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Specific approaches in microscopy samples preparation

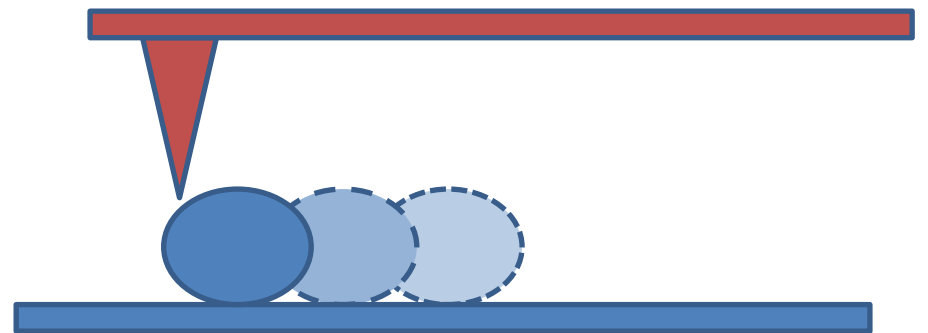
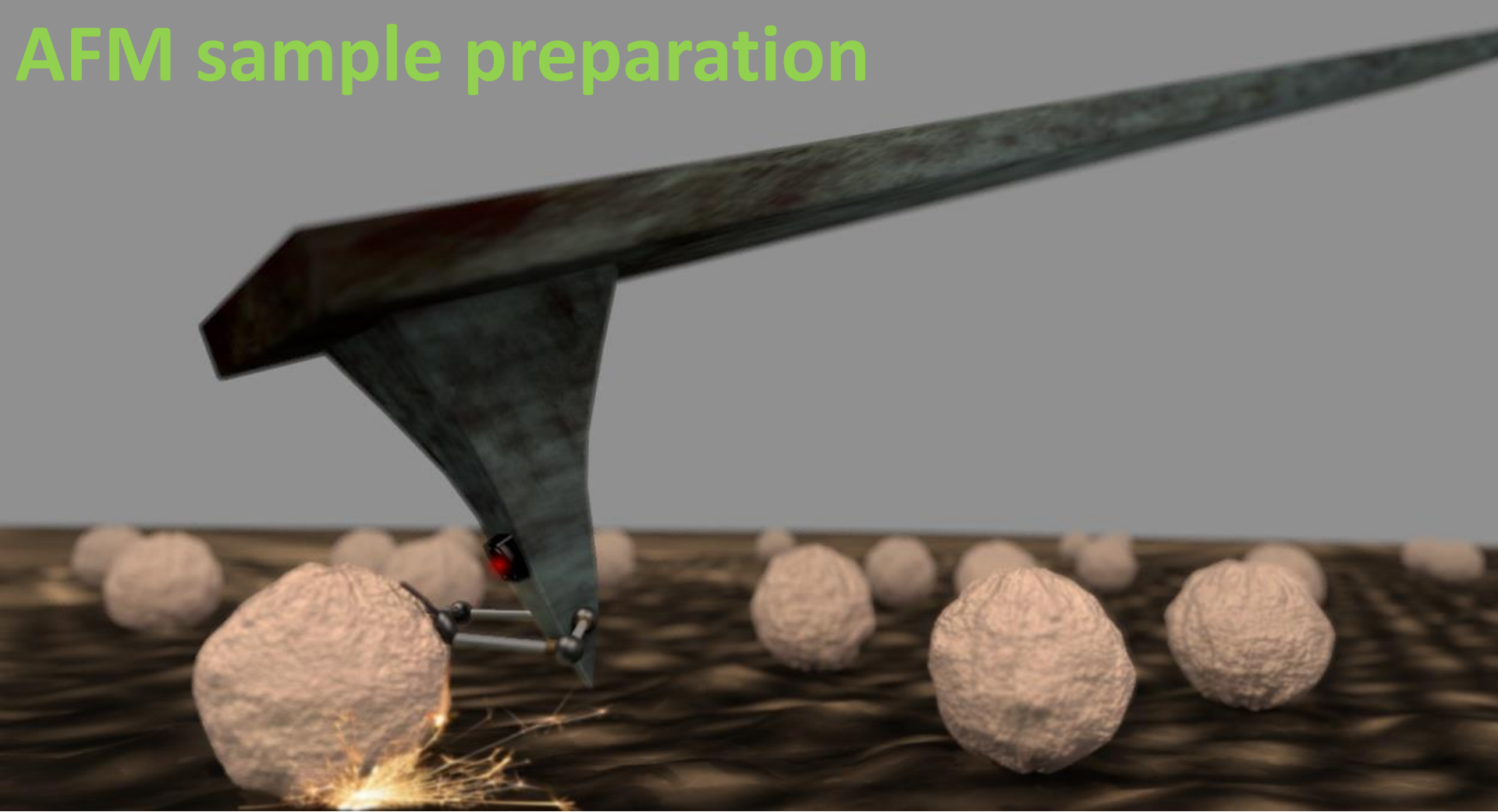
MUNI

Multimodal Microscopy Workshop: Probing the Triad of Structure,
Mechanics, and Chemistry in Biological Systems, 2024, Brno

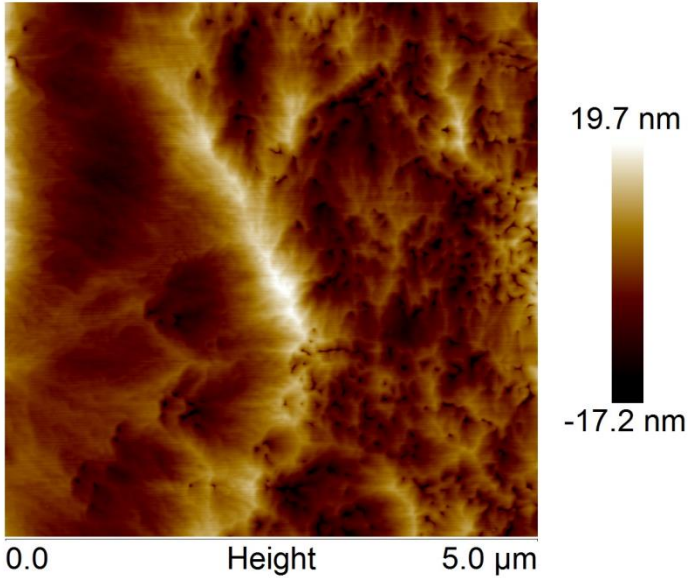
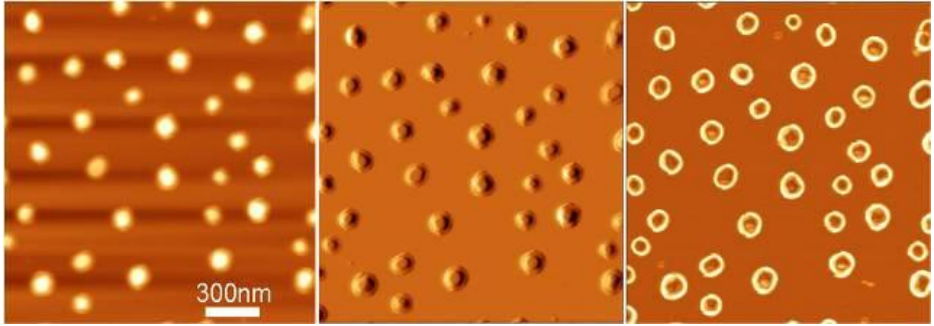


Sample preparation for AFM

AFM sample preparation



Concentration – surface density

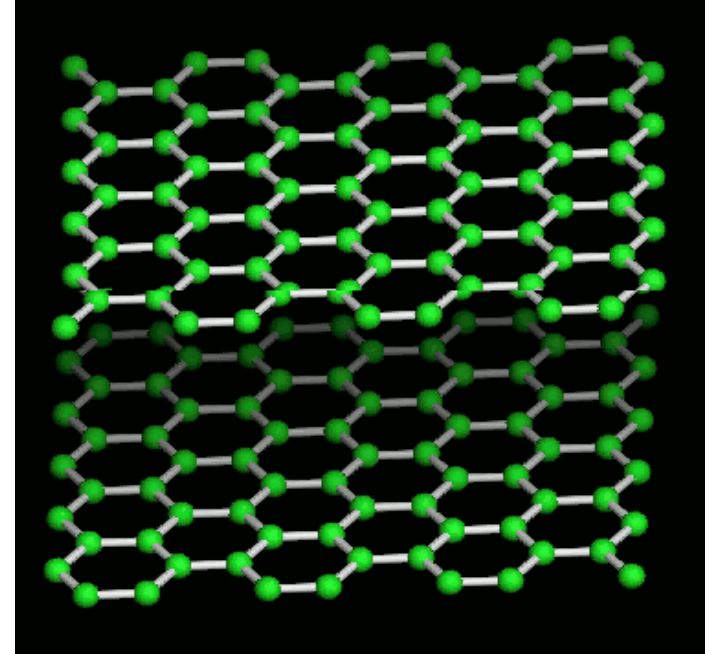


Substrates for preparation of AFM samples

Atomically flat surfaces

1. HOPG Highly Ordered Pyrolytic Graphite

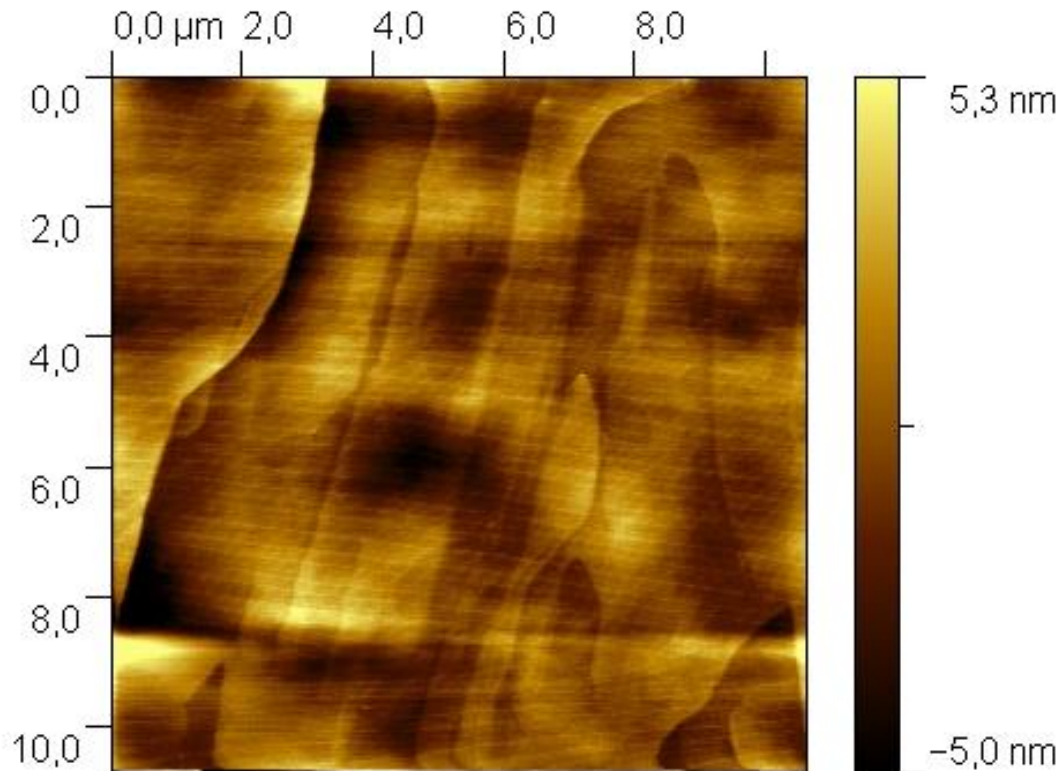
- Kish's graphite, waste in steel production
- Hexagonal planar structure
- C-C bond 142 pm, layer-layer distance 335 pm
- Conductive, highly hydrophobic
- Planar structure
- Synthetic form of graphite, high chemical purity
- Traditionally – substrate for SEM, STM i AFM (→ **conductivity**)
- **Immobilization**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



