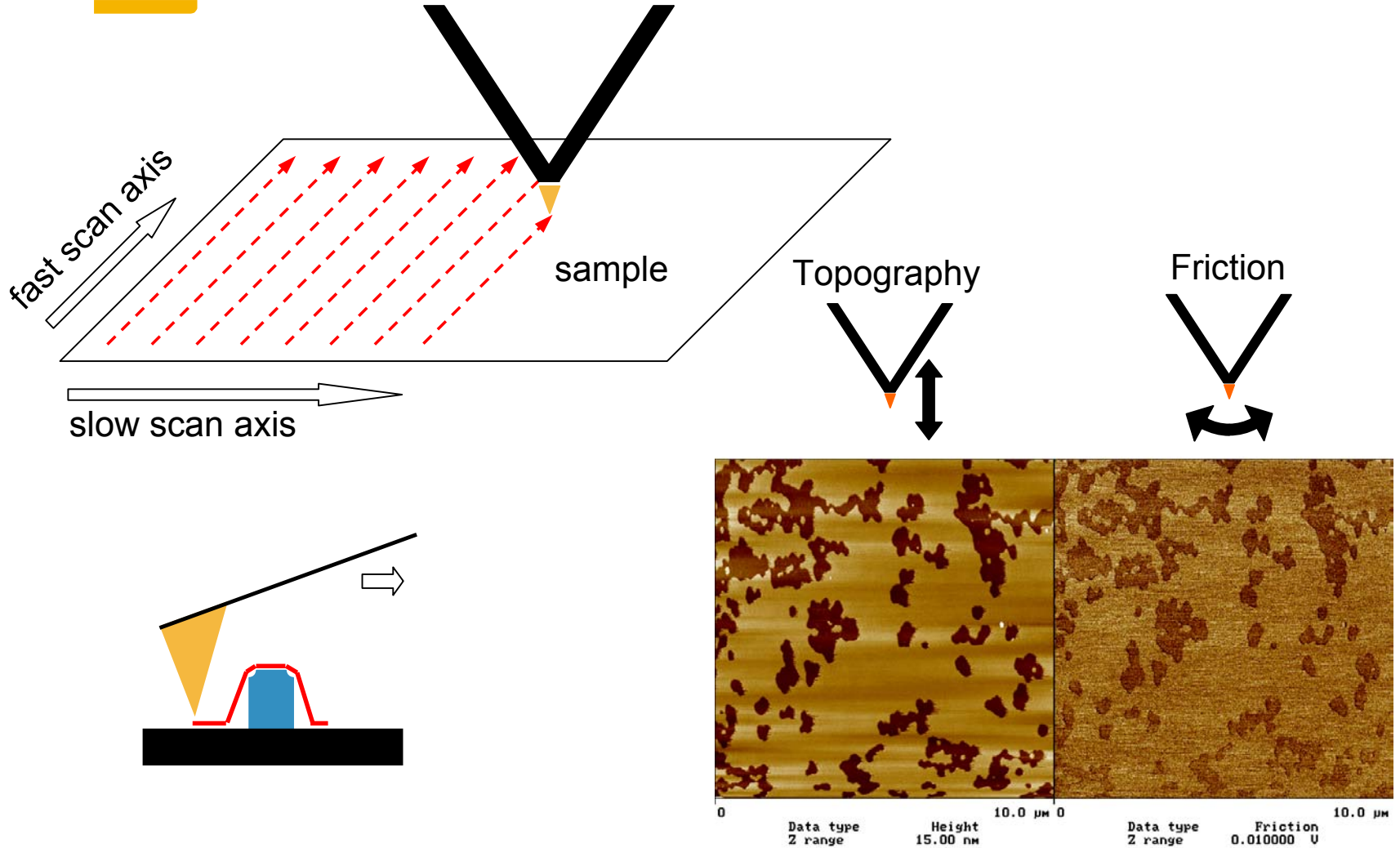
Tip



Spring formula : $F = k x$

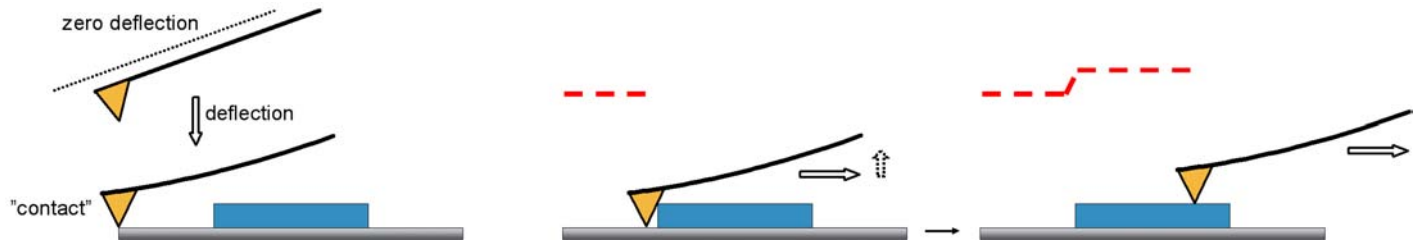
- $k = 60 \text{ mN/m}$
- The sensitivity in the measurement of the deflection is $\sim 1 \text{ \AA}$ \rightarrow forces as small as few pN can be detected
- A force of 100 pN induces a deflection of 1.6 nm
- Typical scanning rates in contact mode: 4-10 Hz
ex: at 5 Hz, 512 lines are scanned in $\sim 100 \text{ sec}$
 \rightarrow low time resolution

