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Sample preparation for AFM microscopy

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EUROPEAN UNION
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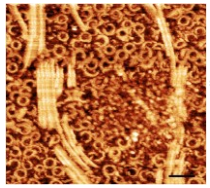
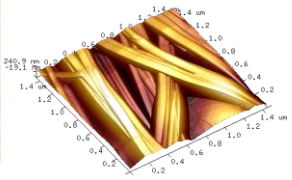
2007–13
OP Research and Development for Innovation

Potential of AFM
in bio-sciences

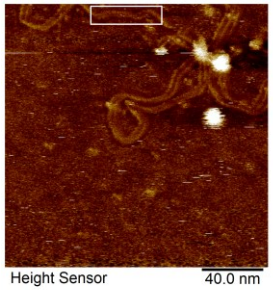
Atomic Force Microscopy (BioAFM)
what is this good for?

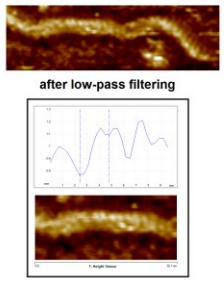


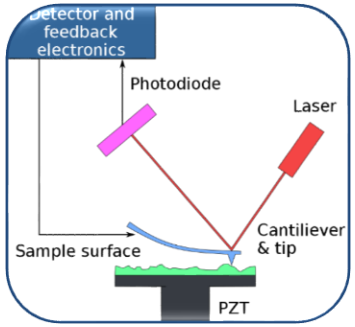


IMAGING

Proteins, DNA, Nanoobjects



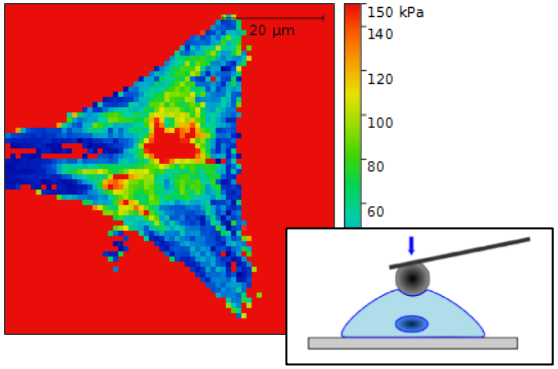




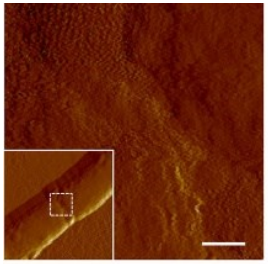
by OverloardQ

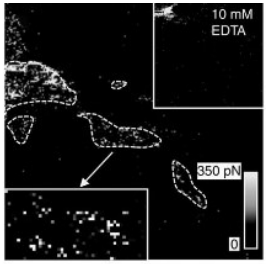
NANOINDENTATION

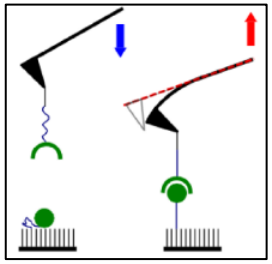
Stiffness mapping



AFFINITY INTERACTION

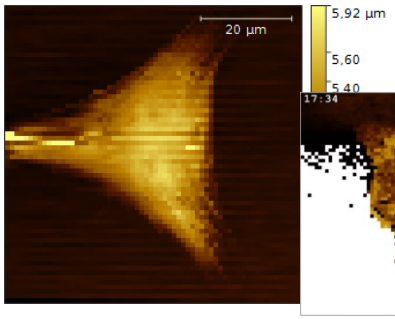


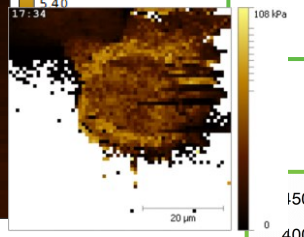


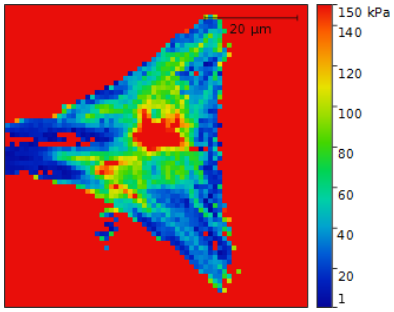


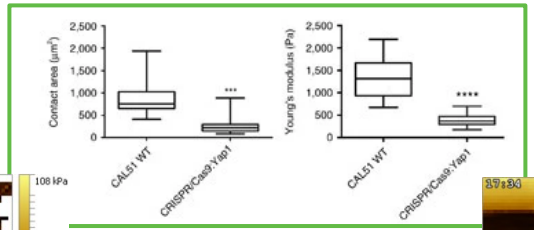
It is good to combine with other techniques

AFM mapping - correlation with fluorescence microscopy

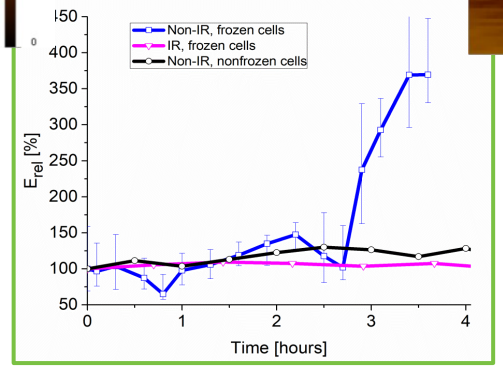




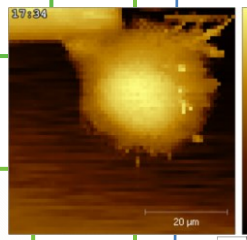


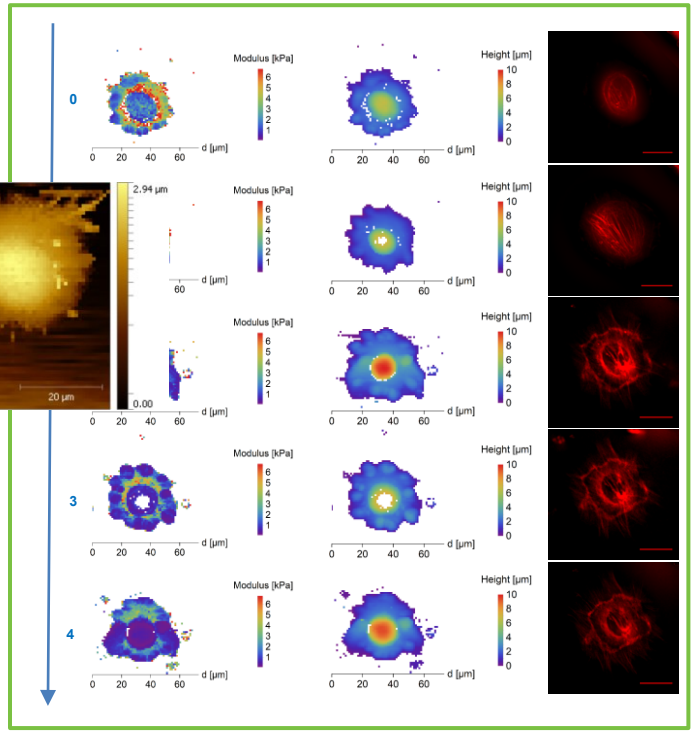


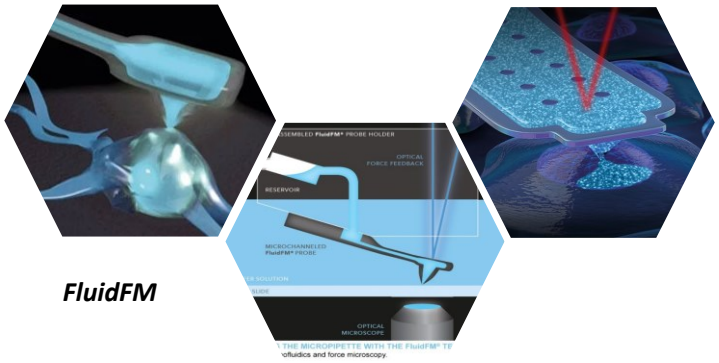
Evalutation - statistics



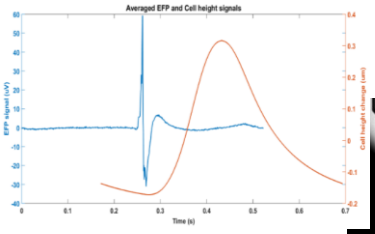
Time-lapsed biomechanics

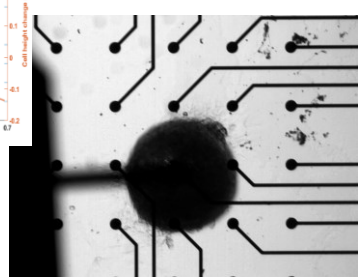






FluidFM

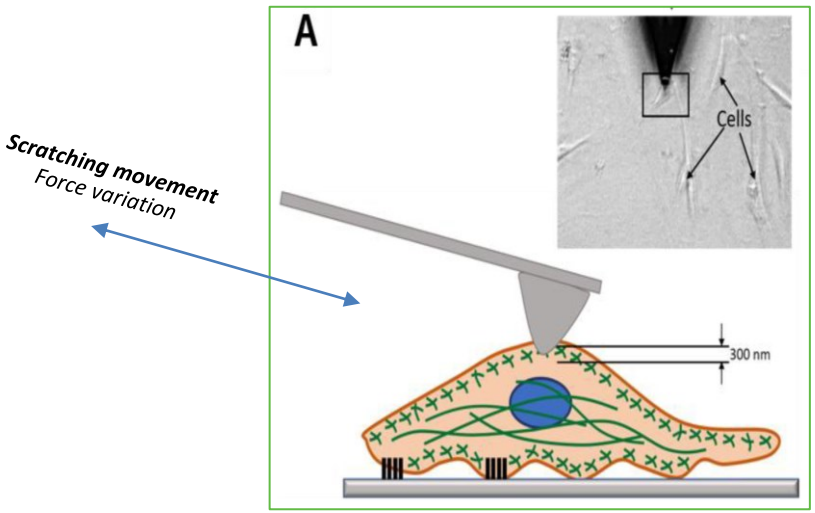


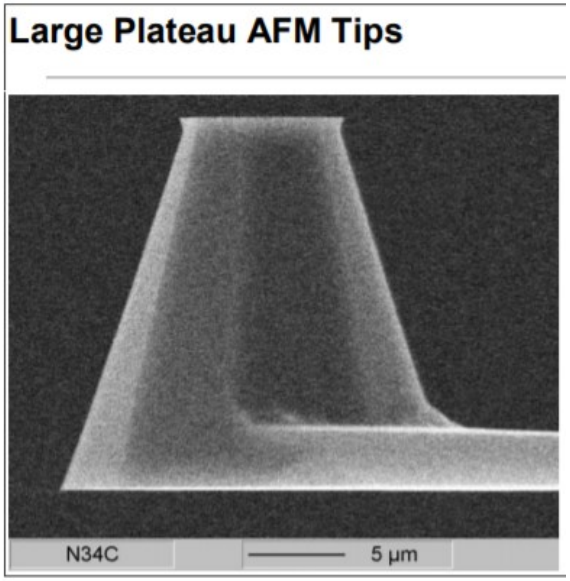


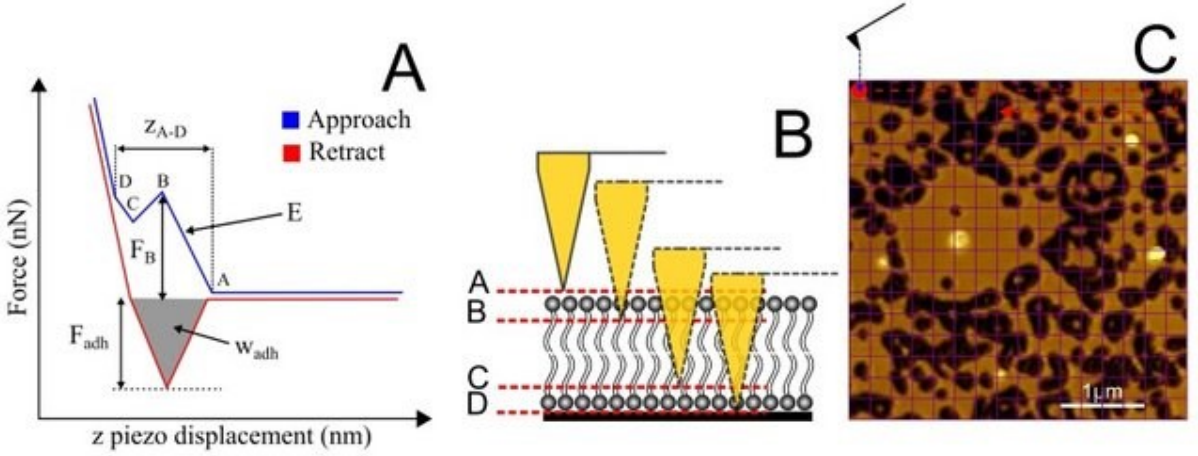
AFM + cellular electrophysiology

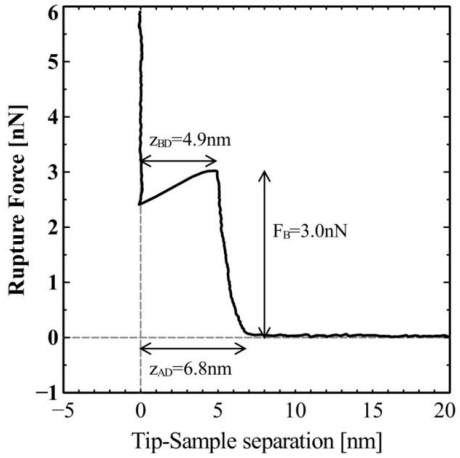
AFM as destructive technique

Cell scratching = cell adhesion

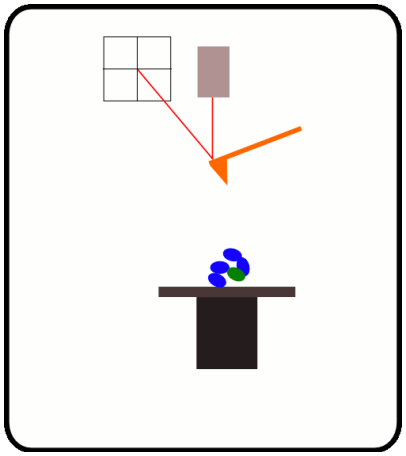


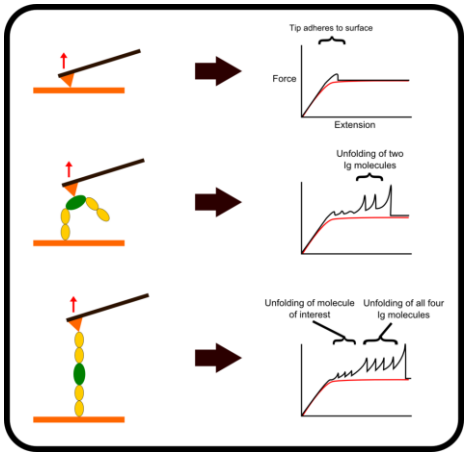


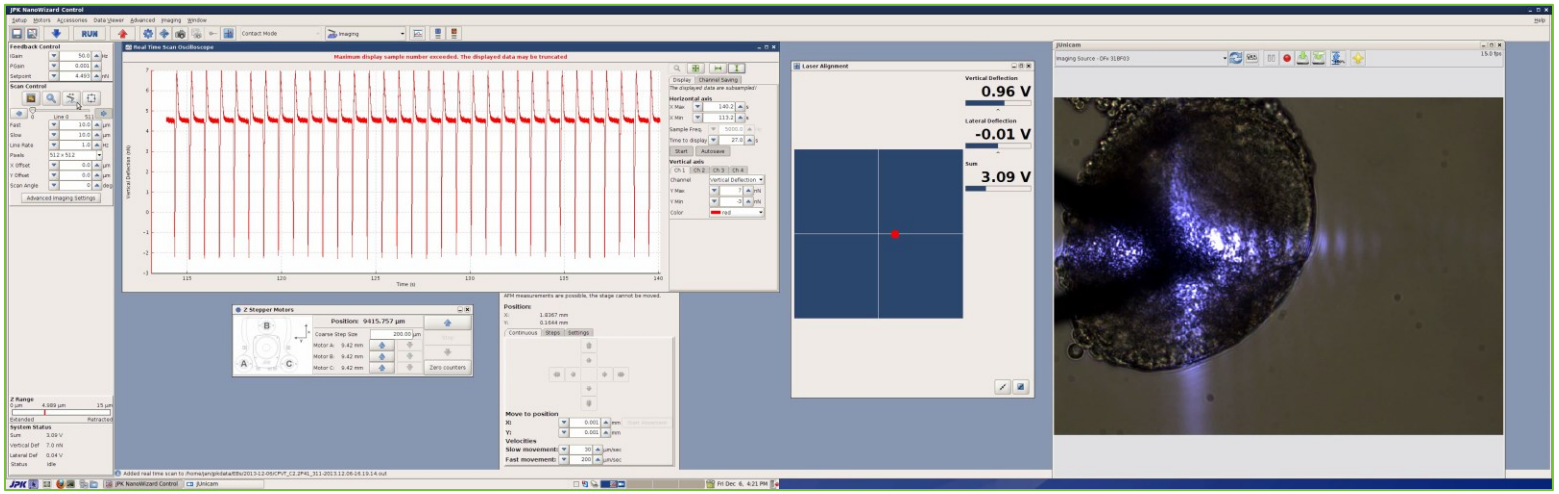




And keep moving!

