ANALYSIS OF FUNCTIONALIZED MICA SUBSTRATES FOR AFM IMAGING OF DNA

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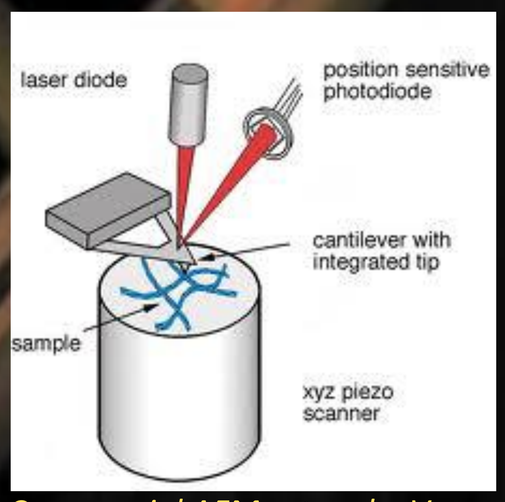
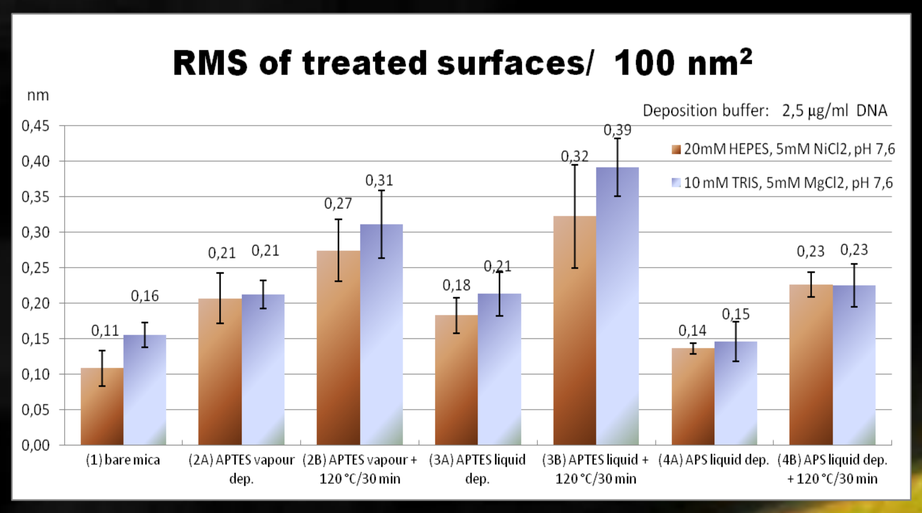


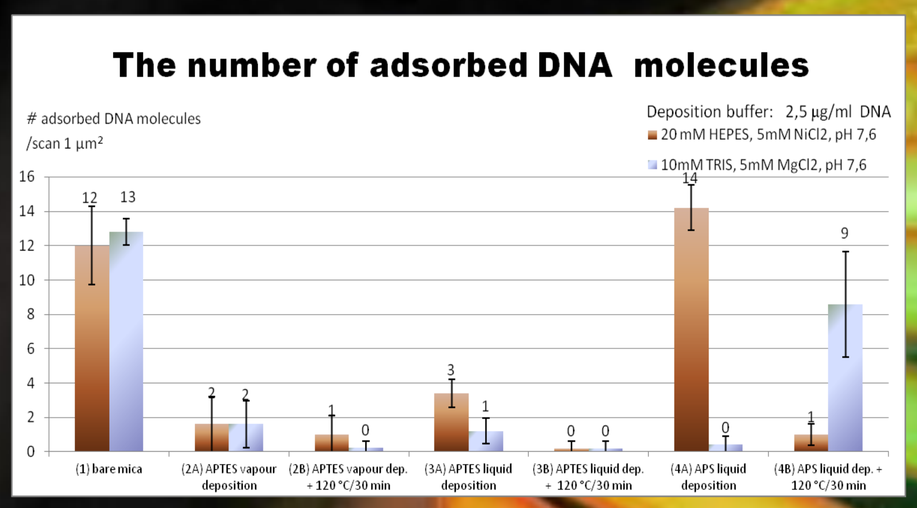
Commercial AFM setup, by Veeco, USA

The scheme illustrates principles of the atomic force microscopy (AFM). Briefly a sharp stylus reads the profile of the sample by raster scanning over the sample. The tip is attached to a cantilever which reads the surface profile due to force interactions with sample surface. The vertical position of the tip is measured by a laser light spot reflected from the cantilever to the position sensitive photodetector.