AFM Imaging

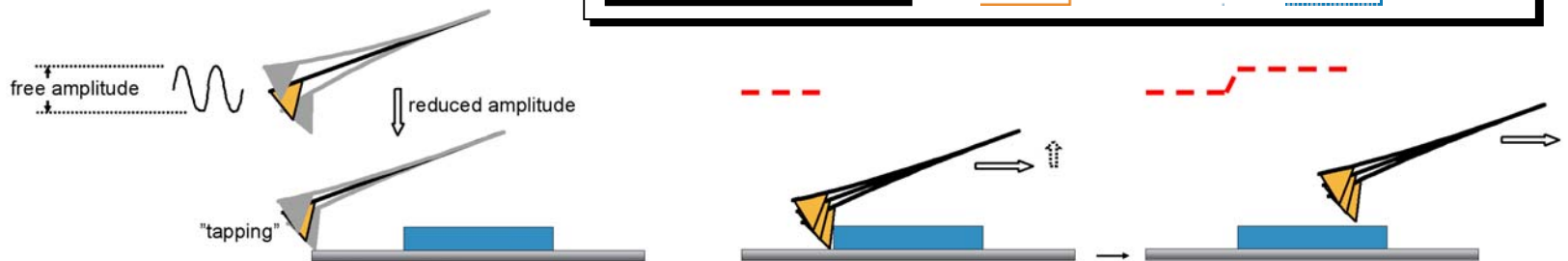


AFM Imaging Modes

Contact Mode



Tapping Mode

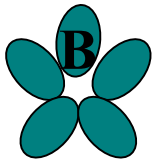


Atomic Force Microscopy in Biology

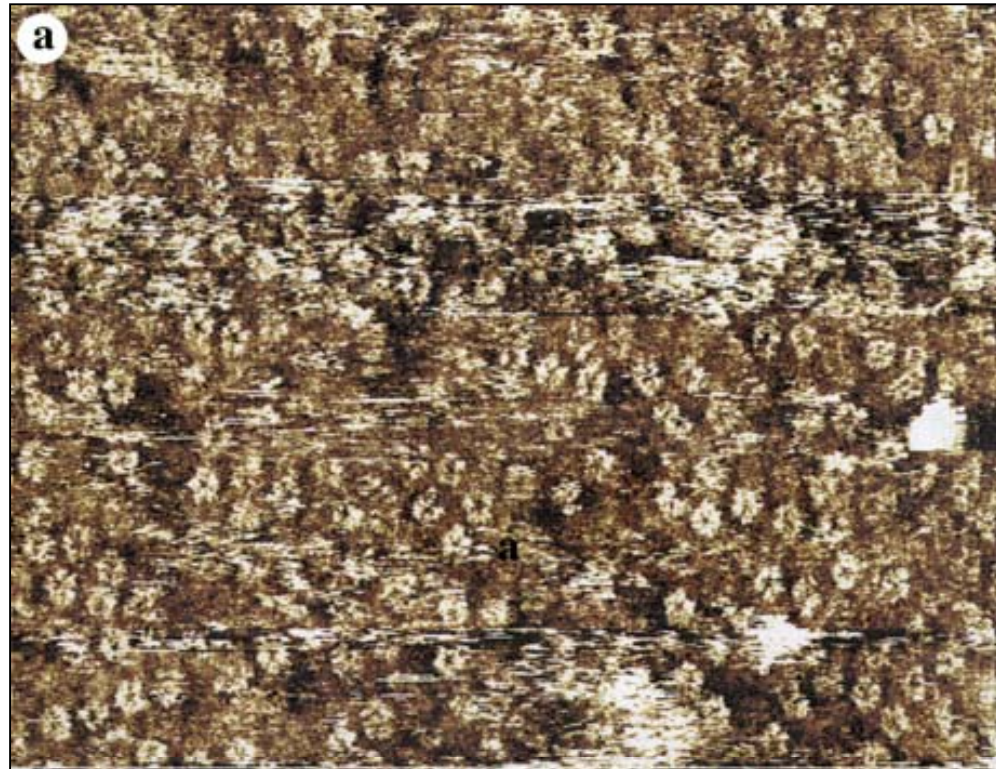
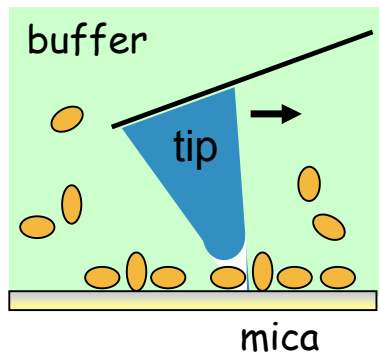
- I- topography of single molecules and molecular assemblies, *in an aqueous environment*, at a resolution from 100 μm to ~ 1 nm
- II- dynamics of molecular processes, *in situ*, in \sim real time
- III- molecular force spectroscopy : characterization of the mechanical properties of molecules

1 - AFM imaging of soluble proteins - *in buffer*

Cholera toxin



- Oligomer B₅
- B = 10,600 Da
- direct adsorption on a mica support
- imaging in liquid



