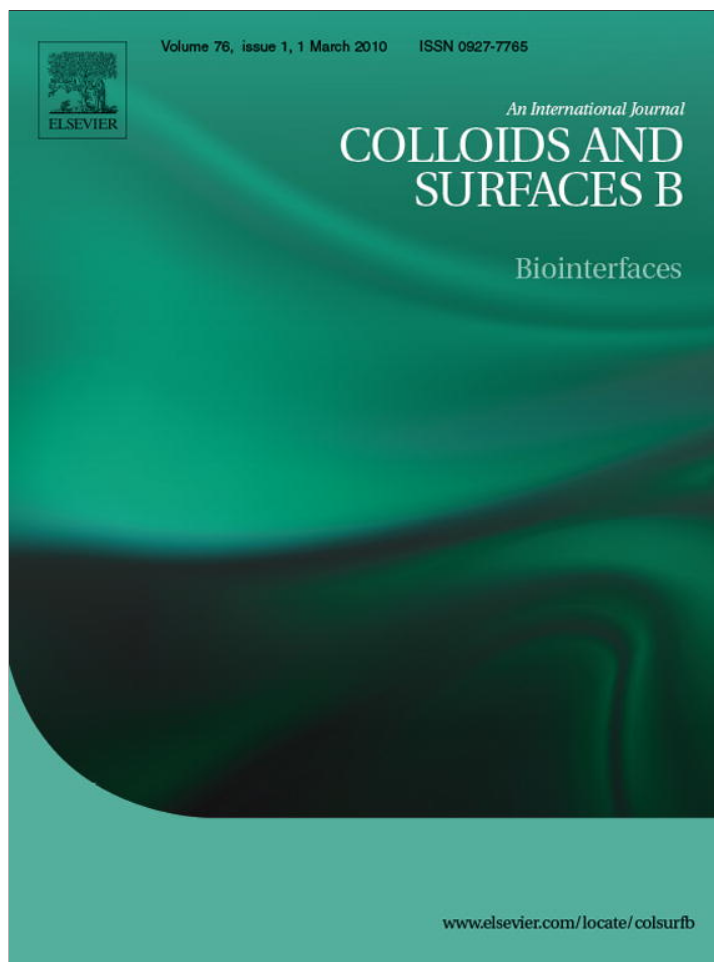


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## The effect of underlying octadecylamine monolayer on the DNA conformation on the graphite surface

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## ABSTRACT

DNA was immobilized on highly oriented pyrolytic graphite (HOPG) surfaces modified in octadecylamine (ODA) vapor. ODA molecules, deposited from the vapor phase onto HOPG form a nanostructured surface, which was utilized as a template for DNA adsorption. Peculiarities of double- and single-stranded DNA adsorption on these surfaces were investigated with atomic force microscopy (AFM) both in air, liquid and under different salt conditions. AFM images of DNA molecules immobilized on octadecylamine modified HOPG reveal a segmented shape of biopolymers: it constitutes straight segments with sharp turns at angles 120° or 60° between them, reflecting the symmetry of the underlying pattern. The analysis of DNA conformations on ODA modified HOPG surface has shown that under certain conditions DNA equilibrates on the surface on the scale of the whole molecule. A persistence length estimate of 97 nm was determined for those molecules. Participation of different forces in the ODA pattern driven DNA assembly is discussed.

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### 1. Introduction

Up to present a lot of self-ordered molecular structures have been developed on different substrates [1–6]. These structures, or molecular nanopatterns, can be utilized as surfaces for immobilization and self-assembly of polymers. These nanopatterns are typically formed by low molecular weight molecules (compared to that of polymers), such as long chain alkanes, alcohols, fatty acids [1,2], porphyrins [3] and their derivatives [4–6], which self-assemble on crystal surfaces such as mica [3], graphite [1,4], Au(1 1 1) [6], MoSe<sub>2</sub>, and MoS<sub>2</sub> [2], though similar assemblies were also observed on non-crystalline substrates, e.g., glass [3].

The importance of the investigation of polymers behavior on molecular templates is connected with the desire for template driven “tuning” of the properties of the immobilized polymer that is of great importance in technological applications. One of the best examples of such “tuning” is manipulation of single DNA molecules on the dodecylamine modified graphite surface by the AFM tip [7]; polymer chains are tailored in such a way that the single macromolecule is immobilized and at the same time can be manipulated with AFM tip without chain breakage.

AFM investigations of template directed self-assembly of polymeric molecules, especially DNA, on crystal surfaces have attracted much attention in the last few years [7–10]. Such self-assembly implies control of polymeric conformations and positions simultaneously and therefore represents a big challenge for molecular design, calling for (bio)technological (e.g., constructing molecular electronic devices and biosensors) and medical (e.g., diagnostics, such as direct DNA sequencing on the surface) applications. In this connection DNA adsorbed onto modified graphite represents one of the most attractive systems for such an investigation due to the particular biological importance and possibilities of this molecule (graphite is the most accessible and easy-to-use conductive support for SPM which is very important if we think about future applications).

Very recently dodecylamine pattern driven self-assembly of DNA was demonstrated on graphite [10]. It took place only on ordered dodecylamine layers, which were formed at temperatures below 30 °C.