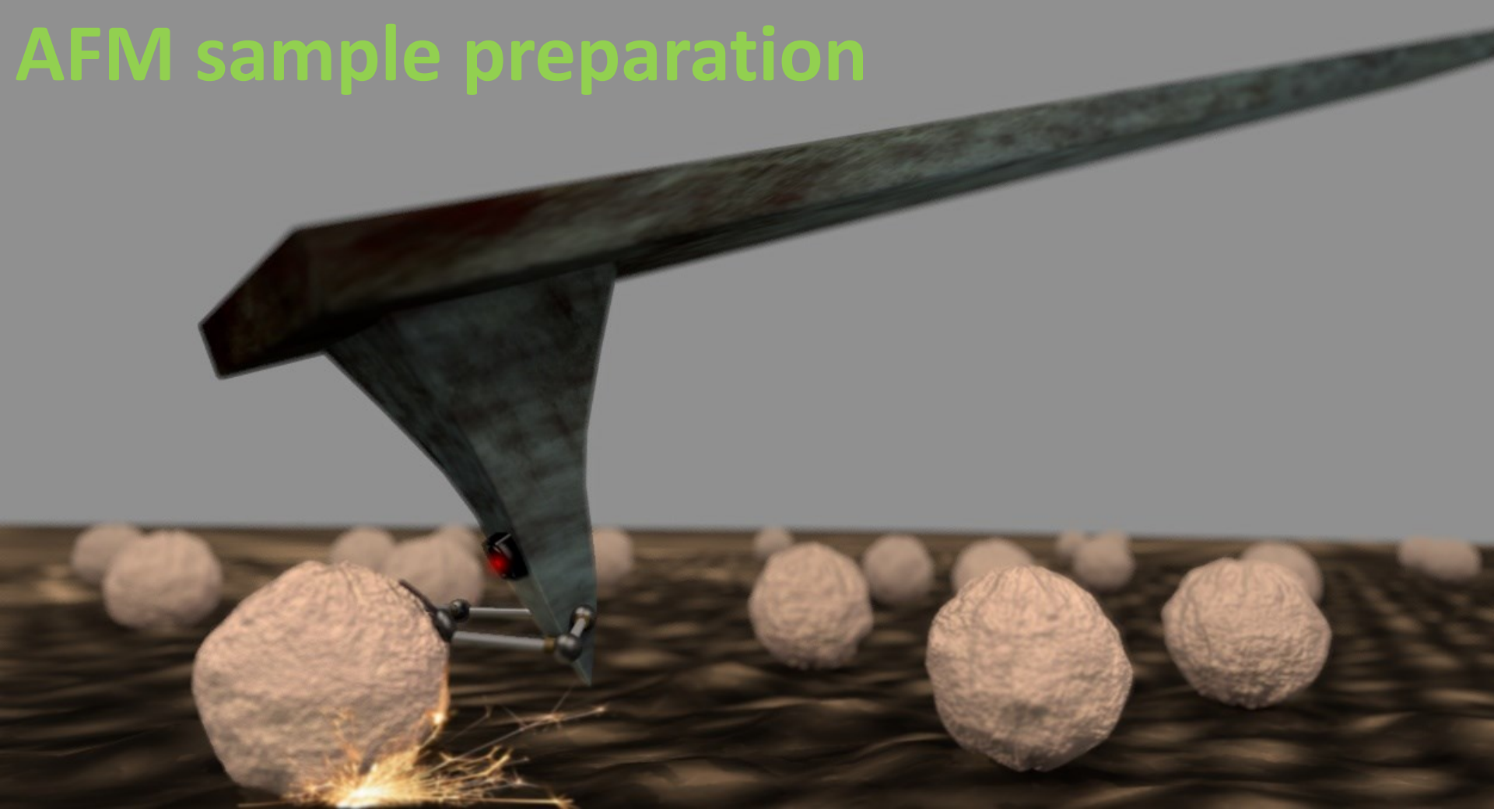


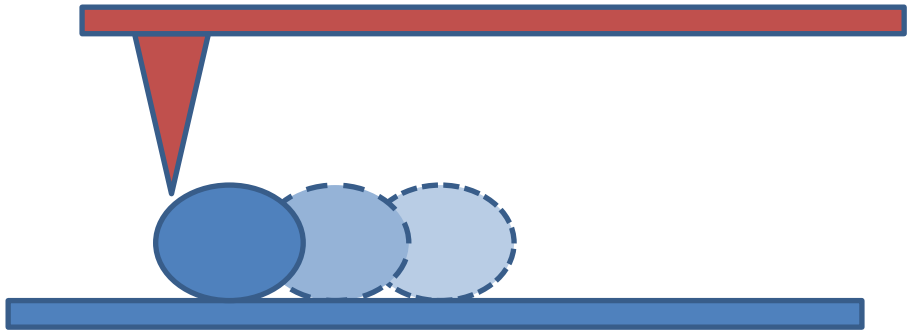




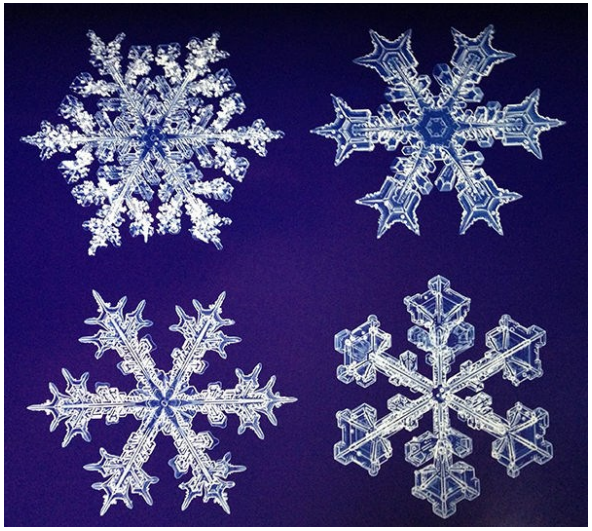
Sample preparation for AFM

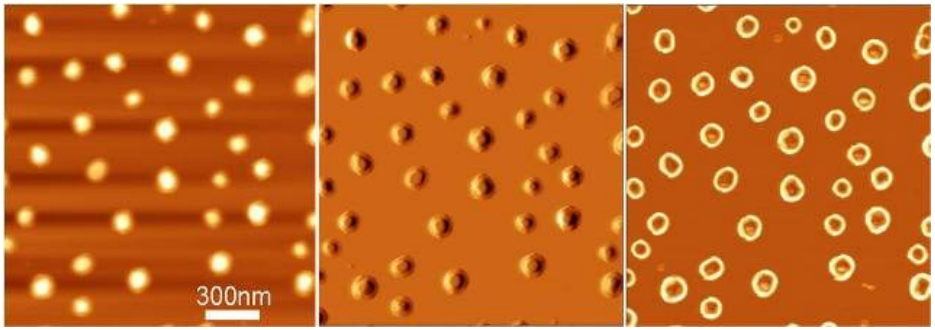
AFM sample preparation

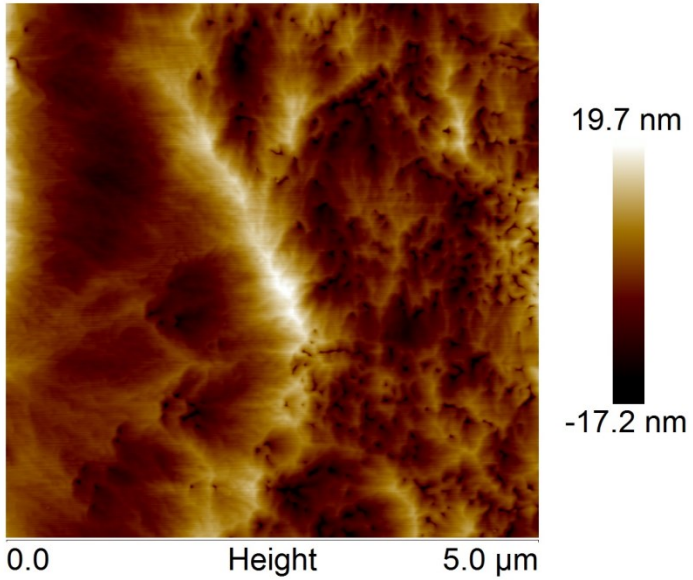




Concentration – surface density



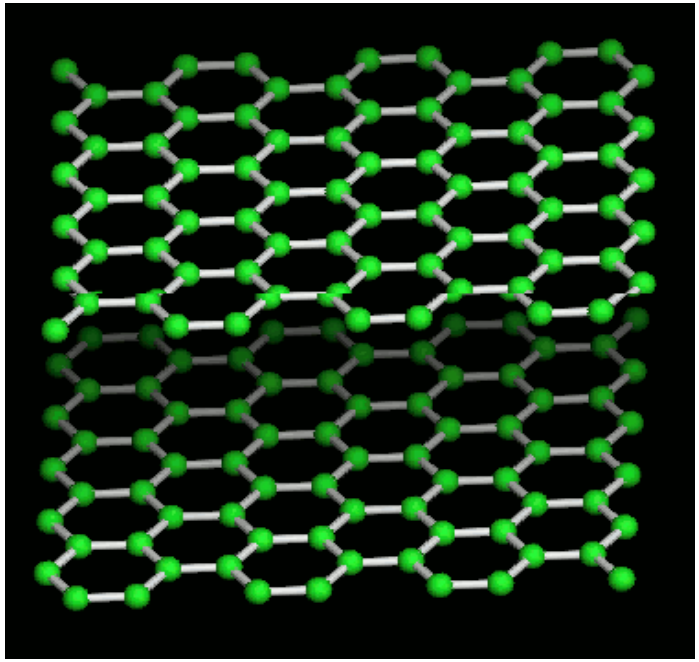




Substrates for preparation of AFM samples

Atomically flat surfaces

1. **HOPG** Highly Ordered Pyrolytic Graphite

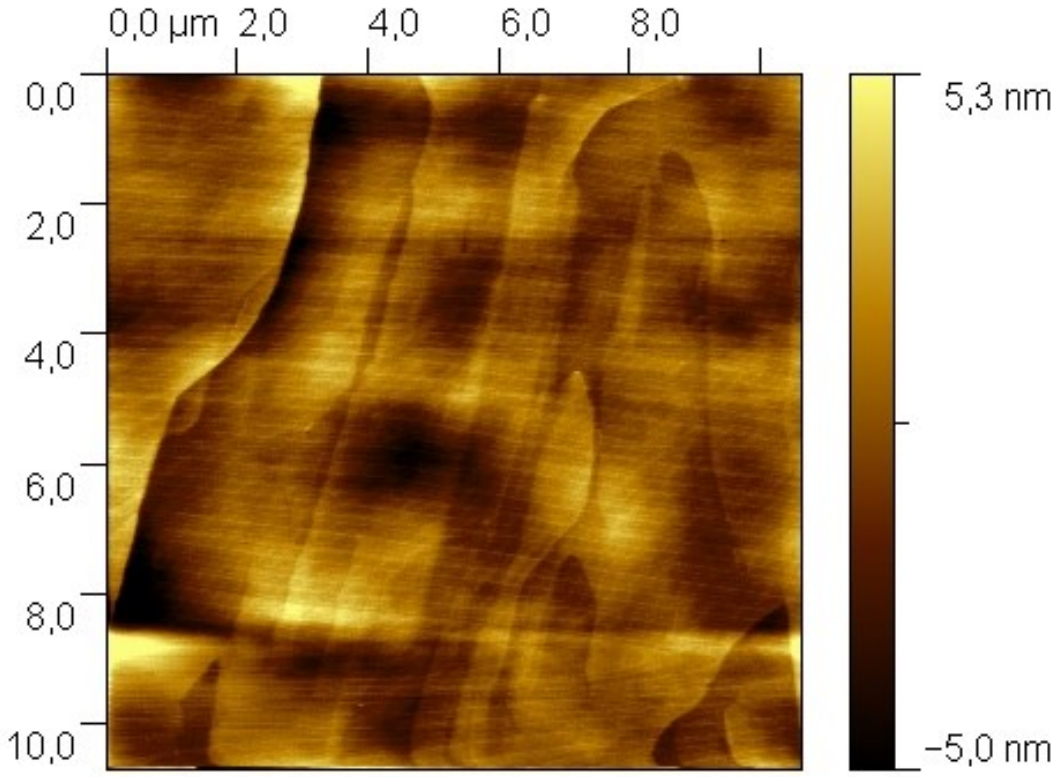


- Kish's graphite, waste in steel production
- Hexagonal planar structure
- C-C bond 142 pm, layer-layer distance 335 pm
- Conductive, highly hydrophobic
- Planar structure
- Synthetic form of graphite, high chemical purity
- Traditionally – substrate for SEM, STM i AFM (→ **conductivity**)
- **Immobilization** – spontaneous adsorption (→ **hydrophobicity**)

