NOTES ON NANO(BIO)TECHNOLOGOGY

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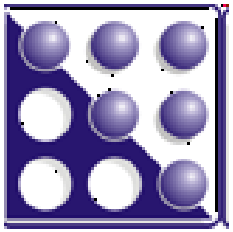


Vienna (Mödling) – 16/10/2013

Who are we since Sept 2010 - organisation

Dept. Nanobiotechnology

Bioarchitectures	**Bio-inspared. Materials**	**Biophysics**
Eva Sinner	Erik Reimhult	Jose. L. Toca-Herrera

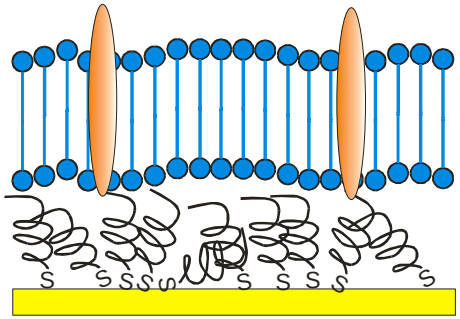
Full Professors: 3
Emeritus Professor: 1
Associate Professors: 4
University Assistants: 7
Administration: 1

Postdocs: 7
PhD students: 16
MSc students: 10
Technical assistants: 7

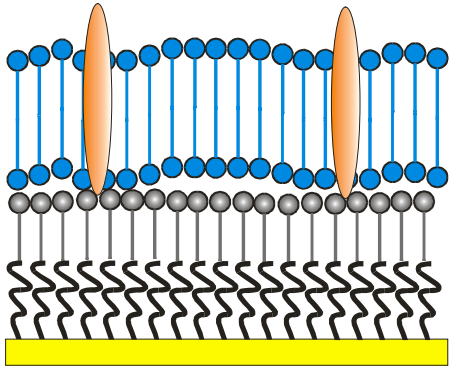
We are also hosting PhD and MSc students of other universities or institutes!

DNBT – Research Eva Sinner

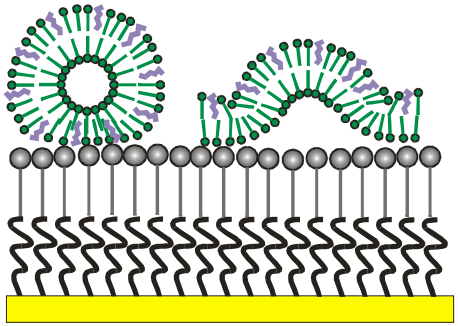
- Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems which display functions that do not exist in nature



- Peptide P19
- CHO membrane (with *hPepT1*)



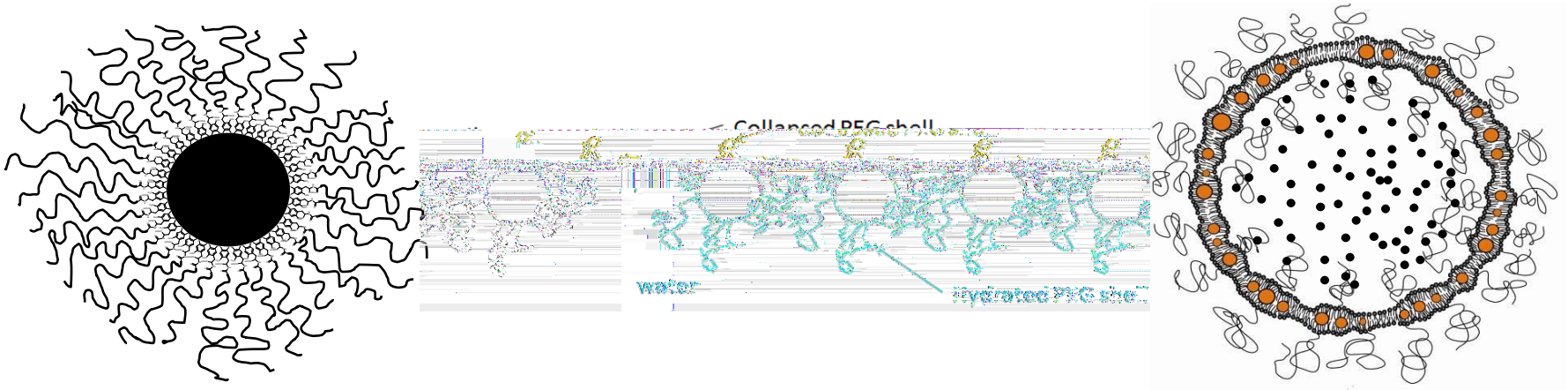
- Octadecanthiol
- Lipid
- CHO membrane (with *hPepT1*)



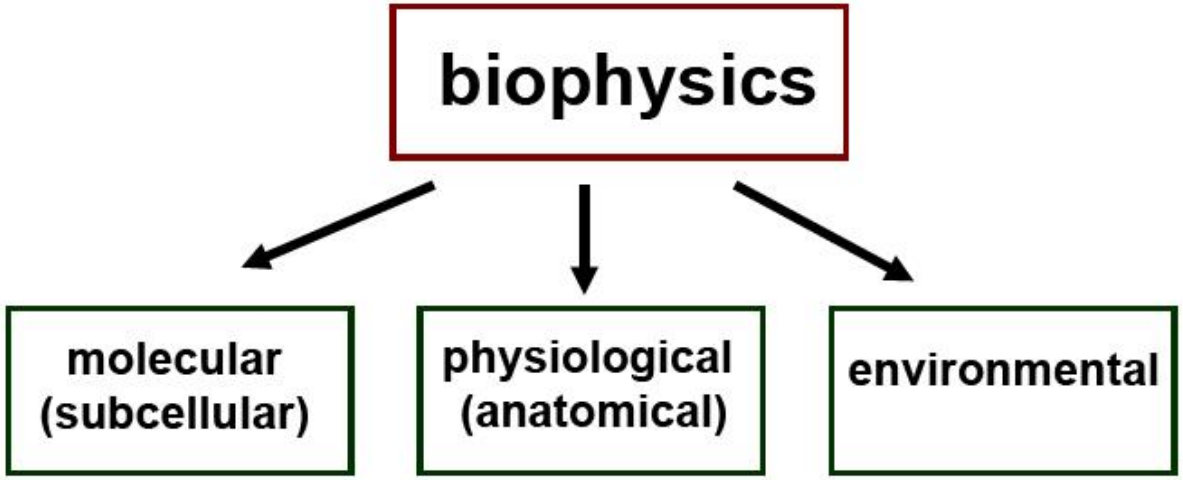
- Octadecanthiol
- Lipid
- PC/Cholesterol vesicles

DNBT – Research Erik reimhult

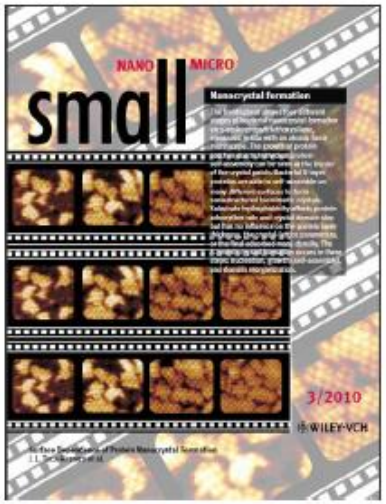
- Supramolecular (bio)materials - nanoscale building blocks enables controlled interfacial assembly
- (Bioinspired) liquid-liquid interfaces allow energy mimization by attaining ordering of adsorbed nanoparticles
- Dispersed nanoparticles can be used as antennas for external actuation of interfacial (membrane) properties without causing structural or environmental degradation



DNBT – Research José L. Toca-Herrera



small → large



Biophysics is an interdiscipinary science where physics, biology, chemistry and mathematics all meet

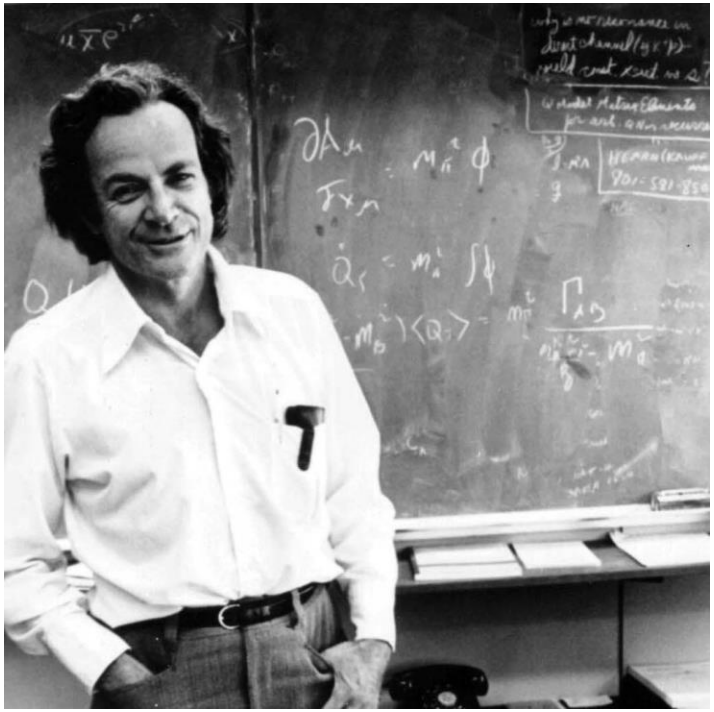
Where are we since sept 2010 ?

Here !!

Muthgasse 11 (2nd floor), A-1190 Vienna

NANO – When?

I want to build a billion tiny factories, models of each other, which are manufacturing simultaneously. . . The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; but in practice, it has not been done because we are too big.
— Richard Feynman (1959), Nobel Prize winner in physics



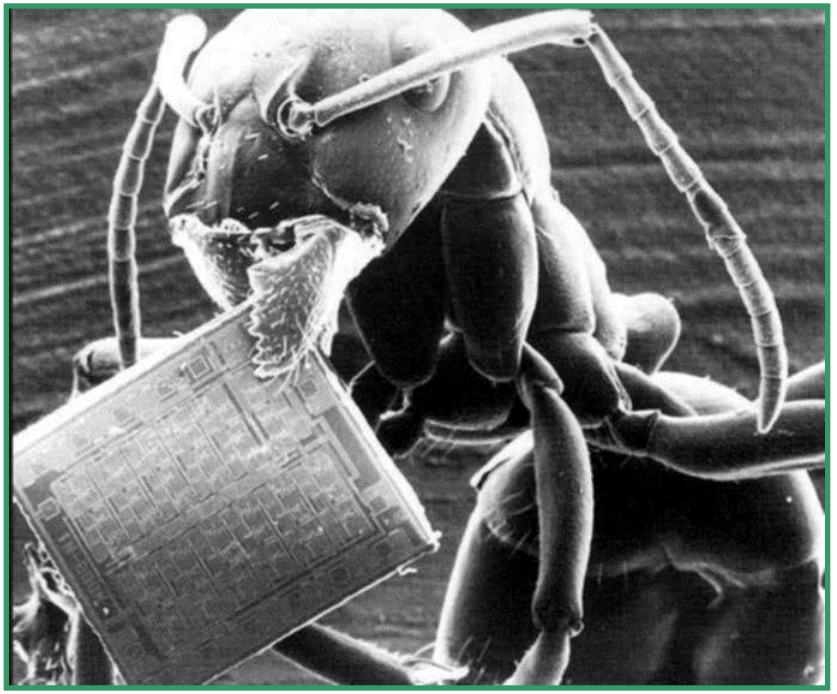
Possible consequences: scanning tunneling microscope and scanning probe microscope, quantum computers….

Link to the lecture: http://www.youtube.com/watch?v=4eRCygdW--c

Definition of NanoBiotechnology

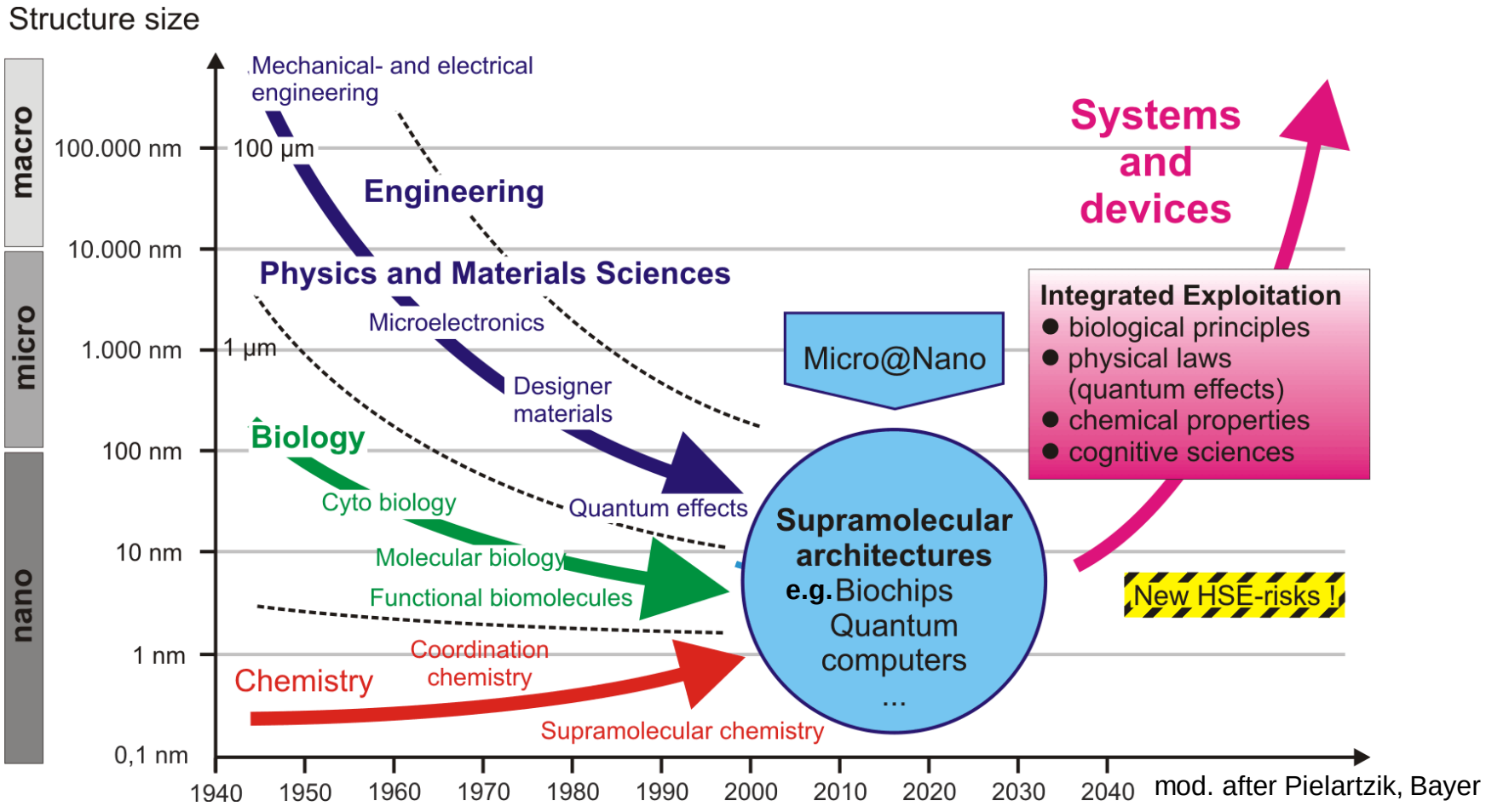
Nanobiotechnology involves the processing, fabrication and packag-ing of organic or biomaterial devices or assemblies in which the dimen-sion of at least one functional comp-onent lies between 1 and 100nm.