Our goal here was to investigate the influence of the molecular pattern on the conformation of adsorbed DNA. We have chosen ODA as modifier due to its known ability to self-assemble on HOPG into patterns, which can direct the adsorption of negatively charged synthetic polymer, poly(sodium 4-styrenesulfonate) (PSS) [7]; polymer chains orient preferably along ODA lamellae formed by alternating amine groups and hydrophobic tails. Knowledge about the behavior of DNA during the adsorption on ODA monolayers on HOPG could be very useful for the characterization of properties

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of the biomolecule on the surface (such as rigidity, elongation) as well as for the development of biotechnological applications. Also it is likely that results obtained from this particular system are representative for many other polymers and patterns under similar conditions.

## 2. Materials and methods

ODA was purchased from Aldrich Company and used as received. We have used plasmid linearized dsDNA with the length 2000 b.p. In one of the presented experiments we used DNA from the Fermentas Company that contains longer DNA fragments (23,130, 9416, 6557, 4361 b.p.).

### 2.1. HOPG modification in ODA vapors

For HOPG modification 2–3  $\mu\text{g}$  of ODA was placed on the object glass and heated up to 100 °C using a heating plate placed in a fume hood. After ODA melting (its melting point is 52.9 °C) freshly cleaved HOPG surface was exposed 2 cm above the liquid ODA droplet for 3–60 s. Afterwards the studied DNA sample was applied on top of modified graphite surface.

### 2.2. Sample deposition onto modified HOPG

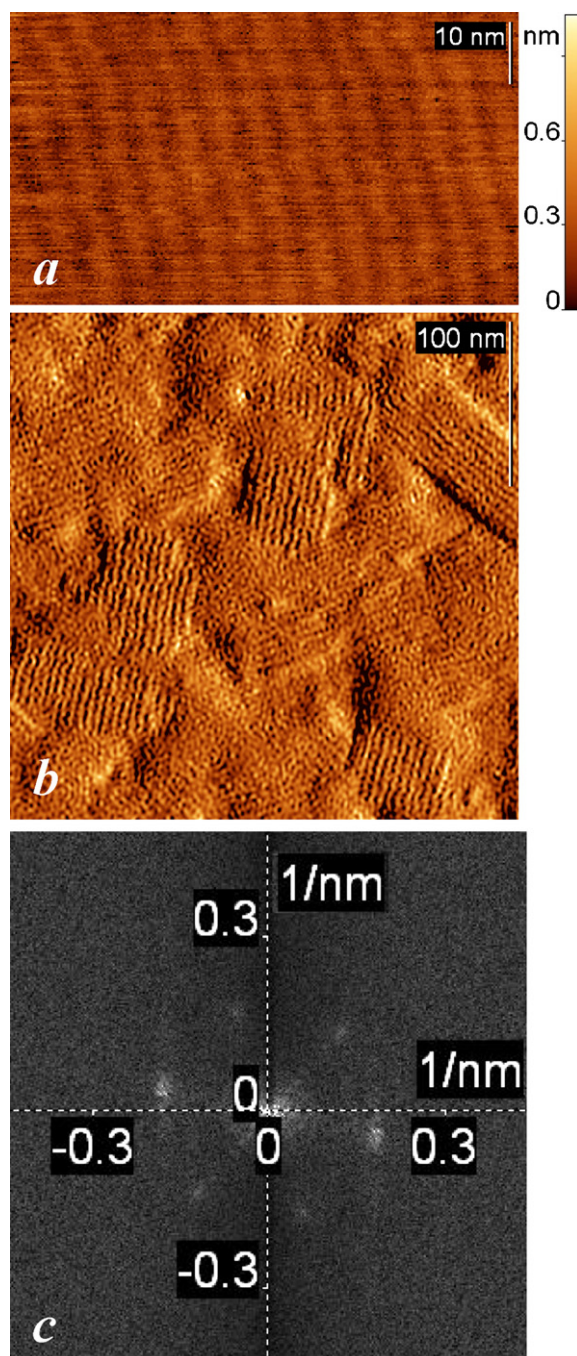
10  $\mu\text{L}$  of 0.1  $\mu\text{g}/\text{mL}$  DNA solution in milli-Q water (or 5 mM  $\text{MgCl}_2$  buffer) was placed on the modified surface and left for 10 min in controlled atmosphere (23 °C, 50% humidity). After that the DNA droplet was removed from the surface with filter paper, the surface was washed with 100  $\mu\text{L}$  of milli-Q water and dried in nitrogen flow. For AFM in liquids instead of drying during the last stage 20  $\mu\text{L}$  of the buffer (10 mM Tris–HCl, 150 mM NaCl, pH 7.5) was deposited on the surface prior to liquid cell mounting. For deposition of single-stranded DNA 10  $\mu\text{L}$  of dsDNA, heated up to 80 °C, was deposited on ODA modified HOPG surfaces and placed in humid atmosphere in thermostat at 80 °C for 20 min. After that the sample was taken out of the thermostat, the DNA droplet was removed from the surface with filter paper, the surface was washed with 100  $\mu\text{L}$  of milli-Q water and dried in nitrogen flow.

