Final Scientific Report

Molecular insight on the interaction between surface-tailored Si/SiO_2 wafers and fibrinogen.

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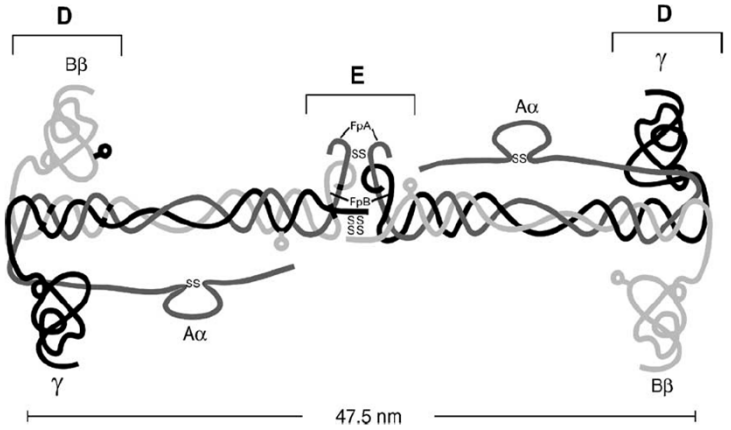
The aim of the visit is to study by Atomic Force Microscopy the morphology of fibrinogen at the surface of Silica wafers and the occurrence of conformational modifications.

Fibrinogen, one of the most important proteins involved in the coagulation process, is composed of a central domain and two lateral domains.
The central domain is the site of interaction with thrombin. After the activation of the coagulation process, thrombin interacts with fibrinogen and results in the cleavage of the lateral peptides from the central domain, and the subsequent formation of fibrin monomer.



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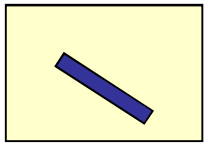
Atomic Force Microscopy

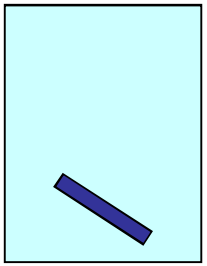
The main goal of AFM for protein structural investigation is the determination of topographical features at near physiological conditions. Other biophysical methods require sophisticated sample preparation procedures, while AFM does not require any special sample treatment.
AFM is one of the most powerful tools for determining the surface topography and forces of native biological systems on a nanometer scale.[*L.P.Silva/Current protein and peptide science, 6 (2005) 387*]
In this study, AFM is employed to monitor fibrinogen coverage of silica surfaces treated to vary their hydrophobicity/hidrophilicity and to probe the conformation of the protein on a molecular level.

Cleaning



1.
10 min in solution of sulphuric acid and hydrogen peroxide.

2.
Washing with ultra pure water and dry with compress air.

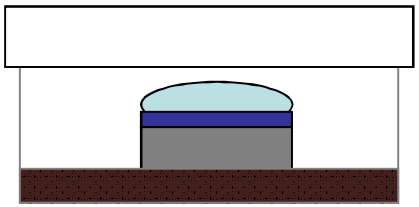


3.
30 min in boiling water.

4.
Drying with compress air.

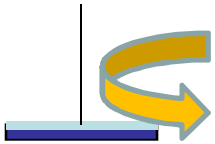
Sample preparation

Clean silicon wafer

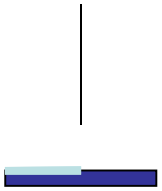


Incubation with protein solution at 37°C for 1 h.

Washing with ultra pure water and drying with compress air.



Surface is partially masked and cleaned with solution of sulphuric acid and hydrogen peroxide.



Washing with ultra pure water and drying with compress air.

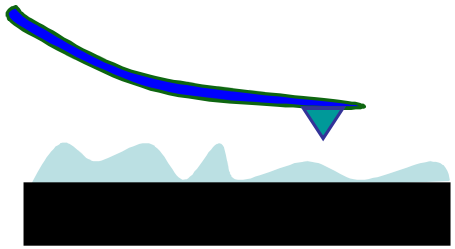
Half of the silica wafer is covered with protein and half is clean.

AFM measurements

Adsorption isotherms

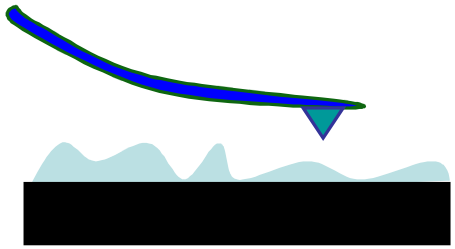
Silica wafers were incubated with different protein concentrations at 37°C for 1h and then washed to remove the non-adsorbed protein.
AFM Measurement were made in the non-contact mode.
From the data obtained there are two important parameters:

- The thickness of protein layer as determined by the value of average height plus the minimum height of the layers.
- The roughness of the protein layer that is evaluated by the value of average height.



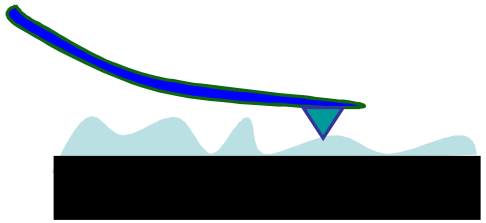
Single molecule images

Silica wafer were incubated with 10 μg/l of fibrinogen at 37°C for 1 h and then washed to remove the non-adsorbed proteins.
The images were acquired with AFM in non-contact mode.



