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# High resolution AFM as a tool for analysis of short-chain DNA structure and properties

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**INTRODUCTION:** Immobilization of biomolecules on surfaces while keeping the maximum conformational flexibility of the molecules is one of the most important techniques for atomic force microscopy imaging. When the DNA molecules are studied with Atomic Force Microscope (AFM) either HOPG (Highly Ordered Pyrolytic Graphite) or freshly cleaved mica is used as s substrate because they show atomically flat surface. DNA molecule can be adsorbed to the graphite surface, however this kind of supporting material is very cost expensive. Mica also shows high flatness, unfortunately it can not be used for DNA immobilization without specific treatment[1], as both mica and DNA are negatively charged in standard buffer solutions. Scanning probe microscopies (SPM) are based on the ability to detect a local property of a surface by means of spatially controlled sensor or probe. Typically, the sensor is scanned over the area of interest so it s only only transiently localized in the neighbourhood of a given point on the surface.

**METHODS:** The AFM images were taken in air as well as in liquid (buffered solution) using the AFM instrument from NT-MDT (Russia) equipped with 'Scanning-by-sample' measuring head allowing highly precise visualization of the surface profile. The whole system was stabilized by the dynamic vibration isolation system TS-150 (JRS Scientific Instruments, Germany). The DNA samples were immobilized to the solid surfaces showing high flatness. Mica and HOPG were used to this purpose. These materials are commonly used in current nanotechnology when biomolecules are needed to be visualized on a solid surface. Various methods were tested with regard to the possibility to capture DNA molecule to a solid support. A simple physical adsorption to HOPG is the simplest one. Efficiency of such process was improved by supporting of capillary forces, when the DNA sample was applied by pressure-print of flat silicon surface. Mica surface was treated in several ways. First, the use bivalent ions was used to make a bridge between DNA and mica surface having the same charge. 1 mM $Mg^{2+}$ was used to capture DNA. However, such a complex is stable only in dry conditons. Nickelous ion of the same concentration were used in a similar way. This kind of ion is able to mediate the DNA-mica interaction even in liquid. Moreover, silanization of the mica surface was employed too. APTES ((3-Aminopropyl)triethoxysilane) was employed to modify the mica surface in order to change its surface charge. Another silanization compound GOPS ((3-Glycidyloxypropyl)trimethoxysilane) was used for covalent attachment of the DNA molecule via formation of peptidic bond with the primary amino-group in the DNA structure.

**RESULTS:** Various approaches in modification of atomically flat surfaces were tested in order to determine not only efficiency of the binding of DNA molecule to such adapted materials but also to immobilize the biomolecules in a native state. It was found, that use of bivalent ions ($Mg^{2+}$, $Ni^{2+}$) is an effective method to capture long-chain DNA. On the other hand, short-chain DNA or even oligonucleotides can not be immobilized using such procedure, because of the DNA self-aggregation. The covalent binding of biomolecules should prefered in this case. Moreover, HOPG substrate was found as an easy-to-use, however very expensive support, showing false results due to breaks in its structure.

**DISCUSSION & CONCLUSIONS:** Even as the above mentioned methods are highly suitable for the DNA binding to solid substrates, there is a need to develop more sophisticated methods. Especially surface protection from non-specific adsorption is essential, e.g. in cases when the DNA molecules should be labelled with either a probe or antibody molecule.

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