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Artificial DNA Patterns by Mechanical Nanomanipulation

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ABSTRACT

A special method, which is a combination of macroscopic "modified molecular combing" and microscopic "molecular cutting", is proposed in this paper. DNA strands are first aligned on a solid substrate to form a matrix of 2D networks. Atomic force microscopy is then used to cut the DNA network in order to fabricate fairly complex artificial patterns. Curved and wavy structures are constituted by a manipulation process based on the elastic behavior of DNA strands. A new phenomenon of physical "folding" of DNA induced by the AFM probe has been found. DNA strands can be converted into spherical nanoparticles and nanorods by the special process of "pushing" during which DNA molecules fold up into ordered structures in air.

There is a growing sense in the scientific and technical community that technologies based on manipulation and assemblance of atoms or molecules could lead to revolutionary industrial processes.[1] To approach this goal, which is ultimately devoted to the generation of nanometer-scale structural and functional elements, many attempts have already been focused on constituting artificial features directly from atoms or molecules.[2−4] Modern biotechnology has made it possible to modify DNA in various ways and with various functional objects.[5−13] Recent findings inspire the imagination with respect to the broad employment of DNA to constitute optical, electrical, or other functional circuits at the nanometer scale. Challenges ahead concern, e.g., the preparation of DNA strands according to a predesigned pattern in order to realize circuit construction and device design.[14] A recent strategy is based on the possibility that nanomanipulation tools such as the scanning tunneling microscope (STM) and the atomic force microscope (AFM) will be able to align DNA molecules to form predesigned patterns.[15,16] Although scientists have made considerable progress in arranging atoms, small molecules, and even nanoscale liquid droplets by STM or AFM to form artificial features on solid substrates,[17−24] manipulation of biomolecules has not yet been realized at sufficient precision because of many problems.[25] In the case of DNA, the optical mapping method was sucessfully used to get high-resolution restriction maps of bacterial artificial chromosomes.[26] However, it is difficult to manipulate this soft and elongated molecule to obtain a regular artificial pattern from its natural random-coil state by solely treating it with a scanned probe.[27] Other methods, however, have been developed to manipulate DNA. These all work at a macroscopic level and cannot lead to the creation of complex patterns at the nanometer scale.[28−32]

In this paper, a special method to form complex nanopatterns of DNA molecules on solid substrates will be introduced. The method is based on the combination of two techniques: "modified molecular combing",[31,33] which is a macroscopic manipulation method, and "molecular cutting", which is a microscopic one. The idea of this combination is similar to that of "digital painting", in which a complex pattern can be obtained from small square pixels. First, a matrix consisting of a 2D DNA network is generated by molecular combing. Then elementary units of the network are cut out and manipulated by the AFM probe to locally form the predesigned pattern.

An important advantage of modified molecular combing is that DNA strands can be aligned on solid substrates along a chosen direction at a very large scale over hundreds of microns. The orientation of the aligned DNA strands depends on the direction of the fluid flow during the process. In some previous work, we succeeded in forming 2D networks of DNA on a 3-aminopropyl triethoxysilane-coated mica (AP-mica) surface.[33] We first aligned DNA in one direction and

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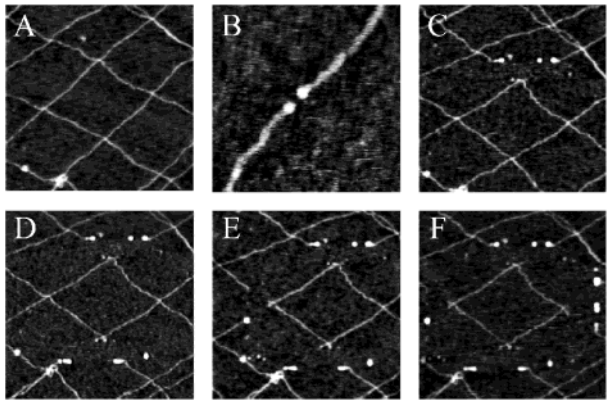