The study of biological systems by AFM requires the immobilization of the sample molecules on a support surface – a substrate. Fixing of the molecule sample to the substrate ensure that the scanning tip does not push the sample out of the scanning area either will not interfere with the sample because of its moving. From this reasons it is obvious that a key step to the successful AFM imaging of DNA is a sample preparation.

Crystal mica, which has atomic level smooth surface, is used as a typical substrate for adsorption of DNA molecules. Freshly cleaved mica stubs have root mean square of surface 0,06±0,01 nm. There are several methods of fixation of negatively charged DNA molecules on mica, whose surface has also a negative charge in water. The simplest technique is a treatment of **bare mica with bivalent cationts** (method 1- bare mica deposition) [1].





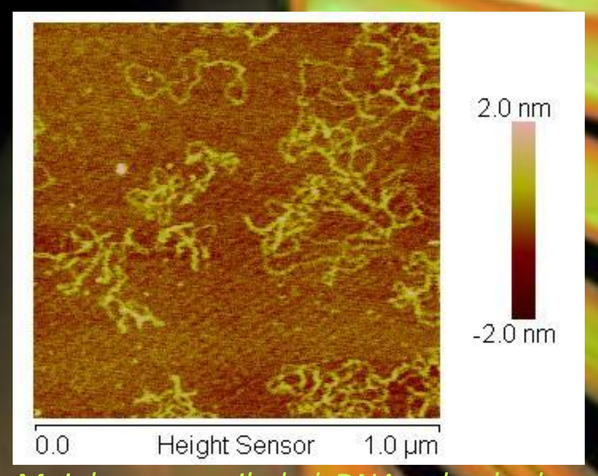
A film of proper chemical layer formed on freshly cleaved mica, known as chemical mica modification is another approach. We choose **APTES mica modification** with aminopropyl-triethoxysilane, CAS: 919-30-2. This alkoxysilane with amino group makes a homogenous layer on support substrate and reverses the mica surface charge through protonated amino groups at neutral pH[1]. Two deposition methods of APTES were investigated: deposition from vapour phase (method 2 – APTES vapour deposition) and liquid phase (method 3 – APTES liquid deposition). An alternative procedure based on synthesized APS, **aminopropyl-triethoxysilatrane,** mica treatment via liquid deposition method[2] was tested as well (method 4 – APS liquid deposition). Silane (APTES) either silatrane (APS) layer formation is strongly affected by relative humidity factor during and after silanization procedure[3]. For this reason mica substrates were investigated either with no further treatment (A) or with middle baking post deposition treatment, baking at 120 °C for 30 min under ambient conditions (B). All surfaces were used for dsDNA deposition on the day of functionalization because surface degraded after a couple of days. The plasmid vector pmaxGFP 0,5µg/ml was purchased as a gift form Dep. of Biophysics, Faculty of Science and diluted with ultrapure water buffer solutions to final concentration 2,5µg/ml for use. APS was synthesized in organochemical lab with no specific requisitions[4], APTES was purchased from Sigma–Aldrich. All AFM images were taken by BIOSCOPE Catalyst, Veeco, operating in ScanAsyst mode in air.

Parameters used for surface analysis:

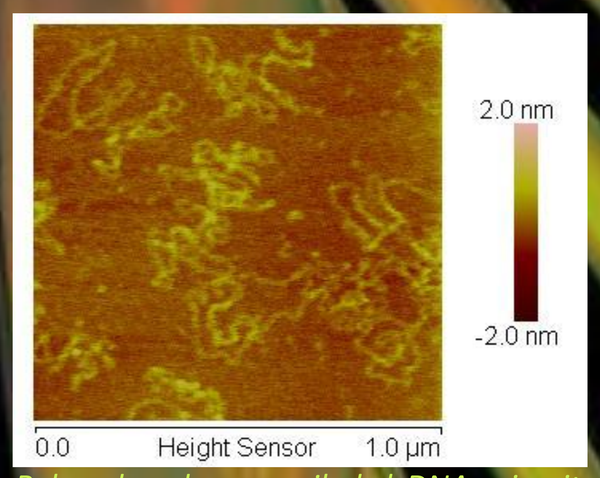
1) **Root Mean Square** (RMS, roughness average) is a term used to quantify the roughness of a surface. Since DNA has diameter of 2,0 nm (in B–form), the substrate has to be as smooth as possible to obtain a good resolution.