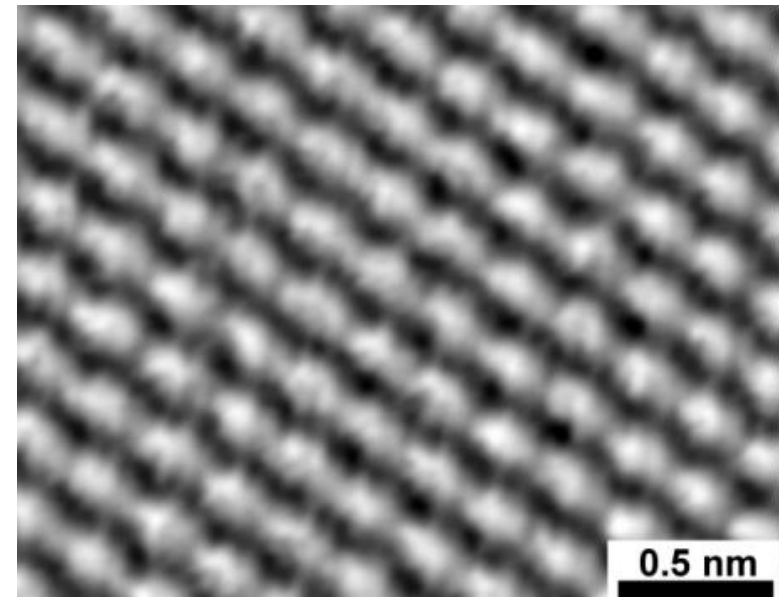
Atomically flat surfaces

1. HOPG Highly Ordered Pyrolytic Graphite

**Large areas
visible layers**



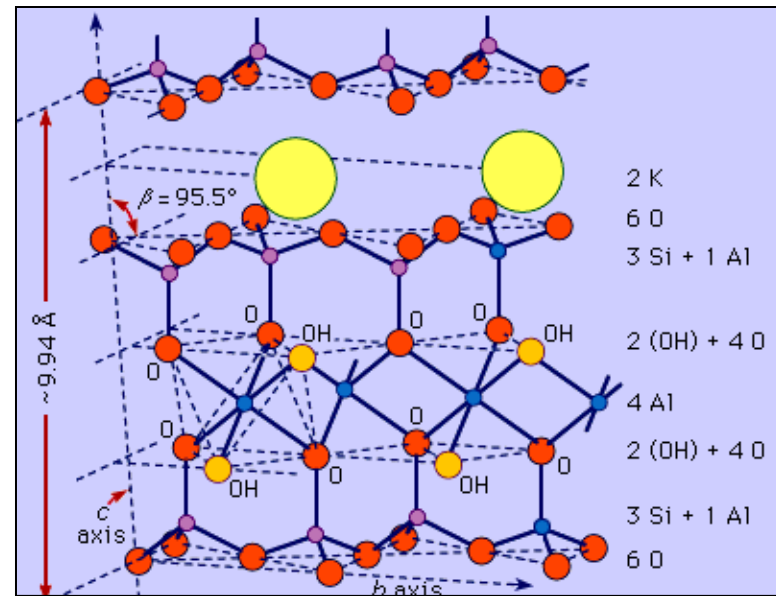
**Small areas
atomically flat**



Atomically flat surfaces

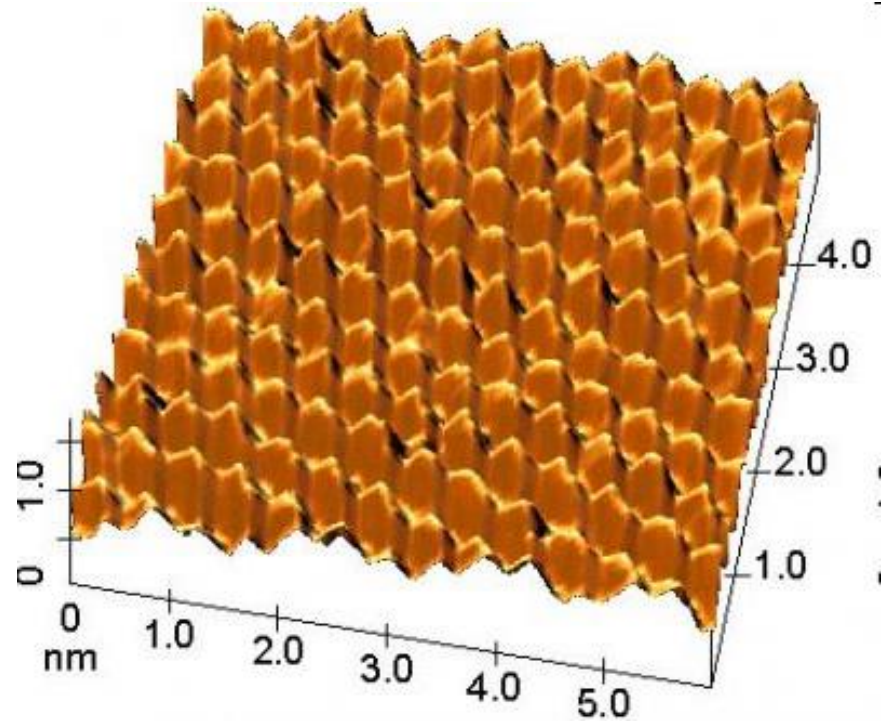
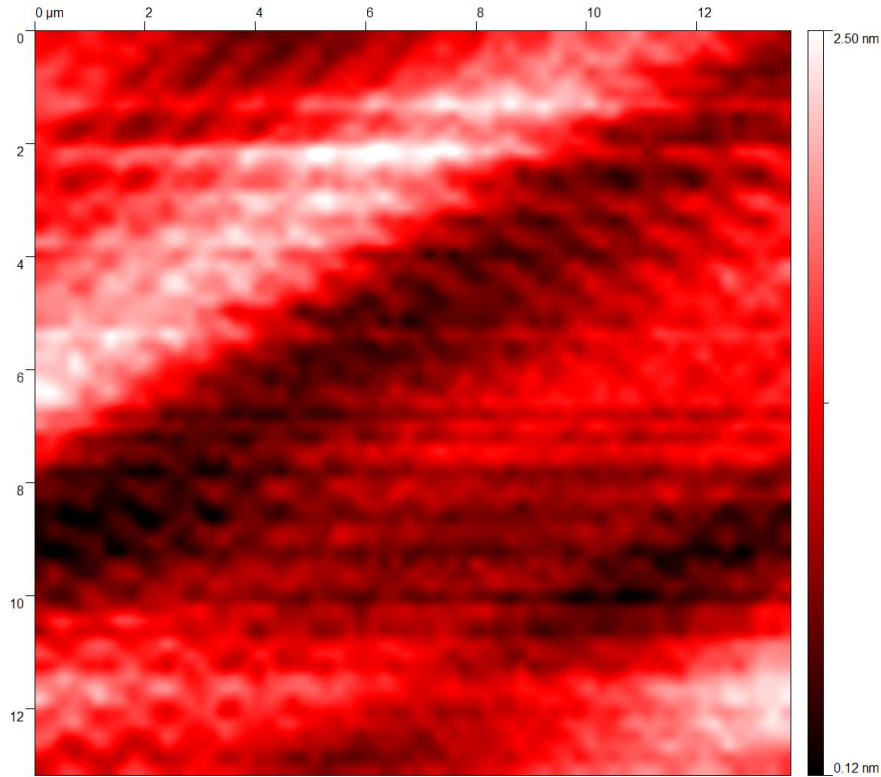
2. Mica (muscovite)

- „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**
- $pK_a \sim 3$, physiological pH \rightarrow negative surface charge
- Mica = silicate, hydrated SiO_2 ($\sim Si-OH$) from the chemical point of view



Atomically flat surfaces

2. Mica (muscovite)



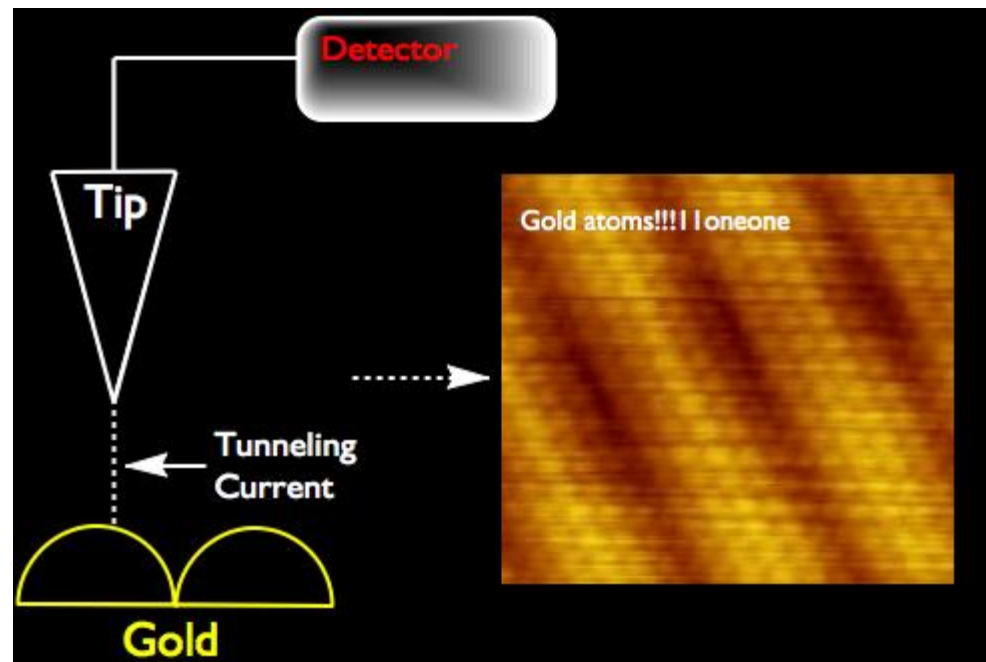
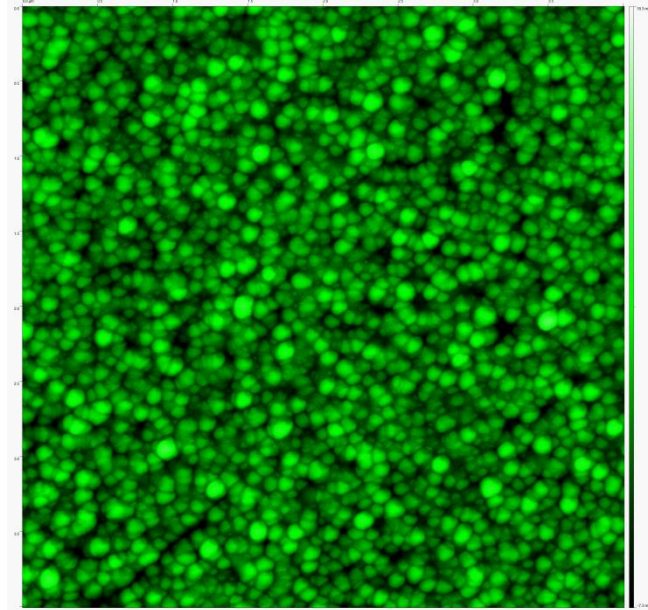
Extremely flat on small and larger areas

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)

