

High resolution imaging of double stranded DNA using Atomic Force Microscopy (AFM)

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Since it was invented in 1986 atomic force microscopy has been widely used to image and study biological objects, including cells, chromosomes, DNA and RNA molecules and proteins [1, 2]. As AFM can be operated in liquid, it has been used to follow some in situ processes under physiological conditions.

In order to successfully image DNA molecules on a mica surface by AFM, DNA molecules need to be immobilized and binding of DNA on mica surface should be moderate. Surface charges of mica and DNA are the same - negative under physiological conditions. Therefore immobilization using bivalent cations (Mg^{2+} , Ni^{2+} , Co^{2+}), binding with ethanolamine and surface silanization (alkoxysiloxane derivatives) were tested and optimized in order to obtain optimal surface structure and density of DNA molecules. The higher ionic radius was found to provide weaker bonds. Vaporous alkoxysiloxane derivatives led to uniform negatively charged mica surface (AP-mica) with strong DNA binding (Fig. 1).

Better quality of displayed DNA was achieved by using the correct setting of the real amplitude cantilever in the AFM spectroscopy. This amplitude corresponds with real size of samples, especially in the x-y resolution. Typical size of scanned area is $1 \mu m^2$, typical length of visualized DNA molecules was 1000 bp with the x-y resolution of DNA equal to 8.1 nm (real size 3 nm).

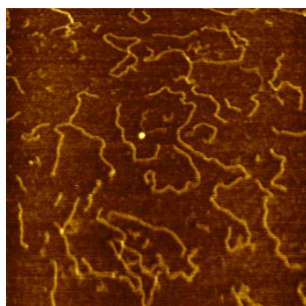


Fig. 1 AFM topography ($1 \times 1 \mu m$) of the dsDNA fragments. DNA was prepared with method „vapour“ APDMES and alkaline catalysis of triethylamine with real amplitude of cantilever 7.9 nm [3].

References

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