# Nanotechnology for Life Sciences

Louis Tiefenauer

Paul Scherrer Institut

Laboratory for Micro- and Nanotechnology

# Nanosciences

## Why is „nano“ important in Life Sciences?

**Medicine:** *Anatomic limitations*
e.g. Capillary pores diameter of 80 nm

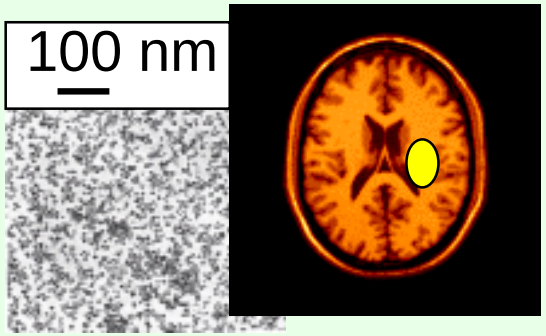
**Biochemistry:** *Single molecules vs ensembles*
e.g. Biorecognition ligand-receptor

**Cell biology:** *Investigation of subcellular functions*
e.g. Cell surface interactions

# Nano-Technology

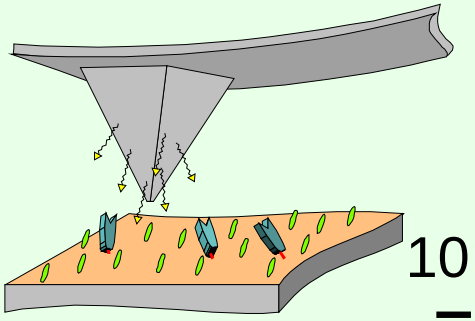
## Nanomaterials

Magnetite nanoparticles
for in vivo diagnosis



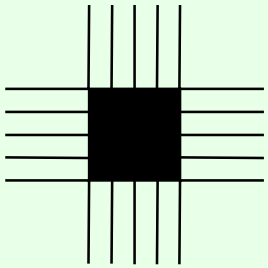

## Methods: AFM

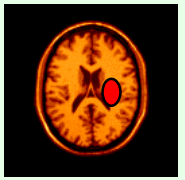
Imaging and functionality of
*individual* molecules



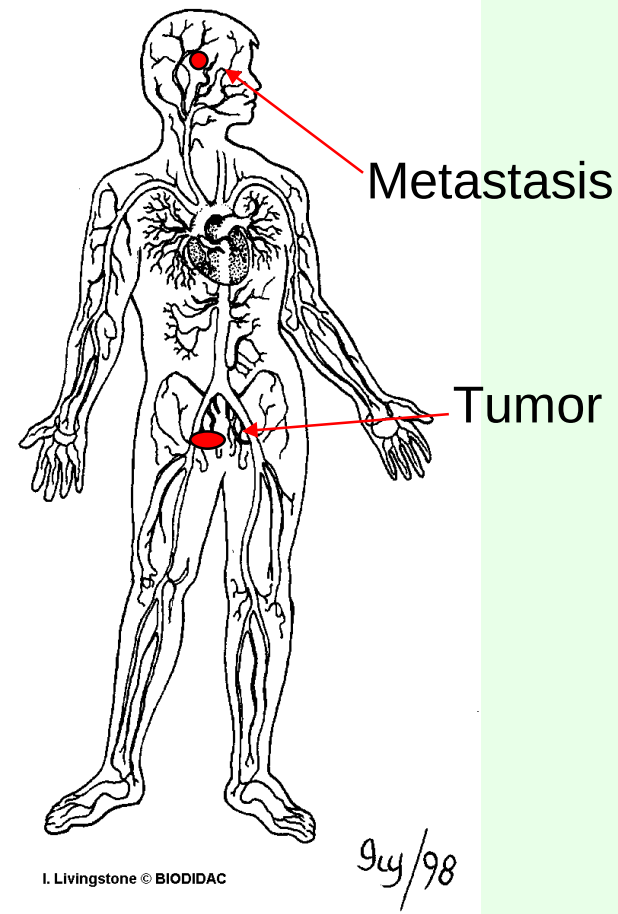

## Nanostructures

Surfaces and devices for
bioanalytics and research

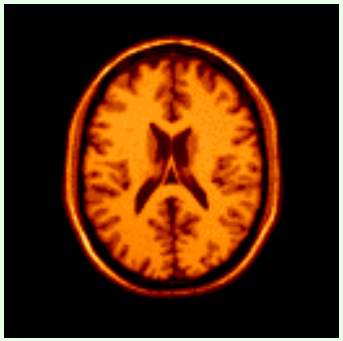


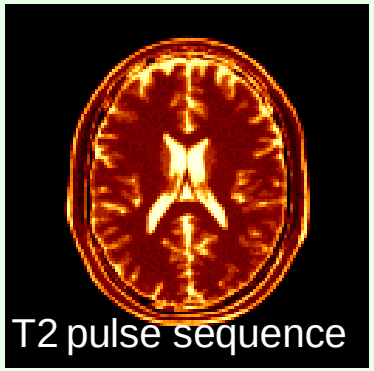


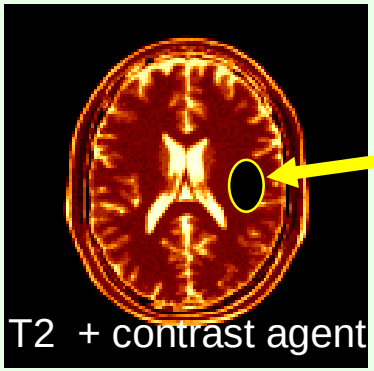
# Nanoparticles for *in vivo* diagnostics



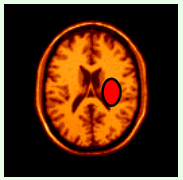

Contrast agent in Magnetic Resonance Imaging (MRI)





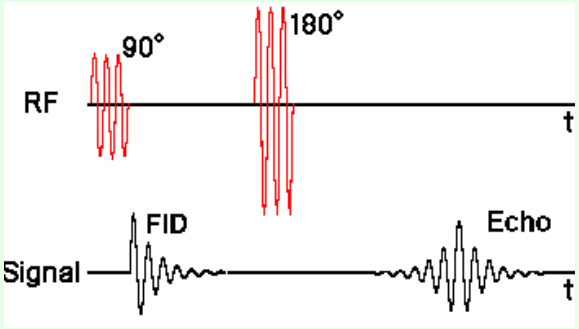

Tumor



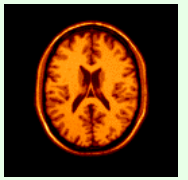
# MRI image contrast

| Tissue | $T_1$ (s) | $T_2$ (ms) | ρ |
|---|---|---|---|
| CSF | 0.8 - 20 | 110 - 2000 | 70-230 |
| White | 0.76 - 1.08 | 61-100 | 70-90 |
| Gray | 1.09 - 2.15 | 61 - 109 | 85 - 125 |
| Meninges | 0.5 - 2.2 | 50 - 165 | 5 - 44 |
| Muscle | 0.95 - 1.82 | 20 - 67 | 45 - 90 |
| Adipose | 0.2 - 0.75 | 53 - 94 | 50 - 100 |

## *Spin echo Sequence*




| Weighting | TR Value | TE Value |
|---|---|---|
| $T_1$ | $< = T_1$ | $< < T_2$ |
| $T_2$ | $> > T_1$ | $> = T_2$ |
| ρ | $> > T_1$ | $< < T_2$ |



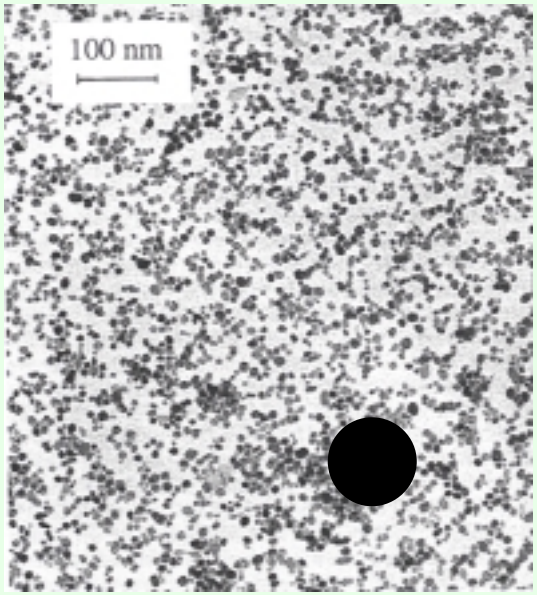
# Nanoparticles used as a contrast agent

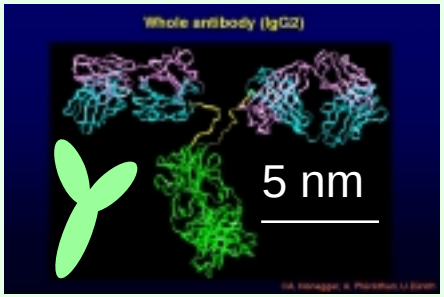
## *Preparation*

$$\mathbf{Fe^{II}/\ Fe^{III}} \xrightarrow[47\ C,\ Ultrasound]{NH_4OH\ /\ H_2O} \mathbf{Fe_3O_4} \xrightarrow[100\ 000\ g]{Centrifugations} \mathbf{Nanoparticles}$$

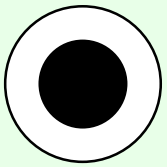
10 nm

## *Functionalization*



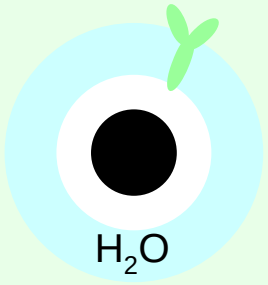

Crosslinked → polypeptide

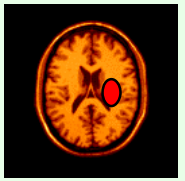


Tumor specific → antibody

## *Functionality*

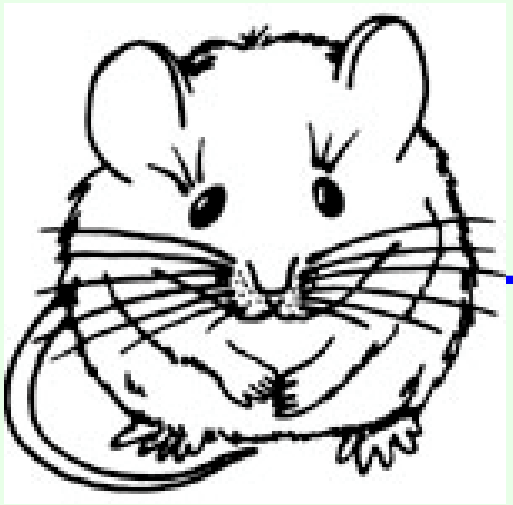
Binding capacity !