Nanobiotechnology is characterized by its highly inter-disciplinary nature and features a close collaboration between life-scientists, physical scientists, and engineers.



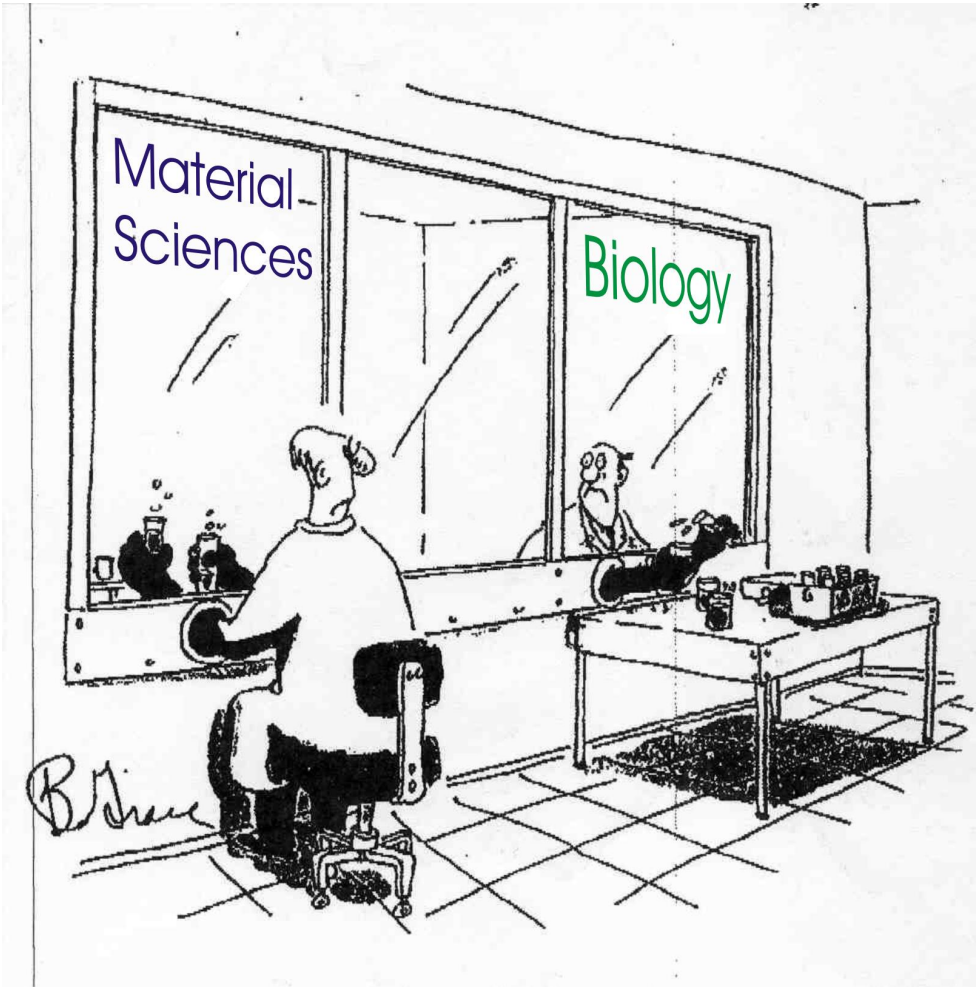
courtesy of FEI-Company, NL

Converging Technologies

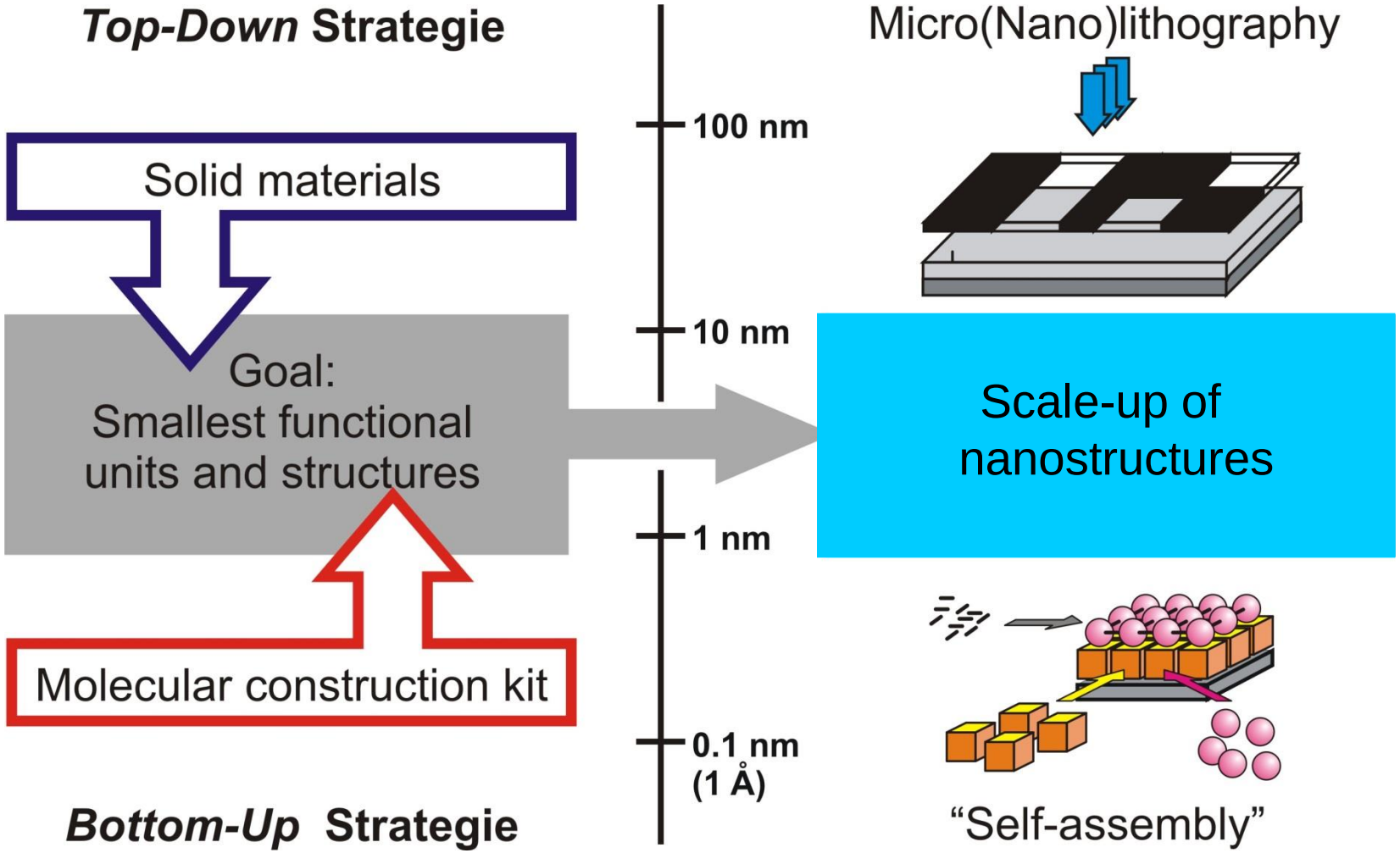


Nano(bio)technology is not a „rebranding“ of older science !
Its influence is revolutionary rather than evolutionary.

Finally, we can say that **Nanobiotechnology** is an Interdisciplinary Field Crossing the Boundaries of Established Disciplines



Nanosciences and Nanotechnology



Generating Supramolecular Structures

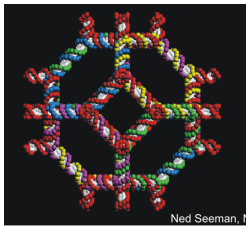
Molecular LEGO™

- Supramolecular design
- Self-assembly of complex structure

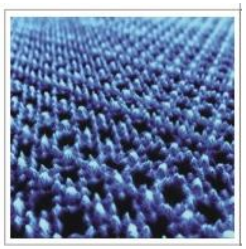
  nature builds complexity in a hierarchial way

  sequential assembly strategies
- Patterning elements
- Functionalization of supramolecular structures
- Quality control and characterization of stuctures

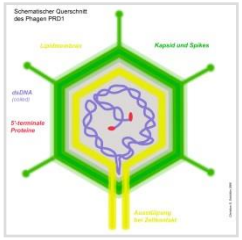
Basic Structures (Patterning Elements) for Generating Complex Supramolecular Structures



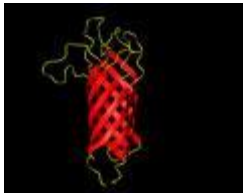
- DNA



- Monomolecular crystalline bacterial cell surface layers (S-layers)



- Phage proteins



- Membrane proteins

In our department we basically give importance to basic building blocks (in a biomolecular construction kit)

- Biological molecules (e.g. proteins, lipids, glycans, nucleic acids)
- Chemically or genetically modified molecules
- Chemically synthesized molecules

We also look for understanding the principles behind self assembly and biological processes

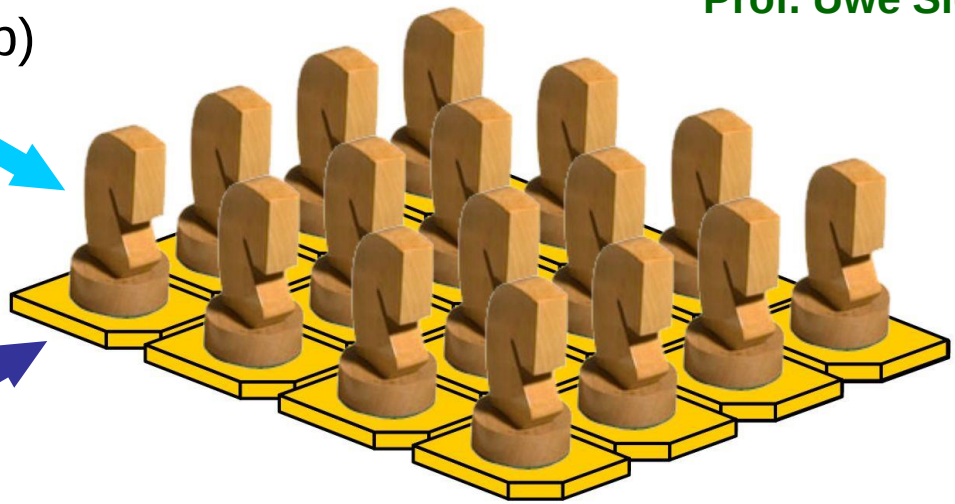
SOME REAL EXAMPLES FROM THE LAB

Our Department has experience on nanobiotechnology using surface protein layers

Prof. Uwe Sleytr

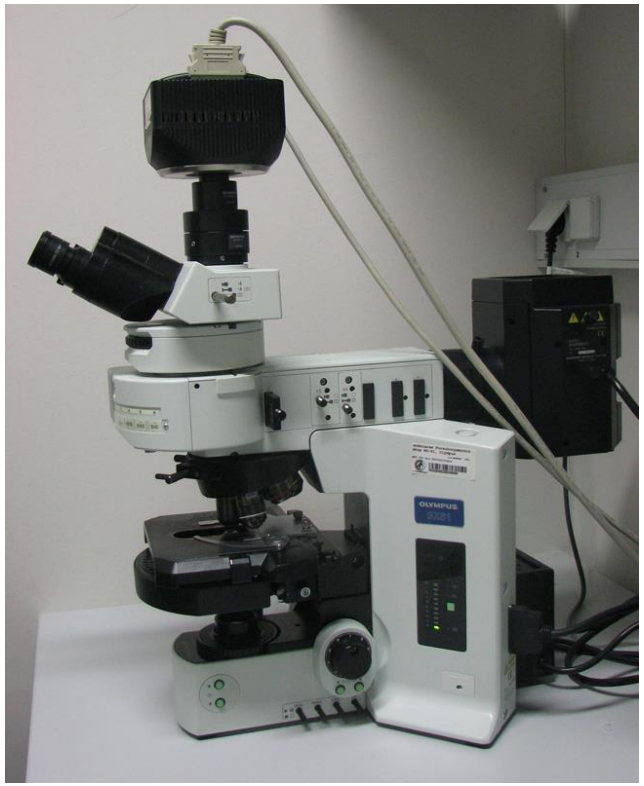
functional groups (e.g. Ab)

assembly
orientation
positioning



S-layer Fusion Proteins

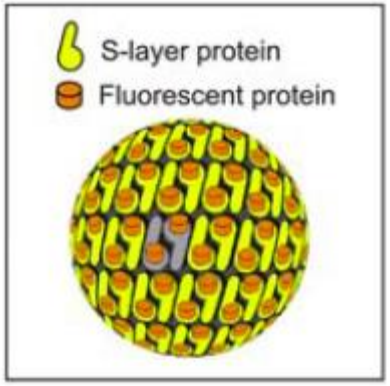
providing a highly ordered functional surface in nanometer-scale