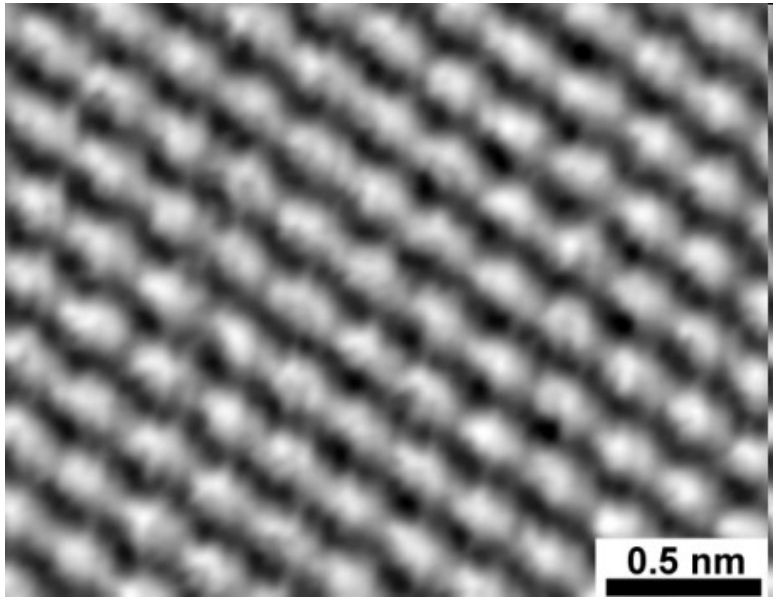
Atomically flat surfaces

1. **HOPG** Highly Ordered Pyrolytic Graphite

Large areas
visible layers

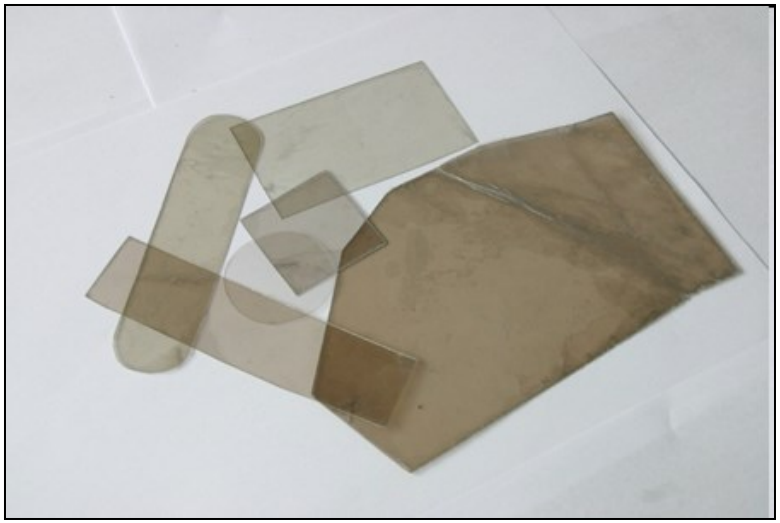


Small areas
atomically flat

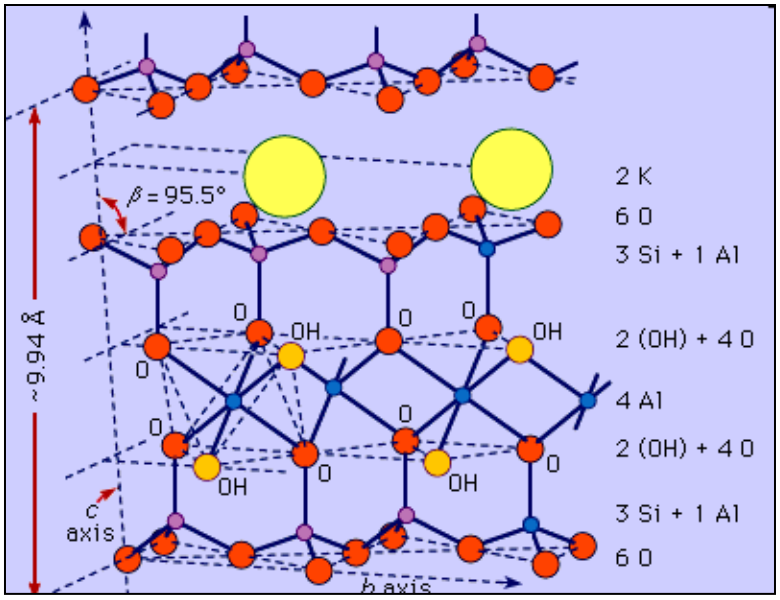


Atomically flat surfaces

2. Mica (muscovite)

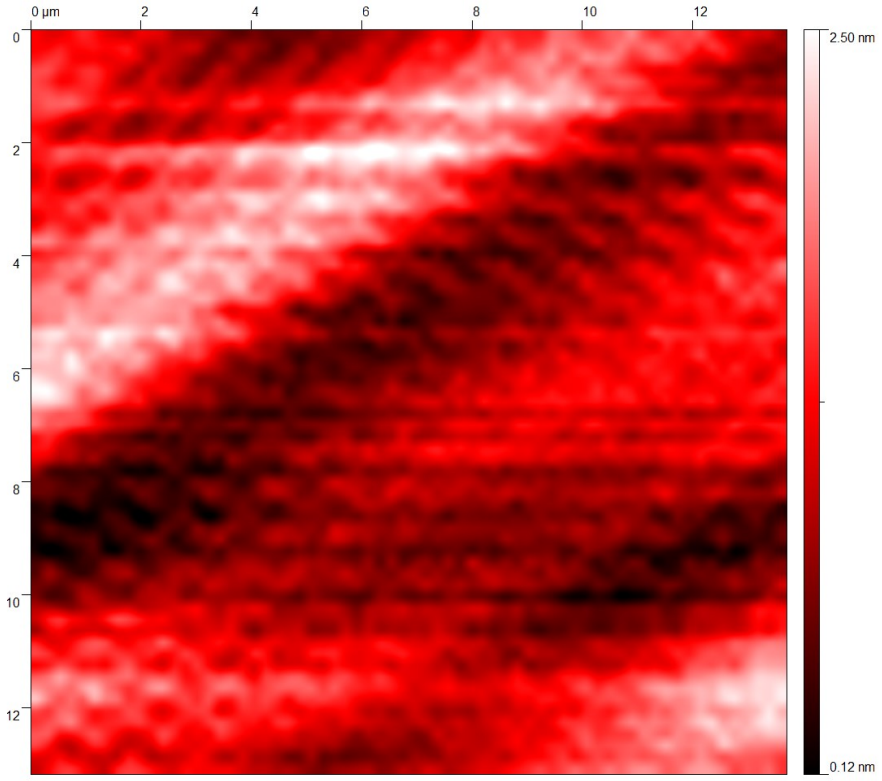


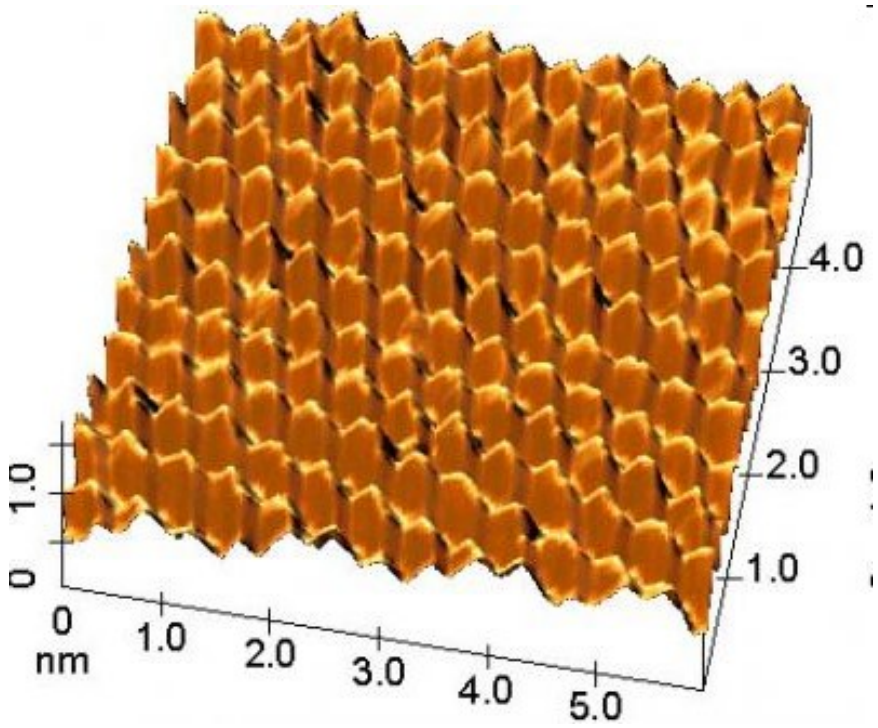
- „Cat's silver", muscovite acc. to city of Moscow
- Chem. structure: $K_2O \cdot Al_2O_3 \cdot SiO_2$
- Hydrophilic surface
- Easy to be modified by chemical synthesis
- Immobilization by **chemical bonding** as well as **ionic interaction**
- pKa ~ 3, physiological pH → negative surface charge
- Mica = silicate, hydrated SiO_2 (~ Si-OH) from the chemical point of view



Atomically flat surfaces

2. Mica (muscovite)





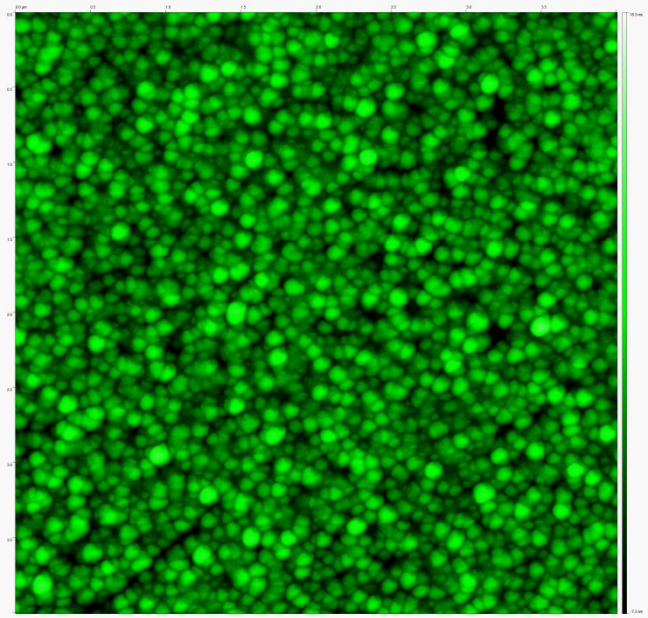
Extremely flat on small and larger areas

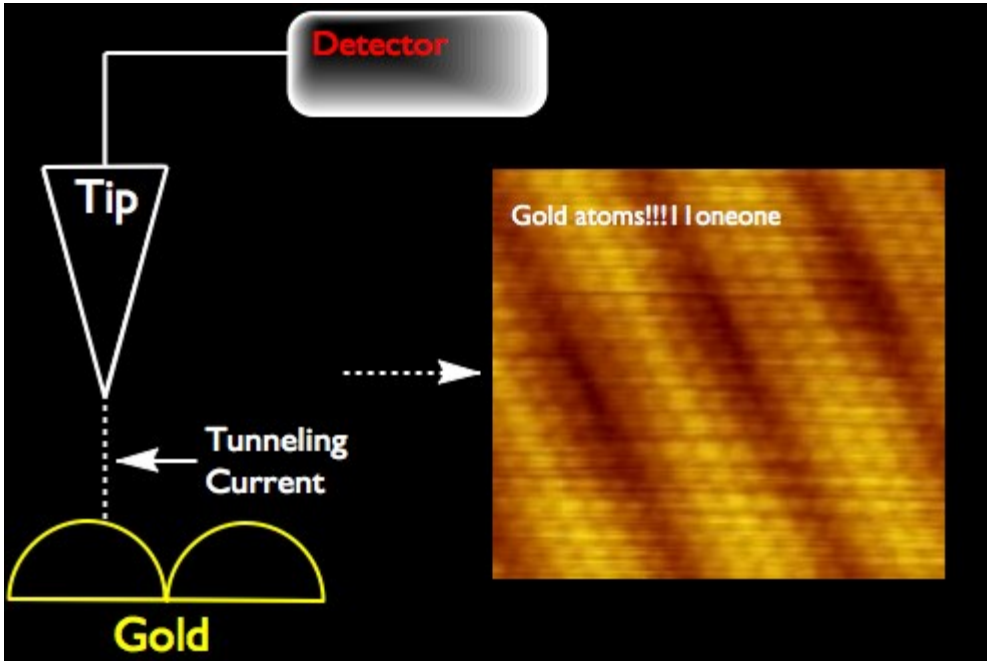
Other surfaces

3. Gold

- Inert metal
- Traditionally in (bio)electrochemistry (i.e. biosensors) - electrodes
- Conductive - STM + AFM
- Hydrophobic: spontaneous non-selective adsorption of molecules (proteins, DNA, …)
- Specific chemical binding of thiols (-SH) – organic molecules + cysteine
- Prepared usually by evaporation
- Adhesion layer for operation in liquids (Al/Cr/Ti)

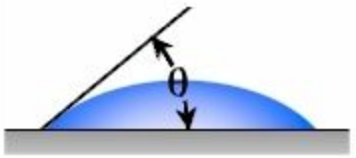
Sputtered gold layer
image by tapping mode AFM



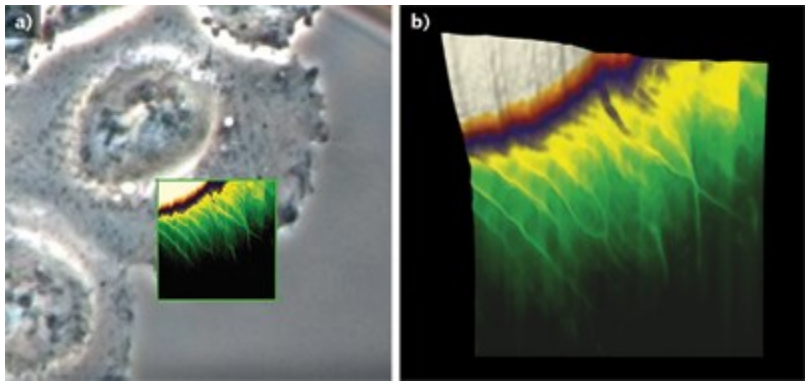


Other surfaces

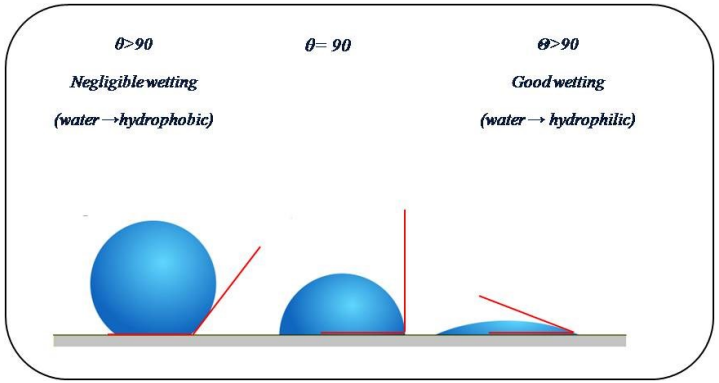
4. Glass



- Amorphous noncrystalline structure
- Lab glass composition: 75% SiO_2 plus Na_2O, CaO, borate and minor additives
- Si-OH → from chemical point of view
- Less hydrophilic comparing to mica
- Roughness much higher comparing to mica (production by pressing)
- **Not** suitable for **individual molecules** imaging with AFM
- Typically used together with optical microscopy – cell compartments, whole **cells**

