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Molecules in Motion: Imaging DNA with the Scanning Force Microscope in Aqueous Solutions

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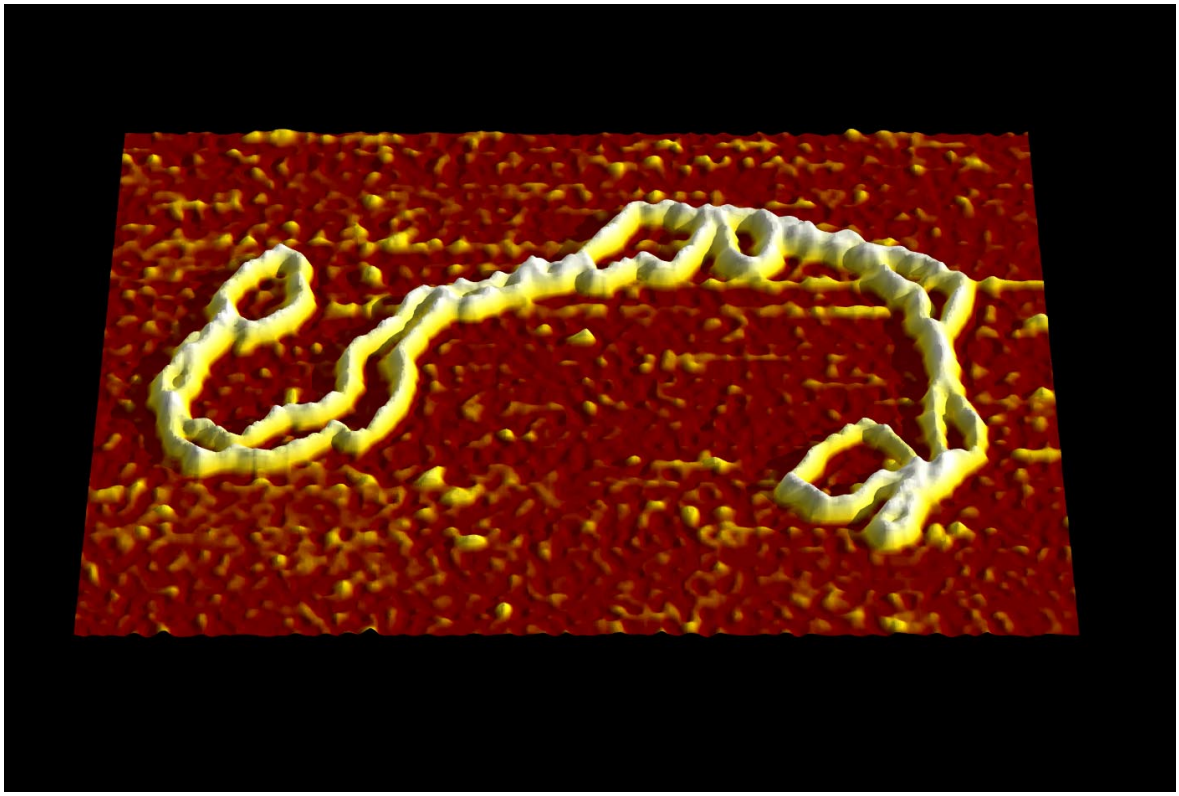
SUMMARY

Scanning force microscopy (SFM) is an emerging technique that provides images of surface bound biomolecules such as DNA in aqueous solutions at nanometer resolution. By imaging in physiological buffers the native hydration state of the DNA can be preserved and artifacts due to drying of the sample are avoided. Under certain conditions the surface bound DNA molecules retain some ability to change their position on the surface, and it is possible to detect conformation changes and dynamic processes on the molecular level. In this report we present SFM images of a superhelical circular DNA plasmid (6800 base pairs or 2300 nm length) and a short linear DNA fragment (600 base pairs or 200 nm length) that have been acquired in liquid.



Introductory figure: Three dimensional surface representation of a plasmid molecule imaged by scanning force microscopy. The image is a magnified view of one of the molecules presented in Fig. 2A.

