Replication of Single Macromolecules with Graphene

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ABSTRACT: The electronic properties of graphenes depend sensitively on their deformation, and therefore strain engineered graphene electronics is envisioned.[1−3] In order to deform graphenes locally, we have mechanically exfoliated single and few layer graphenes onto atomically flat mica surfaces covered with isolated double stranded plasmid DNA rings. Using scanning force microscopy in both contact and intermittent contact modes, we find that the graphenes replicate the topography of the underlying DNA with high precision. The availability of macromolecules of different topologies, e.g., programmable DNA patterns,[4] render this approach promising for new graphene based device designs.[1,3] On the other hand, the encapsulation of single macromolecules offers new prospects for analytical scanning probe microscopy techniques.

KEYWORDS: Graphene electronics, analytical science, mechanical exfoliation, scanning probe microscopy, mica, DNA
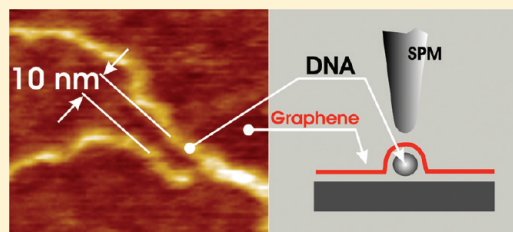
Graphene, a one atom thick planar sheet of carbon, exhibits a unique combination of electronic and mechanical properties.[5] In particular, graphene is very flexible with its electronic properties depending sensitively on its deformation, as evidenced, e.g., by low temperature scanning tunneling spectroscopy on graphene nanobubbles, indicating strong pseudomagnetic fields.[2] In order to control the graphene topography for strain engineered graphene electronics concepts, a lithographically patterned surface may be used.[6] Another potentially attractive prospect is to pattern a surface with nanosized objects like nanoparticles or macromolecules and thereby profile graphenes deposited on top of it. This may allow profiling with higher precision than lithographic methods provide.[7] Magnetic nanoparticles or polyelectrolyte macromolecules provide furthermore the opportunity to control local magnetic and electric fields. Macromolecules offer also the possibility to directly pattern a variety of structures, including lines, curves, circles, or branches.[8,9] However, it is an open question, to which extent the graphene may follow the profiles of the underlying nanostructured surface, given that it does not fully conform to the roughness of silicon oxide.[10]

Muscovite mica, a naturally occurring layered crystal exhibiting macroscopically large atomically flat cleavage planes, is widely used to immobilize macromolecules and other nano-objects for scanning force microscopy (SFM) imaging. Graphene has been argued to adhere stronger to mica than to silicon oxide[11] following closely its atomically flat surface.[12] We therefore exfoliated graphenes onto mica covered with macromolecules in order to determine whether the strong adhesion to the substrate surface would overcome the graphene stiffness to force graphene to replicate the surface topography. We used circular plasmid double stranded DNA (ds-DNA) molecules to structure the surface, since they can be easily processed from solutions and are also easily recognizable by their shape.

Experimental Section. The preparation of plasmid ds-DNA (pUC19, MoBiTec GmbH) on mica (Ratan mica exports, V1

quality) was adopted from ref 13. The molecules were deposited by putting a drop of a ds-DNA buffer solution (20 mM NaCl and 4 mM MgCl$_2$, Sigma-Aldrich water) with a concentration of 2 μg/mL onto a freshly cleaved mica surface for 2 min and spinning it off subsequently. The surface was washed three times thereafter by applying water (Sigma-Aldrich) for a few seconds and spinning it off subsequently. Then the sample was dried on a hot stage preheated to 70 °C for 2 min at ambient.[14]

Thin graphite flakes were peeled from a piece of freshly cleaved highly oriented pyrolytic graphite (HOPG, grade ZYB, Advanced Ceramics) and pressed gently onto the mica surface on the hot stage. Subsequently the mica was removed from the hot stage, and loose HOPG flakes were carefully removed from the mica surface with tweezers. The graphenes were optically detected, and single graphenes were verified with Raman spectra.[15] The number of graphenes in thicker layers was derived from optical and SFM measurements (JPK Instruments, NanoWizard II Ultra). SiN cantilevers were used with typical resonance frequencies of 70 and 300 kHz and spring constants of 2 and 42 N/m, respectively; they exhibit a typical tip apex radius of 7 nm with an upper limit of 10 nm, as specified by the manufacturer (Olympus Corporation). Both cantilever types were used for intermittent contact and contact mode imaging, with the imaging carried out at ambient conditions. Normal forces were estimated based on the spring constants provided by the manufacturer. First-order line subtraction and plane correction were applied to images to compensate for thermal drifts and sample inclination.