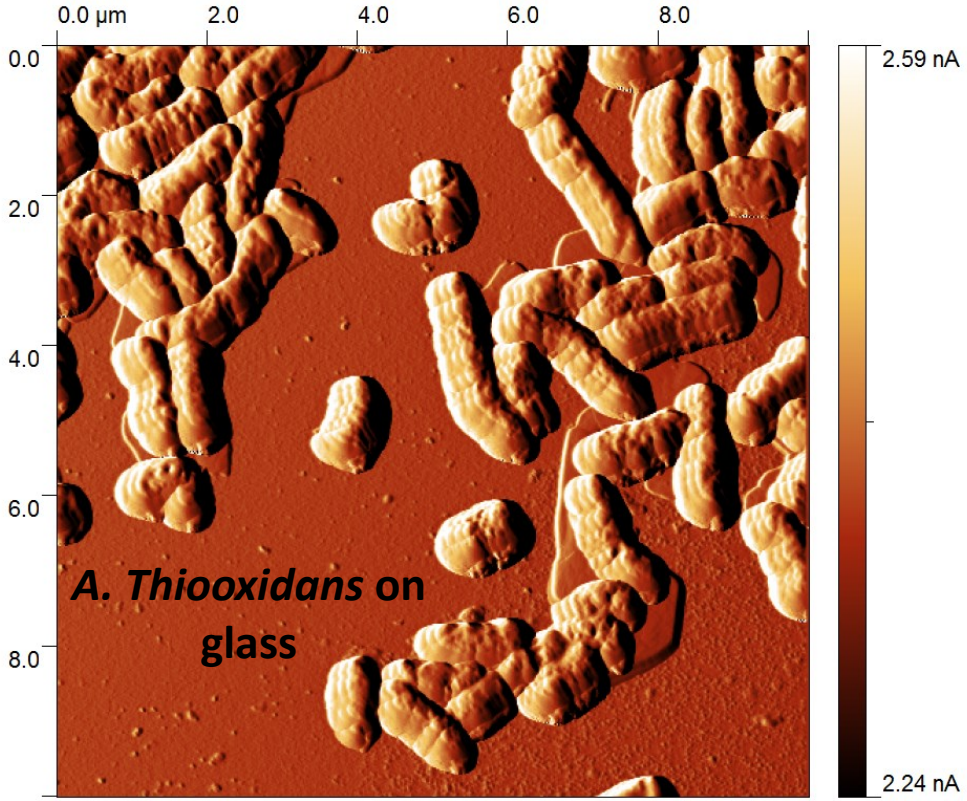


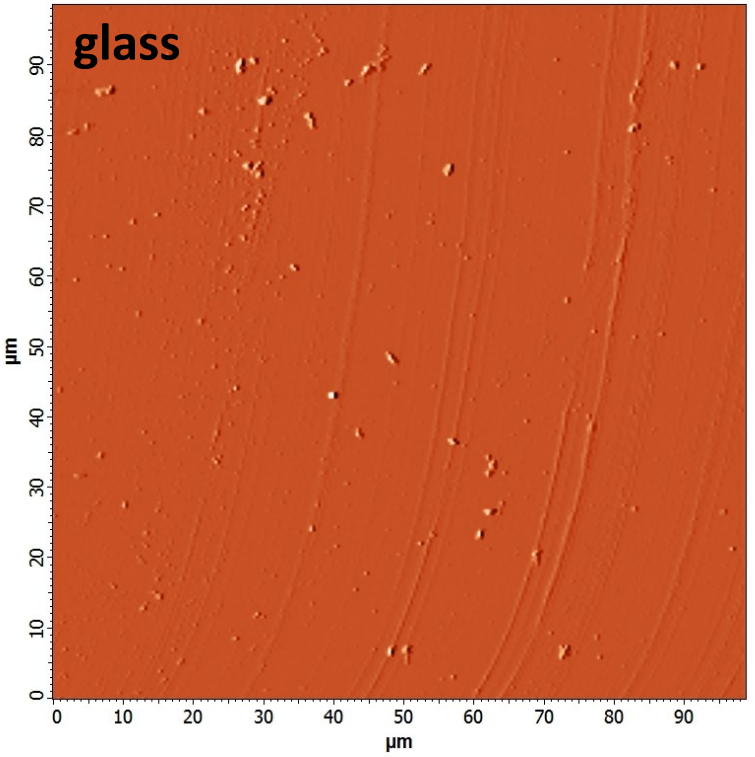
AFM – optical image overlap

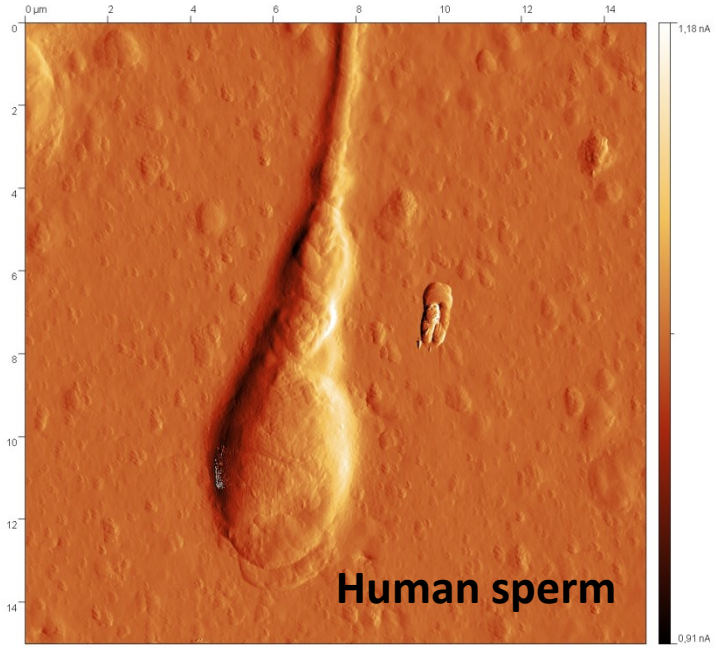


Whole cells on glass

under AFM

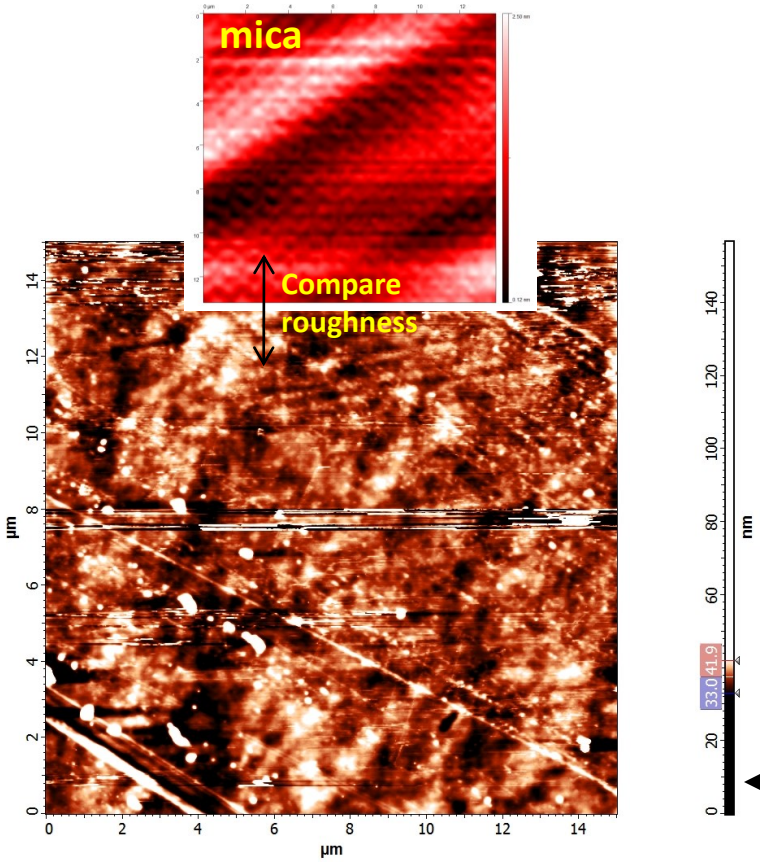


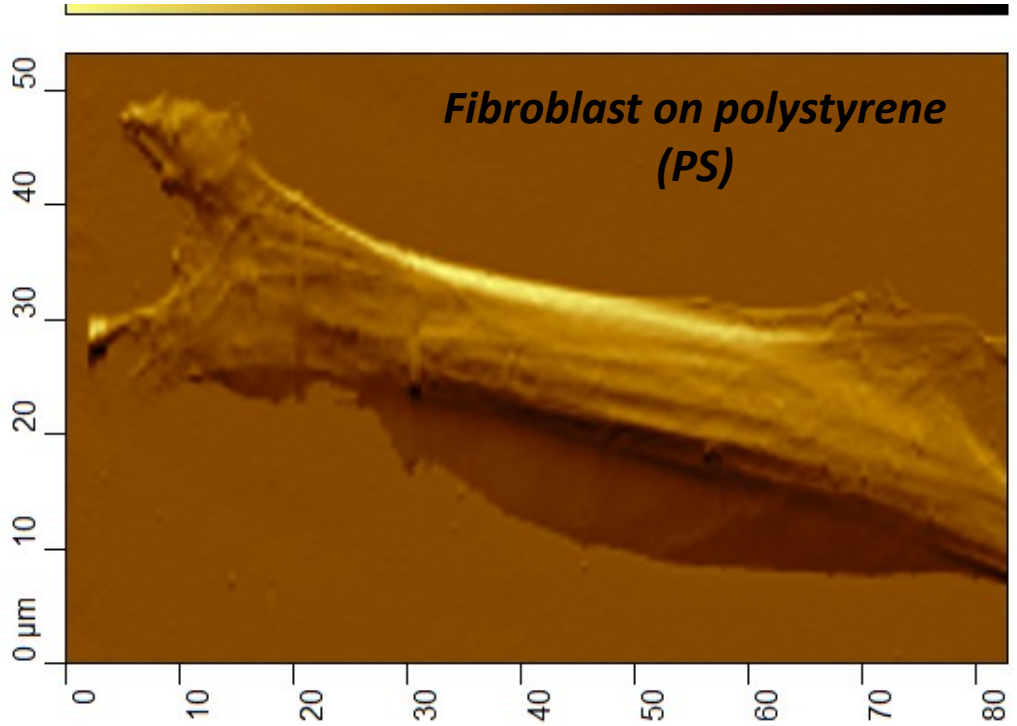




Other surfaces

5. Plastic materials





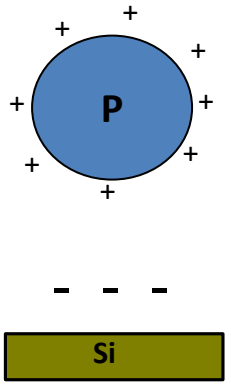
Non-modified polystyrene (PS)

- Most of lab supplies made of plastic (**PP, PE, PS**)
- **No functional groups** to be used in covalent binding
- **PS – hydrophobic** → spontaneous non-specific adsorption of proteins → usually as underlying support (i.e. for cell attachment)

Immobilization procedures

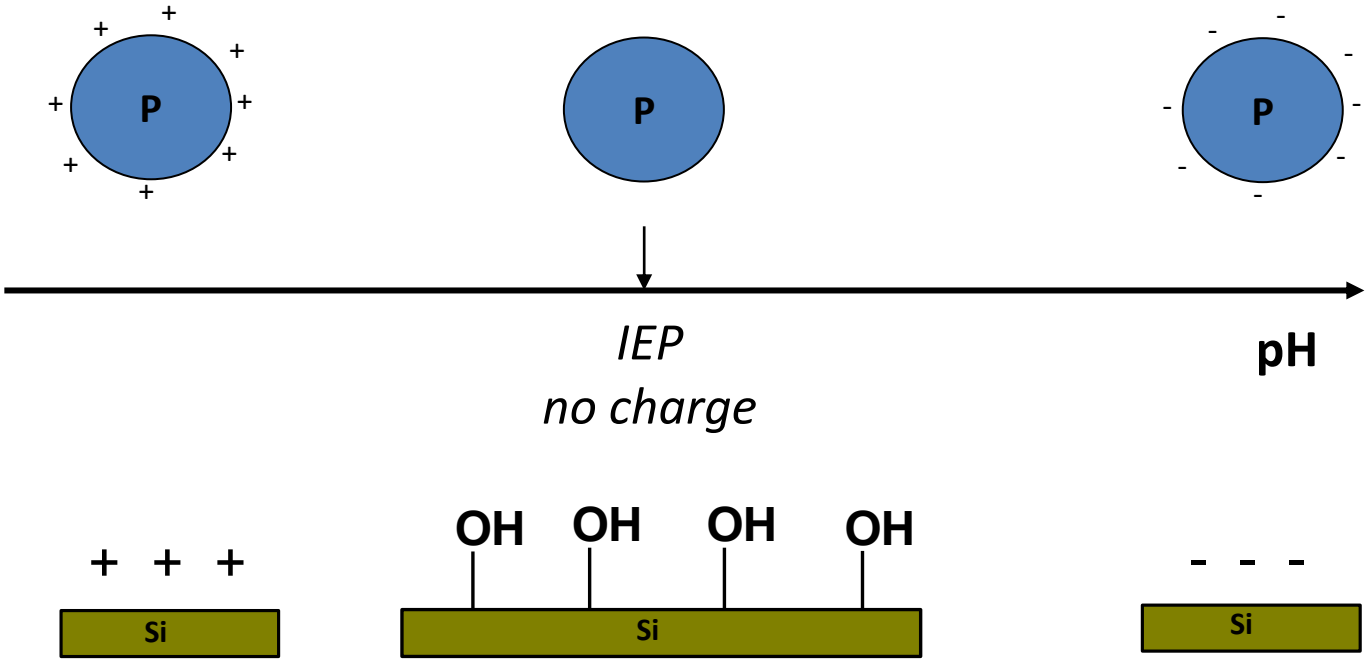
1. Proteins

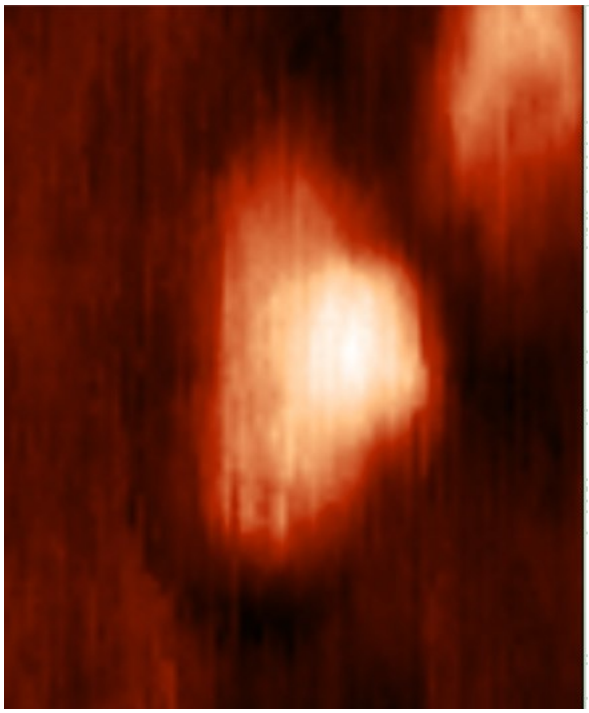
Surface: **mica or HOPG** (extremely flat)

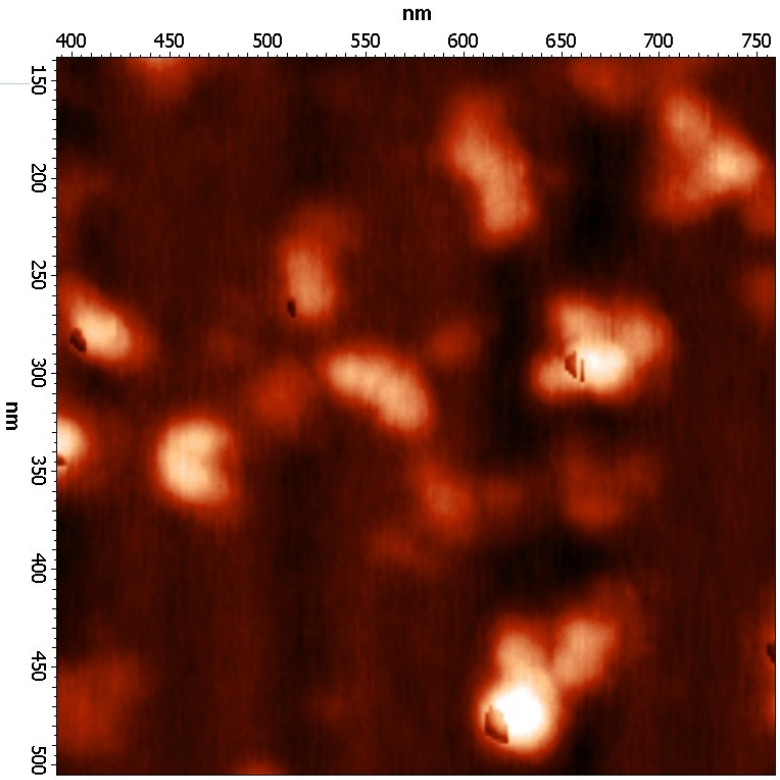


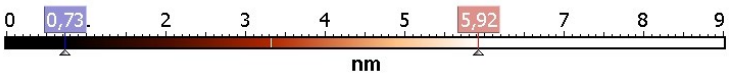
Protein: **charge** is given by IEP + pH

Immobilization on mica: **pKa (mica) < pH < IEP**






nm
400
450
500
550
600
650
700
750
150
200
250
300
350
400
450
500
nm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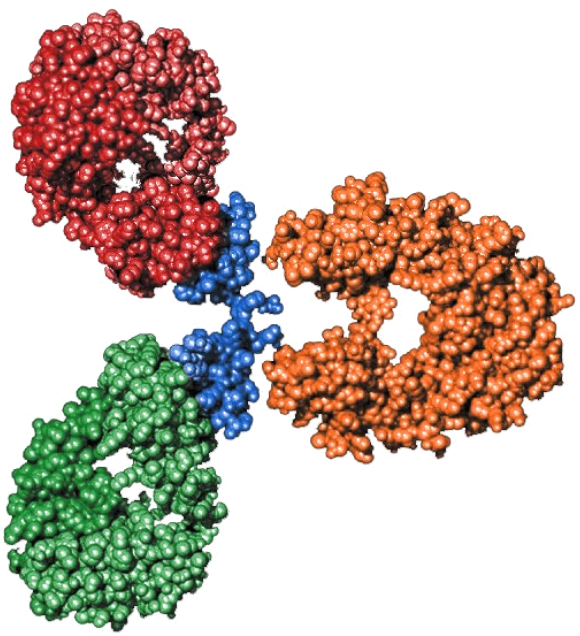
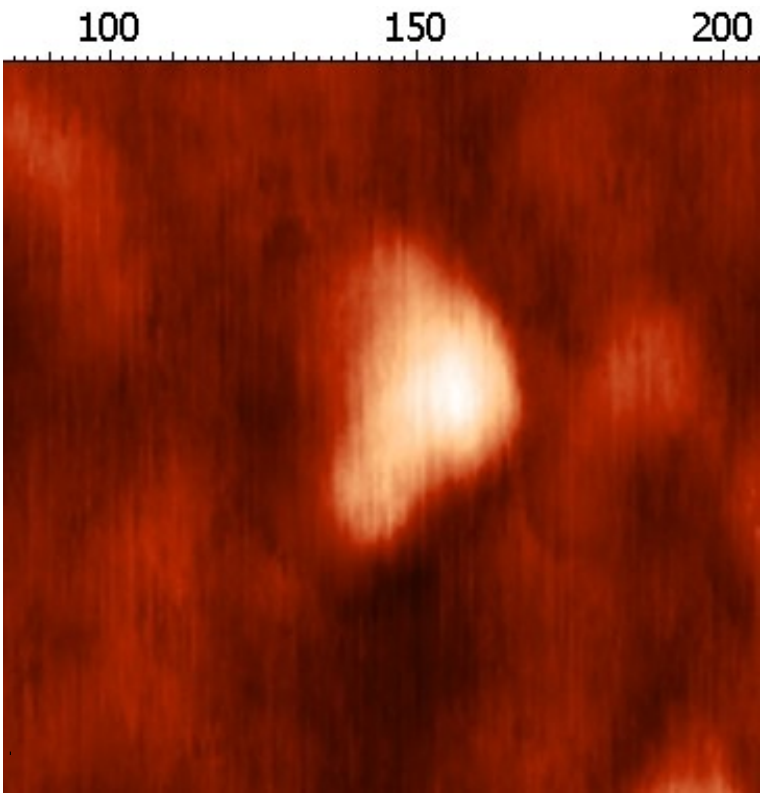
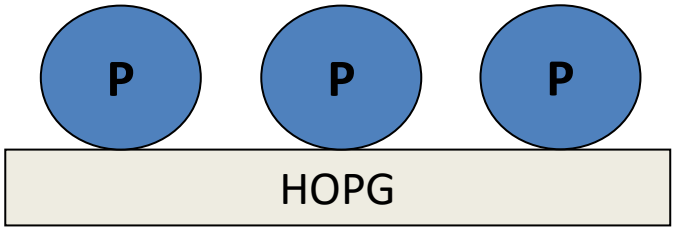
0,73
2
3
4
5
5,92
7
8
9
nm