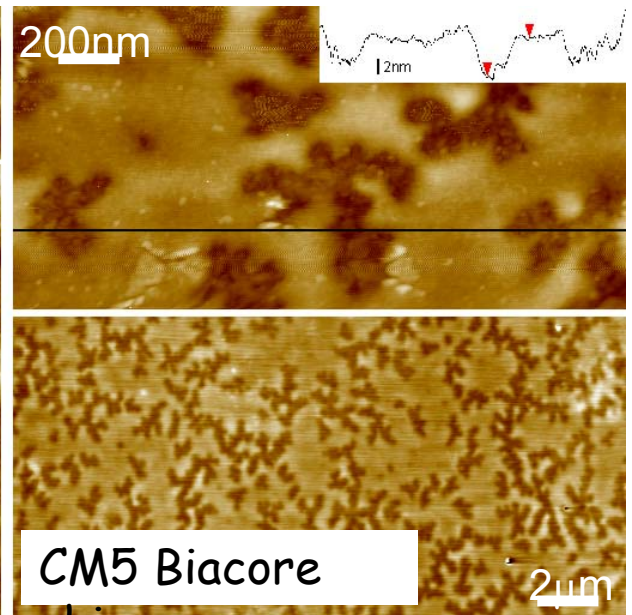
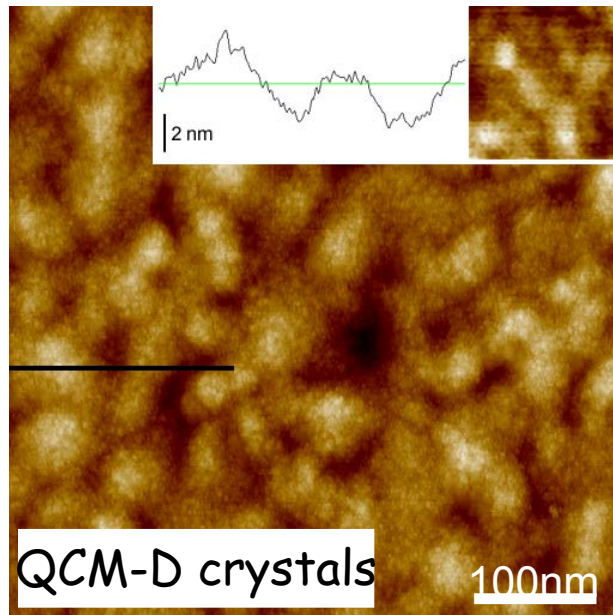
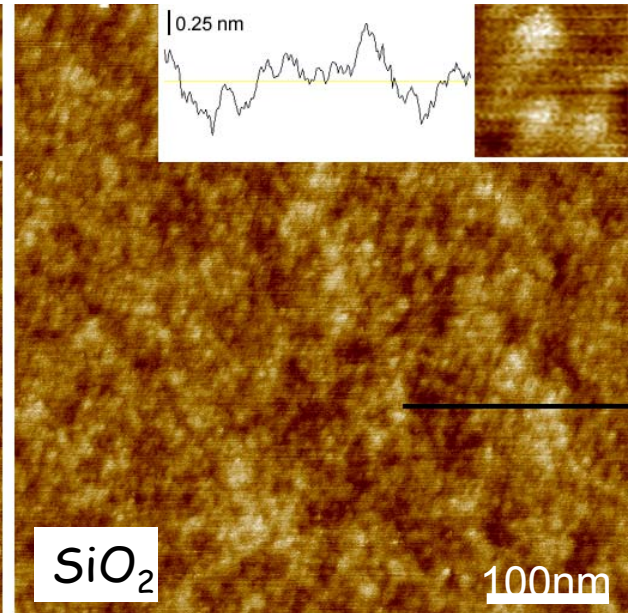
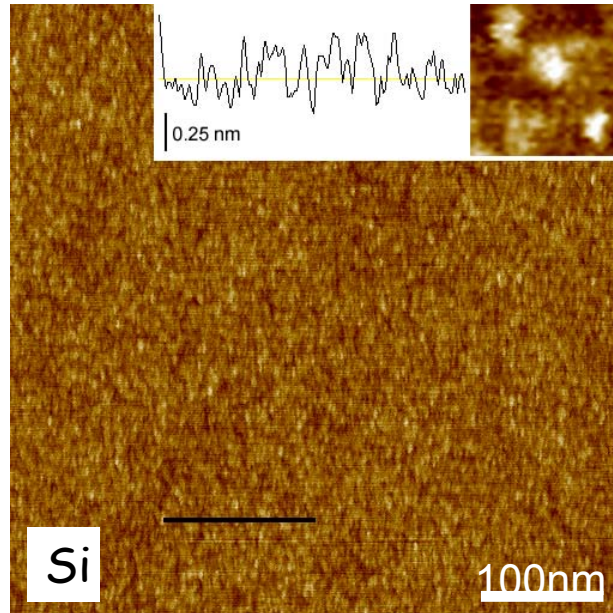
(initial study by Z. Shao et coll. 1995)

Conditions required for "molecular" resolution by AFM :