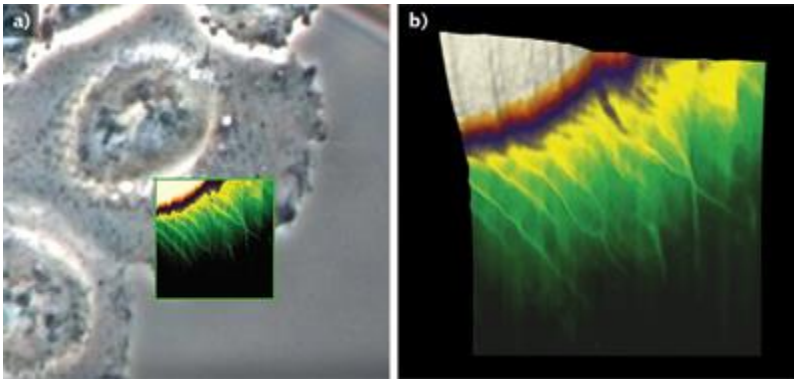
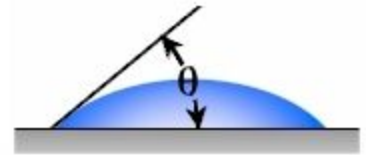
Sputtered gold layer
image by tapping mode AFM



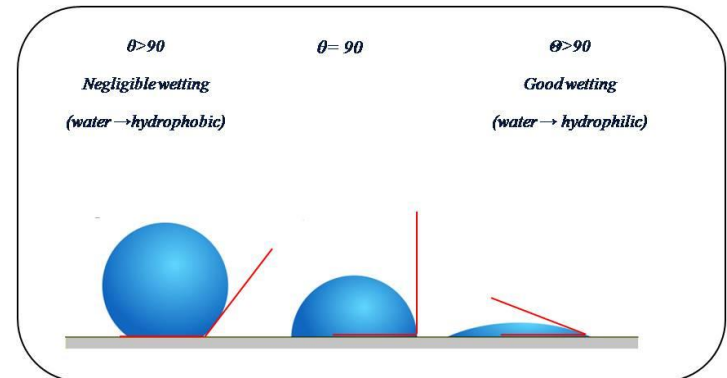
Other surfaces

4. Glass

- Amorphous noncrystalline structure
- Lab glass composition: 75% SiO_2 plus Na_2O , CaO , borate and minor additives
- $\text{Si-OH} \rightarrow$ 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

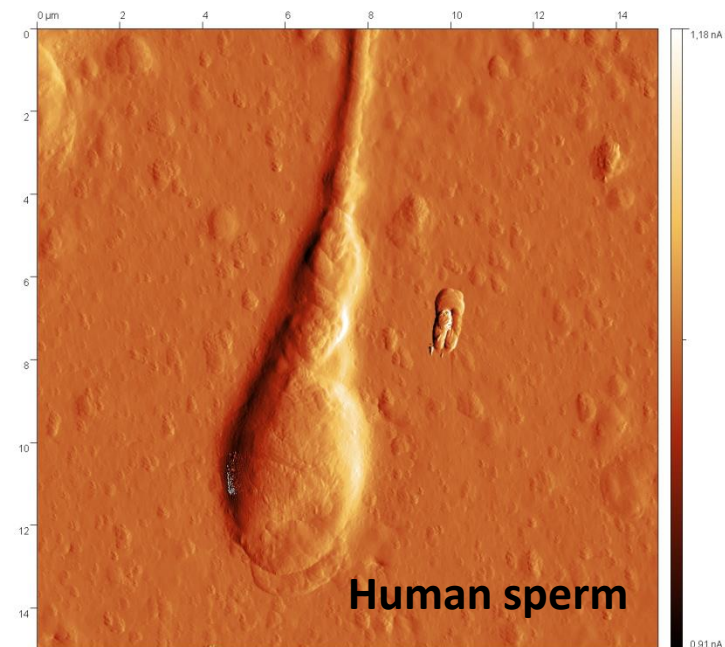
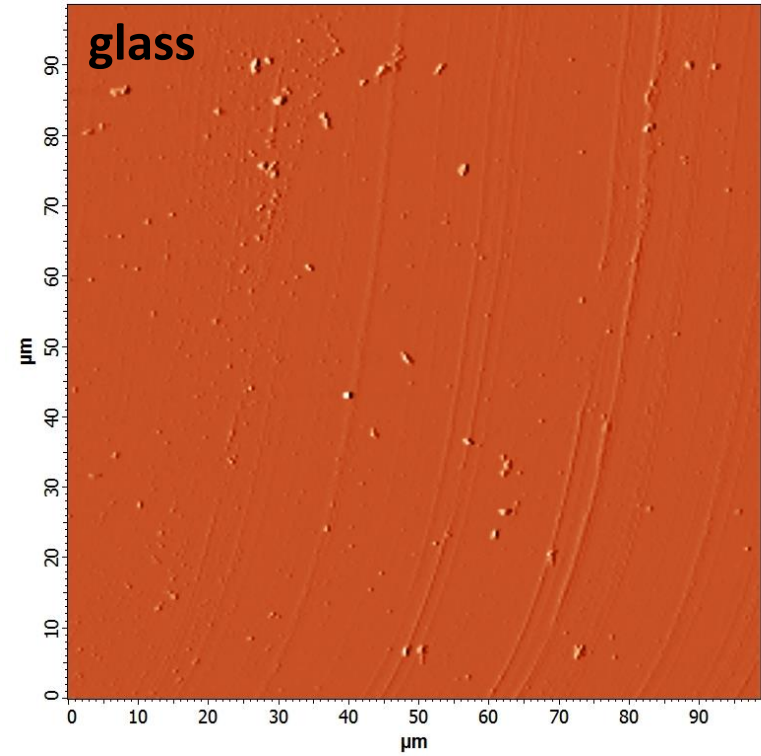
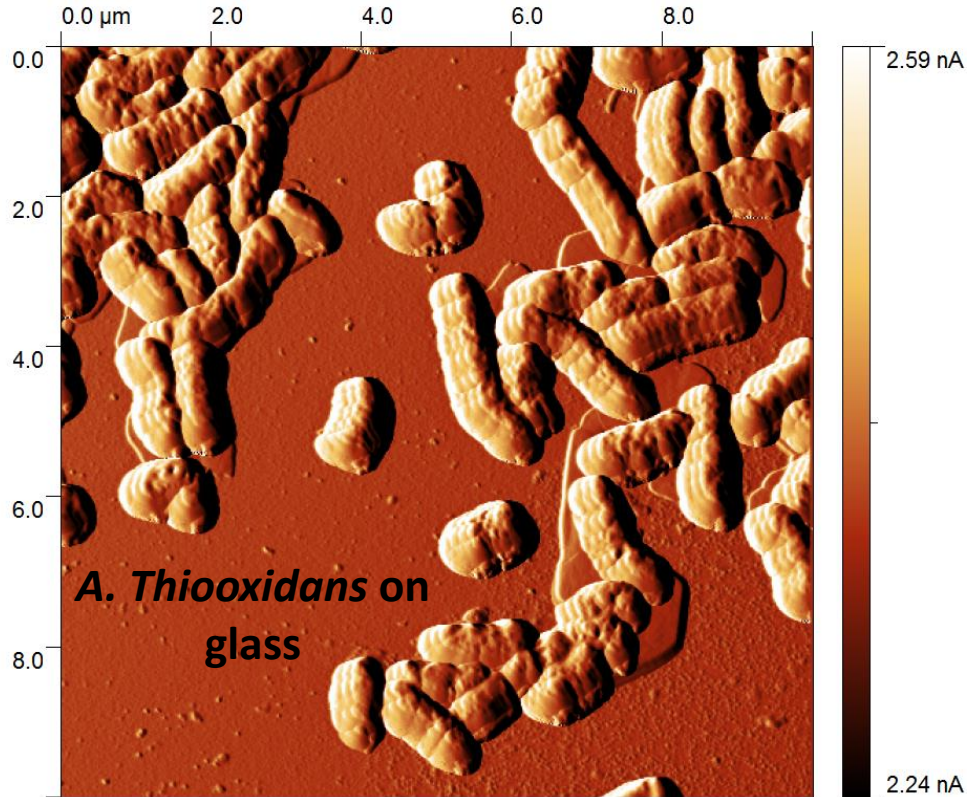


AFM – optical image overlap



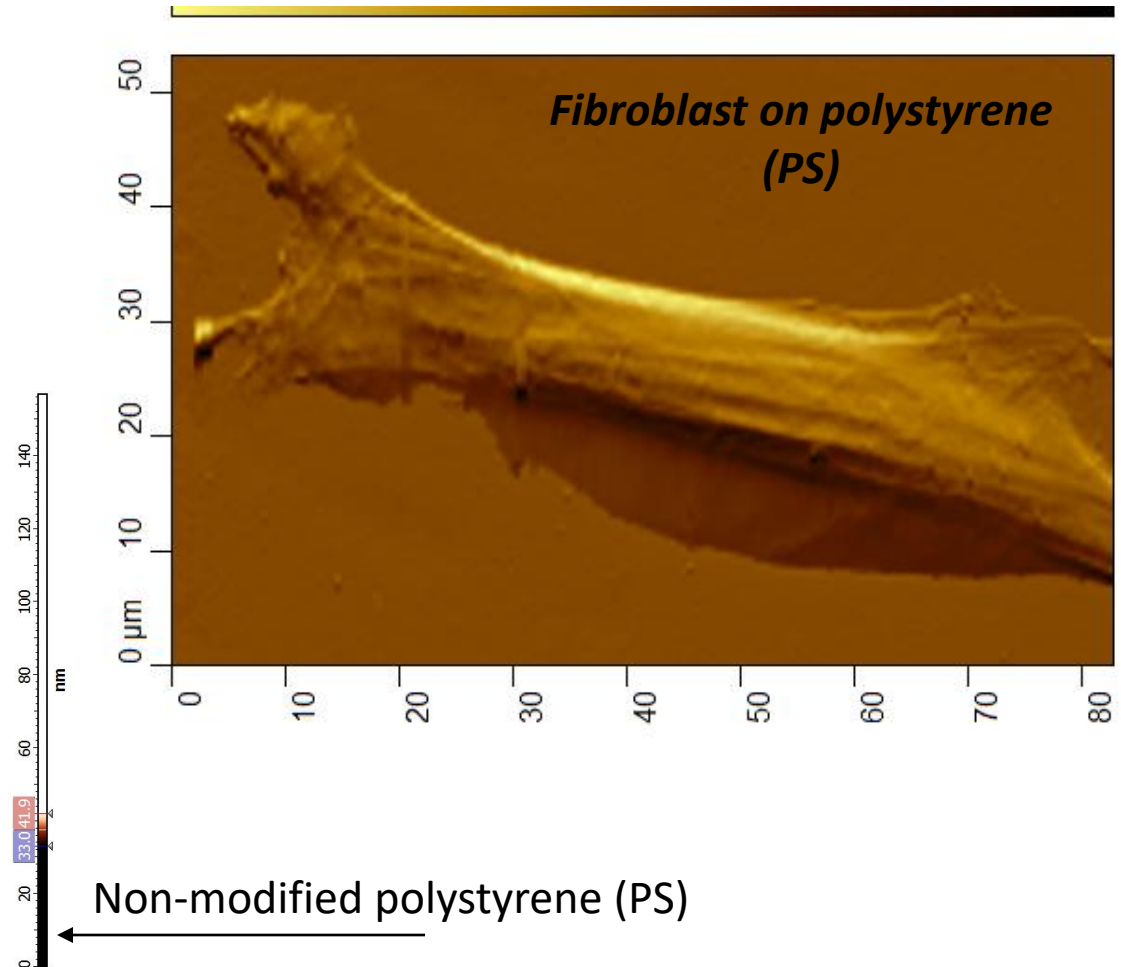
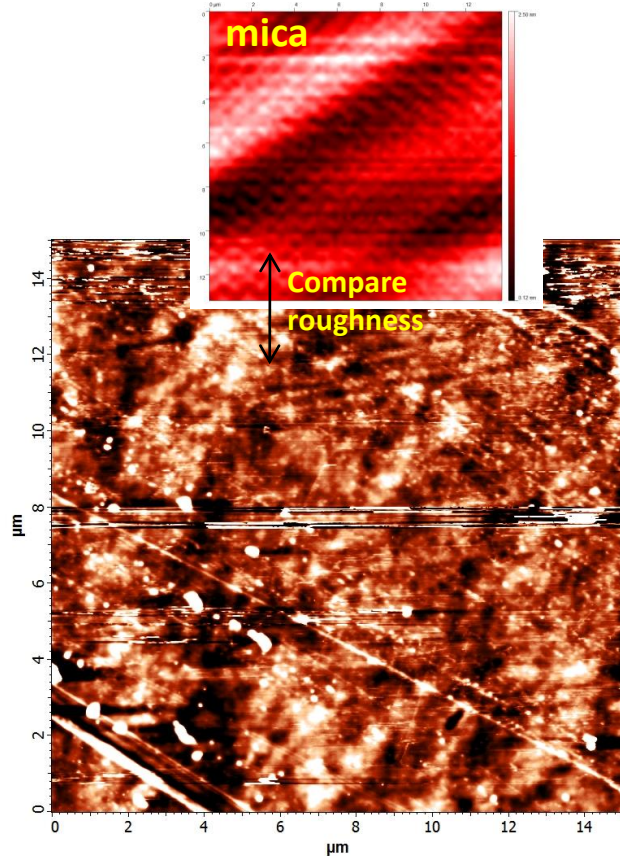
Whole cells on glass

