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Molecular patterns by manipulating DNA molecules

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Manipulating DNA molecules to form molecular patterns on a nanometer scale is a subject with wide prospects. By applying a modified ''molecular combing'' technique and imaging in air with atomic force microscope, we aligned DNA molecules on a mica surface which was chemically modified with a small organic molecule, (3-aminopropyl)-triethanoxysilane. Two-dimensional patterns of DNA molecules were also constructed. © *1997 American Vacuum Society.* [S0734-211X(97)02804-7]

I. INTRODUCTION

Imaging biological macromolecules and supermolecules with atomic force microscopy (AFM) can provide new biological knowledge because of its high resolution for biological samples.[1–4] Additionally, manipulation of individual biological macromolecules is a field of study that is not only significant but also very interesting. The methods of extending and manipulating DNA for various purposes have been reported as follows. Large DNA molecules can be stretched out by flow forces in molten agarose and then rapidly fixed by agarose gelation without application of electrical fields.[5] In order to study the conformational dynamics of an individual DNA molecule, a new technique termed the ''pulse-oriented field'' method was adopted.[6] Bead DNA based optical tweezers were used to measure the elastic properties of individual double-stranded and single-stranded DNA molecule.[7,8] In addition, by first anchoring one end of the molecule in a matrix, DNA molecules were extended and aligned by a receding air–water interface or by electrophoresis. To anchor DNA to a glass surface, researchers first grafted a monolayer of silane molecules onto a substrate surface,[9] then stretched it by a ''molecular combing'' technique or by gel electrophoresis.[10–13]

Extension of DNA is much more important for imaging with AFM which is expected to have potential application in biology. For example, direct mapping of genes or direct sequencing of DNA with AFM requires that the DNA strands be straightened first.[14–16] To directly observe the process of reaction between DNA and other biomolecules, the DNA strands should not be tangled. However, extending DNA for AFM imaging must fulfill special requirements. Usually, biological samples must be strongly attached to an atomic flat matrix surface so that they are immobile in a buffer solution and not swept away during imaging with AFM. Although the mica surface is flat at the atomic level, unfortunately, it is not strong enough to attach biosamples.[17,18] Chemically modified mica, particularly mica silanzed with a monolayer film of (3-aminopropyl) triethanoxysilane (APS), has shown good performance.[19,20] These surfaces have the following characteristics: (i) they meet the requirement of being molecularly or atomically flat; (ii) they have a strong binding ability to DNA; (iii) their preparation is simple and convenient; (iv) they are stable in a buffer solution for a long time.[21] More important, the molecular combing technique, as well as the modified method by Weier,[22] has made it possible to extend large size DNA strands into an aligned form on APS mica.

In this article, we report that large size DNA molecules could not only be aligned in one direction but also constructed into a special two-dimensional pattern on the APS mica surface.

II. MATERIAL AND METHOD

The mica surfaces were chemically modified, using a monolayer film of 3-aminopropyl triethoxysilane (APS, United Chemical Co., Bristol, PA) with a molecular self-assembly method. The detailed method was described in Ref. 10.

Supercoiled lambda DNA molecules were linearized with restriction endonuclease Nae I, both incubated for 60 min at 37 °C. Lambda DNA (48 502 bp) and Nae I were products of Promega Co., USA. Nae I recognizes the special sequence (GCC↓GGC) and produces blunt ends. There is only one recognition site in the lambda DNA molecule to restrict enzyme Nae I. The DNA digested by Nae I was diluted to a concentration of a few nanograms per microliter. For the study with AFM, the samples were prepared by first depositing a small drop (typically 2 $\mu\ell$) of DNA solution onto a clean glass cover slip. The glass cover slip was then carefully placed onto the top of the APS film. The weight of the thin cover forced the solution to spread immediately into a thin layer. After a few minutes, the glass slip was removed and the APS film surface was rinsed with doubly distilled water and dried with a flow of clean nitrogen.

In this study, all images were collected using a Nanoscope III AFM (Digital Instruments, Inc., Santa Barbara, CA) with tapping mode in air.