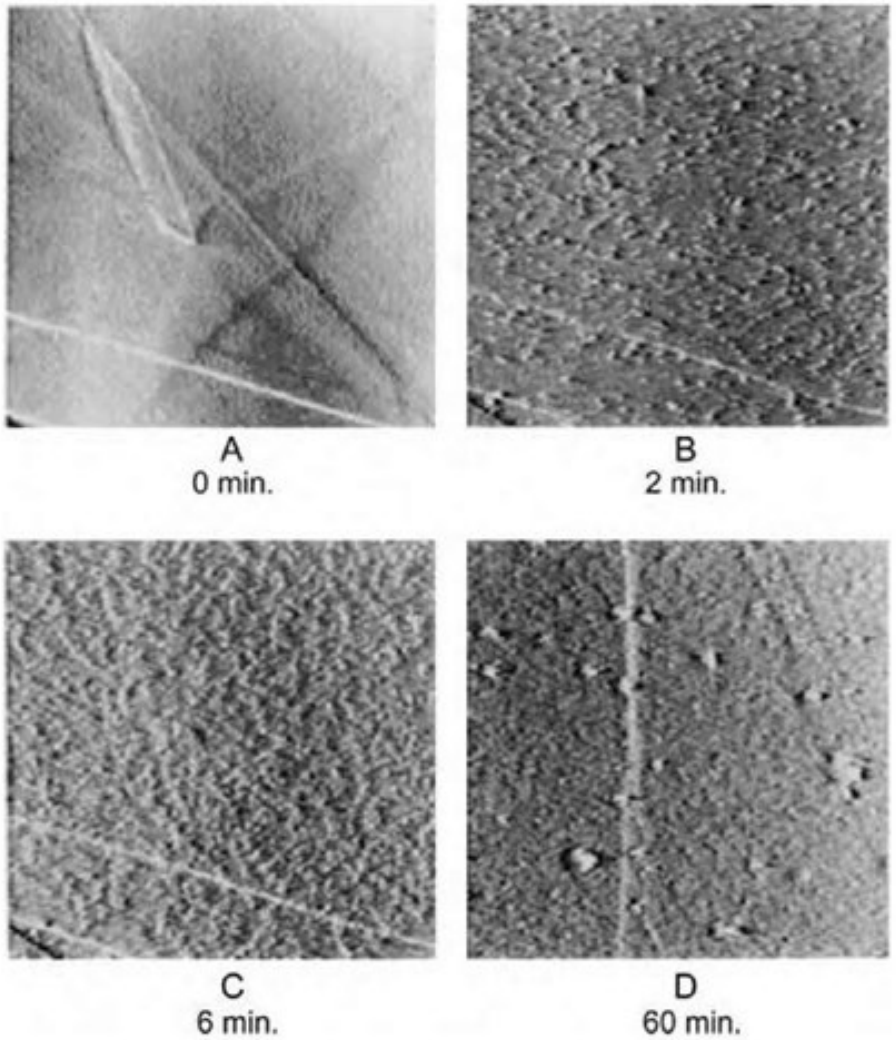



100
150
200

Protein immobilization on HOPG

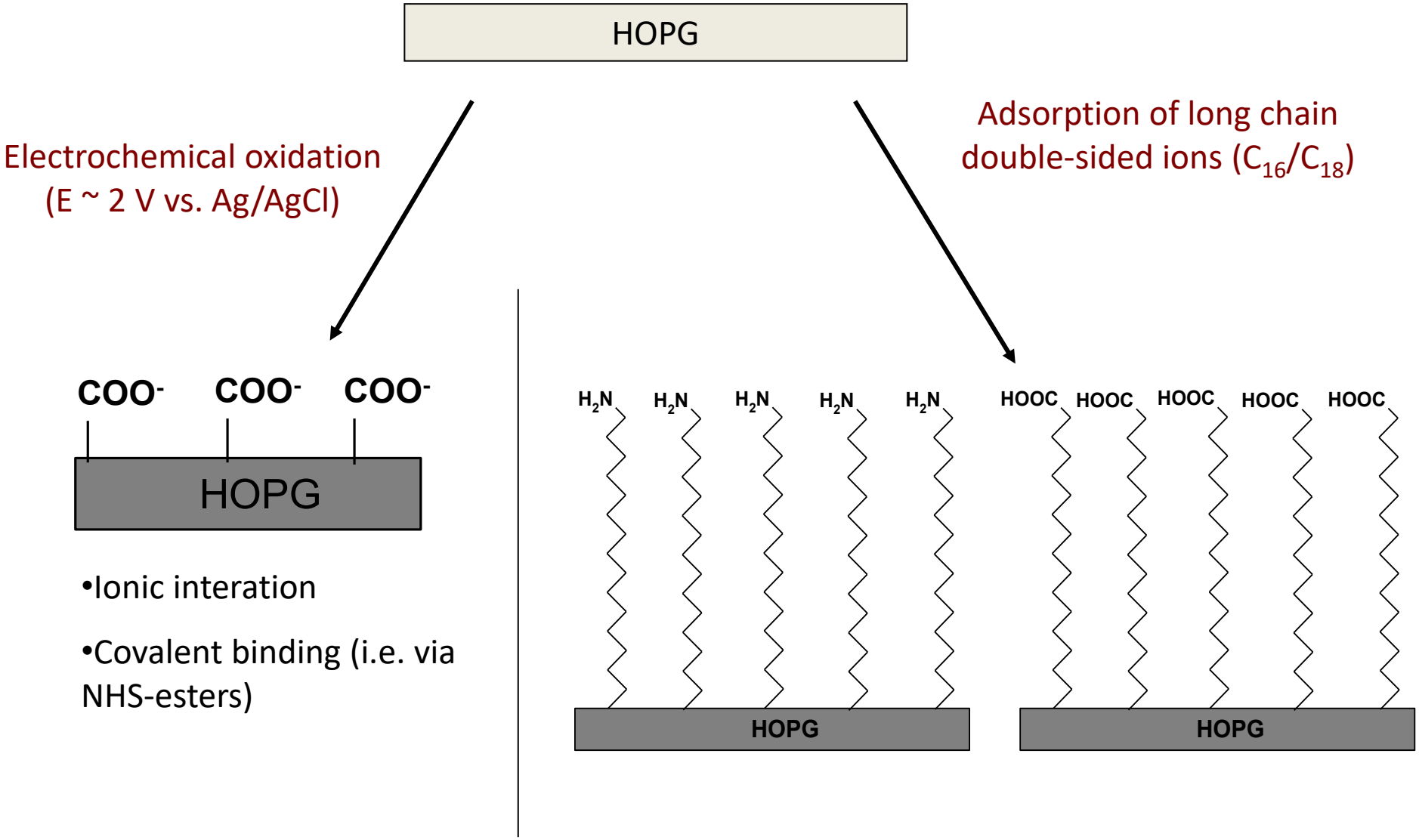
A. **Spontaneous** (non-specific) **adsorption** of protein → hydrophobic surface (best results at zero charge *pH = IEP)*





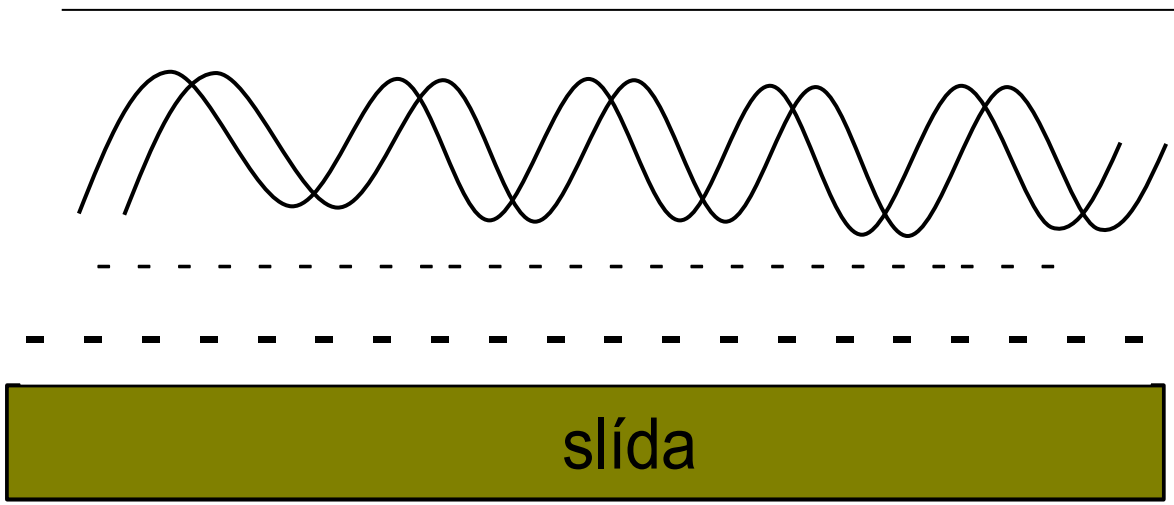
Lysozyme molecules on HOPG

B. **Ionic** (specific) **binding** of molecules → creation of charge/chem. groups on HOPG surface



2. DNA

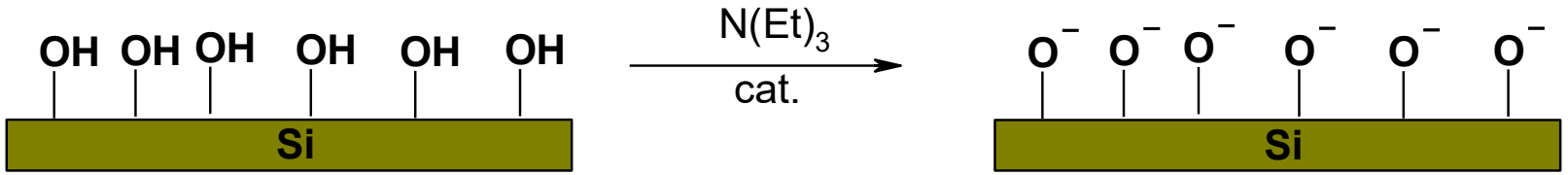
Surface: **mica or HOPG** (extremely flat)



Immobilization problem:

DNA (sugar-phosphate bone) as well as **mica – negative charge** under physiological pH

→ surface introduction of **positive charge**



Silanization

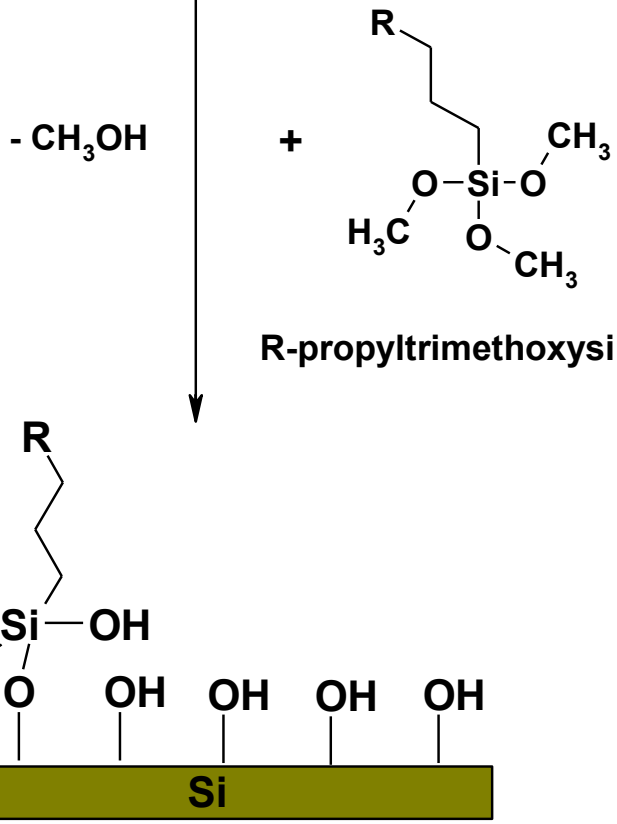
= chemical (covalent) modification of mica surface

- Aim: **introduction** of **functional group**

- Applicable also for: glass, quartz, silicon, titanium, …

- Strong basis catalysis

- Procedure can monitored by water contact angle measurement

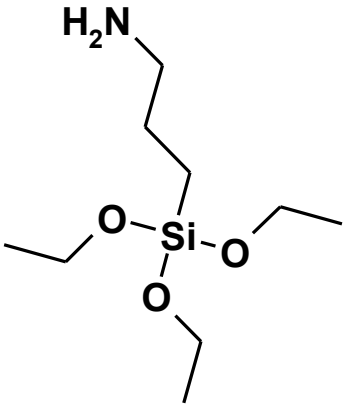


A. DNA on mica

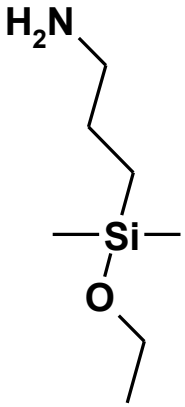
silanization

hydrophobization

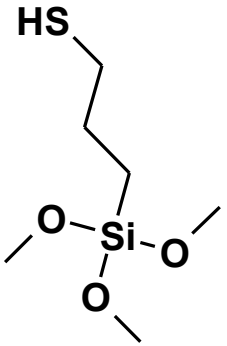
Examples of **alkoxysiloxanes**



(3-Aminopropyl)trimethoxysilane
APTES



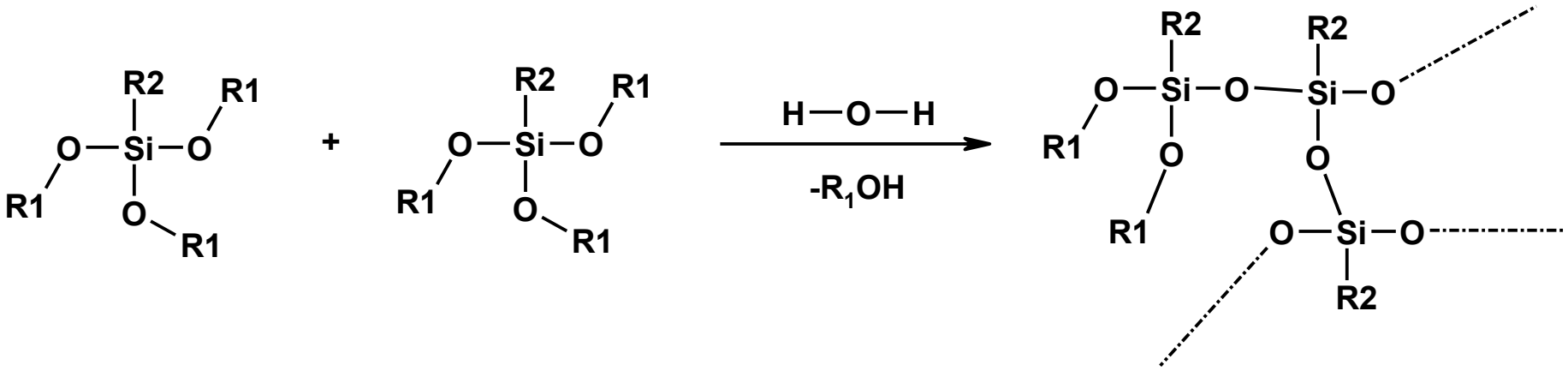
3-(Ethoxydimethylsilyl)propylamine
APDMES



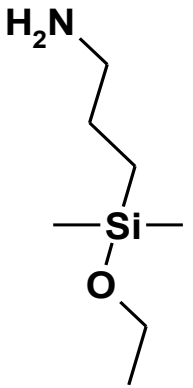
(3-Mercaptopropyl)trimethoxysilane
MPTS

Self-polymerization

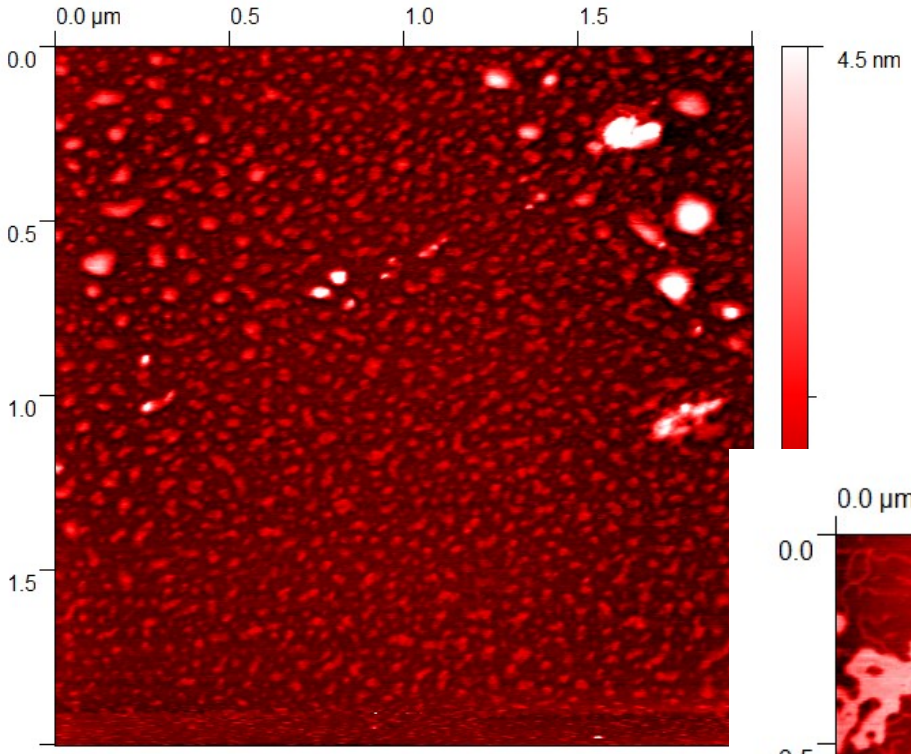
practical complication



- Especially with **APTES** during liquid silanization
- Even vapors of water can cause this effect
- Fixation for **optical** microscopy – **expected** factor
- In contrary – in fixation for **AFM** – very **disturbing**
- Solution:
  - silanization in **vapours** under **vacuum** (i.e. in desiccators)
  - **mono**alkoxysilanes – can not polymerize