Results. The ds-DNA molecules can be readily recognized on topographic SFM images of freshly prepared samples recorded in *intermittent contact mode* (Figure 1a). Strikingly, the molecules appear to be virtually indistinguishable on bare mica and
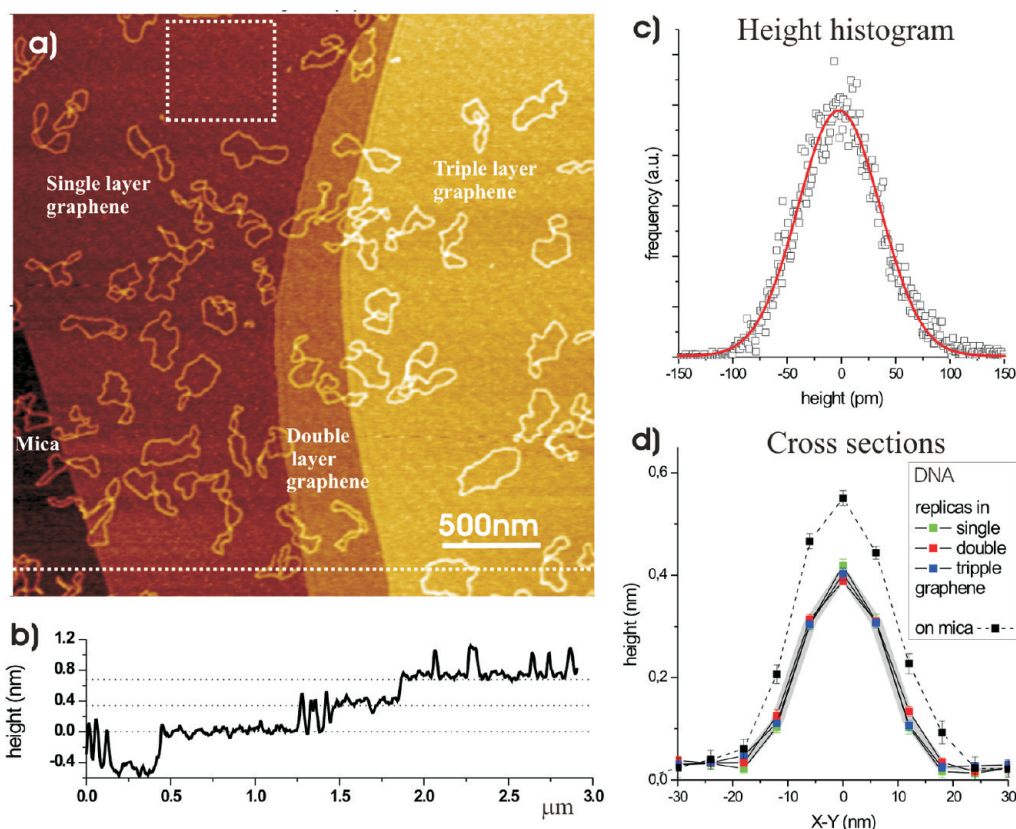
Figure 1. (a) Intermittent contact mode SFM height image of freshly prepared graphene on a mica surface covered with plasmid ds-DNA molecules. The number of graphene layers is indicated directly on the image. Faint periodic stripes visible to some extent throughout all reported images are an instrumental artifact. (b) Cross section along the dotted line in (a); single layer graphene height is assigned to be zero; horizontal dotted lines are guides for the eye at heights of 0.0, 0.34, and 0.68 nm. (c) Height histogram from the area indicated with the dotted rectangle in (a); fitting with Gaussian function (red line) gives standard deviation σ = 38 pm. (d) Averaged cross sections of ds-DNA replicas under different numbers of graphenes and on mica. In order to be averaged, single cross sections were fitted with Gaussian functions and shifted along the x axis to the mean value of the fit. Cross sections were shifted in height by 0.34 and 0.68 nm for double and triple graphenes, respectively, and arbitrarily for mica to match with the base of other cross sections. The gray shaded area is a guide to the eye produced by shifting of an averaged cross section along the x axis by ±1 nm.