### 2.3. AFM imaging

All experiments were carried out using Nanoscope III and IV multimode atomic force microscopes (Veeco Instruments Inc., USA) in tapping (mostly) and contact modes at 23 °C and 50% relative humidity (in the case of ambient atmosphere). Commercial silicon NSC11 (spring constant 48 N/m, resonant frequency 330 kHz) and DP14/HI-RES-C cantilevers (MikroMasch, spring constant 5 N/m, resonant frequency 160 kHz) were used for tapping and CSG01 (NT-MDT, Russia, spring constant 0.03 N/m, resonant frequency 9.8 kHz) for contact mode of scanning. Experiments in liquid employed a tapping mode liquid cell (Veeco Instruments Inc., USA) and silicon nitride cantilevers (Nanoprobe) with a spring constant of 0.6 N/m. The cantilever oscillation frequency was 8–10 kHz. Image processing was performed using the FemtoScan software [11]. The scan rate was typically 2 Hz, the number of lines were 512.

## 3. Results

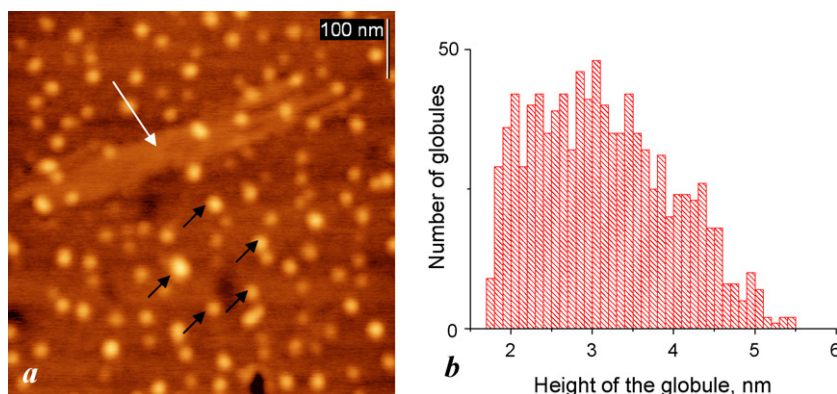
ODA molecules deposited onto HOPG surface from vapor form self-assembled monolayers, which consist of domains with regular topographic structure inside (Fig. 1). According to the work of Severin et al. [7], where a similar pattern was formed after octadecylamine deposition on HOPG from chloroform solution, alkyl chains orient along the substrate axes parallel to each other,



**Fig. 1.** (a) High resolution height AFM image of ODA monolayer on HOPG (inside one ODA domain); (b) AFM amplitude image of ODA monolayer (many ODA domains are inside) and (c) its fast Fourier transformation reflecting 3-fold symmetry. ODA monolayer was obtained on HOPG from vapor.

while the end groups separate into straight lamellae. The sizes of observed octadecylamine domains vary from several thousands to several hundred thousands square nanometers and more. Adjacent domains are shifted by the angle 60° or 120° from each other thus forming 1-, 2- or 3-fold symmetry depending on the chosen region on the surface. Typically, 1  $\mu\text{m}$  AFM images exhibit a 3-fold rotational symmetry of ODA patterns as demonstrated with fast Fourier transformation in Fig. 1c. The periodicity of the octadecylamine 2D crystal constitutes  $5.3 \pm 0.5$  nm, the corrugation in the ODA pattern or stripes (which gives a lower estimate of the thickness of the monolayer) measures about 1 Å. Octadecylamine monolayers are stable in aqueous environment though after exposure to water two





**Fig. 2.** (a) AFM image of octadecylamine modified HOPG exposed to water droplet for 10 min and (b) histogram of height distribution of observed globules. Black arrows denote some of the observed globules, the white arrow 3D lamella.

types of structures appear on the surface according to AFM images (Fig. 2a): globules with heights within the range 2–5 nm (the size distribution is presented in Fig. 2b) and plain elongated structures about 1.2 nm high (see arrows in Fig. 2a). We attribute these features to octadecylamine micelles and 3D lamellae correspondingly (we call them 3D lamellae in order to differ from lamellae formed by the headgroups of octadecylamine in the monolayer on the surface). These are formed from the excess of ODA molecules (not bound with the surface) in aqueous environment due to hydrophobic interactions between the carbon tails of the molecule [12,13]. The observed heights are in a good agreement with the heights of assumed structures taking into account that the length of octadecylamine molecule is about 2.46 nm (distance between two most far hydrogen atoms according to Chem.3D software simulation) and the effect of reduced apparent height due to contact deformations by the AFM cantilever. Formation of these structures can be avoided by HOPG modification without excess of ODA.

AFM images of DNA molecules immobilized on mica using conventional techniques (adsorption from 20 mM KCl, 5 mM MgCl<sub>2</sub> solution) have shown that AFM based contour lengths of the DNA molecules constitute  $640 \pm 20$  nm. This is about 7% shorter than the theoretical value of B-form 2000 b.p. DNA length (680 nm). Similar observation was reported earlier and explained, in particular, by the following reason [14]: in a typical image one pixel corresponds to approximately 4 nm of DNA (12 bp) ( $512 \times 512$  pixels, scan size: 2000 nm). Therefore, DNA bends within this range cannot be resolved. Also we measured contour lengths by extrapolation of the contour with linear segments, which can also shorten the measured contour lengths. The apparent AFM heights and widths (FWHM) of biopolymers on mica constitute  $0.6 \pm 0.1$  and  $7.0 \pm 1.4$  nm (for high resolution cantilevers) correspondingly. These values are comparable with previous findings [15], taking into account that the apparent width may vary due to its dependence on the radius of the used tip.