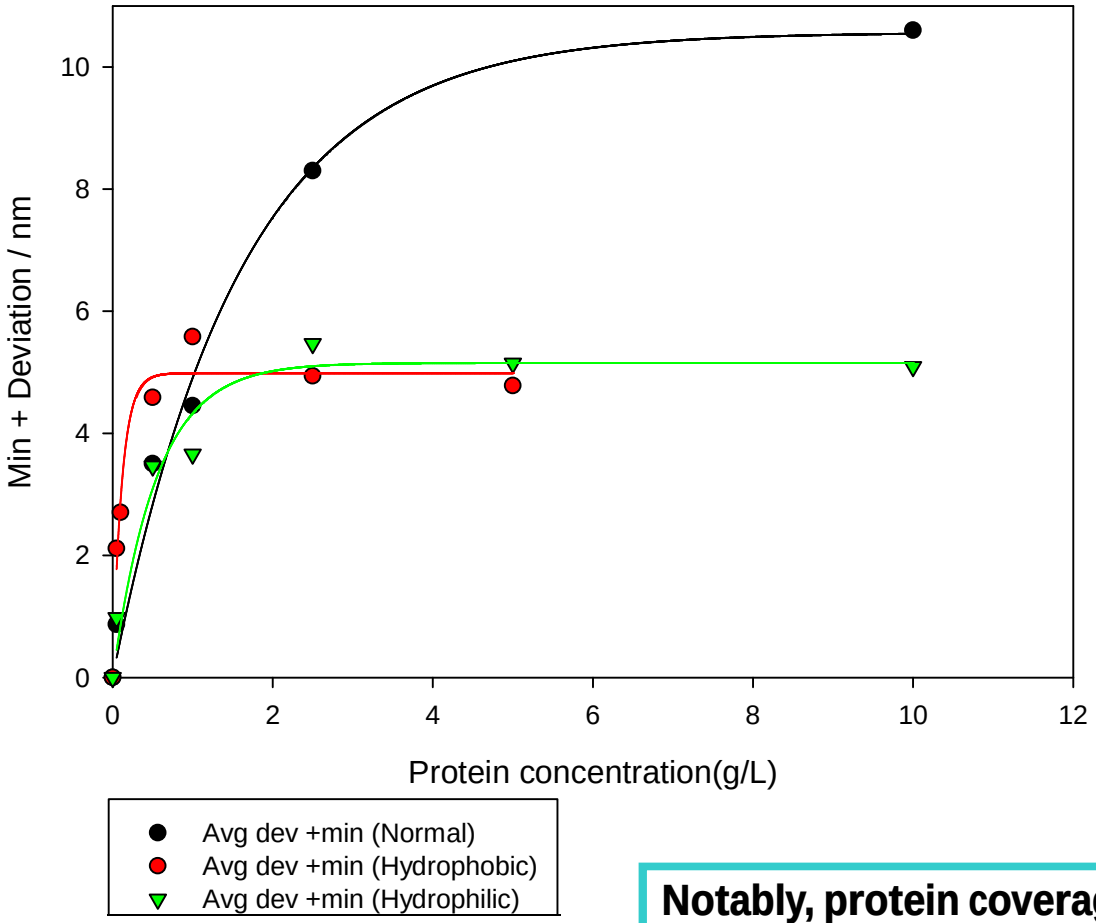
Force curves

Silica wafers were incubated with two different concentrations of fibrinogen (10 g/L and 10 μg/l) at 37°C for 1h and then washed to remove the non-adsorbed protein.
The images were acquired with AFM in contact mode.



RESULTS

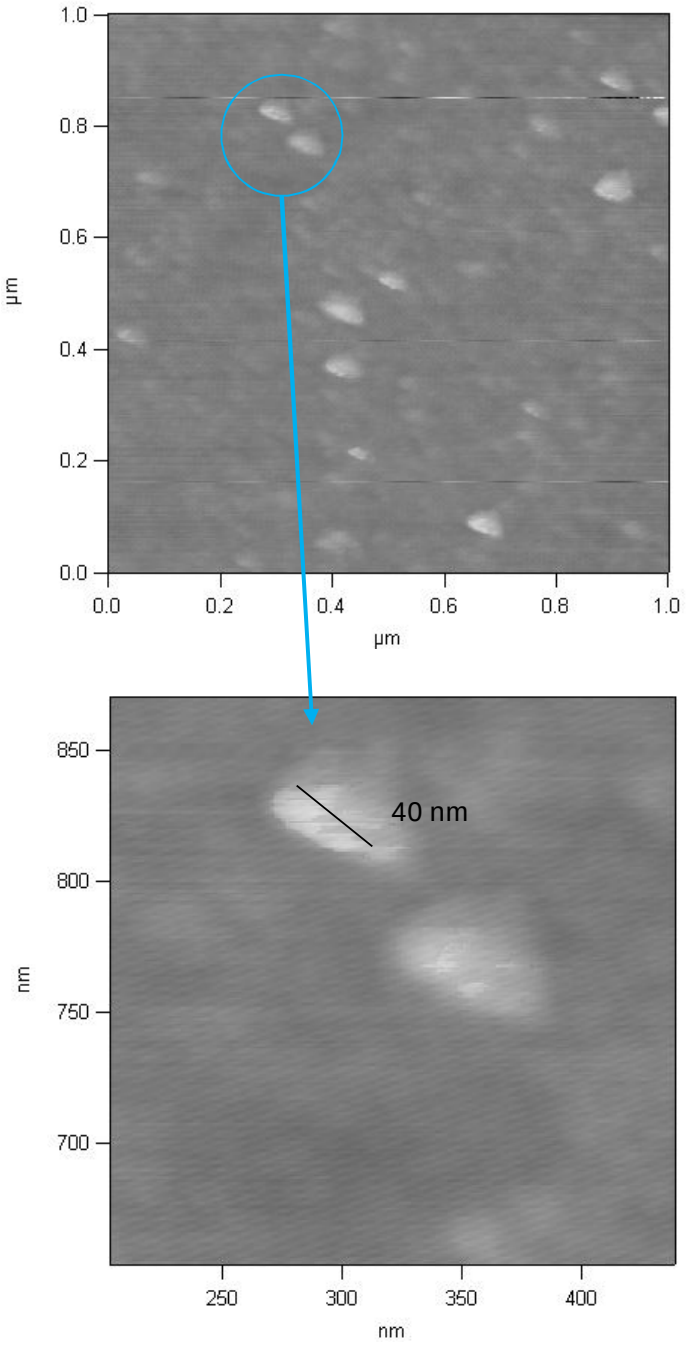
Comparisons of adsorption isotherms

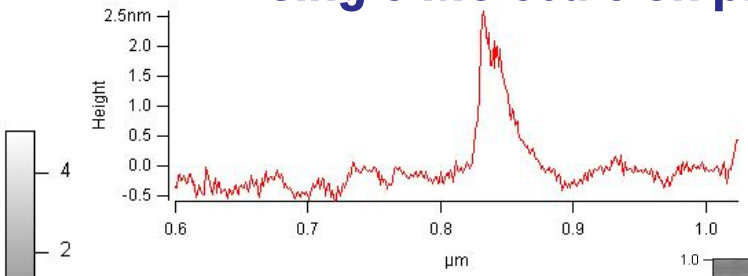


For all substrate types, an increase of thickness is observed, up to concentrations of ~2g/L. At this concentration, the coverage of the hydrophopic (red) and hydrophillic (green) substrates appears to saturate at ~4.5 - 5nm, whereas coverage of the untreated substrate continues to rise with increasing concentration up to ~10nm.

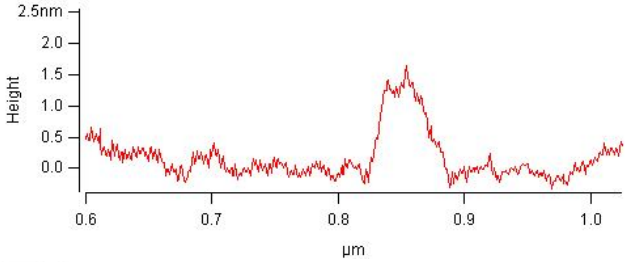
Notably, protein coverage of the pristine wafer is twice that of the hydrophilic or hydrophobic substrates.

