Graphite nanopatterning through interaction with macromolecules

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Protein adsorption on surfaces

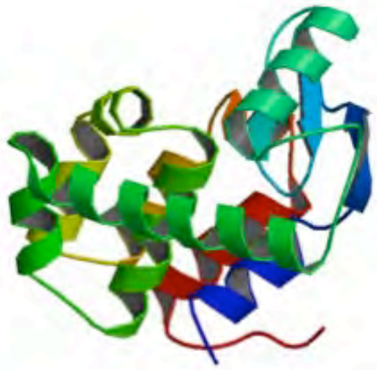
• proteins: polymers characterized by specific intramolecular interactions which result in a well defined 3D structure, essential to ensure protein functionality

• influence of the physico-chemical properties of the surface on the 3D protein structure

➢ HOPG : hydrophobic surface

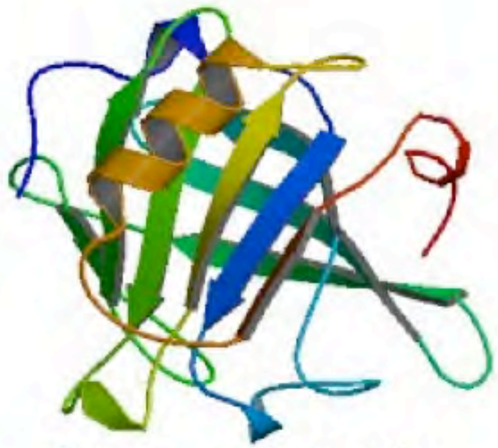
➢ Proteins

insulin



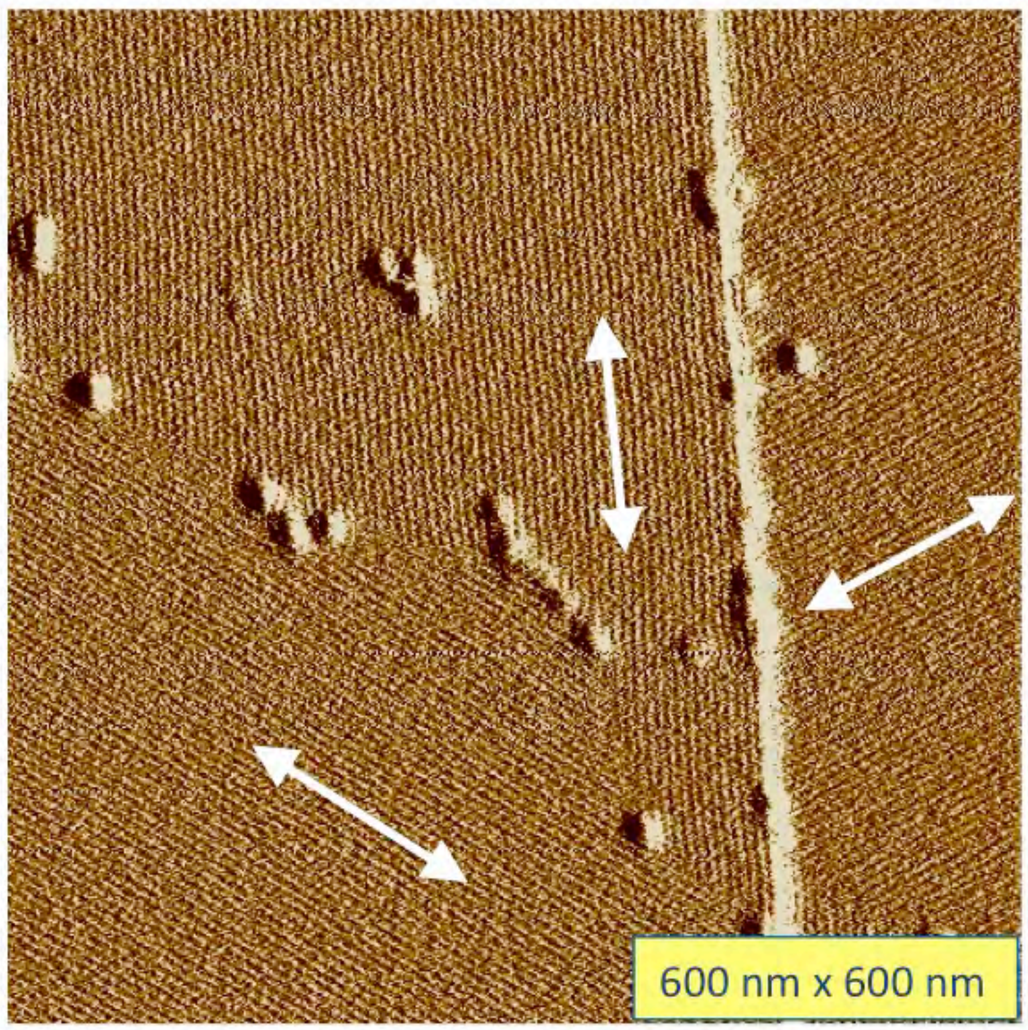
lysozyme

albumin



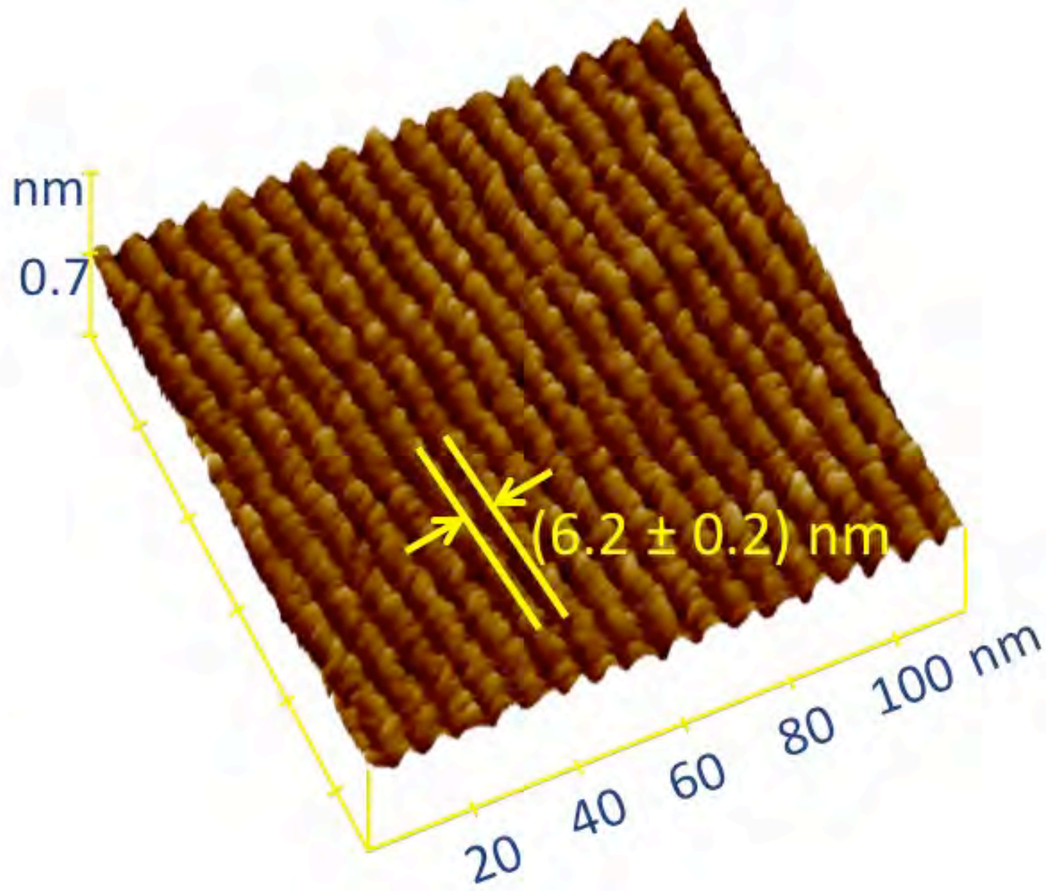
β-lactoglobulin

cytochrome *c*



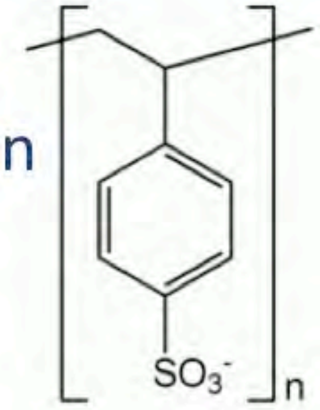
1 hour interaction with a 20 nM lysozyme solution

- ordered domains hundreds of nm in size
- three-fold symmetry
- some globular material

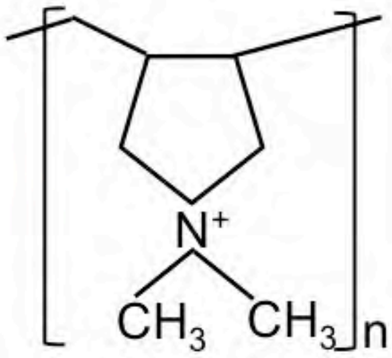


• synthetic polyelectrolytes on HOPG:

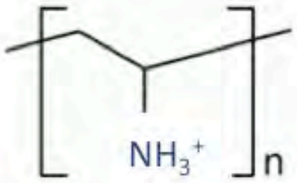
PSS
strong polyanion



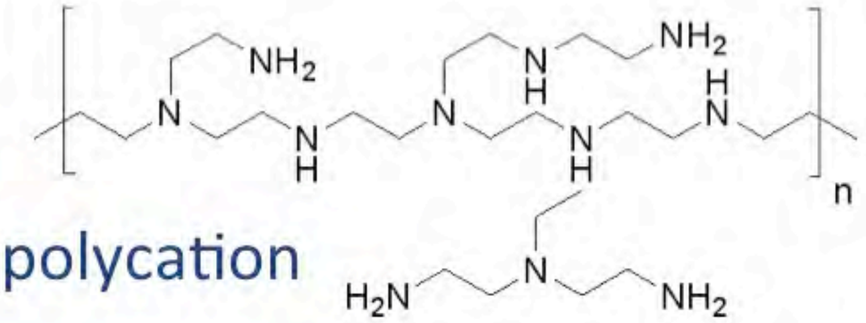
PDMAC
strong polycation



PAH
weak polycation

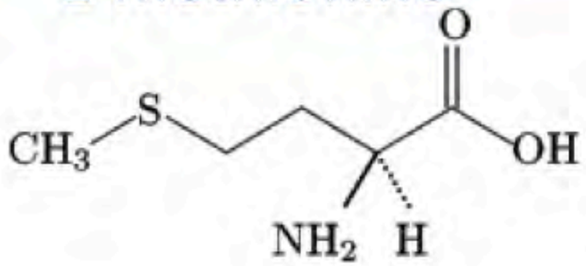


PEI
weak branched polycation

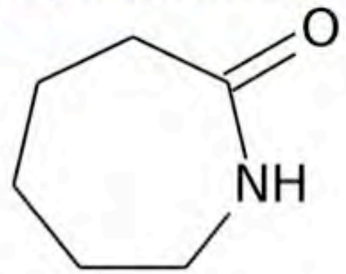


• single monomers on HOPG:

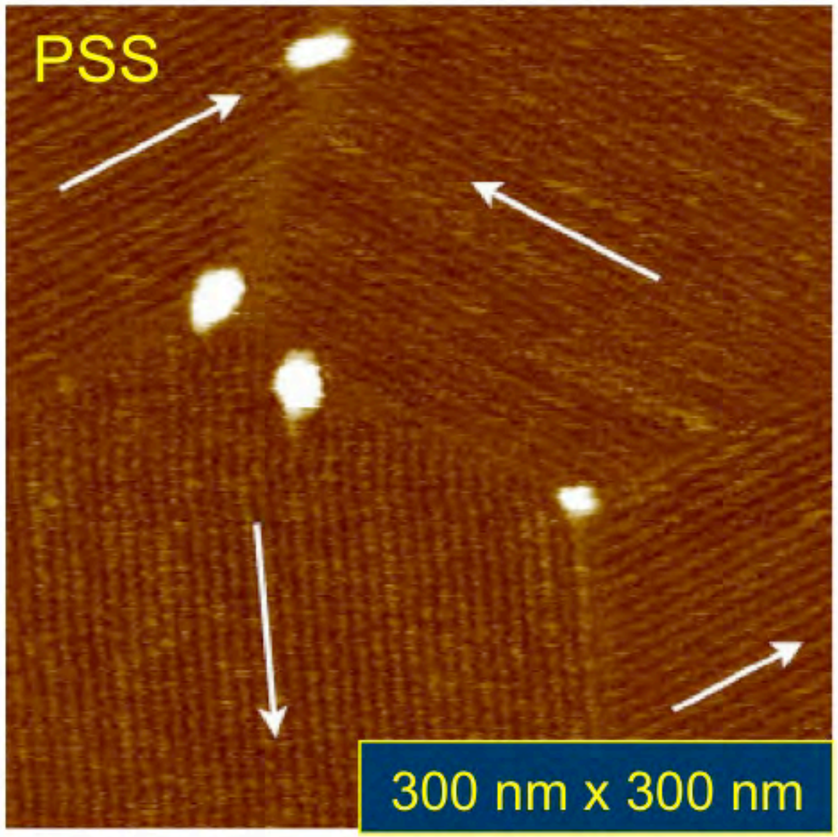
L-methionine

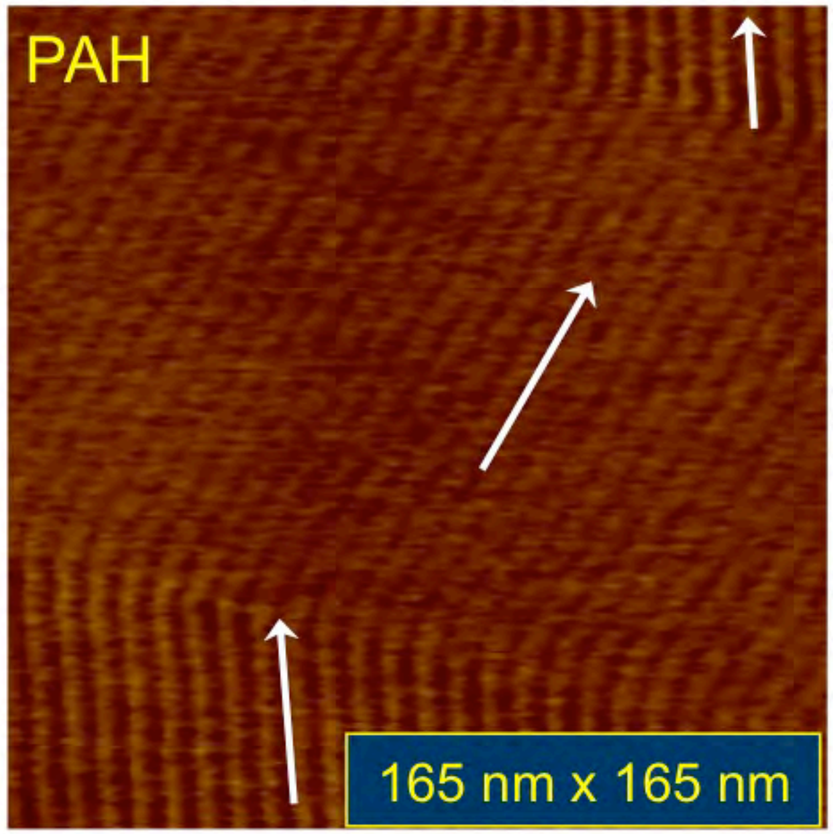


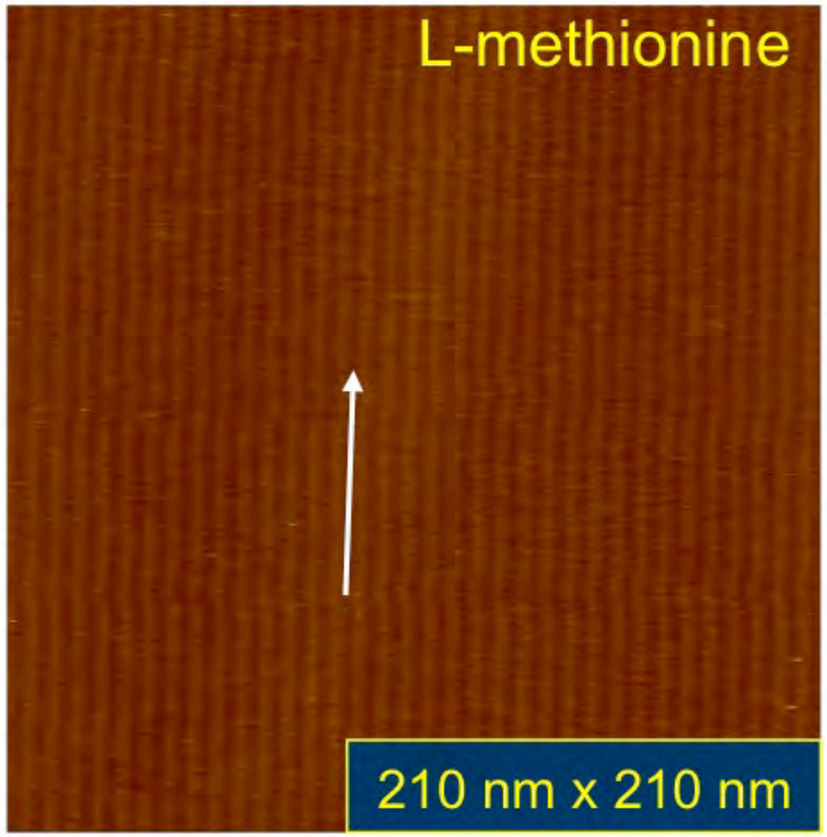
Caprolactam



... as long as **water** is used as **solvent**:



