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Manipulation and Overstretching of Genes on Solid Substrates

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ABSTRACT

A "molecular chip", i.e., a chip on which single macromolecules are freely arranged, is a key for the fabrication of nanoscopic molecular devices, e.g., DNA molecular array chips. We report on a new method for the manipulation of already deposited single macromolecules, which allows to freely position single polyelectrolytes such as DNA on a substrate, to bend and stretch them, to remove stretching defects, and to overstretch double stranded DNA into two parallel single strands.

The direct and fast analysis of single macromolecular chains at the level of their primary chemical structure, such as the nucleotide sequence in DNA, represents a challenge for macromolecular and life sciences. With respect to DNA, a method of direct sequencing would open new opportunities for gene mapping of humans.[1] Different nanoscopic methods are approaching the resolution level required for direct recognition of single bases, including scanning tunneling microscopy (STM),[2,3] tip enhanced Raman spectroscopy (TERS),[4] and their different modifications. Direct sequencing of DNA would require overstretching of the ds-DNA helix into two parallel strands on a surface or fixation of ss-DNA in a fully extended conformation. One promising approach could be to prepare a macromolecular array of single DNA pieces, where the different single polymer chains from the sample are first properly positioned with respect to each other on the surface and then stretched, and subsequently the primary structure is read by some nanoscopic multiarrayed analyzer.

A so-far unsolved problem is the deposition of single macromolecules on the surface in a predefined conformation. Different attempts were undertaken earlier in order to control the macromolecular conformation for different purposes. In bulk liquids, macromolecules such as DNA have been stretched by pulling on one end of the macromolecule with an optical tweezer[5] or an SFM-tip[6] while the other end is fixed either by another optical tweezer or a solid substrate. However, these methods cannot be applied to manipulate macromolecules on solid substrates. Macromolecules can also be deposited on solid substrates in extended, almost linear conformations by "molecular combing".[7] However, this method neither allows to control conformations on the nanometer scale nor provides a means to obtain more arbitrary conformations or to control the exact location of one macromolecule with respect to another one. Also, the maximum stretching force is limited, thus over-stretching of ds-DNA cannot be achieved.

Here we demonstrate a method in order to manipulate macromolecules physisorbed on a solid surface such that they can be freely arranged, and in particular stretched and overstretched on the surface. An important aspect is also the ability to correct structural defects such as deviations from linearity or other desired shapes of the macromolecule after the first assembly. Such correction also requires free operation with a polymer chain.

Considerable progress was made in recent years using various local probe techniques[8] to manipulate nanoscopic objects across surfaces. For instance, single atoms[9] and small molecules[10,11] have been moved across solid substrates using a scanning tunneling microscope (STM) at very low temperatures, and carbon nanotubes,[12] nanoparticles,[13] and dendronised molecules[14] have been manipulated using the scanning force microscope (SFM) under ambient conditions. For a macromolecule, a most critical issue is its interaction with the substrate, which is usually either too weak, causing the molecule to diffuse rapidly across the surface, or too strong, causing a long molecule to break during manipulation.[15]

Long-chain alkanes and alkylated small molecules self-assemble on crystalline substrates such as the basal plane of graphite[16] or transition metal dichalcogenides[17] into monolayers with the alkyl chains oriented along the substrate axes parallel to each other, while the end groups phase separate into straight lamellae (Figure 1).

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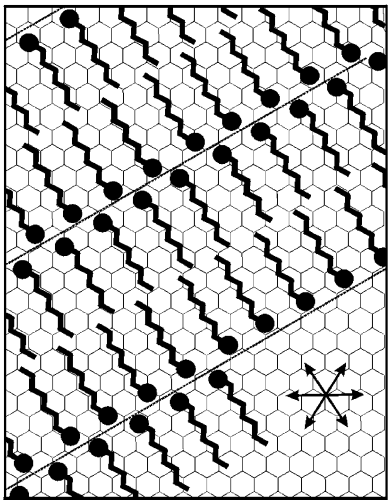

Figure 1. Schematic of a monolayer of alkane derivatives on graphite. Circles denote headgroups and arrows denote the crystallographically equivalent graphite axes with 3-fold symmetry.