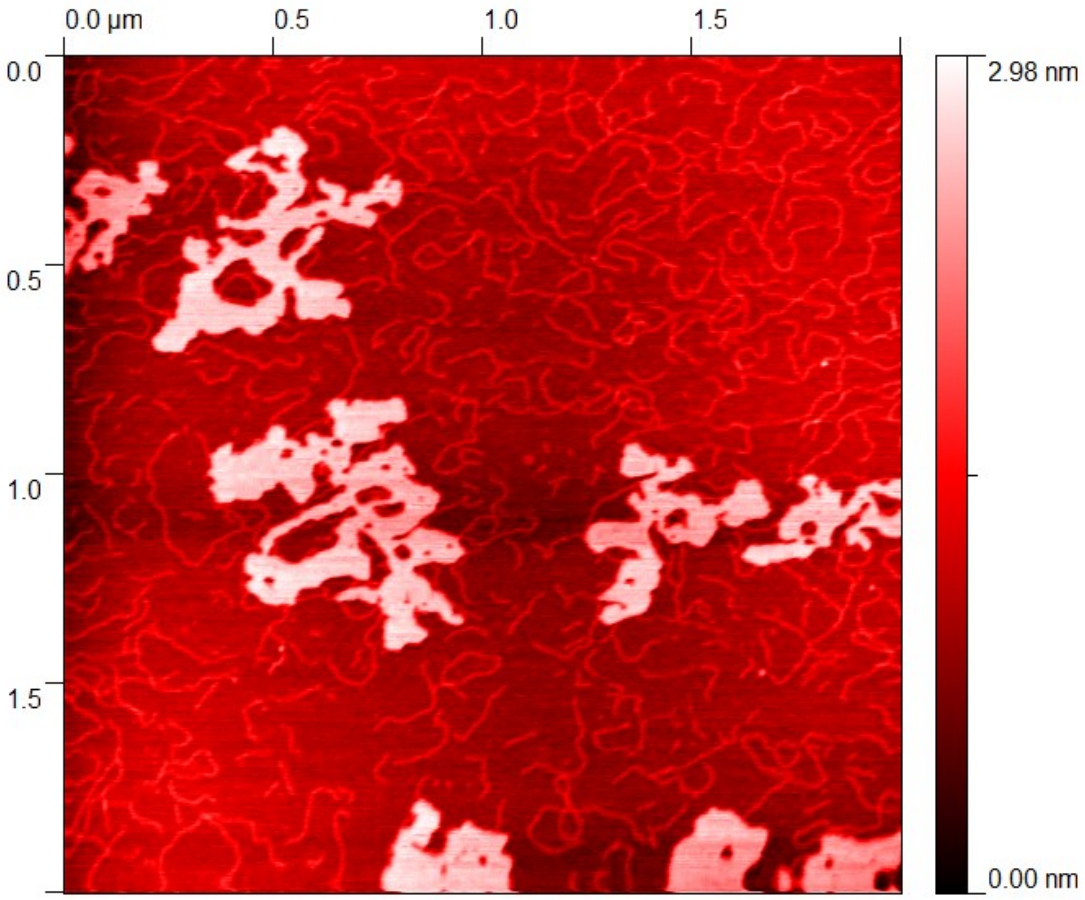


3-(Ethoxydimethylsilyl)propylamine
APDMES



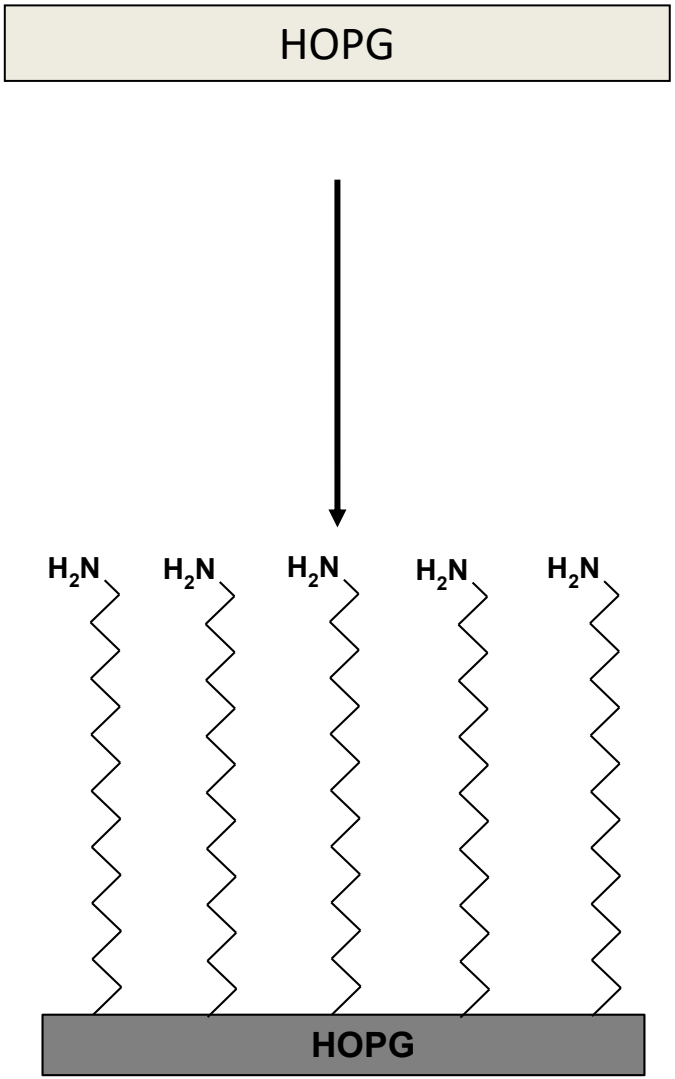
Self-polymerization

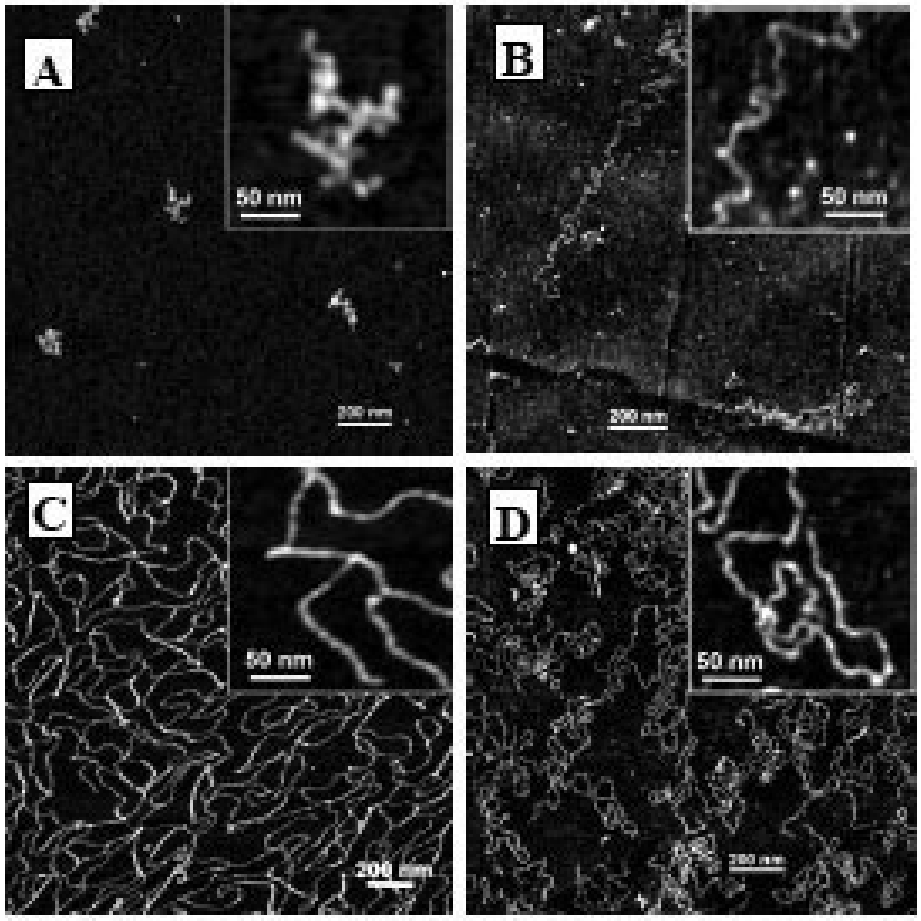
examples



B. DNA on HOPG

Adsorption of long chain double-sided ions (C_{16}/C_{18})

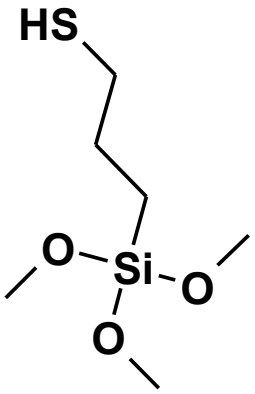




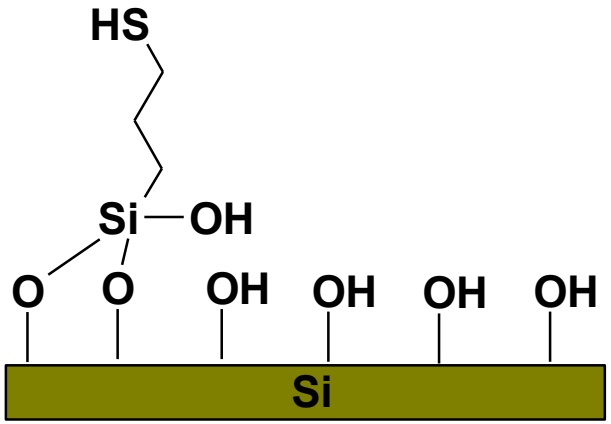
3. Nanoparticles

Substrates for immobilization: **mica** / **HOPG** (smooth surfaces), also gold, glass in selected cases.

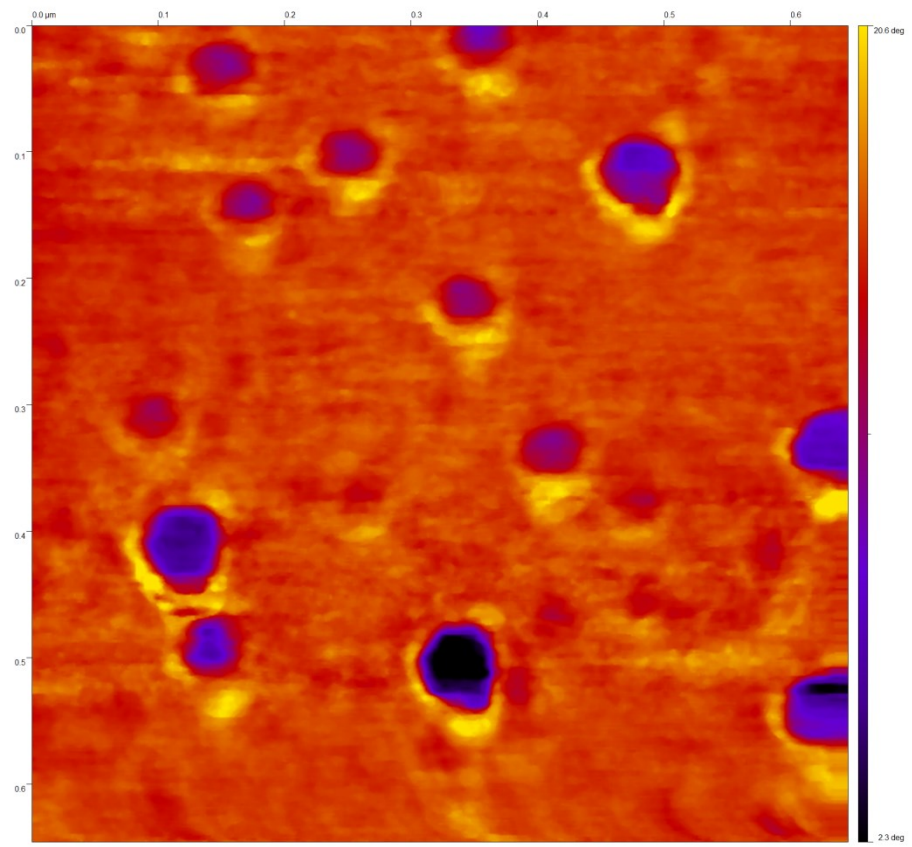
Example: gold nanoparticles (AuNP) mercapto-silanized mica (SH-mica):