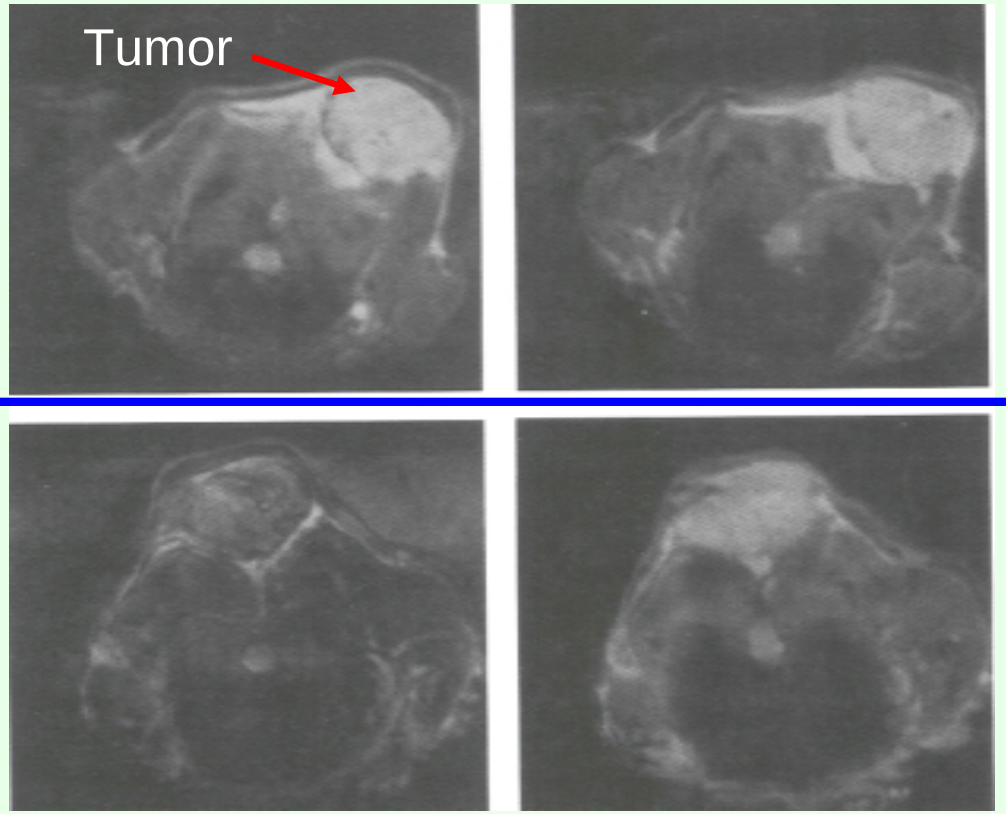




# Localization of tumor tissues

- nanoparticles

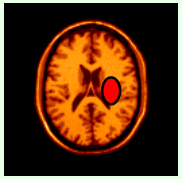





$T_2$ = 65 + - 4 ms

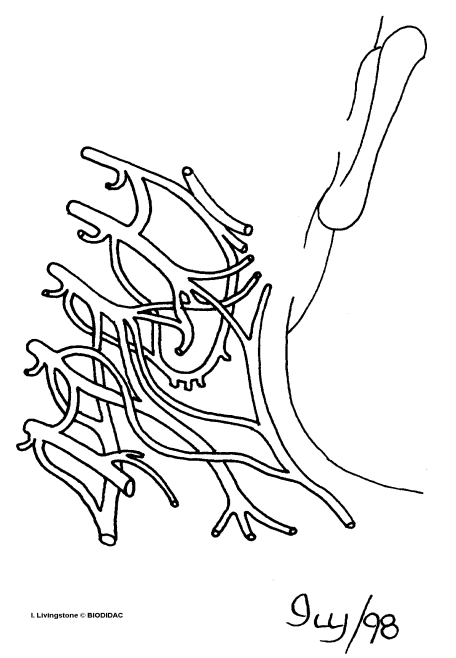
$T_2$ = 56 + - 1 ms

+ nanoparticles

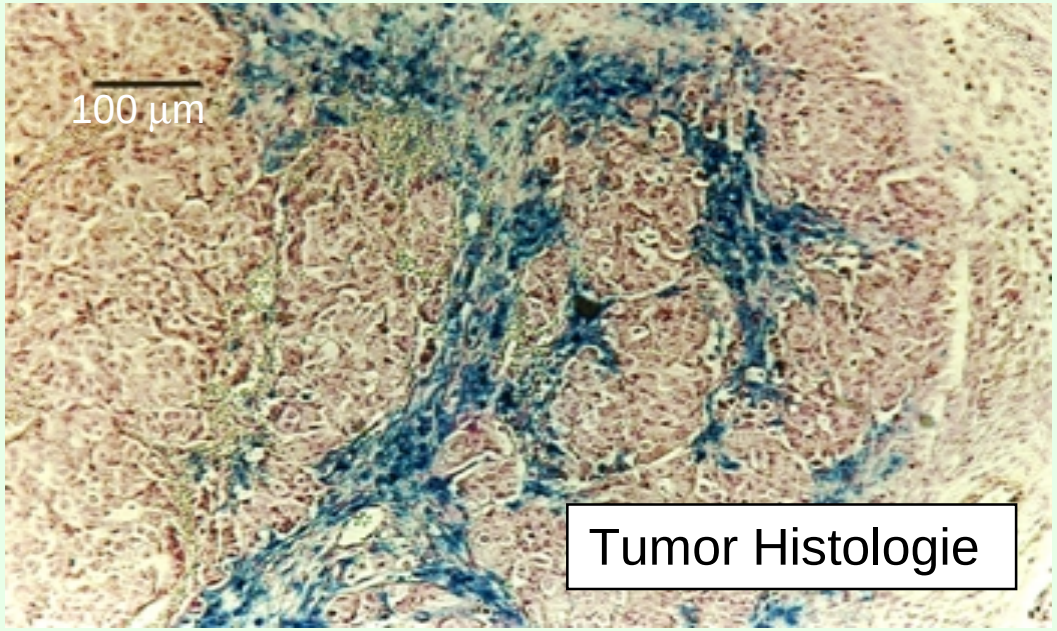


# Results and problems

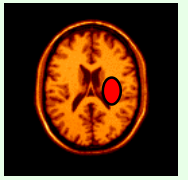
## Inhomogeous distribution of nanoparticles in tumor tissues !




Blood vessels




L. Tiefenauer et al. *Mag. Res. Imag.* 14, 391 (1996)

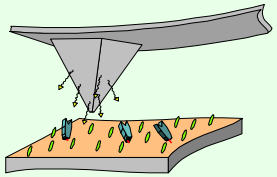


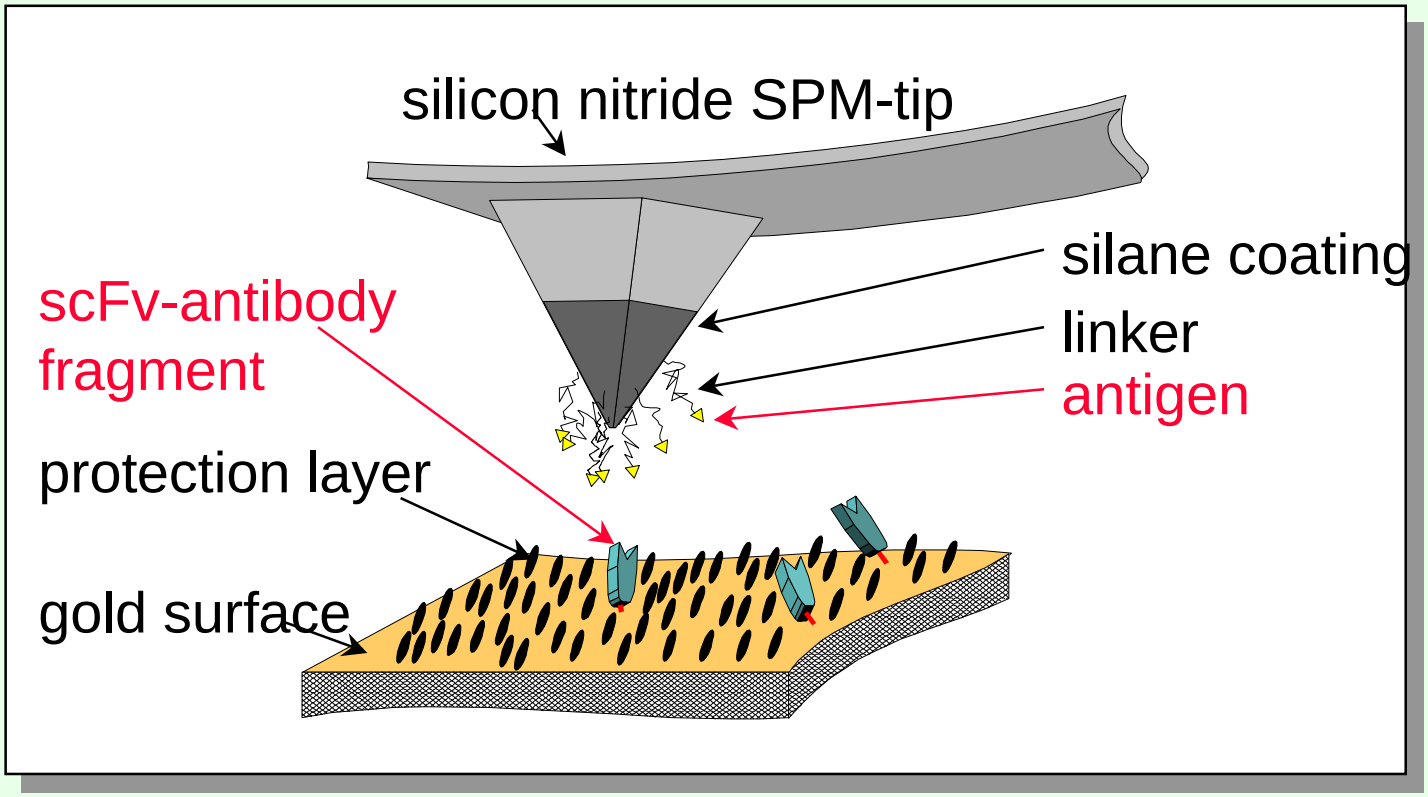
# Nanoparticles for *in vivo* diagnostics

