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DNA Nanopositioning and Alignment by Electron-Beam-Induced Surface Chemical Patterning

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ABSTRACT

Nanopositioning and alignment of arrays of DNA molecules on a surface by combination of high-resolution prepatterning and standard macroscopic deposition is presented. Direct electron beam exposure of a graphite substrate activated by amino groups neutralizes locally the surface charge, preventing the DNA adhesion during the consequent deposition. Because of the high resolution of the electron beam writing process, precise active patches can be created directly on the functionalized surface. Narrow (50 nm) stripe patterns produce both positioning and alignment acting as electrostatic traps for the DNA molecules. The approach is demonstrated using triple- and double-stranded DNA of medium length (350 nm). High yield of alignment and regular arrangement of the deposited molecules are achieved in a simple way within large areas.

Controlled positioning of individual molecules on surfaces constitutes a key element of nanotechnology, making possible the construction of ordered molecular nanosystems that offer functionalities unattainable otherwise. In addition to providing a tool for controllable nanoassembly,[1] such techniques can also yield new platforms for investigating the structure and dynamics of single molecules. The development of molecular positioning methods is particularly interesting in the context of interfacing biomolecules with inorganic nanostructures,[2] a mix that does not occur usually in nature but is of great importance for nanobiotechnology applications.[3] The control over individual molecules during deposition is essential for realizing nanoscale devices such as molecular matrices, biochips, and bioelectronic circuits. Such functional molecular-electronics devices might be based on protein or DNA[1−3] molecules arranged at desired positions on a substrate.

Among the many proposed methods for arrangement of DNA molecules,[4−13] particularly useful are those that employ a combination of top-down nanofabrication using high-resolution substrate patterning with bottom-up self-assembly processes where molecular arrangement occurs in parallel at each particular location. Utilizing such an approach only to a limited extent, the techniques developed so far include a combination of molecular combing with surface prepatterning,[6,7,11] microcontact printing,[9] alignment using electric fields,[12] and resist-based electron beam lithography utilizing fluidic flow alignment.[13] The effective combination of top-down and bottom-up approaches require compatibility of the patterning and the self-assembly processes employed. In particular, a major drawback common to all methods utilizing conventional, resist-based lithography[13] is the limitations in the spatial resolution and the use of surface treatment like development, cleaning, and lift-off, which is not favorable for manipulating biomolecular nano-objects like DNA. In contrast, a direct electron beam writing on a prefunctionalized surface, inducing nanometer-scale local chemical modifications, would fully exploit the high-resolution capabilities of focused electron beam exposure. The prepatterned functionalized surfaces can serve as templates for molecular self-organization, which should provide a fast and easy way of achieving regularity and alignment of molecule arrays starting from chaotic macroscopic molecular ensembles.

In the present letter, we report on a new method for precise positioning and alignment of DNA molecules, based on chemical patterning of surfaces induced by direct electron beam exposure. Construction of ordered arrays of aligned

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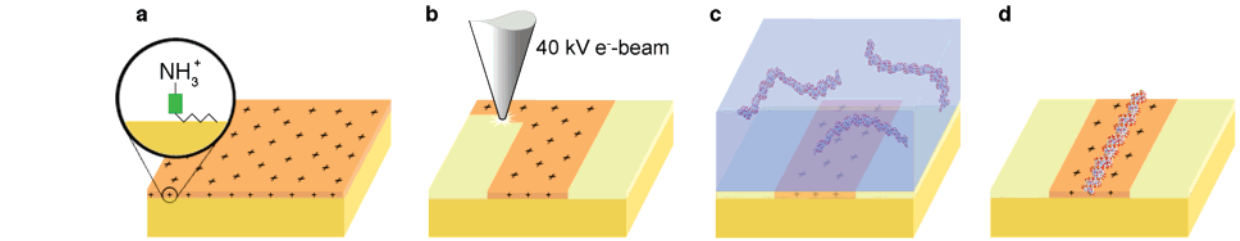

Figure 1. Schematics of the DNA positioning and alignment process. (a) Graphite substrate surface is functionalized to render the surface positively charged with NH_2 groups.[14] (b) Tightly focused high-energy electron beam produces a predefined local neutralization at selected positions.[15] (c) DNA is deposited from a buffer solution.[16] (d) Negatively charged DNA molecules adhere only to the nonexposed, positively charged regions where NH_2 groups remain leading to a predefined arrangement. Once the buffer droplet has been removed, the dry sample is stable for at least 3−6 months.