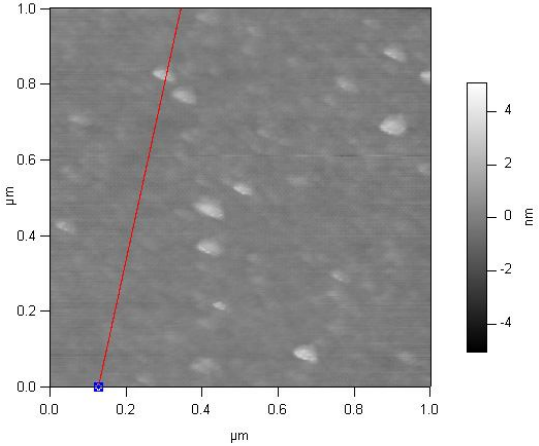
Single Molecule on pristine wafer 10 μg/L (thermal oxide)

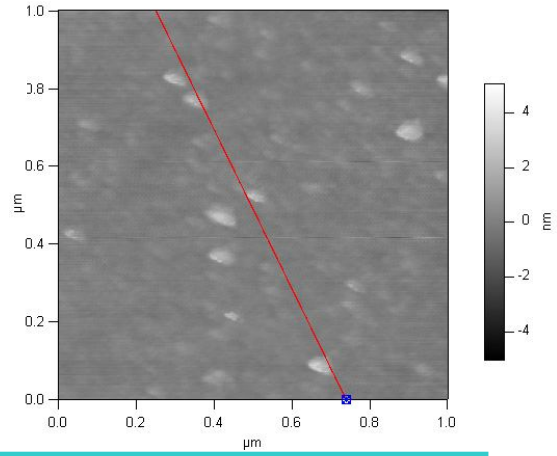




AFM image of some single BPF molecules adsorbed on pristine silica wafer.

