Astrid Kronenberger
School of Engineering and Science

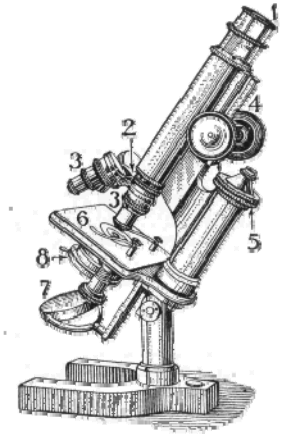
Atomic Force Microscopy
-
Basics and Applications

Summer School June 2006
„Complex Materials: Cooperative Projects of the
Natural, Engineering and Biosciences“

Outline

- Scanning Probe Microscopy
- Atomic Force Microscopy
  - General set-up & operation modes
  - Sample preparation
- Applications in life science
  - Imaging mode
  - Force-distance mode
- Conclusion

Scanning Probe Microscopy (SPM)



~1600 Light Microscope

1938: Transmission Electron Microscope

1964: Scanning Electron Microscope

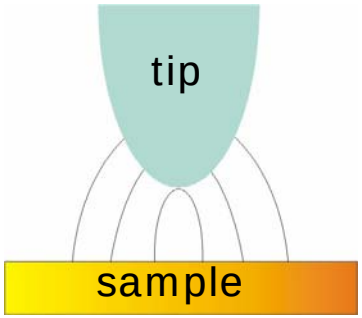
1982: Scanning Tunneling Microscope

1984: Scanning Near-field
Optical Microscope

1986: Atomic Force Microscope
- magnetic force, lateral force, chemical force...

Scanning Probe Microscopy

- Creates images of surfaces using a probe.
- Probe is moved (scanned) over the sample.
- Sample-probe interaction is monitored as function of location.



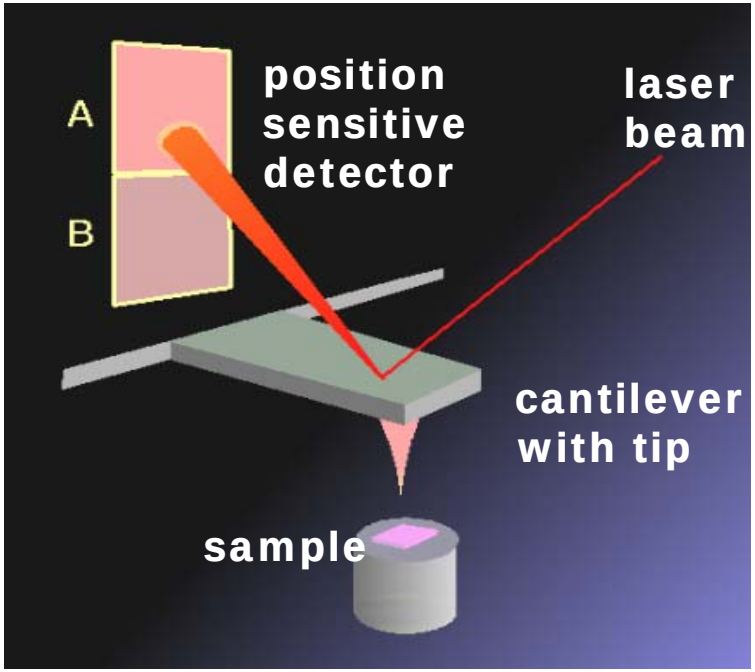
\+ Image resolution limited by probe-sample interaction volume - not by diffraction .

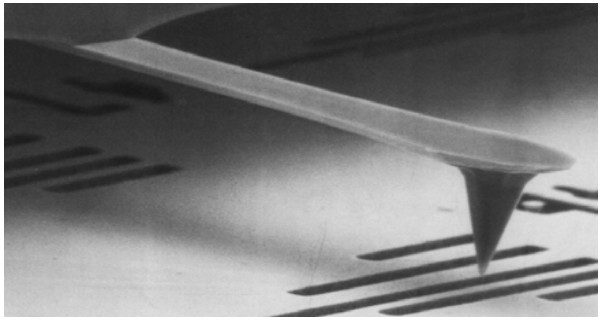
\+ Interaction can modify surface - nanolithography possible.

\- Scanning technique quite slow.

\- Limited maximum image size.

Atomic Force Microscopy





Molecular interaction:

$E = F \Delta s$

$E \sim eV$; $\Delta s \sim Å$

$\Rightarrow F \sim 2 \cdot 10^{-9}$ N

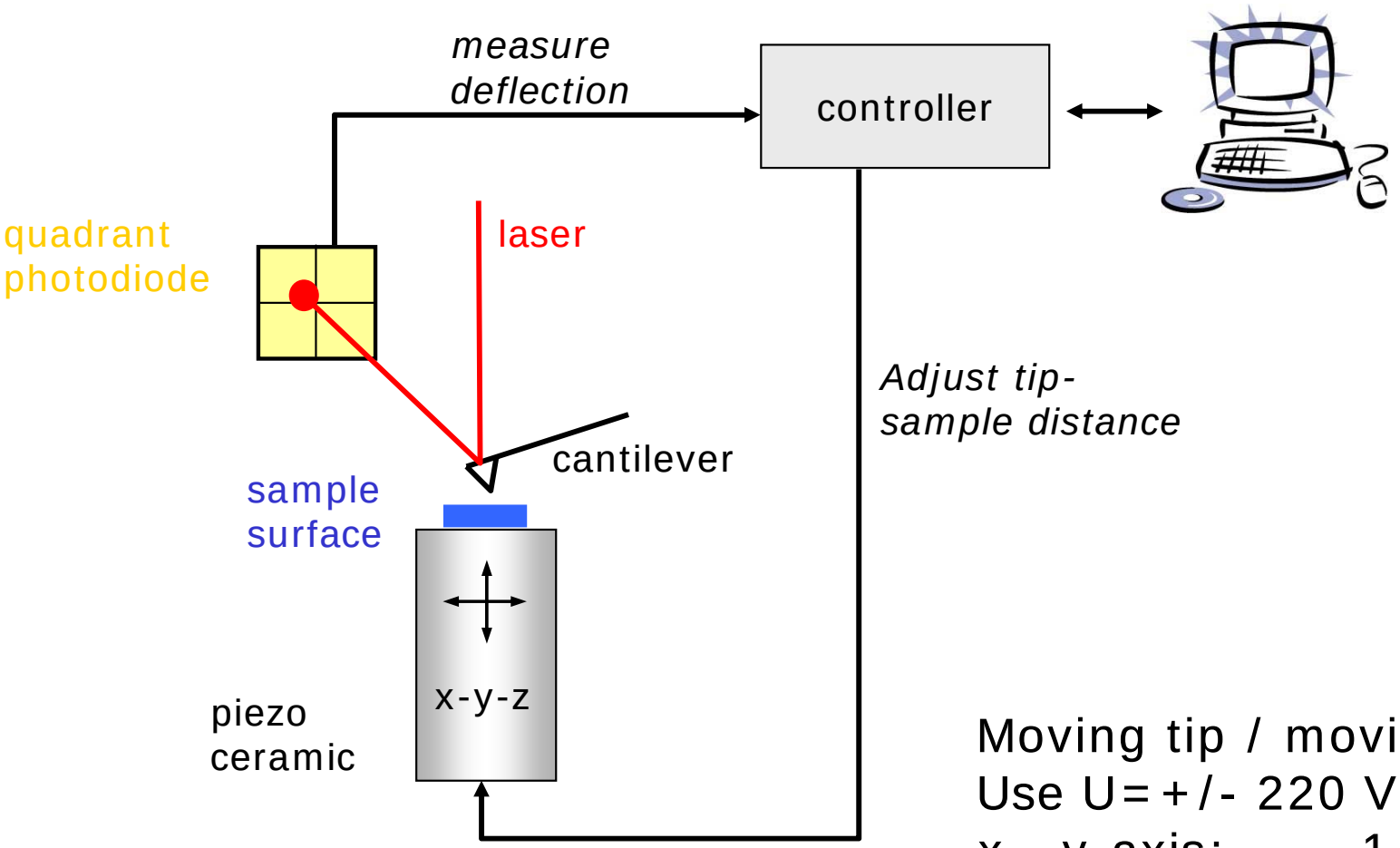
Typical AFM resolution:

x-y: 1nm; z: 0.1nm

Detection:

- sub-Å deflection
- pN forces

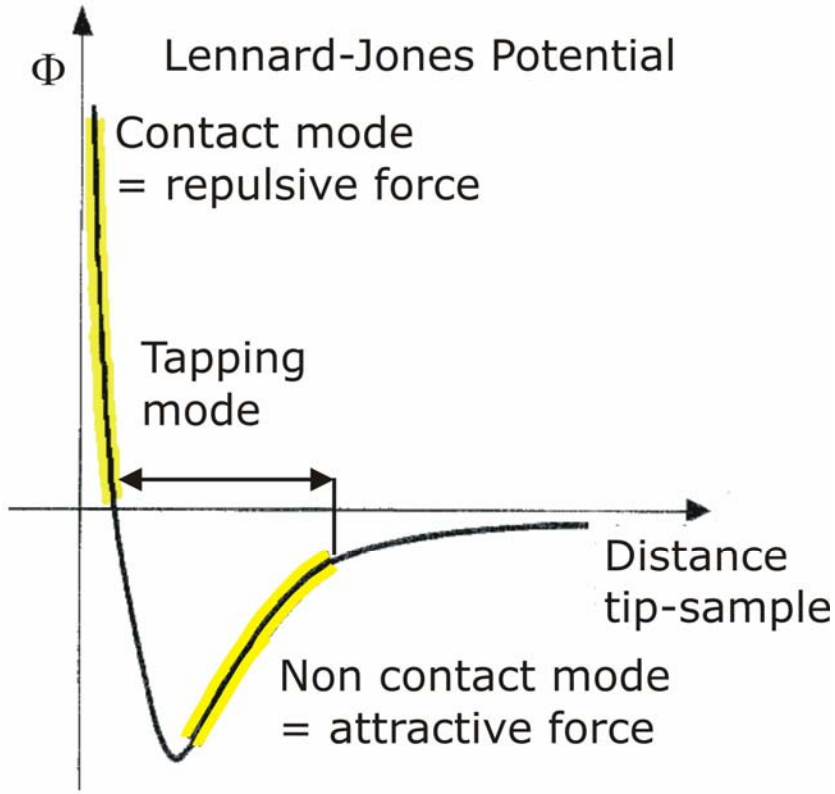
General AFM set-up



Moving tip / moving sample:
Use U= +/- 220 V
x-, y-axis: 1 ...125 µm
z-axis: 1 ... 20 µm
closed / open loop control

Basic AFM modi

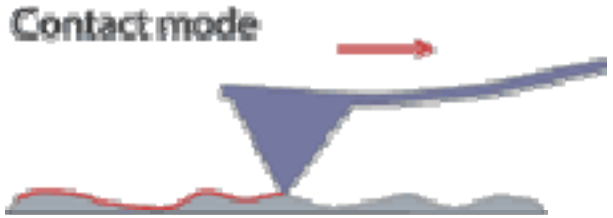
- Imaging mode
  - contact mode
  - non contact mode
  - intermittent / tapping mode
- Force-distance mode
  - force spectroscopy
  - combined imaging & force spectroscopy



Static AFM modi

- Contact mode:
  - tip in continuous contact with sample
  - preferably used for hard samples
  - imaging in air and liquid
  - high resolution

  detect: deflection



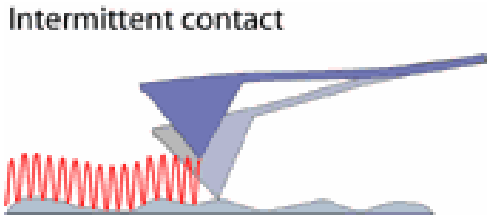
- Force spectroscopy mode:
  - consecutive cycles of tip approach and retract
  - interaction forces between tip and sample are recorded

$F = - k_{spring} \cdot \Delta x$

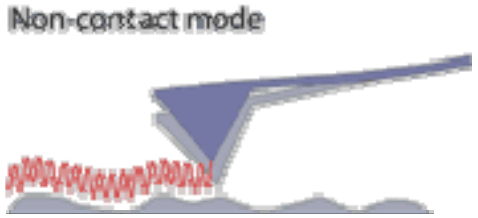
http://www.jpk.com/tutorial/tutorial1.htm

Dynamic AFM modi

- Intermittent/tapping mode:
  - oscillating cantilever, tip touching surface gently and frequently
  - often used for biological samples
  - imaging in air and liquid
  - good resolution



- Non contact mode:
  - oscillating cantilever, tip not in contact with sample
  - used for soft samples
  - imaging in vacuum
  - distance range 50Å - 150Å

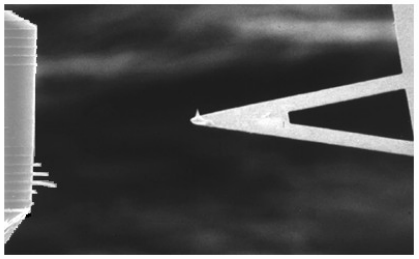


$$\omega = \sqrt{\frac{k}{m_{eff}}}$$

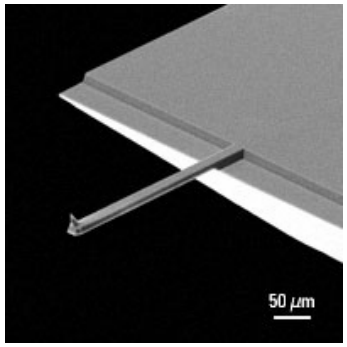
detect: amplitude
phase
deflection

http://www.jpk.com/tutorial/tutorial1.htm

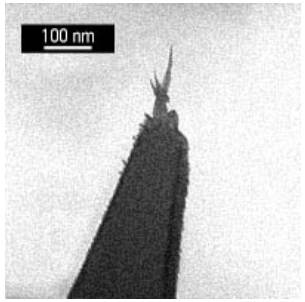
Microfabricated AFM cantilevers



silicon nitride cantilevers



silicon cantilevers

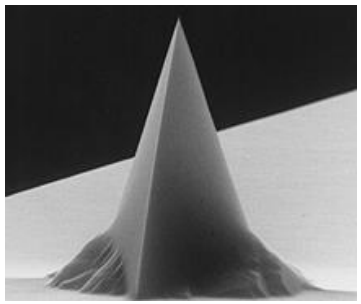


diamond tip

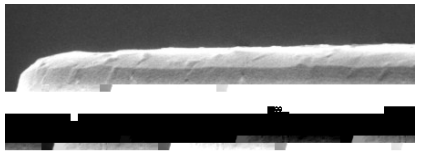
Typical cantilevers:
1µm thick, 100s µm long
k_{spring} ~ 0.01 ... 20N/m
f_{res} ~ 4 ... 400kHz
r_{tip} ~ 1 ... 20nm
reflective backside coating:
- better signal

spring constant:
force resolution
resonance frequency
tip radius:
lateral resolution
tip aspect ratio:
“depth" resolution