under AFM



Other surfaces

5. Plastic materials

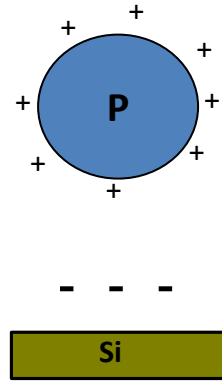


- Most of lab supplies made of plastic (**PP, PE, PS**)
- **No functional groups** to be used in covalent binding
- **PS – hydrophobic** → spontaneous non-specific adsorption of proteins
→ usually as underlying support (i.e. for cell attachment)

Immobilization procedures

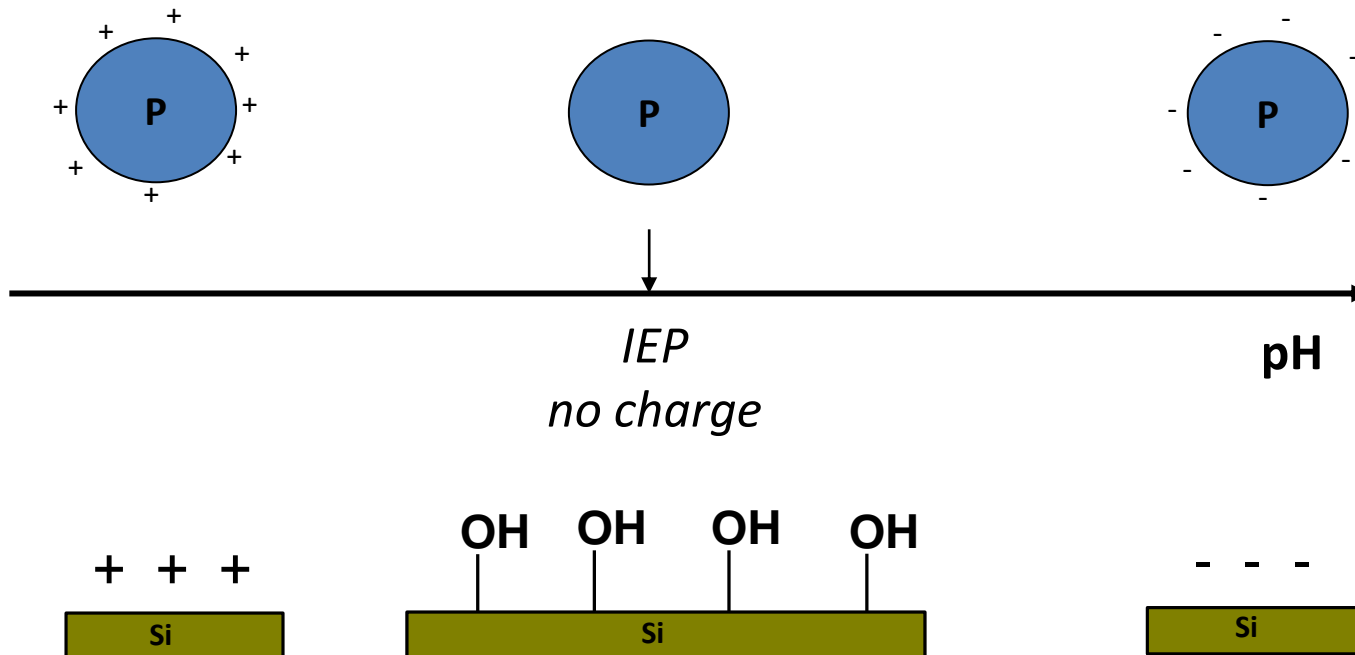
1. Proteins

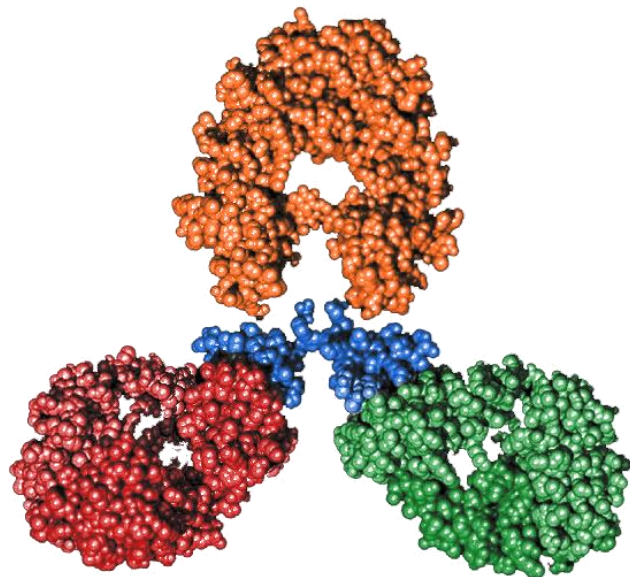
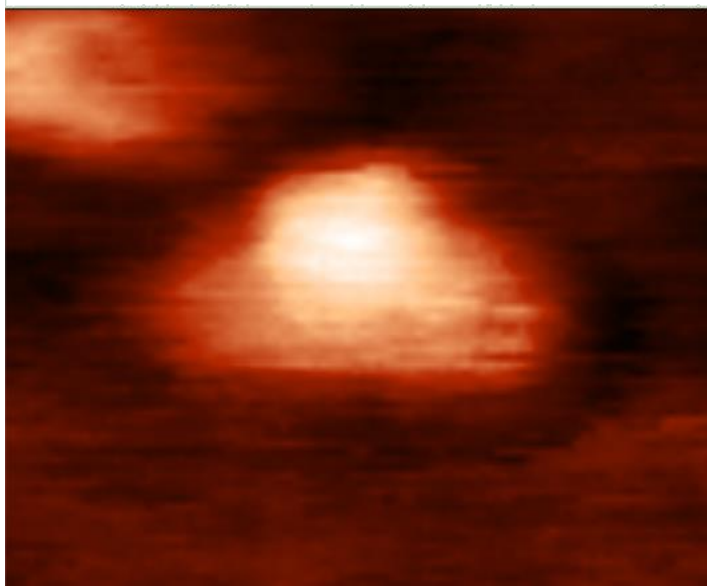
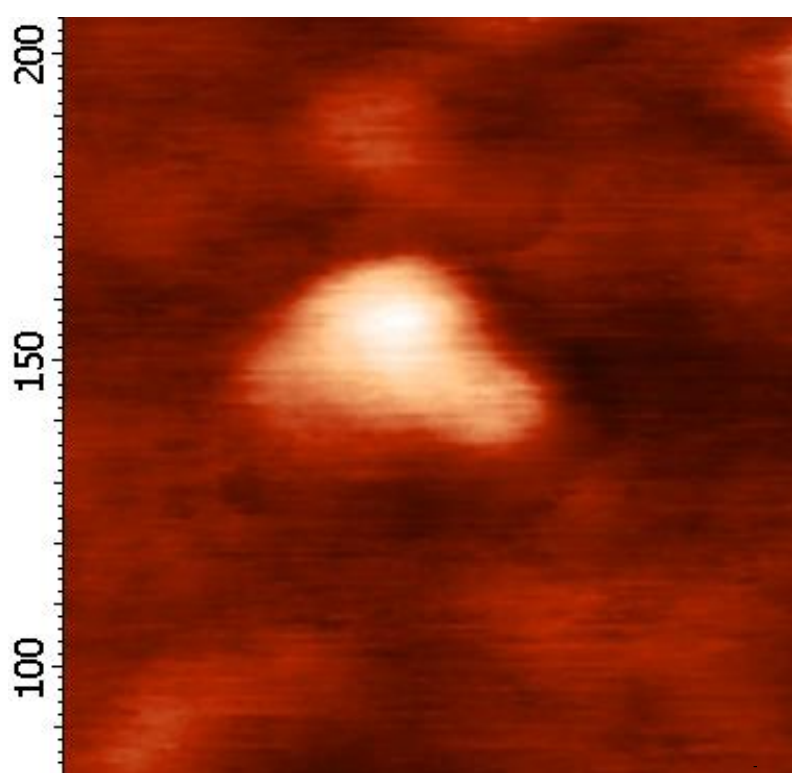
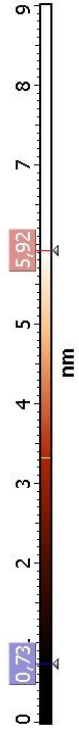
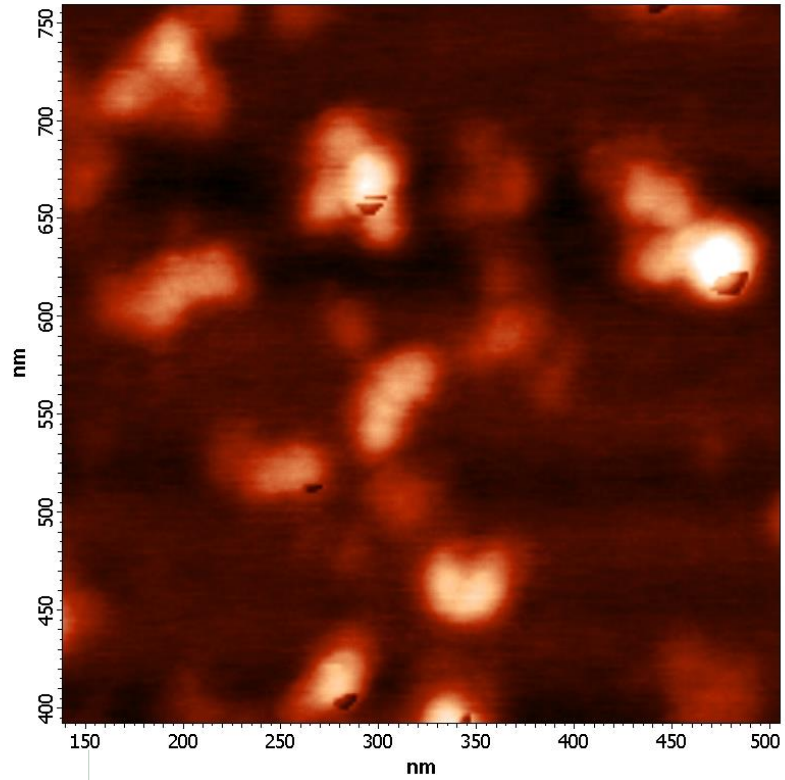
Surface: **mica or HOPG** (extremely flat)