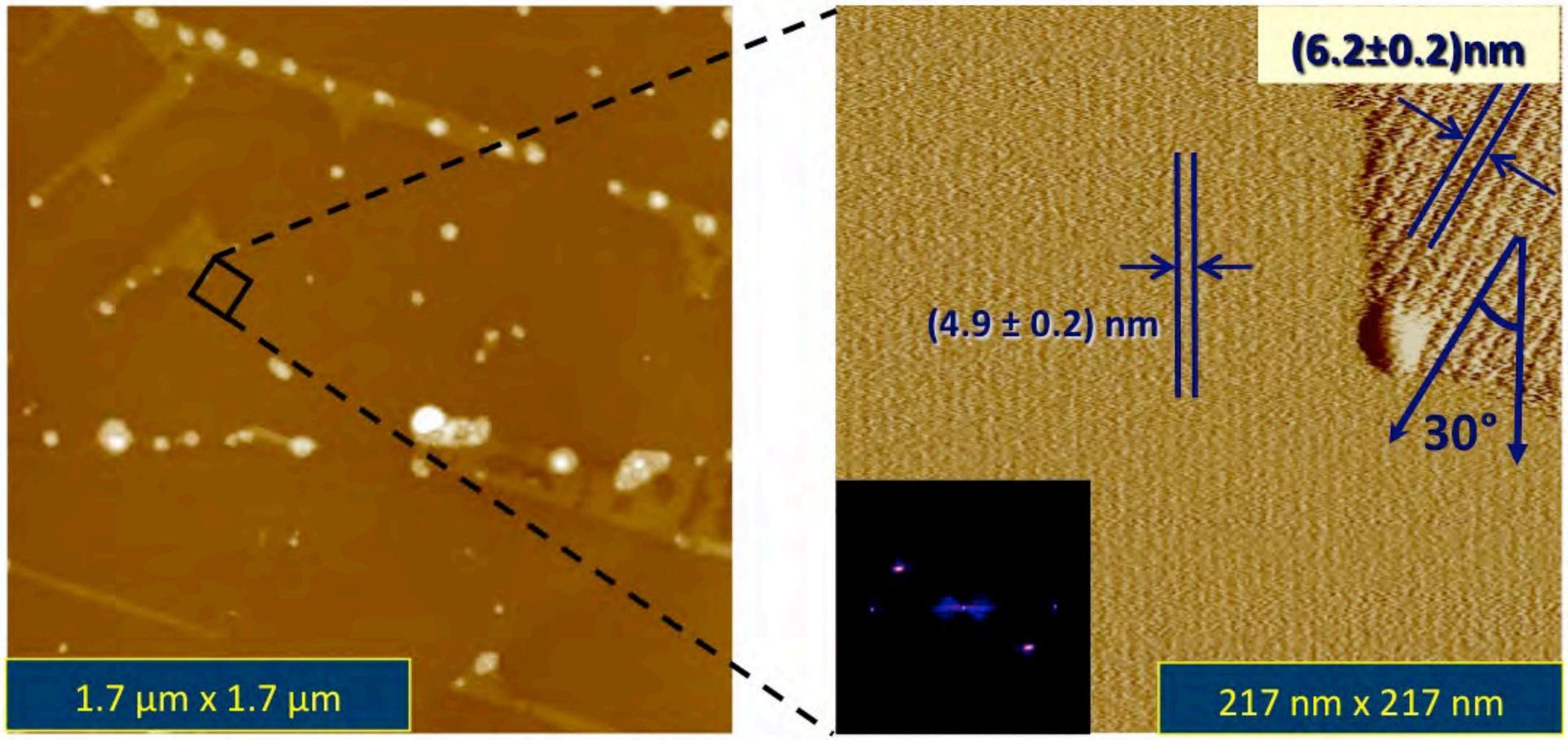




...the **same** striped structure for **all** the investigated molecules

- majority of samples: uniformely covered by patterned domains

- sparsely distributed islands
- a second striped layer characterized by a 4.9 nm periodicity



β-lactoglobulin /HOPG (1 hour interaction with 0.1 nM solution)

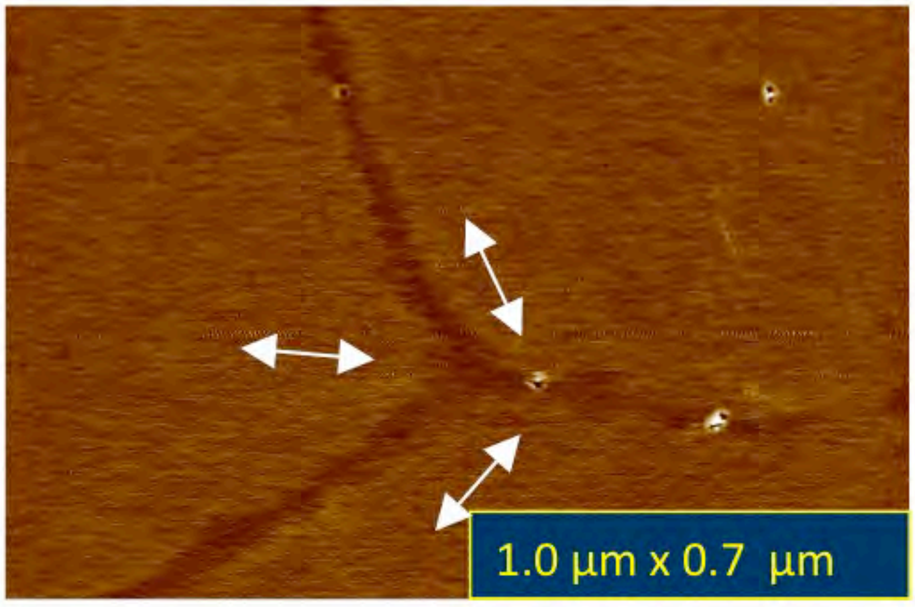
- topmost layer: 6.2 nm periodicity
- second layer: both 6.2 and 4.9 nm periodicities

Origin of the nanopattern?

- nanopattern is formed by **molecules adsorbed** on the surface (a suitable model that can account for all the molecular systems has to be worked out)

- nanopattern is due to a **restructuring** of the graphite surface layers caused by the interaction with solution

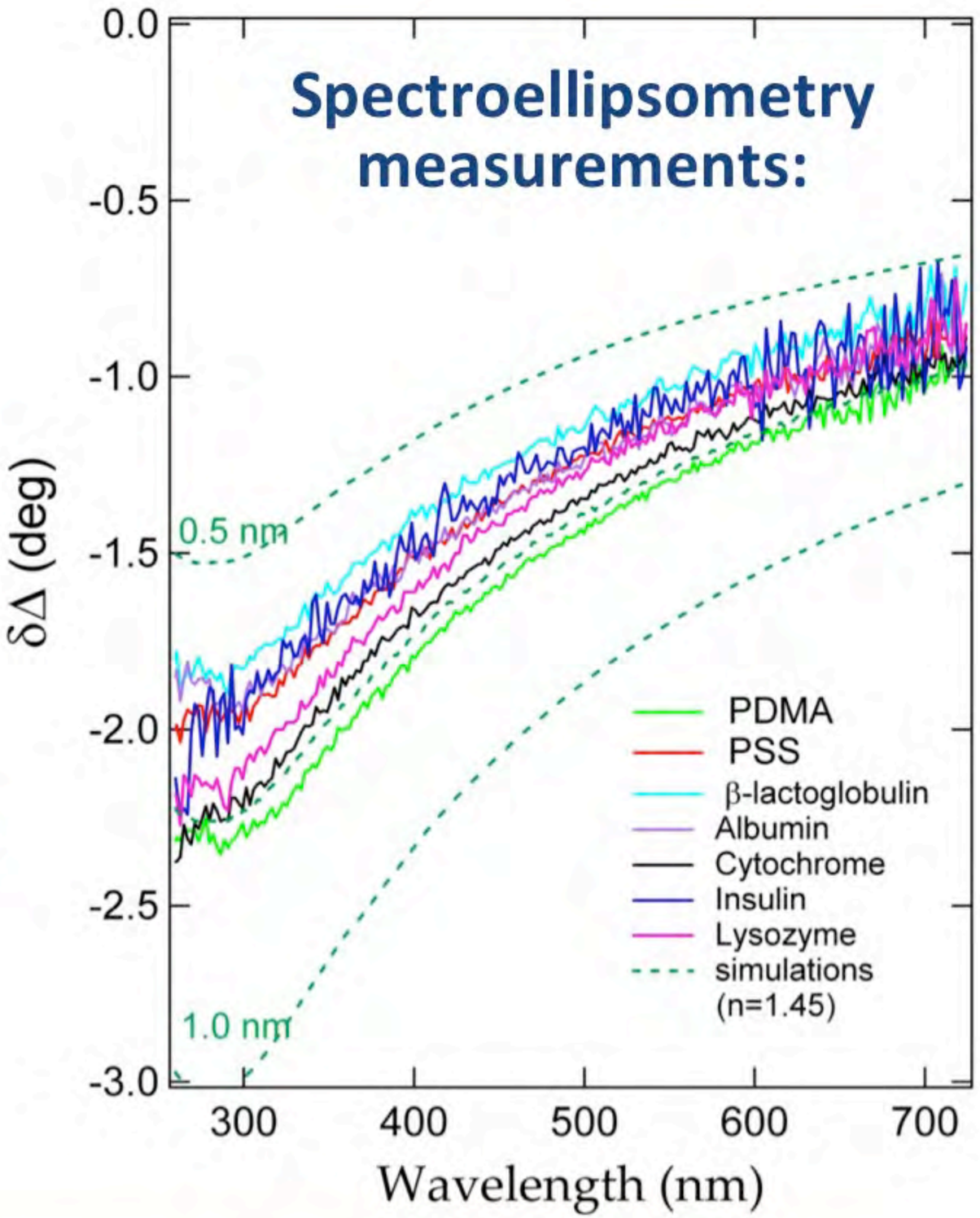
➢ Layer thickness



AFM analysis:

the heights of both upper and lower level striped layers:

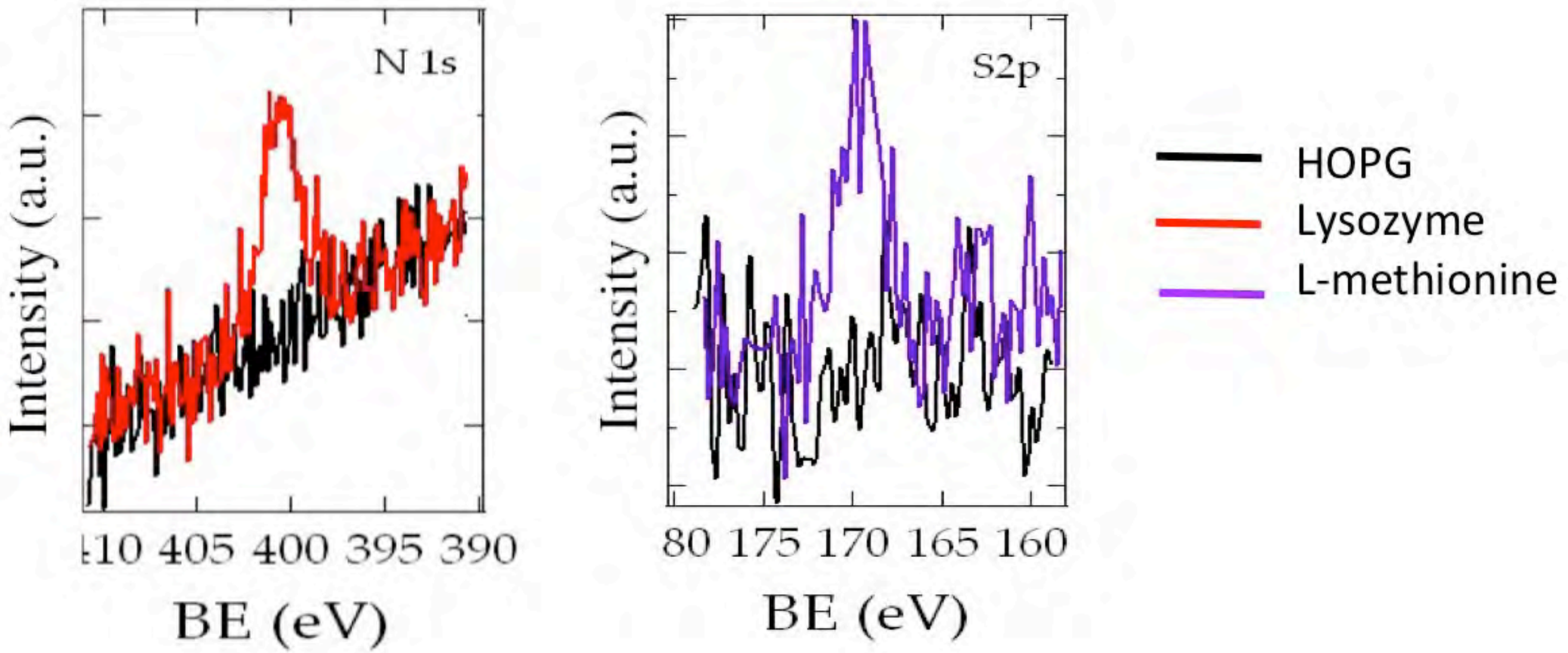
(0.35±0.05) nm



➡ single patterned layer thickness ⟷ single graphite layer

➢ XPS measurements

freshly cleaved HOPG vs. HOPG after interaction with solution



➢ weak molecular-related signals, likely related to globular material

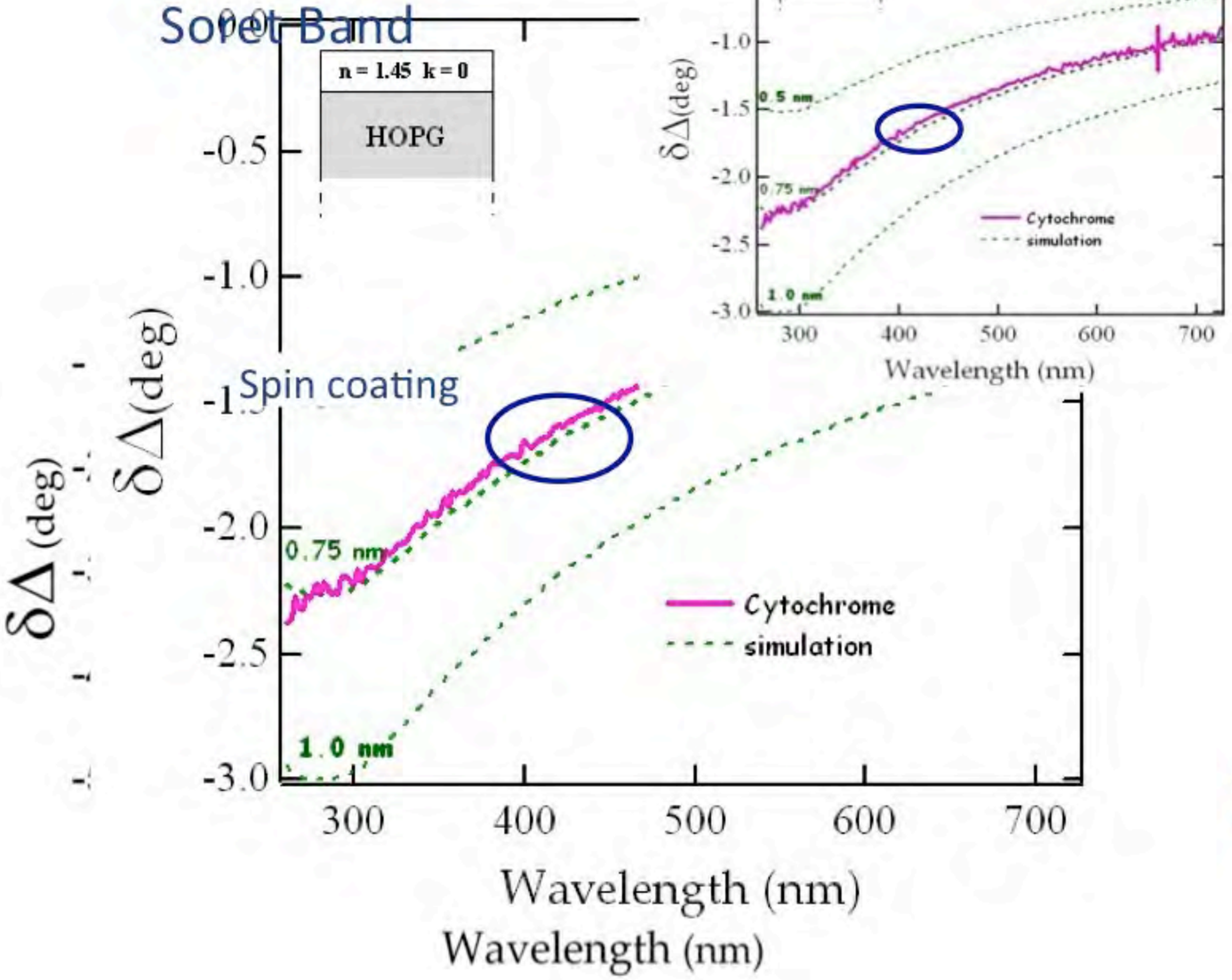
➢ Spectroellipsometry

cytochrome ⟷ porphyrin ⟷ Vis absorption

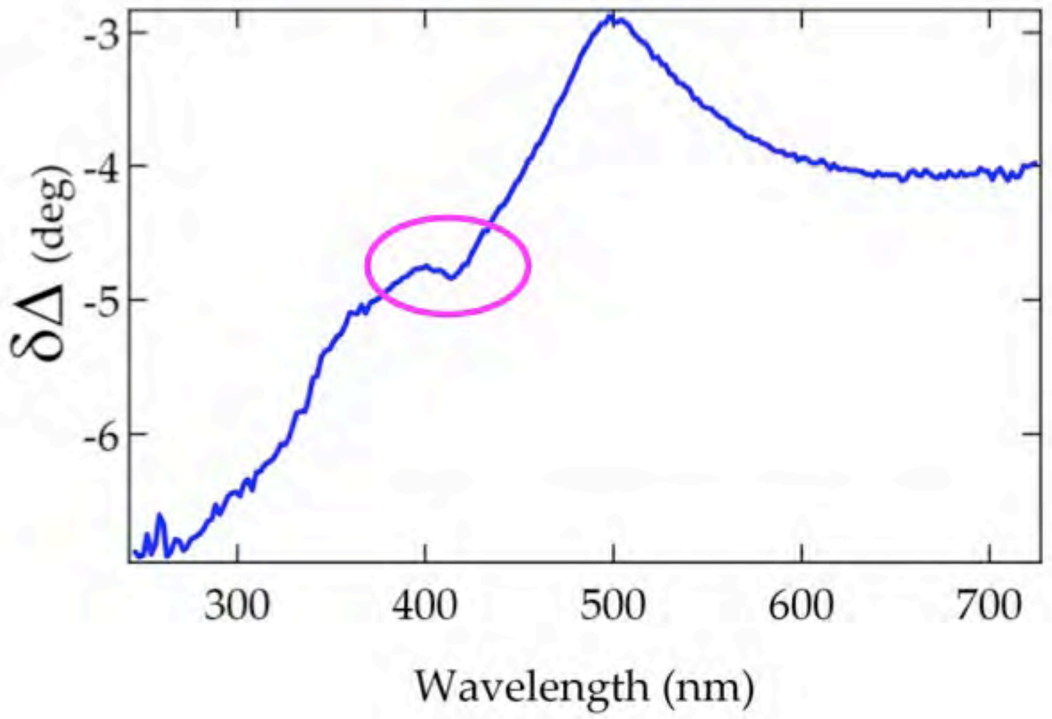
cytochrome/HOPG vs. cytochrome/Au(111)