Figure 1. (A) AFM image of a DNA network on an AP-mica surface using the tapping mode in air. The modified molecular combing technique was used to align the DNA molecules on the substrate. We first aligned the DNA strands along one direction and subsequently along a second direction with a selected angle to the first one. As a result of this 2D molecular combing, DNA molecules are arranged with uniformity at the nanometer scale. The image size is 800 nm × 800 nm. DNA samples were linear lambda-DNA from Sigma (St. Louis, Missouri) with a length of 48.5 kb in TE buffer (10 mM Tris-HCl, pH 8.0, 1mM EDTA). The detailed preparation process of the 2D modified molecular combing method can be found in ref 33. (B) DNA strands can be cut by an AFM tip at a sufficient load. During each line scan, the tip first scanned across the surface in the standard 'tapping mode' and then after scanning back it was lowered down onto the surface by a setting distance resulting from the preliminary line scan. The DNA strand is broken after several such scans at a certain site when the setting distance, which corresponds to the load on the surface, was large enough. A typical cut is shown. The image size is 300 nm × 300 nm. (C−F) Molecular cutting of the DNA network resulted in a square pattern. All image sizes are 800 nm × 800 nm. We first broke a DNA strand at a selected site, such as that in (B), and then swept the residuals away. During sweeping the tip scanned at a load smaller than the cutting threshold.

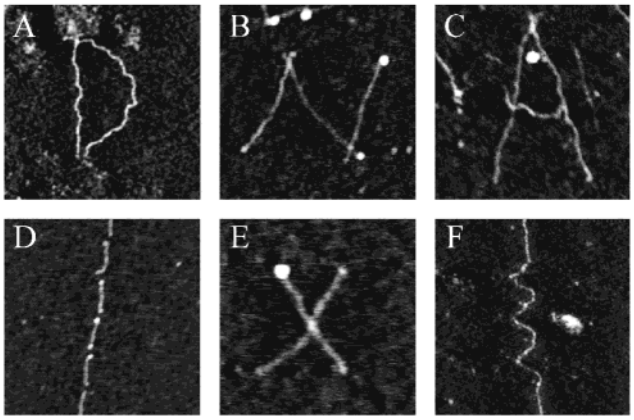

Figure 2. Several patterns formed from DNA molecules at the nanometer scale. The image sizes are (A−C) 500 nm × 500 nm, (D) 1 μm × 1 μm, (E) 200 nm × 200 nm, (F) 500 nm × 500 nm.

subsequently in a second direction. The spacing of the DNA strands in the network could be controlled at the nanometer scale by adjusting the DNA concentration in the solution. Although the 2D network cannot yet be made very uniform, many regions with a sufficiently regular arrangement of DNA strands can be found.

After performing molecular combing, the DNA molecules are no longer random coils and are arranged on the substrate in a fairly uniform way, as shown in Figure 1A. Local patterns can be fabricated from this network by molecular cutting employing AFM. Previous approaches reported by several authors have shown that DNA strands can indeed be broken by an AFM tip.[34]

Upon cutting a DNA strand, the cutting site can be addressed with nanometer precision. In Figure 1B, a typical result is shown. The gap between the two parts is of the same size as the apparent diameter of the DNA strands, indicating that the gap width is determined by the tip shape. If the tip is blunt or contaminated, the gap becomes large and sometimes cutting is difficult to control. The loading forces exerted by the tip can be estimated by measuring the adhesion force between tip and substrate. It is found that there is a threshold for the force necessary to break a DNA strand. The threshold force varied for different tips from 20 nN to 100 nN in the experiments. The mechanism underlying DNA cutting is very interesting but details are beyond the scope of this paper. Some spherical aggregation of material at the two ends of the broken DNA strand is often observed. These clumps of shorter DNA segments at the end of DNA strand are possibly caused by DNA folding during the cutting as will be discussed below.