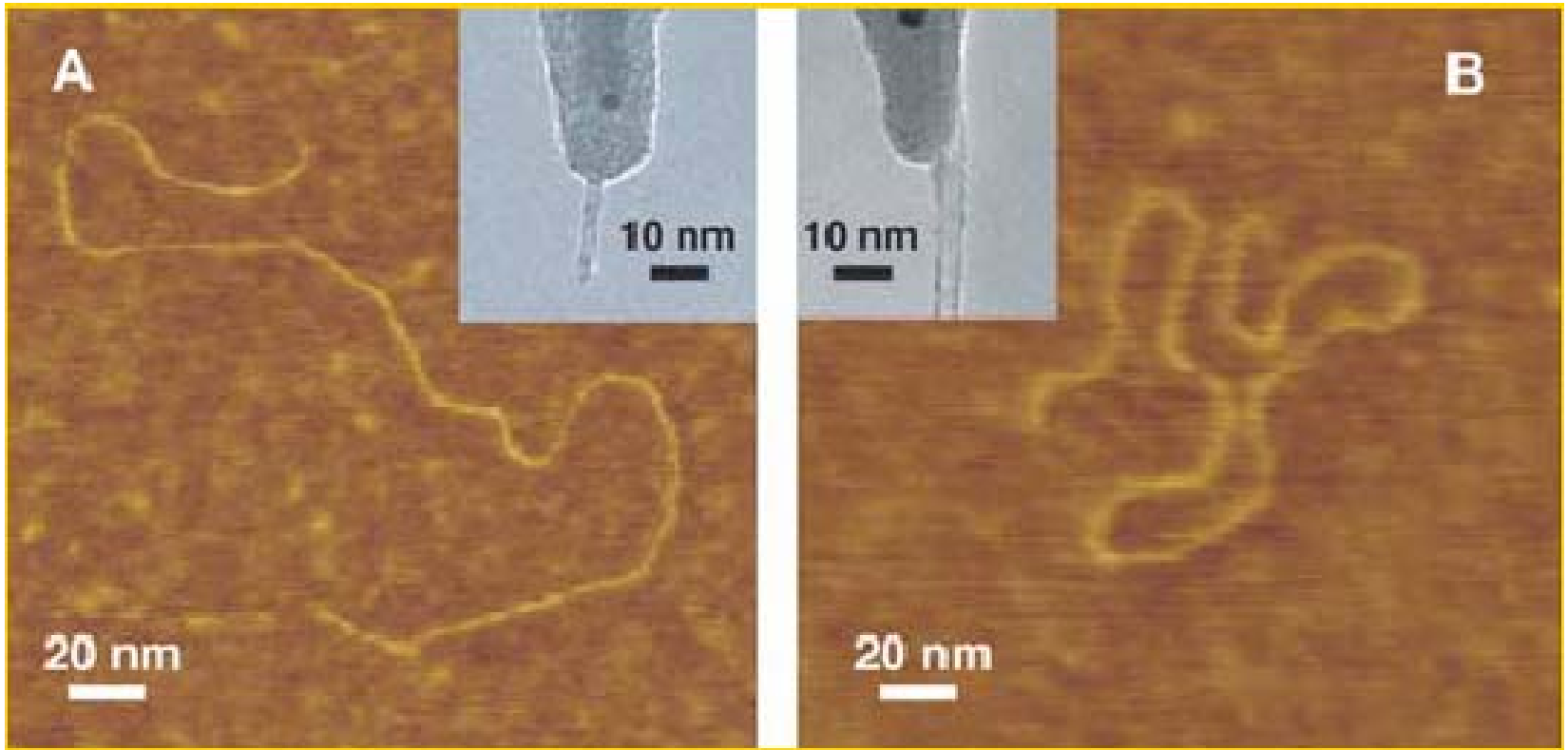
- flat support (near-atomic scale)
- molecules "immobile" on the support

Surface roughness

→ mica,
silicon,
graphite

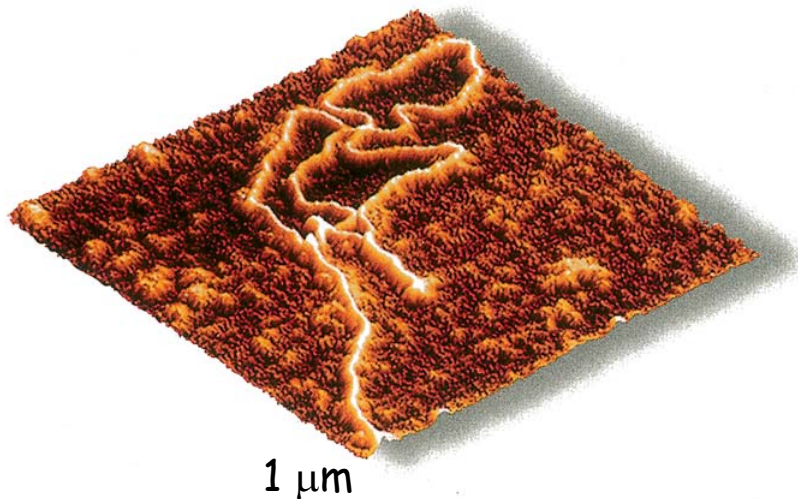


Topography of AFM filaments, in buffer



The tip consists of a C nanotube

Isolated molecules

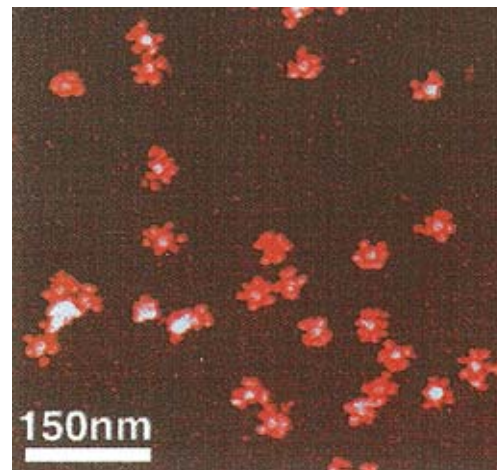


Phage lambda on mica
Tapping mode (air)