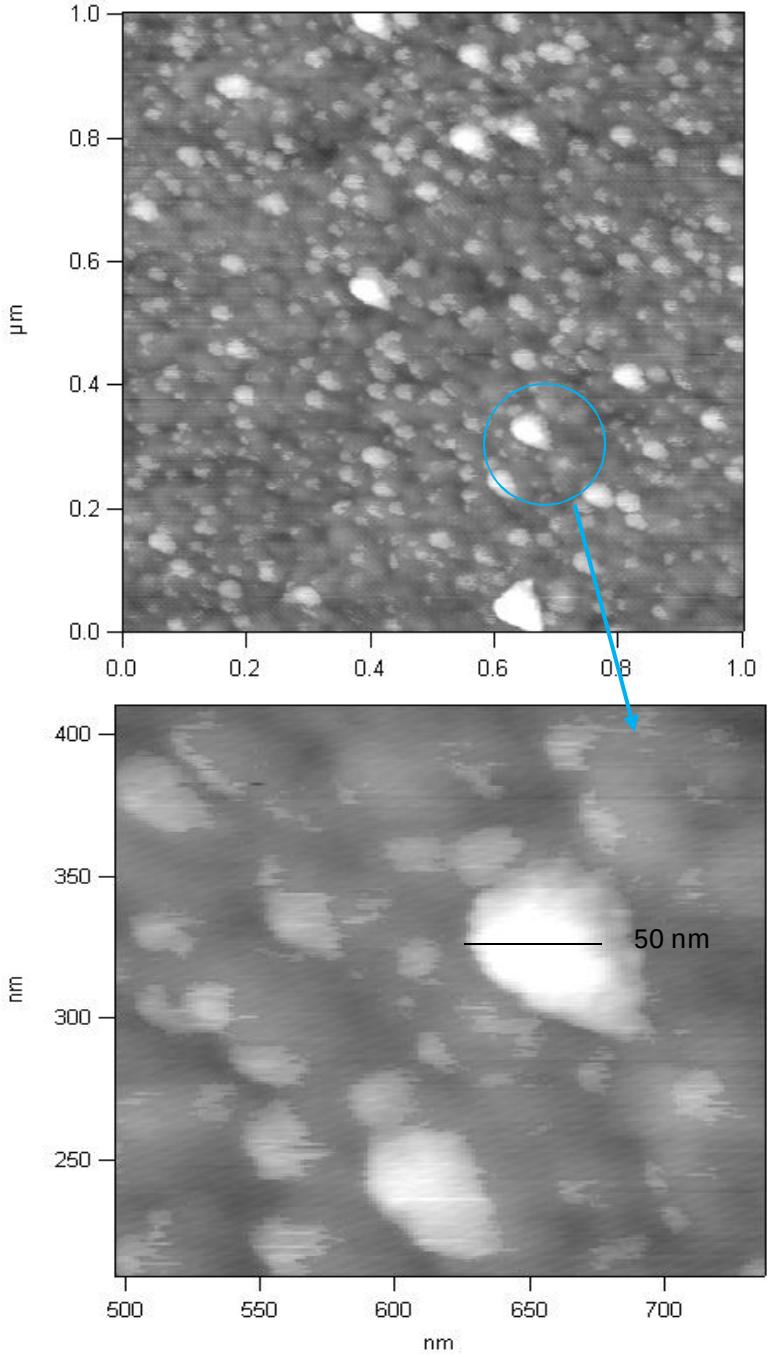




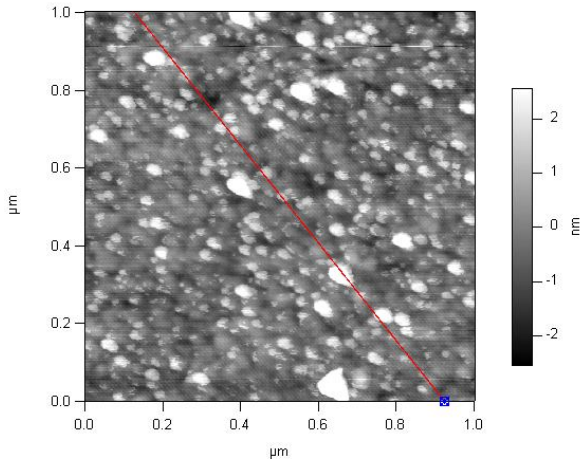


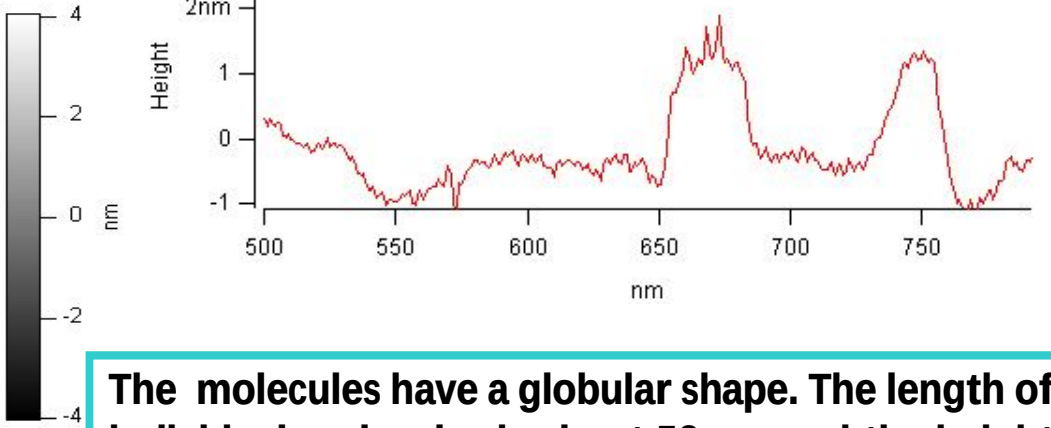
The molecules have a globular shape. The length of individual molecules is about 40 nm and the height of BPF molecules on the surface is about 2.0 nm.

Single Molecule on hydrophobic wafer 10μg/L (Out gassed 800C)



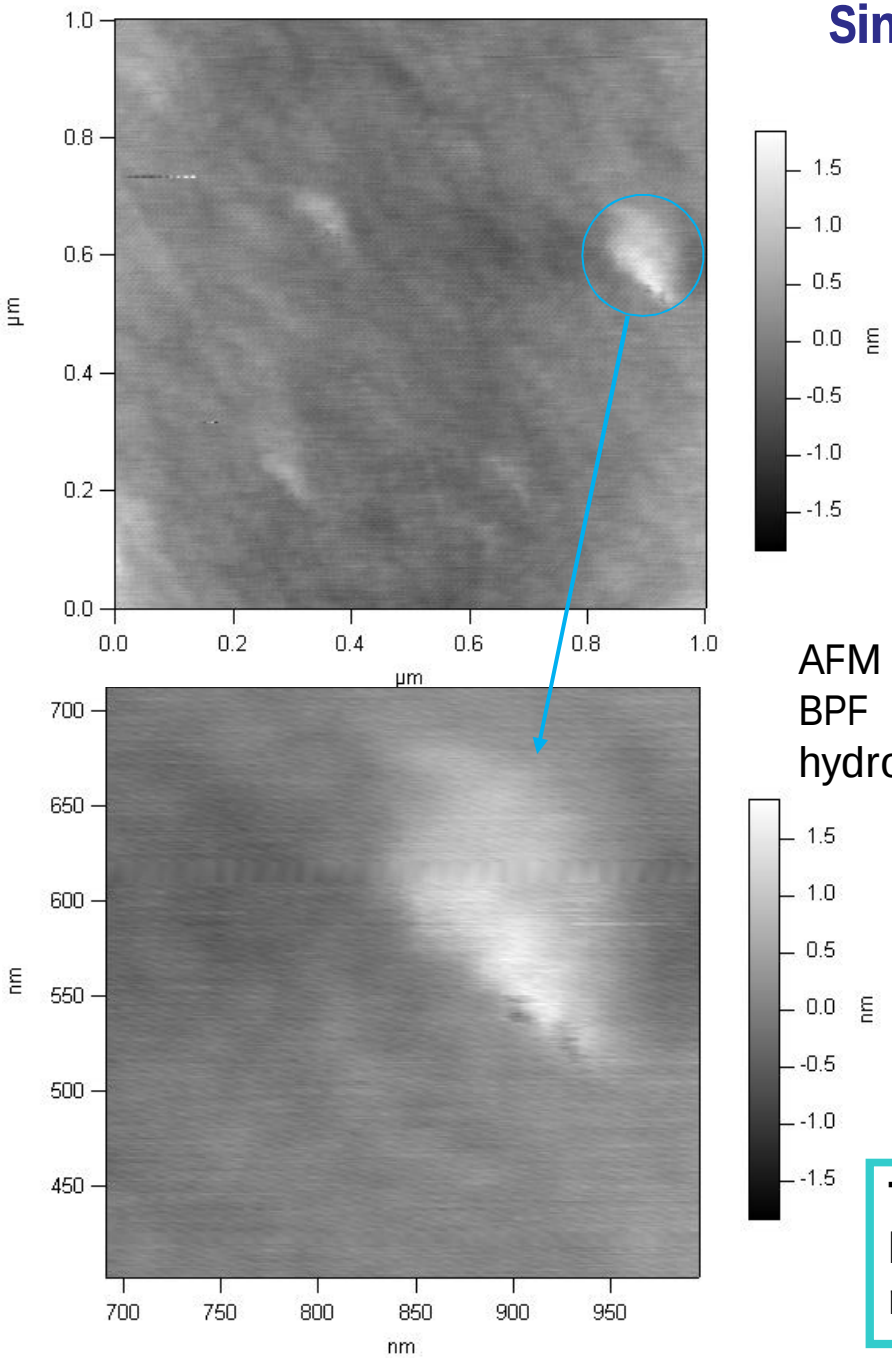
AFM image of some single BPF molecules adsorbed on hydrophobic silica wafer.

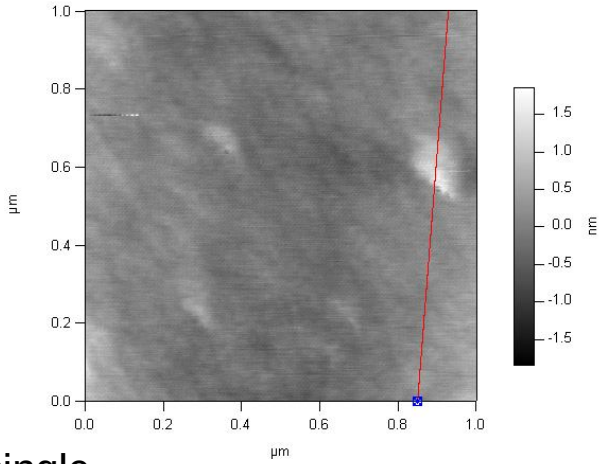




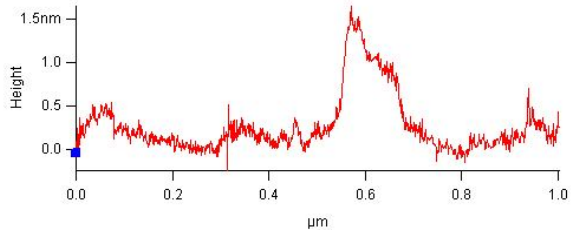
The molecules have a globular shape. The length of individual molecules is about 50 nm and the height of BPF molecules on the surface is about 2.0 nm.