- Particle size as a small virus
- Anatomy limits the distribution of nanoparticles
- Surface coating determines their fate *in vivo*

➡ „Nano“dimensions may be helpful

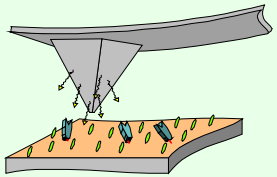
# Force spectroscopy on *individual* molecules

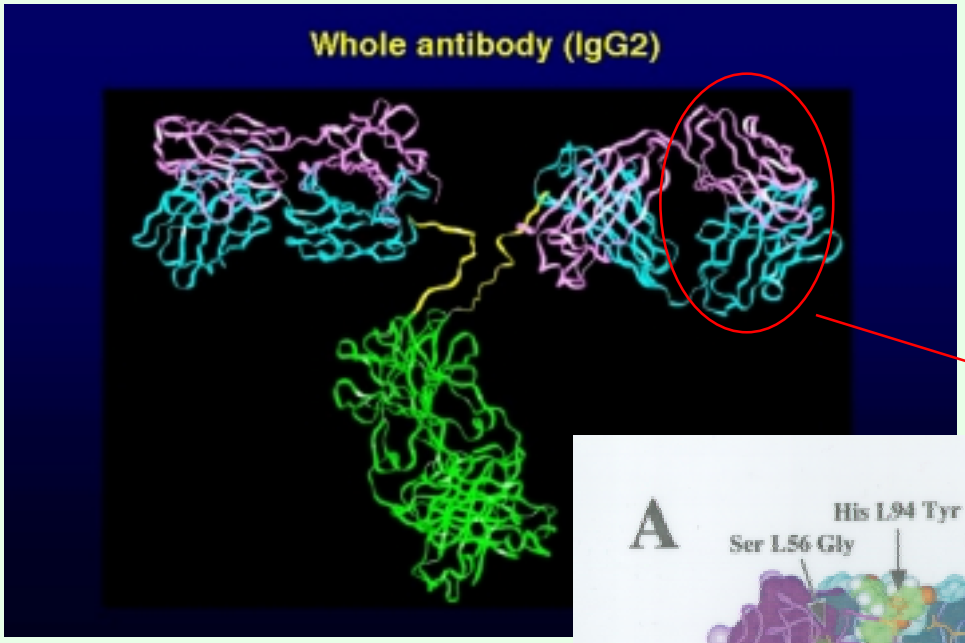




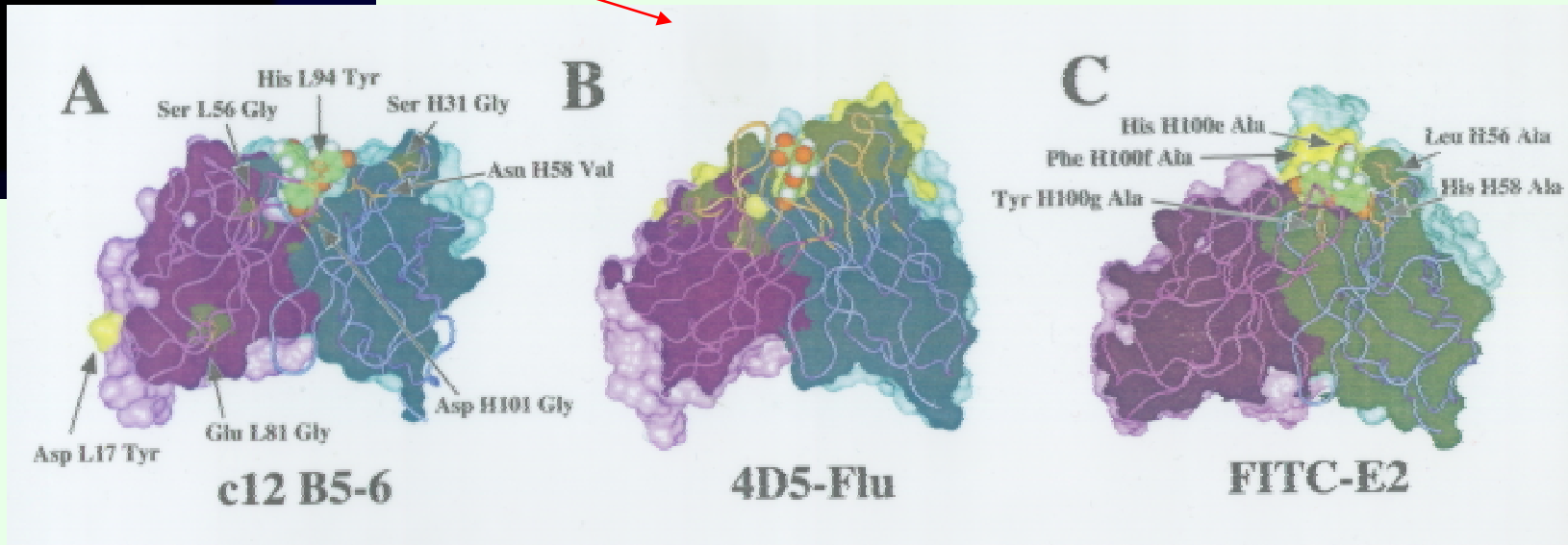

NPF36 R. Ros PSI, collab. A. Plückthun UniZH & Güntherodt UniBS

# Single chain Fv antibody-fragment

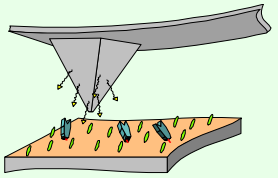


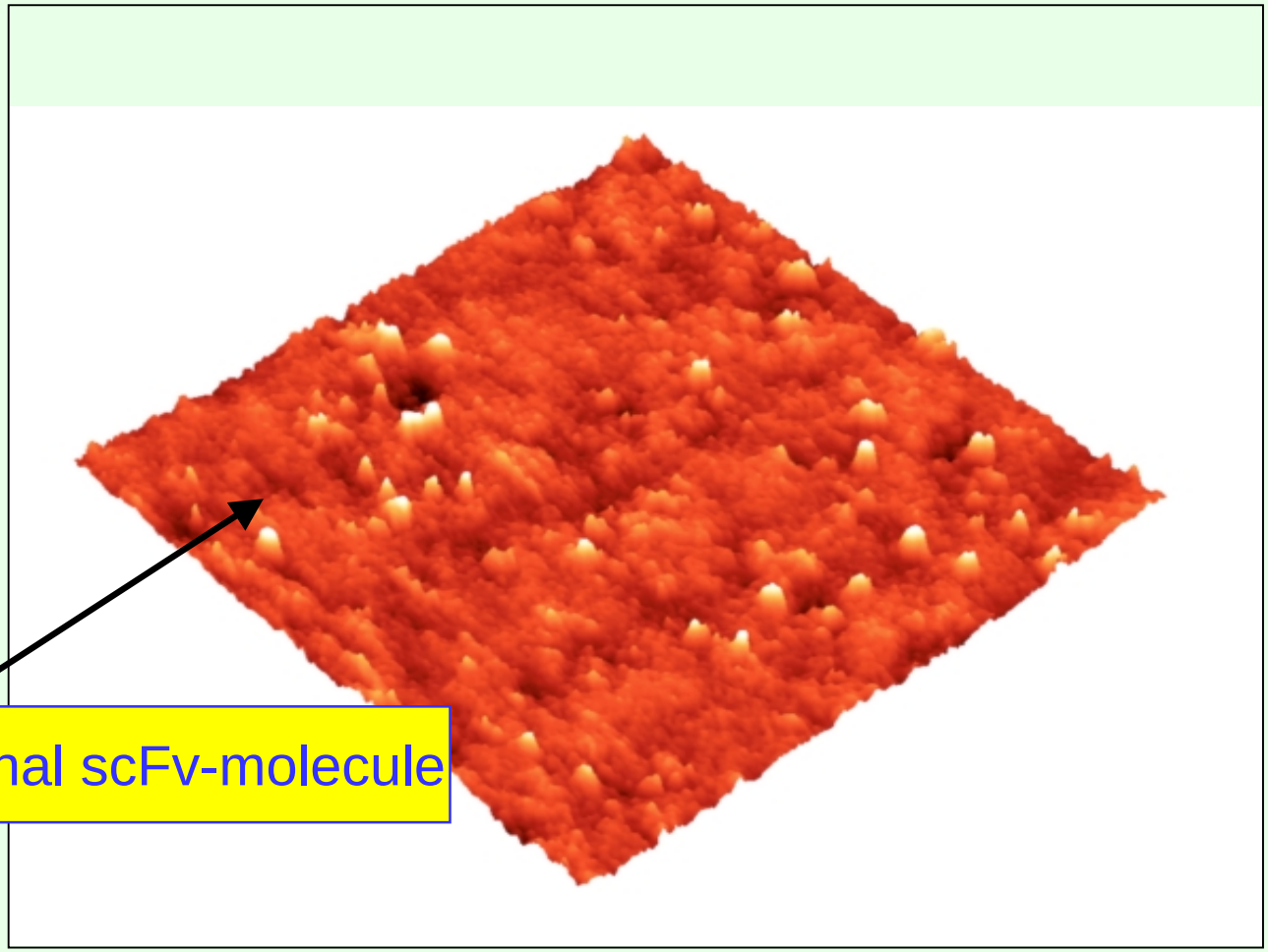



scFv proteins:
mutants and variants

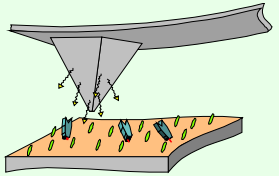