Protein: charge is given by IEP + pH

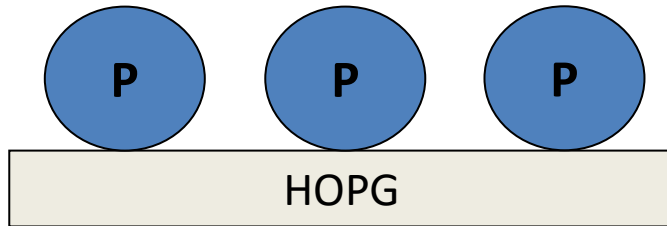
Immobilization on mica: $pK_a(\text{mica}) < \text{pH} < \text{IEP}$



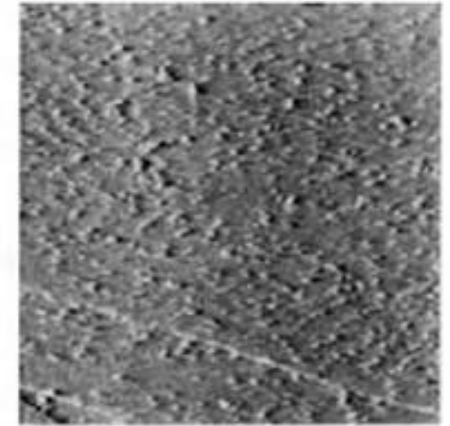


Protein immobilization on HOPG

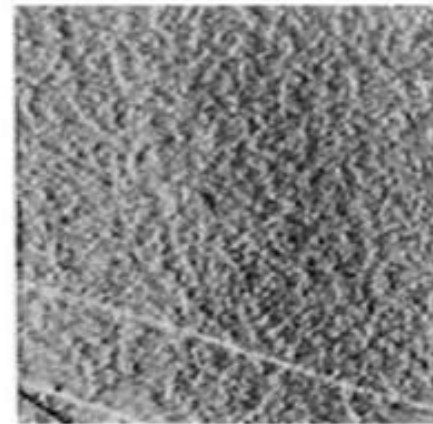
A. **Spontaneous** (non-specific) **adsorption** of protein → hydrophobic surface
(best results at zero charge $pH = IEP$)



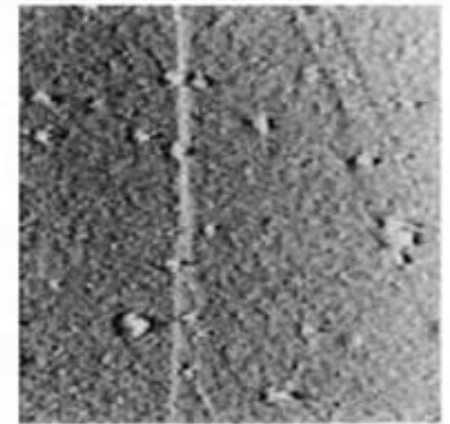
A
0 min.



B
2 min.



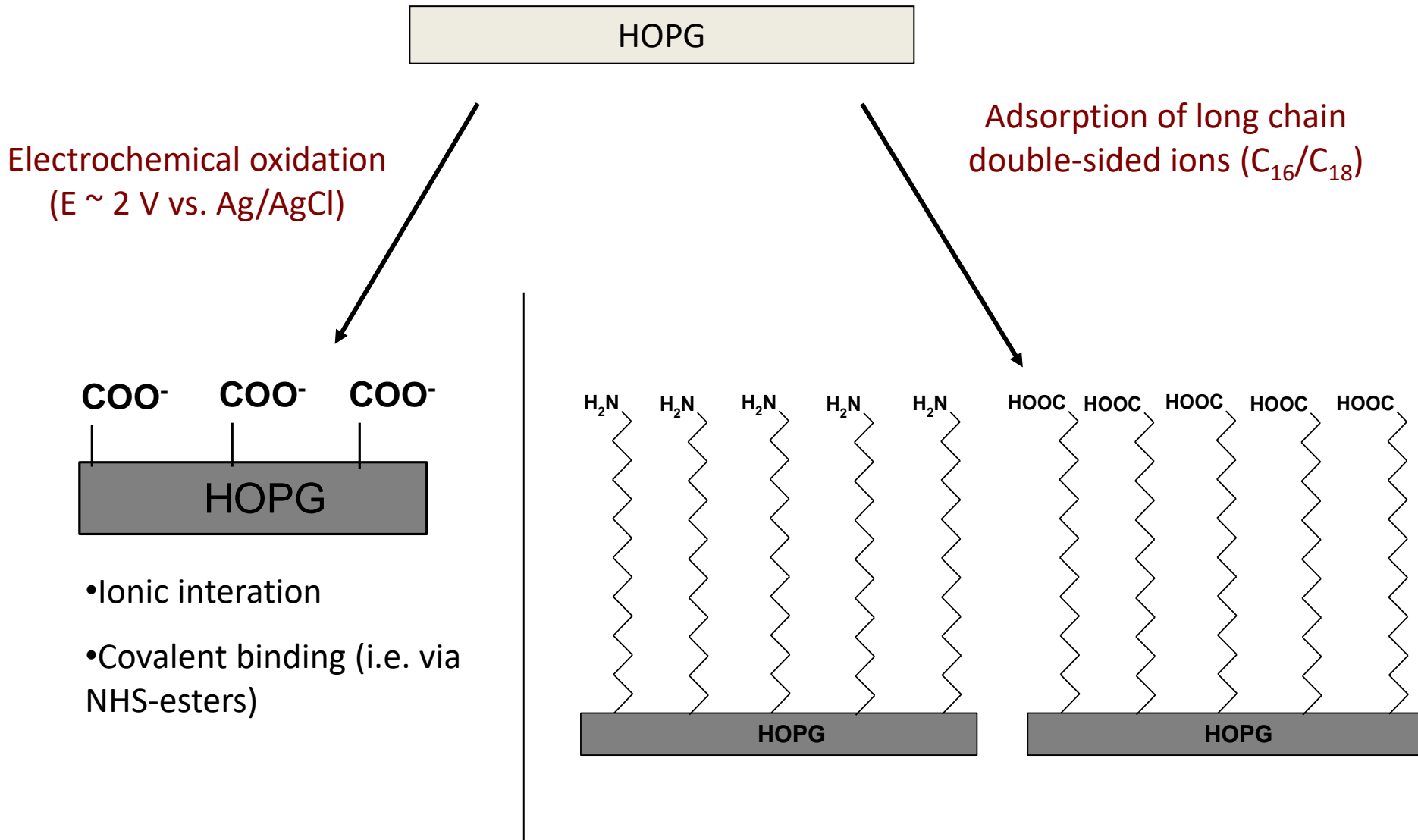
C
6 min.



D
60 min.

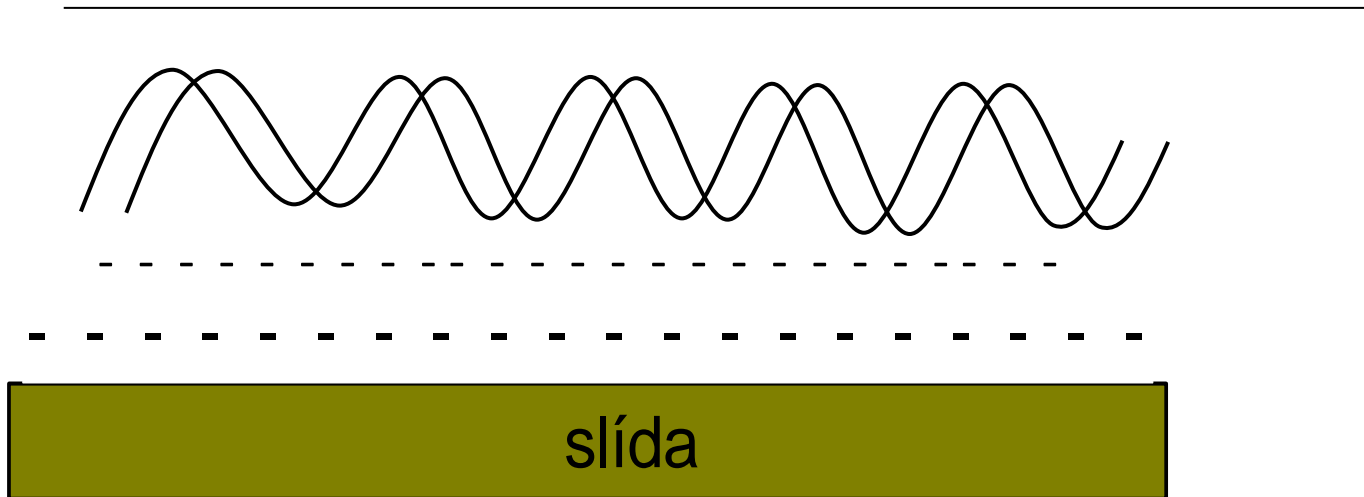
Lysozyme molecules on HOPG

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



2. DNA

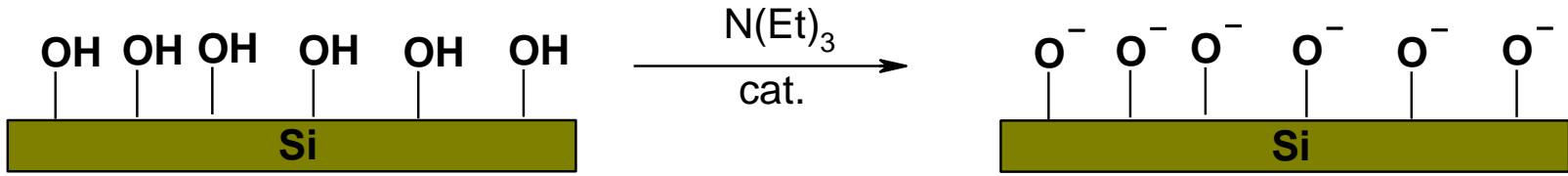
Surface: **mica or HOPG** (extremely flat)



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

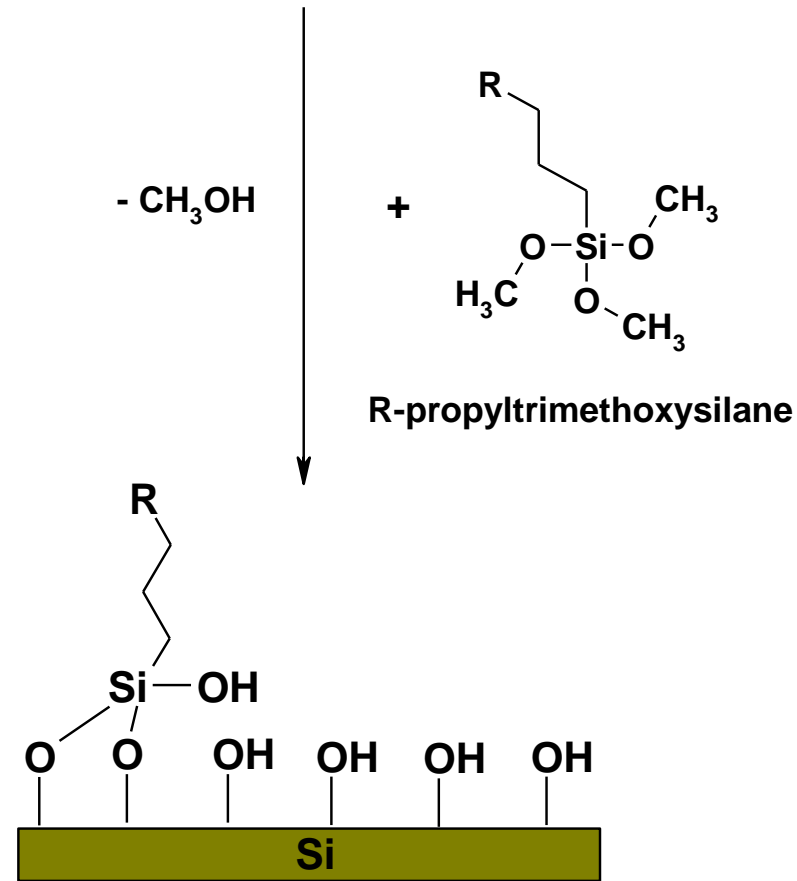
- Aim: **introduction of functional group**