Single Molecule on hydrophilic wafer 10 μg/L (Plasma)



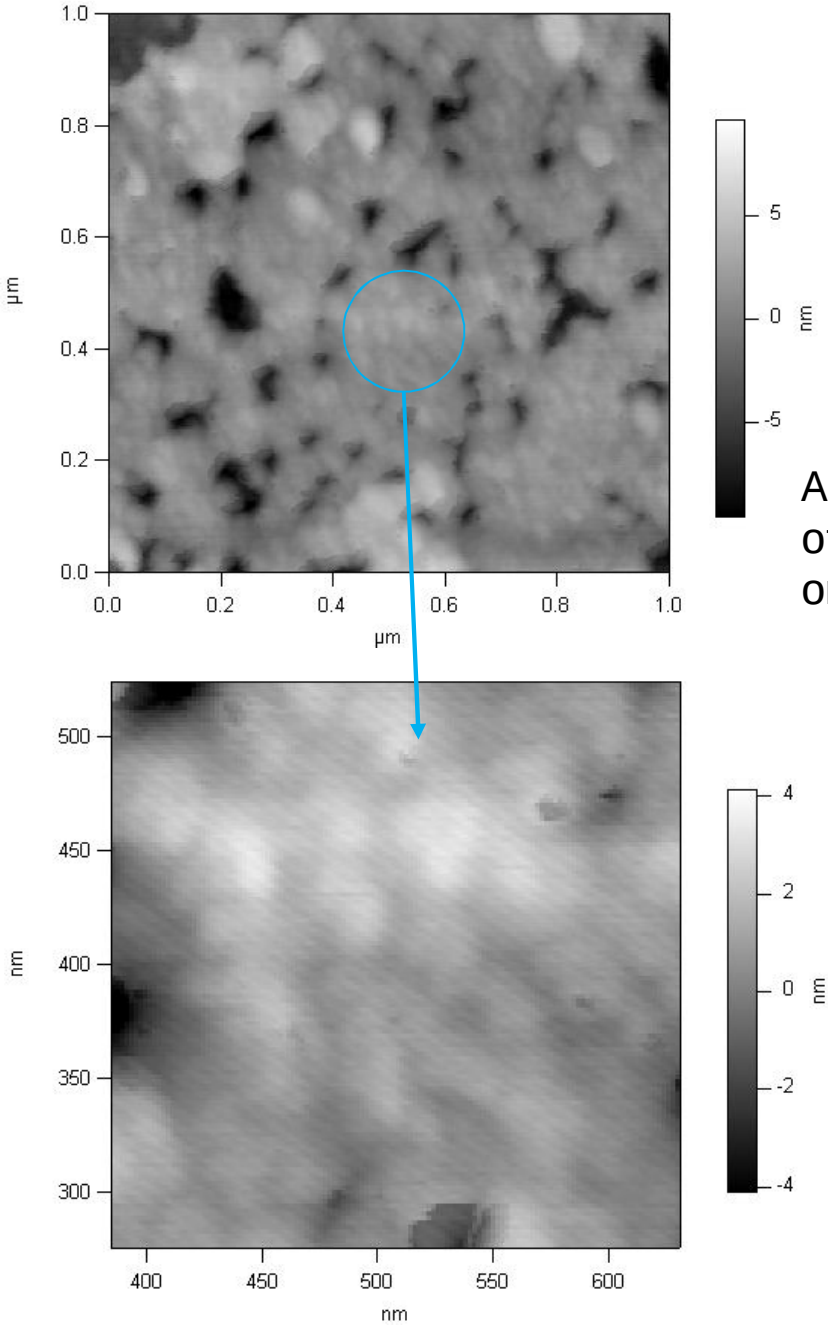


AFM image of some single BPF molecules adsorbed on hydrophilic silica wafer.

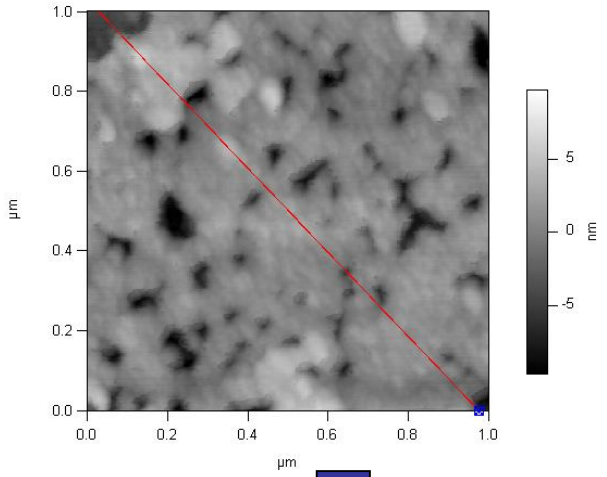


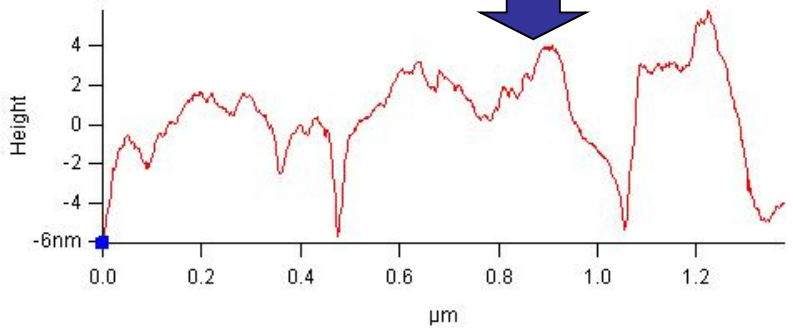
The molecules have a thin, elongated shape. The height of BPF molecules on the surface is about 1.5 nm.