Spin coating
Absence of Soret Band

Soret Band



Soret Band

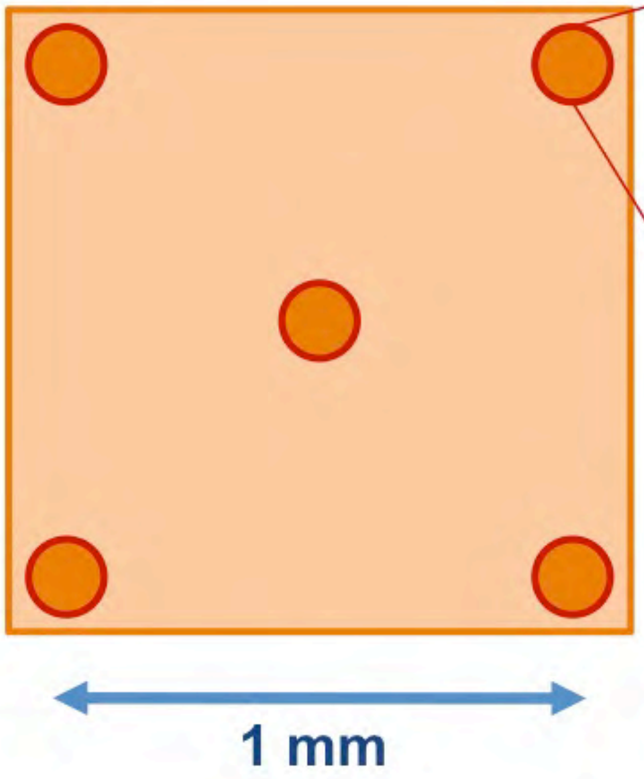


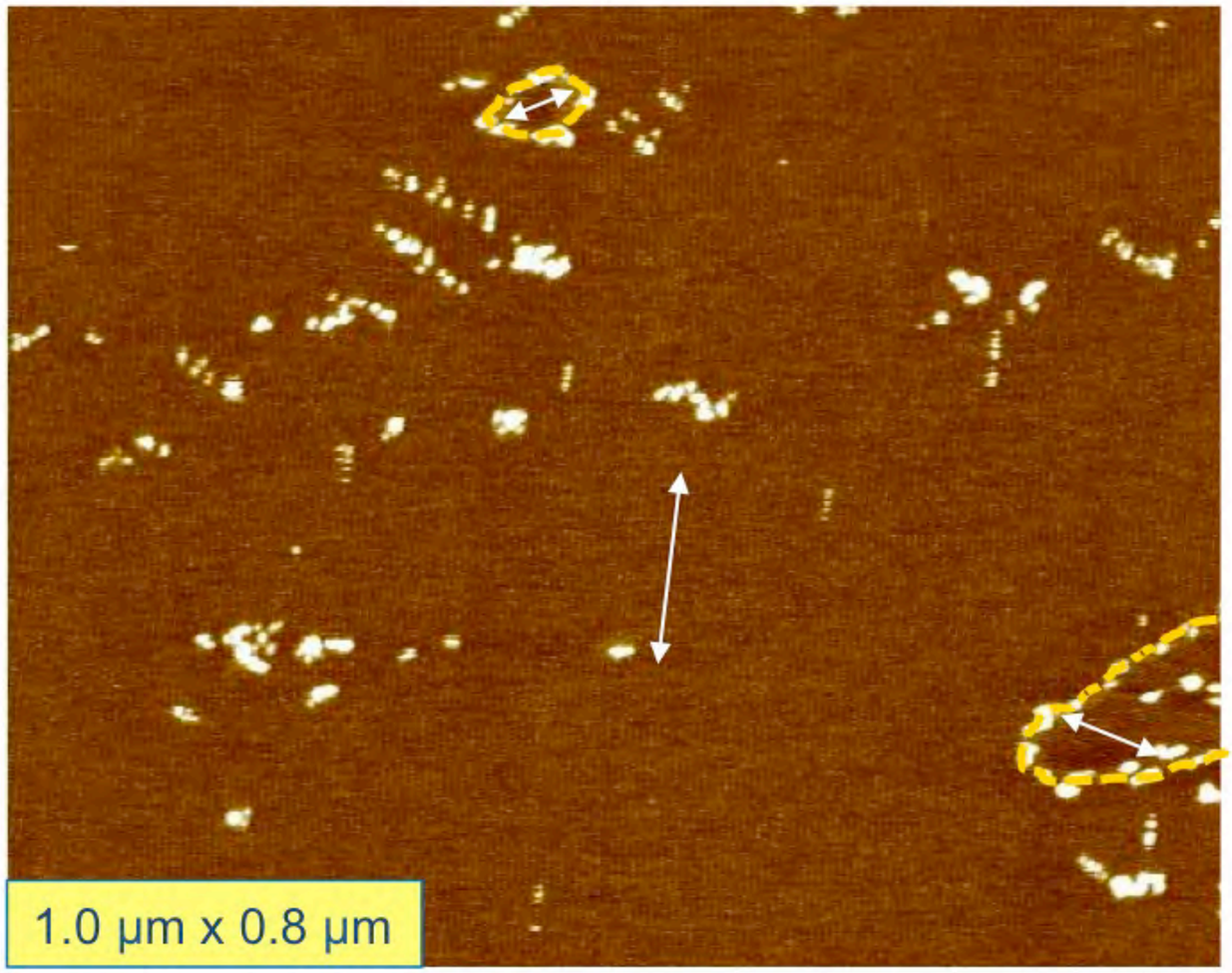
➢ No cytochrome –related absorption on rippled HOPG

- “scratching” experiments

  No way to scrath the pattern by AFM tip

- Single crystal of graphite



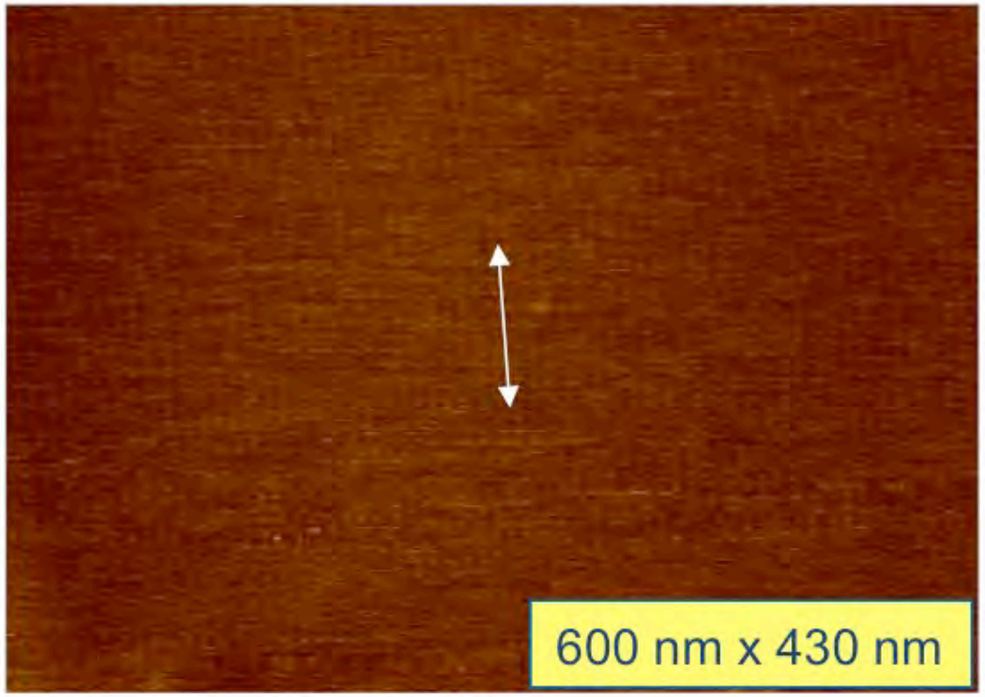


domain orientation preserved over mm distance
→ domain size related to graphite crystallographic quality

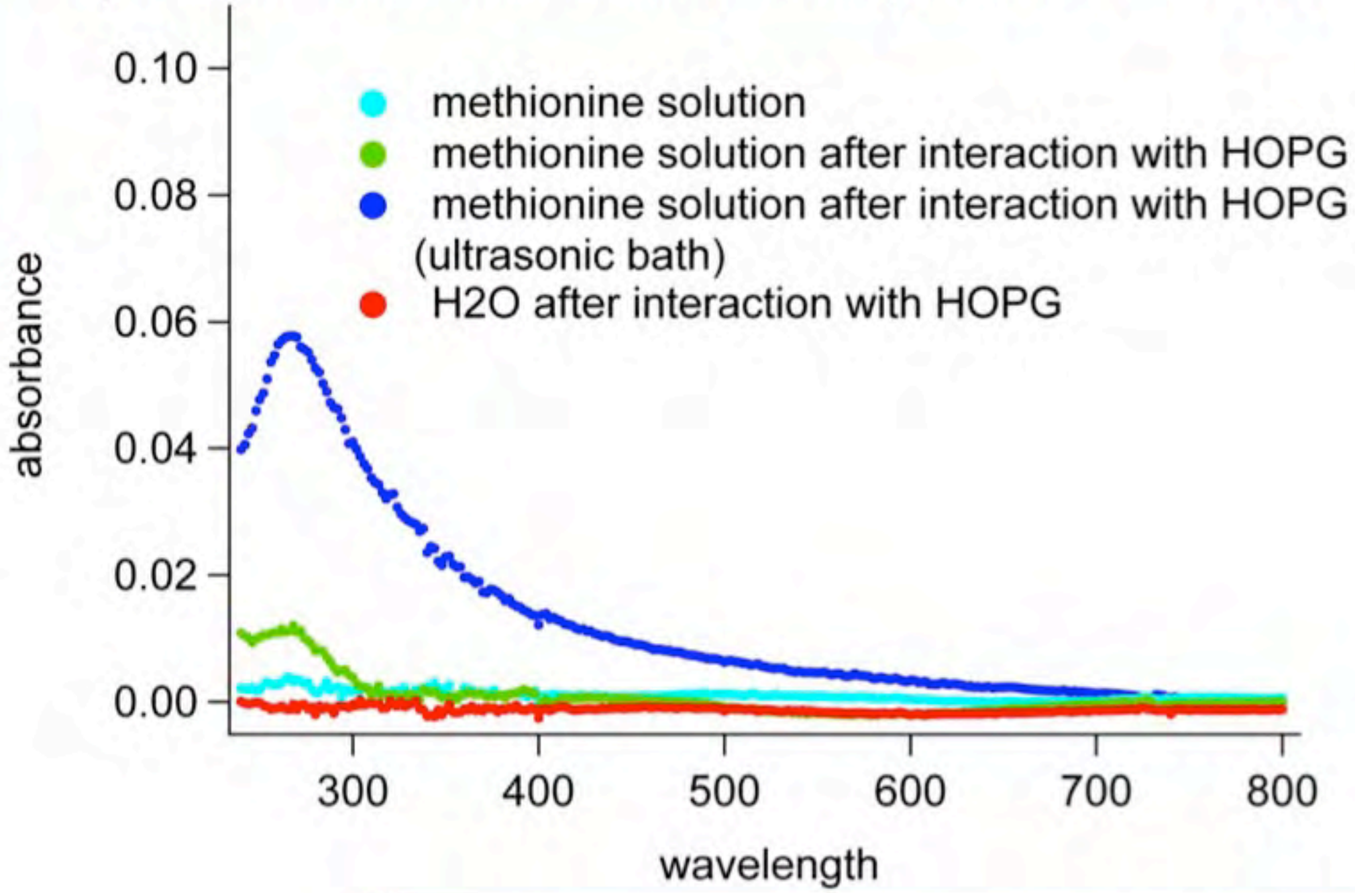
➢Incubation of mechanically cleaved thin HOPG sheets in methionine solution

→ after removal of HOPG sheets from solution

1) HOPG sheets are nanopatterned



2) methionine solution: absorption at 270 nm



…to resume:

Interaction between **aqueous** solutions of **organic molecules** and the **graphite** surface leads to the formation of **nanostructured interfaces**

-- liquid-phase exfoliation of graphene
(Coleman, *Adv. Funct. Mater*. 2009; Laaksonen et al. *Angew. Chem Int. Ed*. 2010)

-- rippling of free-standing and supported graphene
(Meyer et al. *Nature* 2007, Vazquez de Praga et al. PRL 2008, Locatelli et al. *ACSNano* 2010)

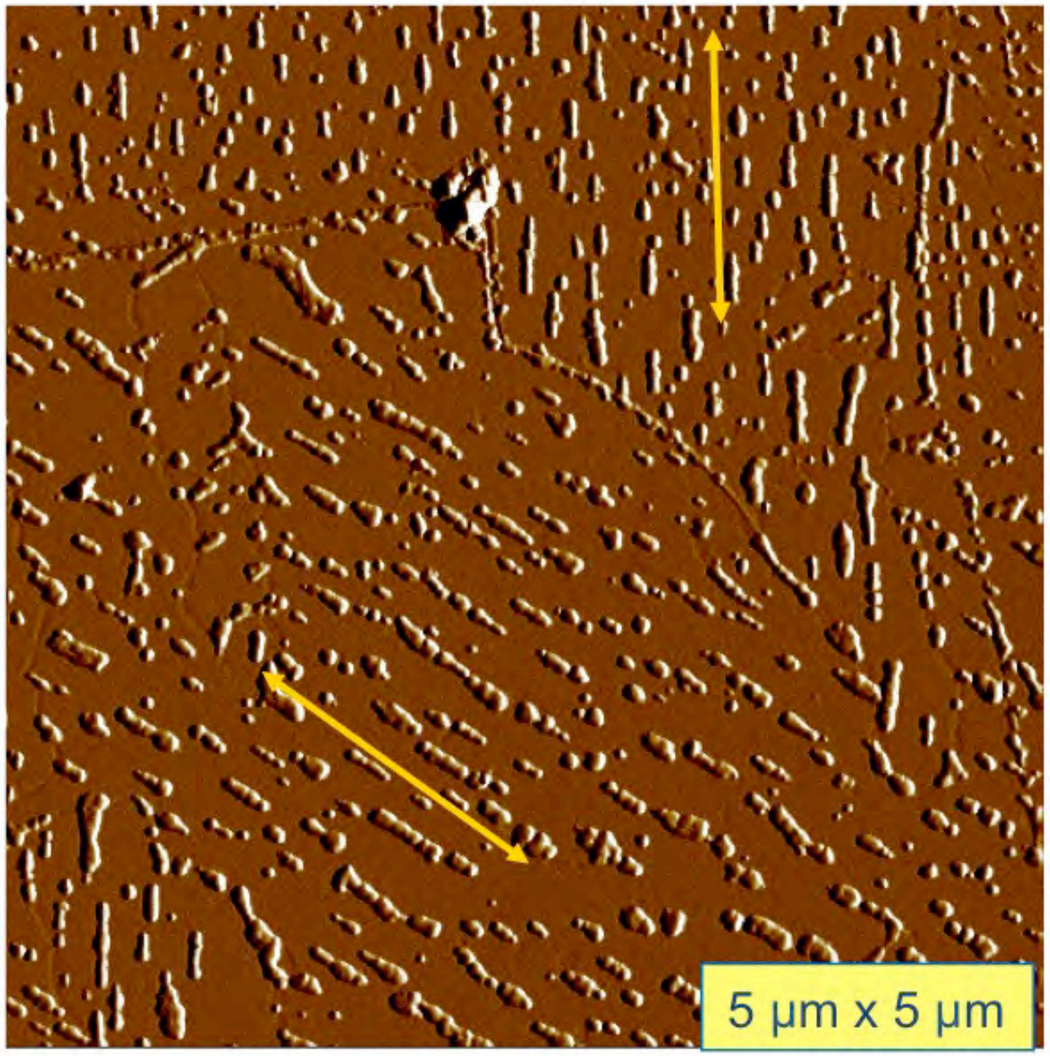
The interaction between the solution and the outermost graphite planes could weaken the interaction between the graphite planes themselves.
These weakly bound layers, "graphene-like" layers, would undergo a rippling process.