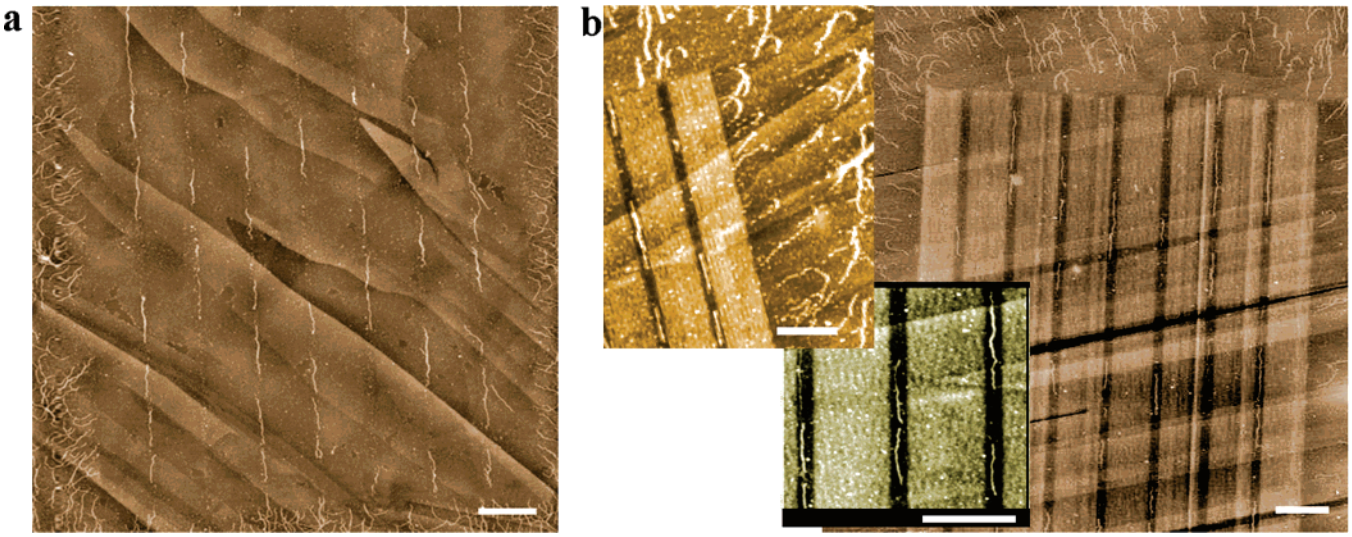

Figure 2. Arrangement and alignment of triplex DNA. (a) AFM image of triplex DNA molecules periodically arranged and aligned conforming to the stripe pattern. The low electron beam exposure dose used in this case does not modify the height of the exposed parts of the polymer, thus the pattern can only be deduced from the distribution of the DNA molecules. (b) AFM images of similar, arranged triplex DNA molecules but on patterns written using a higher (×10) exposure dose. Such a high exposure results in a growth of polymer (height ∼1−2 nm), which renders the exposed pattern visible. The active surface for DNA adhesion is seen as narrow (∼50 nm), darker channels. (Insets represent different samples). All scale bars are 500 nm long.

double-strand and triplex DNA molecules is demonstrated, and the positioning and alignment mechanisms are investigated. The direct electron beam writing on a prefunctionalized surface induces nanometer-scale local chemical modifications. This is combined with a macroscopic deposition of DNA from a buffer solution. The self-assembly of the DNA arrays is driven by a patterned electrostatic field generated above the surface of the substrate due to the patterned functionalized layer. The molecules select the most favorable attachment locations while self-aligning during adsorption. The flexibility of the method opens many possibilities for arranging DNA and other biomolecules in dense patterns of arbitrary configurations.