Imaging of BPF on pristine wafer 1o g/L (thermal oxide)



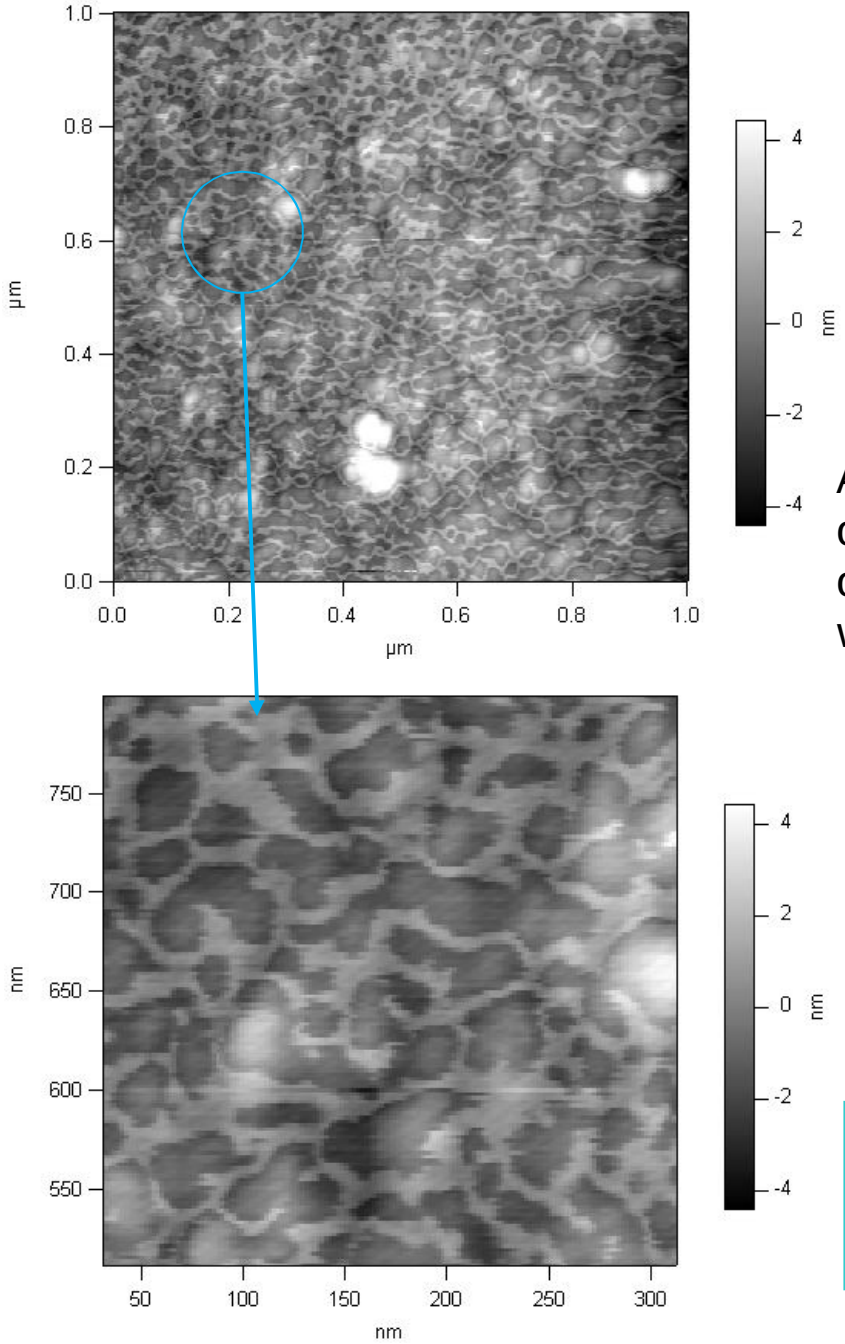
AFM image of multilayer of fibrinogen adsorbed on pristine silica wafer.



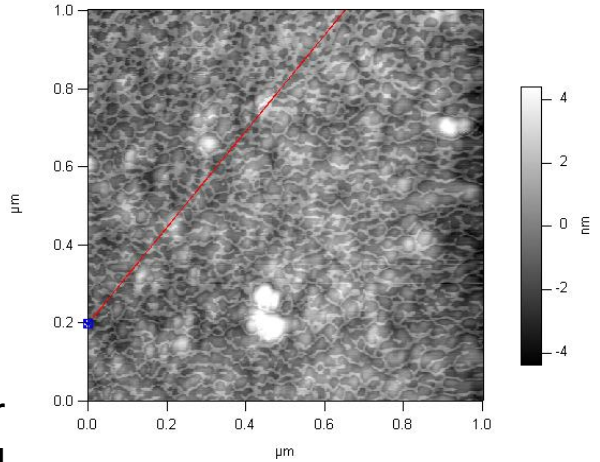


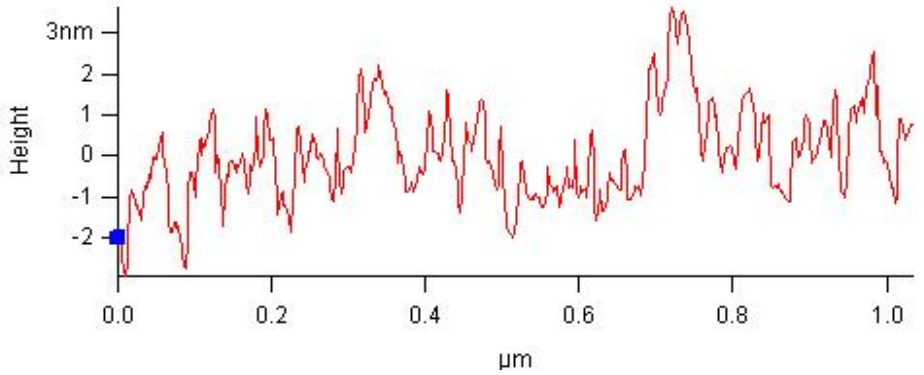
The height of the protein adsorbed onto the pristine wafer at higher concentrations is around 10 nm and the image shows some compact layers, according with the isotherm curve.

BPF on hydrophobic wafer 10g/L (Out gassed 800C)