Our AFM images of DNA molecules immobilized on octadecylamine modified HOPG reveal a segmented shape of the biopolymers: it consists of straight segments with sharp turns at angles  $120^\circ$  (about 90% cases) or  $60^\circ$  (about 10% cases) between them (Fig. 3), reflecting the symmetry of the underlying pattern (see fast Fourier transformation in Fig. 3d). Fig. 3b depicts a long DNA molecule (9416 b.p., Fermentas Company), while all other AFM images of DNA, presented in this paper depict 2000 b.p. DNA. DNA molecules in the AFM images are often accompanied by the micelles (and sometimes 3D lamellae) formed presumably during DNA deposition from aqueous environment as was shown in Fig. 2. Also some of these micelles are bound with DNA molecules (see Fig. 3c, for example), especially at bending points. Similar assemblies of polyelectrolyte molecules (PSS) on ODA monolayers [7] and

of DNA molecules on dodecylamine monolayers [10] were reported and attributed to the potential ripple provided by an amphiphilic monolayer on the basal plane of graphite. The rows of positively charged headgroups are separated by stripes of hydrophobic alkyl chains, providing a surface potential ripple.

In order to investigate how the shape of this potential ripple (or nanotopography of the ODA pattern) correlates with the observed DNA conformations on the surface we scanned our samples at high magnifications (scan size less than  $1 \mu\text{m}$ ) using high resolution tips. In higher resolution AFM images (Fig. 4) the DNA molecules look like a thread with a non-homogenous width and height (which possibly reflects helical DNA structure) and with its contour following the direction of the underlying octadecylamine lamellas. In other words the direction of each straight segment of DNA matches octadecylamine lamellae directions beneath the biopolymer and its contour usually turns at the boundaries of two adjacent octadecylamine crystal domains (Fig. 4). The distribution histogram of the segments' lengths (Fig. 5) shows a maximum at 44 nm (according to a Gaussian fit).

Interestingly, the AFM based contour lengths of DNA molecules constitute in this case  $810 \pm 100$  nm, which is about 20% longer than the expected value for B-form DNA. Such extensions of the biopolymer can be attributed to the partial unfolding of the DNA helices. This suggestion is also supported by splitting of DNA molecules into two single strands, which is observed occasionally in AFM images (Fig. 4a and c). Our observations are similar to the recent findings obtained for DNA adsorbed on dodecylamine monolayer [10]. In these experiments DNA contour lengths were 35% longer than the theoretically predicted length for B-form DNA [10].

The apparent AFM heights and widths (FWHM) of biopolymers on octadecylamine modified HOPG constitute  $1.3 \pm 0.2$  and  $5.7 \pm 1.4$  nm (for high resolution cantilever) respectively, while for single strands (in the regions of unfolded DNA) the obtained values are about 0.8 and 5 nm. In previous studies of DNA on modified HOPG [16] the apparent AFM height and width were also closer to the value of B-form DNA diameter (2 nm). Observed differences in these values for different surfaces could be attributed to strong electrostatic interaction in case of mica that flattens the molecule, to differences in "wetting" with respect to mica, and therefore also to capillary forces. Another aspect could be the salt layer which is deposited on mica, but which is not relevant upon deposition of the ODA monolayer on graphite.

In order to eliminate drying effects, which are possible during DNA adsorption [17] we have also conducted AFM experiments in aqueous buffer solution without the drying stage during preparation (see Section 2 for details). The same effect of template driven assembly of DNA molecules was observed in this case (see Fig. 6). Corresponding AFM images were devoid of micelles and 3D lamel-

lae which should be due to their weaker binding to the surface in aqueous environment than in air. In this experiment the adhesion of DNA molecules appeared also weaker, noticeable from the blurred DNA contours and even small tracks (not shown) in AFM images which is a result of short displacements of biomolecules by the AFM cantilever (while z-resolution of AFM was high enough to distinguish single ODA lamellas).

To study the conformation of single-stranded DNA on octadecylamine modified HOPG we carried out DNA deposition in thermostat at 80 °C. At this temperature dsDNA normally denatures and adsorbs as ssDNA [15]. DNA deposition on octadecylamine modified HOPG at 80 °C results in the formation of a dense network structure with similar orientation effect as for dsDNA (Fig. 7). We suggest that this structure is composed of ssDNA molecules, which is also supported by typical apparent height for ssDNA observed, measuring about 0.5 nm. No secondary structures of ssDNA typical for conventional deposition methods (like Mg<sup>2+</sup> or APTES-treated mica) [15] are observed. The latter fact is in agreement with previously investigated ssDNA deposition onto modified HOPG [15] and on polylysine treated mica [18]. In both papers ssDNA deposition is explained by electrostatic interaction with positively charged substrate surface. This experiment also demonstrates that the ODA assemblies remain stable after its exposition to the aqueous solution at 80 °C.

#### 4. Discussion

According to previous works [7,8] there are two different scenarios of the polyelectrolyte molecules adsorption on the template of amphiphiles. In the first scenario the surface diffusion of the polyelectrolyte molecules is effectively prevented by the deep surface potential ripples or in other words by the nanotopography of the molecular pattern. In this case the conformations of immobilized polymeric molecules could be considered as projections of their 3D conformations in solution to the surface, merely locally

straightened by the surface potential ripple. In the second scenario polyelectrolyte molecules can diffuse and equilibrate at the solid–liquid interface with respect to the nanotopography of the underlying pattern while only the removal of the solvent immobilizes the molecules.

