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Geometric Properties of Covalently Bonded DNA on Single-Crystalline Diamond

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Since the first report about DNA microarrays,[1] research on DNA-modified surfaces either as double-stranded segments or as short single-stranded oligodeoxyribonucleotides has become increasingly active. It is expected to have a broad impact on a number of emerging biotechnologies, such as drug development, DNA sequencing, medical diagnostics, nucleic acid−ligand binding studies, and DNA computing.[2] DNA immobilization techniques have been explored for a variety of substrates, such as latex beads, polystyrene, carbon electrodes, gold, and oxidized silicon or glass.[3] The surface chemistries applied for these substrates do not generally possess all of the desired characteristics of an ideal surface, such as flatness, homogeneity, chemical stability, reproducibility, and biochemical manipulation. In addition, the integration of biofunctionalized surfaces with microelectronics or micromechanical tools, for sensing or gene therapy, attracts increasing attention, shifting activities toward chemical and biological modifications of semiconductors.[4,5] Most of the microelectronic-compatible materials, such as silicon, SiO_x, and gold, show, however, degradation of the interfaces which inhibits the development of integrated sensors.[5]

Diamond became recently a promising candidate for bioelectronic devices as it shows good electronic and chemical properties, is considered to be biocompatible, and can be grown single-, poly-, or nanocrystalline, either by homo- or heteroepitaxy. In 2000, Takahashi et al. demonstrated the covalent attachment of DNA on polycrystalline diamond using a photochemical chlorination/amination/carboxylation of the initially H-terminated surface as initial steps.[6] In the same year, Yang et al. introduced an alternative method to modify nanocrystalline diamond surfaces with DNA.[5,7]

Up to now, however, only macroscopic experiments have been applied to characterize DNA attachment on ultrananno-, nano-, and single-crystalline diamond films.[8] Since the development of DNA-based biosensors requires efficient immobilization of DNA with optimum coverage and orientation, the main objective of this work is the study of hybridized DNA arrays on diamond on a microscopic level. Using atomic force microscopy (AFM) in electrolyte solution, we derive information about topographic and geometrical properties, elucidate bonding arrangement, strength, homogeneity, as well as degree of surface coverage.

DNA bonding has been realized on homoepitaxially grown intrinsic (100) oriented single-crystalline CVD diamond with H-terminated surface. These films have been grown on 3 × 3 mm synthetic (100) Ib diamond substrates. Growth parameters: substrate temperature 800 °C, microwave power 750 W, total gas pressure 25 Torr, total gas flow 400 sccm with 0.025% CH_4 in H_2. To achieve H-termination after growth, the CH_4 is switched off and the diamond is exposed to a pure hydrogen plasma for 5 min with otherwise identical parameter. A detailed discussion of sample properties has been given in ref 9.

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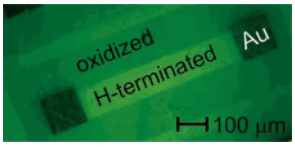

Figure 1. Fluorescence microscopy image of hybridized DNA on diamond using green fluorescence tag for the complementary DNA.

The diamond surfaces are modified by photochemical reactions with 10-amino dec-1-ene molecules protected with a trifluoroacetic acid group (TFAAD). First, 4 μL of TFAAD is spin-coated onto the H-terminated diamond surface at 4000 rpm for 20 s, which results in a 5 μm thick liquid layer. Then, the sample is sealed into a chamber with a quartz window and nitrogen atmosphere, followed by UV illumination for a given period of time. The ultraviolet light is from a high-pressure mercury grid lamp with peak emission at 250 nm and 10 mW/cm^2 intensity. Angle-resolved XPS measurements show oriented TFAAD attachment on diamond with densities in the range 10^{15}cm^{-2}, which has been reported also for nano- and single-crystalline (111) diamond.[8]

DNA has then been bonded to the TFAAD molecules by first deprotecting the protected amine, second reacting with the heterobifunctional cross-linker, sulfosuccinimidyl-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate, and finally reacting with thiol-modified DNA. DNA-modified surfaces were exposed to complementary oligonucleotides that were labeled with fluorescence probes. Figure 1 shows the result in which the diamond films were modified with a distinct sequence (S1 = 5′-HS−C_6H_{12}−T_{15}−GCTTATCGAG-CTTTCG-3′) of DNA. The entire sample was then exposed to the complementary sequence (F1 = 5′-FAM−CGAAAGCTCGAT-AAGC-3′), where FAM indicates the presence of fluorescein phosphoramidite. After the sample was exposed to F1, it shows high fluorescence intensity from regions where the surface was originally hydrogen terminated and then modified with S1. Some intensity is detected also from originally oxidized surface areas. It arises from nonspecific bonding and light-trapping in transparent diamond.