INTRODUCTION

The conformation of DNA depends on the solution environment, and processes such as drying and staining used in classical electron microscopy can induce changes to the native DNA structure. For example, it is known that at low relative humidity (60-75%) natural DNA sequences will undergo a transition from B-DNA (the dominating DNA double helix conformation found *in vivo*) into the A-form. A-DNA is characterized by a different helix geometry and in particular by a shorter axial rise of 0.28 nm per base pair (bp) instead of 0.34 nm/bp in B-DNA. Furthermore, also the DNA tertiary structure is affected by variations from the *in vivo* conditions. The axes of DNA double helices can be twisted so that two DNA double strands are interwound and form a superhelix [1]. Most naturally occuring DNA molecules are superhelical, and this supercoiling is critical for a number of biological processes. The degree of supercoiling, and along with it the DNA tertiary structure, are sensitive to salt, pH, temperature etc. [1]. Thus, controlling these parameters is essential for determining the biologically functional DNA conformation.

Scanning force microscopy (SFM) - also termed atomic force microscopy (AFM) - is a new technique for imaging biomolecules without further fixing or staining, the only constraint being that the molecule is bound to a surface (reviewed in ref. [2]). In a number of studies DNA (e.g. [3, 4, 5, 6, 7]) and also protein-DNA complexes (e.g. [2, 8, 9]) have been visualized successfully. A schematic view showing the SFM liquid cell and some central parts of the instrument is presented in Fig. 1A. An ultrafine sensor tip which typically has a curvature radius between 5 and 15 nm is mounted on the end of a flexible cantilever. The tip is moved in x and y direction over the surface by a piezo scanner. In the so-called "tapping mode" in liquid which was used here, the cantilever is oscillating up and down at a frequency of about 10-30 kHz while it is scanning [10]. The tip makes transient contacts with the sample which lead to a reduction of the cantilever amplitude as compared to its free amplitude, when the tip is not contacting the sample. Any changes in sample height encountered by the tip during the scanning process translate into variations of the cantilever amplitude. These variations are detected by monitoring a laser beam that is reflected on the back of the cantilever with a four quadrant photodiode as illustrated in Fig. 1A. With this setup height changes as small as 0.01 nm can be measured. If a variation of the amplitude is detected the piezo scanner moves the sample in *z* direction up or down until the original amplitude is reestablished.