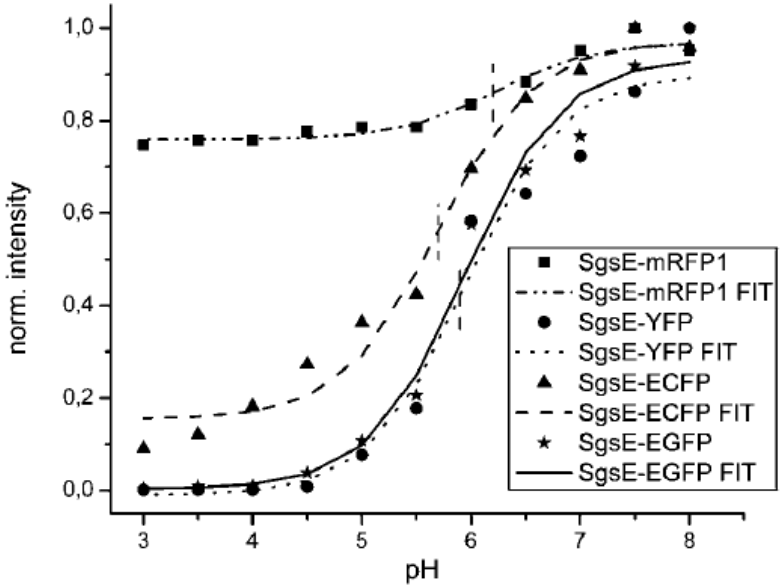


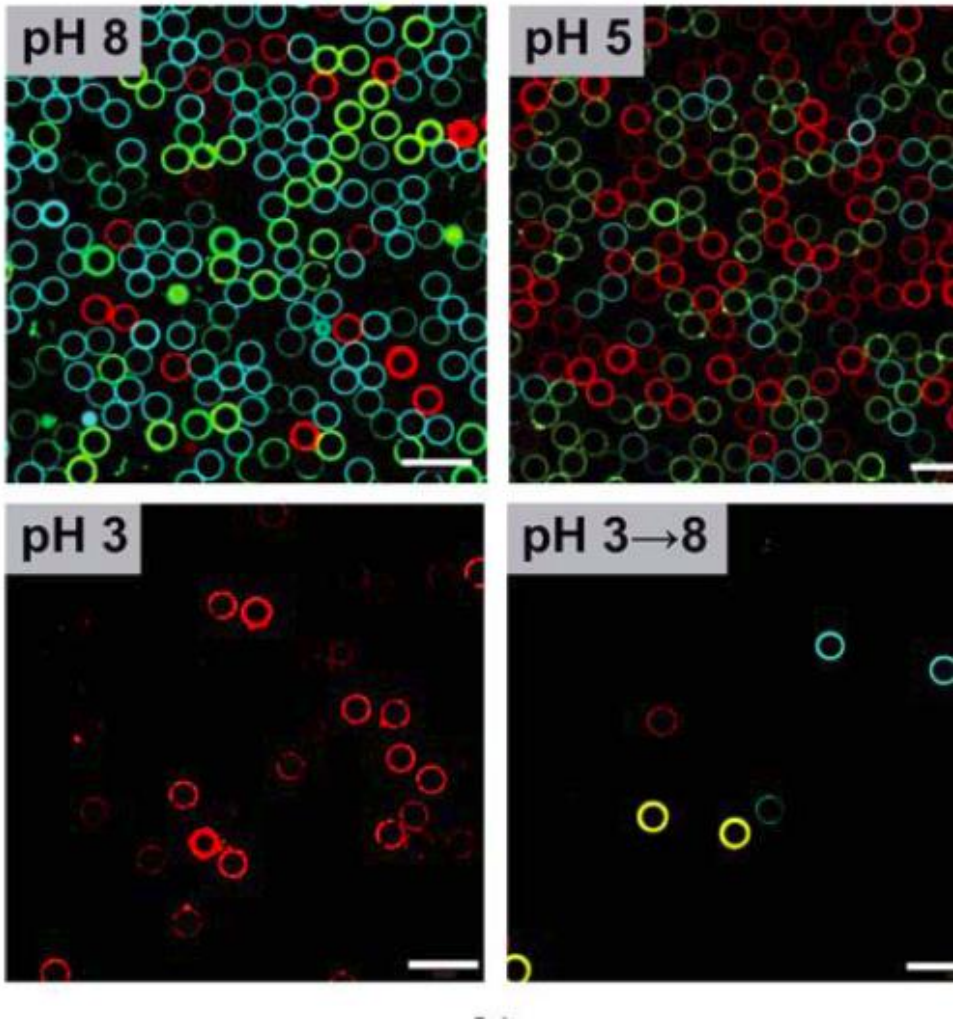
Fluorescence microscopy

Flow cytometry

Preparing particles that are sensitive to changes in pH







Soft Matter 6 (2010) 3809

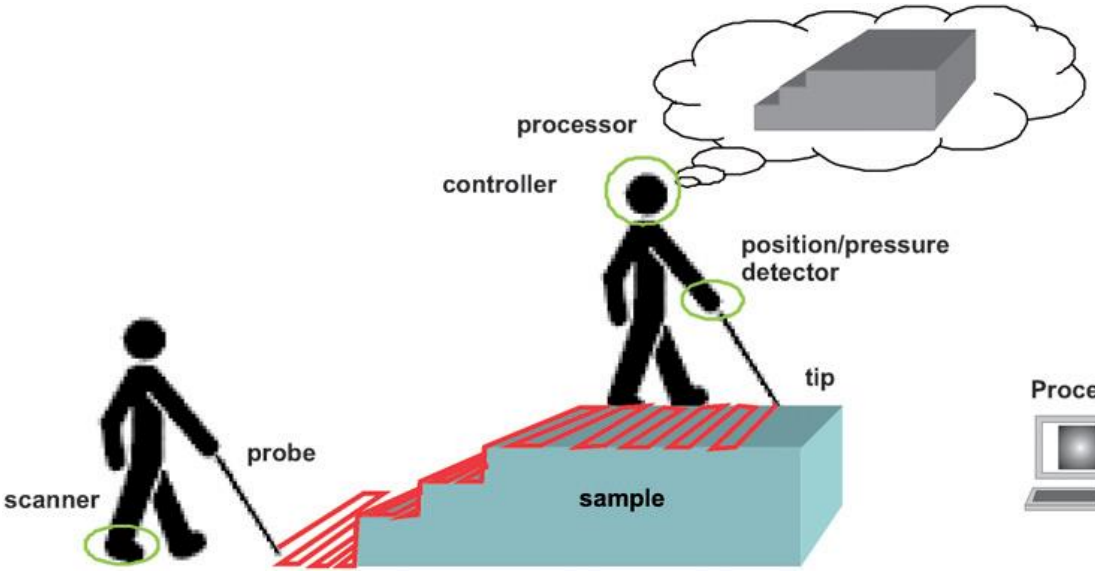
AFMs am DNBT... (surface analysis, mechanics and molecular forces)

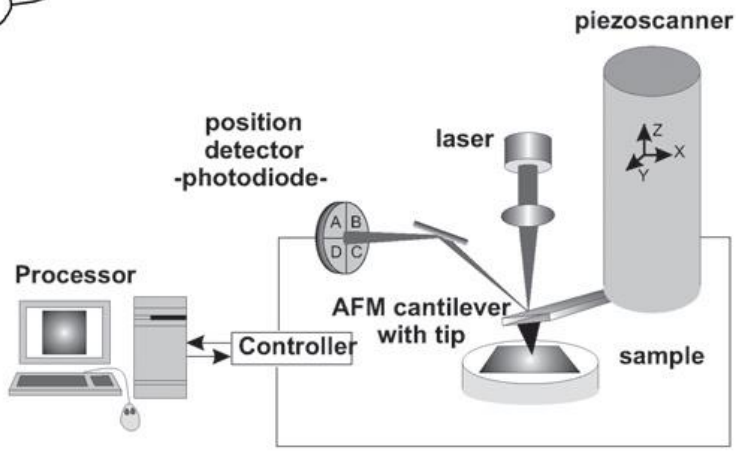
Scanning probe microscope I

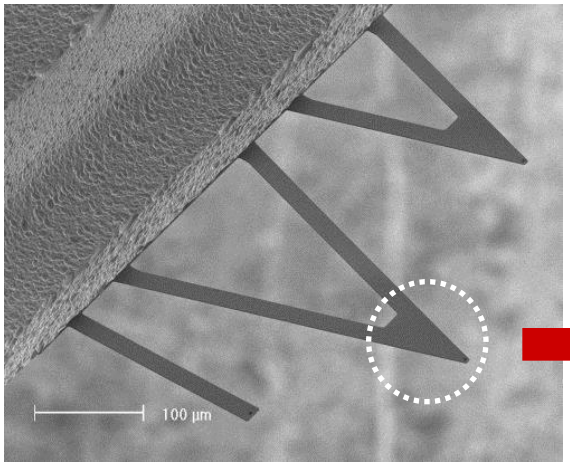
Scanning probe microscope II

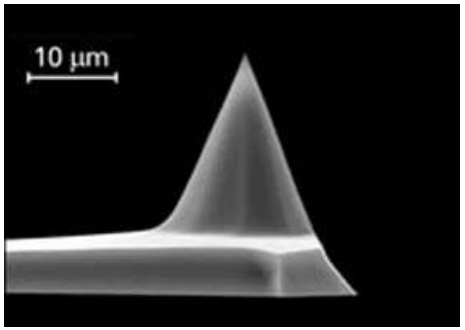
Third SPM will arrived soon!

This thing called AFM...



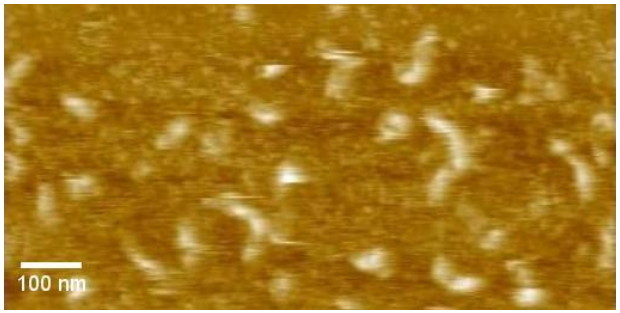






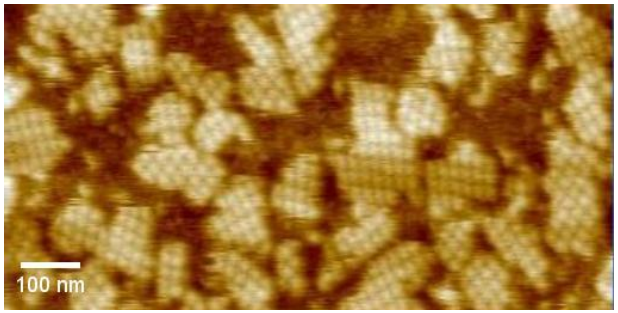
Nanoscale 1 (2009) 40

Bacterial crystals on silanes



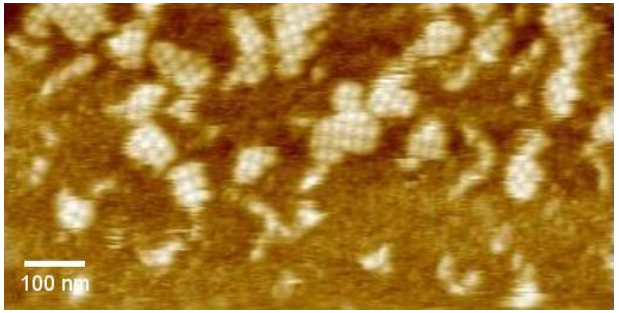
t_0

3^{30}



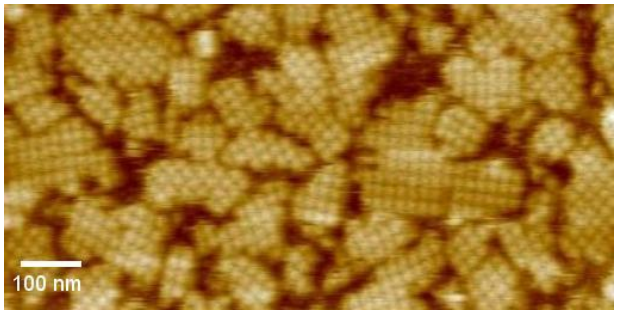
7^{00}

10^{30}



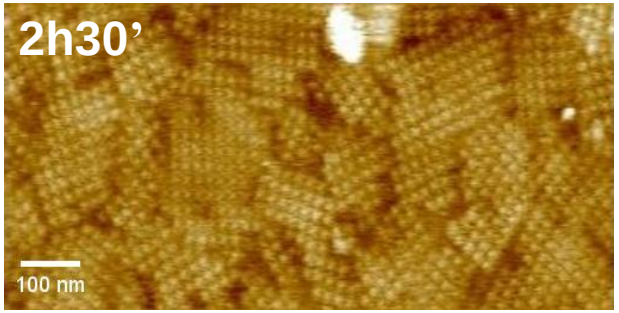
7^{00}

3^{30}



14^{00}

10^{30}



time	SiO_2	OTS	APTS
5’	X	31	21
15’	33	77	68
30’	62	96	83
1h	80	99	85
2h30’	85	99	95
>12h	98	100	100