The steps of the nanoarranging process are shown schematically in Figure 1 (see refs 14−16 for technical details). Our first experiments were performed on poly(dG)-poly(dG)-poly(dC) triplex DNA[17] molecules (1000 triads, ≈330 nm long). Figure 2 shows atomic force microsocopy (AFM) images[18] of such molecules regularly arranged and aligned along the prepatterned substrate motif that consists of narrow (∼50 nm) unexposed stripes, separated by 350 nm wide areas that were exposed to the electron beam. Comparison with the peripheral, nonexposed area (Figure 2a) clearly shows the selectivity of DNA adsorption. In fact, a relatively large area (16 μm^2) is completely free from molecules except within the pattern stripes. This area represents a topographically flat grating composed of *chemically* modified surface stripes. Note that the molecules on the peripheral, nonexposed areas exhibit an isotropic distribution of their orientation. Higher electron beam exposures (×10 times) used in other samples (Figure 2b), result in a growth of 1−2 nm thick polymer film. Importantly, the topography in this case is not the source of the regular alignment of DNA molecules, although it might improve the alignment yield.

The method has then been applied to the case of short double-strand poly(dG)-poly(dC) DNA (dsDNA)[19] (1000 bp, ≈330 nm long). The observed characteristics of positioning and alignment are similar to those of the triplex DNA molecules. However, the triplex molecules are more rigid than dsDNA[17,20] and therefore are straighter and apparently easier to align. Indicative of their higher mechanical flexibility, the dsDNA molecules exhibit short-range wiggling, though they are well aligned globally (Figure 3). This wiggling is also similar to that observed for the nonaligned

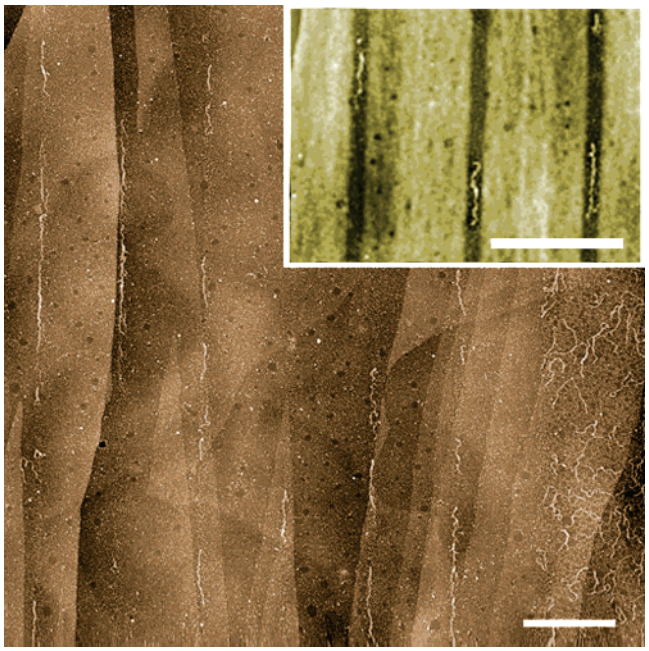

Figure 3. Double-stranded DNA molecules periodically arranged along the horizontal direction and aligned along the vertical direction. The inset shows higher-dose "visible" written pattern. Molecules are more flexible than the triplex DNA, which results in their wiggling in the relatively broad channels. The scale bars are 500 nm long.