2) **Number of DNA molecules attached** to the treated substrate is a most significant sign. As well as a tertiary structure of dsDNA, which should be taken into account. We can see dsDNA deposited on APS treated mica (either HEPES (4A) and TRIS (4B)). DNA adsorbed from HEPES is highly condensed, probably thanks of presence of free alkoxysilanes on the APS layer. In that point of view the cationic deposition method and APS treatment method seems to be the most effective mica modification.

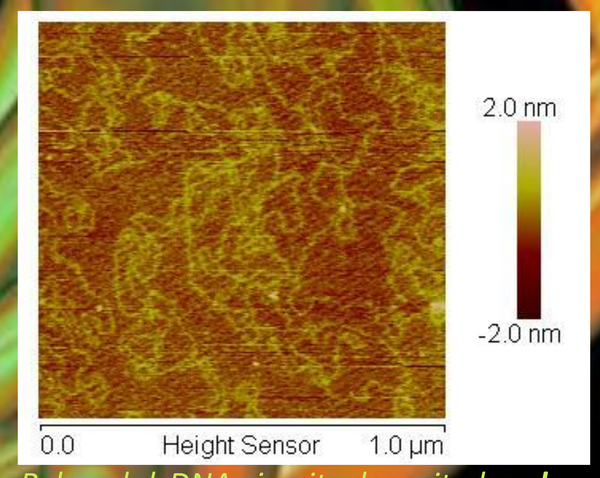
3) **Contamination of surface, reproducibility**. The worst quality and highest number of contamination showed substrate functionalized via **method 3 – liquid APTES**, either non baked and baked surface. **RMS=0,18±0,03 nm and RMS=0,21±0,03 nm for APTES-liquid deposition (3A); RMS=0,32±0,07nm and RMS=0,39±0,07 nm (3B) for APTES – liquid deposition + baking step; HEPES and TRIS deposition buffer respectively. APTES-LIQUID treatment is not suitable for DNA deposition and AFM imaging.**



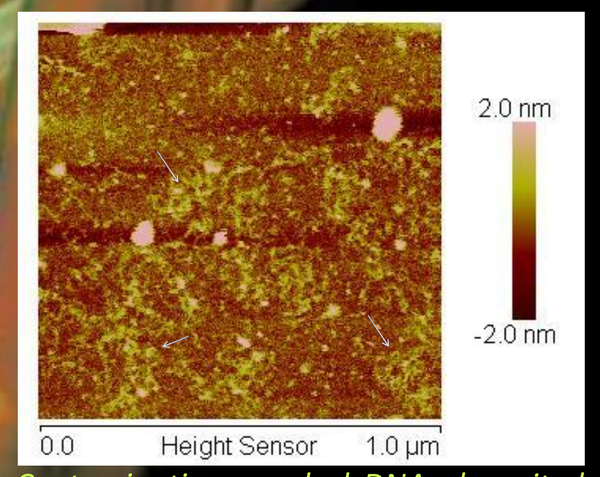
*Mainly supercoiled dsDNA adsorbed on **APS – mica (4A), 20mM HEPES deposition buffer.***



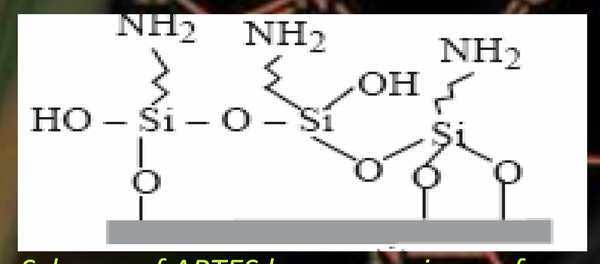
*Relaxed and supercoiled dsDNA circuits adsorbed on **APS–mica (4B), 10mM TRIS deposition buffer.***



*Relaxed dsDNA circuits deposited **on bare mica (1)** cationic assisted deposition method, **10mM TRIS** deposition buffer.*



*Contaminations and dsDNA deposited **on APTES-mica (3B), 10mM TRIS** deposition buffer.*



Scheme of APTES layer on mica surface

AFM image analysis showed sufficient DNA binding to the support surface. DNA binding could cause also distortion of it´s secondary structure. The extent of such distortion depends on the physical and chemical characteristics of the substrate surface and acting forces between the DNA and surface. Therefore the proper choice of a suitable mica modification method depends on further DNA investigation, mainly on to desired degree of fixation.

Acknowledgement to project LF_2012_019

[1] Hansma H.G., Laney D.E., Biophys. J., 1996
[2] Lyubchenko et al., J.Biomol. Struct., 1992
[3] Crampton et al., Langmuir, 2005
[4] Dumitriu A.M.C., Polyhedron 33 , 2012