➢Nanopatterned layers as an intermediate step toward graphene exfoliation

Nanopatterned HOPG as a template

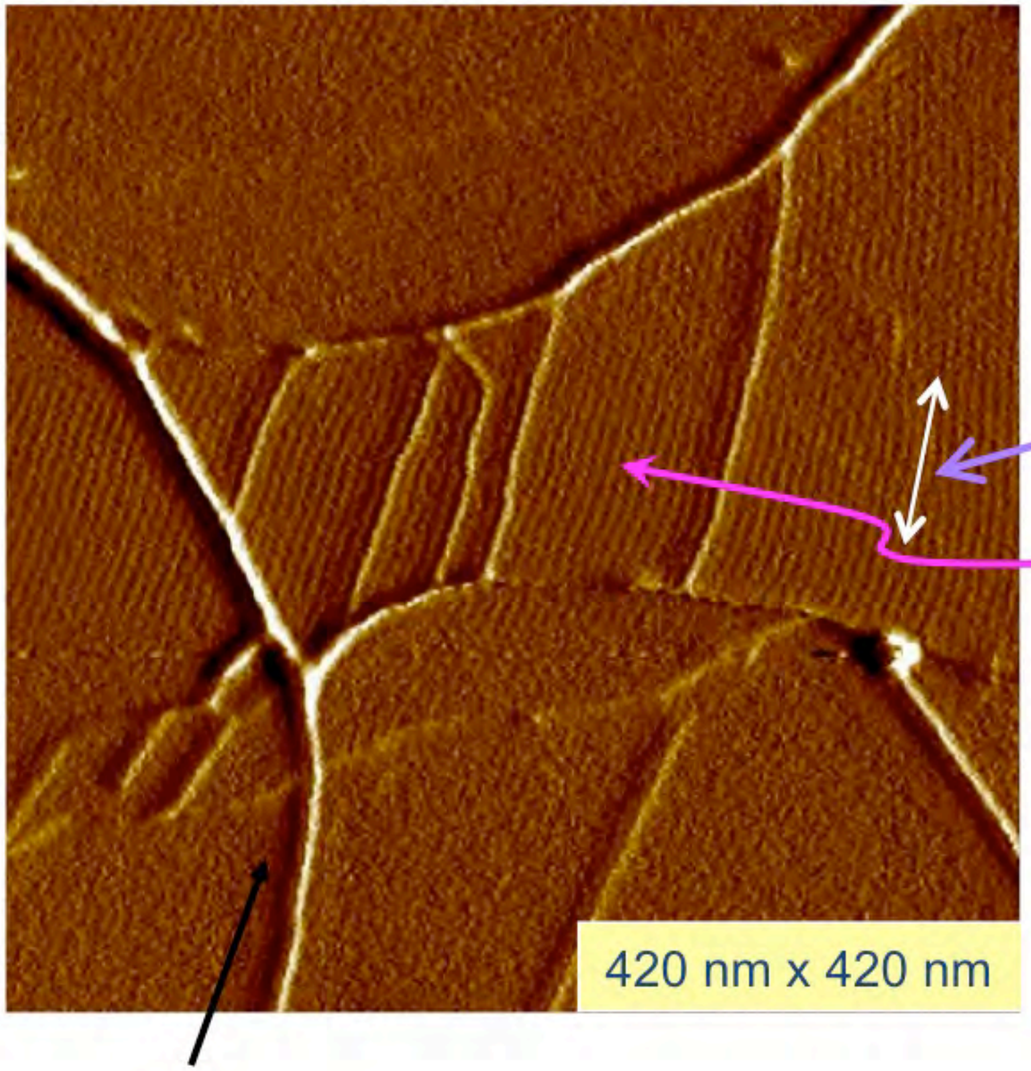
1)Avidin deposition

alignment of avidin molecules along the pattern direction



Nanopatterned HOPG as a template

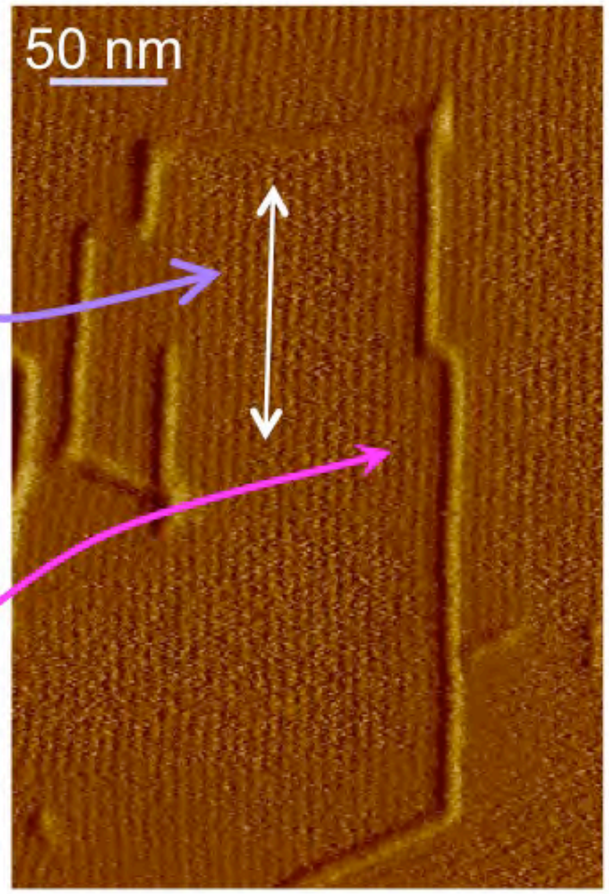
2) Amyloid fibril deposition on patterned HOPG



Mature fibrils
- curved shape
- random orientation

Nanopattern

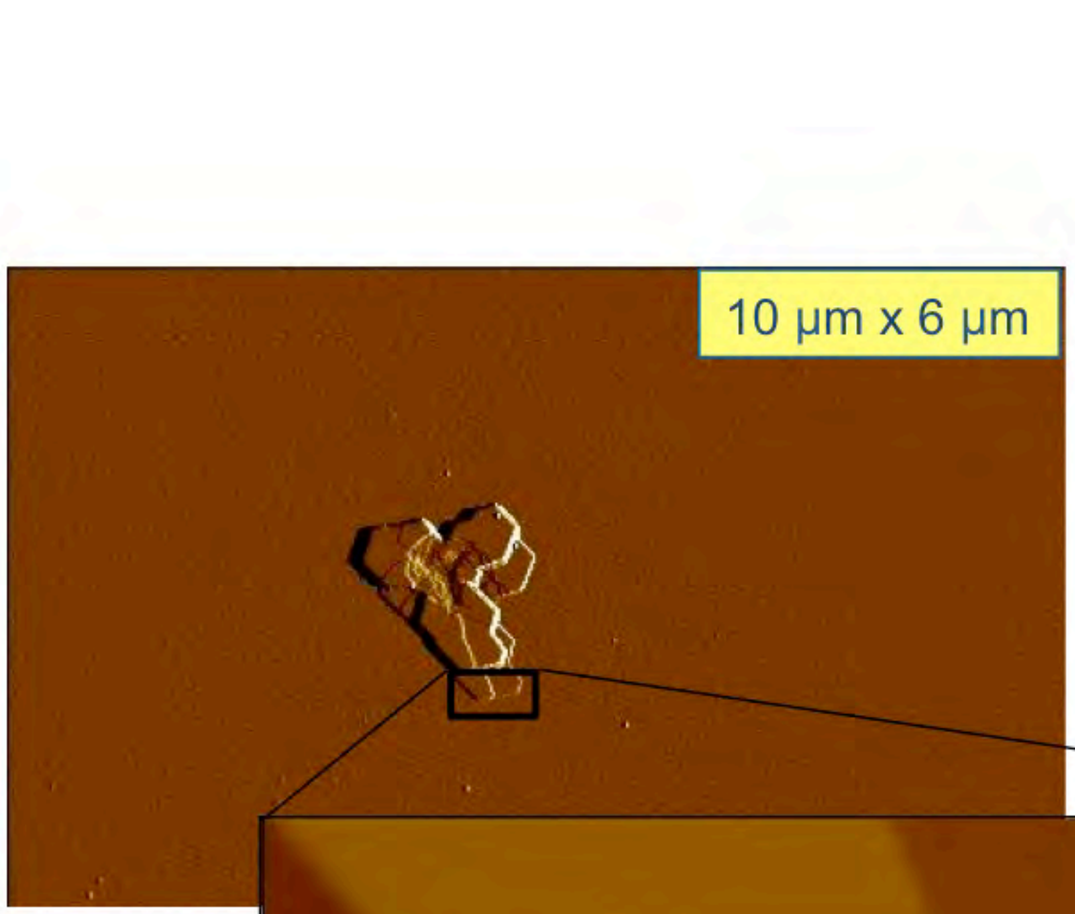
Protofilaments: straight conformation

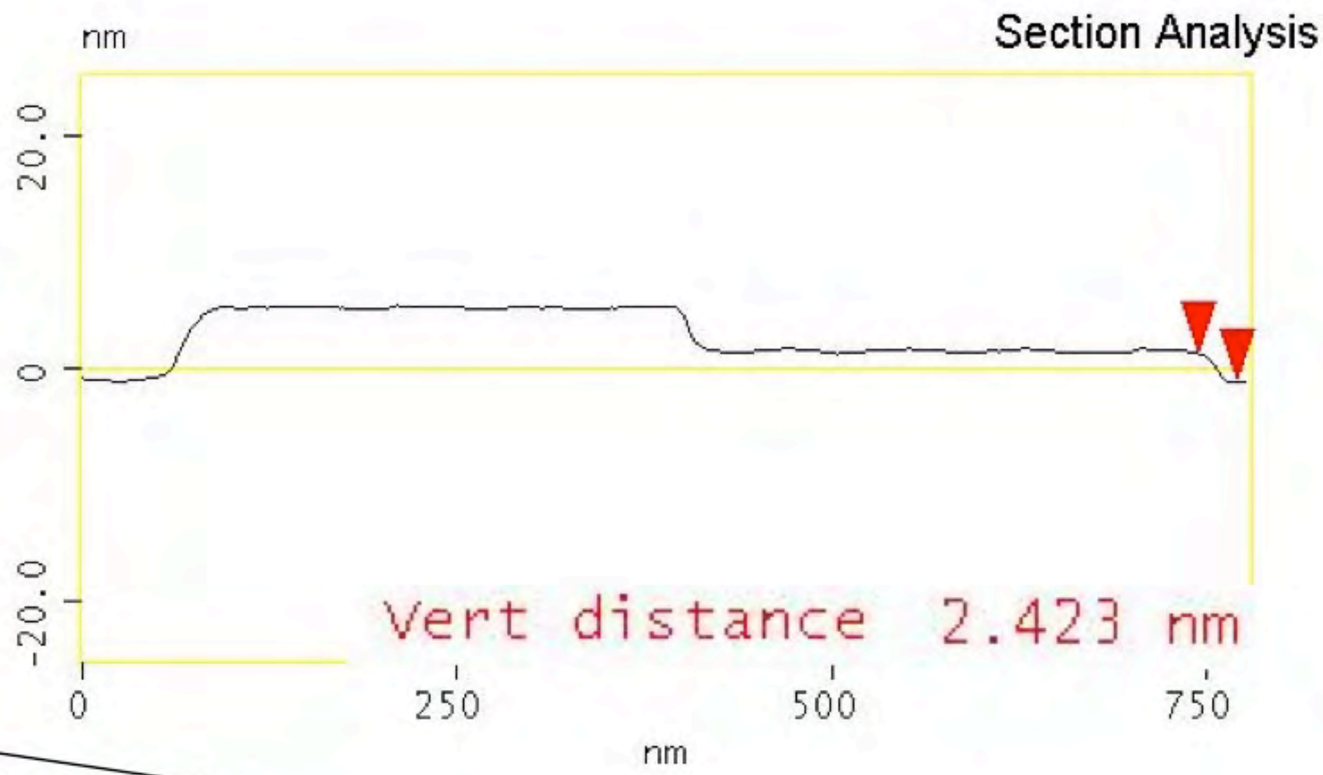


Alignment of functionalized protofilaments: a possible strategy towards biosensor development

Thank you for your attention

Liquid-phase exfoliated sheets deposited on mica

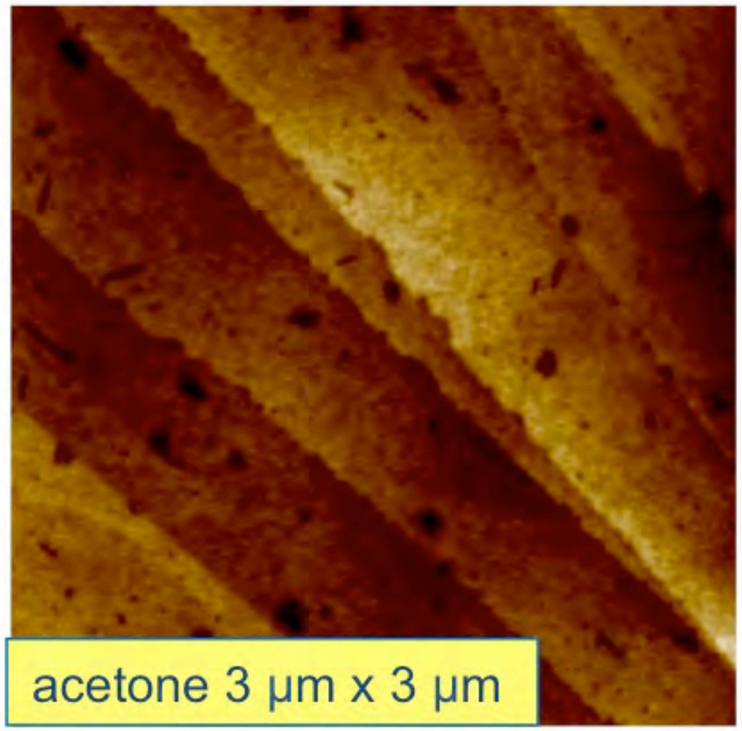




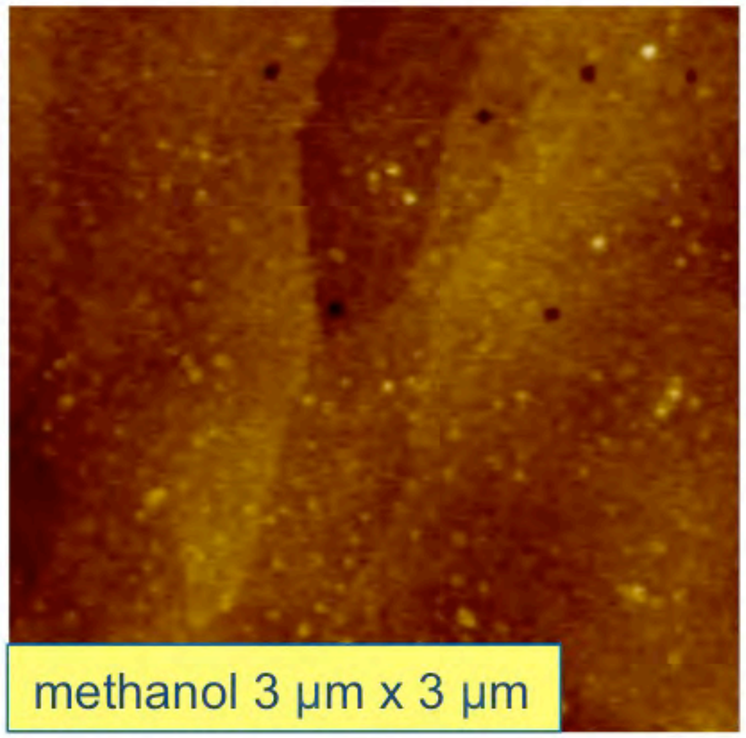
Key role of water as a solvent:

milli-Q water

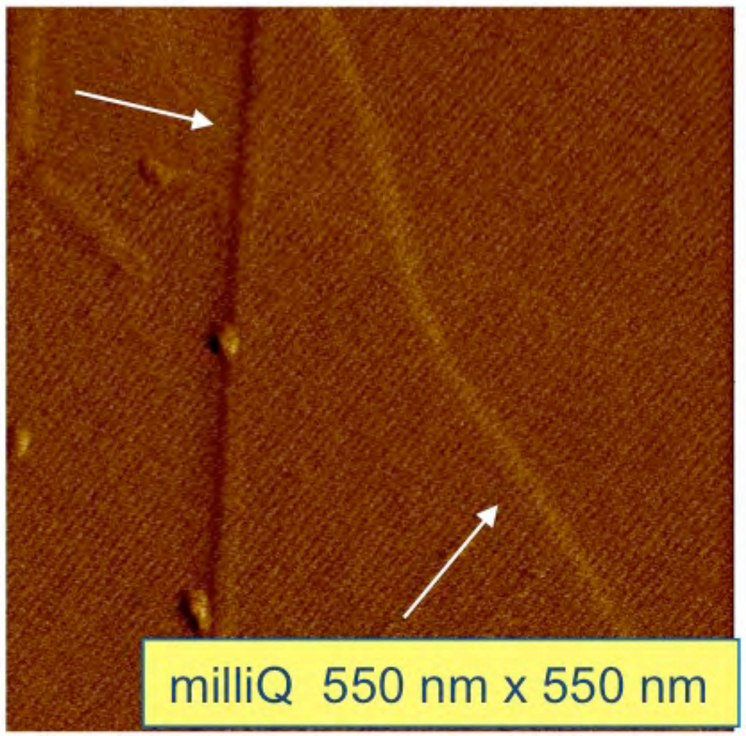
Caprolactam



acetone 3 µm x 3 µm

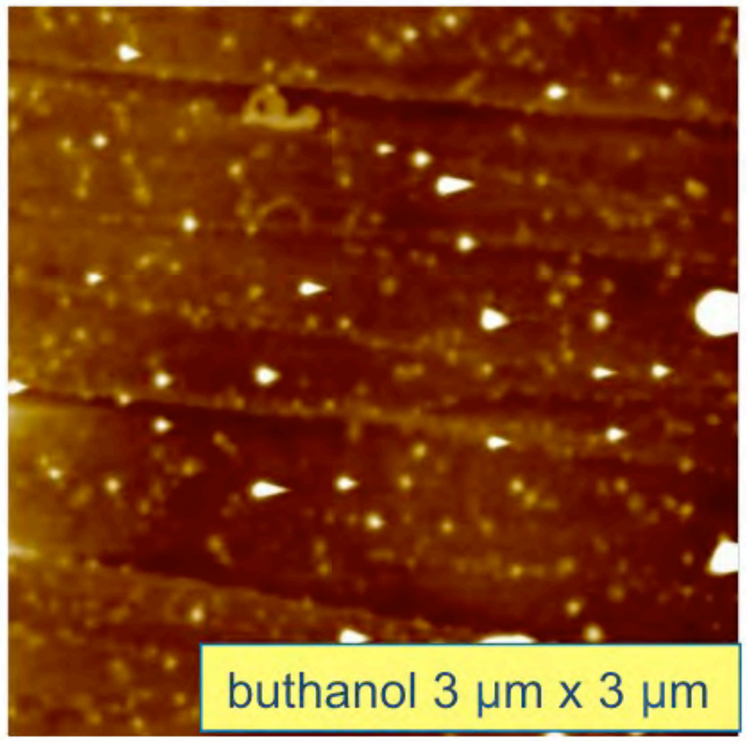


methanol 3 µm x 3 µm

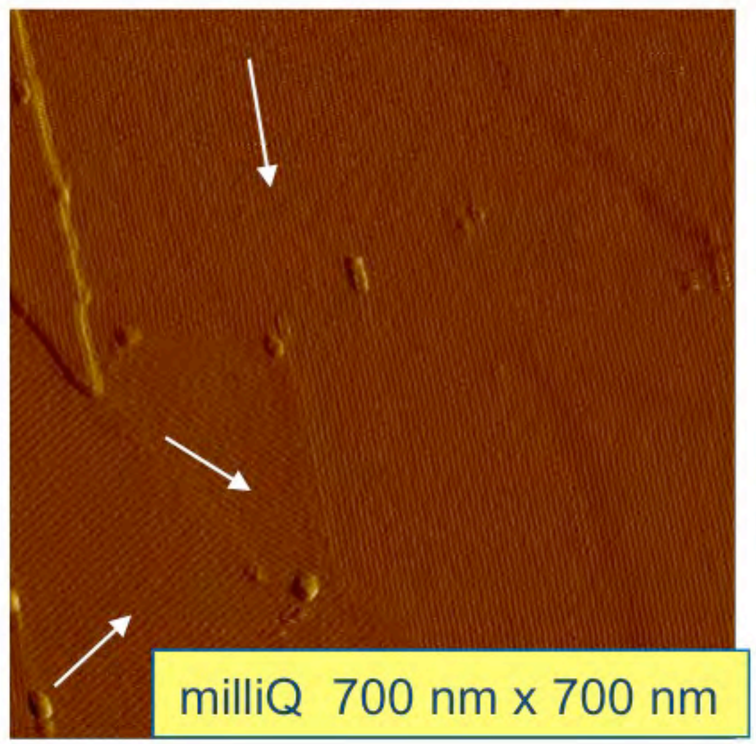


milliQ 550 nm x 550 nm

L-methionine

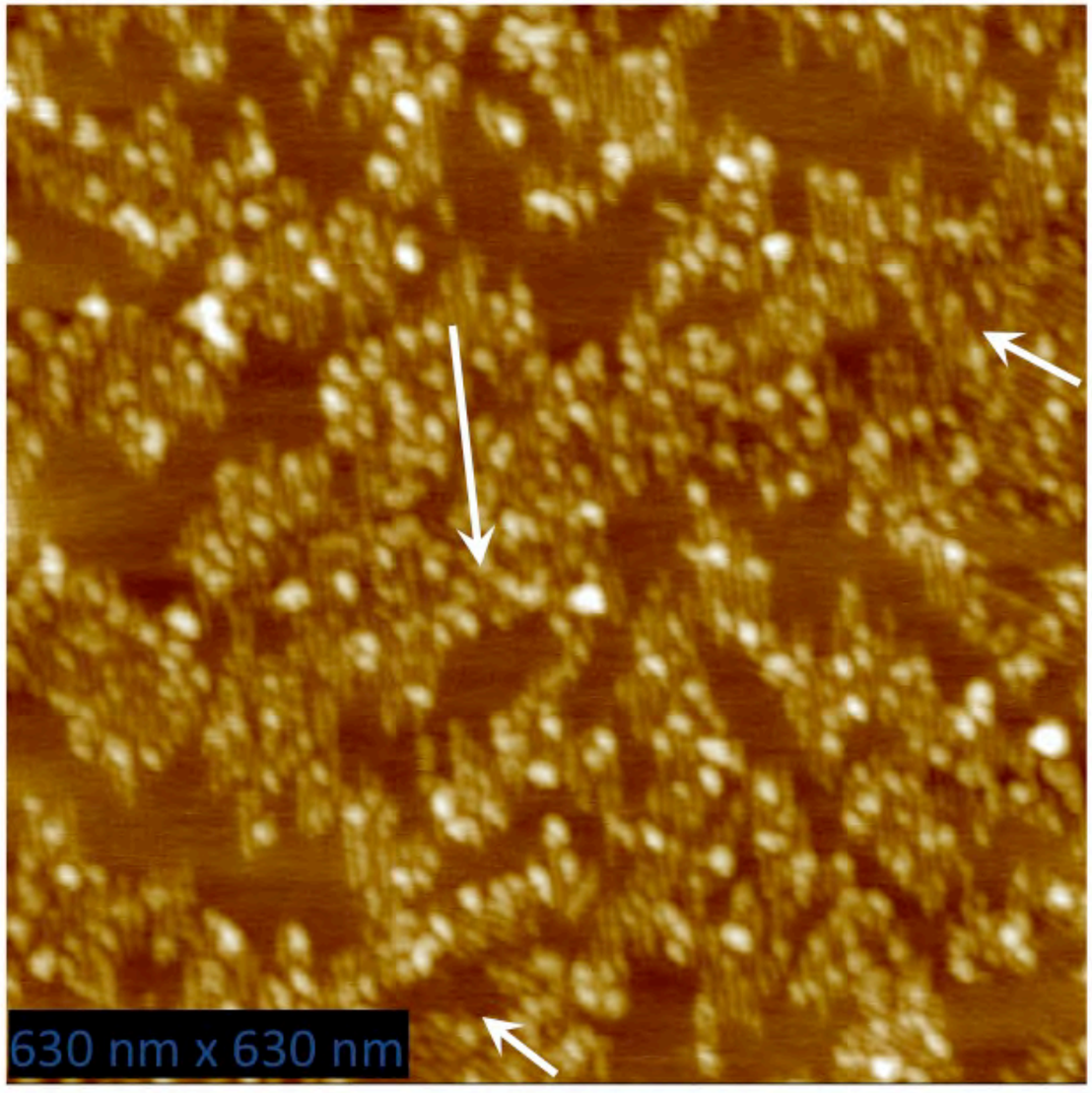


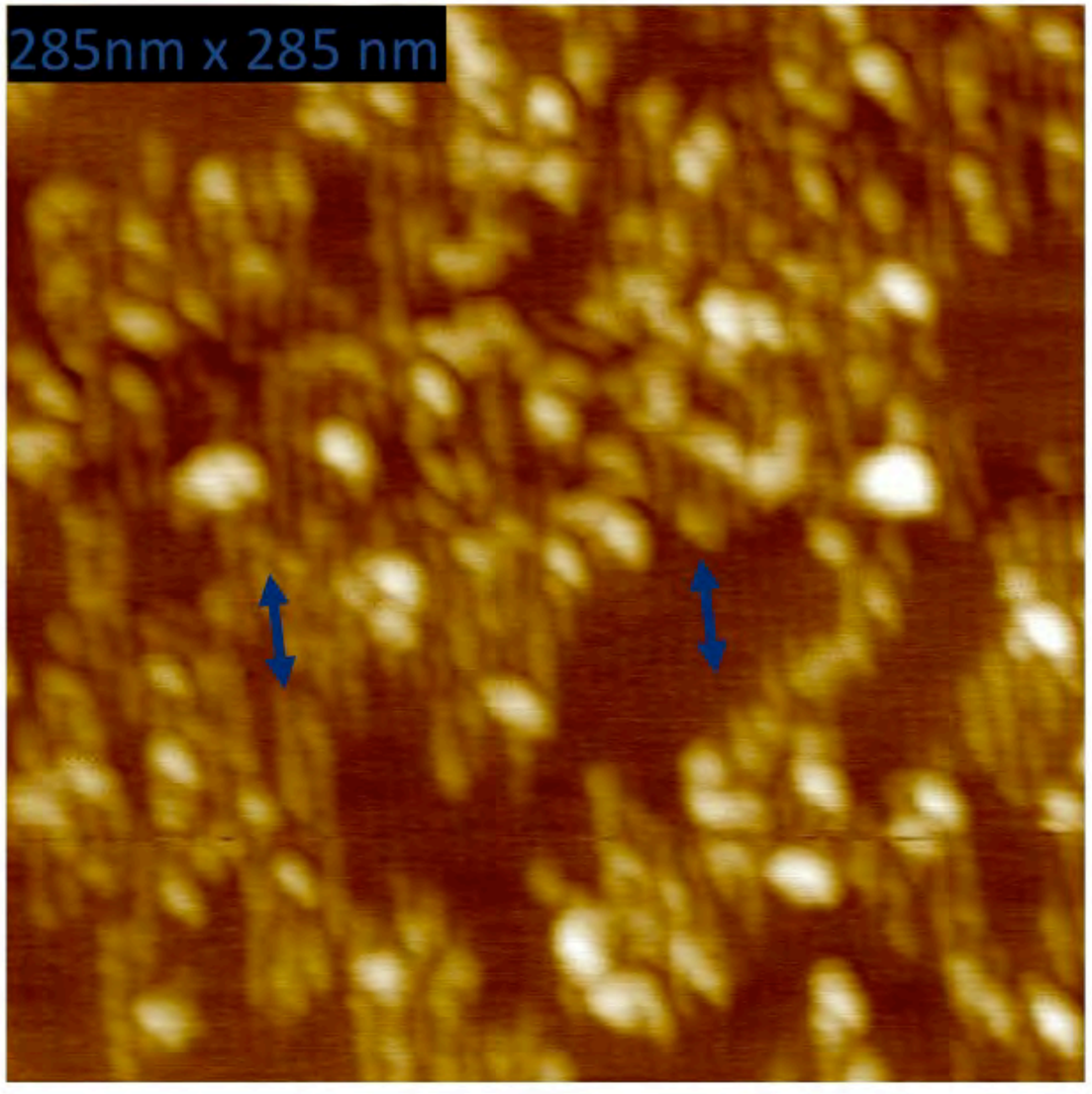
buthanol 3 µm x 3 µm



milliQ 700 nm x 700 nm

PAH at pH 12





rod-like structures:

- length ~ 40 nm
- thickness ~ 0.45 nm

PAH in an almost extended configuration aligned on nanopattern

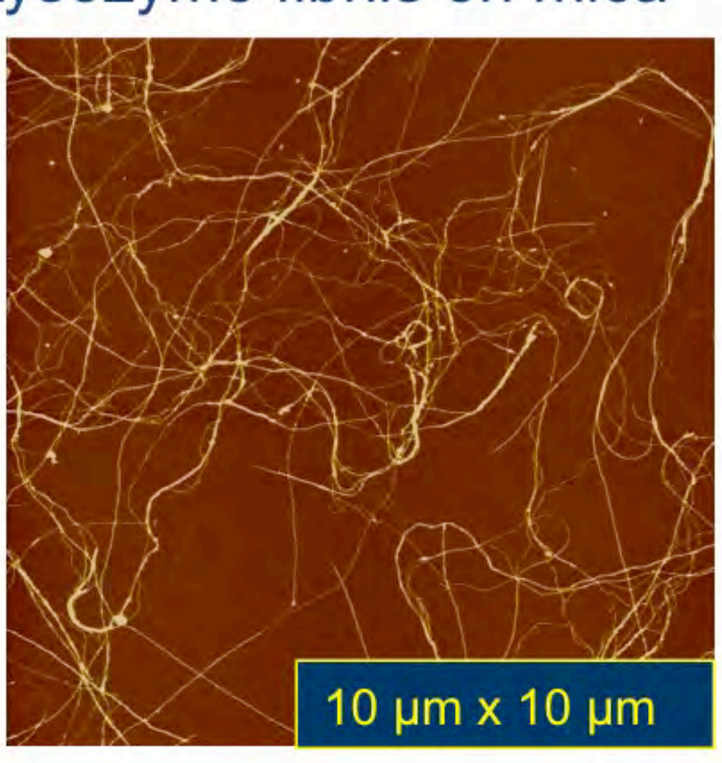
Amyloid fibril adsorption on nanopatterned HOPG

Two-fold interest of amyloid fibrils:

-biomedical
important pathologies are related to amyloid fibril deposition in tissues

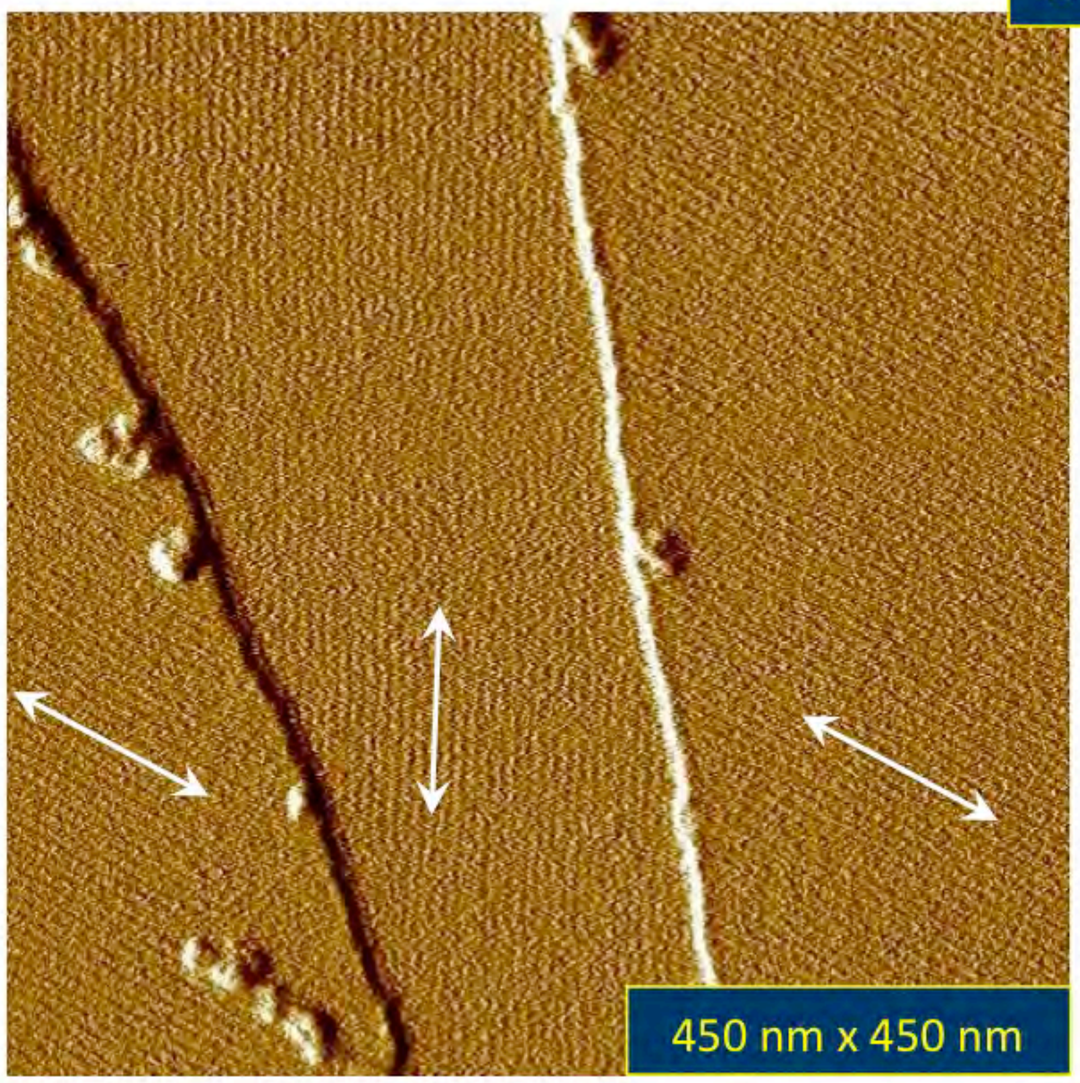
-nanotechnological
fibrils as new materials (high mechanical strength, scaffolds for conductive nanowires….)

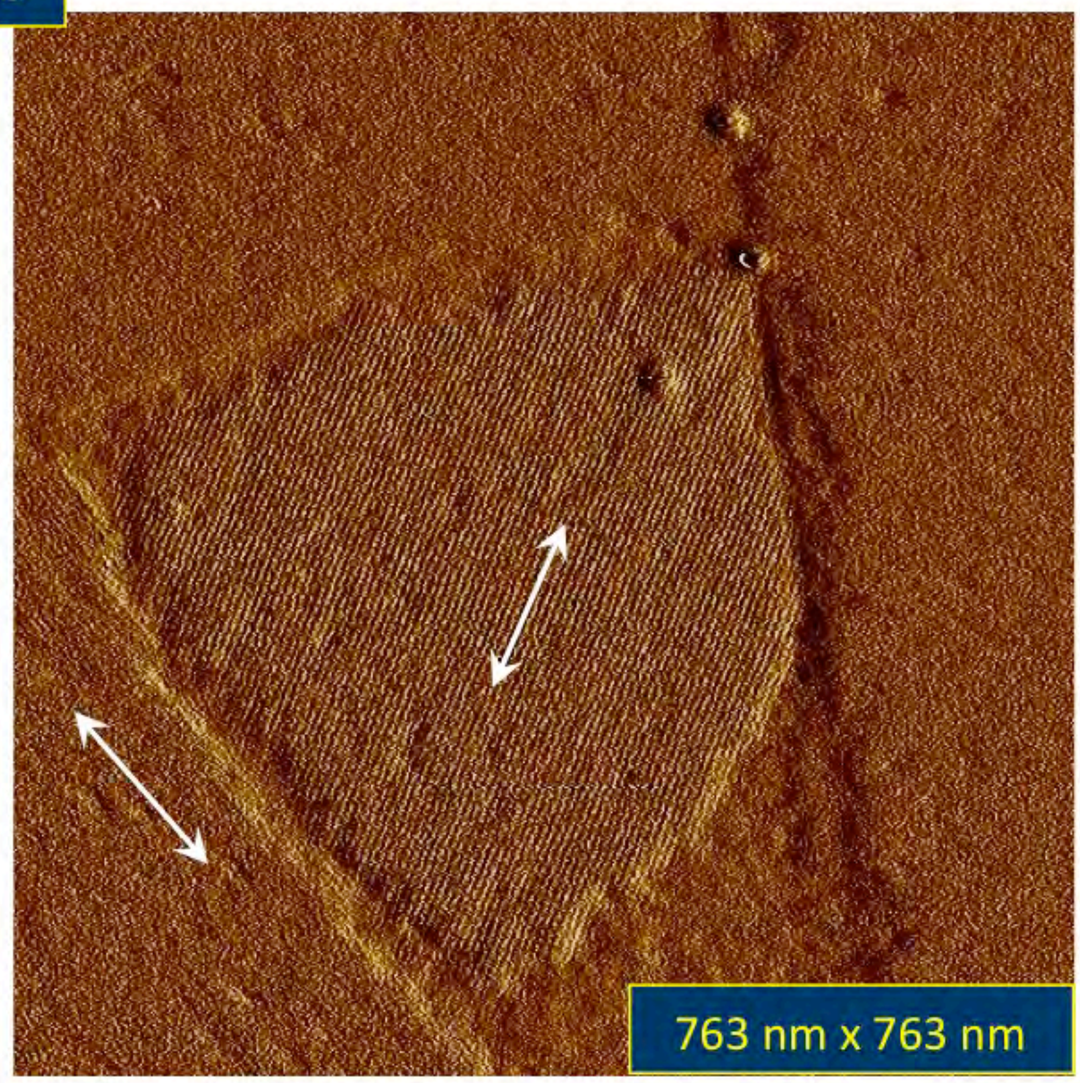
Lysozyme fibrils on mica



Effect of interaction time

Insulin, lysozyme and β-lactoglobulin after 1 minute interaction: coexistence of well ordered domains with domains "under construction"