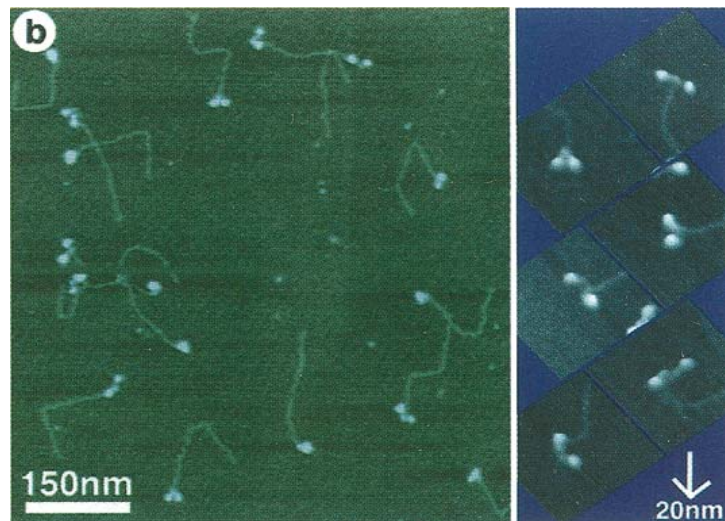
Myosin

Z. Shao FEBS Lett. 430 (1998) 51

Cryo-AFM

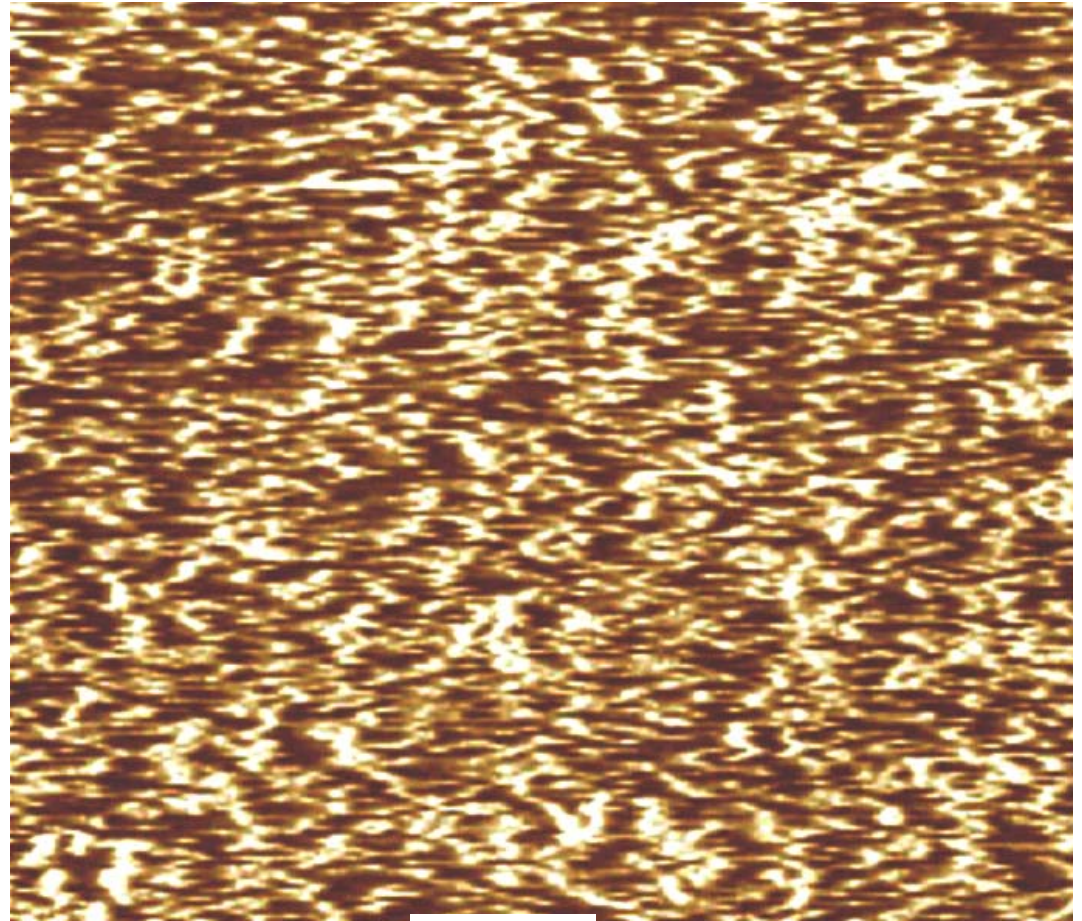


Immunoglobulins IgM on mica



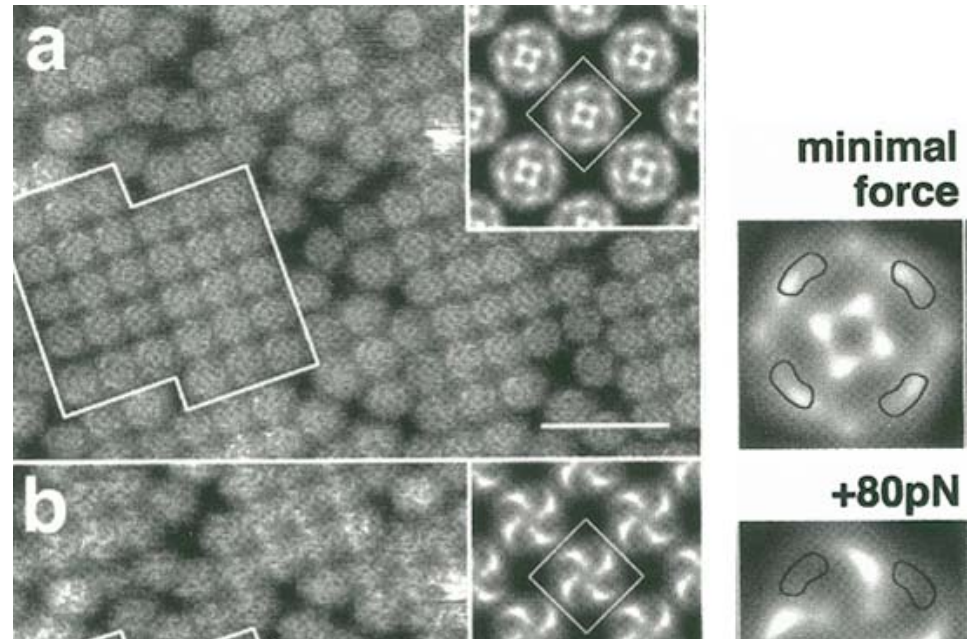
BUT: AFM imaging of isolated molecules is very difficult/risky/hazardous

2D protein matrix
at max. density