While our AFM images of DNA molecules adsorbed on ODA modified HOPG do not reveal many self-crossings, which are the typical features for 3D to 2D projection of the molecular conformation, we do not consider the first scenario for this adsorption. Therefore we can attribute DNA adsorption in our experiments closer to the second scenario though our AFM experiments in the liquid cell showed that weak adsorption of biomolecules to the template takes place prior to the removal of the solvent.

The second scenario implies that DNA adsorption can be characterized by two different length scales: on a large length scale biomolecules have equilibrium 2D-conformation, which is locally straightened by the template on a short length scale. The length of straight segment depends on two main factors: the size of the underlying ODA domain (local interaction of biomolecule with the template plays a big role on this scale) and the internal elastic energy of the biopolymer. On large ODA domains we observe a significant increase of the lengths of the straight segments, as in Fig. 4a, where one segment measures 340 nm (while its most typical value according to the distribution presented in Fig. 5 is 44 nm). In the fields with small ODA domains and a large number of domain boundaries within the regions typically covered by one adsorbed DNA molecule, the internal energy of the molecule plays a greater role during adsorption, i.e. molecules choose those directions of the allowed ones (according to local interaction with the pattern), which mostly correspond to its equilibrium 2D configuration. The possible scheme of ODA pattern dependent DNA conformation generalized from high resolution AFM images is shown in Fig. 8, where black lines demonstrate the direction of ODA pattern stripes inside the domain and the cyan curve symbolizes the DNA molecule. The presence of different angles for the DNA segments within one ODA

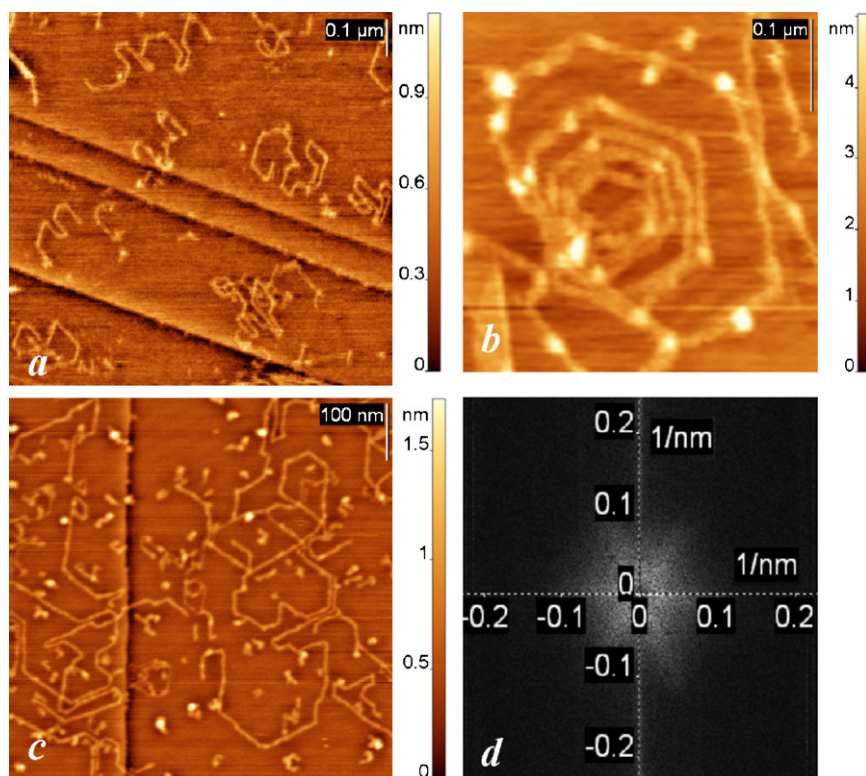
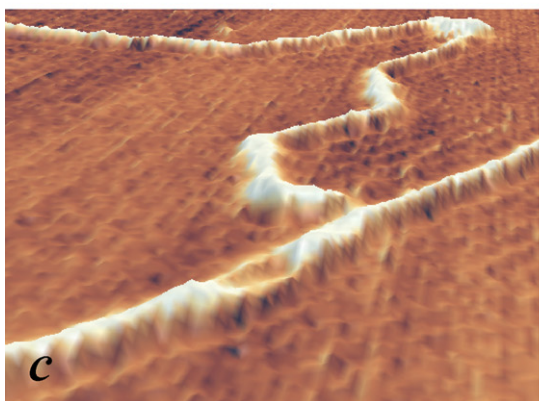
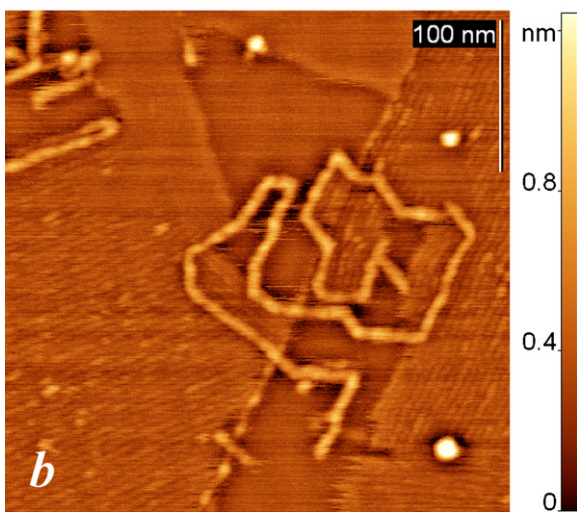
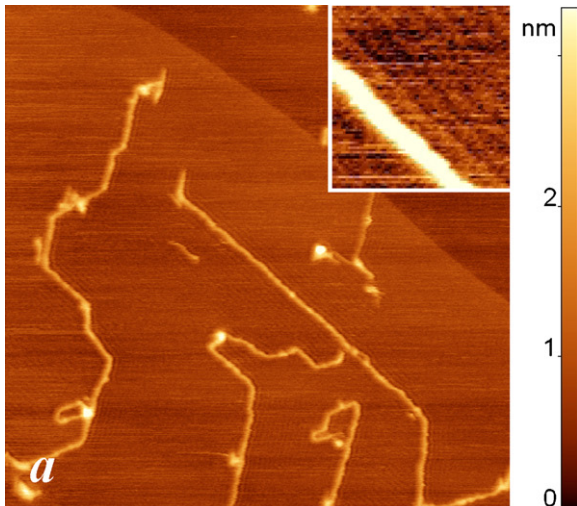