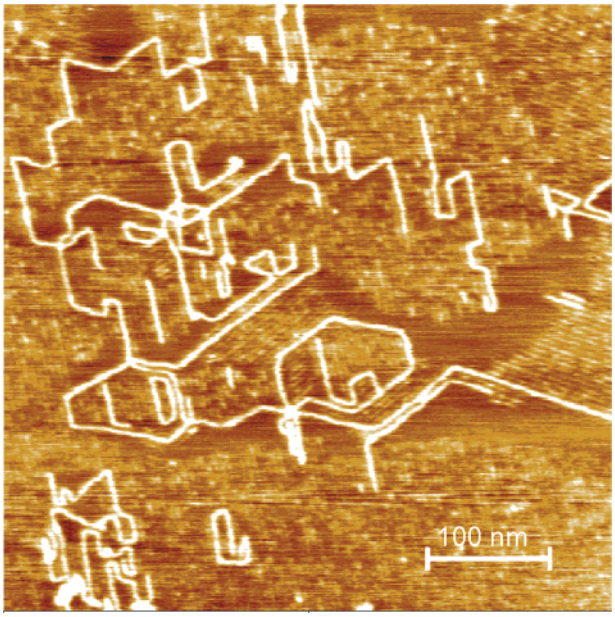

Figure 2. SFM image of poly(sodium 4-styrenesulfonate) (PSS) on the basal plane of graphite modified with $CH_3(CH_2)_{17}NH_2$ molecules, which form lamellae that can be readily visualized with tapping mode SFM. The periodic stripes with 3-fold symmetry are lamellae formed by the coating molecules, while the bright stripes are PSS molecules adsorbed along the lamellae. Imaging was performed by SFM in tapping mode using a multimode head (Digital Instruments Inc., Santa Barbara, Ca.) and Olympus microcantilevers with a typical resonance frequency of 300 kHz and a spring constant of 42 N/m.

These lamellae can serve as soft nanoscopic "rails". Rows of headgroups, which may be positively or negatively charged, are thereby separated by rows of hydrophobic alkyl chains. The chemical nature of the headgroups and the length of alkyl chains define a surface potential ripple, which may be used to orient single polymer molecules on a dry surface.[18] In the present work, monolayers or submonolayers of alkyl-derivatives on graphite or molybdenum disulfide were first prepared by spin coating from chloroform solution. Subsequently, polyelectrolytes were adsorbed from aqueous solution (milliQ purified water) by applying a droplet to the surface and then blowing it off with air. For instance, a positively charged polyelectrolyte (poly(allylamine hydrochloride) (PAH)) was adsorbed to fatty acid ($CH_3(CH_2)_{17}$-COOH) monolayers, while negatively charged polyelectrolytes (ss-, ds-DNA and poly(sodium 4-styrenesulfonate) (PSS)) were adsorbed to alkylamines ($CH_3(CH_2)_nNH_2$, with $n = 11$ or 17). Figure 2 displays an SFM image of PSS macromolecules adsorbed to alkylamines with the main polymer chain oriented preferably along the alkylamine lamellae. Each macromolecule exhibits straight segments and occasional abrupt jumps from one lamella to another, reflecting the 3-fold symmetry of the lamellar arrangement. Contrary to the case of a pure graphite surface, the PSS molecules do not aggregate, and therefore almost every molecule can be traced from one end to the other. The adsorption behavior of the other investigated polyelectrolytes is similar. These results are attributed to the nanopatterned surface providing a potential ripple, along which the polymer orients.

At room temperature, any attempt to manipulate, with an SFM tip, polymers with a contour length beyond 200 nm on a graphite surface coated with a monolayer of CH_3-$(CH_2)_{17}R$ (R = COOH or NH_2) led to breakage of the molecules. For the shorter alkyl chains, e.g., $CH_3(CH_2)_{11}$-NH_2 with a bulk melting point of only 31 °C, we could not observe lamellae with the SFM, which may be due to an increased molecular mobility within the monolayer[19] and correspondingly to a lower 2D density. Still the polyelectrolyte molecules are adsorbed individually onto the substrate and still the 3-fold symmetry appeared. However, the length of the straight segments is reduced. Most interestingly, manipulation with the SFM tip allows to displace the whole molecule without its rupture, which we attribute to the softness of the short-chain monolayer near room temperature.