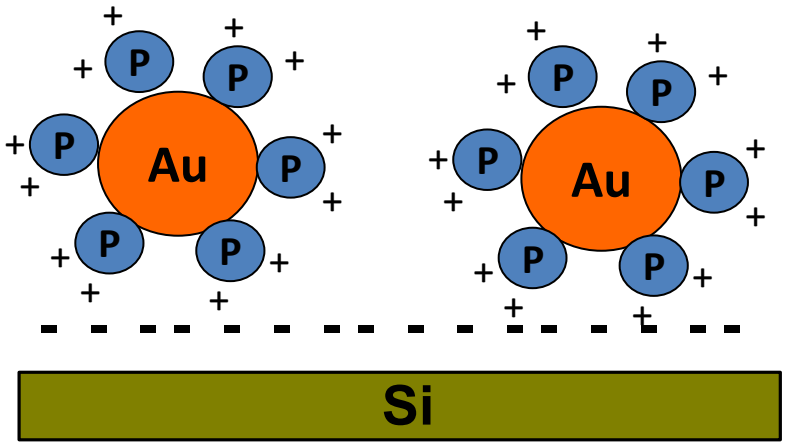


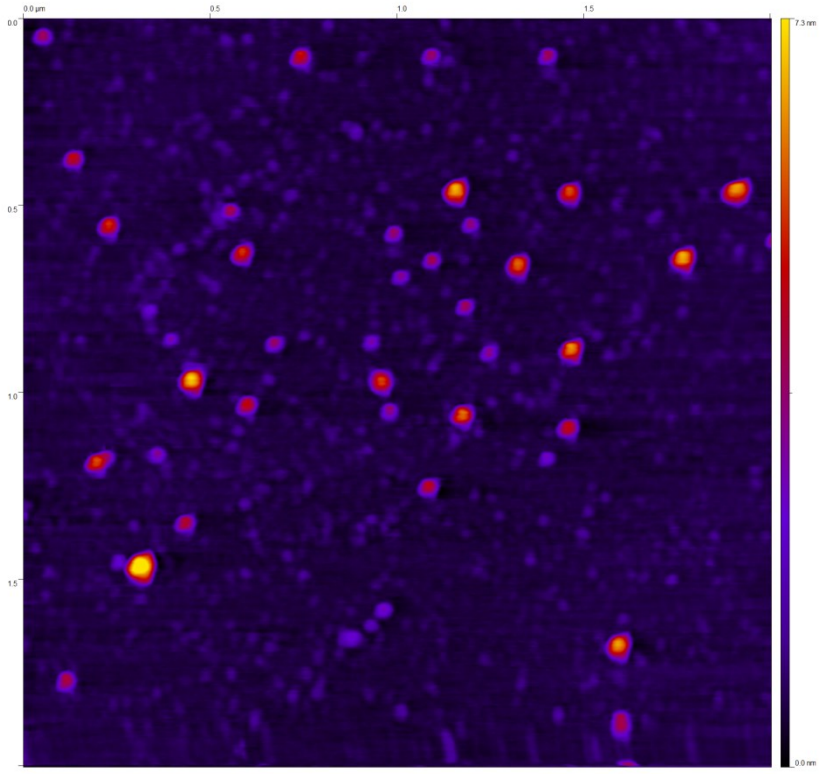
(3-Mercaptopropyl)trimethoxysilane
MPTS



SH-mica

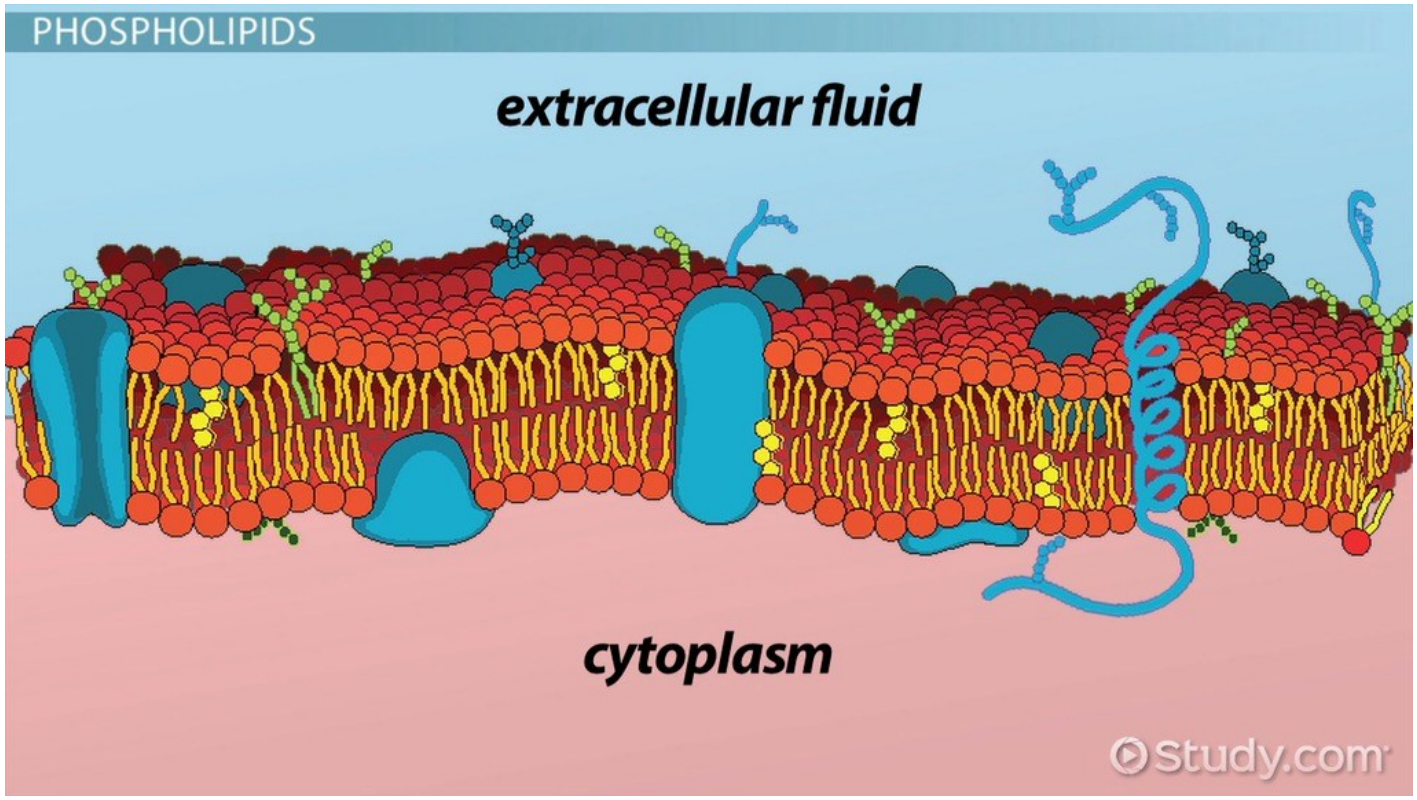


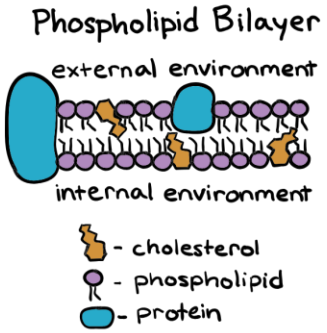




Gold nanoparticles (**AuNP**)
conjugated with **protein** molecules:
protein = immobilization bridge

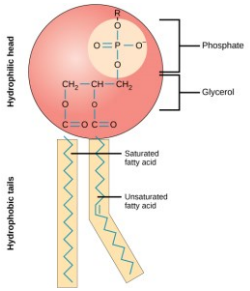
4. Supported lipid bilayers (SLBs)

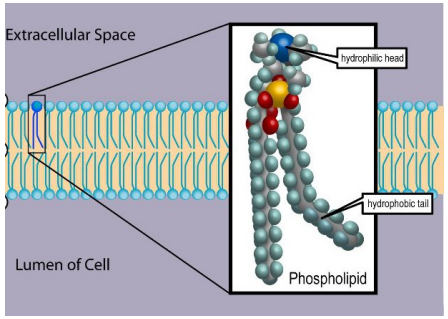




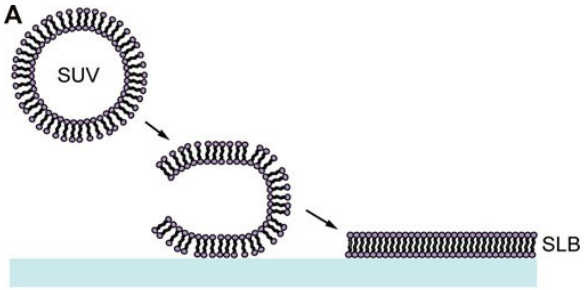
4. Supported lipid bilayers (SLBs)

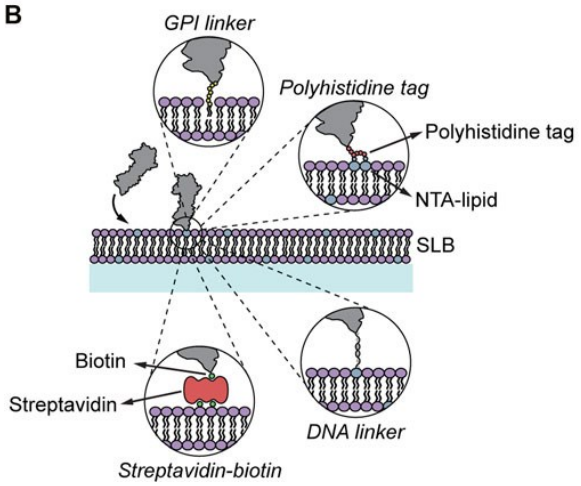
cell-membrane-mimicking platforms on solid surfaces and integrated with a wide range of surface-sensitive measurement techniques.



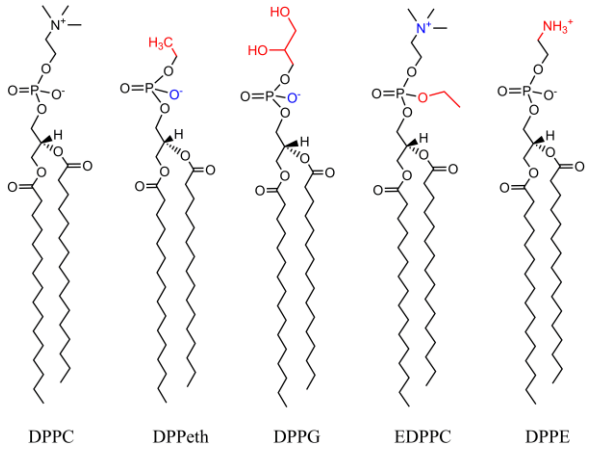


SLB formation:



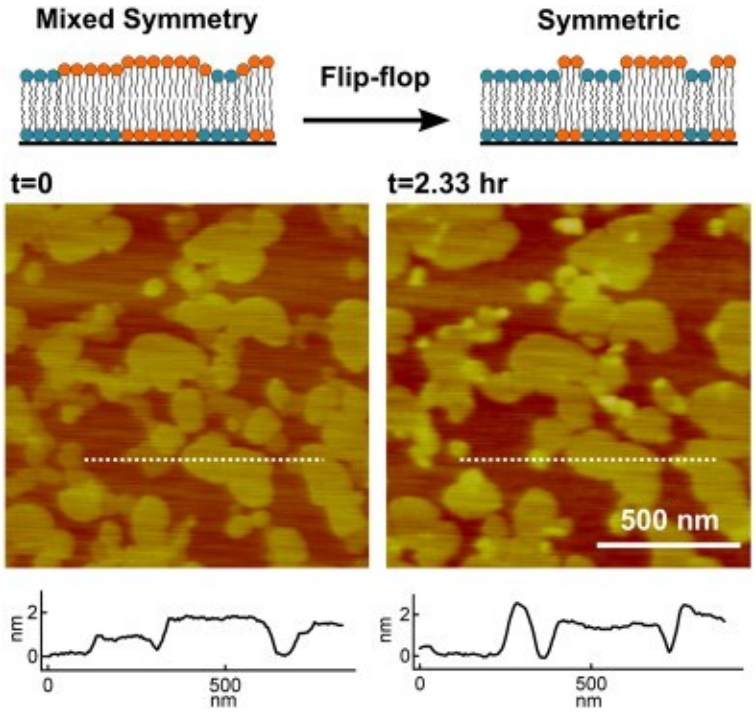


Head group → immobilization



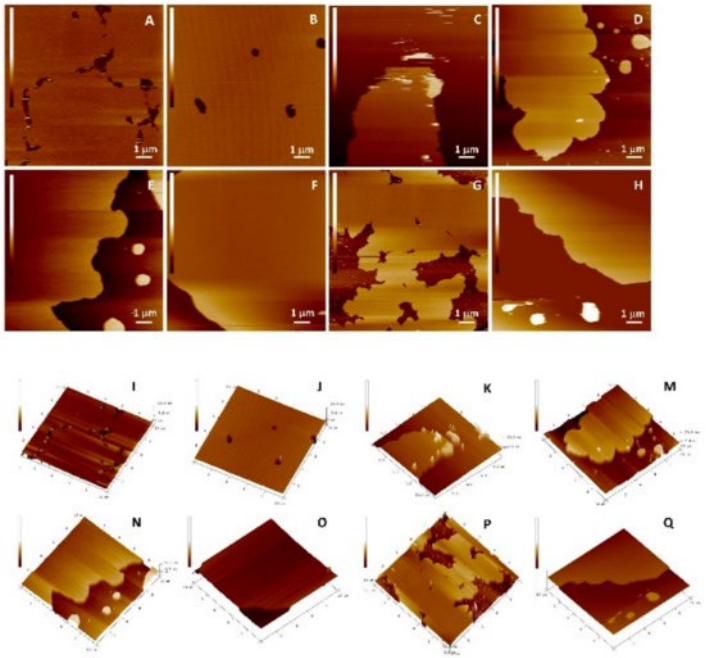
4. Supported lipid bilayers (SLBs)

AFM experiments



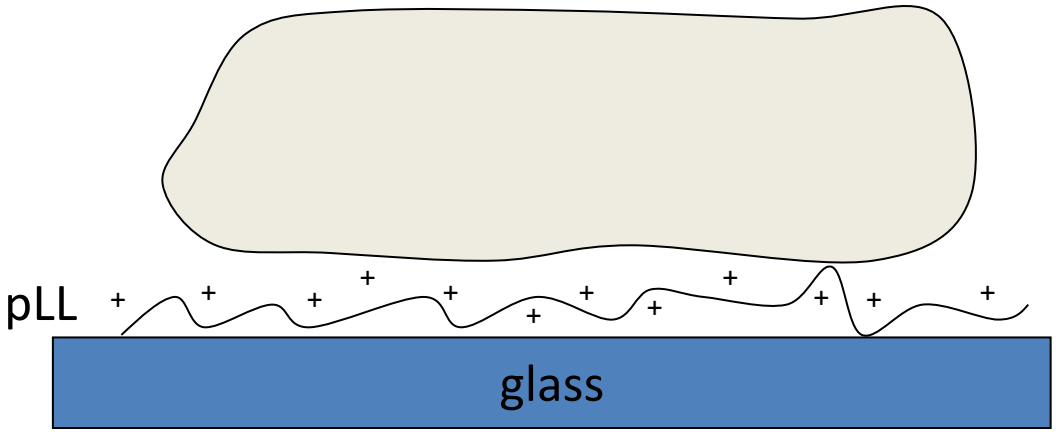
Bilayer remodelling

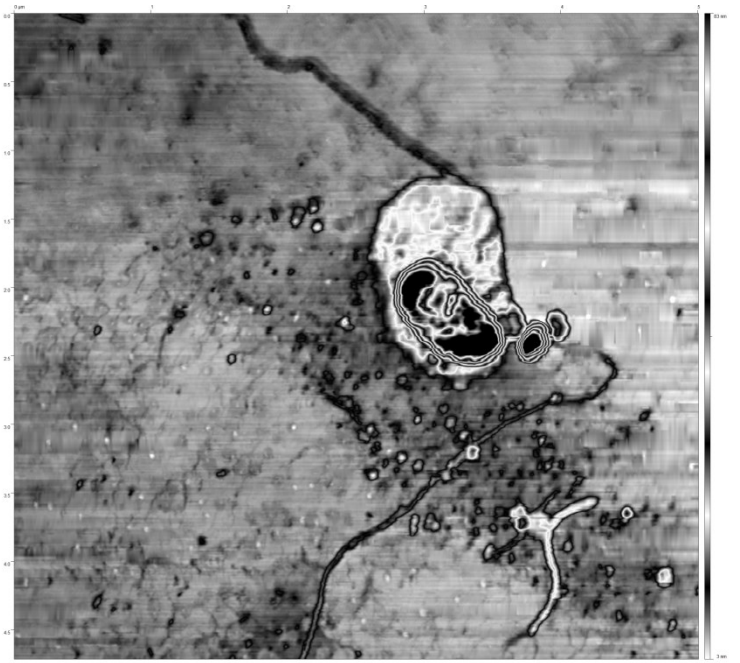
Bilayer peroxidation



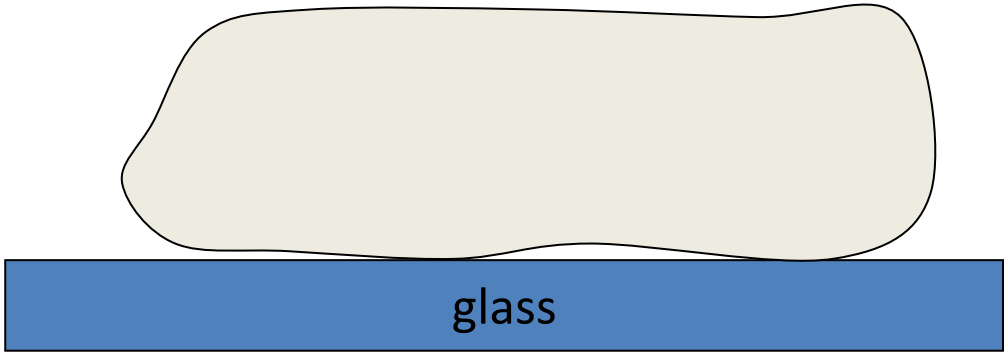
3. Bacteria, spores

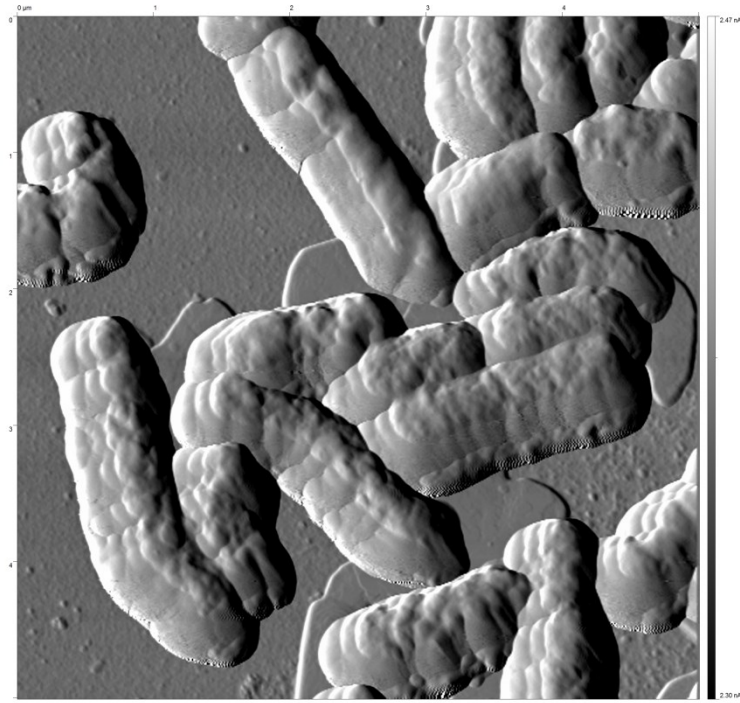
Protein adhesive layer, i.e. pLL
(poly-L-lysine → introducing positive charge)





Standard coating on glass

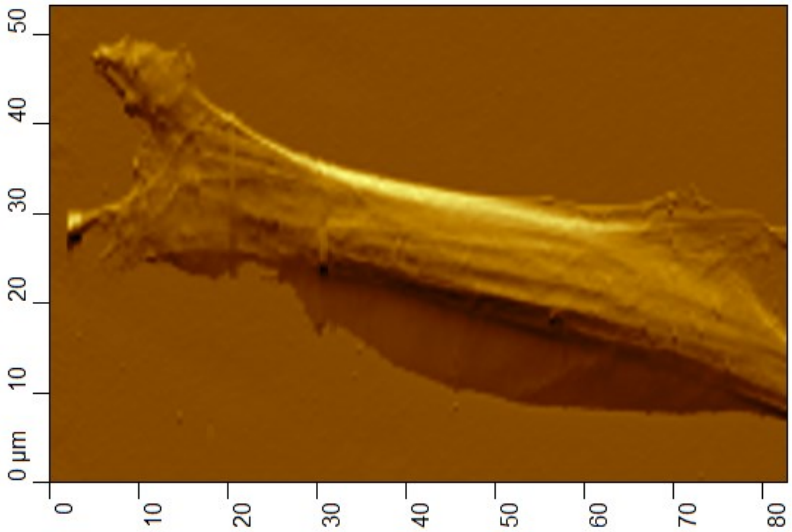


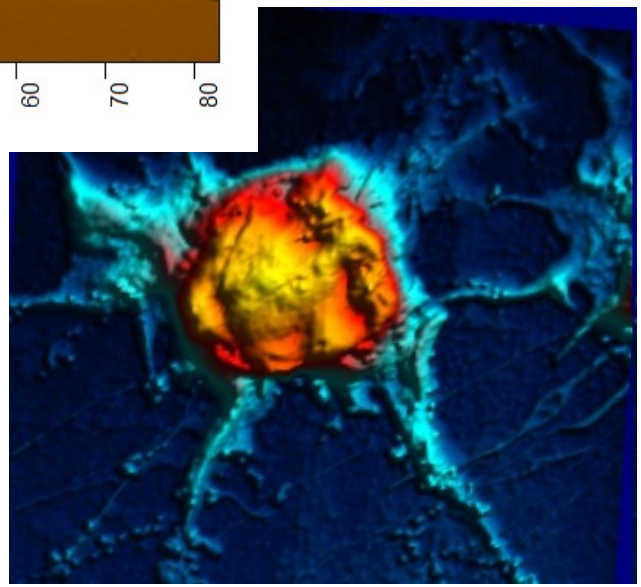


5. Eukaryotic cells

A. Standard culturing on polystyrene dishes

Adhesive protein layers usually takes place (i.e. pLL, RGD adhesion factors, fibronectin, etc.)