Fig. 3. (a–c) AFM images of DNA molecules, immobilized onto ODA modified HOPG from water solution; (b) a longer DNA molecule. (d) Fourier spectrum of an AFM image (c), demonstrating 3-fold symmetry of adsorbed DNA molecules.

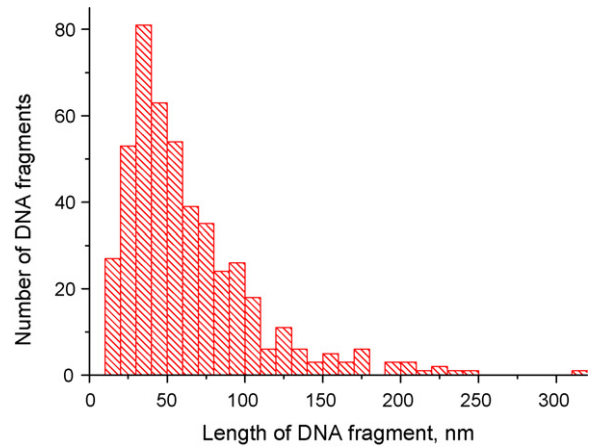




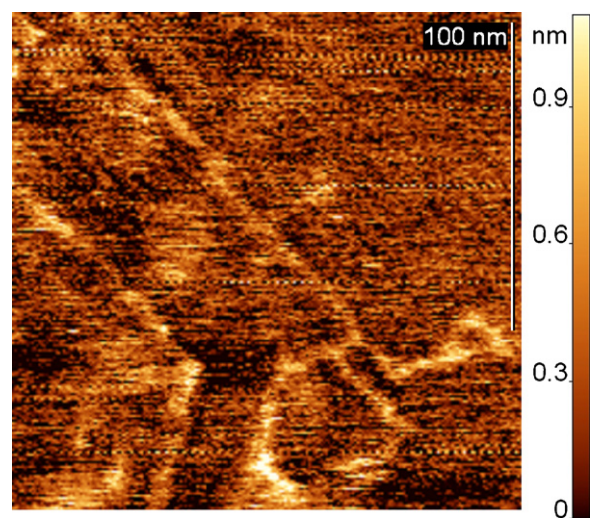
**Fig. 4.** High resolution AFM images of DNA molecules, immobilized onto ODA modified HOPG. Inset: zoomed region from the center of the image; (c) quasi 3D AFM image, demonstrating partial DNA unfolding. Image sizes are (a) 520 nm × 520 nm (inset 40 nm × 40 nm), (b) 340 nm × 340 nm and (c) approximately 200 nm × 200 nm × 2 nm.

domain reported for PSS [8] was attributed to possible reorientation of amphiphile molecules in the close vicinity of the polymer backbone that is not resolvable with AFM.

In order to point out whether the observed DNA shape reveals 2D equilibrium conformation on the large scale we analyzed the angles  $\theta$  between tangents to the shape of DNA segments, separated by a certain distance  $l$  along the molecule as described by Frontali et