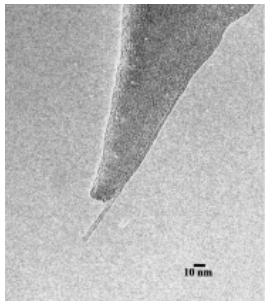
$$k_{spring} = \frac{E \cdot w \cdot t^3}{4 \cdot l^3}$$



standard tip



sharpened tip



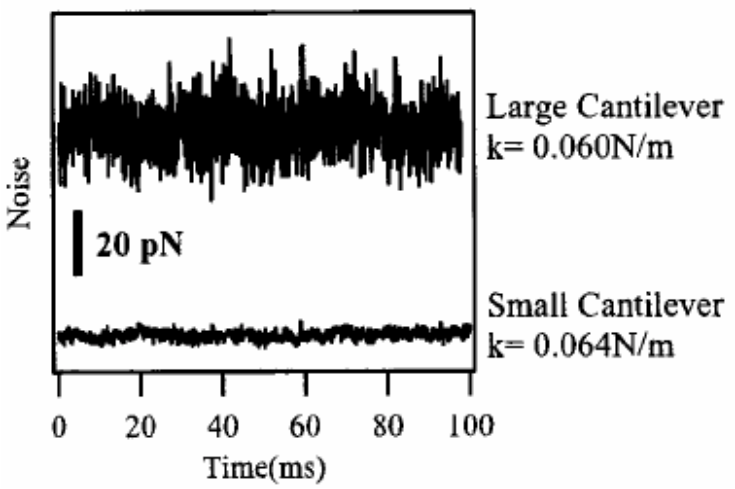
nanotube tip

Cantilever calibration and signal-to-noise ratio

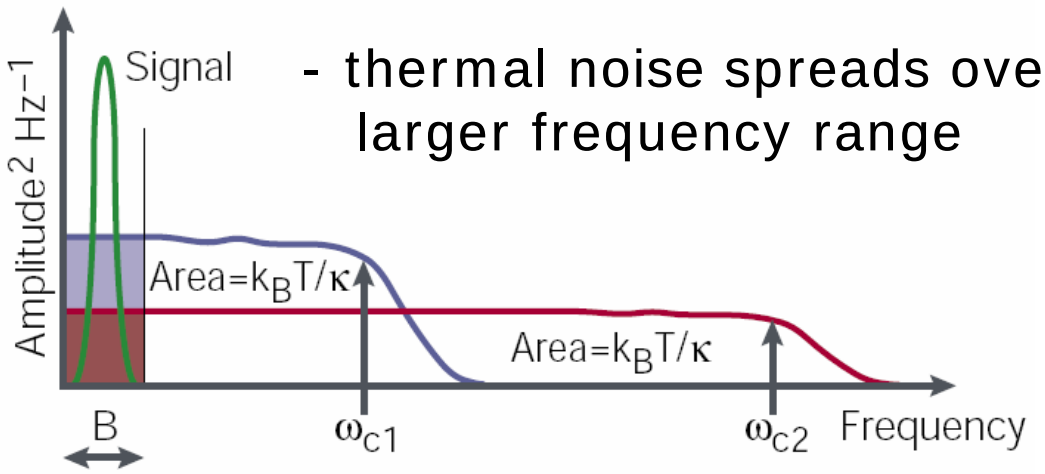
- Thermal noise:

Equipartition theorem

$$k_{spring} \cdot \langle \Delta x^2 \rangle = k_B T$$



- Comparison:
  small – large cantilever
  $k_{small} = k_{large}$



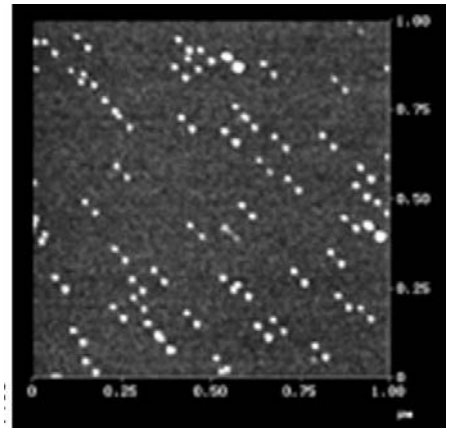
- thermal noise spreads over larger frequency range

small cantilever:
- better signal-to-noise
- faster measurements

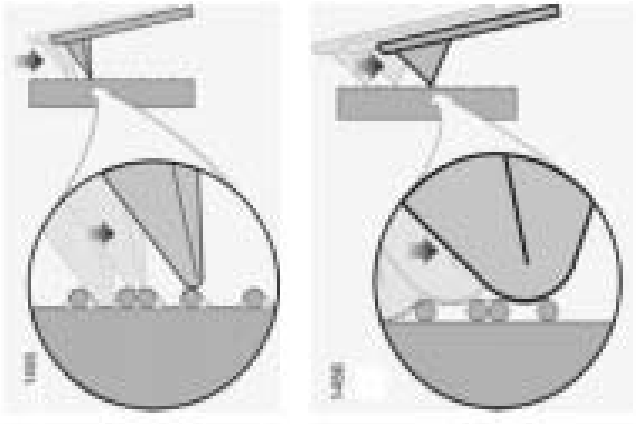
Viani et al, *APL* 1999

Bustamante et al., *Nature Rev. Mol. Cell Biol.* 2000

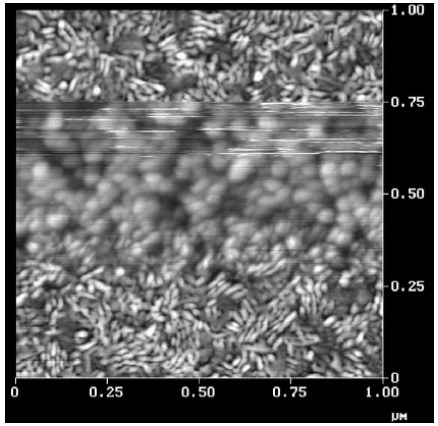
Imaging artefacts

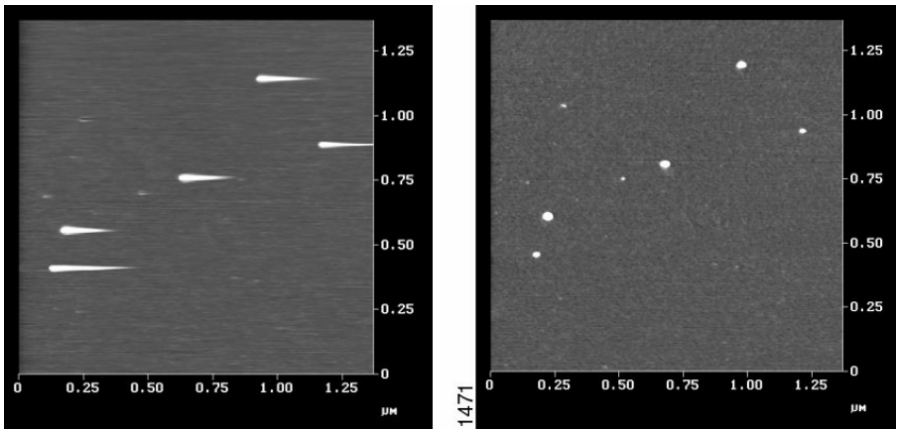


- Appearance of objects
  - tip radius
  - aspect-ratio
  - double tip
  - blunt tip



- Blurred images
  - contamination
  - feedback adjustment
  - interference
  - noise
  - thermal drift
  - static charging





Sample preparation

- Suitable substrate

  flat and rigid

  – mica (atomically flat, hydrophilic)

  – SiO_2, glass (nm roughness, hydrophobic)

  – ultraflat gold (stripped gold)