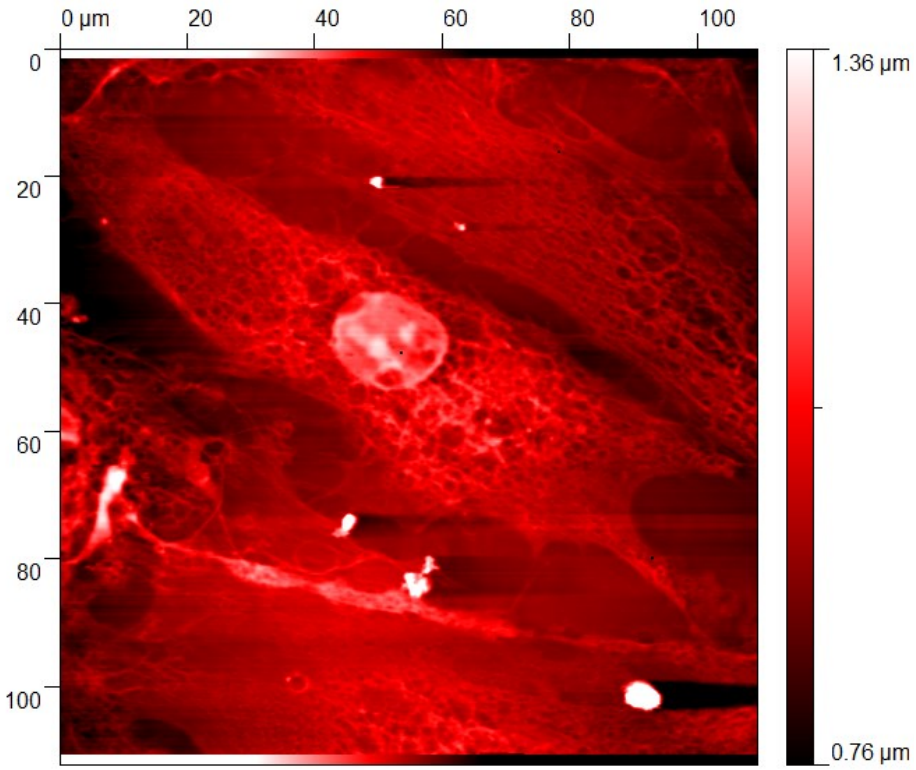


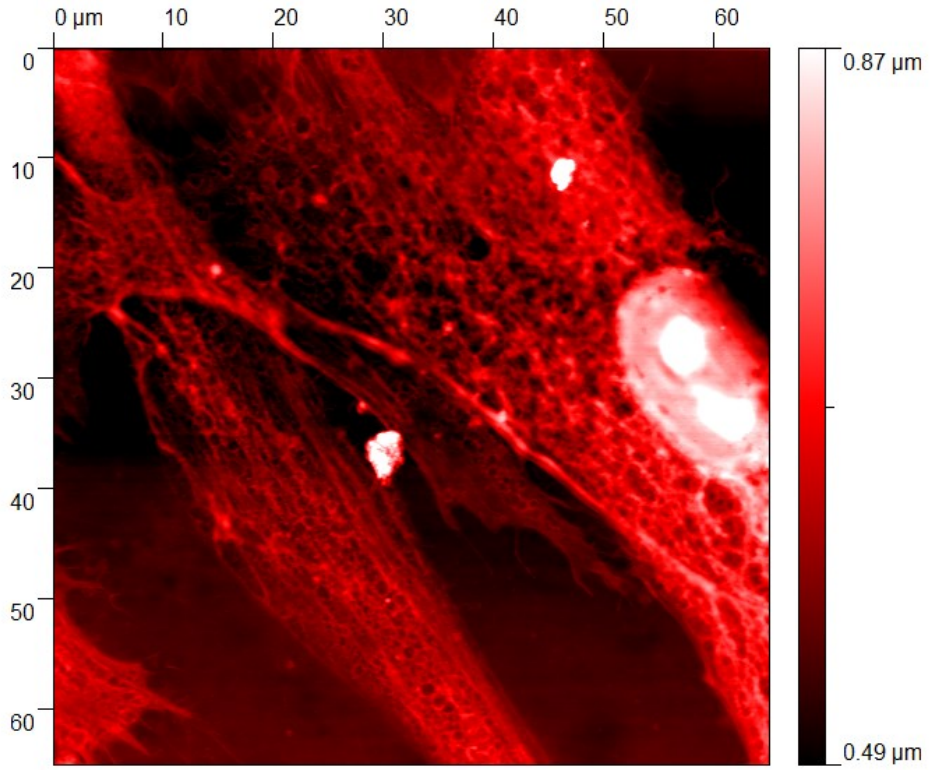
Cell culturing equipment

BioAFM incl. Petri dish **heater** for in-vitro imaging of **cell cultures**

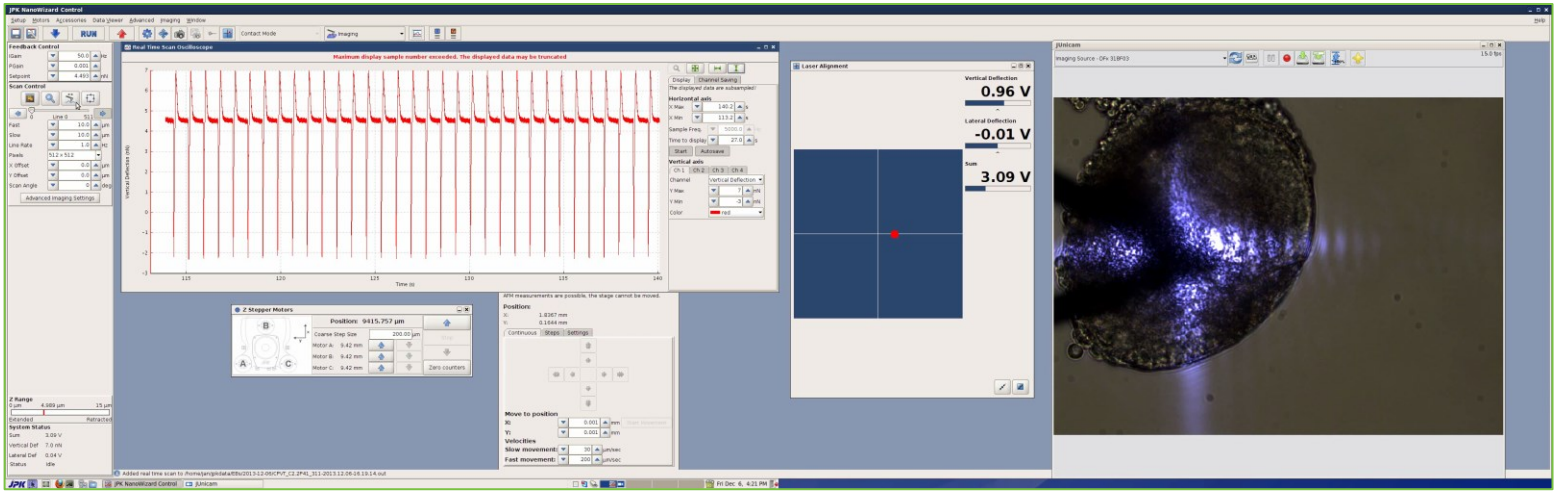
B. Fixation agents

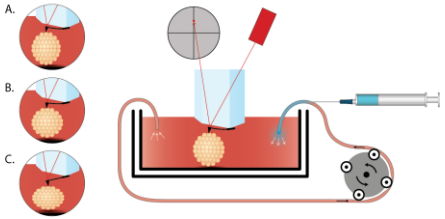
- Adhesion of cells out of incubator (37°C, 5% CO_2) is mostly problematic
- Allows study of cells in long term periods after removal from incubator
- Cell wall destruction
- Example: EtOH, acetic acid, paraformaldehyde, glutardialdehyde

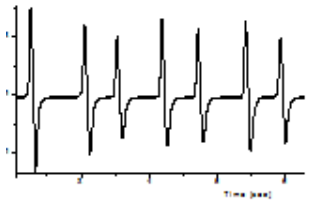




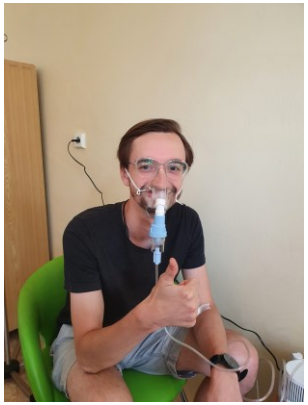
5. Organoids = cell clusters



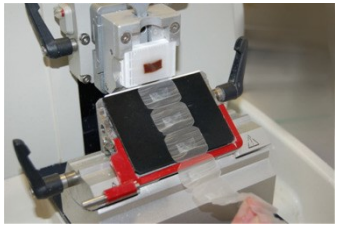




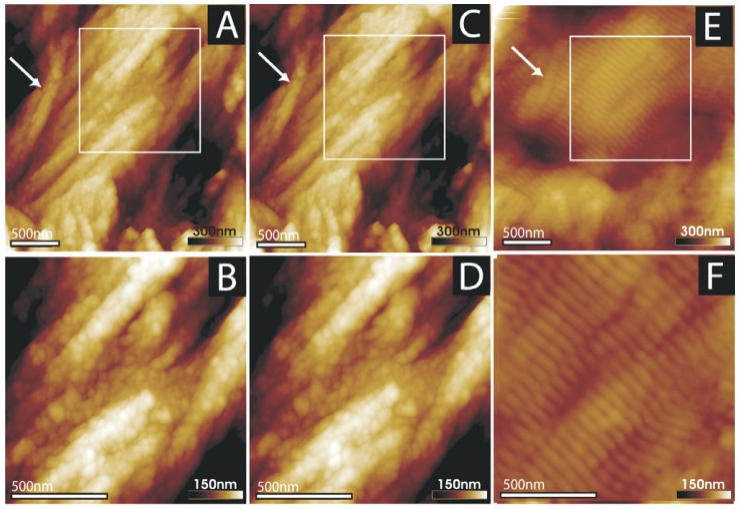
MCG = mechanocardiogram



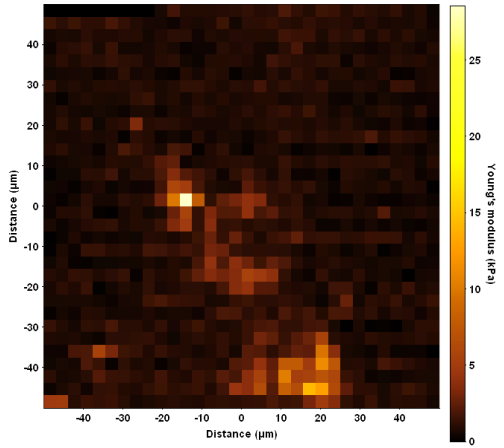
5. Tissue slices



bones



+ combination with AFM topography

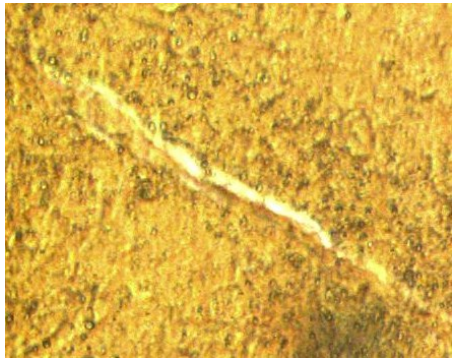


Liver cirrhosis

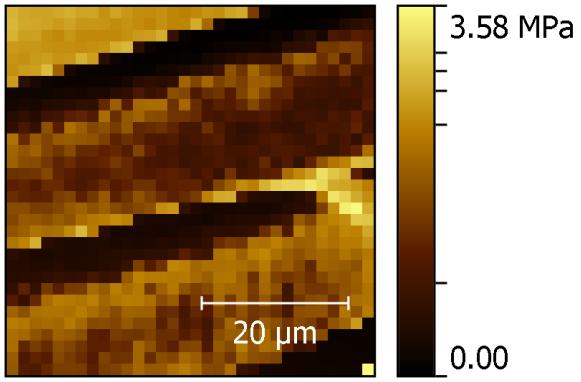
Correlation of

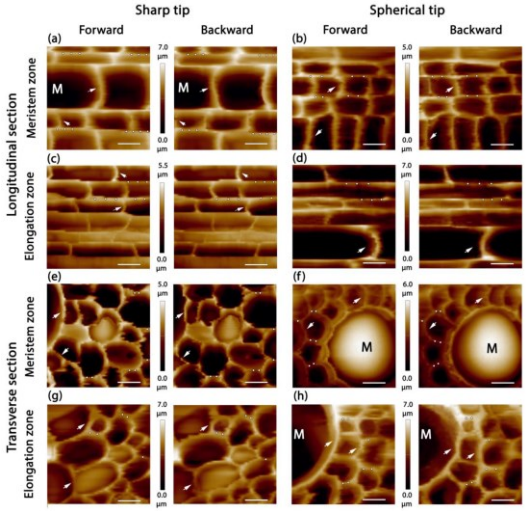
Collagen fibers by polarized microscopy

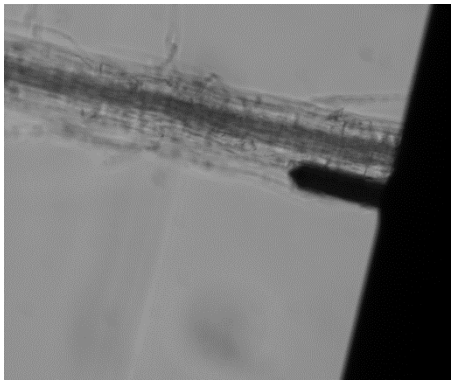
AFM nanoindentation

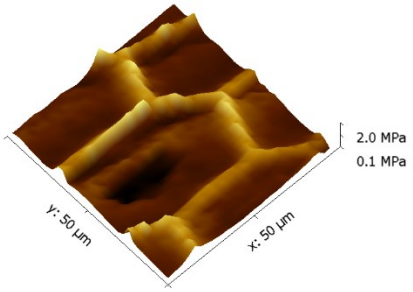


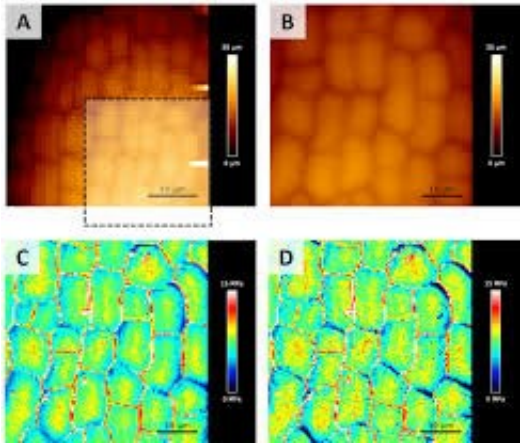
5. Whole organisms











Plant samples

(hypocotyl)

Thank you for your attention!