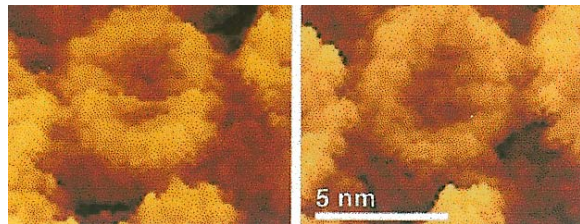


1 μm

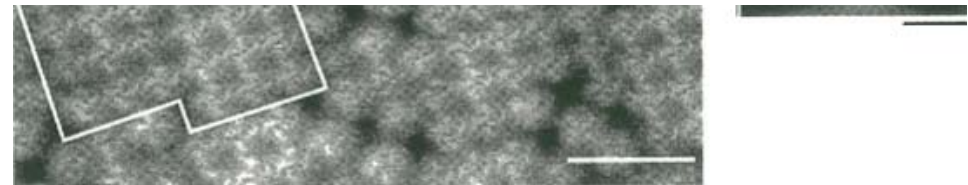
2- AFM of (membrane) protein 2D crystals



The cohesion of the 2D assembly ensures ~ immobilization



FO-subunit III of chloroplast ATP synthase consists of 14 subunits

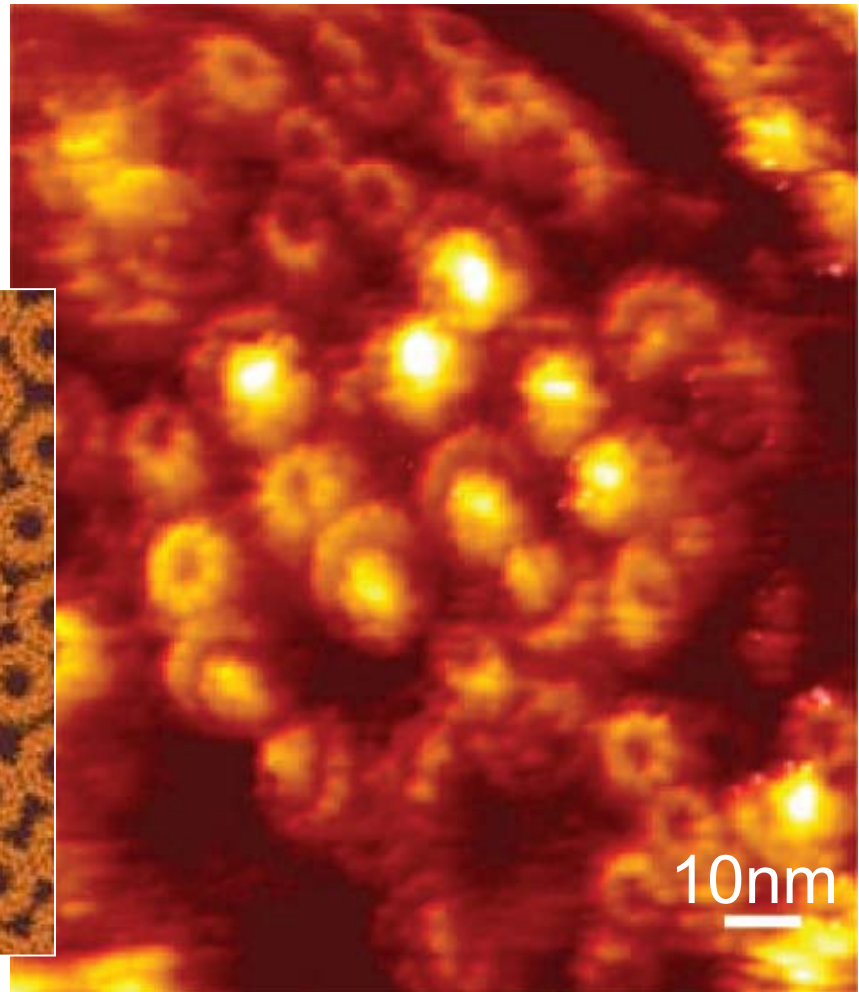
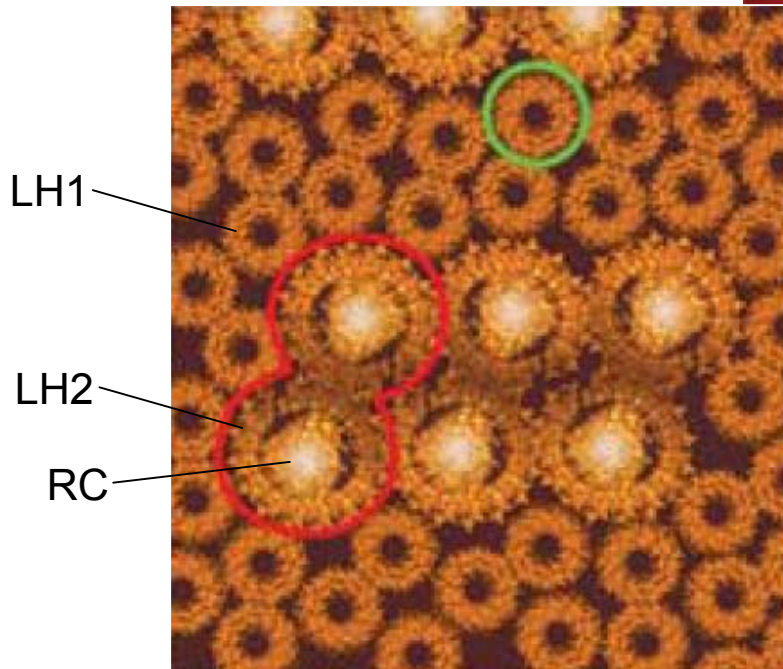