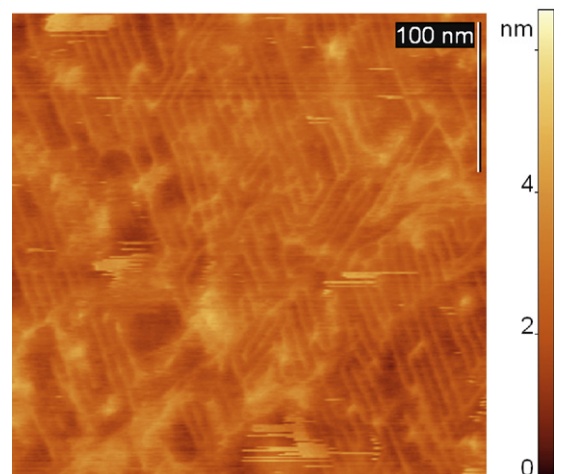


**Fig. 5.** Distribution histogram of straight segments' lengths of DNA immobilized onto ODA modified HOPG.

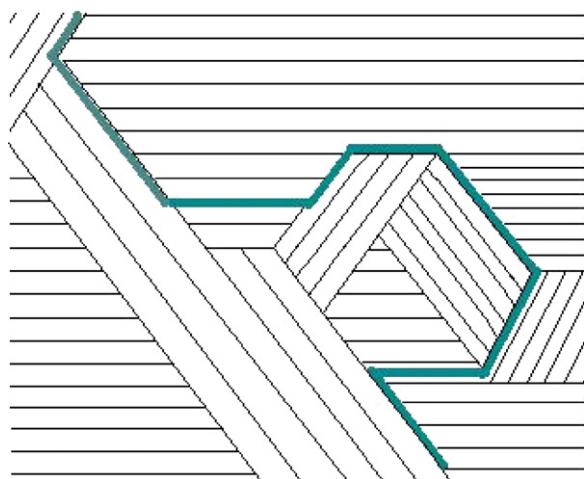


**Fig. 6.** AFM image of DNA molecules, immobilized onto ODA modified HOPG in buffer (10 mM Tris–HCl, 150 mM NaCl, pH 7.5) in a liquid cell.

al. [19]. Because equilibrium conformation implies Gaussian behavior of the angle  $\theta$ , ratios between higher moments provide a quick test of the basic hypothesis, i.e. a Gaussian distribution of  $\theta$ . In this case,  $\langle \theta^4 \rangle / \langle \theta^2 \rangle^2 = 3$ , and  $\langle \theta^6 \rangle / \langle \theta^2 \rangle^3 = 15$ . In order to satisfy the



**Fig. 7.** AFM image of ssDNA molecules, immobilized onto ODA modified HOPG at 80 °C.



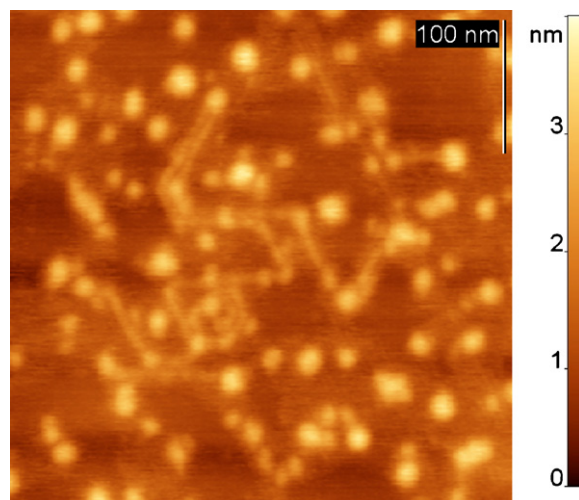
**Fig. 8.** Proposed scheme of ODA pattern driven assembly of DNA molecule, generalized from AFM images. Thin lines denote the direction of ODA stripes within a domain; the thick curve denotes the contour of a DNA molecule.

**Table 1**  
Values of  $\langle \theta^4 \rangle / \langle \theta^2 \rangle^2$ , and  $\langle \theta^6 \rangle / \langle \theta^2 \rangle^3$  calculated as described by Frontali et al. [19] for two different series of experiments.

Adsorption type/moment	$\langle \theta^4 \rangle / \langle \theta^2 \rangle^2$	$\langle \theta^6 \rangle / \langle \theta^2 \rangle^3$
Type 1 adsorption (ODA modification time 5 s, Fig. 9a)	3.3	14.5
Type 2 adsorption (ODA modification time 20 s, Fig. 9b)	1.8	3.6

condition of large length scale we used  $l=650$  nm for this testing which is much higher than the typical segment length (44 nm). On the other hand the chosen value is not as big as the mean DNA length which allows us to increase the statistics efficiently [19].

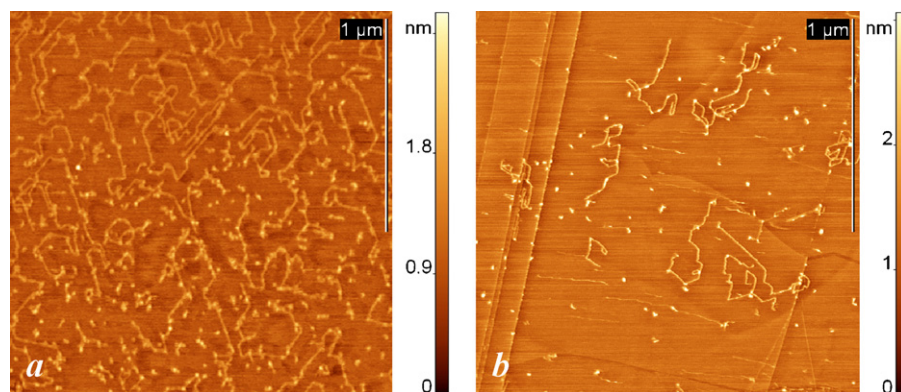
The analysis of  $\theta(l)$ -distribution for two different series of AFM images of immobilized DNA has shown that two types of adsorption take place in our system. When ODA modification in vapor lasted for 5 s (Fig. 9a), the calculated ratios  $\langle \theta^4 \rangle / \langle \theta^2 \rangle^2$ , and  $\langle \theta^6 \rangle / \langle \theta^2 \rangle^3$  (number of molecules  $n=20$ ) were in a good agreement with expected values for normal distribution (Table 1). Therefore we can speak about 2D equilibrium deposition on the large scale. Then the persistence length of DNA that can be calculated from the relation  $P=l/\langle \theta^2 \rangle$  [19], constitutes approximately 97 nm. For comparison, the persistence length of DNA, defined by the same procedure from electron microscopy images varied from 56 to 132 nm depending on the ionic strength of the solution [19]. Oppositely, when ODA modification time was 20 s (Fig. 9b) the  $\theta$  angle distribu-