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

- Strong basis catalysis

- Procedure can be monitored by water contact angle measurement

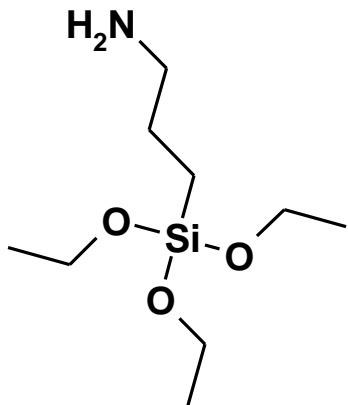
A. DNA on mica



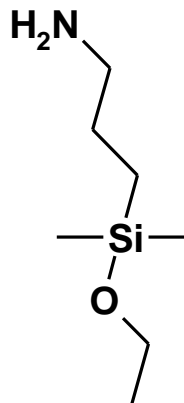
silanization
 $\xrightarrow{\hspace{2cm}}$
 hydrophobization



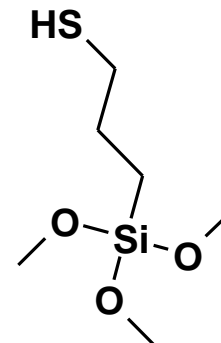
Examples of alkoxysiloxanes



(3-Aminopropyl)trimethoxysilane
APTES



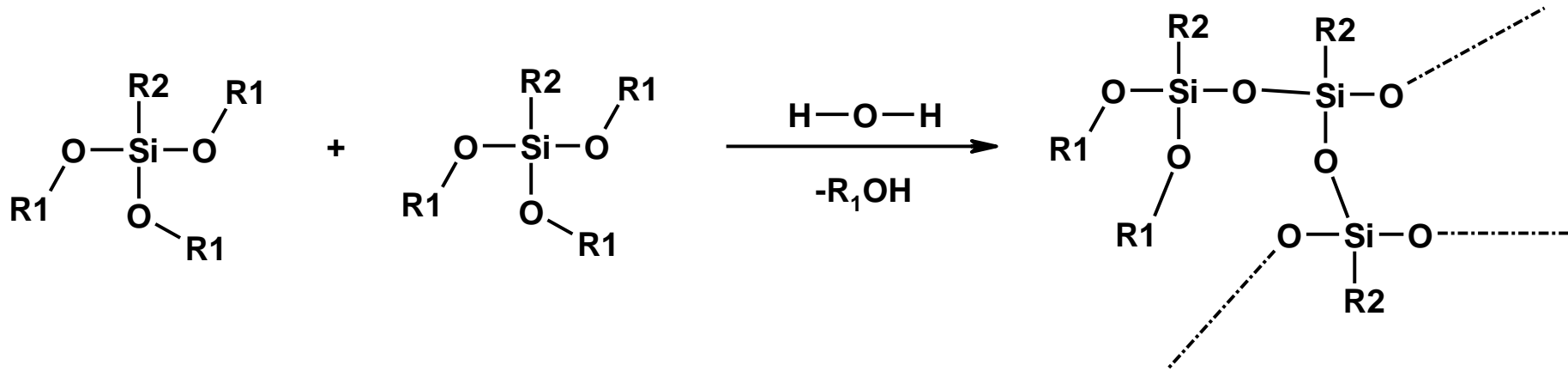
3-(Ethoxydimethylsilyl)propylamine
APDMES



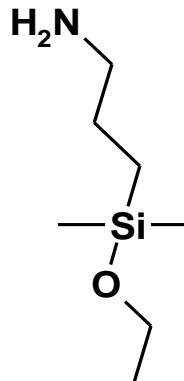
(3-Mercaptopropyl)trimethoxysilane
MPTS

Self-polymerization

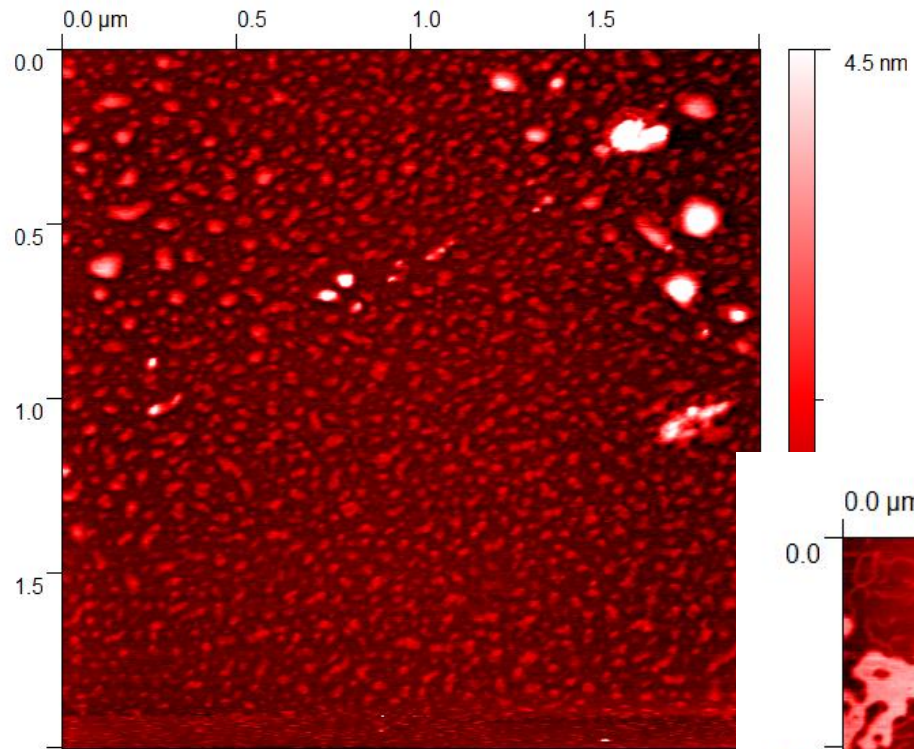
practical complication



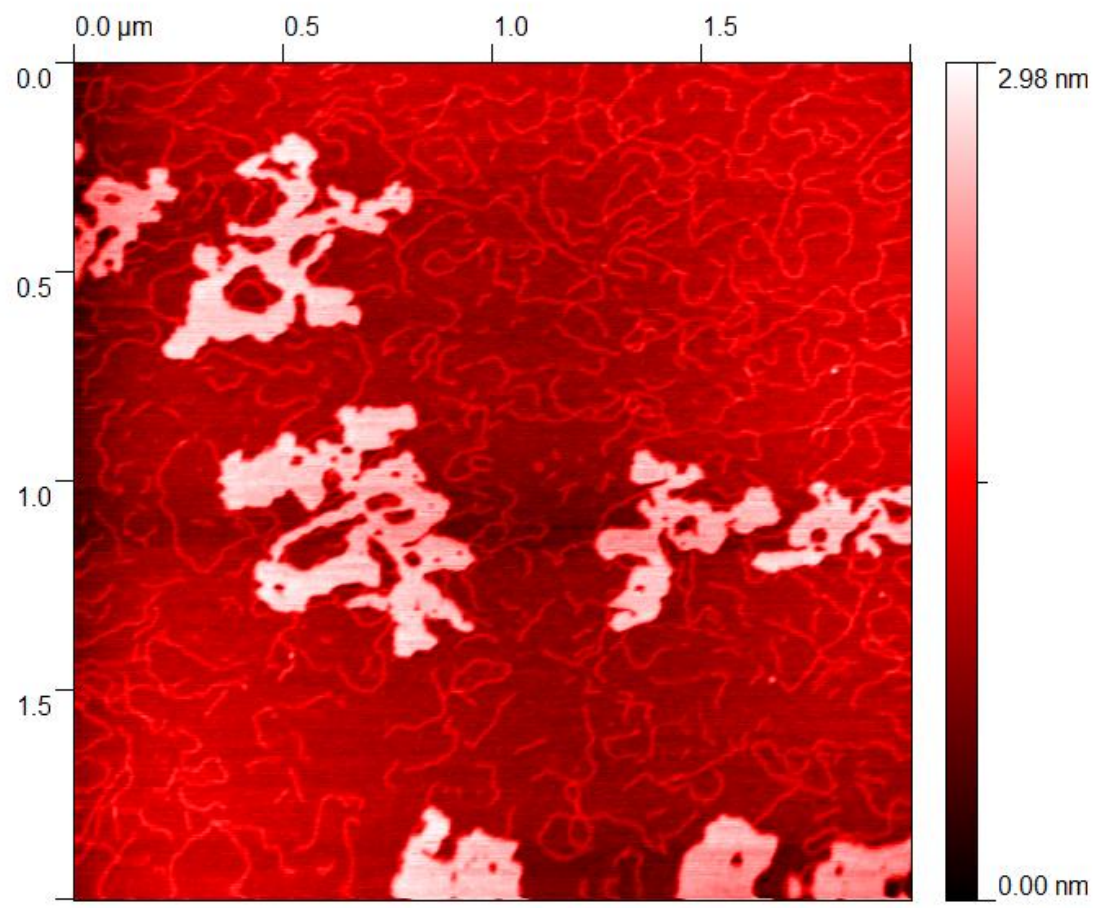
- Especially with **APTES** during liquid silanization
- Even vapors of water can cause this effect
- Fixation for **optical** microscopy – **expected** factor
- In contrary – in fixation for **AFM** – very **disturbing**
- Solution:
 - silanization in **vapours** under **vacuum** (i.e. in desiccators)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