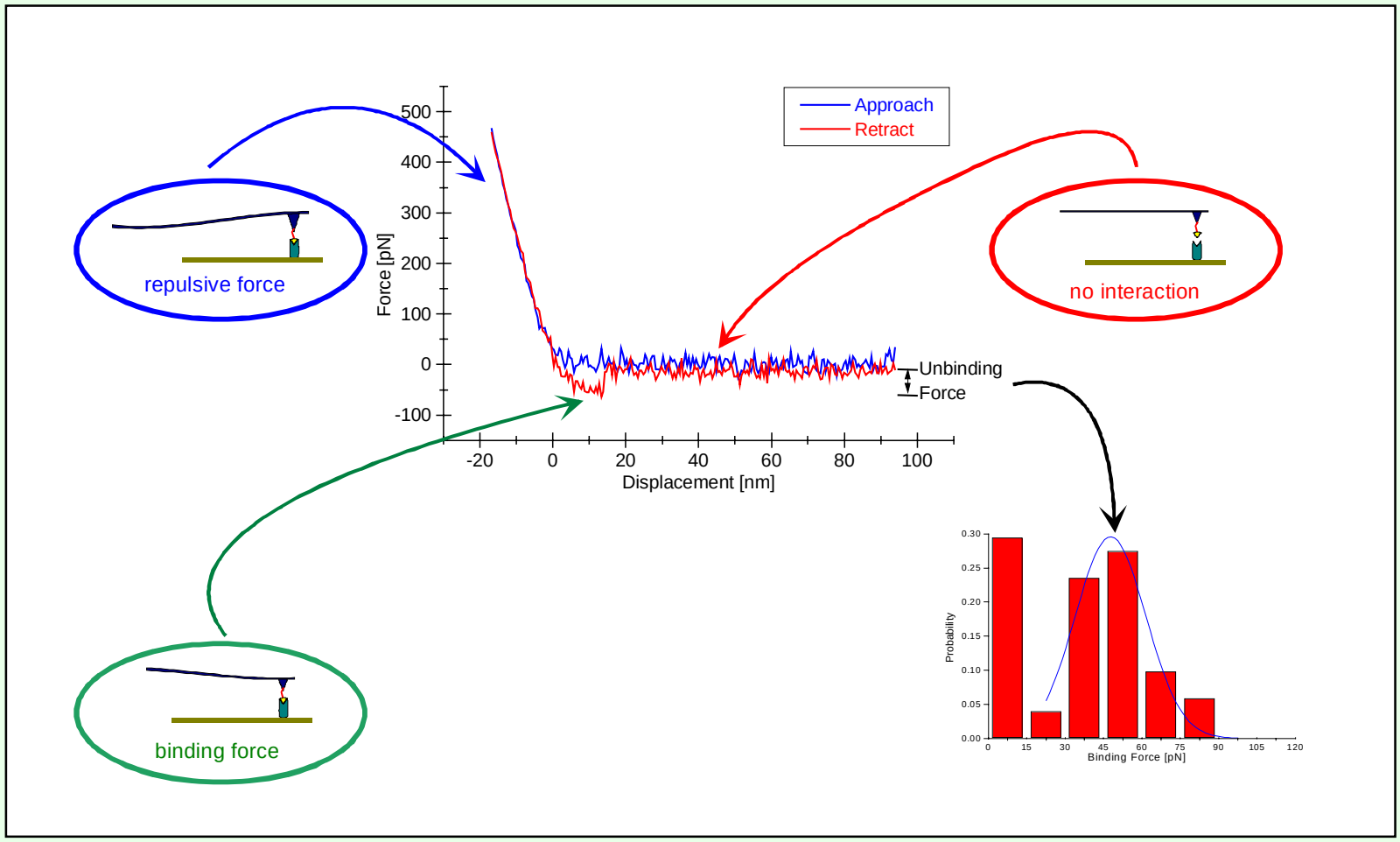
# Surface preparation and analysis

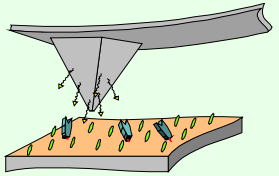




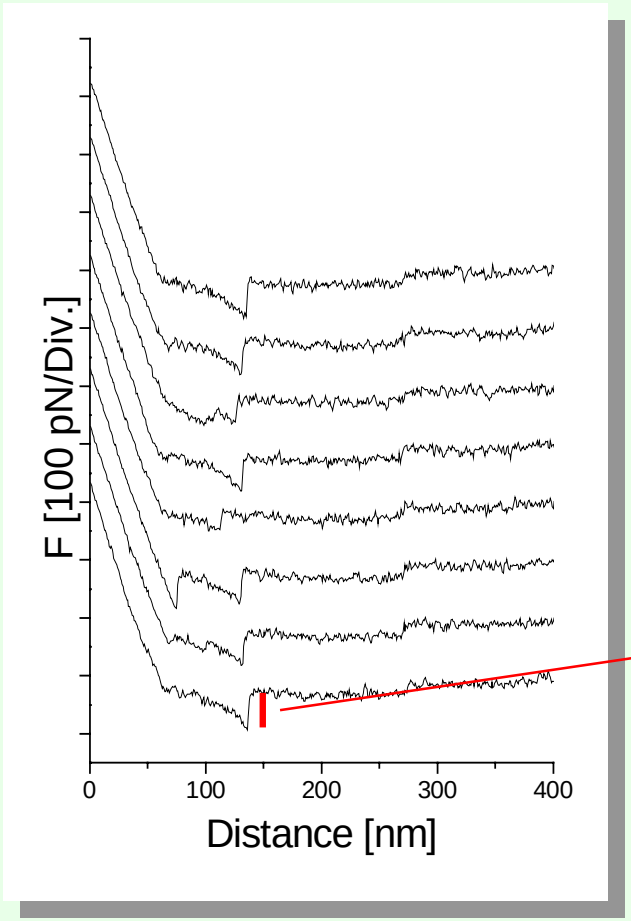
# Force spectroscopy method

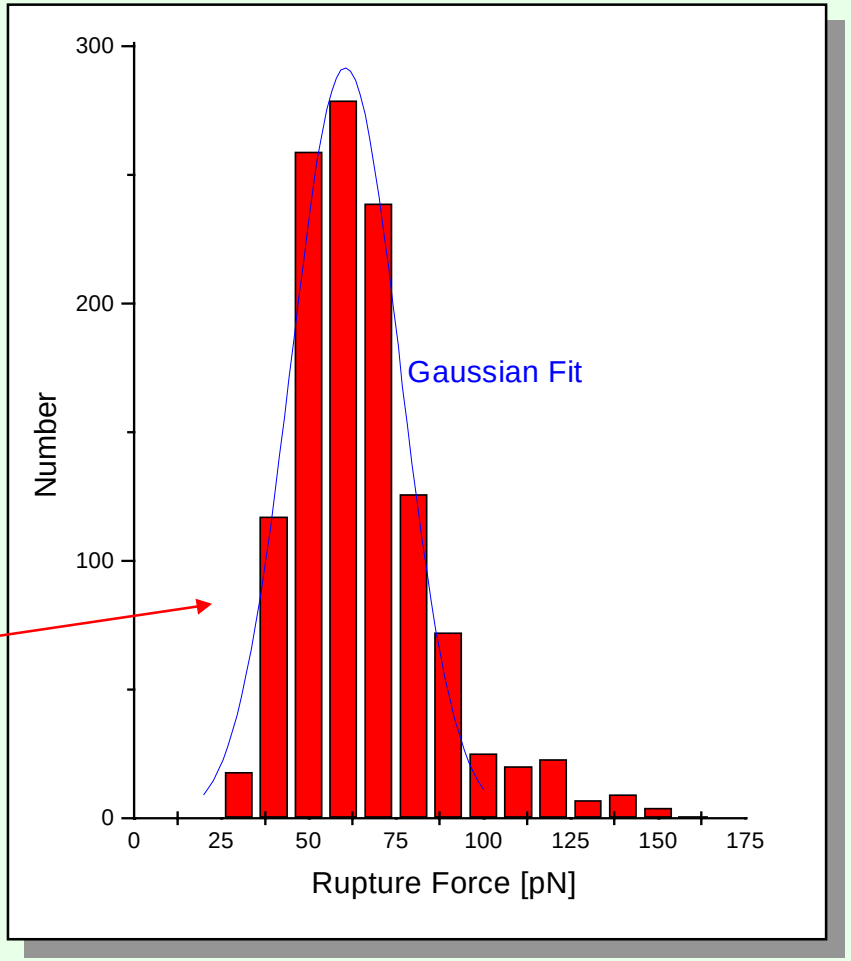






# Rupture force measurements



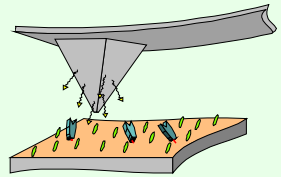
# Kinetics and thermodynamics

$$K_D = \frac{k_{off}}{k_{on}} \quad \text{and} \quad \Delta G = RT \ln K_D$$

## scFv proteins

$k_{off}$: 1.4 $10^{-4}$ - 4.7 $10^{-1}$ $s^{-1}$

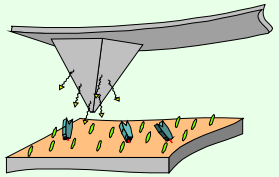
$k_{on}$: 0.3 - 11.9 $\mu M^{-1}$ $s^{-1}$