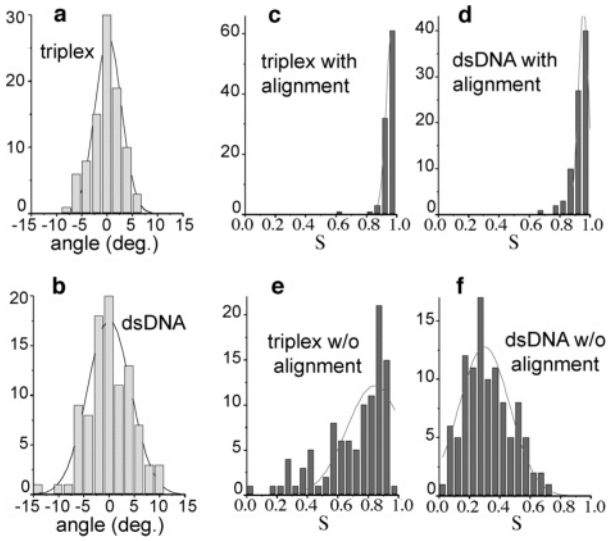

Figure 4. Statistical analysis of DNA alignment. (a,b) Angle deviation with respect to the direction of the stripes. (c−f) The distributions of the ratio *S* (end-to-end distance divided by the contour length) of (c,d) aligned and (e,f) nonaligned molecules: (c) aligned triplex molecules (mean = 0.97, SD = 0.03); (d) aligned dsDNA (mean = 0.96, SD = 0.03); (e) nonaligned triplex molecules (mean = 0.84, SD = 0.18); (f) nonaligned dsDNA (mean = 0.3, SD = 0.17). The sharpness of the distributions (c,d) indicates a very good linear alignment even for the more flexible dsDNA. (About 100 molecules have been measured for each histogram).

dsDNA. It is therefore reasonable to suggest that the molecules are aligned without overstretching, maintaining their "natural" structure. Thus, the immobilization process is not expected to affect their intrinsic properties.

Contrary to fluidics-based methods,[4,6,8,11,13] the predefined self-organization described here allows in principle for the exclusion of flow-related effects from the deposition process. As observed in the AFM studies of different samples, our alignment procedure was successful even when the direction of the nitrogen gas flow used during sample drying was oriented at 45−60° to the stripes direction. This implies that other surface patterns, not necessarily stripe-shaped, could also be employed in such positioning and alignment scheme (see Supporting Information).

The images in Figures 2 and 3 clearly show a very high selectivity of DNA adhesion and alignment within the chemically modified substrate patterns. The chemical nature of the selective positioning is attested to by the fact that similar results were obtained using different electron beam doses, independent of the topography of the prepared pattern. However, we have observed that in the case of strong transversal liquid flow that might occur during the last (drying) stage of the deposition process DNA molecules might get detached from the patterned surface. This effect was stronger in the case of low-exposure dose ("invisible" pattern), suggesting that the low-height (1−2 nm) "walls" produced by the highly exposed patterns can prevent such detachment.

To quantify the characteristics of DNA alignment, we have analyzed the statistics of molecule directionality and straightness (Figure 4). The angle distribution (Figure 4a,b) peaks at 0° (the stripe direction) with a standard deviation of only ∼2.5° for the triplex and 4.2° for the dsDNA molecules within the whole patterned area. This is very different from the nonpatterned areas, where the angular distribution is isotropic. It is also worth noting that we do not observe any angles beyond ∼8° for the triplex molecules (Figure 4a), which would correspond to the maximum angle that a "rigid stick" of 350 nm length could reach within a ∼50 nm wide active path.

The straightness of the aligned molecules is described by the parameter *S* (end-to-end distance divided by the contour length) for each molecule (Figure 4c−f). It assumes a value approaching unity for rodlike molecules and 0 for perfectly circular or tightly wound molecules. Thus the *S* parameter is useful in the quantification of the overall alignment on a straight-line pattern (the short-range wiggling of the molecules will not have any significant effect on *S*). When measured on a conventional nonpatterned substrate, the triplex molecules are straighter than dsDNA (Figure 4e,f) with mean *S* values of 0.84 and 0.3, respectively. With the alignment, the situation changes remarkably (Figure 4c,d), and we obtain very narrow distributions with practically the same *S* values of 0.97 for triplex and 0.96 for dsDNA, that is, both very close to 1. The important narrowing of these distributions for aligned molecules confirms a high degree of the alignment and straightness in both cases, in spite of a big difference in the inherent mechanical properties. Note that the direct contour length measurement of the molecules gives 333 ± 32 nm for the aligned triplexes and 357 ± 26 nm for the aligned dsDNA, which within an experimental error is identical to 330 ± 30 nm and 353 ± 25 nm, respectively, for the nonaligned molecules on the conven-