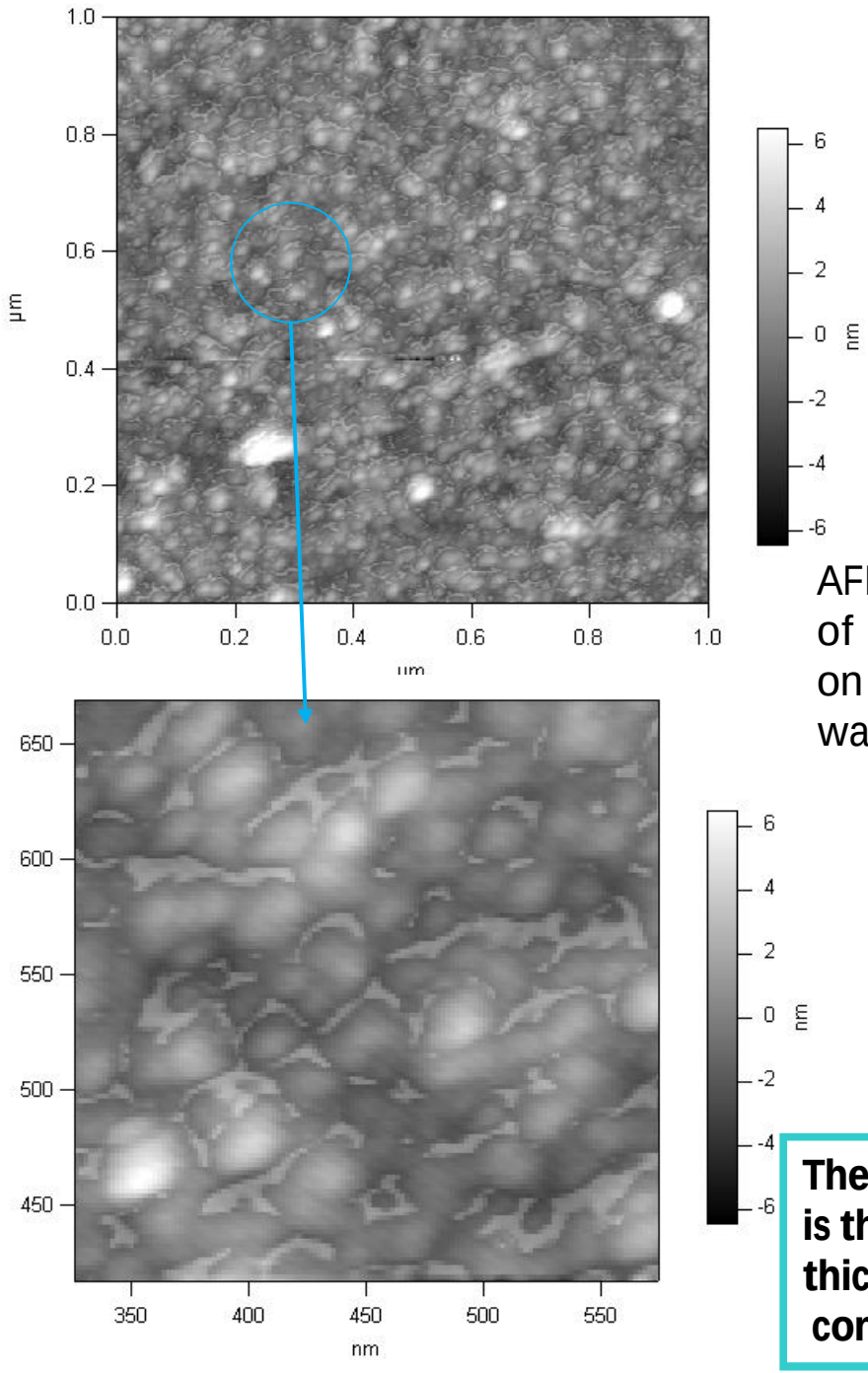
AFM image of multilayer of fibrinogen adsorbed on hydrophobic silica wafer.

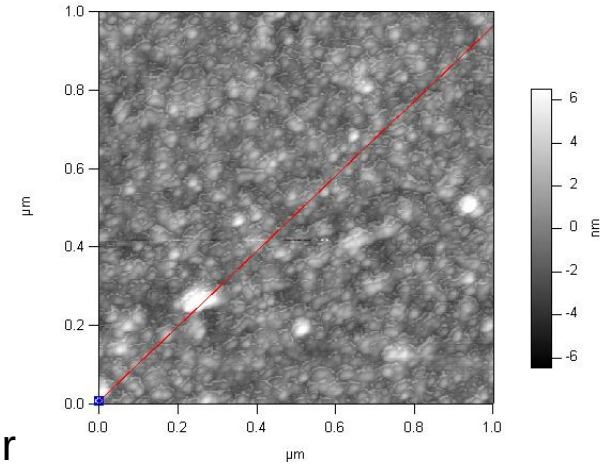




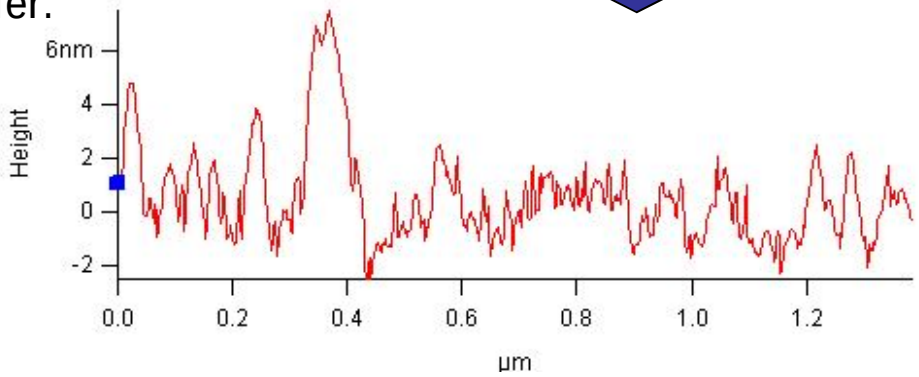
The height of the protein adsorbed onto the hydrophobic wafer at higher concentrations is around 5 nm, according with the isotherm curve.

BPF on hydrophilic wafer 10g/L (Plasma)



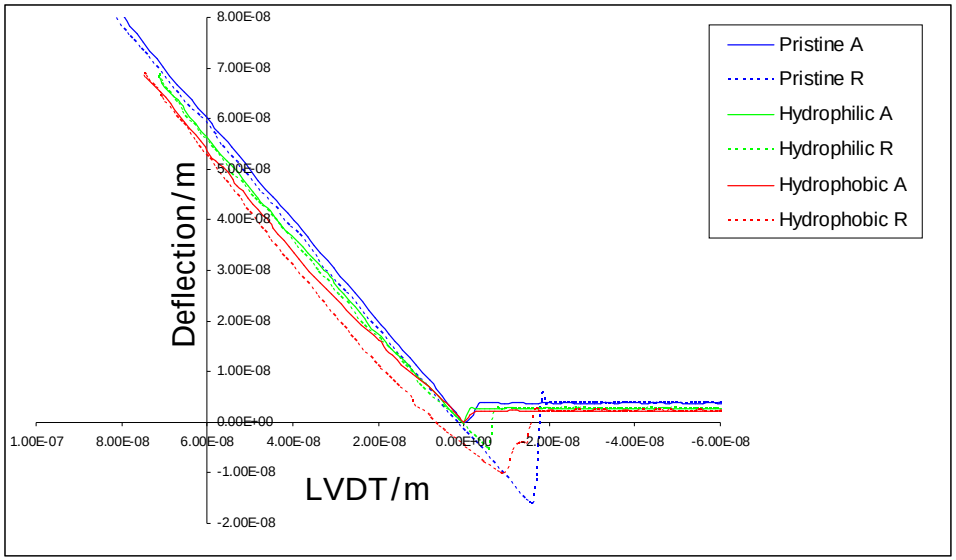


AFM image of multilayer of fibrinogen adsorbed on hydrophobic silica wafer.