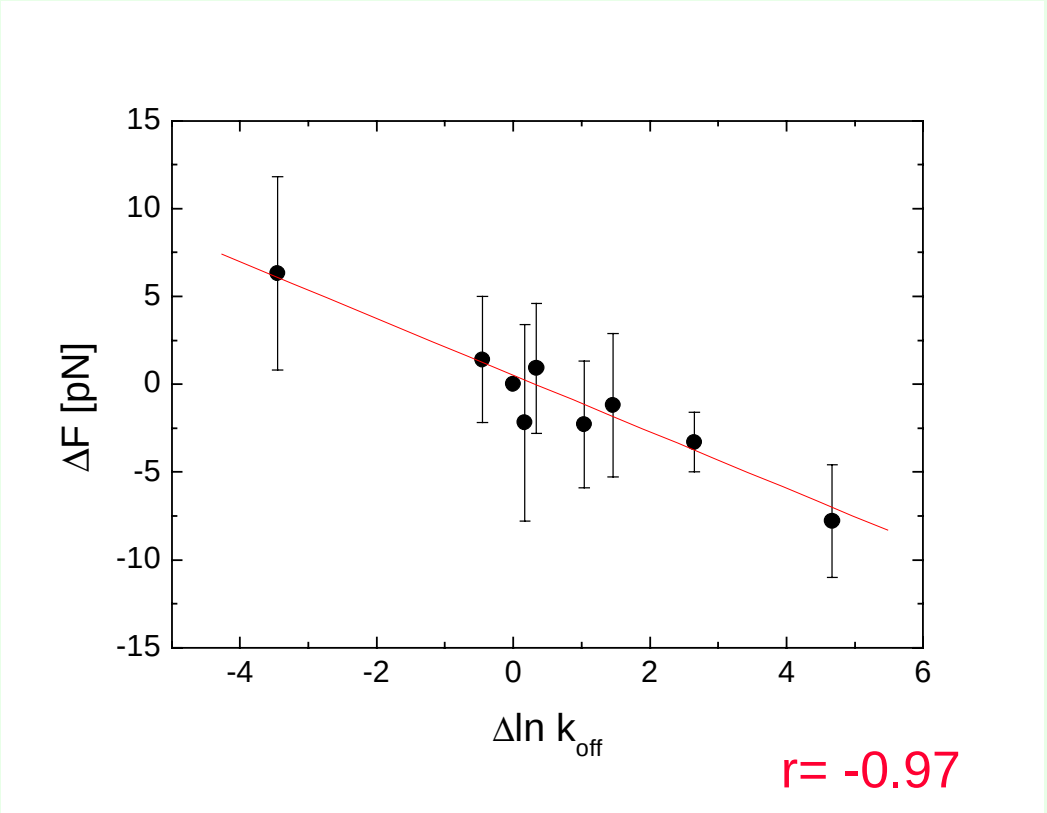


# Summary of the data

| **Antibody** Type | **F** pN | $k_{off}$ $10^{-3}$ $s^{-1}$ | $K_D$ nM | $\Delta G^{\#}$ $kJmol^{-1}$ | $\Delta H^{\#}$ $kJmol^{-1}$ |
|---|---|---|---|---|---|
| **AB1** | 55 | 4.4 | 1.1 | 27.7 | 111.3 |
| **AB2** | 52 | 5.2 | 2.7 | 27.2 | 105.9 |
| **AB3** | 58 | 6.2 | 9.6 | 26.9 | 105.6 |
| **AB4** | 55 | 12.5 | 1.8 | 25.1 | 115.0 |
| **AB5** | 54 | 19 | 7.9 | 24.1 | 87.7 |
| **AB6** | 47 | 470 | 15.0 | 15.8 | 77.7 |
| **AB7** | 58 | 2.8 | 1.5 | 28.9 | 99.1 |
| **AB8** | 63 | 0.14 | 0.1 | 36.1 | 105.0 |
| **AB9** | 54 | 62 | 9.7 | 21.1 | 93.4 |

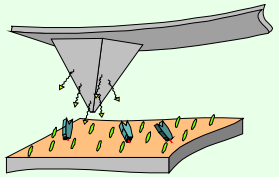
# Results: correlation of ΔF to off-rate

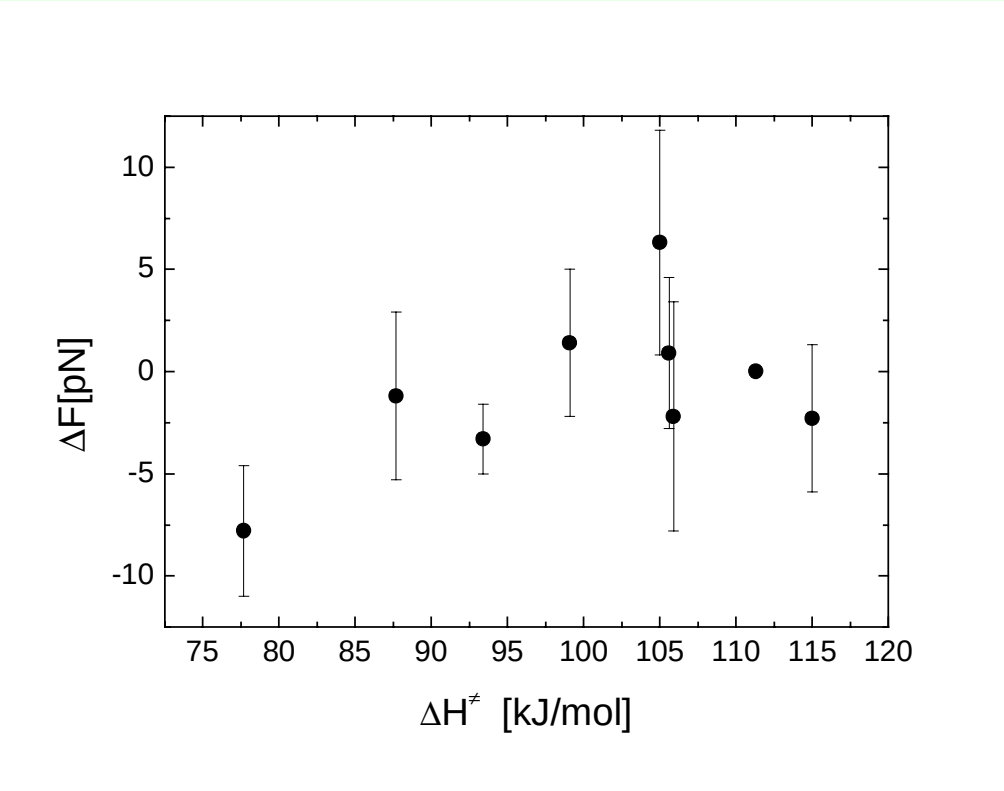




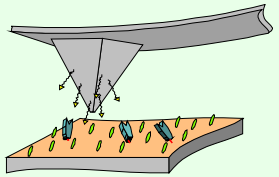

F. Schwesinger et al. *PNAS* 97, 9972 (2000)
R. Ros et al. *PNAS* 95, 7402 (1998)

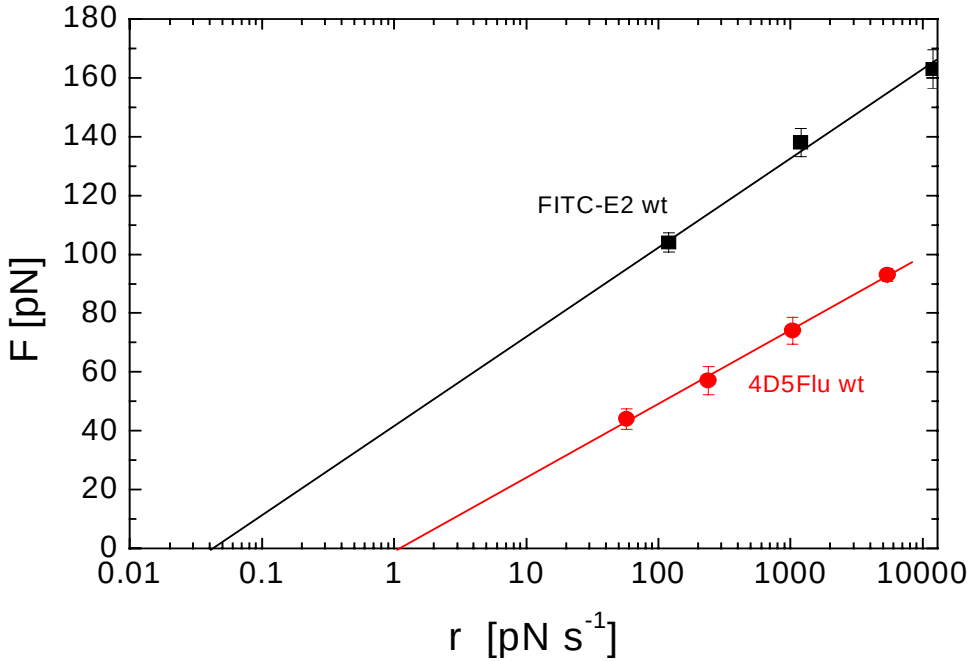
# *No* correlation to free activation enthalpie $\Delta H^{\neq}$





# Dissociation under external force






$$F = \frac{k_B T}{x_\beta} \ln \frac{r_f x_\beta}{k_B T k_{off}}$$

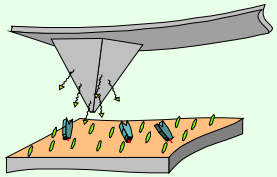
E. Evans and K. Ritchie;
Biophys.J.;72:1541-1555; 1997.

off-rates:

FITC-E2 wt → 0.003 ± 0.002 $s^{-1}$ (0.004 $s^{-1}$)

4D5Flu wt → 0.10 ± 0.05 $s^{-1}$ (0.062 $s^{-1}$)

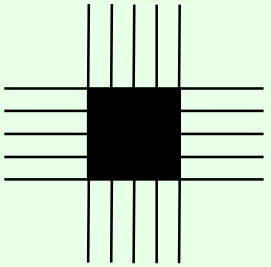
# Force spectroscopy on *individual* molecules



- Characteristics of *individual* molecules measured
- Correlations to ensembles of molecules
- Sensitivity of force spectroscopy method evaluated

„Nano“methods provide
a new analytical quality

# Nanostructures



## Surface nanopatterning

Pattern definition: E-beam writing, stamping

Material structures: topographical, chemical contrast

Molecules: adsorption, covalent bonds

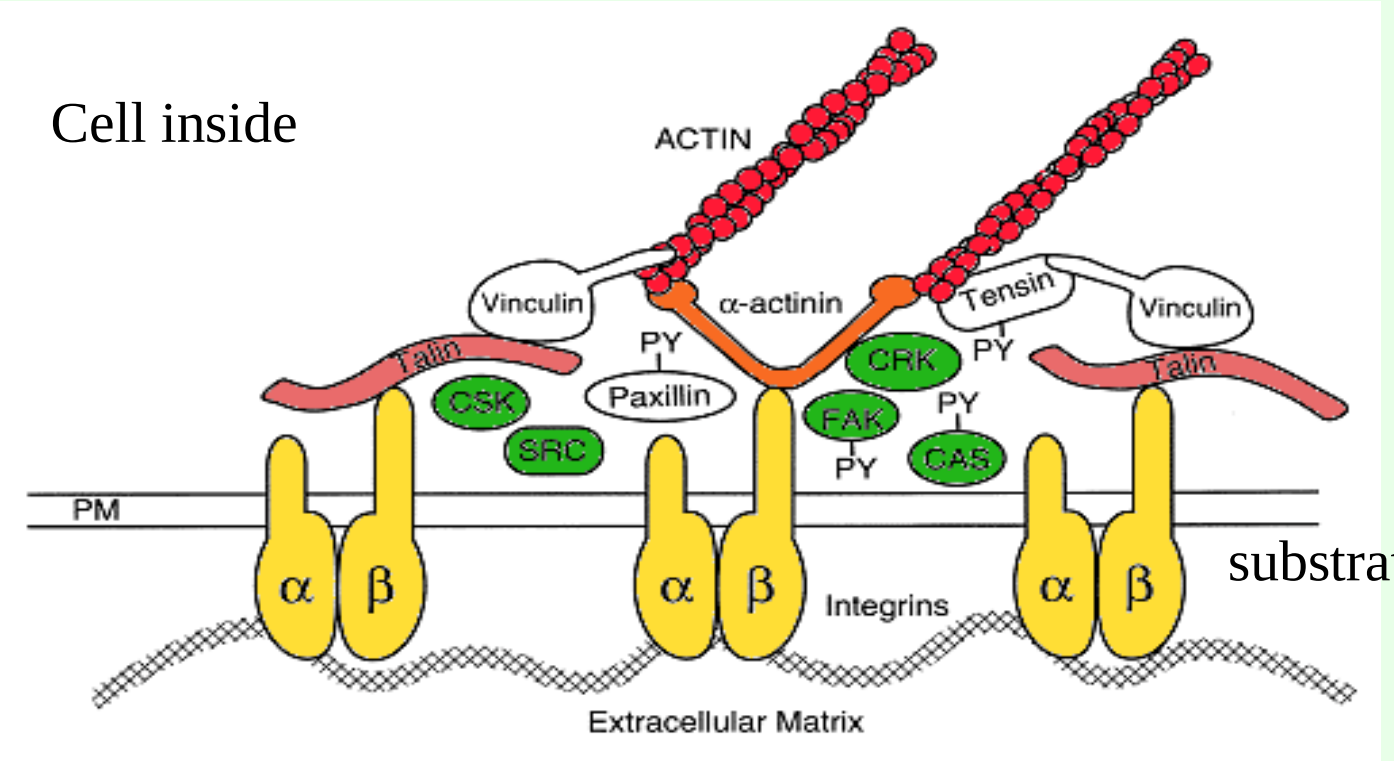
## Nanodevices

Micro- and Nanostructures

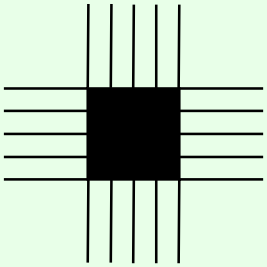
3D-structures

# Cell surface contact

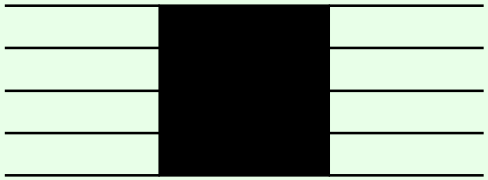
*Hypothesis:* Nanostructures influence cell functions