3-(Ethoxydimethylsilyl)propylamine
APDMES



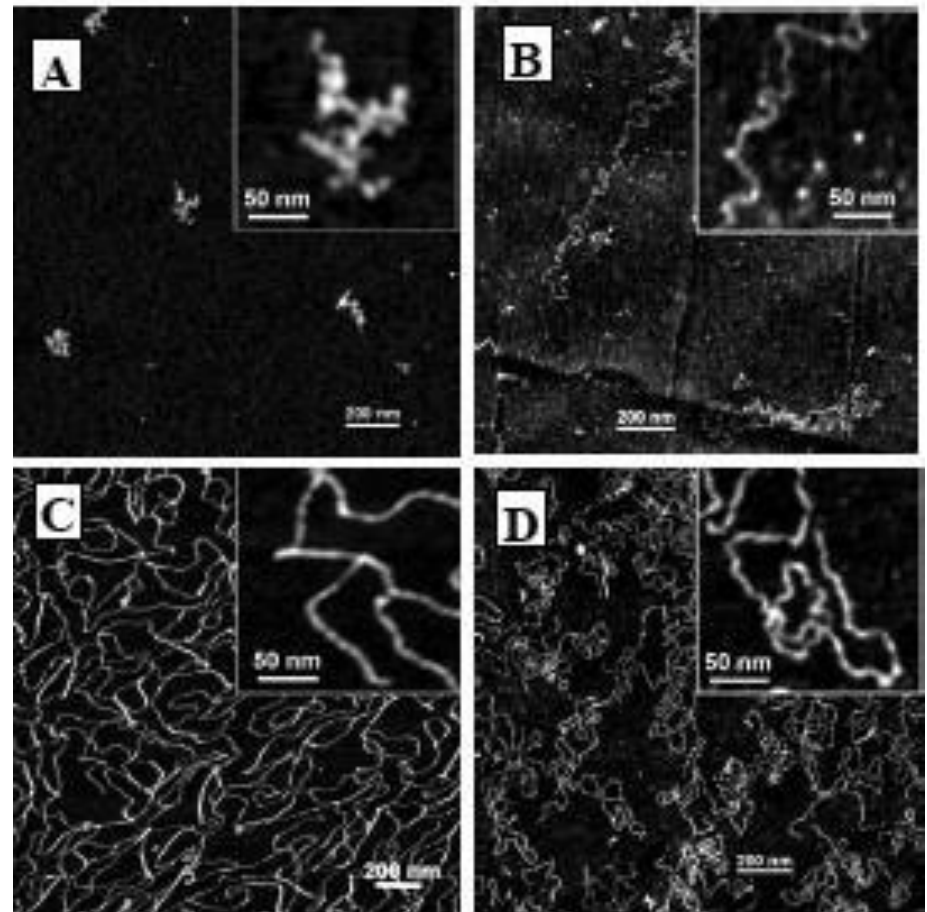
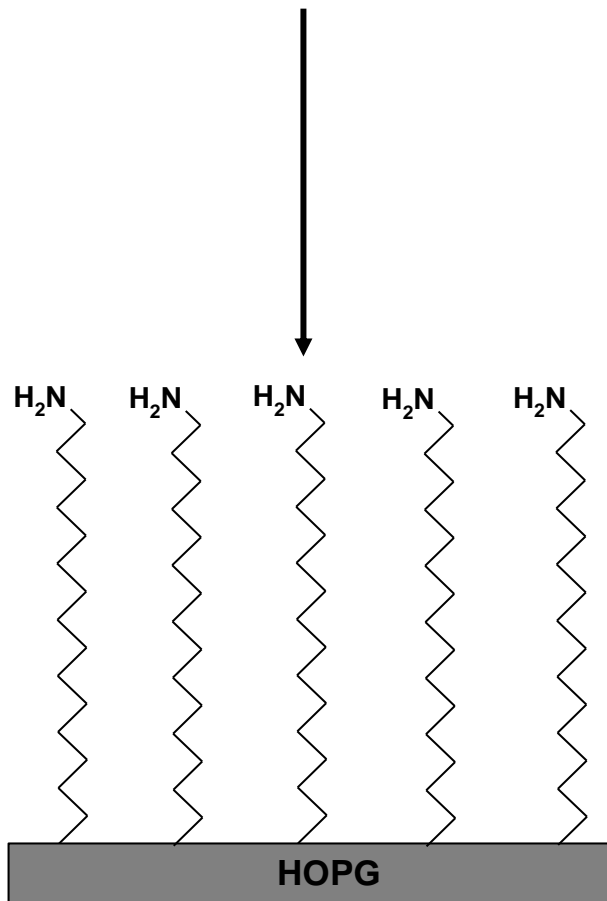
Self-polymerization *examples*



B. DNA on HOPG

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

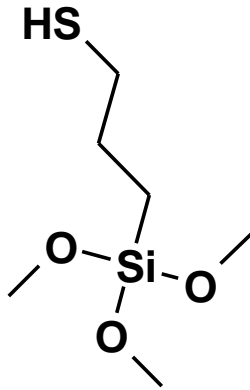
HOPG



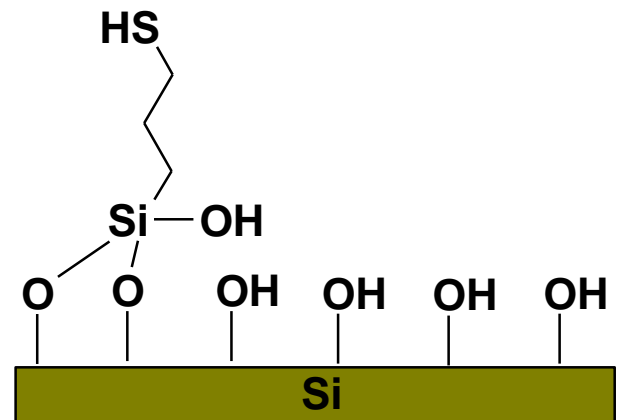
3. Nanoparticles

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

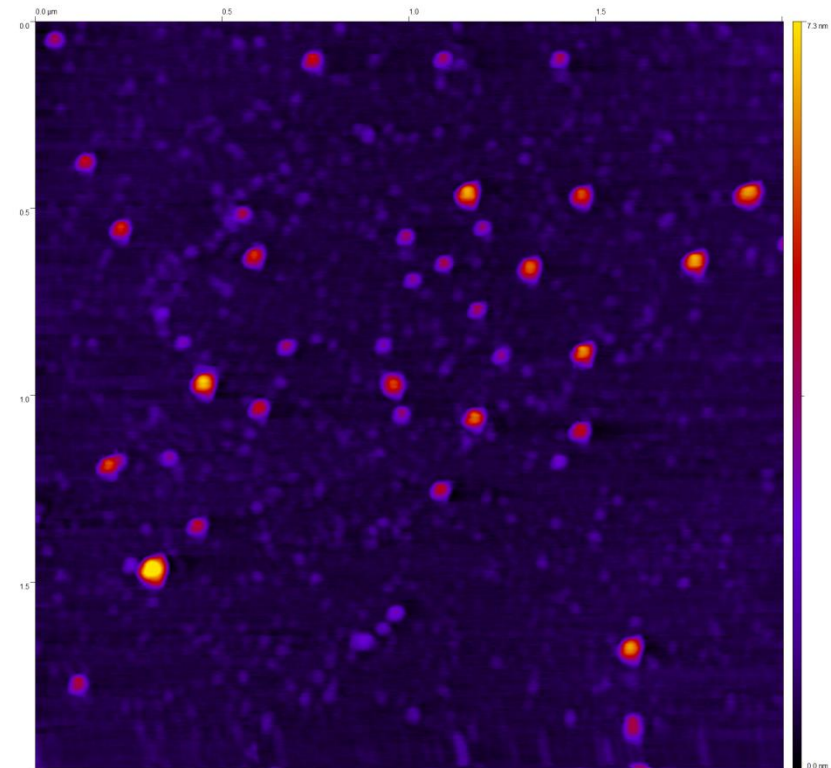
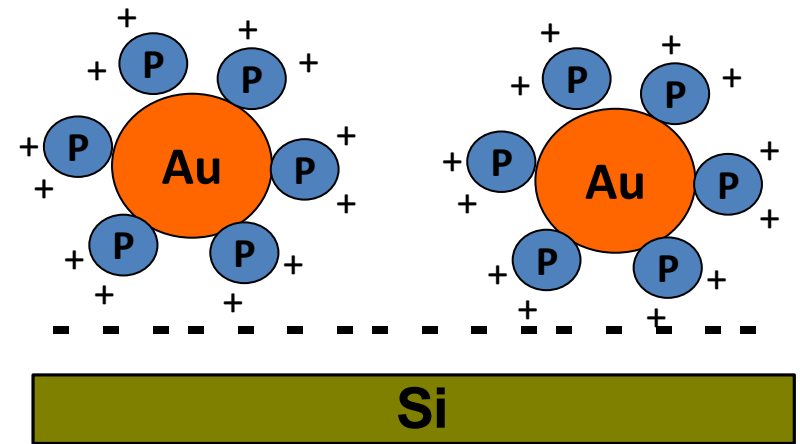
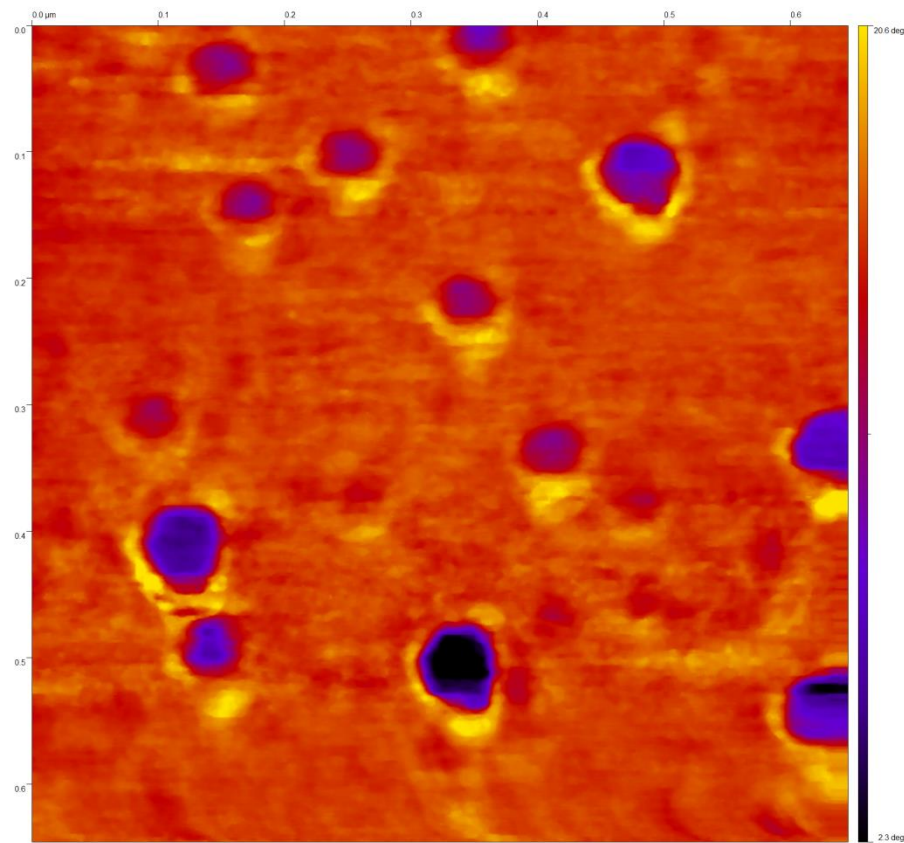
Example: gold nanoparticles (AuNP) mercapto-silanized mica (SH-mica):