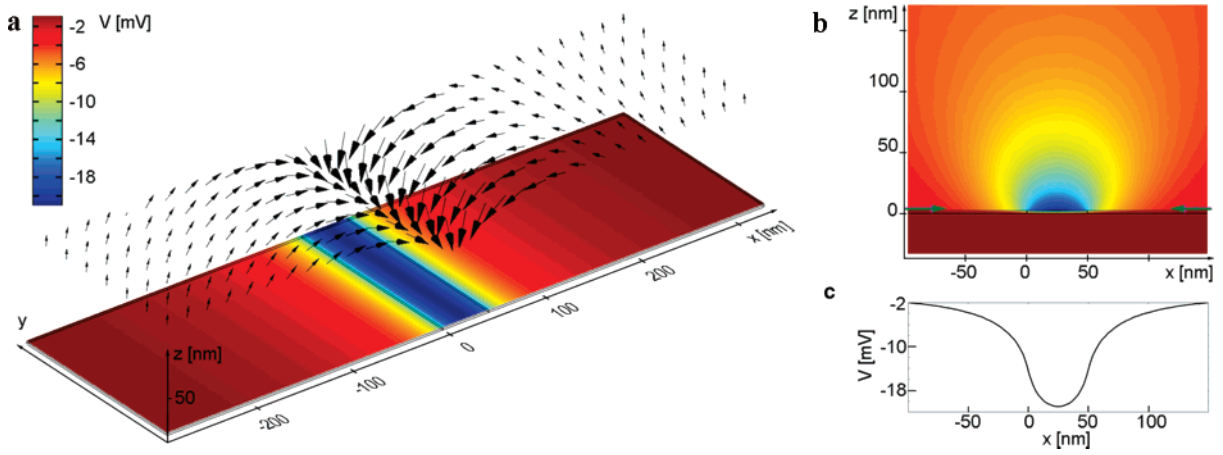

Figure 5. Electrostatic potential and attractive force F_e. (a) Colors: distribution of the potential V in the plane (XY) of the positively charged stripe taken 1 nm above it. Arrows: normalized electrostatic force distribution at 100 nm above the stripe (note that right above the stripe this distribution is essentially the same). (b) Out-of-plane (XZ) potential distribution. (c) X-profile of the potential at 1 nm above the stripe (position denoted by arrows in b).

tional nonpatterned substrate. This indicates that no overstretching of the molecules is occurring during their alignment.

To get insight into the possible mechanisms of alignment process, we have analyzed the electrostatic forces arising from the field induced by a positively charged, 50 nm wide and infinitely long stripe covered with the graphic modifier (GM).[14] Representative results[21] are shown in Figure 5, which depicts the electric potential V and the force field distributions in the plane of the substrate, above the GM-coated surface. It is clearly seen that the charged stripe produces a potential well in its vicinity with a local minimum at its center. Note that the DNA deposition is done from a buffer solution with an ionic strength of 1 mmol/dm^{-3}. This leads to a Debye-Hückel screening length[22] of 310 nm and therefore to an efficient electrostatic attraction up to ~300 nm above the stripe, where molecules experience the potential well. The dissociation of the DNA phosphates groups ($pK_a = 2-3$) is complete in a buffer solution with pH = 7.4; therefore, we can assume a one electronic charge per base. On the other hand, the GM has $pK_a \sim 5$ leading to relatively weak ionization at pH = 7.4. Moreover, as evidenced experimentally, its activity decays with time (time constant of ~2 h). Thus, the stripe is considered to bear only ~0.1−0.3% of its initial surface charge density (a value of 0.1% is taken into account in Figure 5). A 1000 bp molecule in the vicinity of the stripe will therefore experience an attractive force of $F_e \sim 3-10$ nN at 100 nm above the stripe and $F_e \sim 30-100$ nN at 1 nm above the stripe. The shape of the potential well is such that for a landing molecule, the most favorable location (lowest potential energy) is the center of the stripe. Therefore, it is expected that a landing molecule will tend to straighten, aligning itself segment by segment along the stripe. In addition, because the focused electron beam of our scanning electron microscope (SEM) has a Gaussian distribution of width ~20−30 nm and the exposures were considerably long it is further expected that the potential well is even more tapered at its edges due to the gradient in exposure dose. Thus, the molecules are expected to align preferentially along the central axis of the stripe, as observed experimentally.