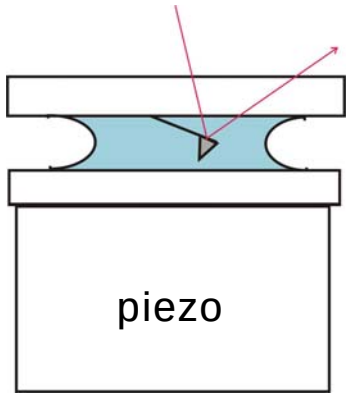
- Immobilisation of sample

- Typical sample size

  – Scanning surface: ~1cm²

  – Scanning tip: ~ Petri dish

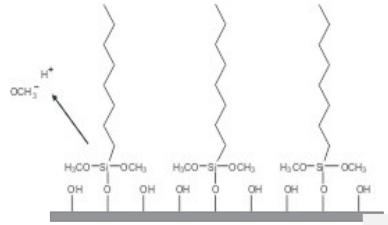
  – Liquid sample: 1 ... 100µl



Surface-/ Tip-Functionalisation

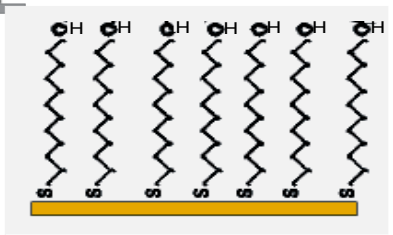
- Surface modification

  self assembling monolayers (SAM)
  - silanes on glass- and Si-surfaces
  - thioles on Au-surfaces



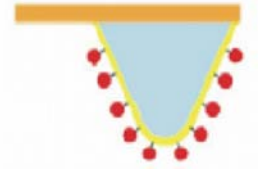
SAM formation

silanes

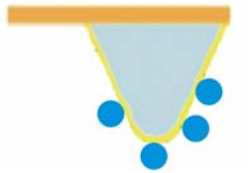


thioles

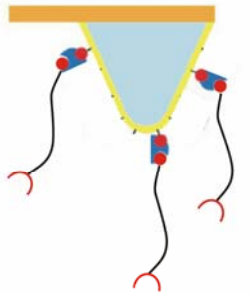
- Tip modification
  - Adsorption of molecules from solution e.g. proteins



  - Decrease AFM tip radius with attachment of molecules or nanotubes

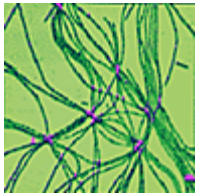


  - Attachment of linker molecules e.g. PEG linker for antibodies, crosslinker for SH-, NH-groups

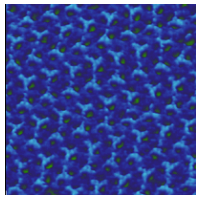


Applications

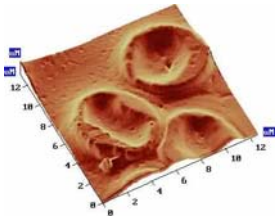
Lifescience



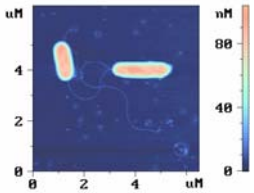
Actin filaments



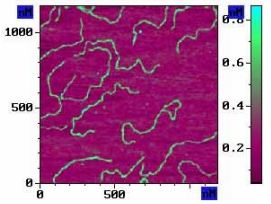
Proteins



Erythrocytes

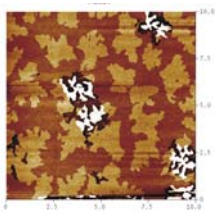


Bacteria

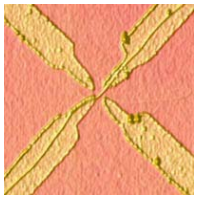


Linearised DNA

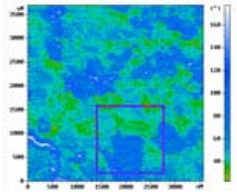
Materials and Surface Science



Organic film

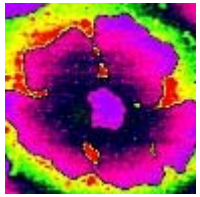


Transistor



Ferroelectric domains

Triblock co-polymer film

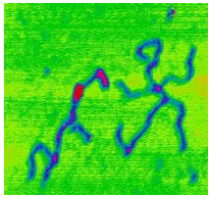


Polymer

Nanolithography & Nanomanipulation

Polymer film engraving

Anodic oxidation



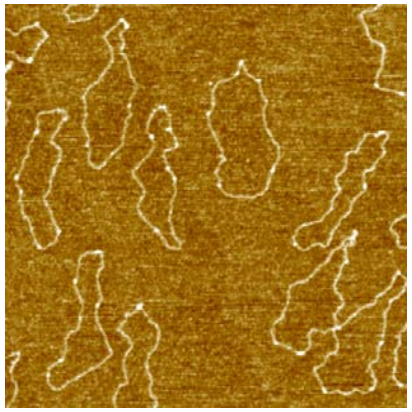
DNA on mica

http://www.jpk.com/
http://www.veeco.com/nanotheatre/
http://www.ntmdt.ru/Scan-gallery/

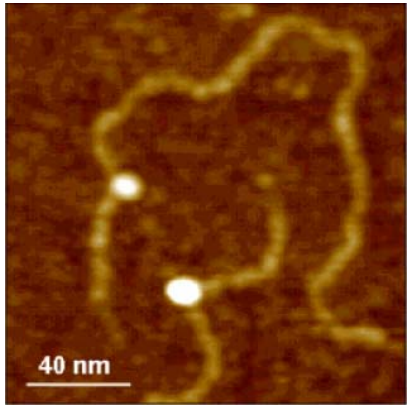
Imaging of isolated molecules in air

Biomolecular structure

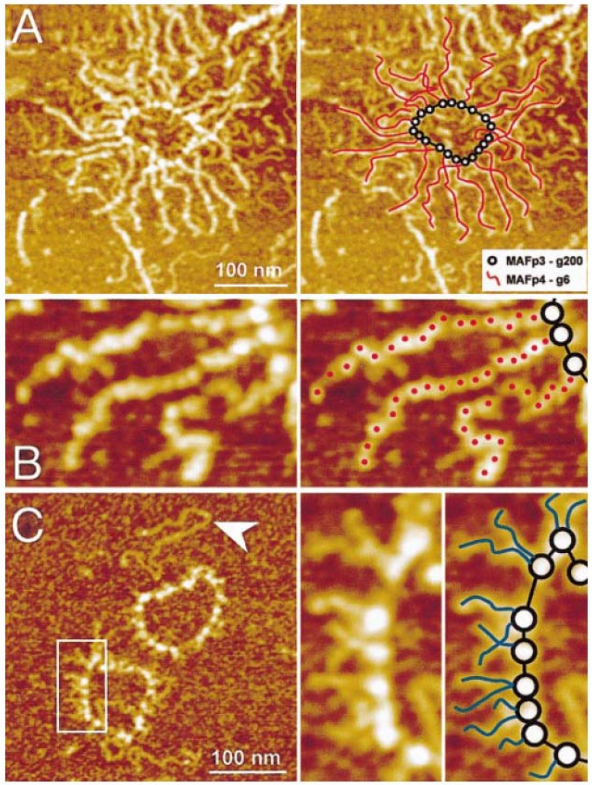
Imaging DNA-protein complexes on aminoterminated mica



DNA plasmids



λ-DNA restriction enzyme complex (*Hae* III restriction endonuclease induces bending at GGCC)



Supramolecular structure of aggregation factors of marine sponges (*Microciona prolifera*) (circular proteoglycans)
MAFp3 : self-interaction between MAF
MAFp4 : binds cell surface receptors