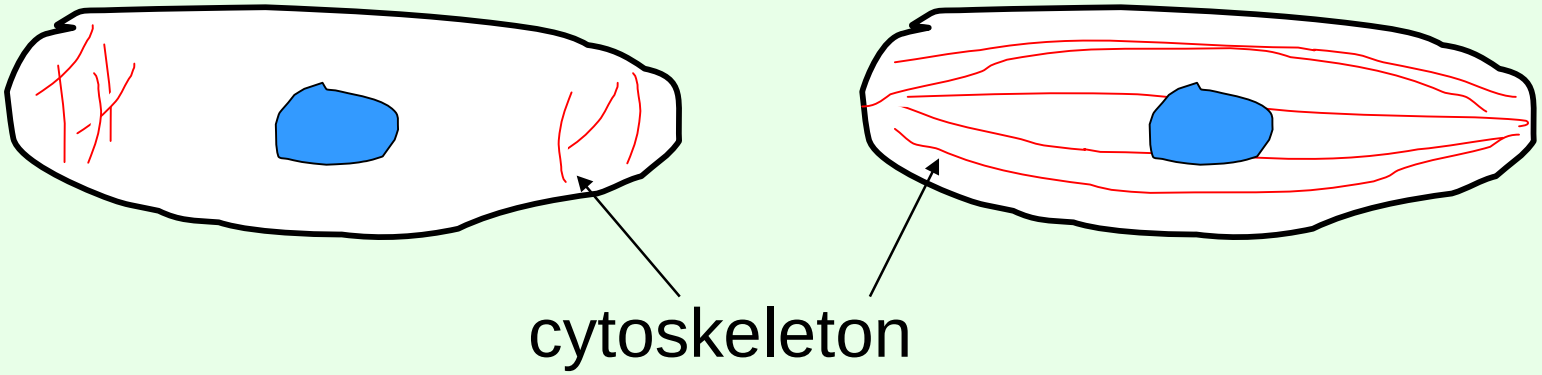


# Nanopatterns and cell function

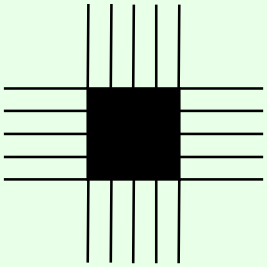


## Nanopatterns



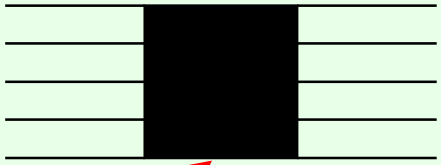


# Design of nanostructures

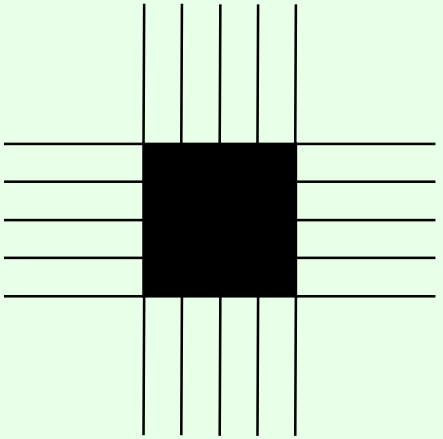


Anisotropic

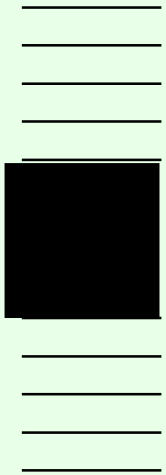
Isotropic



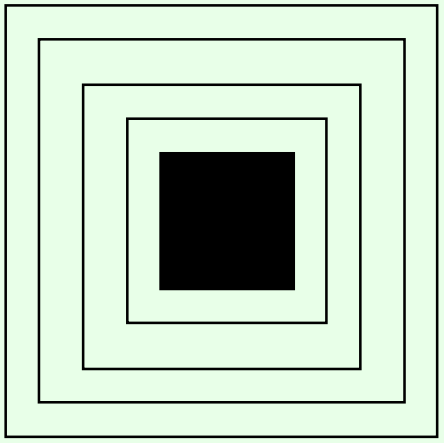
Connected



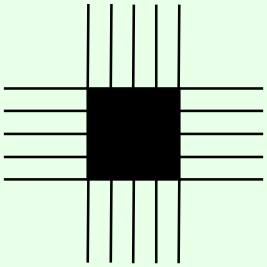
Pad for cells



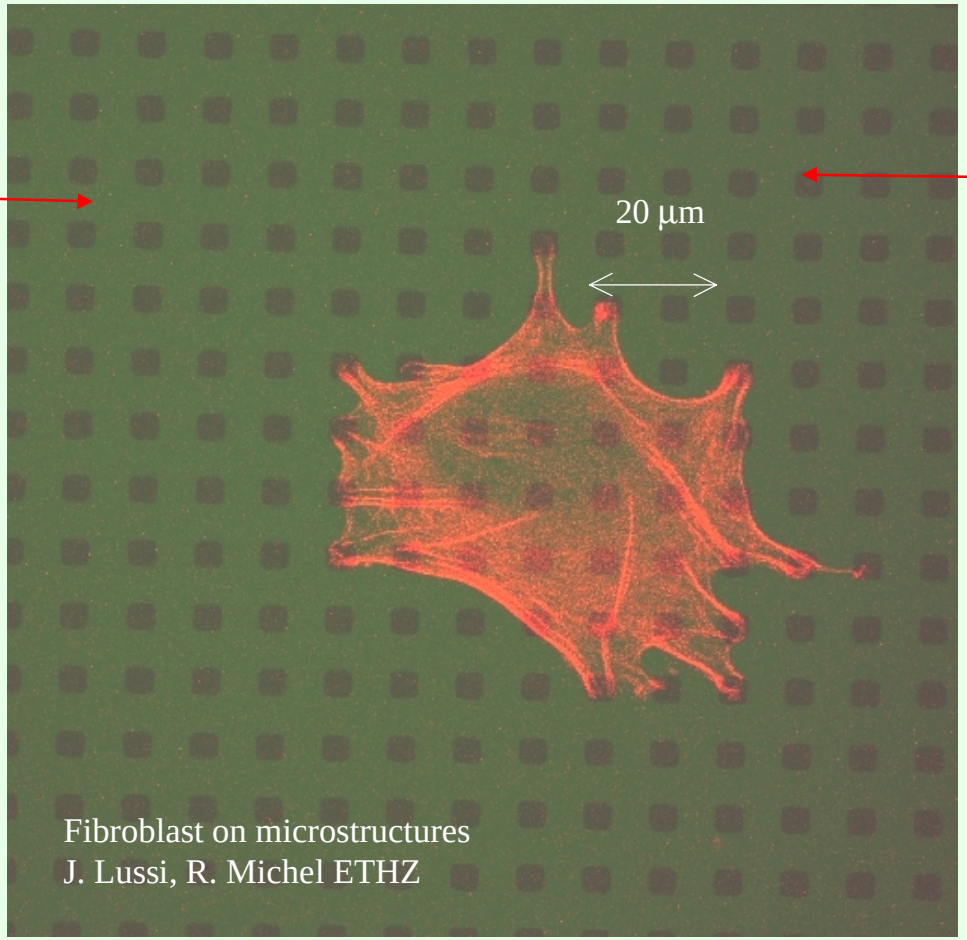
Unconnected



# Results on microstructures

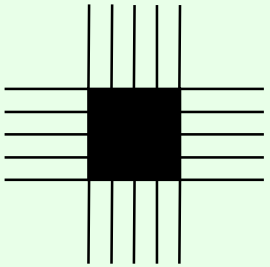


$SiO_2$, PLL-PEG
cell repellent

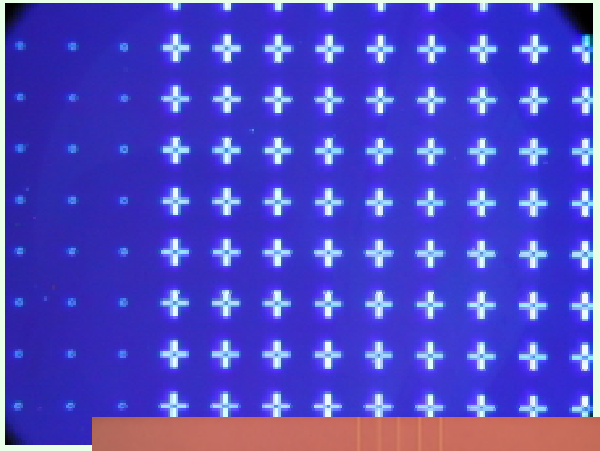



$TiO_2$, DDP, laminine
cell attractant

# Nanopattern generation

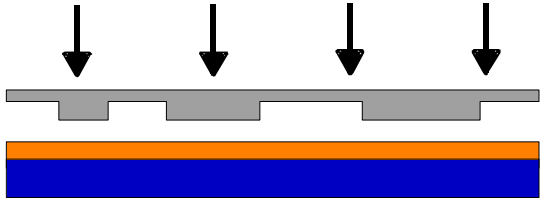


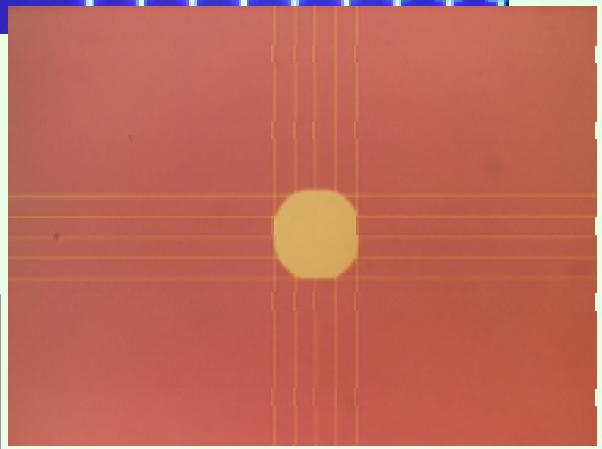
**Stamp production**
e-beam in Si



**SAM of cell adhesive molecules**

**Pattern transfer**





**Cell studies**

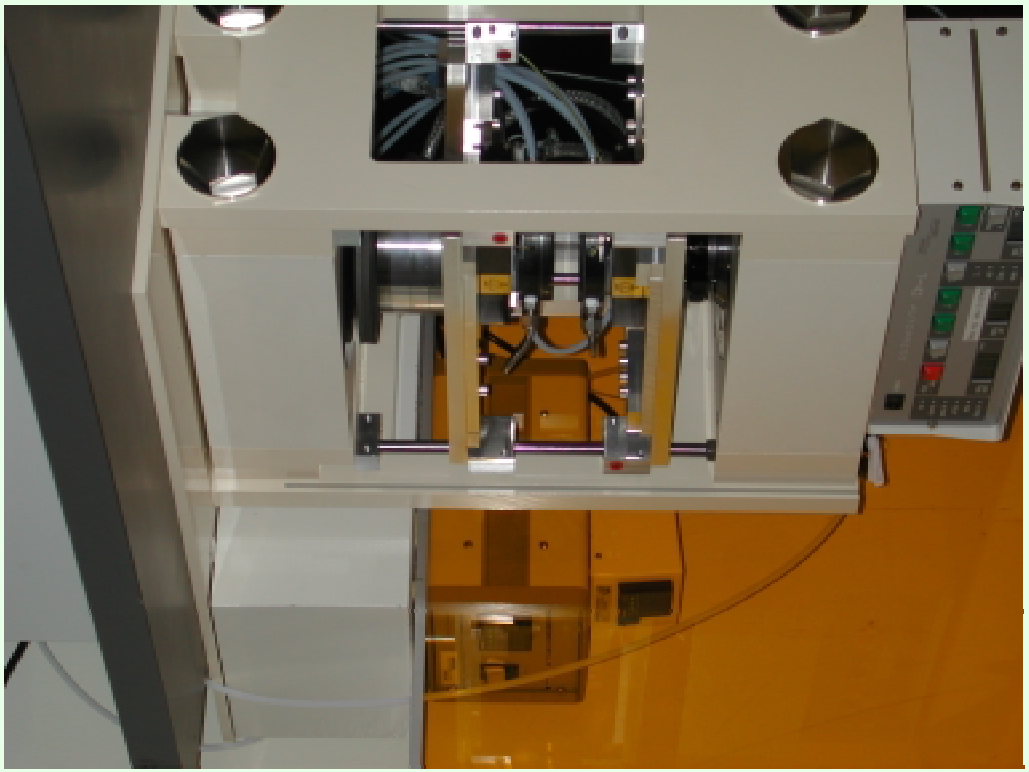
**Structure etching** (RIE)
Material contrast

$TiO_2$ on $SiO_2$

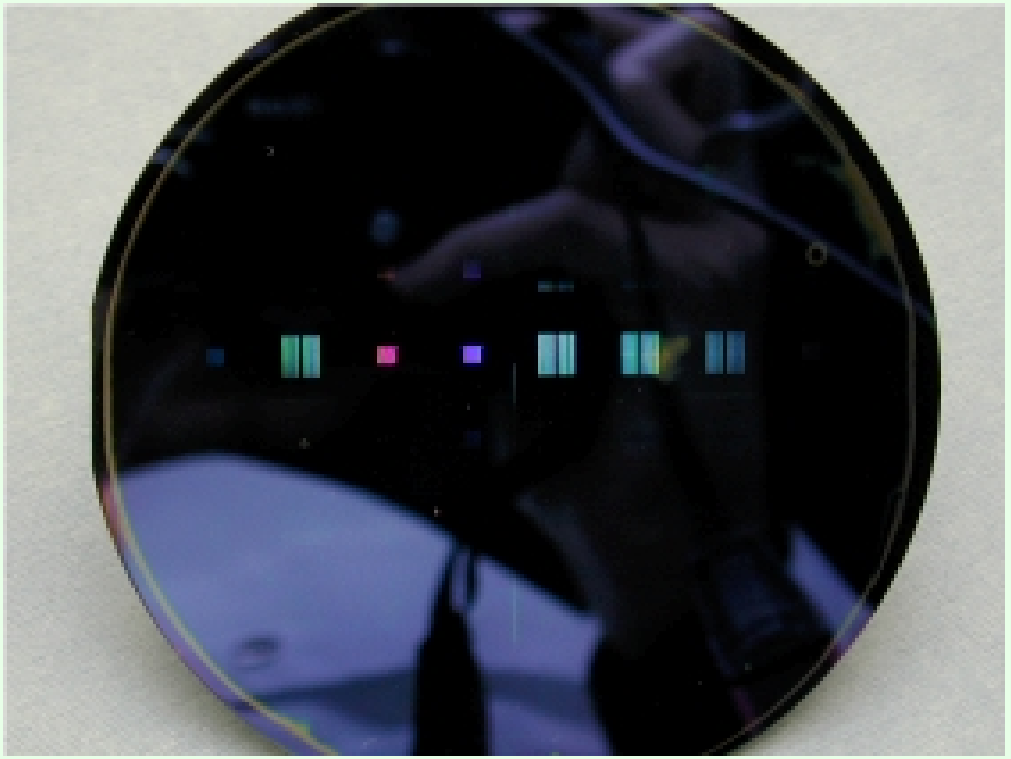
# Nanostructuring on whole wafers :