III. RESULTS AND DISCUSSIONS

Figure 1(a) showed the typical one-dimensional DNA pattern. In this AFM image, the strands were well distributed on the APS film in a parallel fashion. The condition to form a

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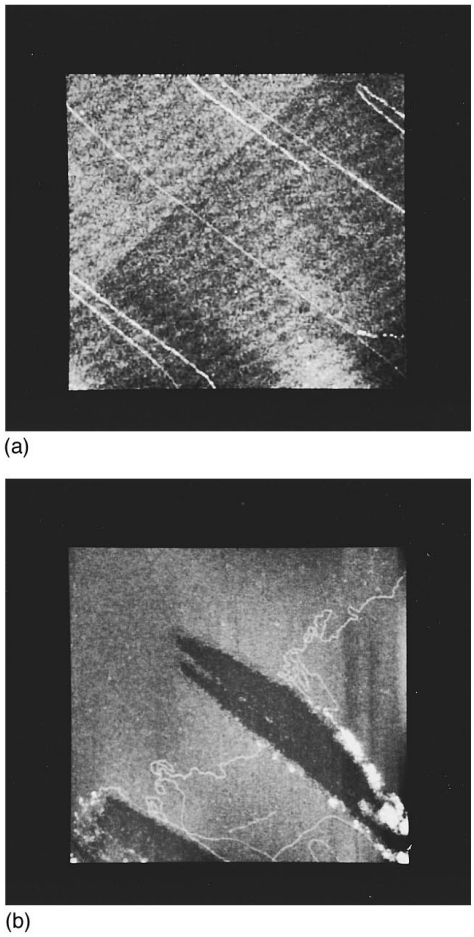

FIG. 1. AFM images of one-dimensional DNA strands aligned on the APS film surface by applying a molecular combing technique. (a) Scanning range: 10 μm×10 μm, z range: 0–2 nm (b) DNA strands on APS film with flaws. Scanning range: 1.1 μm×1.1 μm, the z range: 0–6 nm.