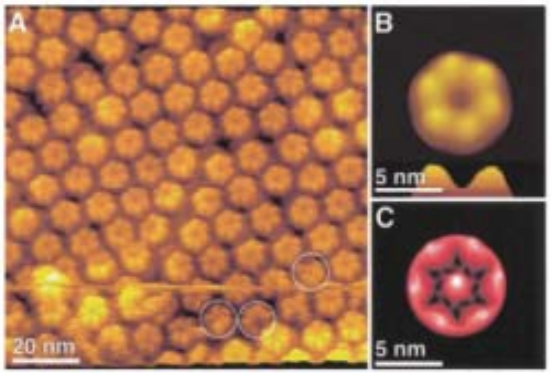
Anselmetti et al., *Single. Mol.* 2000
Jarchow et al., *J. Struct. Biol.* 2000

Imaging of 2d crystals in liquid

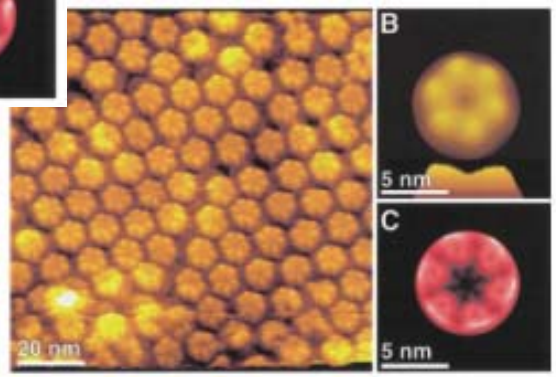
Conformational changes

Extracellular connexon surface.

contact mode measurements in buffer solution

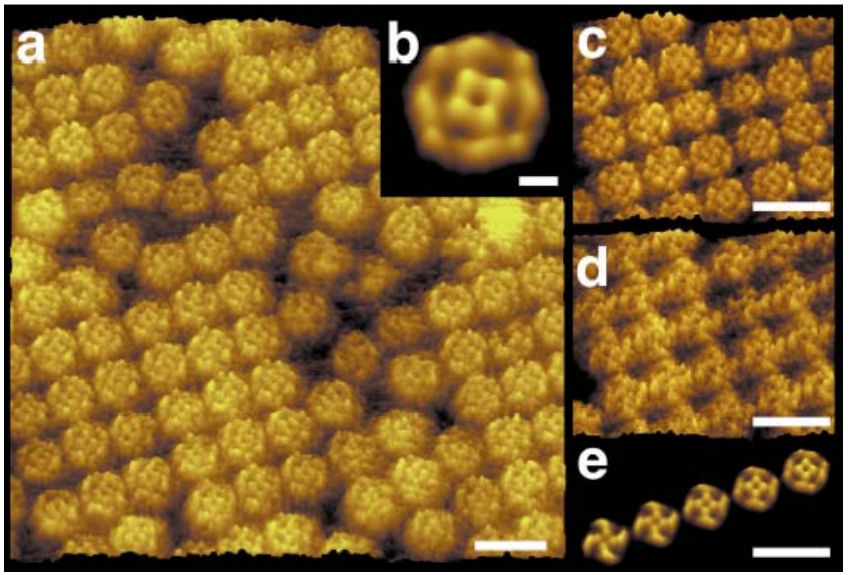


Without Ca^{2+} open channel 1.5nm



With Ca^{2+} closed channel 1.5nm

20nm



10nm

Extracellular aquaporin surface.

contact mode measurements in buffer solution

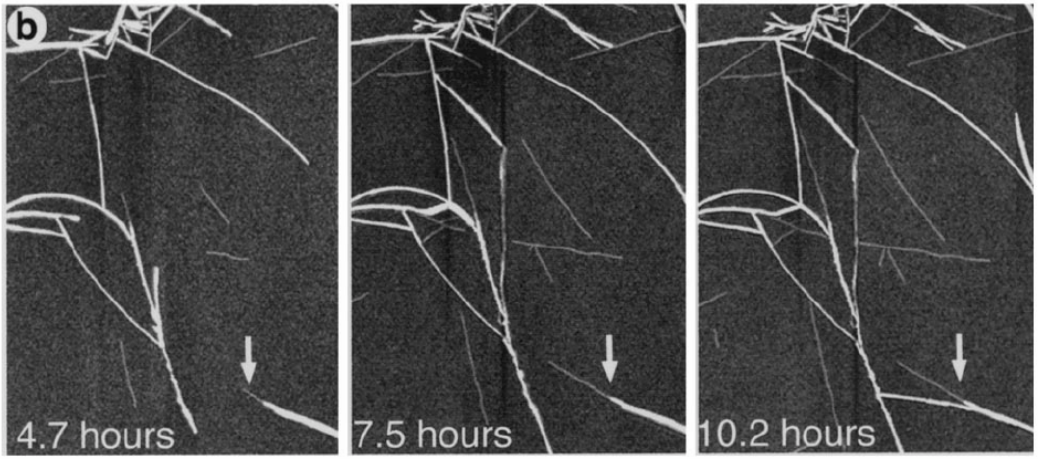
Change of conformation of tetramers

c) Minimal force

d) Maximal force

Müller et al., *EMBO J.* 2002
Scheuring et al., *Single Mol*. 2001

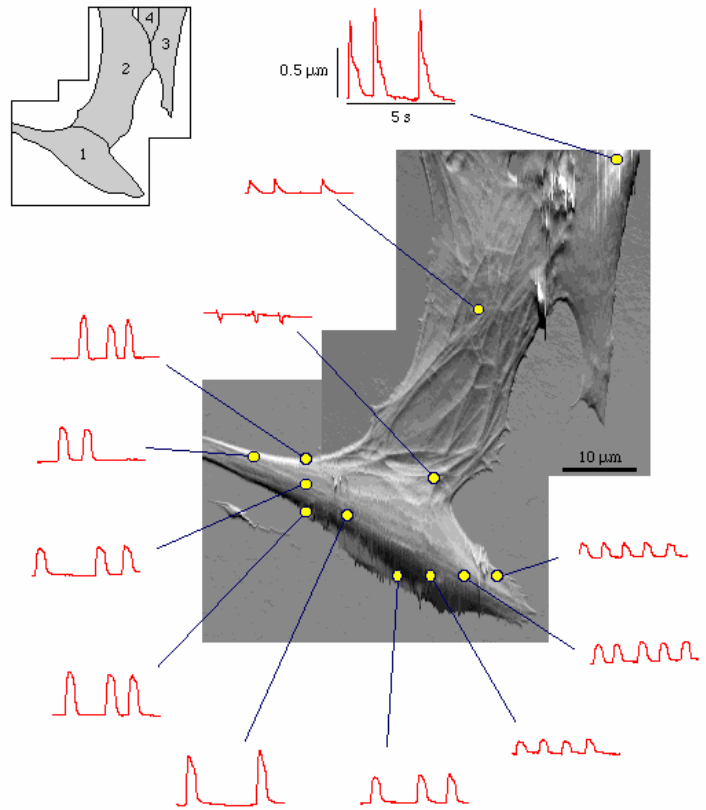
Imaging of cells and long term processes

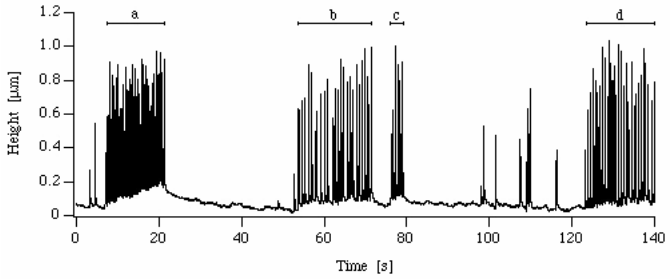


Time-lapse AFM for imaging growth of amyloid fibrils (synthetic human amylin)