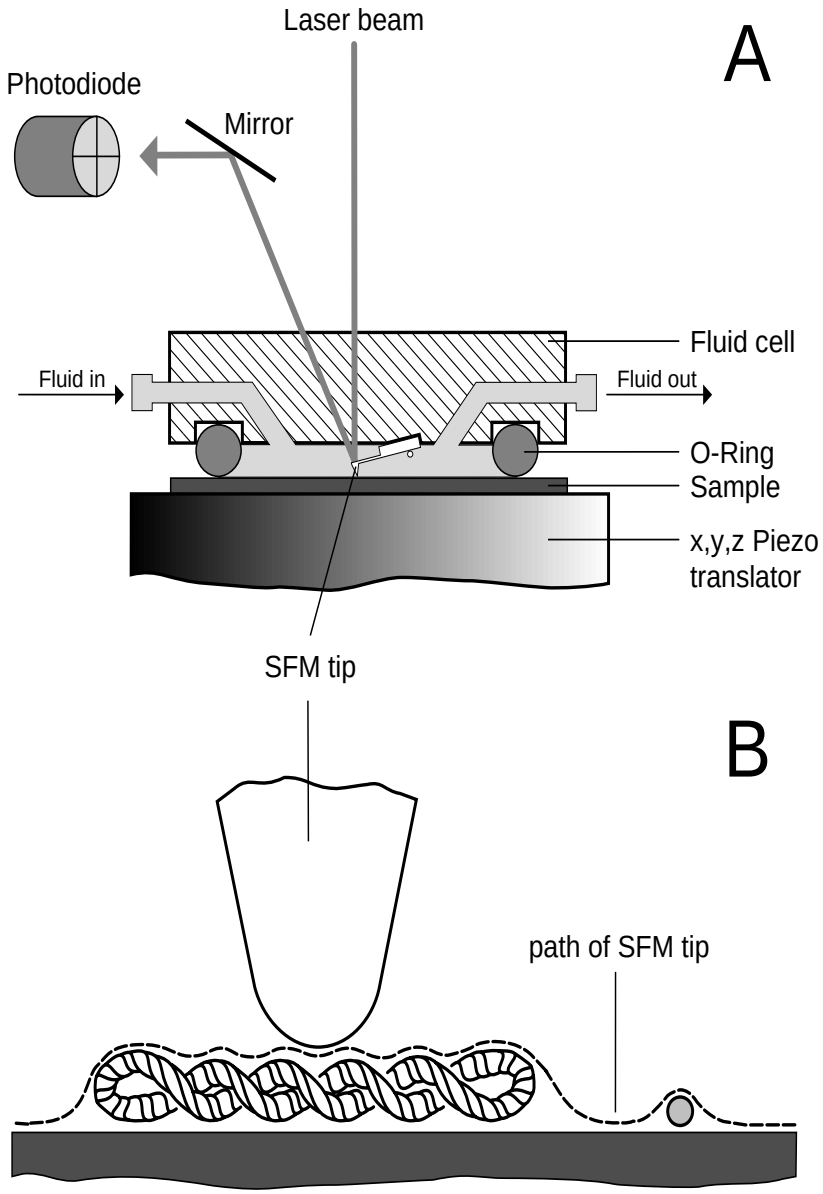


Fig. 1. Schematic diagram of SFM in liquid. (**A**) Cross section of the fluid cell. (**B**) Path of the tip during the scanning process. To the left a scheme of a superhelical plasmid is shown and to the right a cross section through the DNA double helix is presented. The large size of the tip as compared to the dimensions of the DNA double helix leads to an apparent DNA width in the image that is larger than the true DNA double helix diameter.

Thus, the force between tip and sample is held constant during the scan and a topographic image of the surface is acquired from the movements of the scanner in *z*-direction. In order to capture images in liquid a special fluid cell is used (Fig. 1A). An O-ring seals the cell with the sample surface so that the liquid is confined to a volume of about 30 µl. Solutions can be exchanged by carefully flushing them through the cell via a syringe and tubing connected to the cell. The resolution of the SFM image is limited mostly by the size of the scanning tip.

Compared to the dimensions of the DNA double helix, the tip is relatively large. This leads to some distortion of the true dimensions of the DNA in the SFM image as illustrated in Fig. 1B. With a curvature radius of 10 nm for a parabolic tip as it has been used here the theoretical resolution is 6.4 nm for objects of equal height [2].

RESULTS AND DISCUSSION

The SFM images were obtained with a Nanoscope III from Digital Instruments (Santa Barbara, CA, USA; European distributor office in Mannheim, Germany). The preparation of the DNA samples and further technical details are described in ref. [6]. In Fig. 2 images of a 6.8 kb plasmid at native superhelicity are displayed.