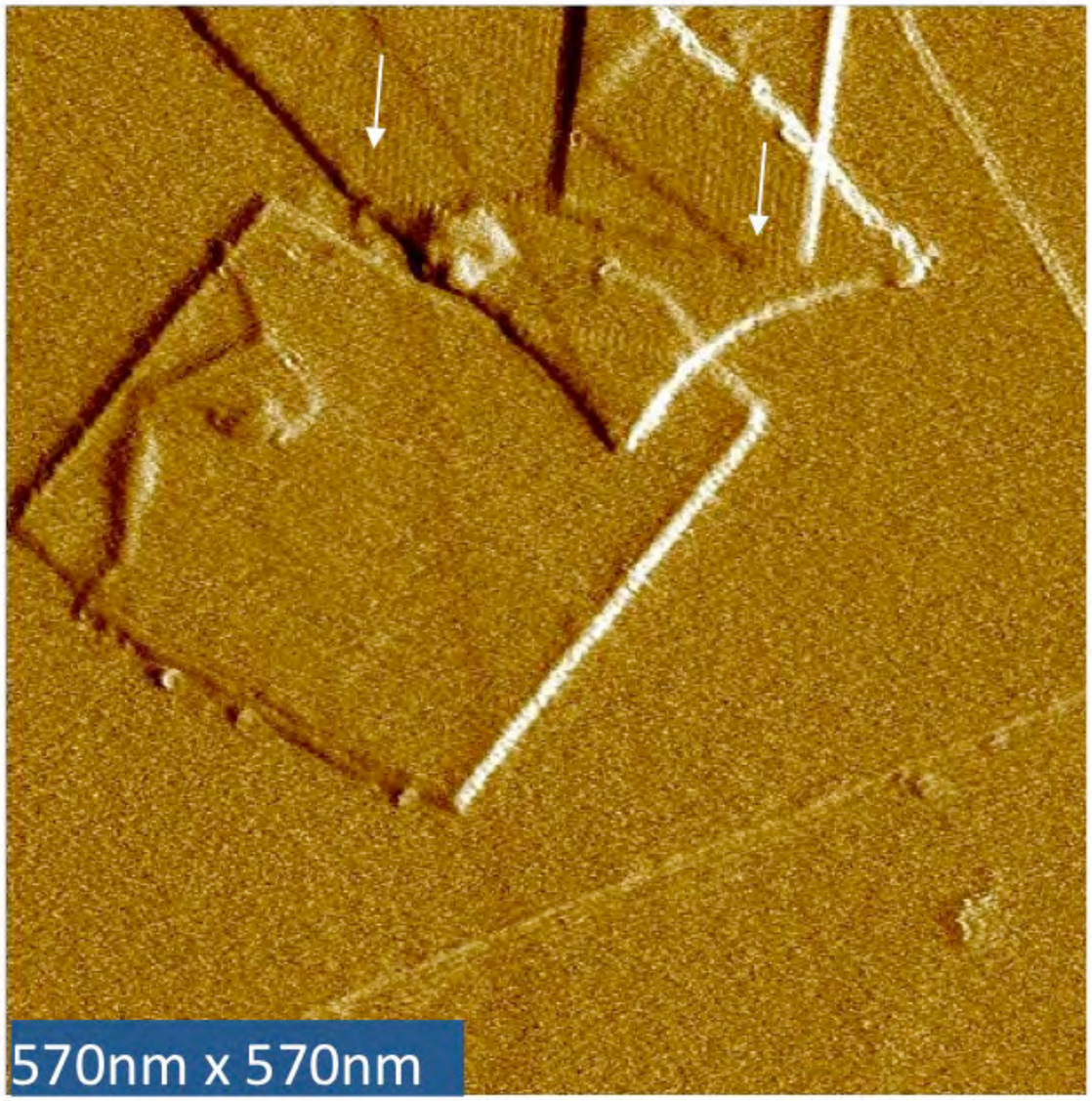


Lysozyme/HOPG interaction

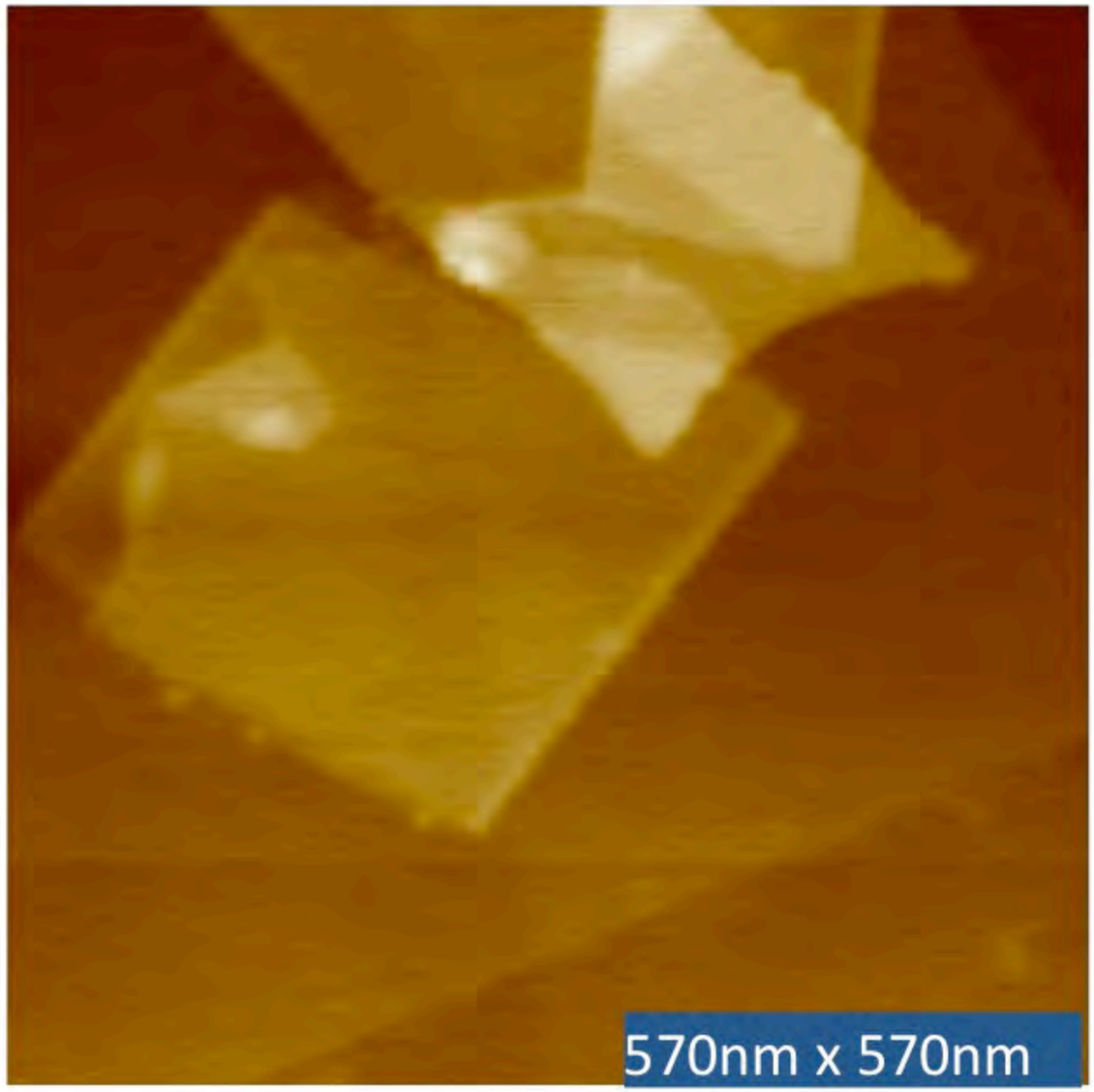
✓ **STABILITY in UHV**

compact layer with preserved periodicity

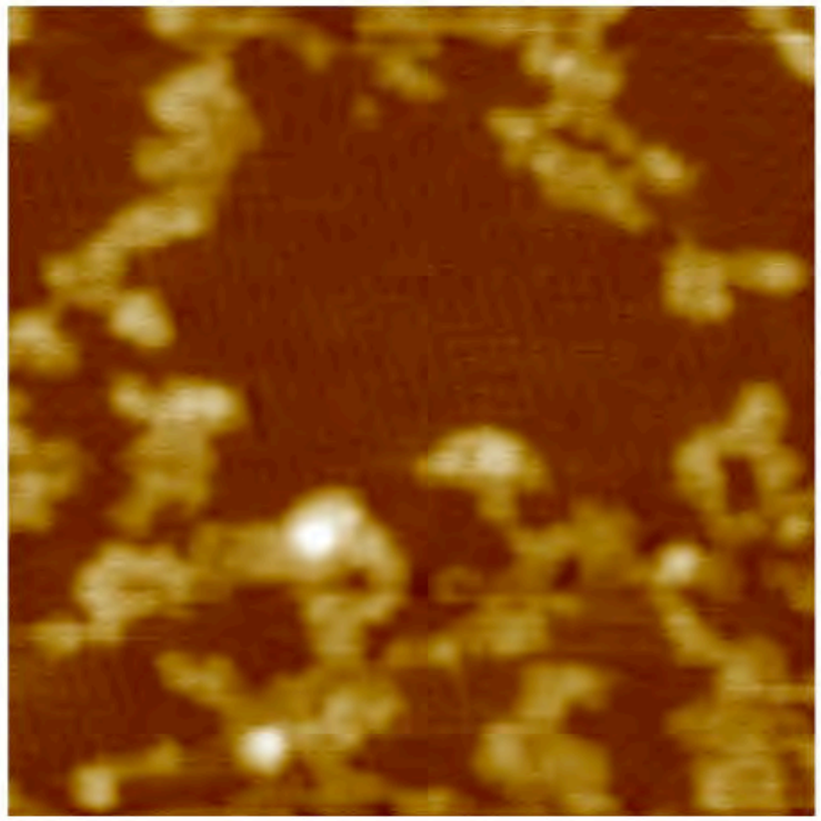
✓ **THERMAL STABILITY**

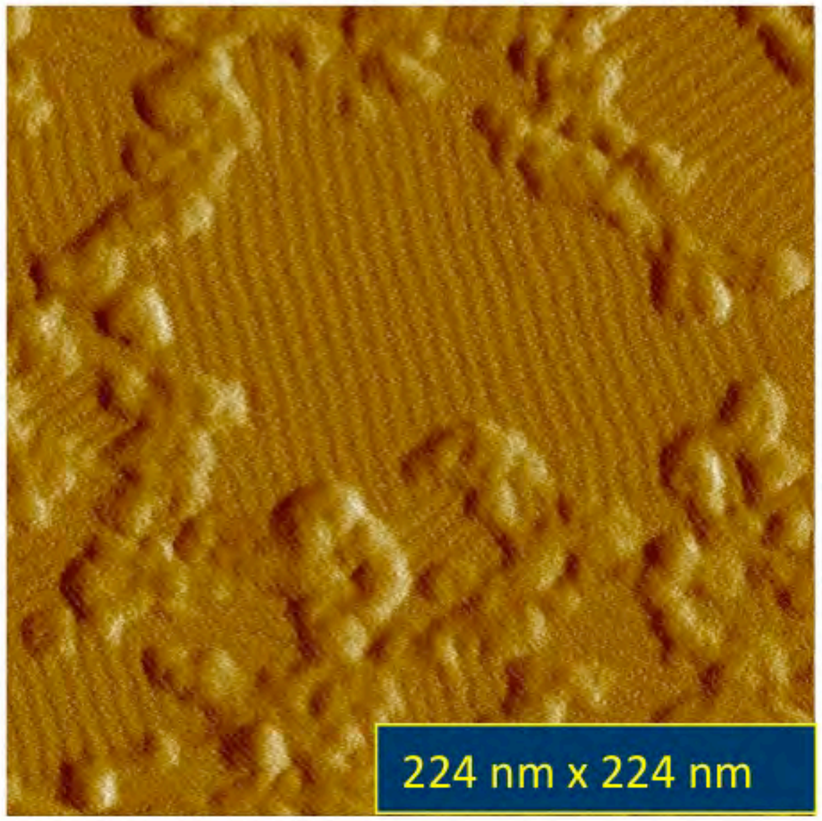


70°C x 1h



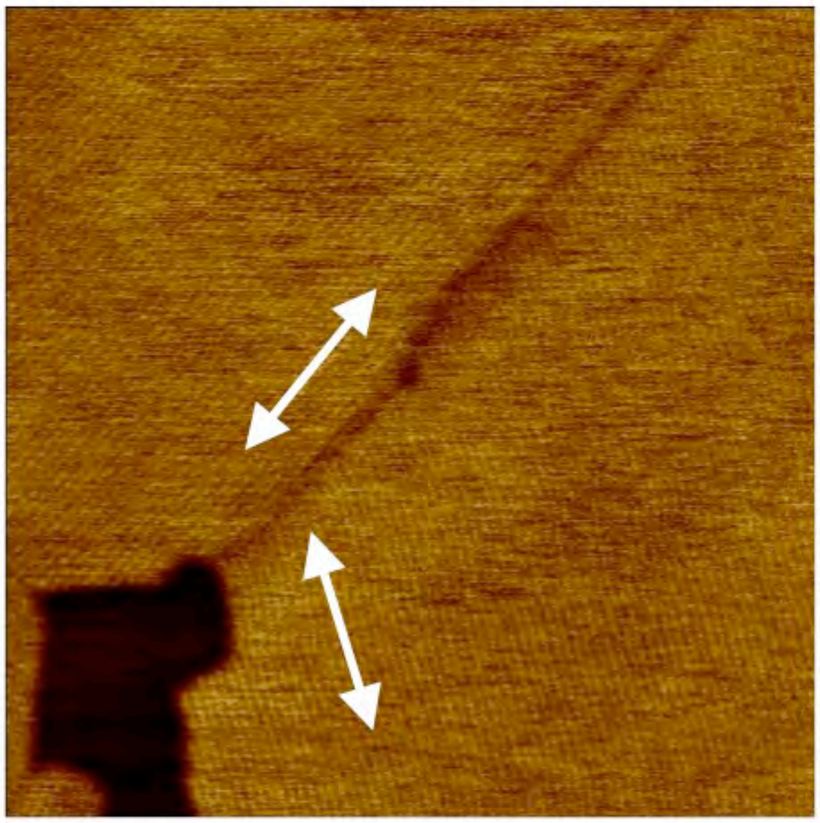
Effect of protein concentration on globular material amount