Goldsbury et al., *J. Mol. Biol.* 1999

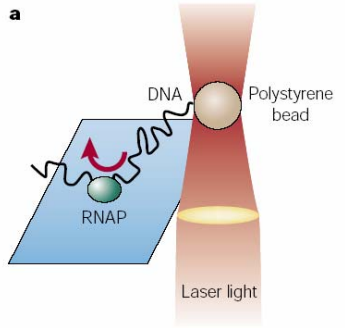
Measuring the heartbeat of single cells
(chicken cardiomyocytes)





Radmacher, Uni Bremen

Forces in molecular biology



optical tweezers

pN

1000

500

100

50

10

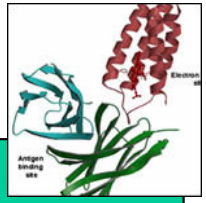
1

covalent bond

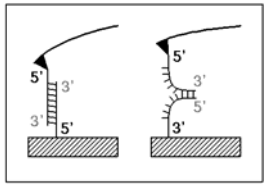
rupture forces

selectins

biotin - avidin

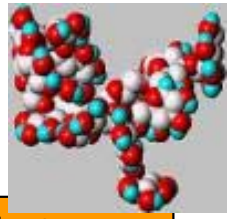


antigen - antibody



unzipping DNA
GC -
AT -

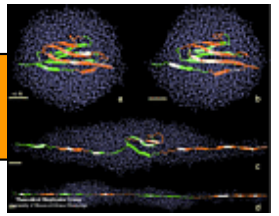
typ.at 0.1 - 5 nN/sec



elasticities

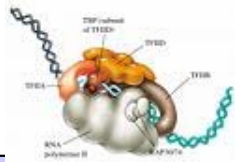
dextran bond flip

unfolding titin



extracting bacteriorhodopsin

DNA B-S transition

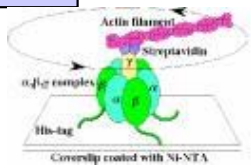


extracting forces

extracting lipids

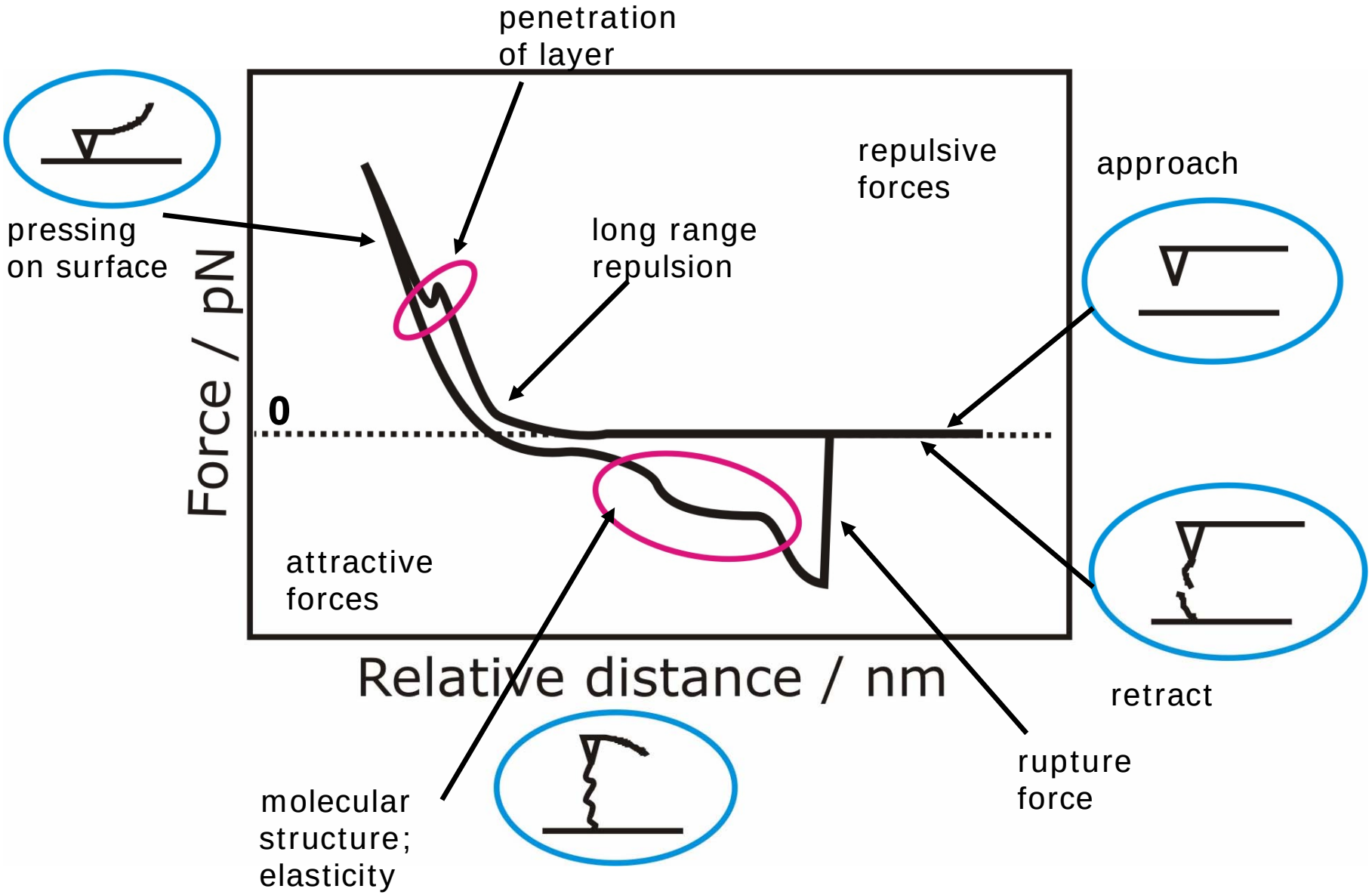
RNA polymerase

motor proteins



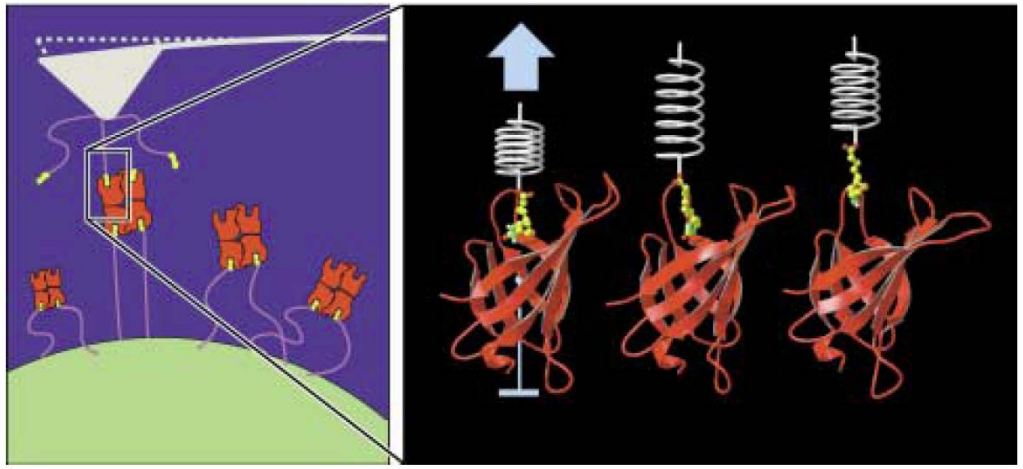
active forces