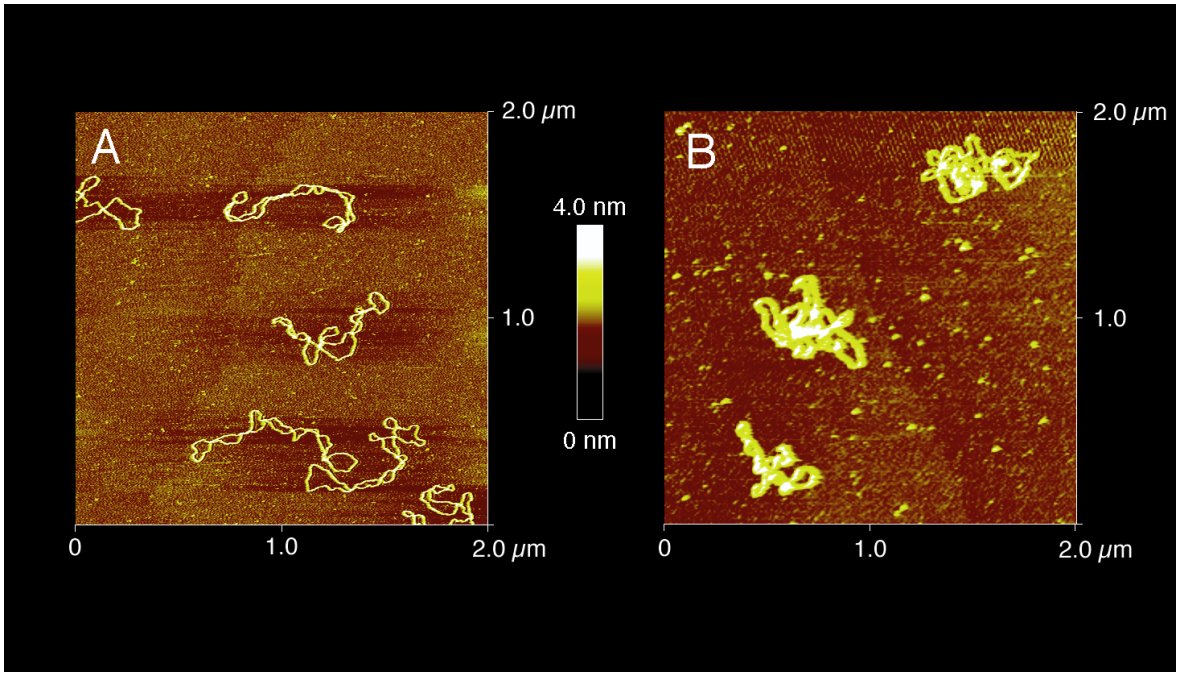


Fig. 2. SFM images of a 6.8 kb plasmid at native superhelical density scanned in a solution of 1 mM $MgCl_2$. The different colors correspond to different heights with the color code given in the middle of the image. (**A**) Deposition in the presence of Mg^{2+}. (**B**) Deposition in the presence of Ni^{2+}.

The samples were deposited onto freshly cleaved mica either in the presence of 10 mM Hepes/KOH, pH 8.0, 10 mM $MgCl_2$, 30 mM NaCl and 0.01% NP-40 (Fig. 2A), or in a

buffer containing 10 mM Hepes/KOH, pH 8.0, 20 mM $MgCl_2$, 100 mM K-acetate, 1 mM $NiSO_4$ and 0.01% NP-40 (Fig. 2B). The divalent Mg^{2+} or Ni^{2+} cations are necessary to bind the DNA to the negatively charged surface of freshly cleaved mica. After deposition of the samples the mica was dried in a stream of nitrogen to fix the conformation of the DNA. We have observed that drying of the samples prevents the molecules from moving on the surface and changing the conformation, as opposed to samples that are directly injected into the fluid cell (see below). After drying, the samples were rehydrated in a solution of 1 mM $MgCl_2$, and the images shown in Fig. 2 were captured. It can be seen that the conformation of the superhelical plasmid depends strongly on the buffer conditions used for the deposition: The plasmids either adopt an extended plectonemic conformation with only a few branches and long regions of interwound DNA strands (Fig. 2A), or are in a more compact rosette like conformation (Fig. 2B). It is likely that the type of divalent cation (Mg^{2+} versus Ni^{2+}) is crucial for the conformation of the DNA superhelix that is visualized by SFM. Binding the DNA via Mg^{2+} to the surface is relatively gentle and slow, and the DNA can reequilibrate on the substrate under the conditions of deposition used here [4]. The images obtained represent a lowest energy conformation similar to that of the molecules in solution [4]. On the other hand the Ni^{2+} mediated interaction between mica surface and DNA is significantly stronger than with Mg^{2+} [7] which could lead to "trapping" the DNA in a conformation that will resemble more a projection of the 3D conformation to two dimensions without reequilibration of the molecule on the surface [4].

The images displayed in Fig. 3 were obtained by injecting a 2.5 nM solution of a linear 600 base pair DNA fragment in 5 mM Hepes/KOH buffer, pH 8.0 supplemented with 1 mM $MgCl_2$ directly into the fluid cell onto fresh Ni-treated mica as described in ref. [5]. This procedure avoids drying the DNA and leads to a relatively weak attachement of the DNA to the surfaces: Upon inspecting succesive images 1 to 4, it can be seen clearly that the DNA fragment in the center is changing its position on the surface. Discontinuities in the path of the DNA as evident in image 2 and 3 indicate that the DNA in this region is transiently not in contact with the surface. Thus, the DNA has retained some ability to move on the surface and conformation changes can be observed under these conditions.