graphene covered areas. In particular, surface coverage, molecular conformations including occasional self-crossings of the ds-DNA backbone, and heights and widths of the molecules did not noticeably vary between bare and graphene-covered areas. The surfaces of graphenes on mica between the DNA molecules were rather smooth (Figure 1). Rare cases of substantial height corrugations of graphene covered areas can be attributed to folds, cracks, and other defects of graphene, which typically occur on graphenes prepared by mechanical exfoliation. The standard deviations, σ, of height histograms from different flat areas in between the DNA molecules varied between 36 and 42 pm, with no substantial difference in roughness of mica and graphene-covered mica. While the height difference between successive graphene steps was 0.34 ± 0.01 nm (Figure 1b), closely matching the interlayer crystal spacing, the step height between graphenes and mica varied substantially, depending on scan conditions and cantilever, which we attribute to SFM tip—surface interactions.[16]

The ds-DNA molecules adopt equilibrated conformations defined by the high DNA stiffness[13] and their circular form. The contour length of the molecules as measured from the SFM images of the graphene replicas is 868 ± 6 nm, similar to the B form length in solution (886 nm). The height of the molecules on mica (Figure 1d) as measured from the topographic images is substantially smaller than the known ds-DNA diameter in

solution (~2 nm). The apparent width of the ds-DNA molecules varied from tip to tip, but it was always exceeding the ds-DNA diameter in solution, which is to be expected for tips with apex radii exceeding substantially the molecular diameter, as discussed in detail below. The apparent widths at half-maximum of the ds-DNA graphene replicas were similar to the ones on mica. Interestingly, the averaged cross sections of the replicas do not depend on the number of graphene layers (Figure 1d).

The roughness of the flat areas as imaged in intermittent contact mode on graphenes between the ds-DNA molecules increased within the first day after sample preparation and remained steady over the course of weeks (Figure 2c). High-resolution images imply that the increased roughness is due to small and shallow plateaus with a lateral extent on the order of 10 nm and a height varying in the range of 0.09−0.16 nm, depending on scan parameters and cantilevers used. Note that in intermittent contact mode SFM also phase images often reveal contrast between graphene flat areas and DNA replicas (Figures 2d), which will be discussed below.