Small 6 (2010) 40

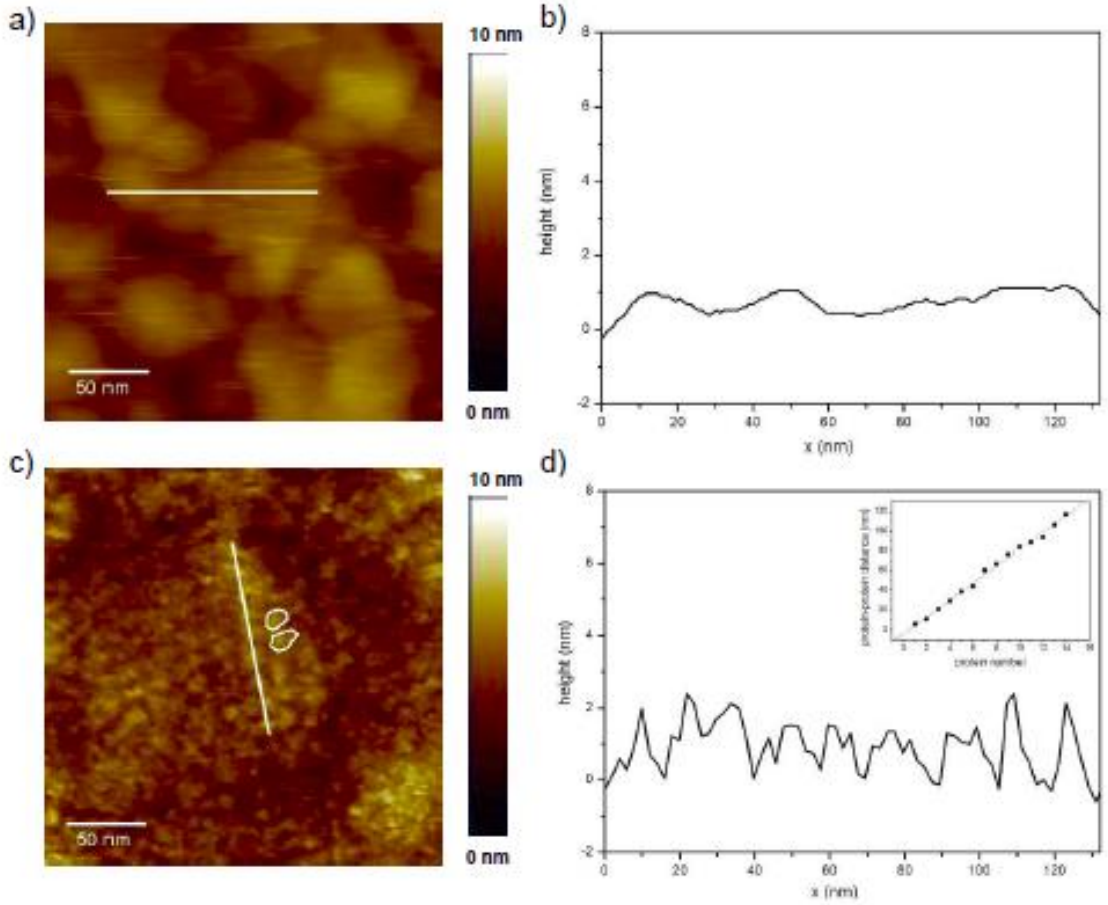
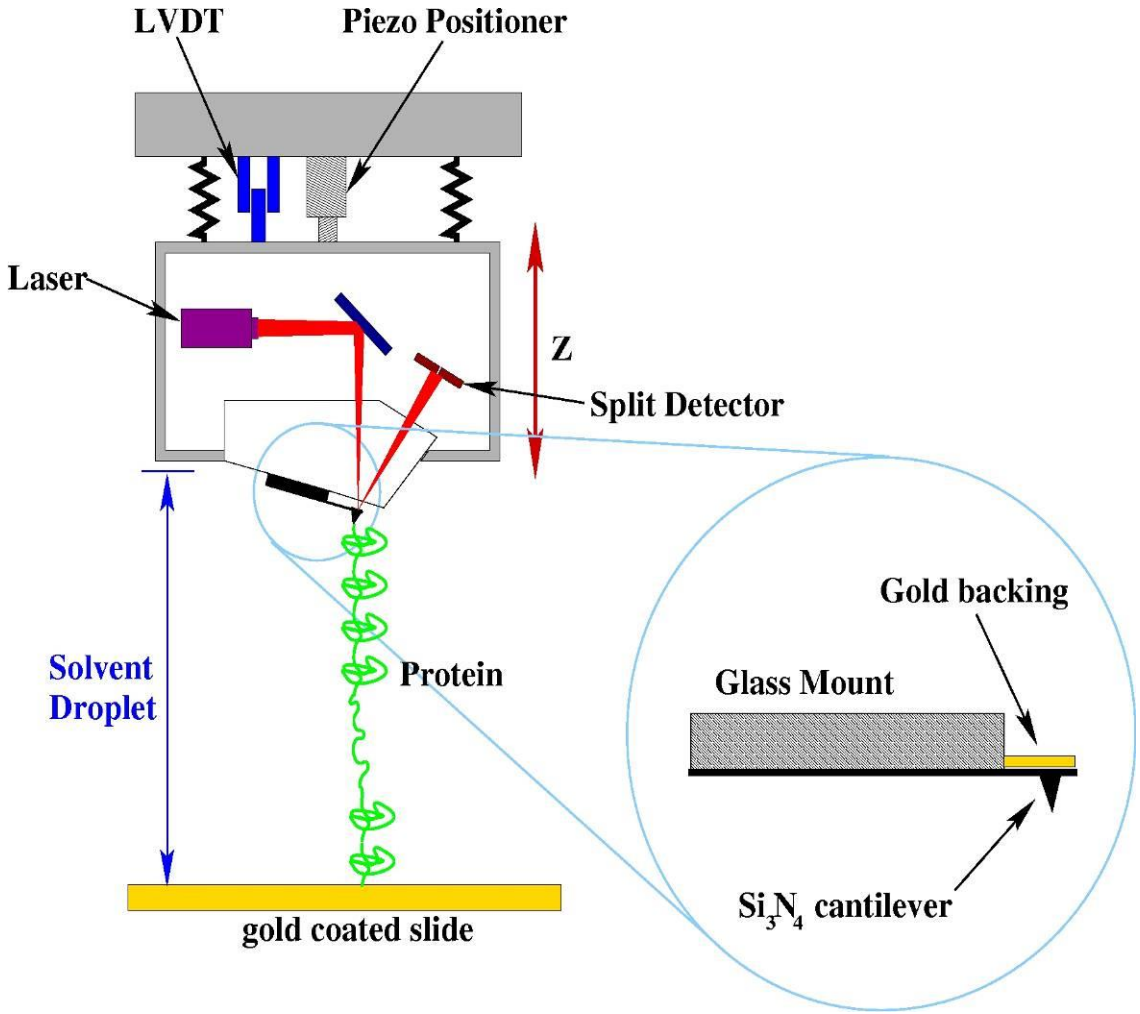
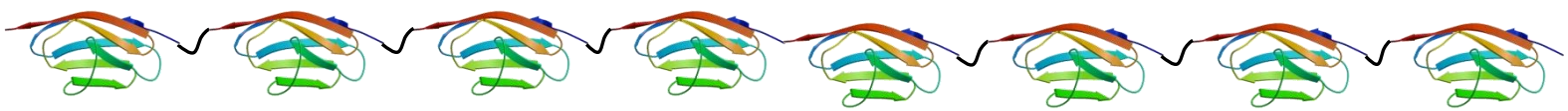


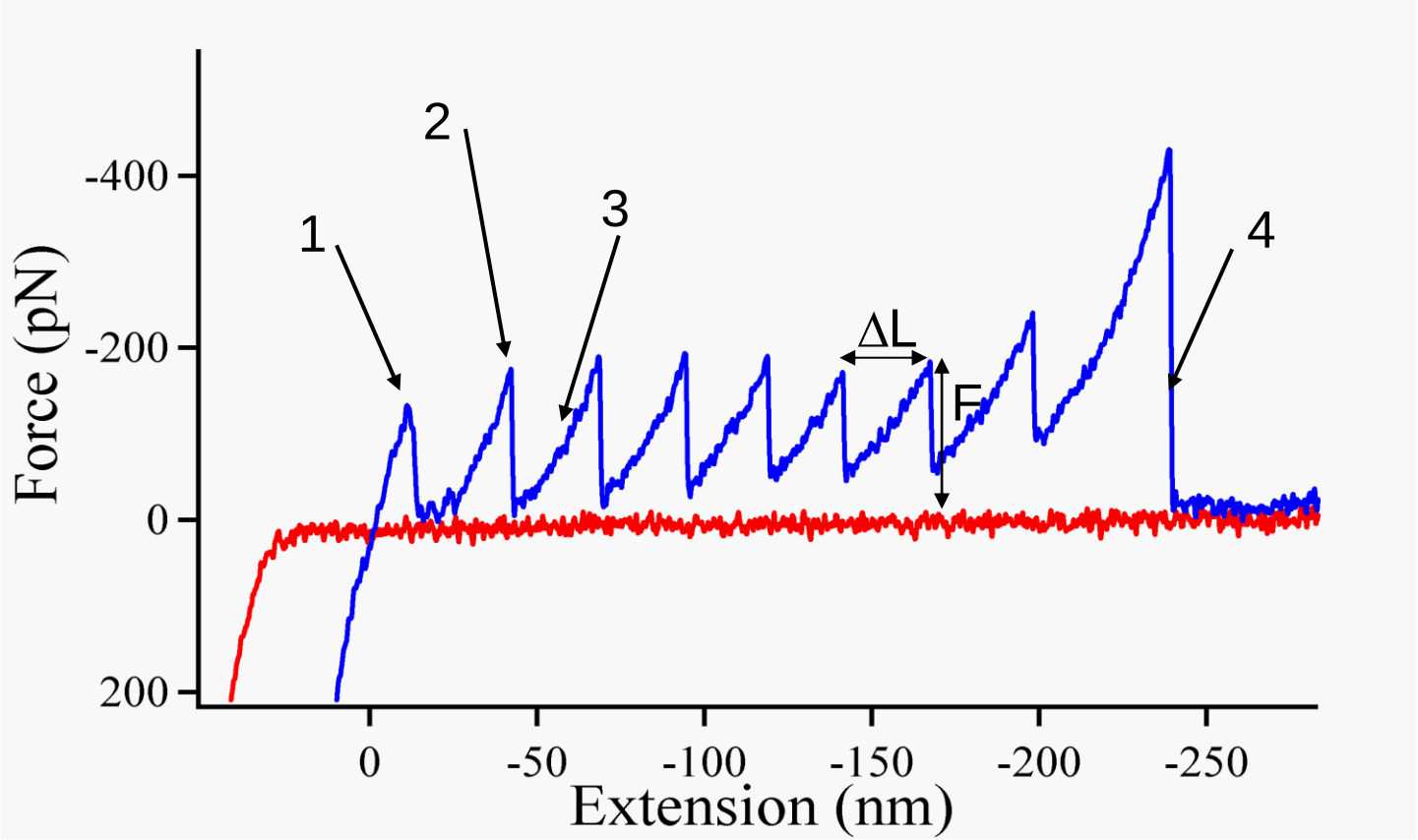
Figure 7.6. a) AFM height image (250 x 250 nm^2) of glutaraldehyde functionalized gold substrate. The white cross section shows the profile of gold/glutaraldehyde surface that is plotted in figure b), c) AFM height image (250 x 250 nm^2) of immobilized HSA with glutaraldehyde. The white circles indicate single HSA proteins and the white cross sections show the albumin profile which is plotted in d). The profile analysis carried out at different points of the surface show that the average height of HSA molecules is 1.8 nm. The inset in figure d) shows the protein-protein distance as a function of protein number. The data were fitted with a linear equation. The slope gives information about HSA width. (These analyses were carried out at 50 different points).

Aitziber Eleta, PhD Thesis, 2010

AFM as a mechanical machine (on proteins)





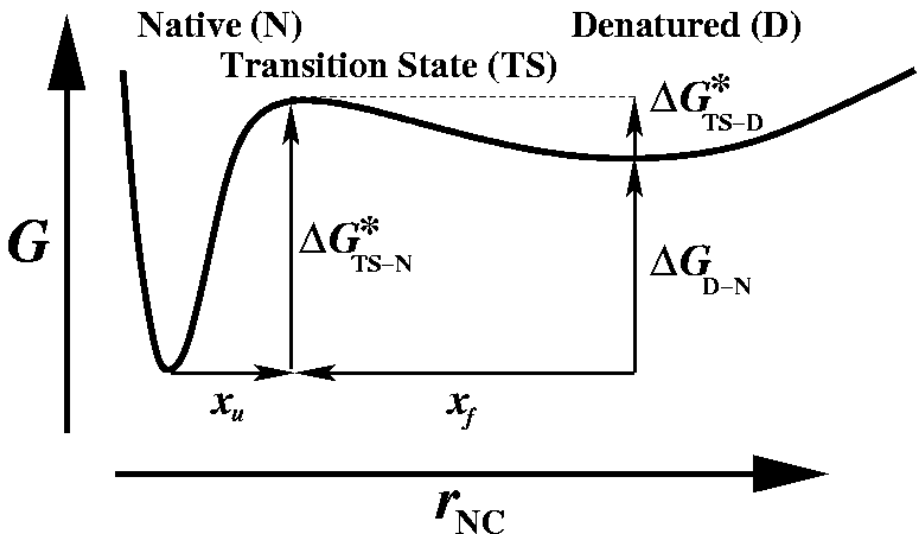


1. Non-specific adhesion 2. Unfolding of one domain

3. Unfolded protein stretching 4. Protein detaches

AFM as a mechanical machine: the AFM offers…

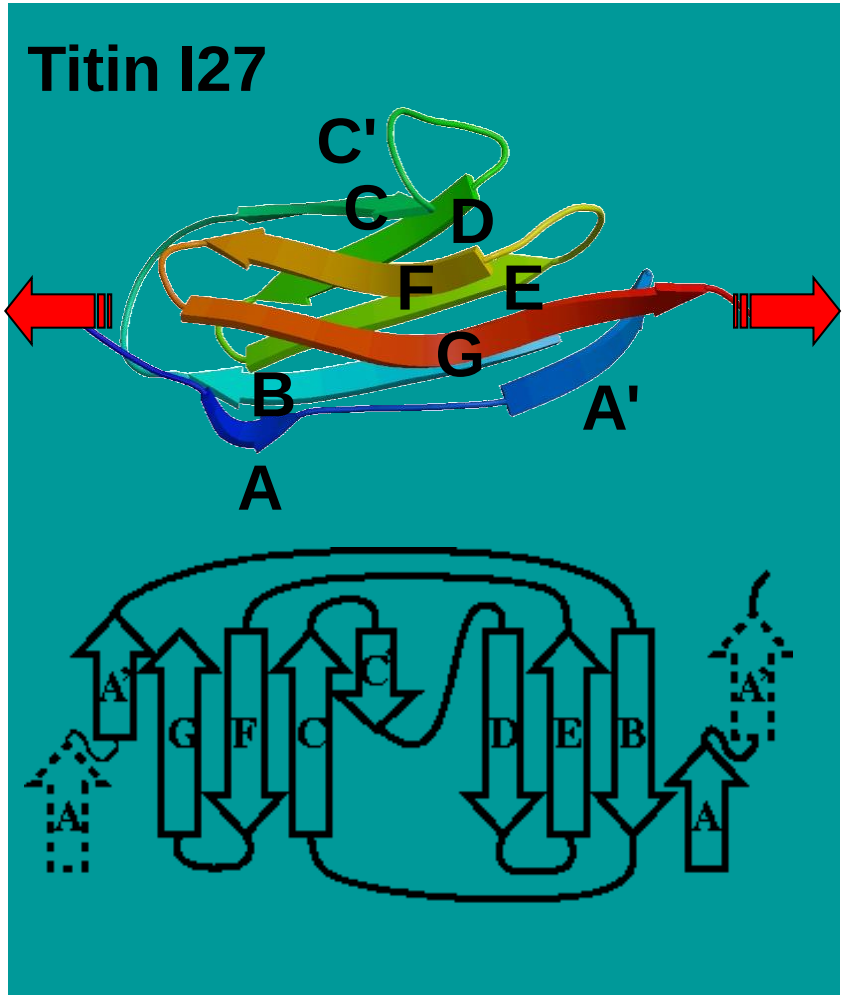
- Alternative to conventional methods of denaturation (e.g. heat, acid, chemical denaturant).
- Single molecule experiment.
- Well defined reaction coordinate.
- Direct comparison with all-atom MD simulation.
- Possibility to observe rare events