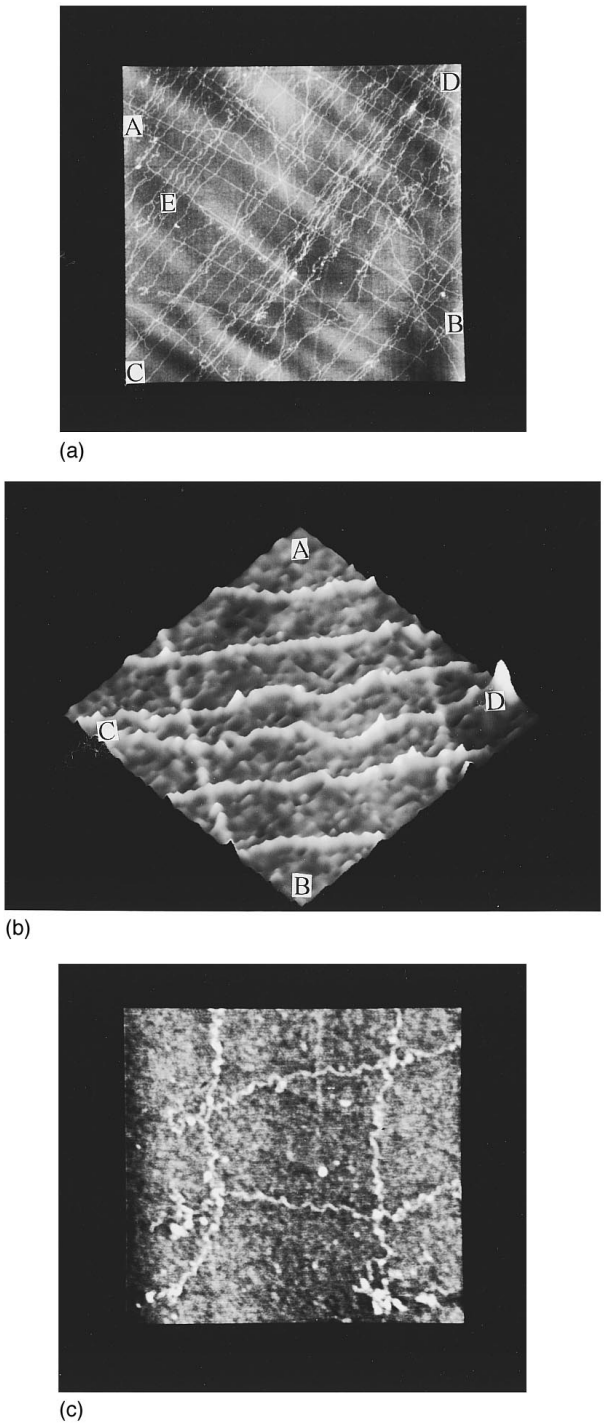

FIG. 2. AFM images of two-dimensional DNA pattern aligned on the APS film. (a) Two-dimensional DNA network in a large scanning range: 10 μm×10 μm, (b) highlight view extracted from the E area in (a), and (c) a typical Chinese character well.

very straight DNA pattern was very subtle. Sometimes the roughness of the substrate surfaces influenced the spreading of the solution and resulted in many tangled DNA strands.[23] We found that the DNA strands could be made straighter by applying extra pressure with a finger on the glass cover slip while combing. Here, we refer to the pressure from the finger as an extra force. The reason for the pressure effect might be as follows. DNA solution can be spread by the gravitational force (the weight of the glass slip itself) and the wetting force between the two surfaces with water inside. If the spreading speed was very slow, the DNA molecules had more opportunity to be adsorbed in a small area on the APS mica surfaces, resulting in tangled fashions. The extra force could enhance the spreading speed of the solution and the straighter DNA patterns could be easily obtained.

In some cases the mica surface was not fully covered by APS film as shown in Fig. 1(b). The black flaw is the bare mica surface since the APS only forms a monolayer on mica substrates. The thickness of the APS film can be measured from the height difference between the APS area and the bare mica area. In this experiment it was about 0.6 nm. It was thought that the APS molecule attached chemically on a mica surface by an oxygen bridge between a silicon atom and the mica surface.[9,19] Theoretically, the monolayer film thickness can be obtained by calculating the sum of all relative covalent bond lengths. It is about 1.0 ± 0.1 nm.[24] The 0.6 nm thickness measured by AFM here might come from the fact that the film is soft and may easily be flattened by the

AFM tip. Another possibility is that the APS molecule is not fully upright on the mica surface.

We noticed that the DNA strands only extended over the APS film and did not enter the flaw where the mica was not covered by APS, illustrating the different adsorption of DNA on different surfaces. The adsorption between DNA and the mica surface is weaker than that between DNA and the APS film.

Figure 2 contains AFM images of two-dimensional DNA molecule patterns. To construct the patterns, we used the following procedures. First of all, a monolayer of APS film was grafted on a mica surface; second, the DNA molecules were aligned on it in the AB direction, rinsed, and dried; then we aligned the DNA along the CD direction, rinsed, and dried again. This result shows that the APS film is a significant contributor to aligning DNA and constructing the DNA network. Figure 2(a) is a two-dimensional network on a large scale. Figure 2(b) is a highlight view in a small range extracted from the *E* area in Fig. 2(a). Figure 2(c) is a typical Chinese character ''well.''

In view of the strong adsorption, the DNA strands aligned on the APS film were difficult to rinse away, therefore, the APS mica system provided a suitable method for direct mapping of genes and direct sequencing of DNA with AFM. It is useful not only to obtain a low ''background noise'' sample for AFM imaging but also to prepare two-dimensional DNA networks. The mechanism of adsorption in this system is not clear. One possible mechanism might be the interaction between DNA and APS film by electrostatic attraction. The APS molecules were grafted onto the mica surface and the up ends were amine groups-NH_3^+ in water solution at $p\mathrm{H}=7$ while DNA is usually negative charged.[10] Though the strength of the attraction would be very weak at each attachment site, it would be enormous for a long strand molecule because of frequent points of attachment along its full length.

IV. SUMMARY

By applying a modified molecular combing technique, we aligned DNA molecules on APS mica surfaces and imaged in air with AFM. Two-dimensional patterns of DNA molecules could be constructed and a typical Chinese character well was formed. Alignment of DNA strand may have potential applications in constructing a molecular electric circuit and precision gene mapping.

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