The topography of a single layer graphene imaged in *contact mode* with normal forces up to 30 nN readily reveals confined ds-DNA molecules (Figure 2a). The graphene replicas of ds-DNA molecules are somewhat narrower and lower when measured in contact mode than in intermittent contact mode (Figure 2c).
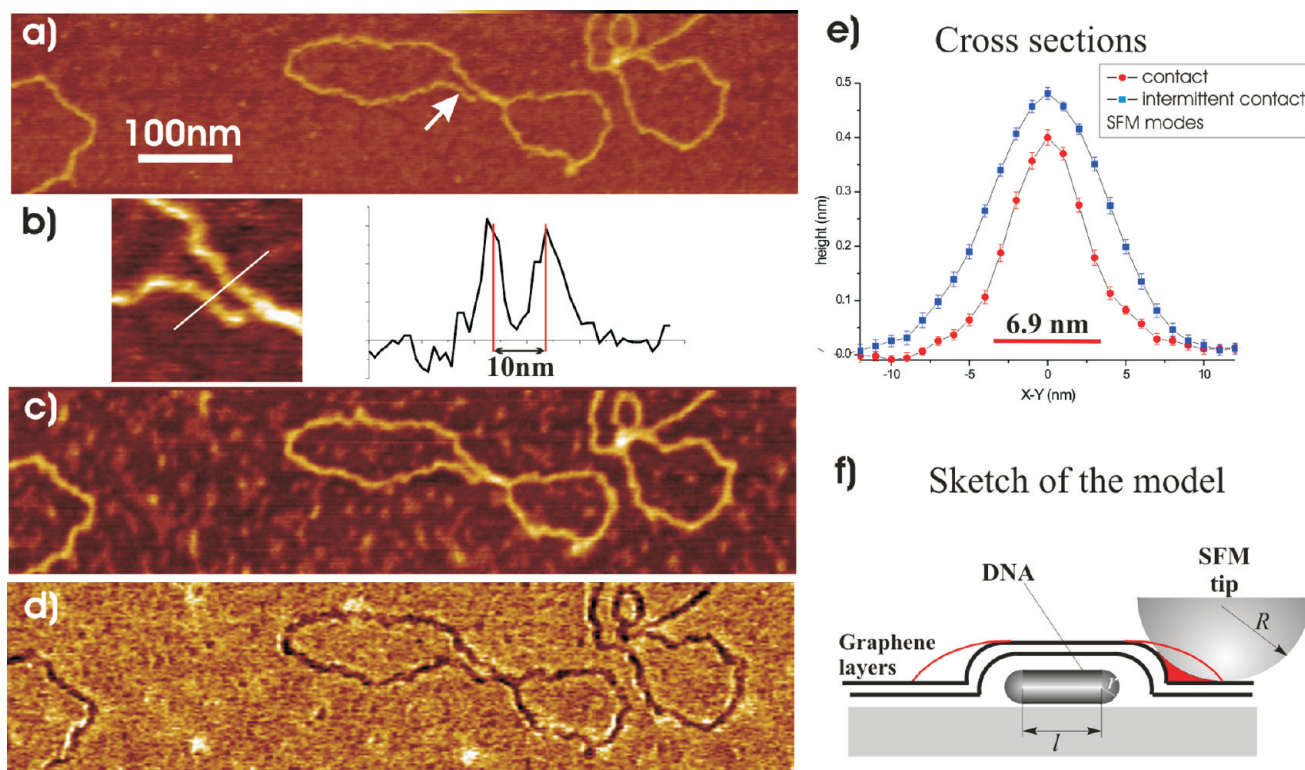
Figure 2. Images taken on a 6 day old sample in the area of a single graphene. (a) Topography image recorded in contact mode under a normal force of 25 nN. Arrow indicates an area enlarged on (b) with two resolved DNA strands running nearly parallel to each other at a distance of 10 nm, as visualized by the inserted cross section. (c) Intermittent contact mode topography image acquired a few minutes after (a) with the same tip. (d) Intermittent contact mode phase contrast image of the same area. (e) Averaged cross sections of DNA molecules measured in contact mode (a) and intermittent contact mode (c). The red line length is 6.9 nm, which is the apparent width of DNA under single graphene estimated from a model sketched in (f) (see Discussion).

Any attempts to image ds-DNA molecules in contact mode (with the same cantilevers) on bare mica failed, i.e., successive imaging in intermittent contact revealed the ds-DNA molecules to be scratched away by the imaging attempts in contact mode. This indicates enhanced protection against wear provided by the graphene, which we attribute to the low friction of graphene.[11] When an attempt was made to image DNA replicas under a single graphene in contact mode at an order of magnitude higher normal forces, the DNA molecules under the graphene were also scratched away. Graphene areas between ds-DNA molecules appear to be substantially smoother on the topographic images recorded in contact mode.

Discussion. Let us first point out that our results demonstrate graphene profiling with a line width of not more than 10 nm, as evidenced by the SFM images clearly resolving the graphene topography profiled with two ds-DNA strands running nearly parallel to each other at a distance of 10 nm (Figure 2b). However, due to the finite radius of the SFM tip apex,[17] lateral dimensions of objects are generally overestimated, since the tip starts to interact with the imaged object before the tip apex encounters it (see the red line in Figure 2f). Therefore, SFM images provide only an upper limit for the lateral size of the objects.

In order to improve on this, in the following we first assume cross-sectional shapes for the ds-DNA and the SFM tip and then demonstrate that the apparent width of graphene DNA replicas is consistent with the graphenes following closely the topography of DNA on the surface. We assume the ds-DNA cross section to be rectangular with a length l, capped with hemicircles with the radius r (Figure 2f). Assuming further that the tip is capped with a