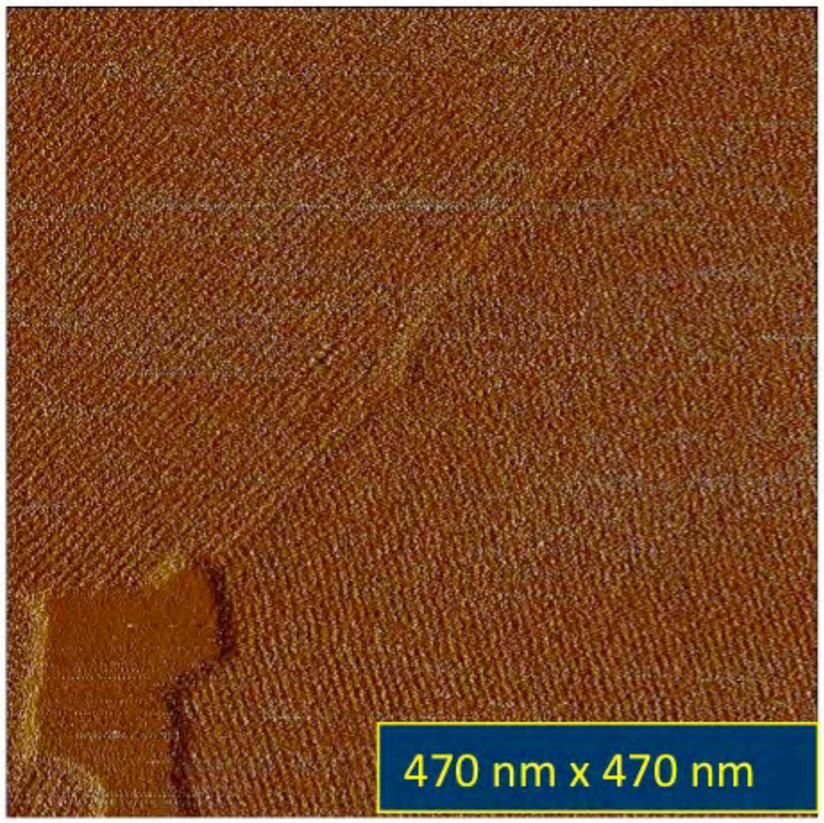




Insulin/HOPG

1 µM
- relatively small domains
- presence of globular material

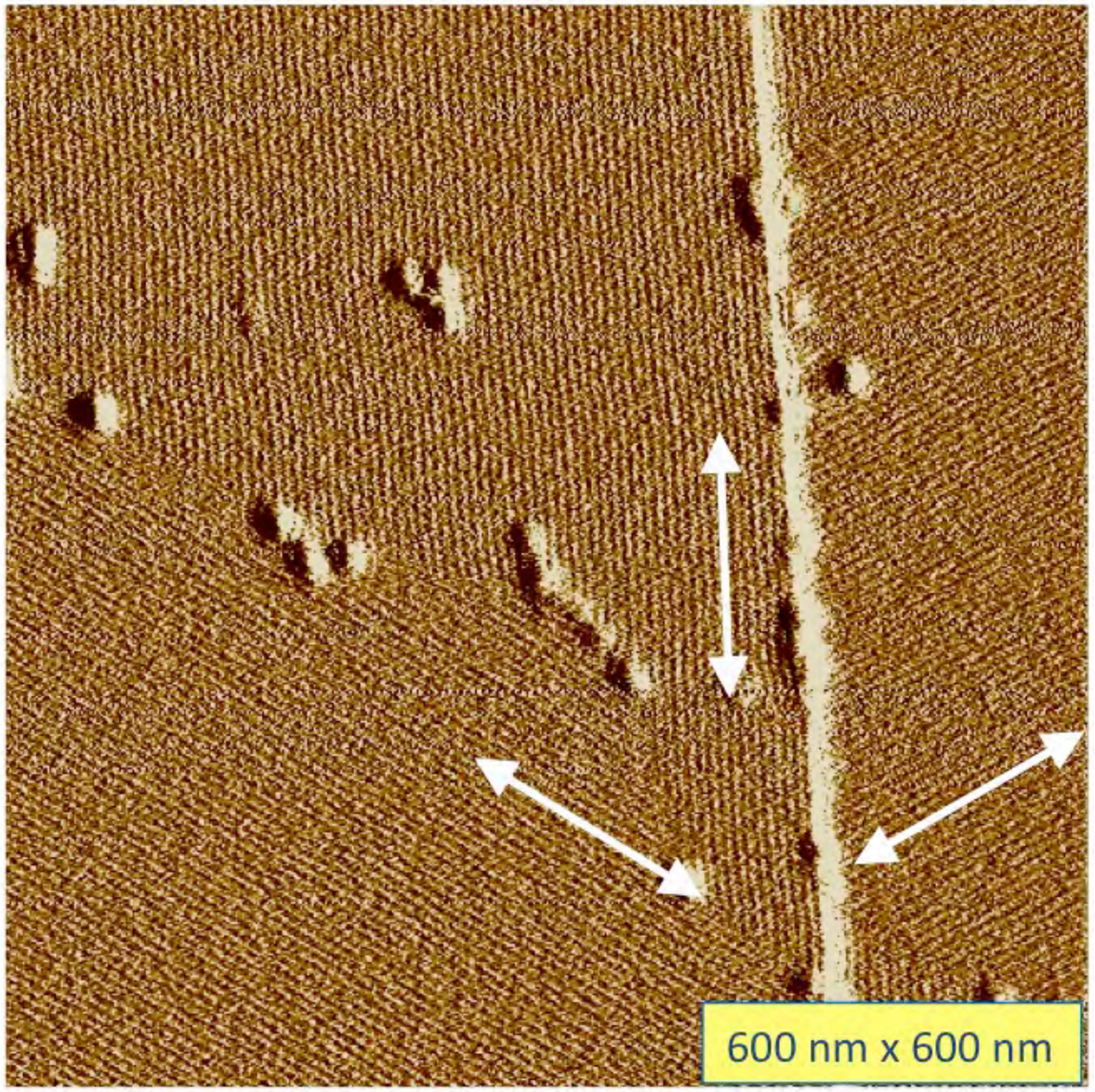




decreasing concentration

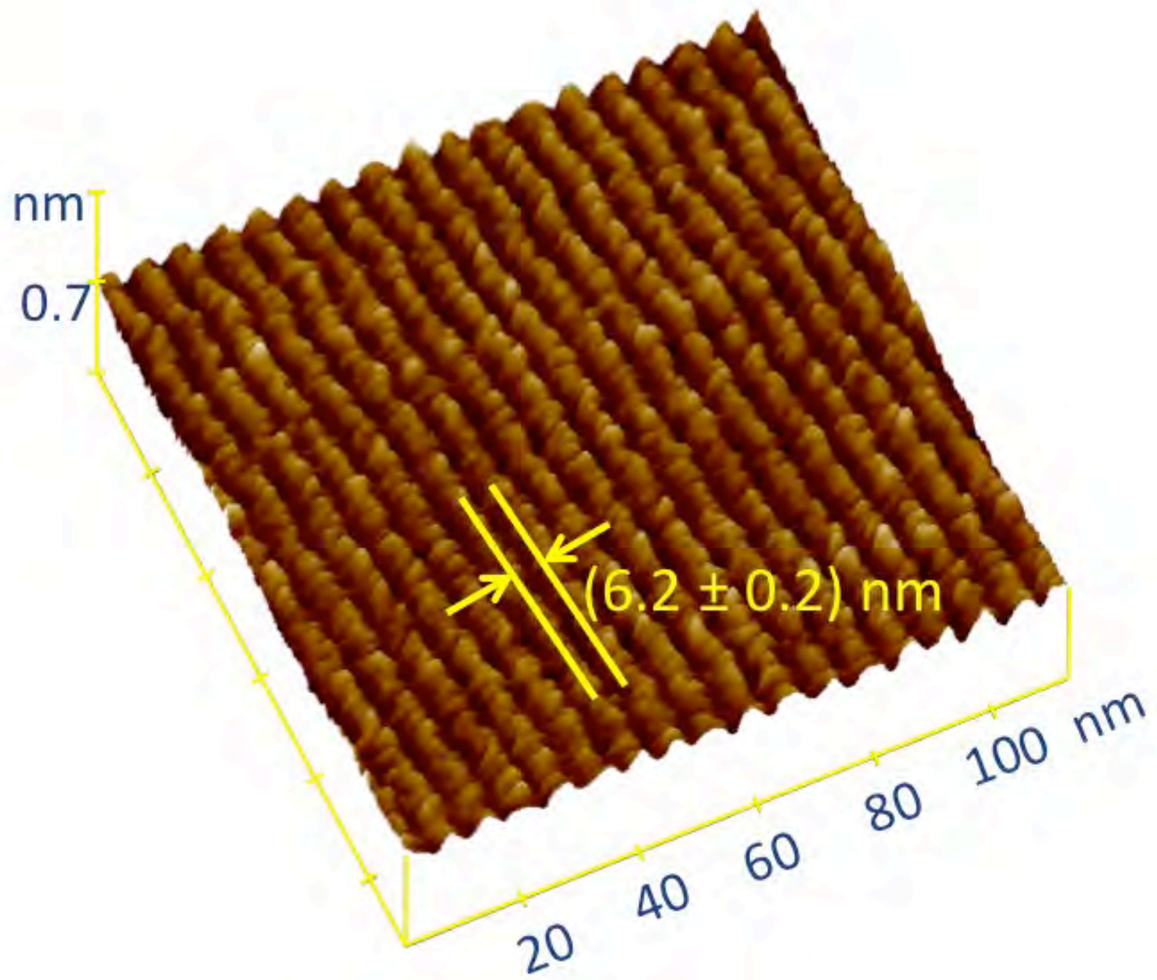
10-100 nM
- large ordered domains

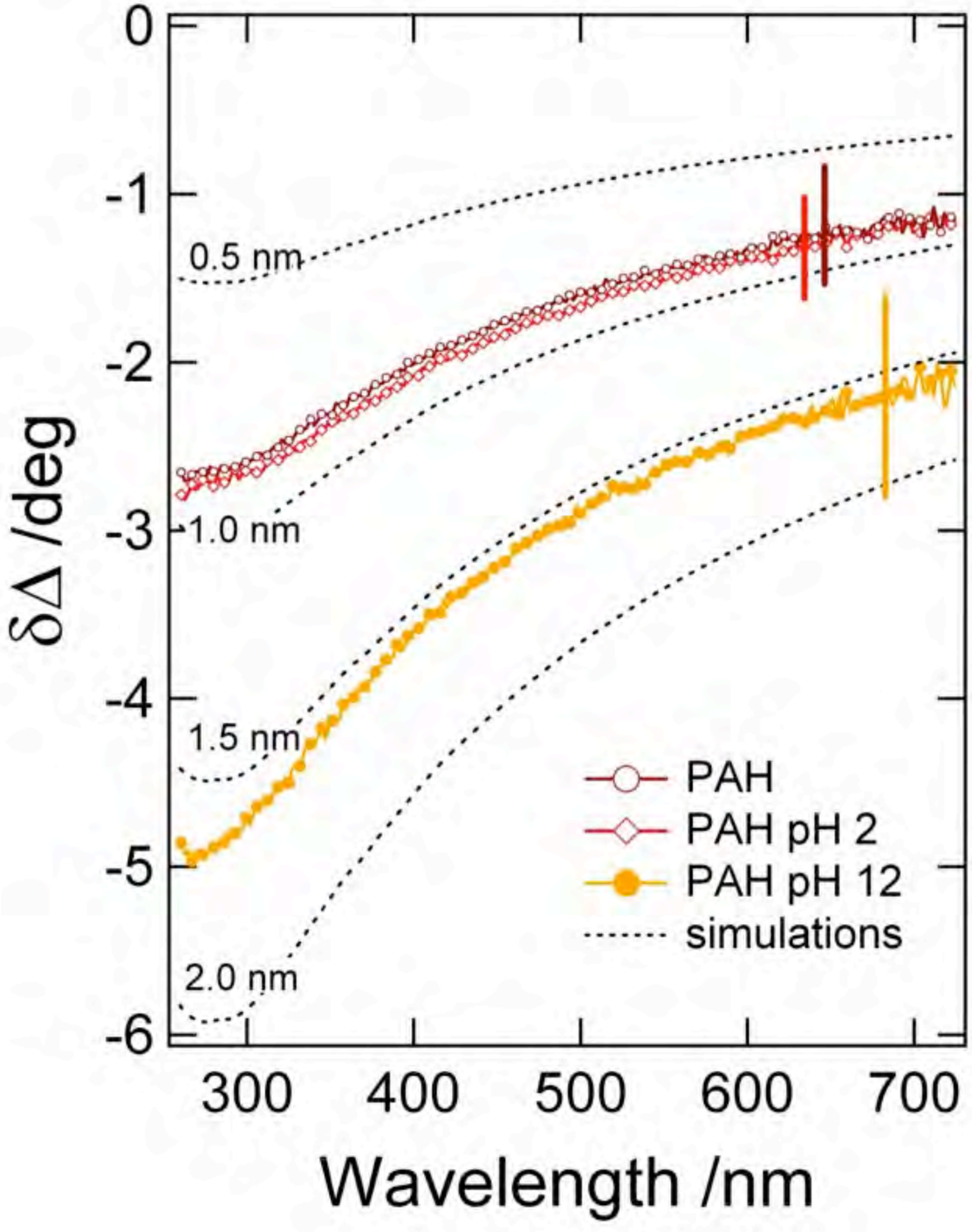
Lysozyme on HOPG

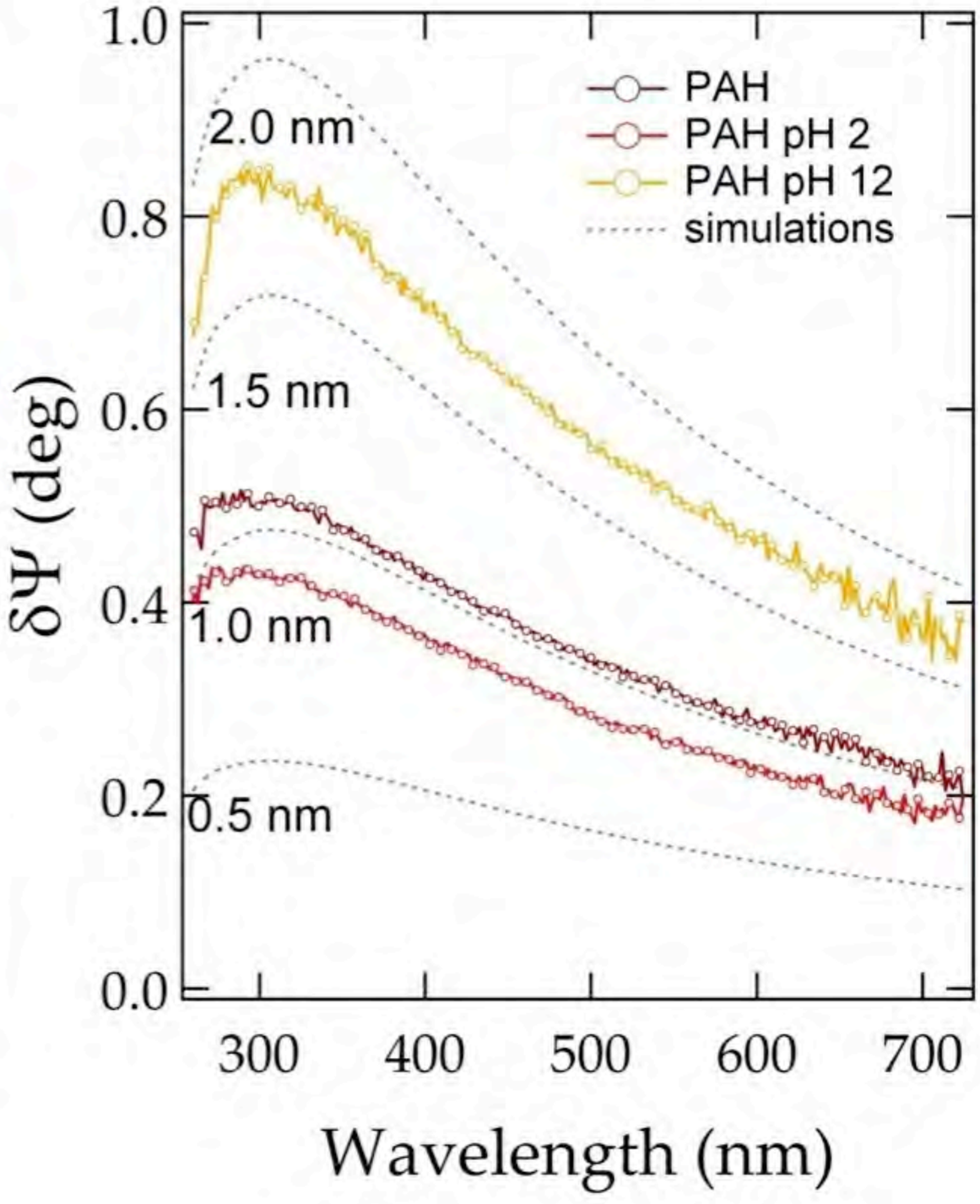


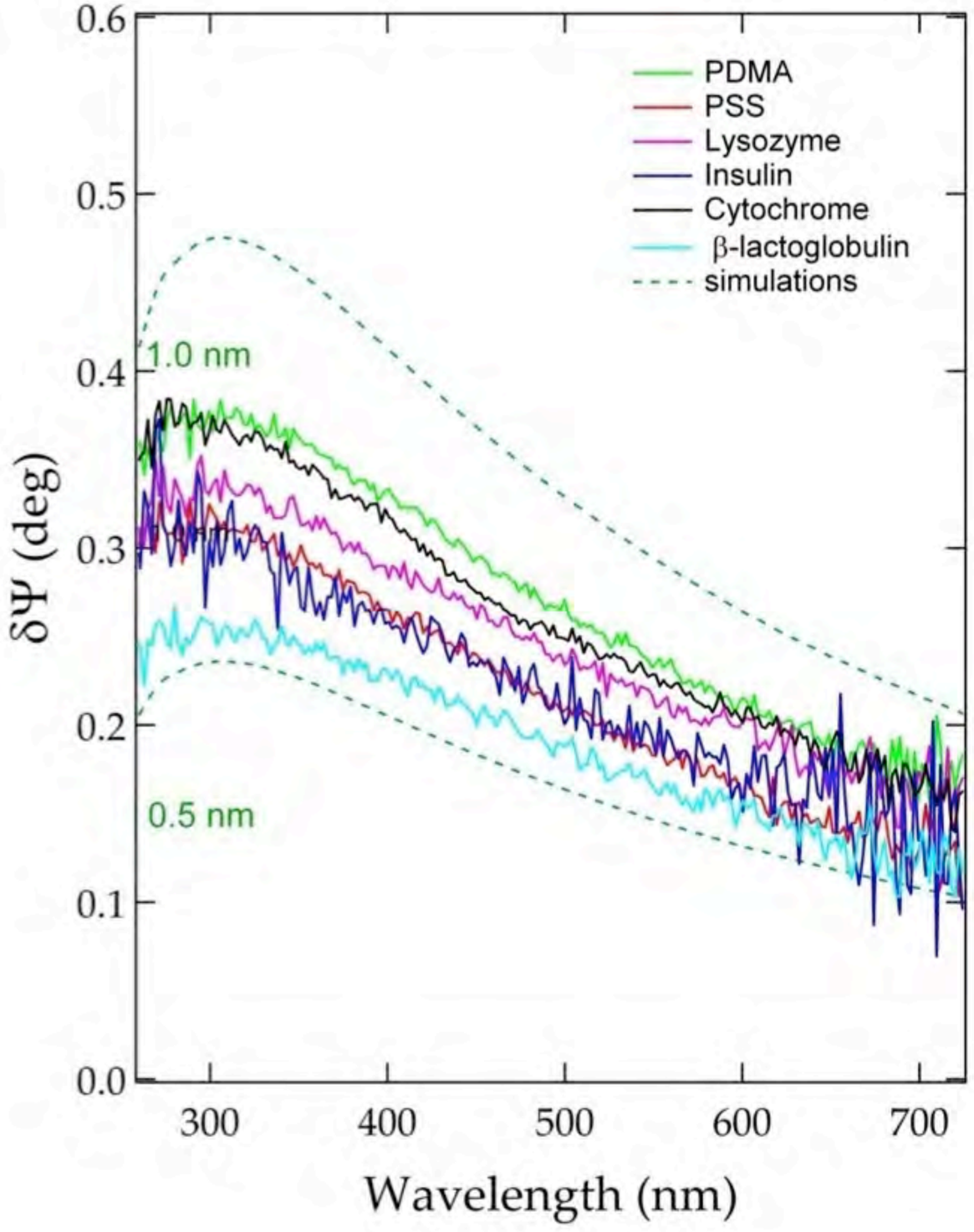
1 hour interaction with a 20 nM protein solution

- ordered domains
  hundreds of nm in size
- three-fold symmetry
- some globular material