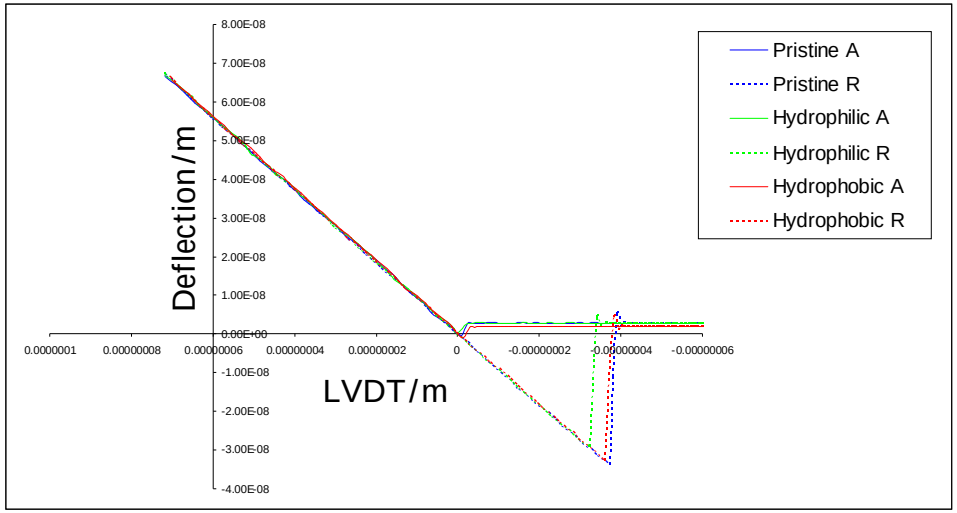


The height of the protein adsorbed onto the hydrophilic is the lowest of them all, it does reach the same thickness as the hydrophobic but it takes a larger protein concentration to form a complete layer.

Force curves

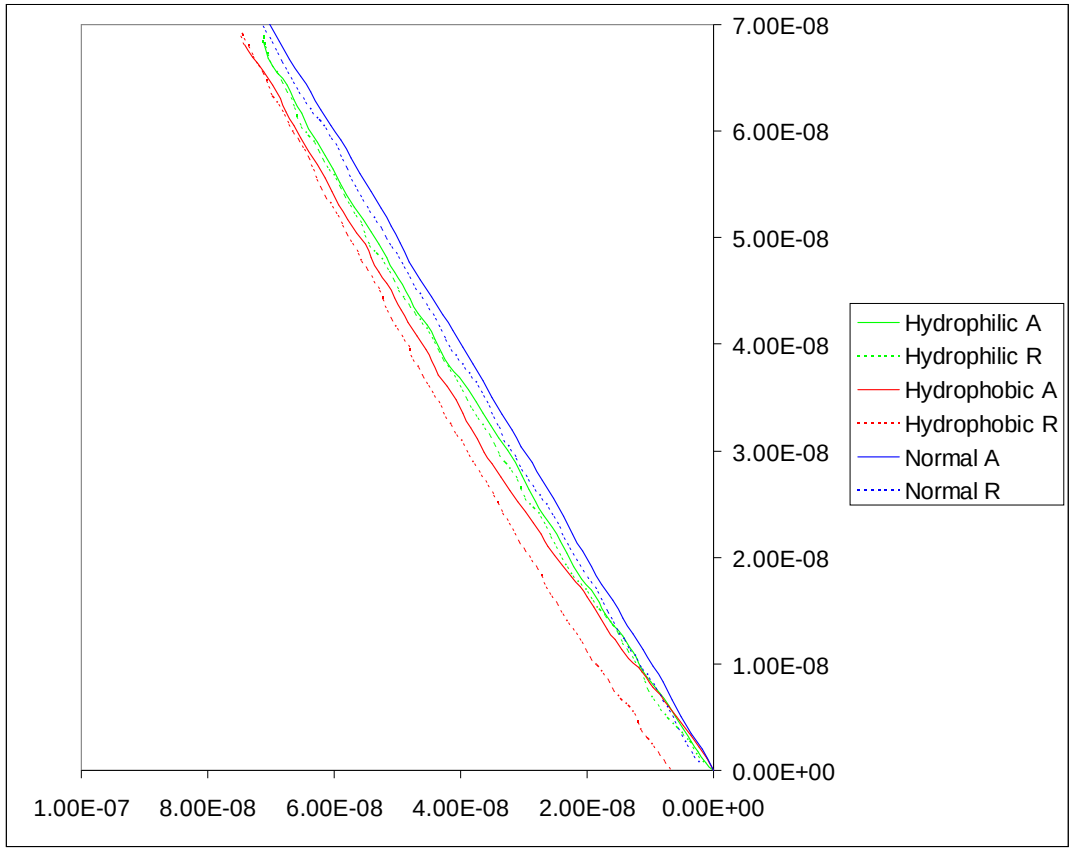


The graph shows the force curves acquired after the adsorption of 10 g/L of fibrinogen in approach (A) and retraction (R).
Those of the pristine wafer (blue) have a shape very similar to the pristine but shifted: the position and the shape shown are typical of a strong attraction between the surface and the tip.
The curves for the hydrophobic (red)indicated less attraction than for the pristine and in this case there is also a typical shape due to multiple tip snaps (when the scanner pulls away from the surface and the tip remains in contact, bending the cantilever).
The hydrophilic (green) indicates the lowest attraction force between tip surface protein, in accordance with the other results.



The graph shows the force curves acquired after the adsorption of 0.01 mg/L of fibrinogen.
For the pristine wafer (blue), the curve has position and shape that are typical of a strong attraction between the surface and the tip.
The hydrophobic (red) indicate less attraction but the shape is similar to the pristine wafer.
The hydrophilic (green) shows the lower attraction.

Surface Hardness



The graph shows the linear region of the force curves acquired after the protein adsorption (10 g/L) .
The slope of the curve reflects the hardness of the sample surface. In this case the hydrophobic sample has a surface softer than both the hydrophilic and pristine wafers.

CONCLUSION

- Wafers with different hydrophilicity show different degrees of adsorption.
  At higher protein concentration (10 g/l), there is a significant difference between the pristine wafer and the other two samples. The thickness of fibrinogen layer on this wafer is about 10 nm while for the hydrophobic and the hydrophilic wafers it is around 4 nm.
  The trend of adsorption thickness becomes similar for all sample at intermediate concentrations.
  At low concentration (1 g/l) there is a difference between the hydrophilic wafer and the other two wafers. The height of the fibrinogen layer on this wafer is about 4 nm while for the pristine and the hydrophobic is around 2.5 nm.

- The conformation of the fibrinogen changes after the adsorption on wafers with different hydrophilicity.
  The imaging of fibrinogen single molecule shows different size, shape and height for all the samples.

- The force curves give information about the protein adsorption strength to the wafer.
  The force required to remove the protein from the wafer is different for all the sample: on pristine wafer there is the strongest protein adsorption and on hydrophilic the weakest.