AquaporinZ-His₁₀ tetramers imaged at minimal force (a, 80 pN) and higher Force (b, +80 pN)

A. Engel & D. Mueller group, Biozentrum Basel

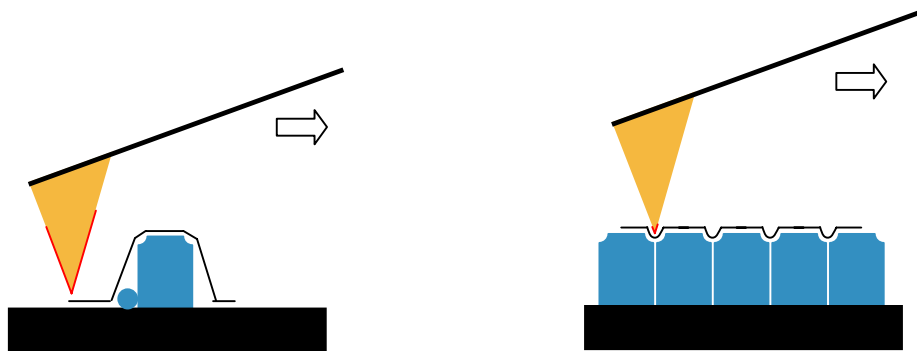
AFM Imaging of non crystalline protein assemblies

Native photosynthetic membranes



AFM Resolution

- Height resolution $\approx 0.1\text{nm}$
- Lateral resolution: Atomic resolution for **FLAT** and hard samples can be achieved
- Soft samples limit lateral resolution (1..10nm or less)
- Rough surfaces limit lateral resolution
- Low time resolution (min)