(3-Mercaptopropyl)trimethoxysilane
MPTS



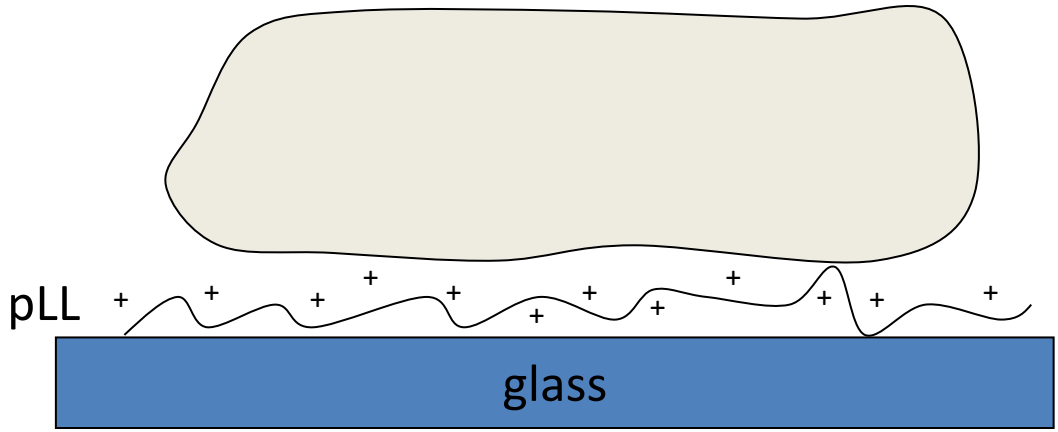
SH-mica



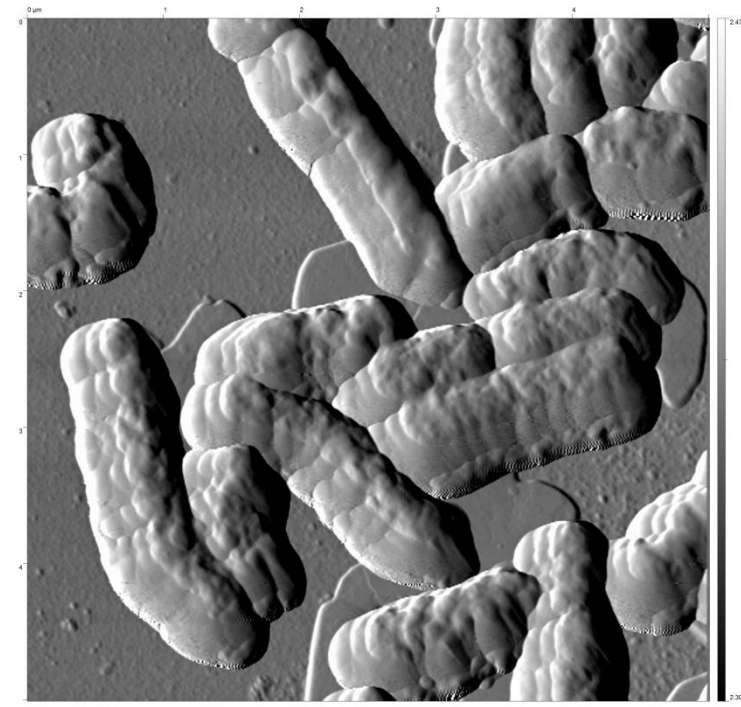
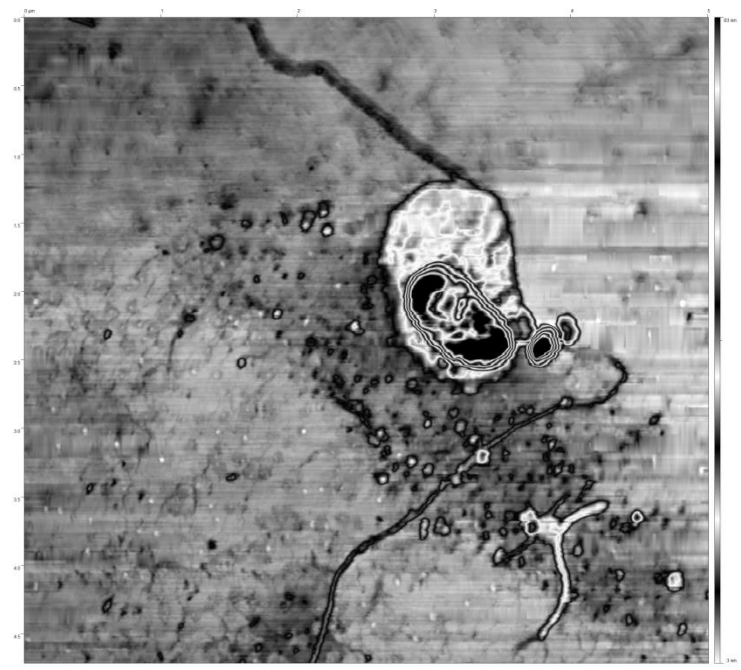
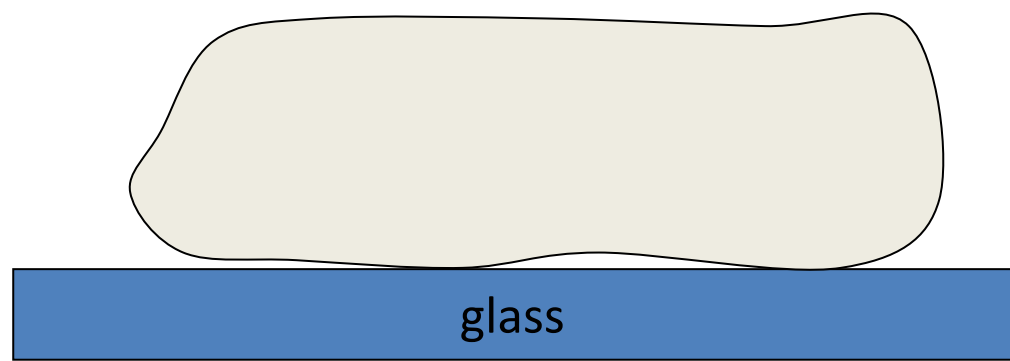
Gold nanoparticles (**AuNP**)
 conjugated with **protein** molecules:
protein = immobilization bridge

3. Bacteria, spores

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



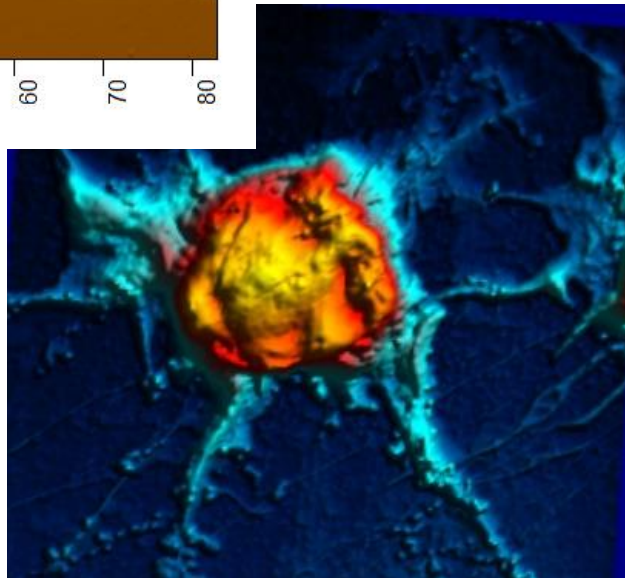
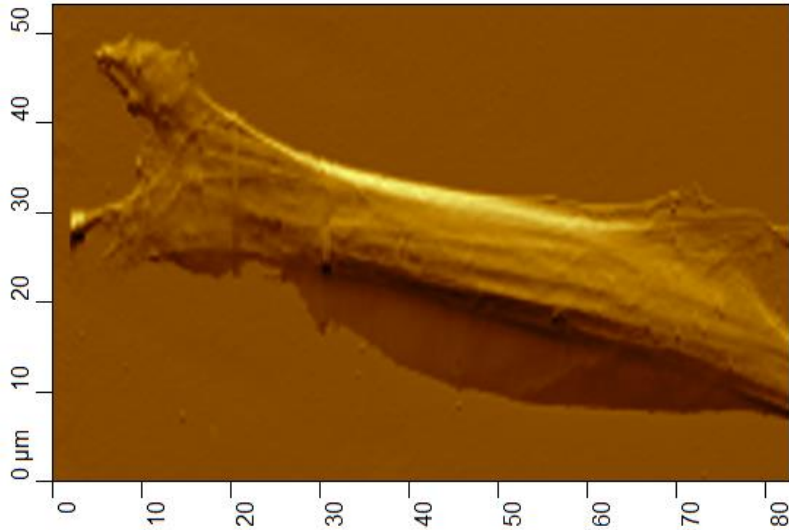
Standard coating on glass



5. Eukaryotic cells

A. Standard culturing on polystyrene dishes

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



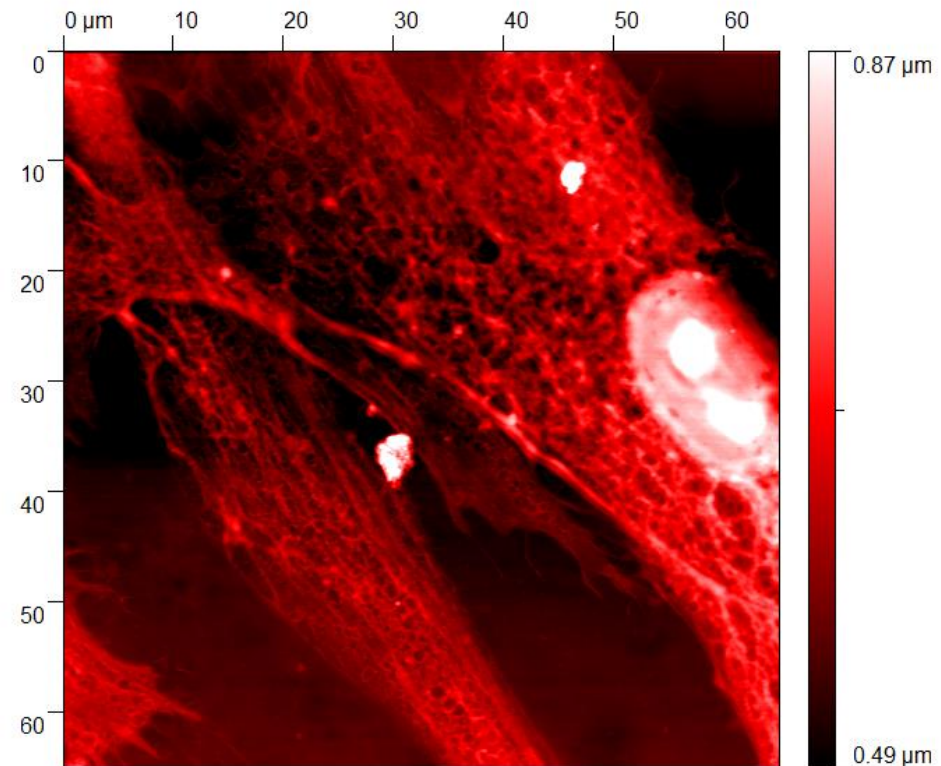
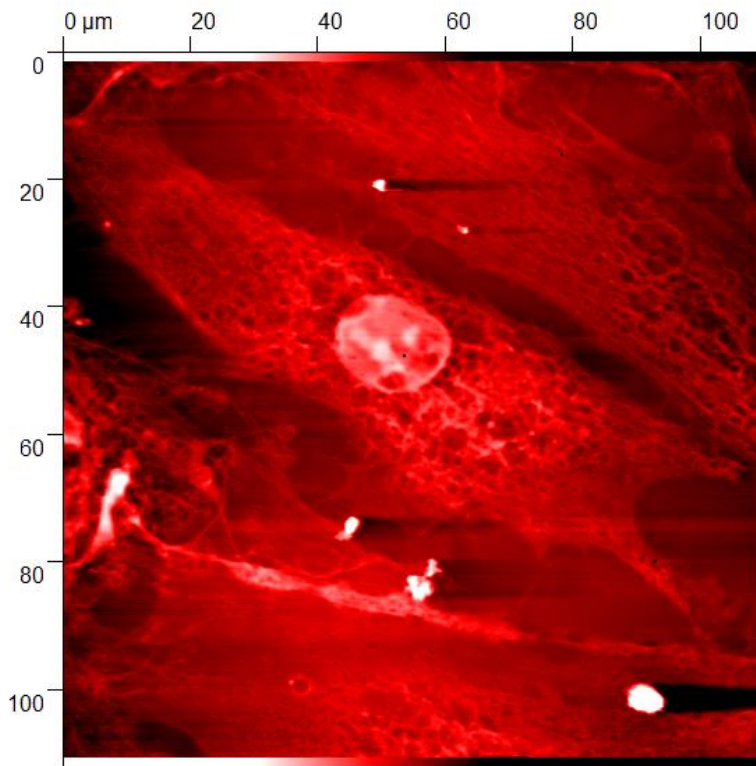
Cell culturing equipment



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

B. Fixation agents

- Adhesion of cells out of incubator (37°C, 5% CO₂) is mostly problematic
- Allows study of cells in long term periods after removal from incubator
- Cell wall destruction
- Example: EtOH, acetic acid, paraformaldehyde, glutardialdehyde





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Thank you for your attention!