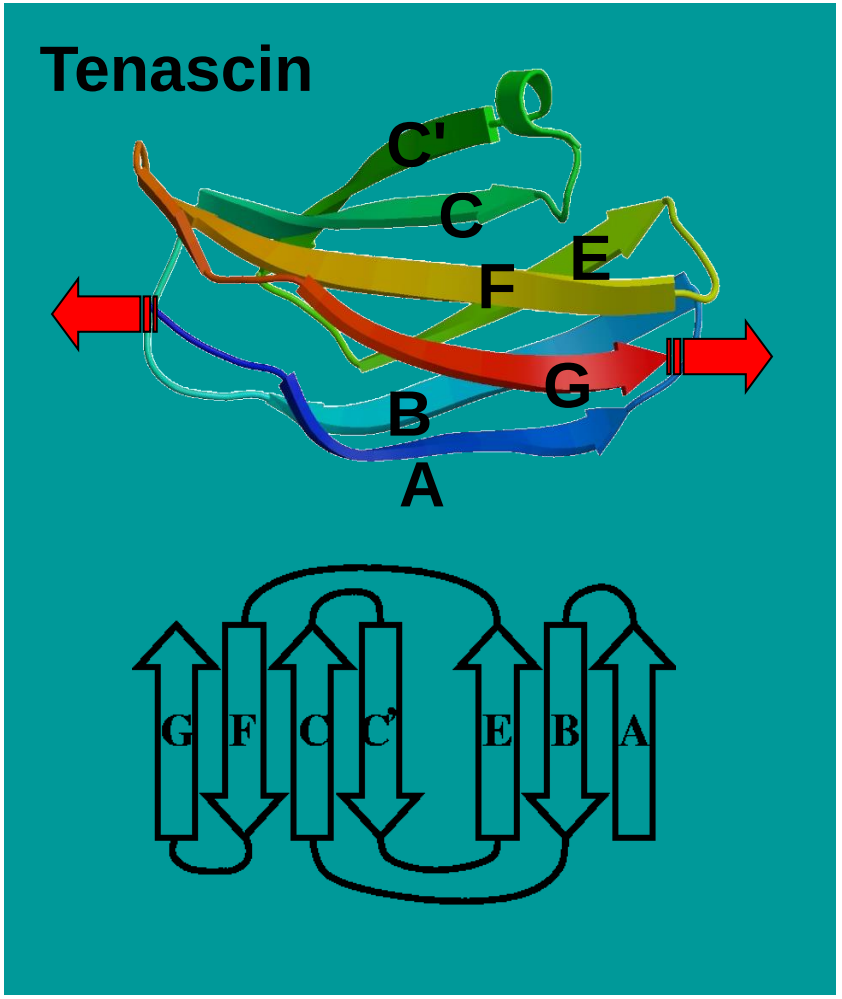


$$k_u(F) = \nu\kappa \exp(-\beta(\Delta G^*_{TS-N} - Fx_u)) = k_u(0)\exp(\beta F x_u)$$

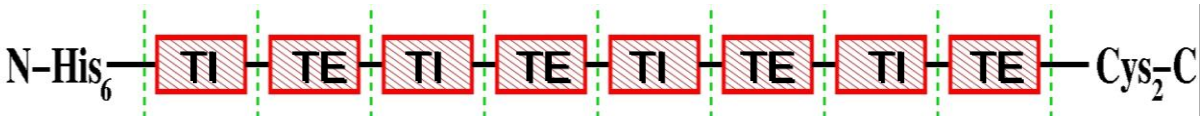
Analytical Chimica Acta, 479 (2003) 87

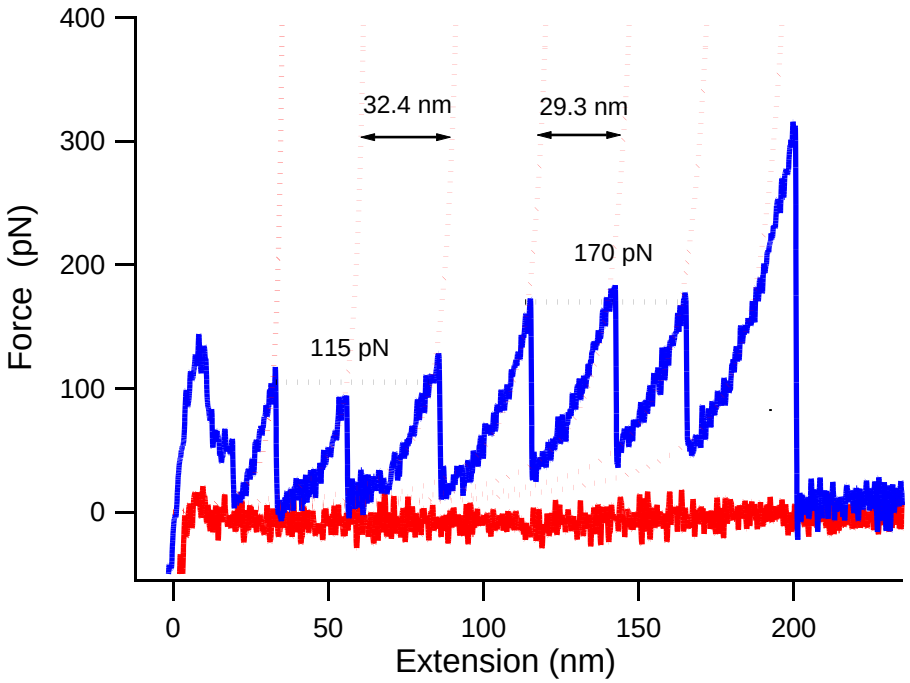
Tenascin and titin I27 have a similar fold

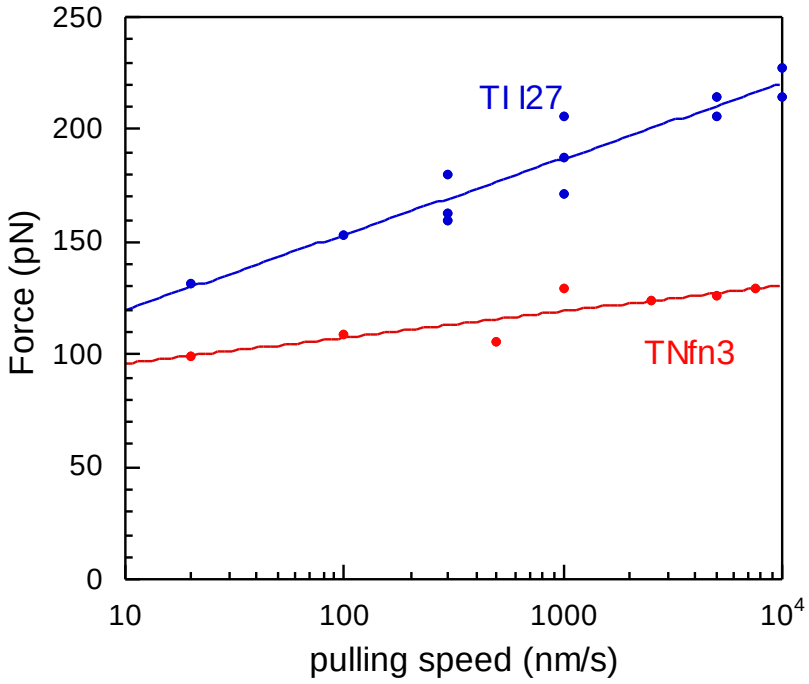




“Similar” proteins unfold mechanically in a different way







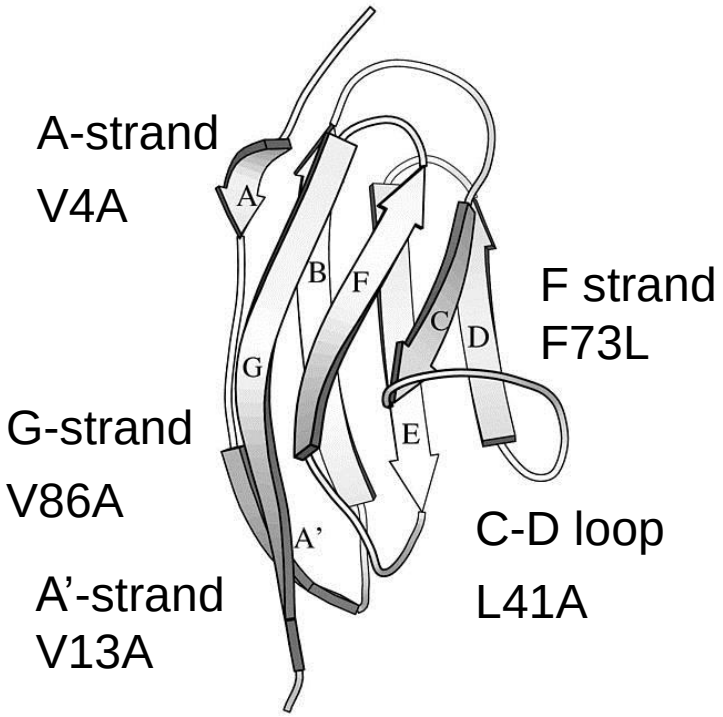
X_u (tn) = 0.7 nm, X_u (titin) = 0.3 nm

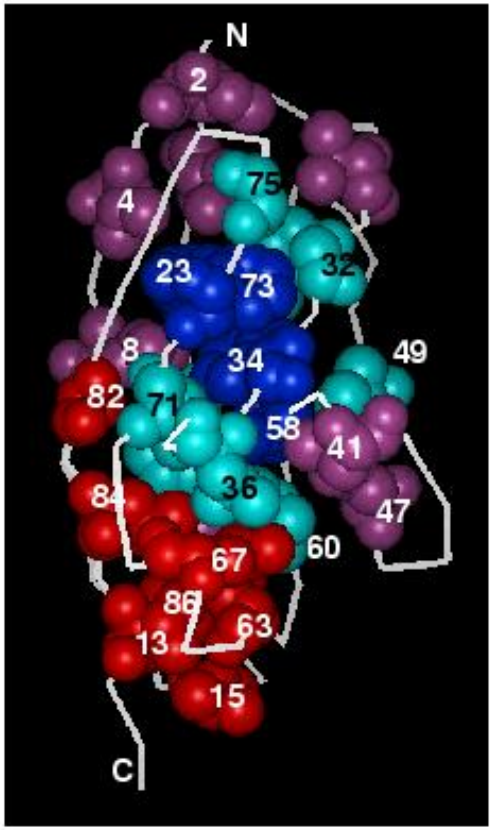
K_{off} (tn) = 8×10^{-9} s^{-1}, K_{off} = 4×10^{-5} s^{-1}

Protein Science, 11 (2002) 2179
PNAS 99 (2002) 12143
Nature, 442 (2003) 446

Choice of mutants is critical and deliver information about protein stability

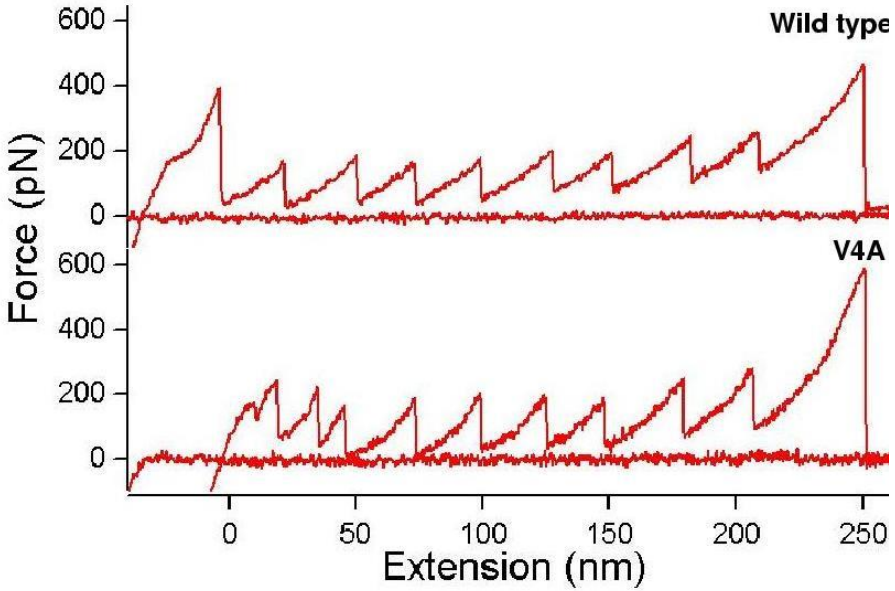
Conservative, non-disruptive, significantly destabilising

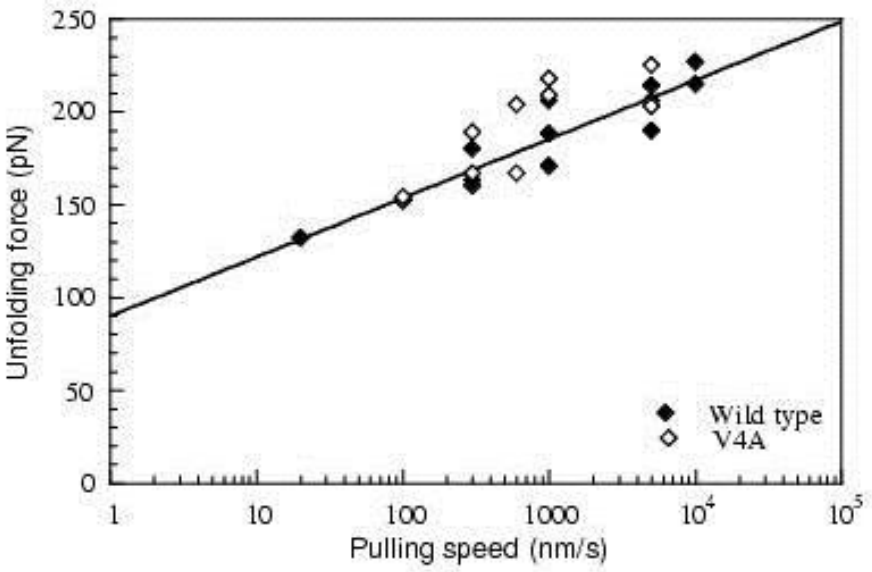




Evidence from mutation

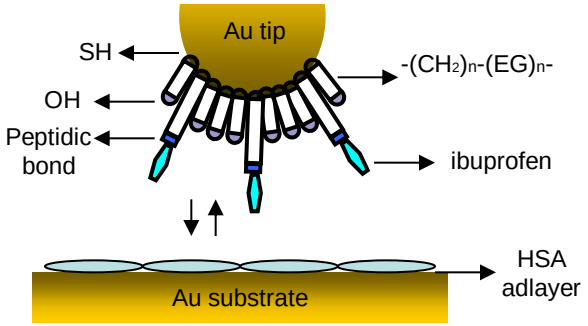
When we pull a protein with a destabilising mutation in the A strand (V4A) it does not affect the unfolding forces at all