Figure 3a displays the image of four ds-plasmid DNA molecules (Mobitec GmbH, Göttingen) adsorbed to the modified substrate. From the digitized images, their contour lengths were determined as 792, 803, 812, and 859 nm, respectively, which is a little less than the expected 913 nm (corresponding to 2686 base pairs). We attribute the difference to the nonideally flat adsorption of some segments. Moreover, there are some short sections, where the double strand has separated into two single strands, which apparently are caused by the interaction with the substrate. To stretch subsequently two of the molecules with the SFM, the tip was brought into contact with the substrate and then moved from within a molecular ring outward in four directions as marked in Figure 3b. Subsequent imaging in the tapping mode reveals an increase of the contour lengths of these molecules to 1048 and 1114 nm, respectively, which is about 15 and 22% longer than the fully extended B-form ds-DNA. Figure 3c displays the same molecules stretched further into a triangular shape with contour lengths of 1131 and 1220 nm, corresponding to 24 and 33% overstretching, respectively. Note the sharp curvature at the upper right edge of the lowest triangle, which indicates a radius of curvature below the resolution of the image, i.e., below 3 nm. Also

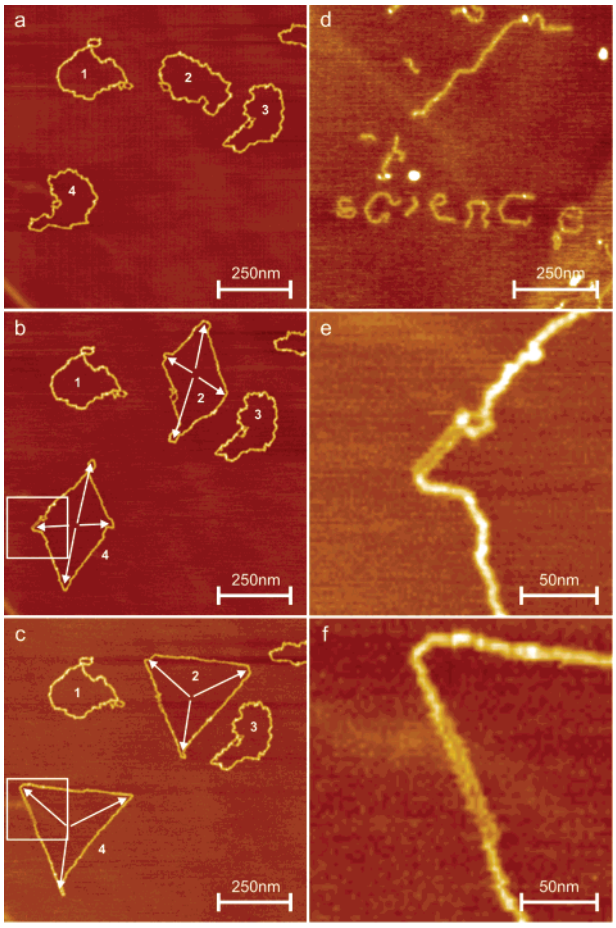

Figure 3. SFM images of dsDNA adsorbed on a graphite surface modified with $CH_3(CH_2)_{11}NH_2$ molecules. Manipulation was performed by bringing the tip in contact with the surface and moving it in the desired direction, using homemade manipulation hardware and software: (a) ds-plasmid DNA molecules as deposited; (b) after stretching two of them (no. 2 and 4) along the white arrows; (c) after manipulation of the same molecules into triangles; (d) seven letter word written with polydisperse sample of linear dsDNA; (e) zoom of the square marked in (b), revealing two separated single DNA strands; (f) zoom of the square marked in (c), revealing the same section as in (e) but now with two fully extended single DNA strands.

note the two parallel single strands on the left side of the lowest triangle (Figure 3c and f).

Moreover, the manipulation with the SFM tip on a properly tailored substrate allows also to displace the whole molecule without its rupture. Free arranging and shaping of polymer molecules is evidenced in Figure 3d, which displays the arrangement and shaping of seven ds-DNA molecules of a polydisperse sample. Similarly we have manipulated ss-DNA and PSS molecules. ds-DNA has also been manipulated on molybdenum disulfide. Both ds-DNA and PSS with contour lengths of up to 2 μm have been moved as a whole. In all these cases, we attribute the immobilization of the macromolecules to the interaction of the charged polymer backbone with an oppositely charged row of headgroups in the surfactant monolayer as in a polyelectrolyte-amphiphile complex.

In conclusion, we have demonstrated that the interaction between a solid substrate and a polymer chain can be tailored in such a way that the synthetic or naturally occurring single macromolecule is immobilized, and at the same time can be manipulated with a scanning force microscope tip without chain breackage. Combined with spectroscopy of fluorescently labeled single molecules,[20,21] this will provide new opportunities to correlate macromolecular conformation with spectroscopical properties. Precise positioning and stretching of DNA molecules combined with ultrahigh-resolution methods such as STM and TERS[2−4] will provide intriguing new opportunities for direct sequencing of DNA. Moreover, free 2D molecular shaping should provide a means to fabricate different 2-D molecular architectures, such as electronic circuitry from single macromolecules.

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