## hot embossing technique

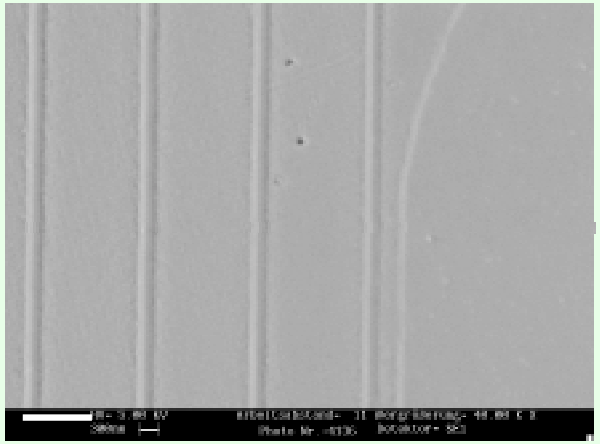
Press

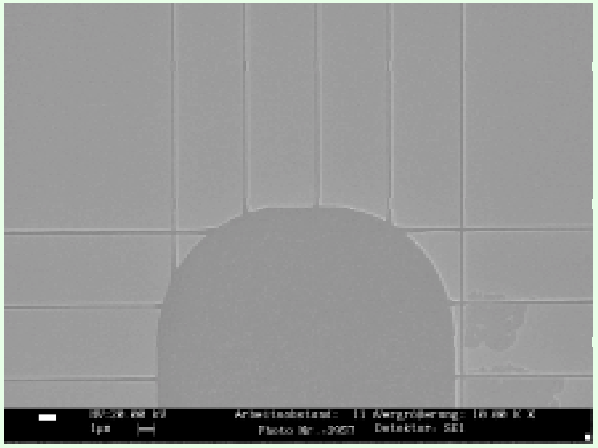


Structured wafer



# Topography

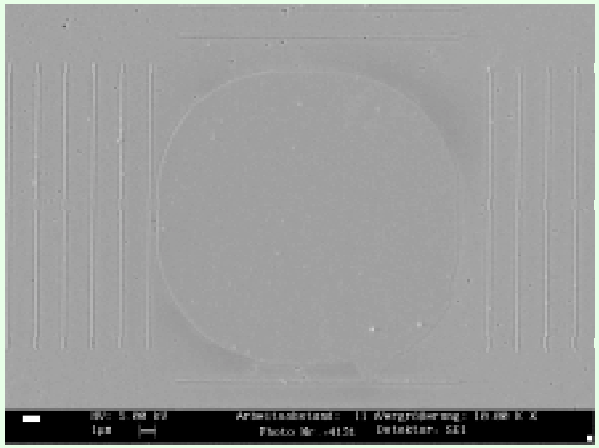


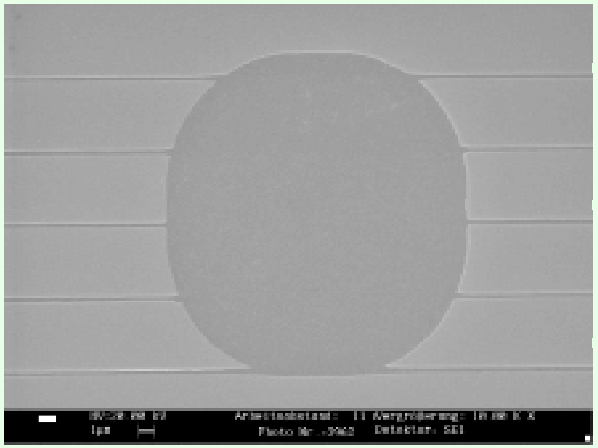


Hills

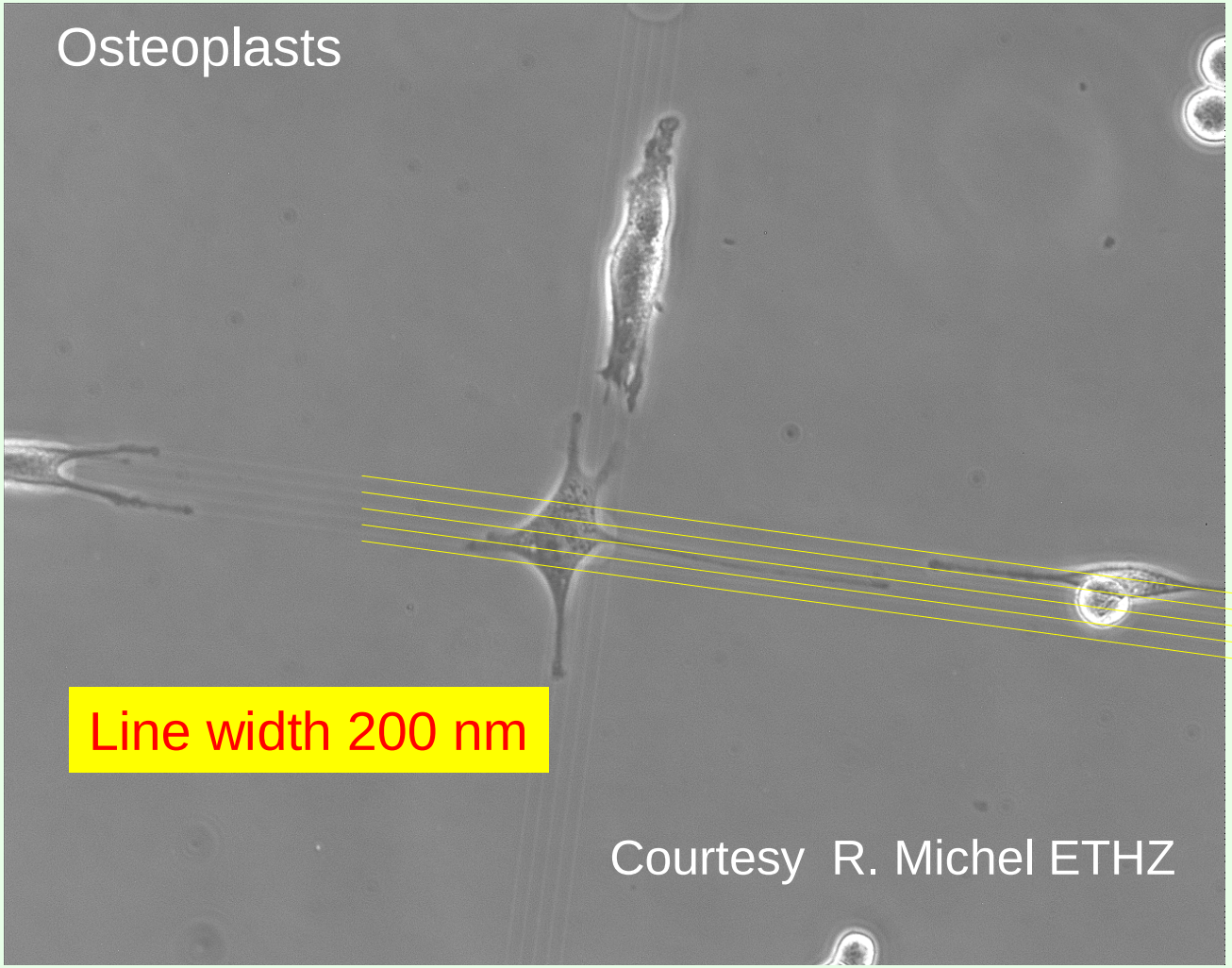
scale bar 1 µm

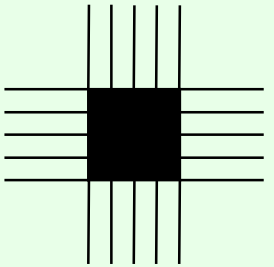
Valleys





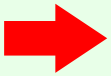
# Cell reaction to nanostructures





# Nanostructuring

- A resolution below 100 nm in nanopatterns can be achieved
- Mass production  is needed  for cell-surface investigations
- Material contrast can be used to achieve a chemical contrast



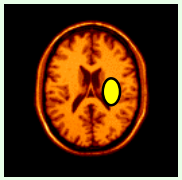
„Nano“structuring opens new capabilities to investigate cell-surface interactions

# Nanotechnology in Life Sciences

# Summary

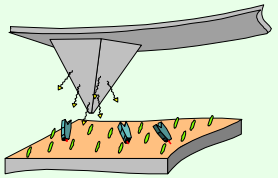
**Medicine**

*Nanoparticles as contrast agent for in vivo diagnostics or therapy*



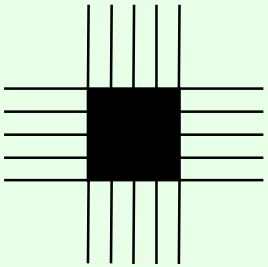
**Biochemistry**

*Single molecules investigation*



**Cell biology**

*Nanopatterns influence cell adhesion and function*



# Nanotechnology

## in Life Sciences

**What can we learn?**

*Interdisciplinary:*
**Physics**, (bio)chemistry, biology, medicine

*Scientific hypothesis:*
Multifactorial, molecule-based, surface structure-related

View: nanometer sized structures

*Applications:*
Research > diagnostics > therapeutics

# Acknowledgements

**Nanomaterials**

Guido Kühne, Andreas Tschirky, J.-J. Hefti

**AFM**

Robert Ros, Falk Schwesinger, Thorsten Strunz

**Nanostructures**

Brigitte Ketterer, Laura Heyderman, Marcus Textor

**Funding**

SNSF, KTI