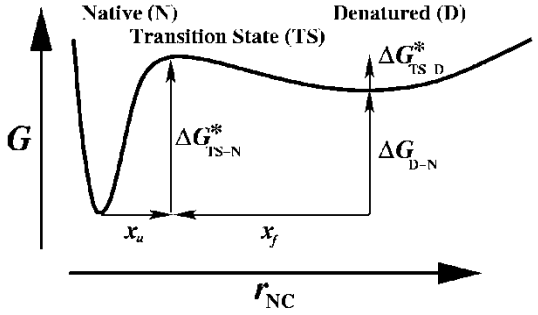


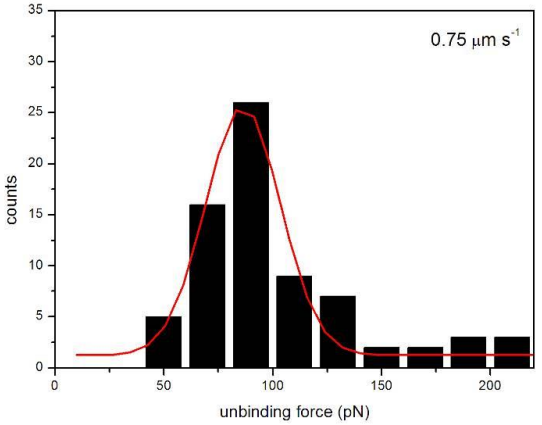


Journal of Molecular Biology 322 (2002) 841

Forces between single molecules





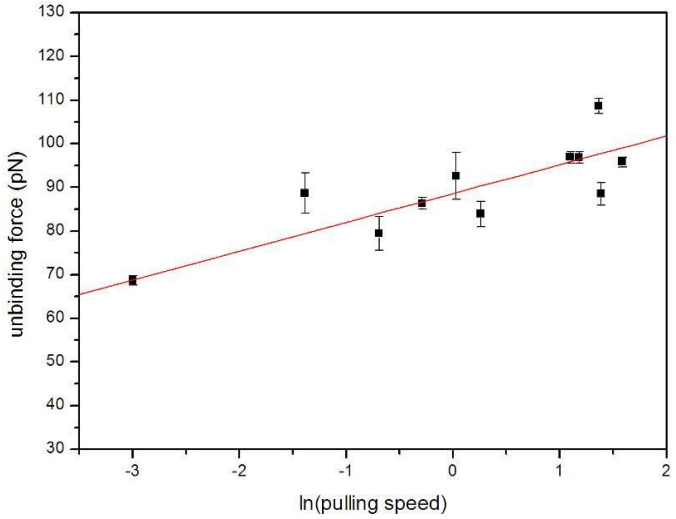


$$F = \left(\frac{k_b T}{x_u}\right)\ln\left(\frac{x_u k_e}{k_b T K_{off}}\right) + \left(\frac{k_b T}{x_u}\right)\ln v$$

X_u = 0.6 nm

K_{off} = 0.055 s^{-1}

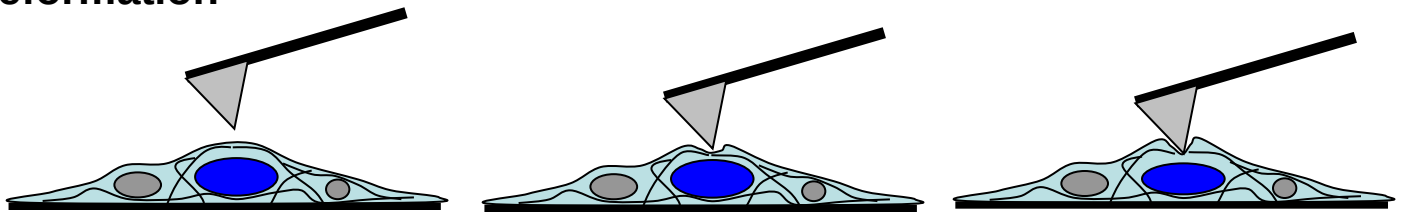
Possibility of molecular recognition !!



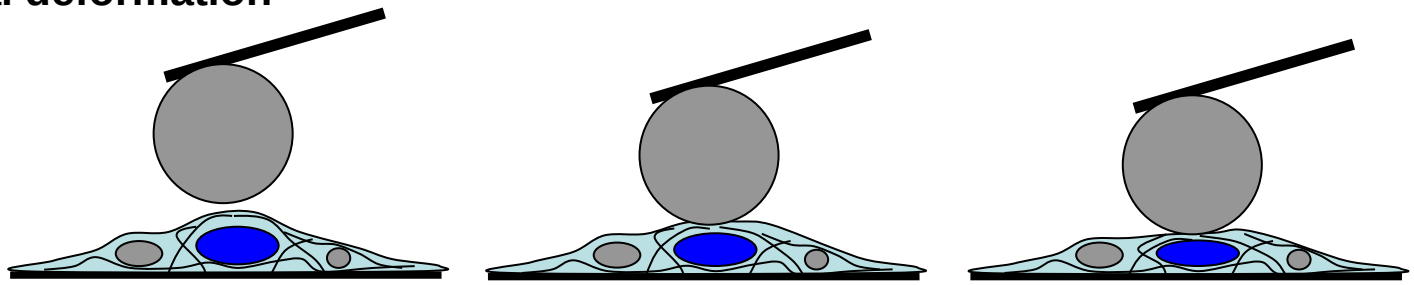
Aitziber Eleta, PhD Thesis, 2010

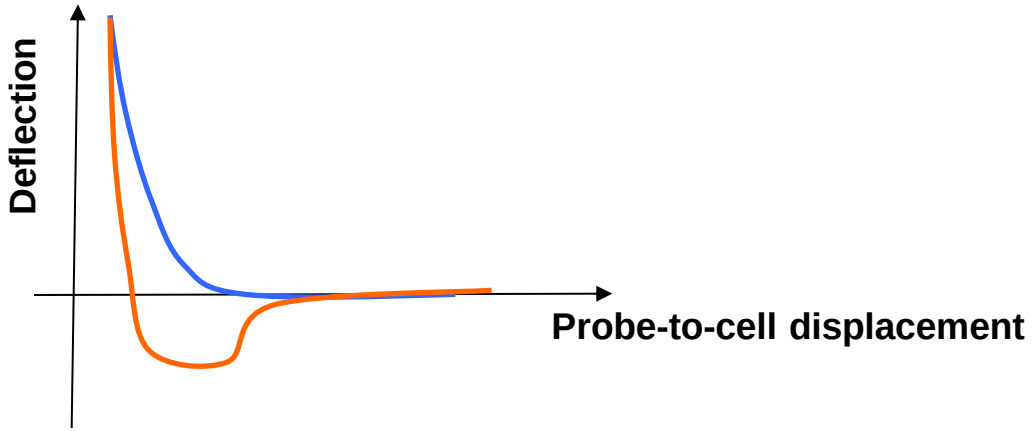
AFM – mechanical machine on cells

Local deformation



Global deformation





MOTIVATION !!

- Alternative AFM-based approach that takes into account

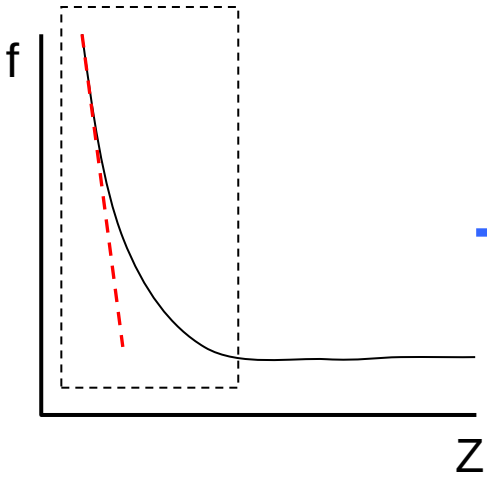
  Cell complexity

  3D heterogeneity

- Use it as a diagnostic tool for cell type/malfunction ???

  correlation mechanical properties – cell function ???

Force-distance based approach: cell elasticity



+ **CONTACT AREA [contact mechanics of a purely elastic body]** = **YOUNG'S MODULUS [elasticity parameter]**

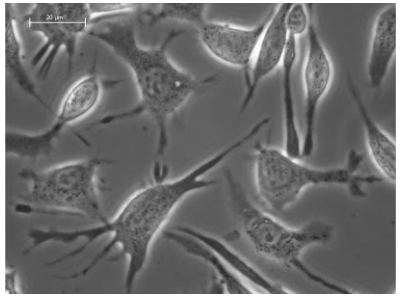
- small deformations (1-5%)

- cells = purely elastic bodies

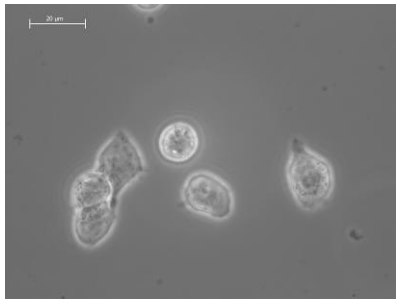
$$F = Cte * E * I^2$$

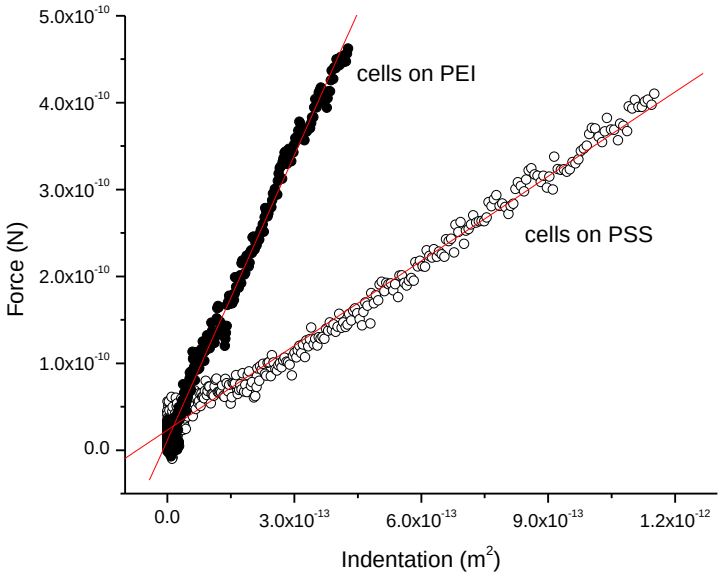
Force-distance based approach - HepG2 cells

PEI



PSS





- small deformations
- cells = purely elastic bodies

- 191 ± 14 Pa (PSS)
- 941 ± 58 Pa (PEI)

Microscopy Research and Technique 72 (2009) 957

Substrate influences SHAPE

SHAPE ⇒ different Young Modulus

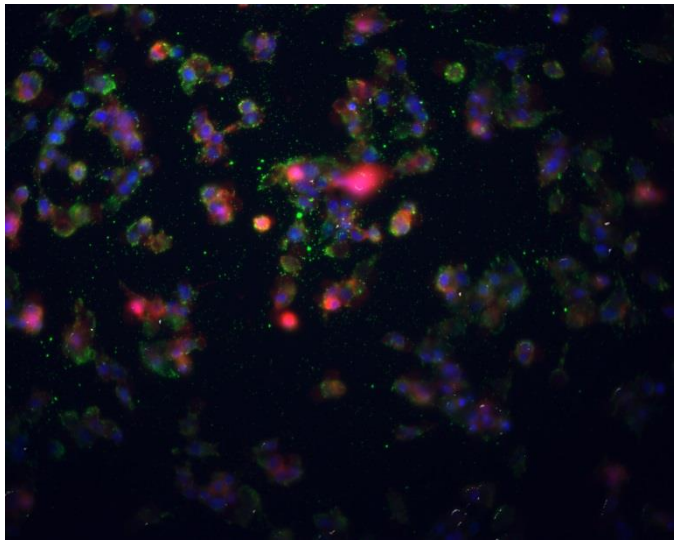
⇓

the membrane should change its permeability

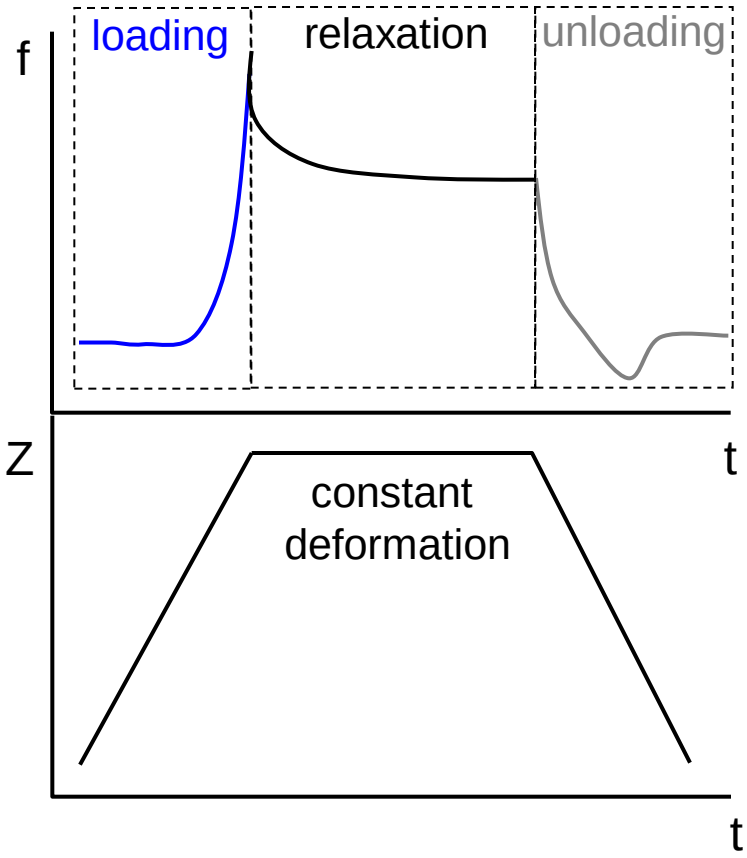
Particle uptake by Hep G2 cell cultured on polystyrene sulfonate

Particle size: 49 nm and 240 nm

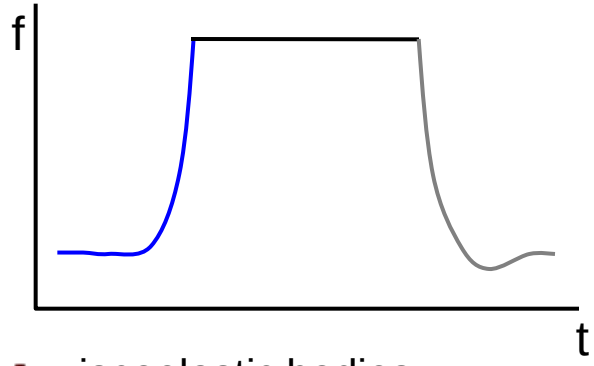
Research on going….