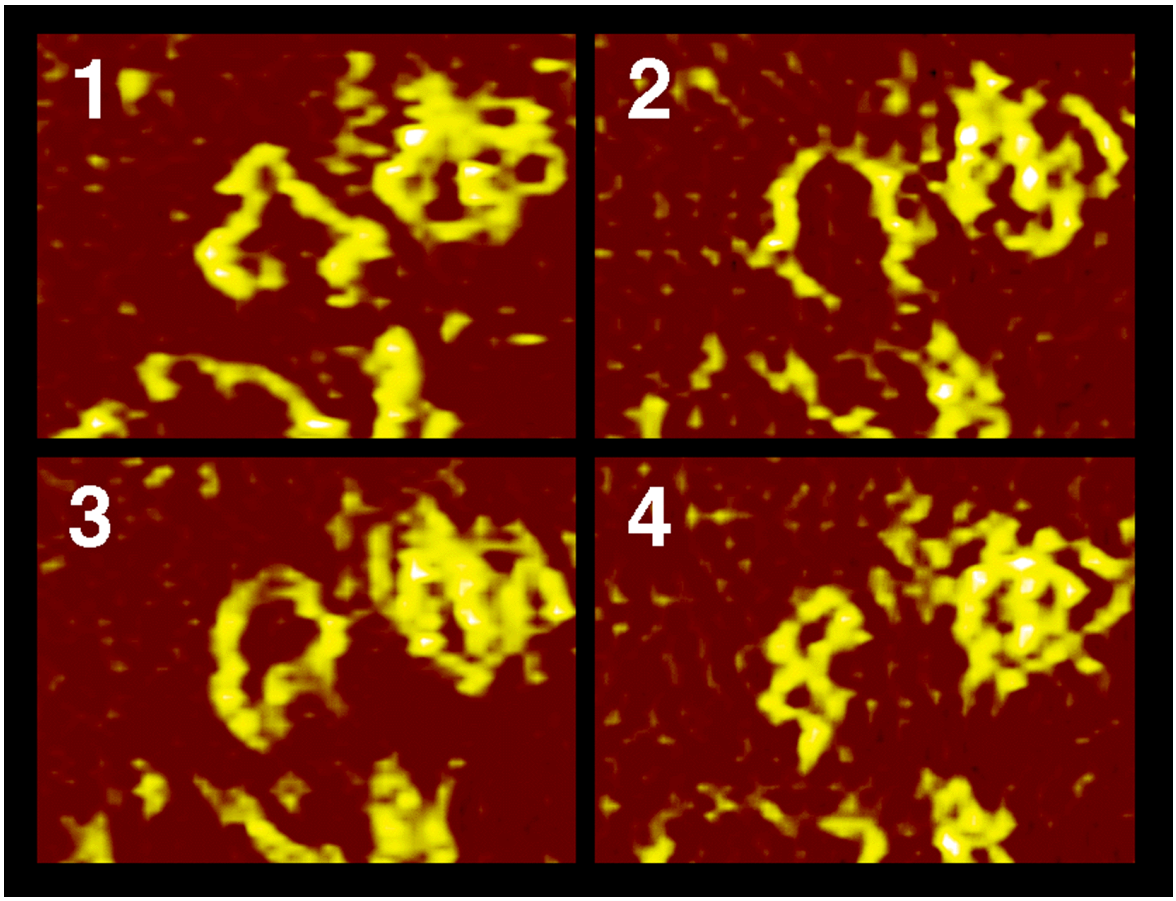


Fig. 3. DNA in motion. Images number 1 to 4 were captured sequentially about 8 min apart. The image size was 220 x 290 nm.

In summary, scanning force microscopy provides unique possibilities for the analysis of the DNA conformation at nanometer resolution. Our results indicate that the buffer composition used to prepare the samples affects the DNA superhelix structure. SFM imaging can be done in physiological buffers which preserves important features of the native conformation, that might not be visible with dried samples. This is going to be even more important for studies of proteins and their interaction with DNA, since it is known that drying of protein samples completely disrupts the protein tertiary structure. In addition, we have demonstrated in experiments with a short 600 bp linear DNA fragment that DNA molecules can be imaged under conditions where they still have some ability to change their location on the surface. This has been reported previously [3, 5], and reveals the potential to detect conformation changes, induced by varying the solution conditions within the SFM liquid cell. In a recent SFM analysis of *E. coli* RNA polymerase [9], the movement of the enzyme during

transcription has been visualized. We anticipate that new and exciting details on the dynamics of biological processes will result from this type of SFM studies in the next years.

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