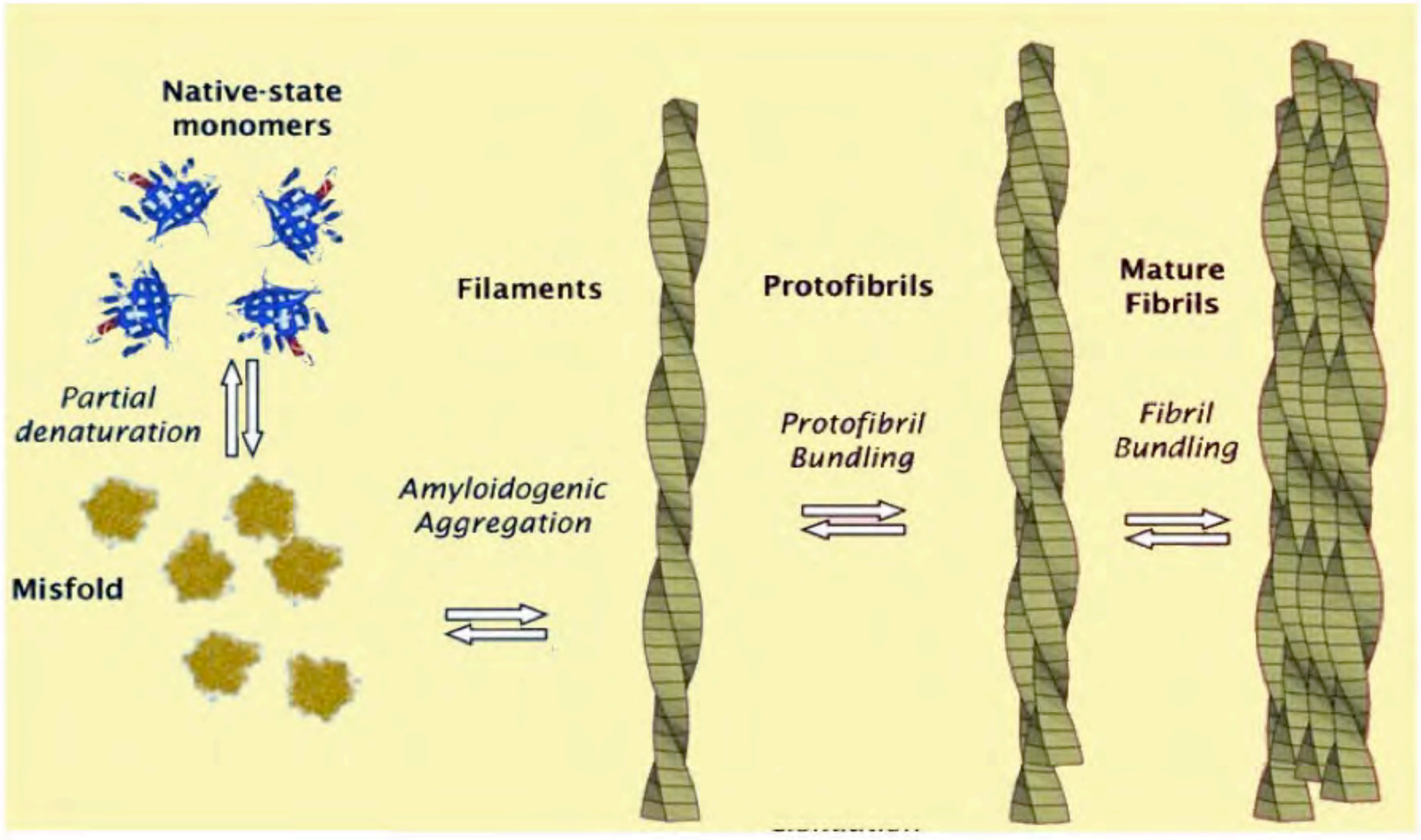



0
-1
-2
-3
-4
-5
-6
δΔ /deg
0.5 nm
1.0 nm
1.5 nm
2.0 nm
PAH
PAH pH 2
PAH pH 12
simulations
300
400
500
600
700
Wavelength /nm

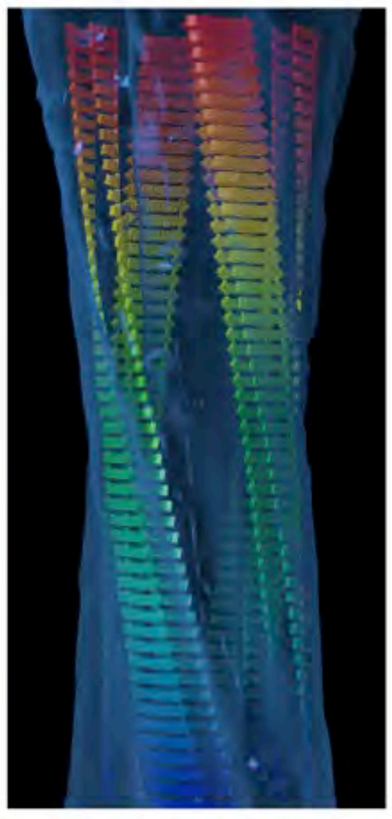

1.0
0.8
0.6
0.4
0.2
0.0
δψ (deg)
2.0 nm
1.5 nm
1.0 nm
0.5 nm
PAH
PAH pH 2
PAH pH 12
simulations
300
400
500
600
700
Wavelength (nm)


0.6
0.5
0.4
0.3
0.2
0.1
0.0
δΨ (deg)
1.0 nm
0.5 nm
PDMA
PSS
Lysozyme
Insulin
Cytochrome
β-lactoglobulin
simulations
300
400
500
600
700
Wavelength (nm)

Protein self-assembly into non-native structures: fibrillar aggregation scheme



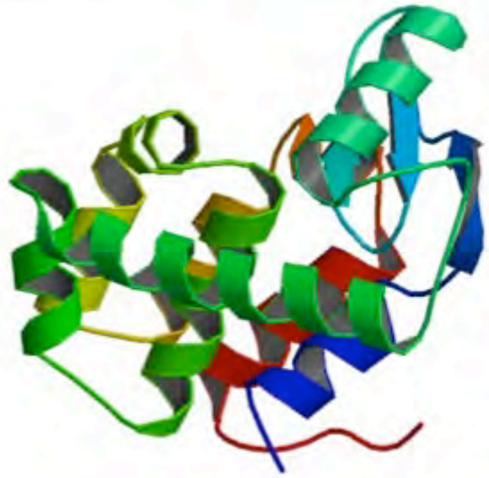
cross-β structure



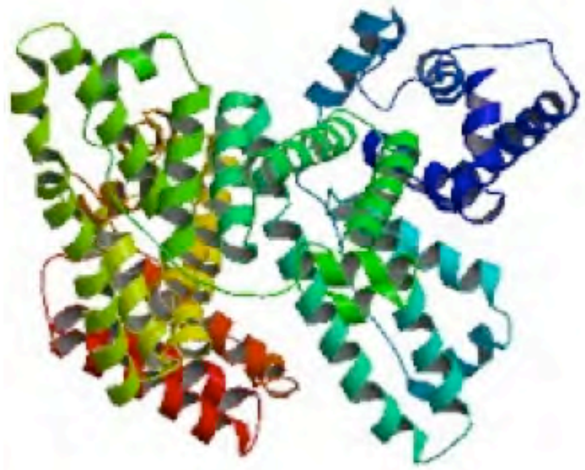
protofilament width
3 nm

the proteins

Bovine pancreas insulin
chain A 21 AA
chain B 30 AA
Mw 5.7 kDa

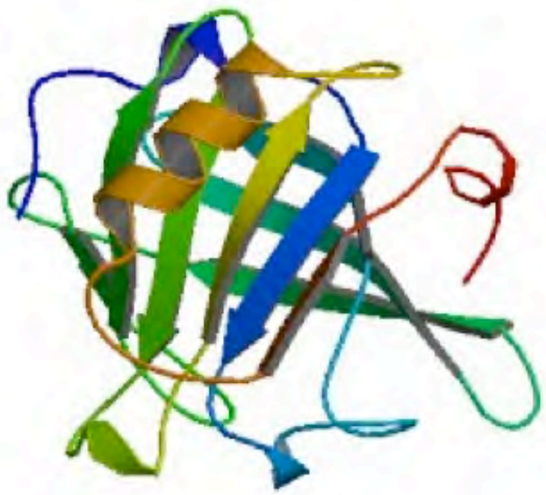


Hen egg white lysozyme
129 AA
Mw 14.7 kDa



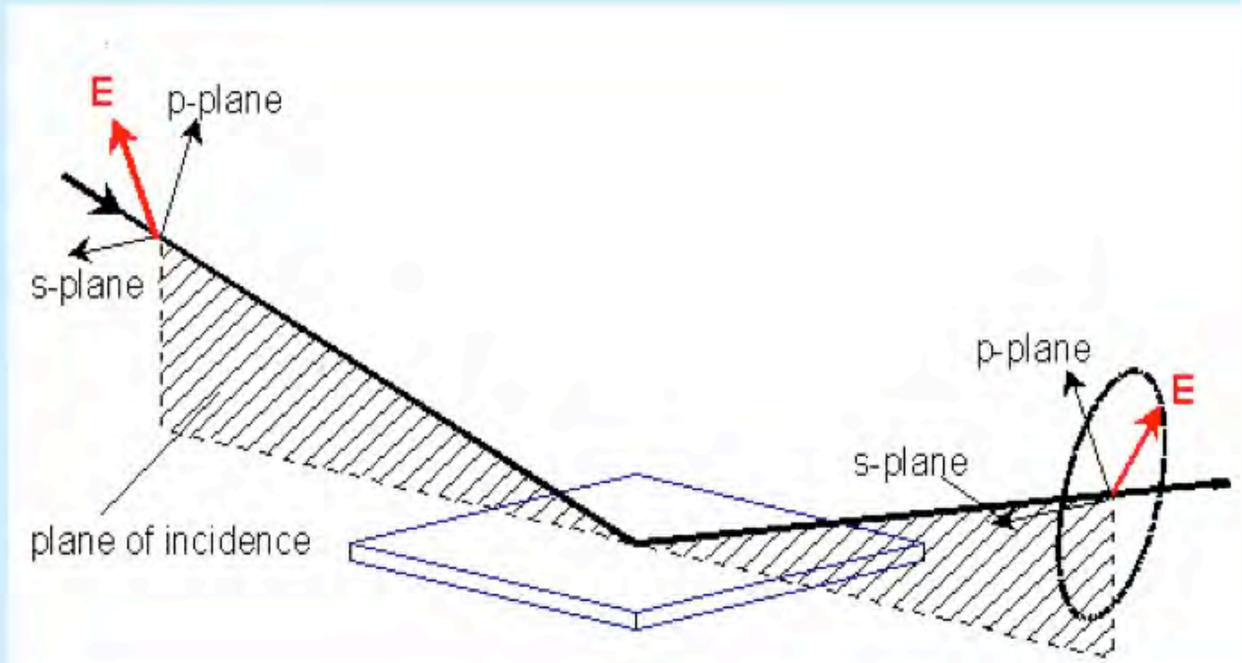
Bovine serum albumin
(BSA)
585 AA
Mw 66 kDa

Cytochrome *c*
108 AA
Mw 12.6 kDa



Bovine β-lactoglobulin
162 AA
Mw 18.4 kDa

Standard Spectroscopic Ellipsometry

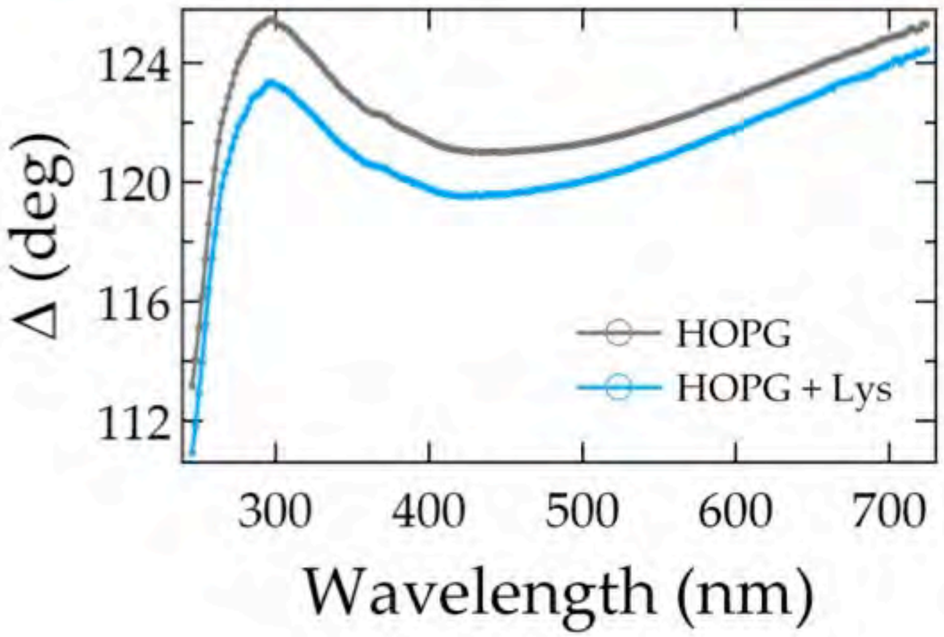


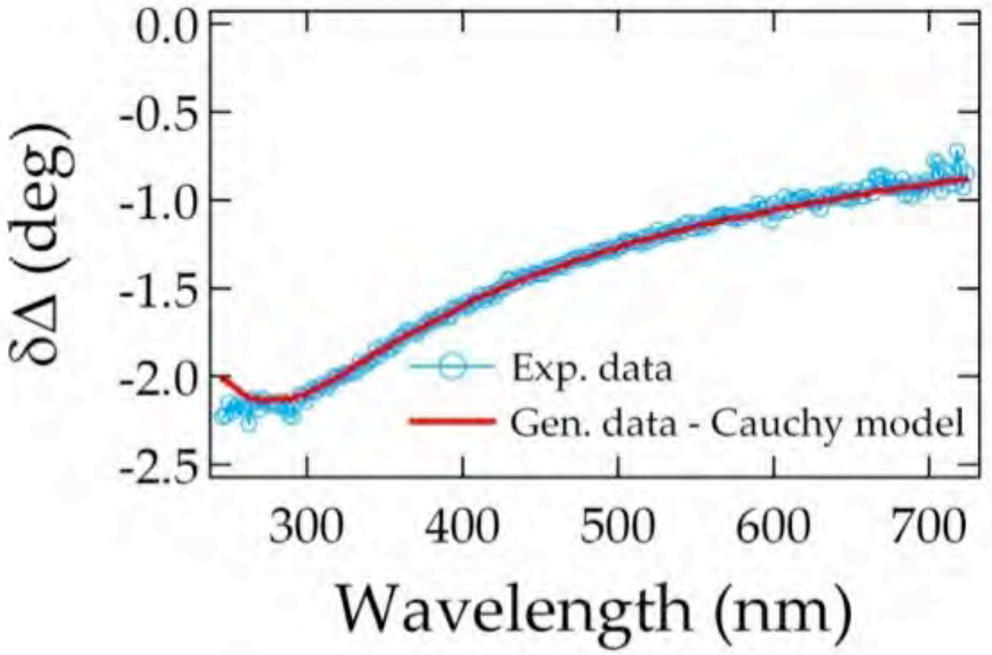
$$\rho = \frac{R_p}{R_s} = \left|\frac{R_p}{R_s}\right| e^{i(\delta_{rp} - \delta_{rs})} = \mathrm{tg}\Psi e^{i\Delta}$$

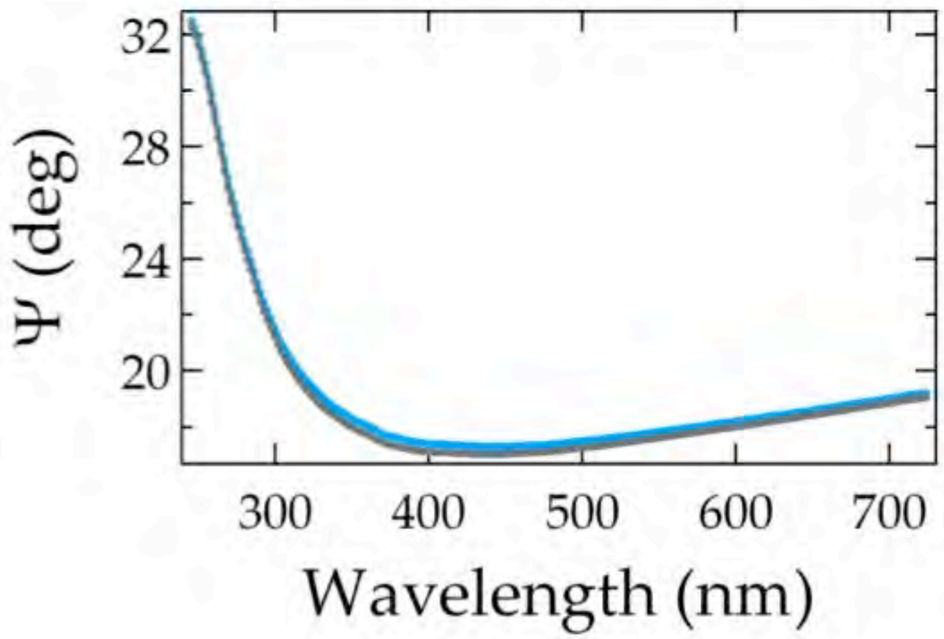
$$\Psi = \mathrm{arctg}\left|\frac{R_p}{R_s}\right|$$

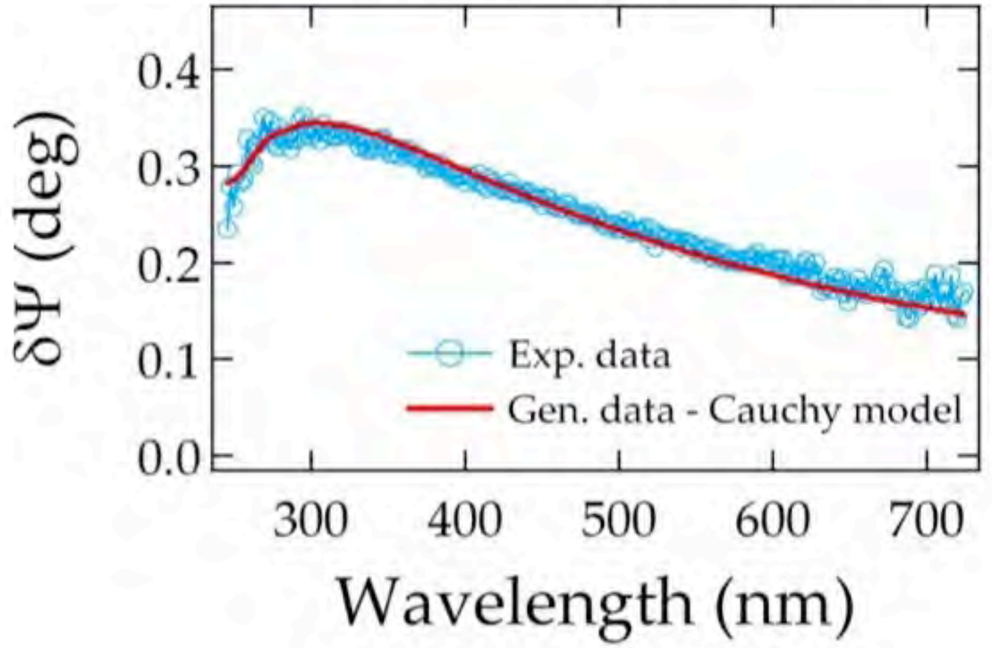
$$\Delta = \delta_{rp} - \delta_{rs}$$

Layer thickness: spettroellipsometry evaluation









Cauchy model: $n = A_n + B_n/\lambda^2$