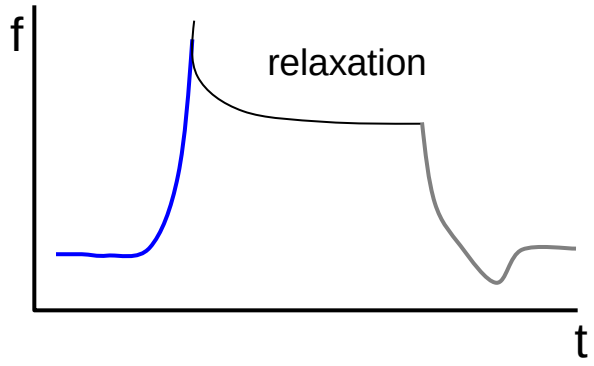
Force-time based approach: relaxation



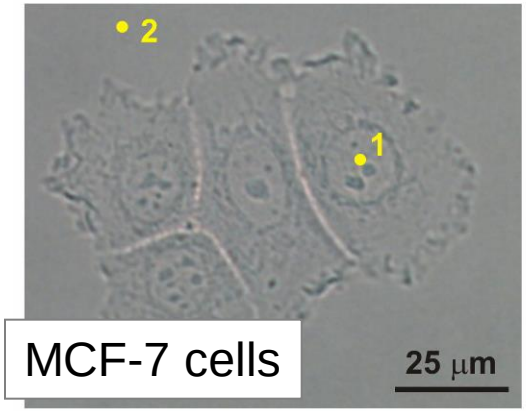
- purely elastic bodies

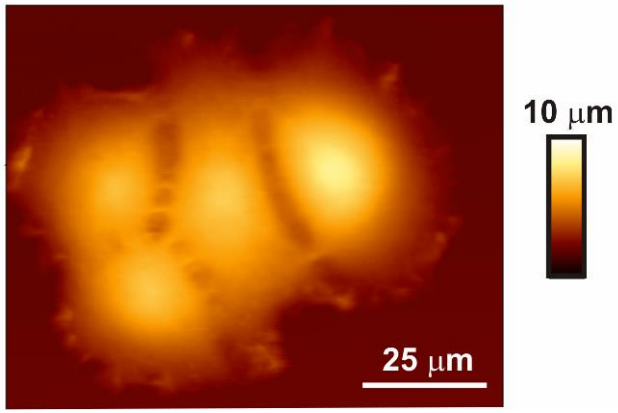


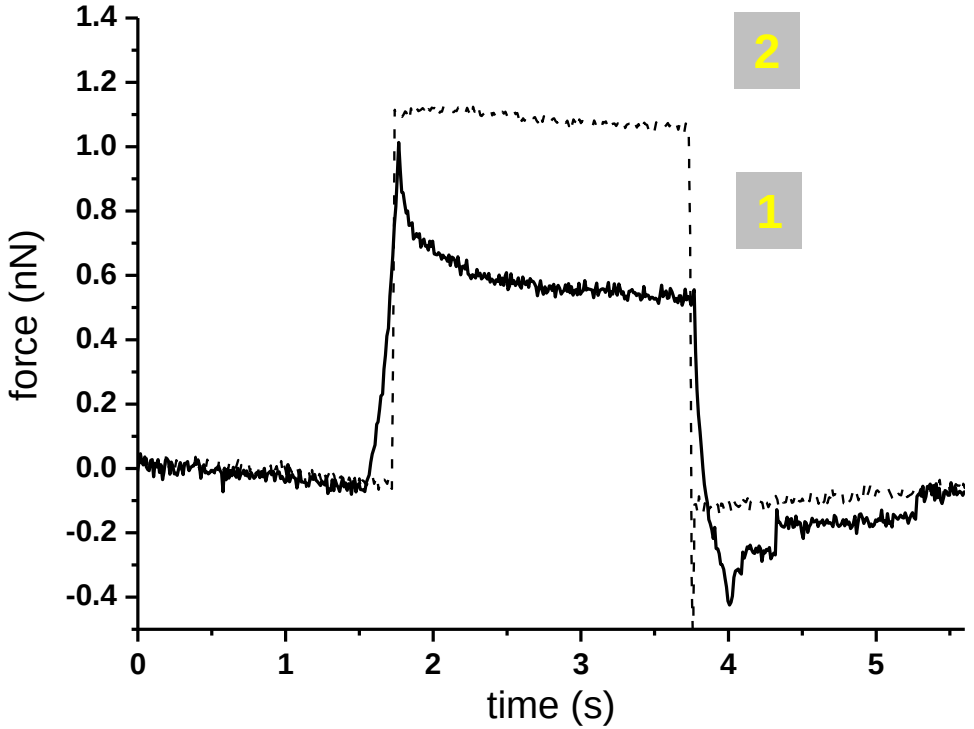
- viscoelastic bodies



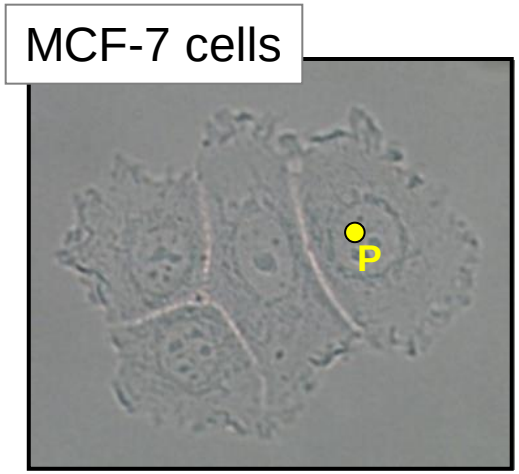
Force-time based experiments: how do MCF-7 cells relax

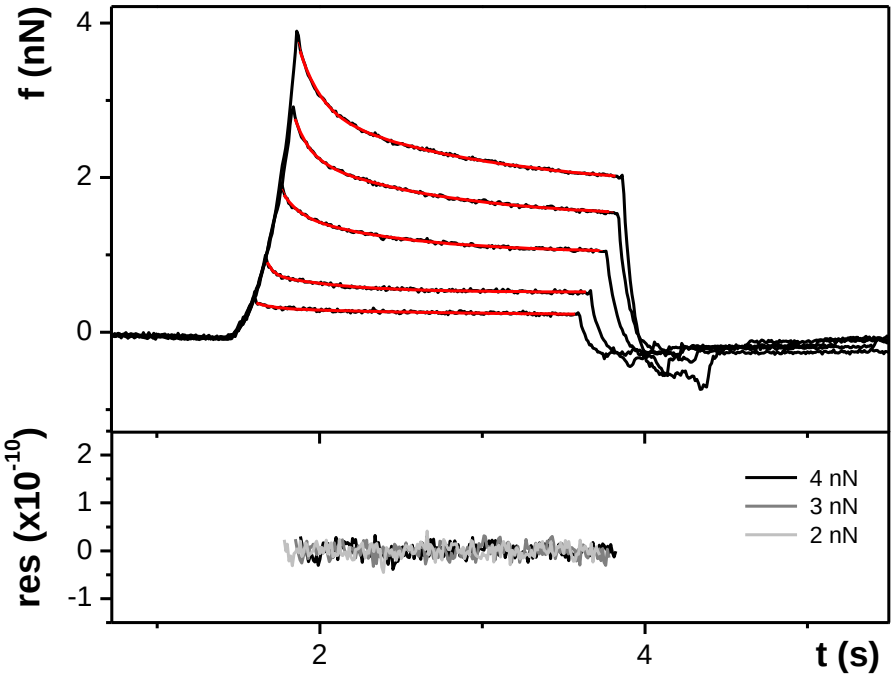






Force-time based experiments: bi-exponential behaviour



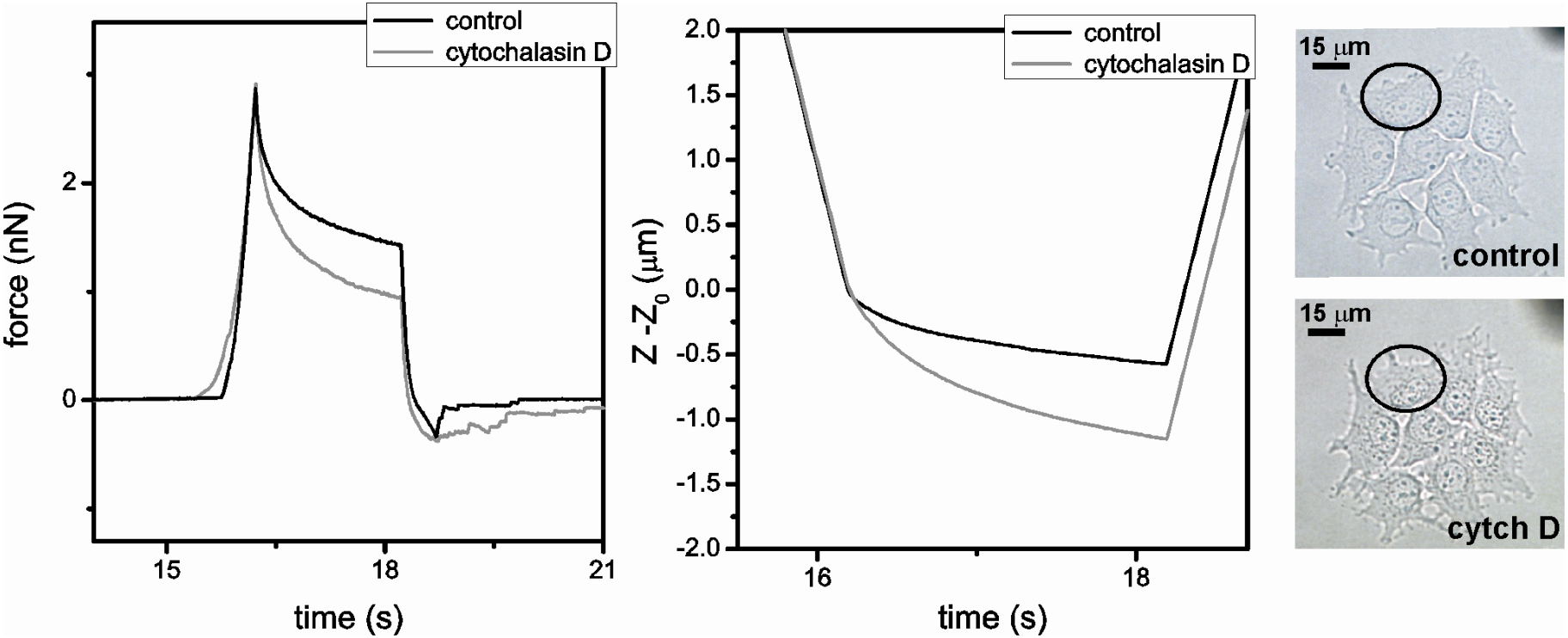


- bimodal decays: the proposed model fits reasonably well (r > 0.8)

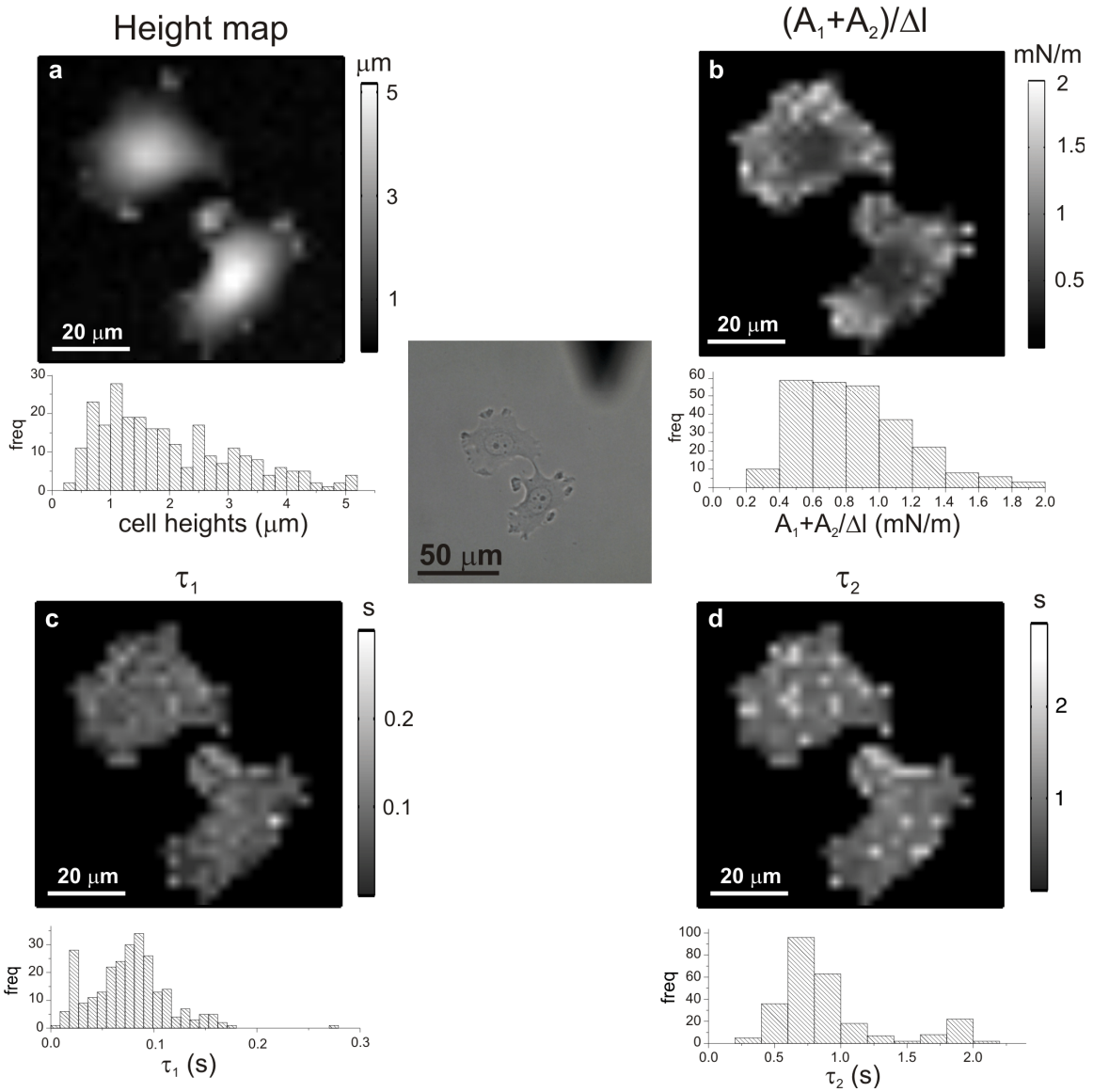
- two simultaneously-occurring processes are detected

Stress relaxation and creep – actin-depolymerizing drug

Cytochalasin D disrupts the actin cytoskeleton
(E2 and $\eta 2$ should be more affected)



Stress Relaxation Microscopy (STREM): imaging decay parameters



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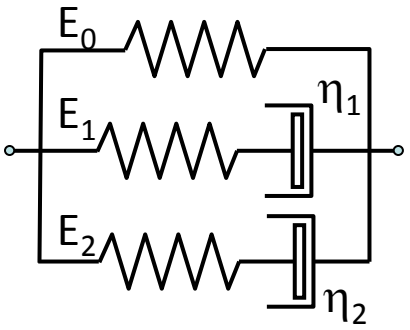


Zener's model (Riande E. et al, *Polymer viscoelasticity, Stress and strain in practice*. Marcel Dekker 2000)