Interpretation of force-distance curves

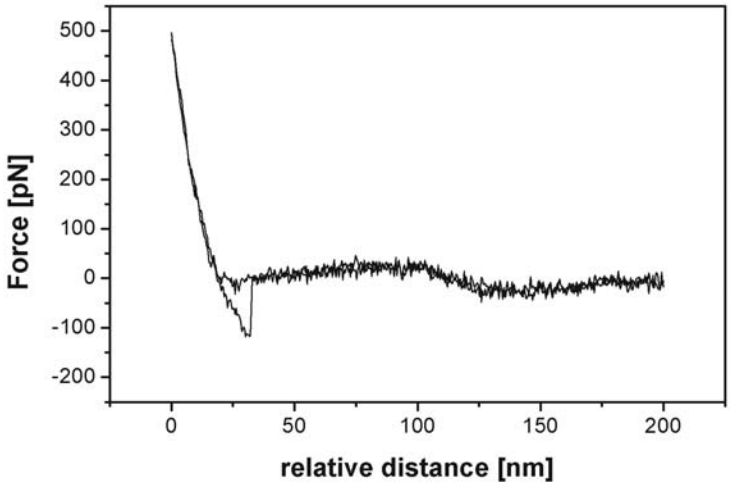


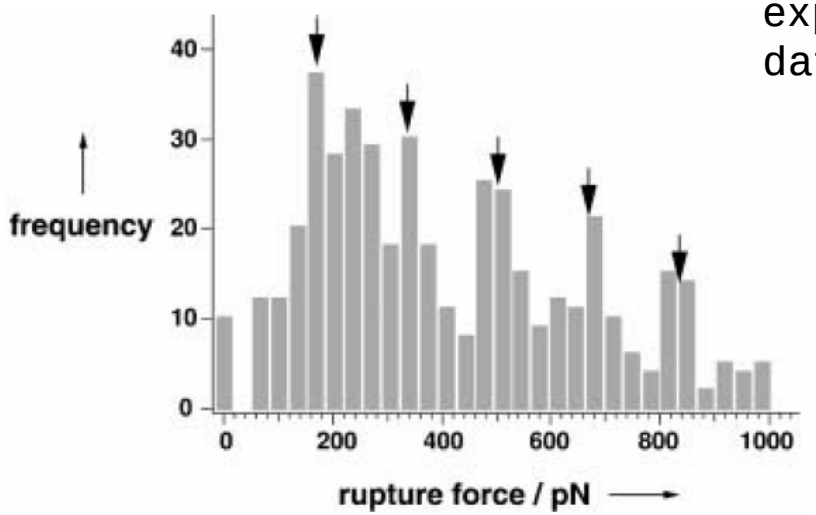
Rupture forces

biotin - avidin interaction



MD simulation

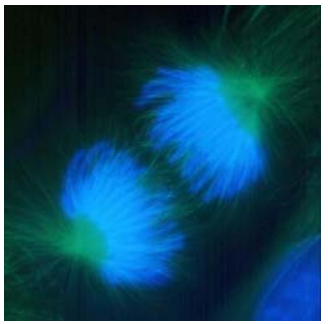




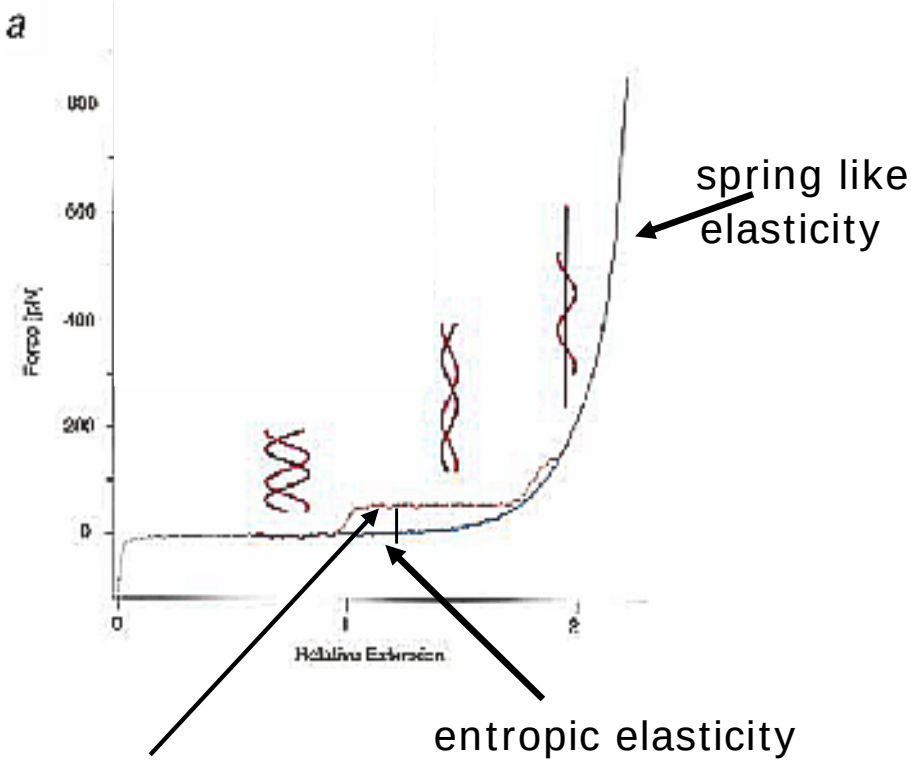
experimental data

Gaub group, *Science* 1994
Grubmüller et al., *Science* 1996
Fritz at al., *J.Str.Biol.* (1997)

Molecular elasticities



Experimental data

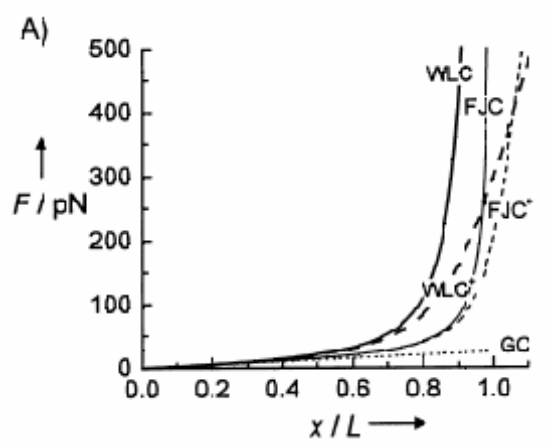


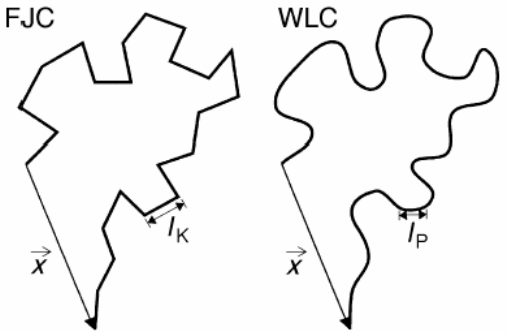
spring like elasticity

entropic elasticity

BS transition
F ~80 pN

Modelling data





Persistance lengths:
ss DNA: p ~ 1nm
dS DNA: p ~ 50nm
Polypeptide: p ~0.4nm

Force-distance curve =
fingerprint for polymers

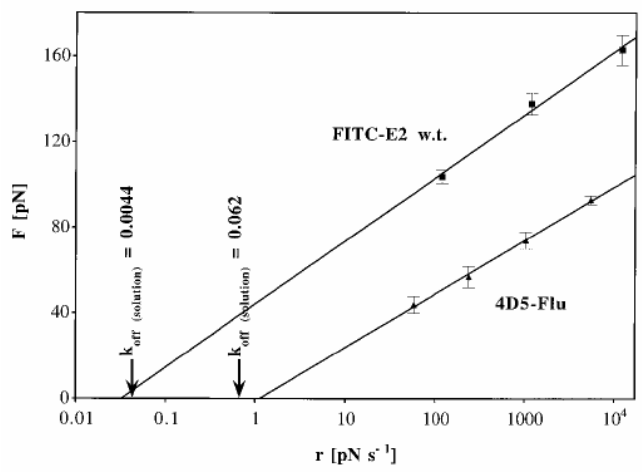
http://en.wikipedia.org/wiki/Anaphase
Rief et al. 1999
Janshoff et al., 2000
Fuchs et al., Angw.Chem. Int.Ed. (2000)