**Fig. 10.** AFM image of DNA molecules, immobilized onto ODA modified HOPG from 5 mM  $MgCl_2$  solution.

tion does not show Gaussian behavior (see Table 1, number of molecules  $n=10$ ). In this case the adsorption process is not completely equilibrated and, probably, kinetic trapping of DNA by ODA surface plays a bigger role. Though our presented analysis is rather rough due to limited statistics, it reflects the real mechanism of adsorption in our samples and helps to understand it better. The reason for observing different types of adsorption may be connected with different quantity of free, unbound to the substrate ODA, e.g., it was shown that unbound surface modifier such as aminopropyltriethoxysilane can act as condensation agent for DNA [20].

One question still remains about the nature of the forces, which result in DNA adsorption along the lamellae of the ODA pattern. As we can see from our obtained AFM images DNA interacts not only with 2D ODA patterns but also with 3D ODA structures, like micelles. The main candidate for the type of interaction is electrostatics because of the oppositely charged DNA molecules and lines of amine groups in the ODA pattern. Electrostatic interaction was considered as the main driving force for adsorption of negatively charged DNA on dodecylamine modified HOPG [7] and "GM" modified HOPG [15]. In order to weaken the influence of electrostatic forces during adsorption we immobilized DNA from 5 mM  $MgCl_2$  solution which resulted in AFM images also revealing template driven DNA adsorption (Fig. 10). Together with the observations of 20% elongation and partial unfolding of DNA molecules and preferable binding of ODA micelles at DNA bending points, at places with maximal local tension, we assume that some other forces may also



**Fig. 9.** AFM images of DNA, adsorbed onto ODA modified HOPG in (a) equilibrium and (b) non-equilibrium conformation. ODA modification lasted for (a) 5 s and (b) 20 s.



play big role in this adsorption and that these forces imply participation of DNA bases. First of all this may be hydrophobic interaction between exposed DNA bases and carbon tails of ODA pattern. Previous studies have shown that exposed hydrophobic core material of DNA can interact with underlying hydrophobic substrates like HOPG [21,22]. Also hydrophobic interaction was assumed to be the reason of DNA adsorption on HOPG during DNA ordering into hexagonal structures on HOPG after sample rotation [23].

Nitrogen atoms of DNA bases may also form hydrogen bonds with nitrogen atoms of amine groups in the ODA structures. Due to the high density of the exposed amine groups in the ODA lamellas and their vicinity to partially unfolded DNA molecules, H-bonds formation is likely to occur.

So, the assembly of DNA molecules is most likely influenced by both physical (van der Waals, electrostatic, hydrophobic) and chemical (hydrogen bonds) interactions. DNA molecules align to the nanogrooves formed by the self-organization of the ODA molecules. Electrostatic interaction between the ODA pattern and DNA makes it possible to adsorb DNA in an extended form onto the surface while the nanotopography (potential ripple) modulates the shape of adsorbed molecules and defines the segmented conformation which we observe in our AFM images.

## 5. Conclusions

In this work we have shown that ODA molecules deposited onto HOPG by evaporation self-organize in the pattern similar to that obtained by ODA deposition from chloroform and ethanol solutions. The obtained monolayer contains domains with three possible directions of the ODA assembly, revealing up to 3-folded symmetry. ODA pattern is stable in aqueous environment up to 80 °C though excess of ODA leads to the formation of micelles and 3D-lamellas due to hydrophobic interaction of ODA molecules in water. DNA molecules immobilized onto the ODA structures reveal an extended shape consisting of straight segments (typical length is 44 nm) with sharp turns at angles 120° (about 90% cases) or 60° (about 10% cases) reflecting the symmetry of the underlying pattern. The mean length of DNA adsorbed on ODA modified HOPG is about 20% longer than expected value for B-form DNA which was attributed to be the consequence of partial DNA unfolding. Our results also show that DNA deposition on ODA modified HOPG at 80 °C results in the formation of a dense network of extended ssDNA molecules with similar orientation effect as for dsDNA. No secondary structure of ssDNA is observed.

According to our analysis of DNA shapes two types of conformation of DNA on ODA patterns were found in our experiments. The first type implies scenario of 2D equilibrium DNA adsorption on the scale of a whole molecule and local straightening of the polymer by

the potential ripple. The second type of observed DNA conformation was formed during non-equilibrium deposition.

The obtained results show the possibility of utilization of ODA modified HOPG surfaces for alignment of biological polymers and for other biotechnological applications. This molecular layer is an interesting candidate for suitable immobilization of DNA for scanning tunnelling microscopy studies, where one has to deal with stronger electric fields.

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