Figure 1C−F shows how a square unit is cut out at the network in Figure 1A. We first break a DNA strand at a selected site, such as that in Figure 1B, and then sweep the residuals away. During sweeping, the tip scans an area at a load smaller (usually 10 nN smaller) than the cutting threshold. Since the DNA is weakly adsorbed on the surface it can be swept out of the scanning region. Various complex patterns could be formed in this way as shown in Figure 2.

It has also been found that DNA strands are highly elastic and can bear a large change in their shape. By a fairly delicate operation, a straight DNA strand was manipulated by the AFM tip to form the wavy structure shown in Figure 2F. Interestingly, the DNA strands do not shrink back but stay on the surface after the deformation process. By precise determination of the respective lengths, the curved part of the DNA strand can be shown to be about 1.6 times longer than the straight part. This might have been caused by an overstretching of the strands leading to an irreversible change of the DNA helix structure.[30,35] The overstretched DNA strand is then stabilized on the substrate surface due to the friction force between DNA molecules and the substrate. Such a manipulation, based on the elastic behavior of DNA molecules, is of fundamental importance in forming more complex patterns beyond the simplicity of straight 2D units. The DNA deposits are in any circumstance very stable. We performed the described experiments as well on samples prepared one year before and stored in a desiccator. No obvious differences were found in the behavior of the stored DNA strands with respect to freshly prepared samples.

DNA molecules can also be converted into spherical nanoparticles and nanorods by tip-induced folding. One interesting phenomenon which we found in our experiments is that DNA can fold during a controlled pushing by the AFM tip. The pushing process is similar to that of sweeping. A

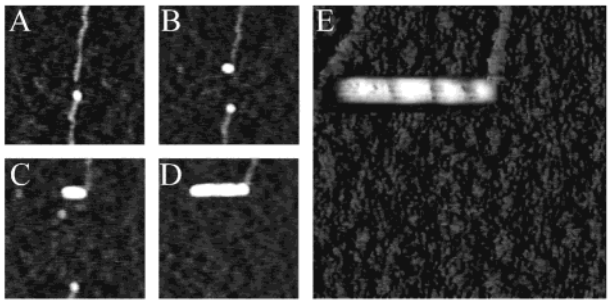

Figure 3. DNA strands can fold up to form particles and thick rods stimulated by pushing with the AFM tip. (A−D) Pushing process showing the folding procedure of a DNA strand. The images were taken after different pushing distances. (E) A 600 nm DNA strand folded up to form a thick rod. All image sizes are 300 nm × 300 nm.

DNA strand can be pushed forward along the vertical scan direction and folded up if we start from the broken site and scan along the horizontal direction, i.e., nearly perpendicular to the orientation of the DNA strand. The pushing operation can be performed step by step at a precision of about 10 nm, depending on the tip size. The length of folded DNA strand fractions can be controlled by adjusting the scanning distance in the vertical direction. In doing so, small DNA fractions could be converted into spherical nanoparticles and long strands into thick rods as shown in Figure 3A−D. In one approach, a 600 nm long DNA strand was converted into a 150 nm long rod. In another, a 2500 nm strand formed a 200 nm long rod. It seems that the rods are at least partially ordered since a periodic structure resembling a helix structure is observed in the longitudinal direction. The periodicity obtained along the rods is not always the same for different experiments, indicating that the structures formed are probably quite complicated. Sometimes the DNA strands are broken during pushing, and sometimes the pushing operation takes place smoothly throughout a very long distance up to several microns. It seems that the local adhesion of the DNA strands to the substrates, the tip shape, the environmental parameters, and the direction of pushing play a role in the DNA folding process. Further quantitative exploration is clearly needed in order to understand the detailed mechanism.

It is, however, worth noticing that the DNA molecules pack themselves in air. This observation can be very helpful in studies on the packing procedure of DNA molecules, which is one of the important issues in biophysics. It is further worth mentioning that there is no reason not to apply the AFM-based procedures described in an ambient buffer solution, which is more relevant to biological investigations. It is obvious that the physical folding induced by the AFM tip, which can be controlled with very high spatial precision, can be extended to studies on other linear macromolecules.

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