semisphere, and that the graphene layers follow precisely the shape of the molecule, the apparent width of the DNA molecules can be estimated to be $w = 4(r(R + d))^{1/2} + l$, where R is the radius the SFM tip and d is the graphene thickness, estimated to be the number of layers, n, times 0.34 nm, i.e., the bulk interlayer spacing. The height of graphene DNA replicas should match closely the height of the encapsulated DNA (Figure 2f). Heights of ds-DNA molecules in SFM images in the range of 0.4 nm, i.e., much smaller than their diameter in solution ($\sim$2 nm), were previously reported and attributed to flattening of the molecules due to the interaction with the surface;[13] in our case this may be additionally enhanced by the pressure developed by the graphene. Moreover, DNA molecules may be buried in a layer of water molecules and salts, as will be discussed below, which effectively reduces the height of the DNA replicas in graphene. We estimate r to be half of the ds-DNA apparent height, i.e., $r = 0.2$ nm. The width of the DNA, l, we assume to be equal to the width of DNA in solution, i.e., $l = 2$ nm, which is only a rough assumption, since a flattened molecule should become also wider. Assuming $R = 7$ nm, i.e., a typical tip radius, we obtain for $n = 1$ an apparent width $w = 6.9$ nm, which correlates well with the DNA cross section imaged in contact mode (Figure 2e) and which is consistent with the assumption that graphene layers follow quite precisely the shape of the molecule. Three graphene layers should increase the apparent width w to 7.1 nm, i.e., add 0.2 nm in comparison to single graphene, i.e., the apparent width of DNA replicas in a few layer graphenes should not vary largely with the number of graphene layers for $R \gg 0.34n$, with this inequality being certainly true for the cantilevers we used. Thus,

the observed independence, within the experimental error, of the cross section of DNA graphene replica on the number of graphene layers supports the assumption that the subsequent graphene layers follow the topography of the first layer.

Since model calculations, per se, do not allow unambiguous deconvolution of an object shape due to a certain volume inaccessible to the tip (red shaded area on Figure 2f), we provide in the following an additional argument for the replication of DNA by the graphenes. The deformation energy of graphene conforming to DNA must be compensated by the adhesion of graphene to the surface. While, the stiffness of a multilayer graphene grows nearly proportional to the number of layers,[18] the adhesion energy should be largely independent of the number of graphene layers, due to the short range of the van der Waals potential and the graphene charge screening.[19] The proportionality of the stiffness to the number of graphene layers would increase the topography aberration for thicker graphene layers, which, however, we do not observe. This can be explained with a high adhesion energy of graphenes to the surface which forces graphenes to follow the DNA topography.

The ds-DNA graphene replicas appear to be broader and slightly higher when imaged in intermittent contact mode, in comparison to contact mode imaging (Figure 2e). One may attribute the difference to a liquid-like layer enclosing the ds-DNA, which is indiscernible under high forces developed during contact mode imaging. The ds-DNA molecules were deposited onto mica from an aqueous solution containing a mixture of salts, which are responsible for binding the ds-DNA to the mica surface.[20] Even intense washing with water does not remove the salts from the surface.[13] Evidently, graphenes encapsulate both DNA and this mobile ionic layer, which then assembles into small islands under graphenes over the course of a day. The mobility of the layer may be increased by the pressure developed by the SFM tip, since the islands become invisible in contact mode. It is reasonable to expect a higher concentration of the ions along the ds-DNA backbone due to its polyelectrolyte nature.[21] Thus, the contrast, which ds-DNA molecules exhibit in intermittent contact mode phase images, may be attributed to the compliance of the fluid cushion surrounding the DNA molecule and the resulting difference in the energy dissipation of the SFM tip oscillation.[22] In addition to the salt traces on the surface, there may be water molecules and contaminations resulting from the sample preparation from aqueous solution and at ambient.

Conclusions. We demonstrate that single and few layer graphenes mechanically exfoliated onto mica surfaces covered with individual ds-DNA molecules can be profiled with a line spacing of 10 nm. Given the broadening of the SFM image due to the finite tip radius, we conclude that the graphenes replicate the topography of the DNA molecules with even higher precision. The graphene replication of self-crossings of the DNA backbone implies the intriguing opportunity to design graphene profiling with, e.g., programmable DNA patterns.[4]

Moreover, we find that graphene provides enhanced protection of DNA molecules to shear forces exerted during scanning force microscopy in contact mode. In addition, graphene will act as a surface protective layer against the ambient, e.g., against oxidation, since it is impermeable to gases.[23] Taking into account both the high electric conductivity of graphene and its extremely small thickness, this offers new prospects for scanning probe microcopies and spectroscopies, such as scanning tunneling or tip enhanced Raman spectroscopy for analyses of both locally deformed graphene and confined molecules.

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