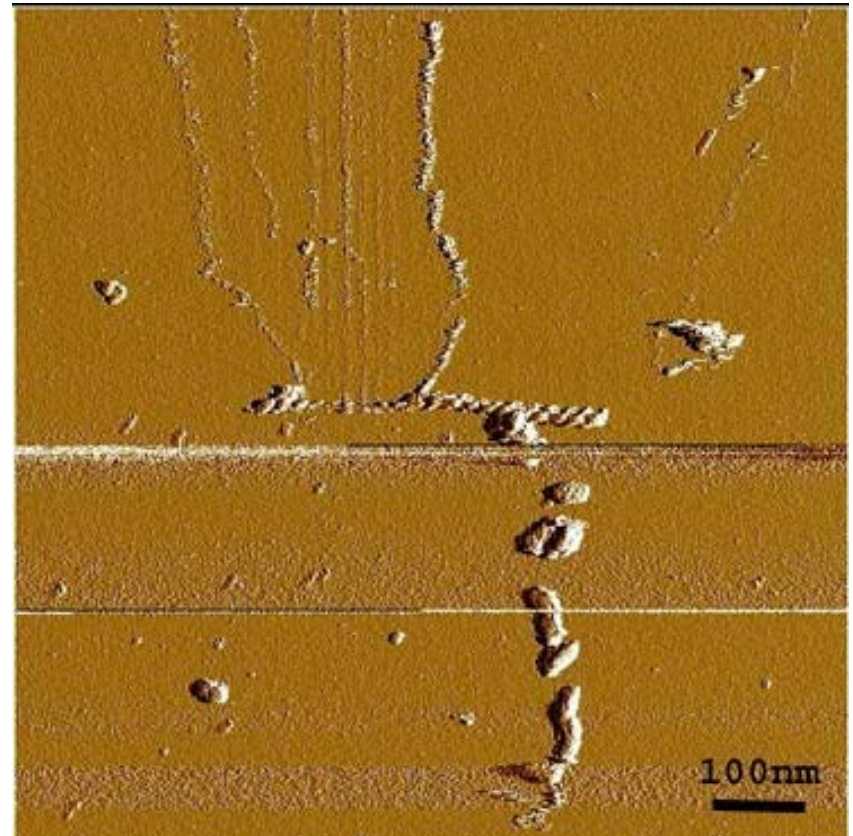
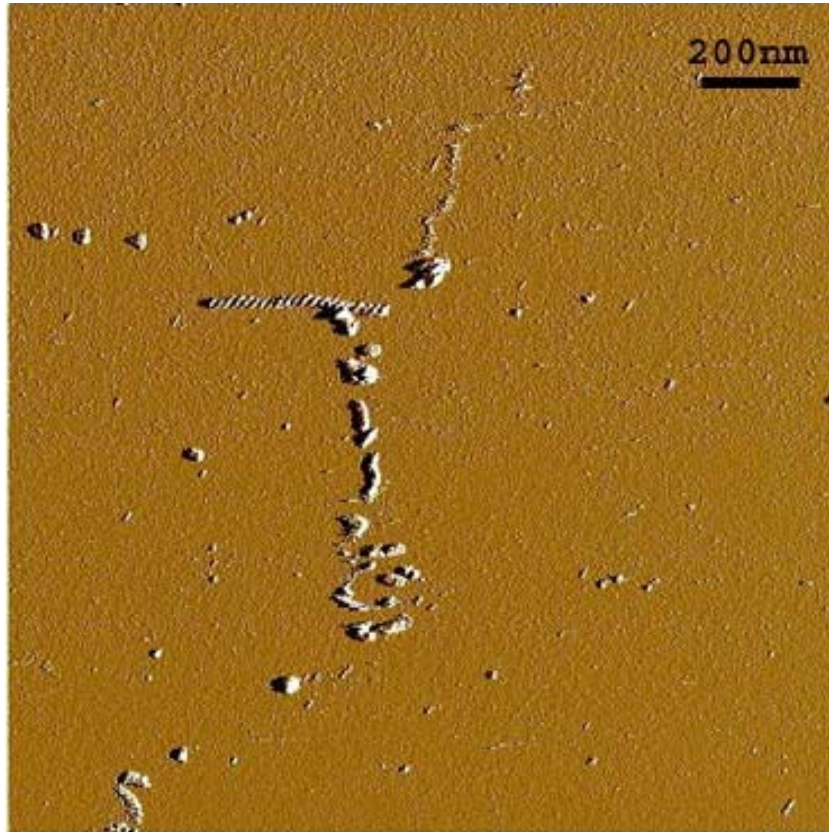


II- Dynamics of molecular processes, *in situ*, in ~ real time

- 1- Oligomerization of the $A\beta_{1-40}$ amyloid peptide
- 2- Formation of supported lipid membranes and
2D ordering of proteins on supported lipid membranes
(Course 3)

4- Tip-induced damages

Ex 1 : Destruction of amyloid fibers by the AFM tip



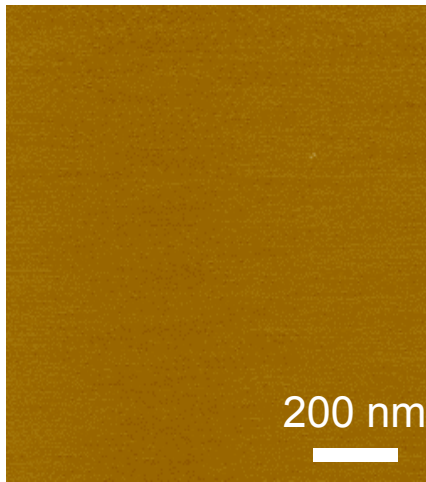
Fast scan : $\leftarrow \rightarrow$
Slow scan : \downarrow

Slow scan : \uparrow
Distance between "traces" : 20nm

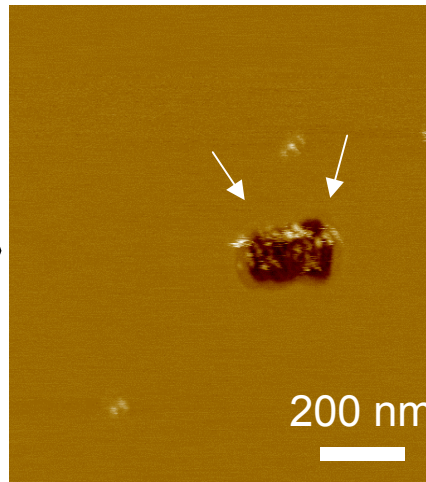
\rightarrow Information on the fiber structure

Ex 2 : Formation of a hole in a supported lipid bilayer

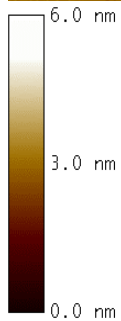
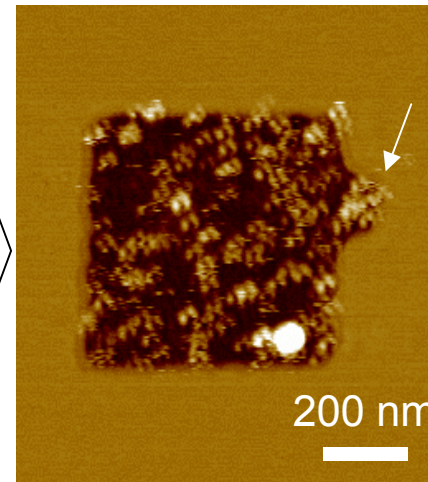
SLB – defect free



Point defects



Patch defects



	SLB: DOPC-DOPS(4:1)
	protein: B5
	support: SiO ₂