Computations of fluid dynamics, performed to simulate the effect of the drying process, show that the drag force from the flow is very low, ~5 pN. On the other hand, the meniscus receding perpendicular to the 330 nm long molecule would exert a force proportional to the water surface tension of $F_m \sim \alpha L \sim 20$ nN.[23] Thus, the electrical force F_e near the surface is sufficiently large to keep the molecules near the bottom of the potential well. At the same time, within the nonpatterned area, F_e is uniform and the molecules can be transported by means of the receding meniscus. Indeed, a trace of such "flow" is found experimentally in the periphery of the patterned region (see Figure 2a).

In conclusion, we have demonstrated a method for controlled positioning and alignment of individual DNA molecules based on the local charge neutralization of a functionalized surface achieved via a high-energy electron beam. The high resolution achievable by direct electron beam writing allows the fabrication of patterns of extremely localized electrostatic potential wells. Using such electrostatic traps, we were able to produce a predefined positioning and alignment of DNA molecules on large substrate areas by simple deposition from a macroscopic buffer droplet. Thus, the process relies explicitly upon "directed" self-organization on a chemically predetermined pattern. The morphology and location of the molecules on the surface after the deposition do not change over a relatively long (3−6 months) period of time. Because the position and the form of the immobilized molecules are known from the beginning via the designed pattern, the deposited objects may be addressed afterward. Such technique might serve as a basis for manufacturing of nanoscale molecular or bioelectronic devices based on DNA and other biomolecules. The latter may be constructed using a designed pattern directly, or it might also be based on DNA-based programmed assembly[24,25] with very high precision and large pattern flexibility. Preliminary observations also suggest that the presented

method may be applied to the self-assembly of sphere-shaped proteins where a single pattern cell corresponds to the particular protein diameter thus organizing a predefined biomatrix.

Supporting Information Available: Figure showing the positioning of triplex DNA molecules within cross patterns. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) The modification of the highly-oriented pyrolytic graphite (HOPG) substrate and the results of dsDNA and triplex DNA adsorption on it have been reported (ref 20). Briefly, freshly cleaved HOPG (NT-MDT, Russia) surfaces were incubated with the GM solution composed of a short hydrocarbon chain terminated by an amino group $(CH_2)_n-(NCRH_2CO)_m-NH_2$ (Nanotuning, Chernogolovka, Russia: http://www.nanotuning.com) during 15 minutes. The surface was then blown dry with N_2 gas.
(15) The electron beam lithography system used in the experiments was based on a modified SEM JSM 6400 (JEOL, Japan) operated via a PC-based controller NPGS 9.0 (Nabity Lithography Systems, U.S.A.). The electron beam produced by the LaB_6 source was of 40 keV, 100−350 pA. Typical exposure times were 50−170 μs per point for "invisible" patterns and 500−600 μs per point for "visible" patterns. The distance between neighboring exposure points was chosen to be small enough (∼10 nm) to ensure good point-to-point overlap and hence the continuity of the written pattern. The area doses were thus in the range of 5−50 mC/cm^2.
(16) The DNA was deposited on the patterned samples from a buffer solution (Tris-Ac, pH = 7.4) diluted by commercial deionized clean water (Fluka). A small droplet (10−20 μl) containing DNA at concentrations of ∼0.5−2μg/ml was deposited on the functionalized surface and incubated at room temperature for 15−20 minutes. Then the droplet was carefully blown away by a nitrogen flux. The DNA deposition was performed within maximum one hour from the sample functionalization due to the degradation of the GM activity with time.
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