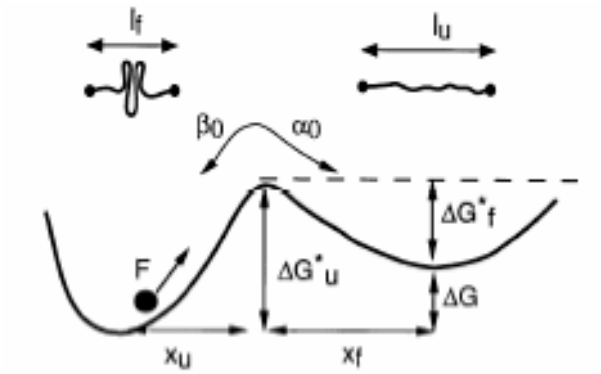
Connection of rupture force to biochemical data

Chemical reaction: $A + B \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} AB$

$$K_d = \frac{k_{off}}{k_{on}}$$

$$\Delta G_{bind} = RT \ln(K_d)$$





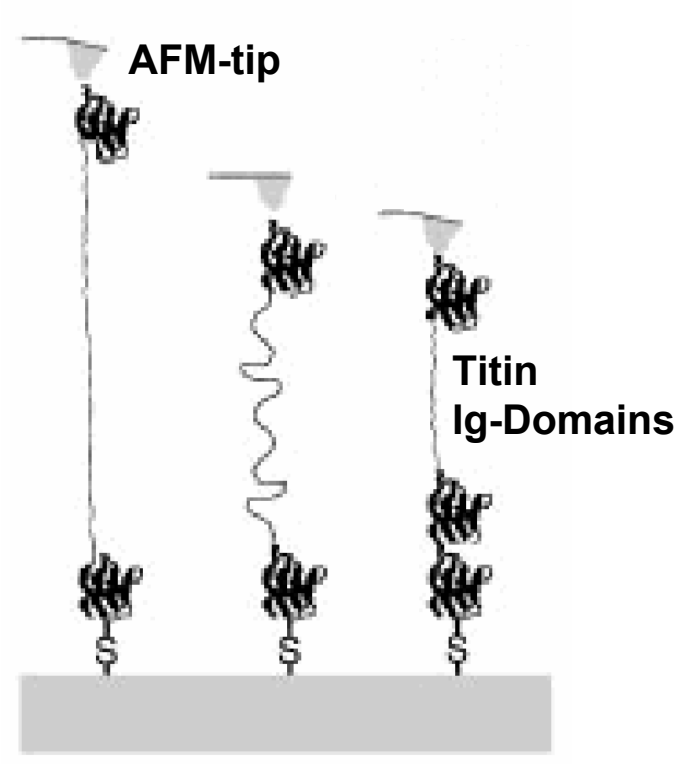
- rupture force influenced by pulling velocity
- force decreases energy barrier for unfolding
- $k_{off}(F) = k^0_{off} \cdot e^{\frac{F \cdot x}{k_B T}}$

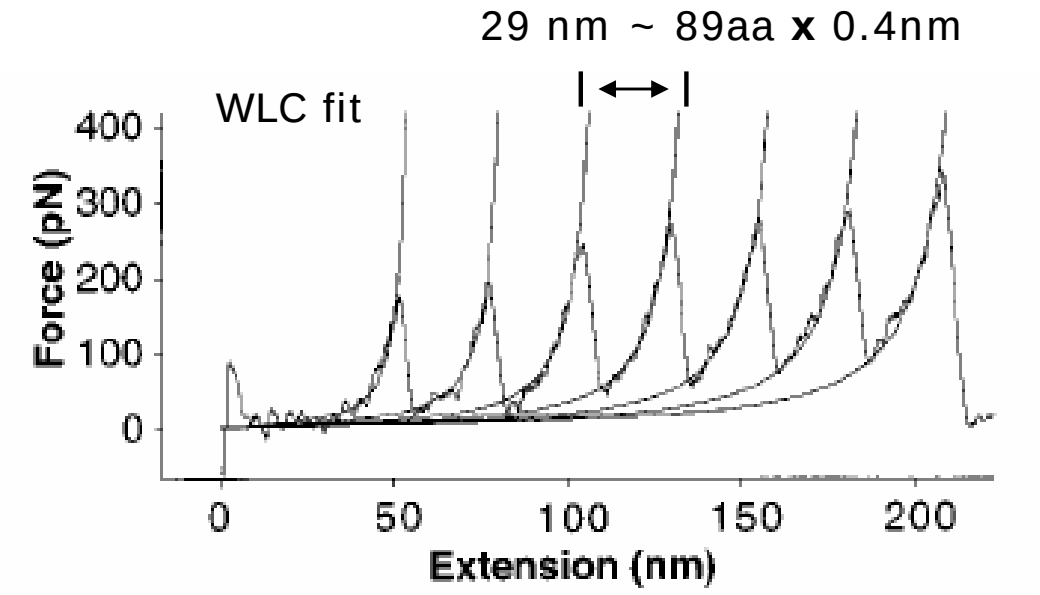
  off-rate increased by force
- rupture / unfolding forces correlate with k_{off}

Schwesinger et al., *PNAS* 2000
Rief et al., *PRL* 1998

Unfolding proteins

- Gain new insights in protein folding
- Novel design strategies for glue

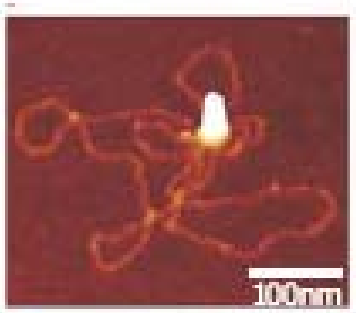




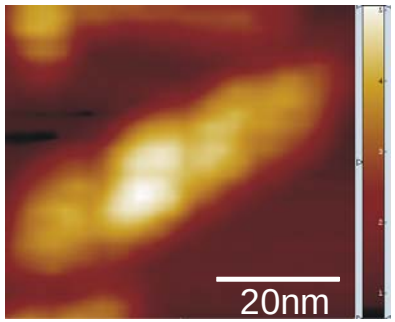
Rief et al., *Science* 1997

AFM @ IUB

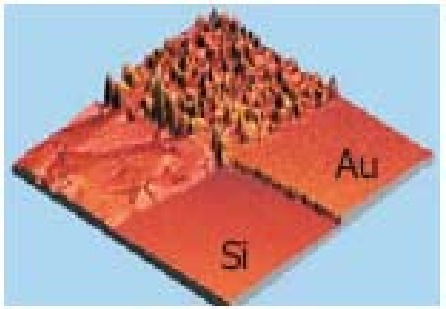
- Investigation of DNA - protein interaction
  S. Maurer



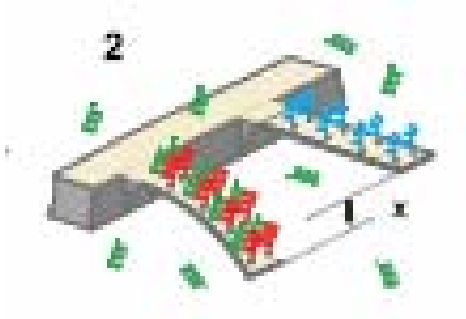
- Investion of proteins & liposomes
  A. Kronenberger



- Investigation of thin organic films
  Prof. V. Wagner



- Investigation on cantilever sensor arrays
  Prof. J. Fritz



Conclusion

AFM is a versatile tool to investigate

- topography of surfaces
- properties of surfaces
- properties of single molecules
- forces within molecules

But: always consider experimental conditions and artefacts on measurements!

Thanks!