For a constant strain/deformation

$$\ddot{\sigma} + A\dot{\sigma} + B\sigma = r_0\varepsilon_0$$

and the solution gives

$$\sigma(t) = E_0\varepsilon_0 + A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2}$$

$$\tau_1 = \frac{\eta_1}{E_1}, \tau_2 = \frac{\eta_2}{E_2}$$

STRESS RELAXATION

A_1, A_2, τ_1, τ_2,ε_0

For a constant stress

$$r_2\ddot{\varepsilon} + r_1\dot{\varepsilon} + r_0\varepsilon = B\sigma_0$$

and the solution gives

$$\varepsilon(t) = \frac{\sigma_0}{G_1} + C_1 e^{-x_1 t} + C_2 e^{-x_2 t}$$

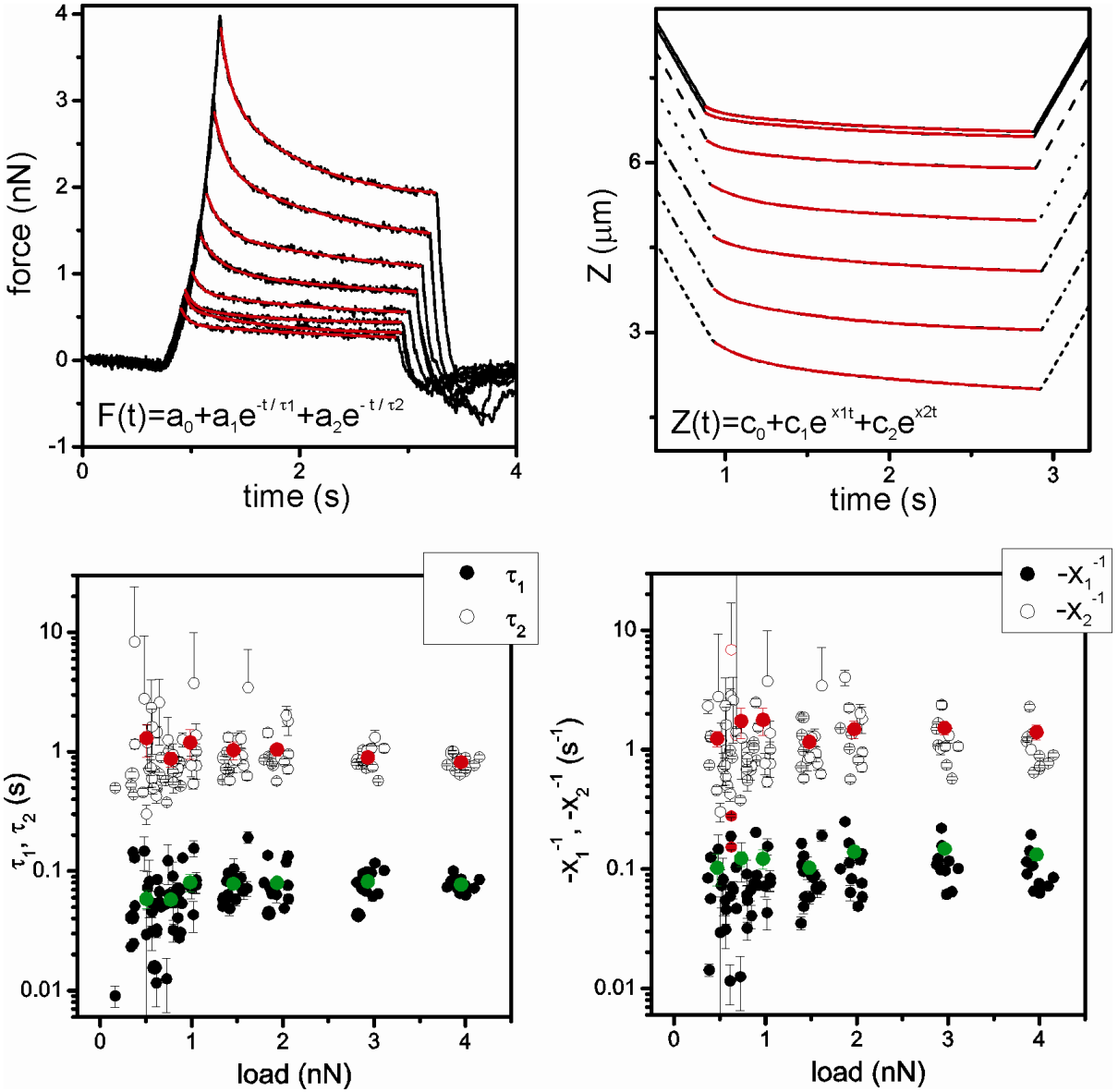
$$x_1 = \frac{-r_1 + \sqrt{r_1^2 - 4r_0 r_2}}{2r_2}, x_2 = \frac{-r_1 - \sqrt{r_1^2 - 4r_0 r_2}}{2r_2}$$

CREEP

C_1, C_2, x_1, x_2,σ_0

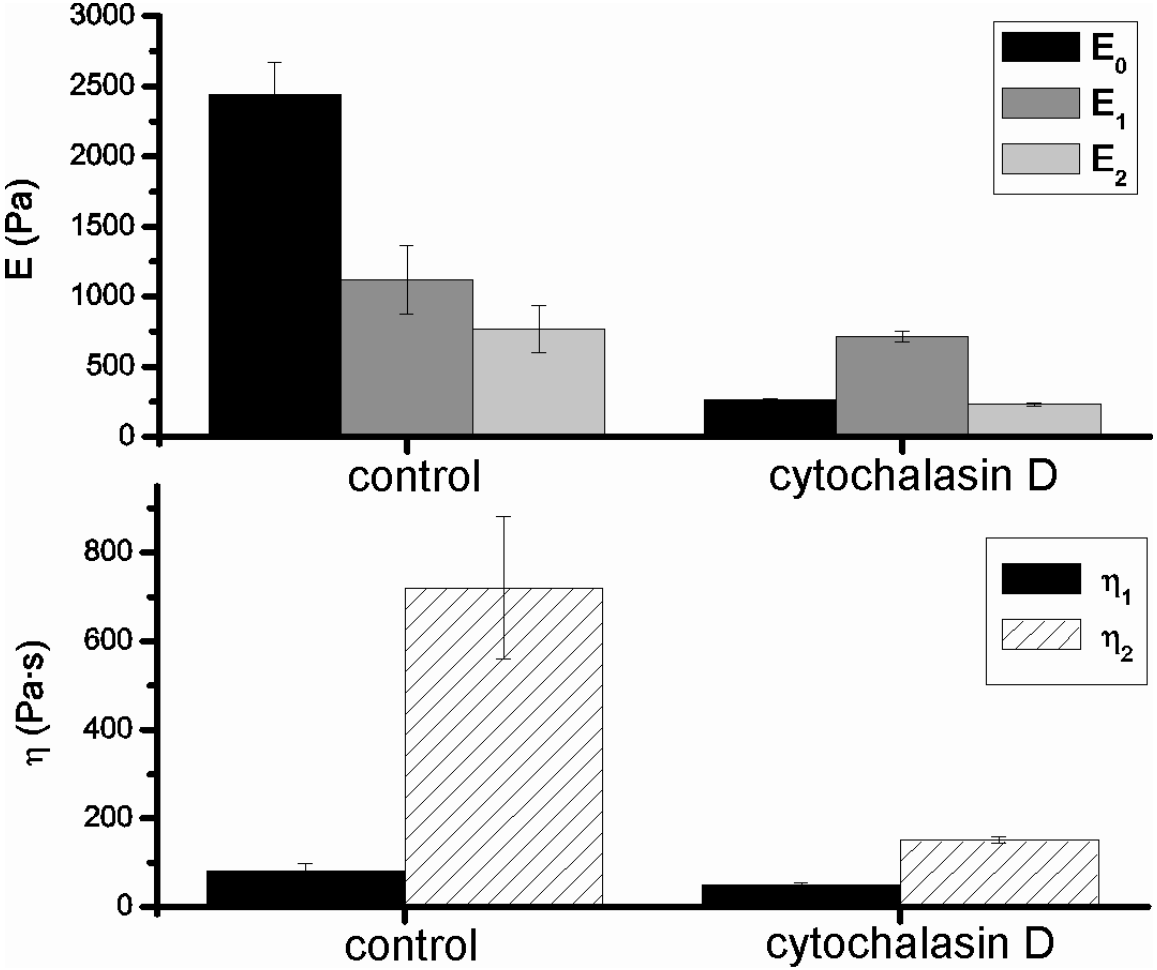
Elastic modulus, relaxation time and viscosity ???

Force-time and height-time based experiments



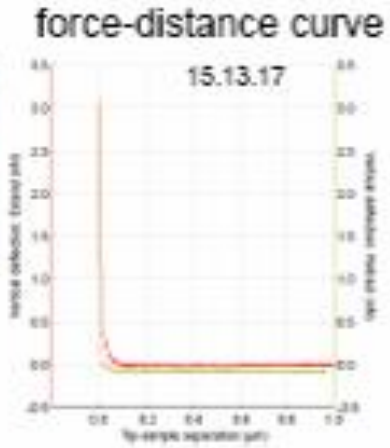
Nanotechnology 21 (2010) 445101

Stress relaxation and creep – actin-depolymerizing drug



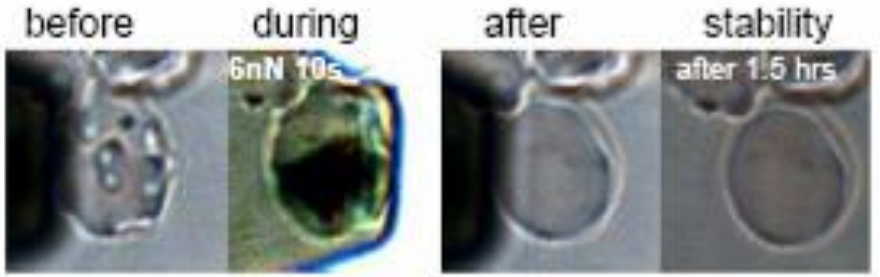
We are happy: This is nice and problematic at the same time!

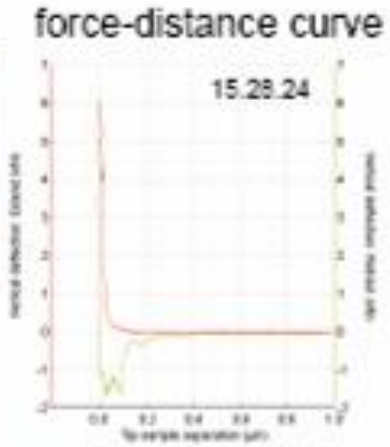
Making RBC look younger – inducing cell shape change



summary

3 nN for 10s, repeated 10x, may not cause shape change (shape changes were sometimes observed at 3 nN)





summary

6 nN for 10s causes shape change (positive control for the 3 nN experiment)

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What could be going on…

-echinocytes were formed by storage of RBCs in plasma, followed by rinsing with phosphate buffered saline

-application of 6 nN or more causes a shape change

-application of 3 nN or less typically does not

- same effect is observed when the ATP-dependent translocases are inhibited (with vanadate)

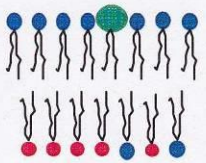
-the smoother cell shapes are stable for periods of several hours

Red blood cells and their shapes:

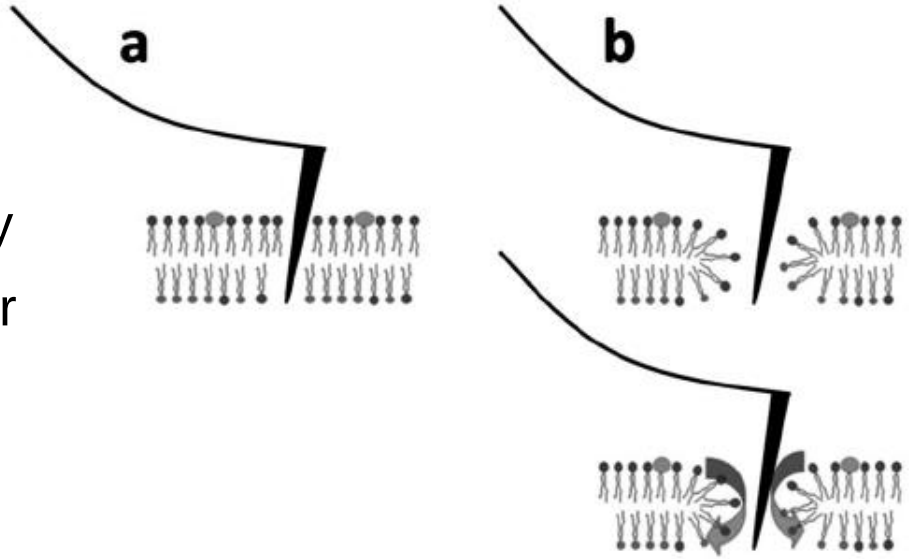
Healthy red blood cells in vivo have a biconcave disc shape.

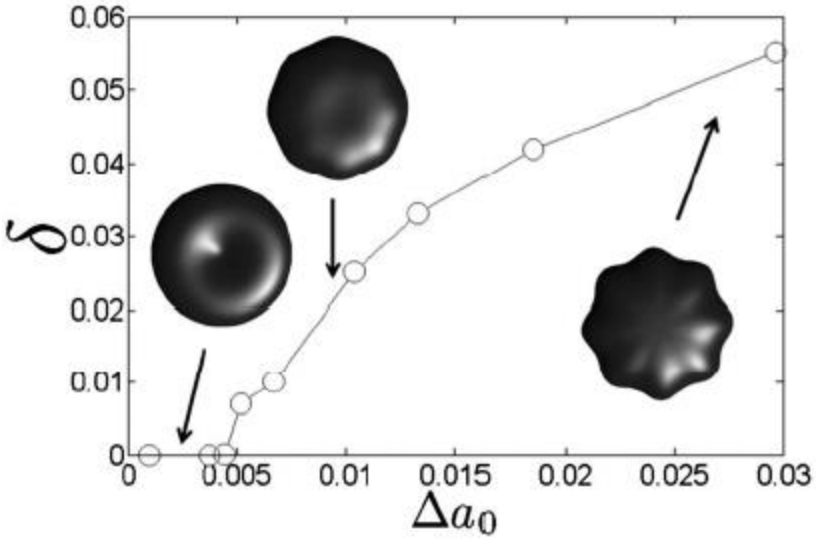
photograph (light microscope)

The disc shape is associated with minimisation of the bending energy[1,2] that arises because the lipid bilayer is asymmetric. The relative areas of the inner and outer monolayers also affects cell shape[3,4].



The disc shape is associated with minimisation of the bending energy that arises because the lipid bilayer is asymmetric





$$E_c = \frac{\kappa}{2}\int_{S_0}(\lambda_1\lambda_2 - 1)^2 \mathrm{d}S_0 + \frac{\mu}{2}\int_{S_0}\frac{(\lambda_1 - \lambda_2)^2}{2\lambda_1\lambda_2}\mathrm{d}S_0$$

$$E_b = \frac{\kappa}{2}\int_{S}(H - c_0)^2 \mathrm{d}S + \frac{\kappa_A \pi}{2AD^2}(\Delta A - \Delta A_0)^2$$

Soft Matter 9 (2013) 6430

Conclusions are boring…there are not any today!

The people who contributed to this presentation…

Dr. Susana Moreno-Flores

Dr. Birgit Kainz

Dr. Rafael Benitez

Dr. Maria Vivanco

Mag. Batirtze Prats

Dr. Kathryn Melzak

Thank you for listening!!!