The DNA-functionalized and -hybridized surfaces are characterized by AFM measurements in 2X SSPE/0.2% SDS (sodium dodecyl sulfate) buffer solution. The buffer solution enables DNA to assume natural conformation and avoids effects of water meniscus around the AFM tip. Surface morphologies are investigated in oscillating-mode (OM-AFM) and contact-mode AFM (CM-AFM). Mechanical properties of surfaces are characterized by variation of loading force on the AFM tip in the CM-AFM regime. Doped silicon AFM cantilevers were used. Their typical spring constant was 3.5 N/m. Cantilever resonance frequencies were 75 kHz in air and 30 kHz in buffer solution.

By scratching the DNA layer in CM-AFM, the force required to penetrate and remove DNA molecules can be determined, giving

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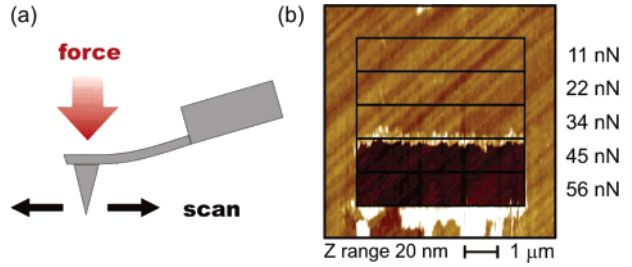

Figure 2. (a) Scheme and (b) results of CM-AFM scratching experiments on DNA-functionalized diamond in buffer solution.

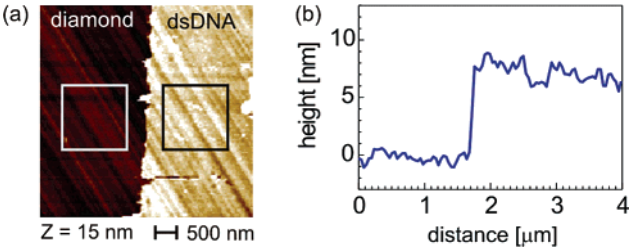

Figure 3. (a) Optimized OM-AFM measurement at the boundary of cleaned diamond surface and double-stranded DNA molecules bonded on single-crystalline diamond. The squares denote the regions where AFM phase shifts were evaluated. (b) AFM height profile across the boundary reveals a DNA layer thickness of 76 Å.

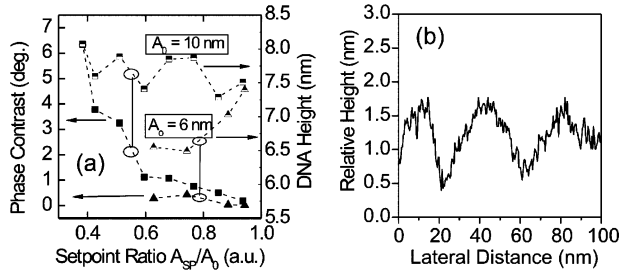

Figure 4. (a) AFM set-point ratio dependence of the AFM height and phase contrast across DNA-functionalized and cleaned diamond surface for free oscillation amplitudes (A_o) of 6 and 10 nm. (b) AFM height profile showing a dense DNA layer with height modulations of ±5 Å.

insight into the mechanical stability of bonding. Scratching experiments were performed at 11, 22, 34, 45, and 56 nN in buffer solution, as shown in Figure 2. For each force, an area of 1 × 5 μm has been scratched (scan rate 10 μm/s). Only for forces larger than 45 nN could a 100% clean area be achieved, indicating a strong bonding of DNA to diamond.

The above experiment was performed on the initially hydrogen-terminated diamond. DNA molecules on oxidized diamond surface (identified by a weak fluorescence in Figure 1) were removed by CM-AFM forces <10 nN, indicating that bonding of DNA on oxidized diamond is noncovalent.

By measuring across the boundary between DNA-functionalized and cleaned diamond surface using OM-AFM, the DNA layer thickness can be obtained as shown in Figure 3. OM-AFM is preferable to CM-AFM on soft layers as the tip−surface interaction can be minimized by monitoring the phase shift of the cantilever oscillations.[10] The phase shift was measured as a function of the set-point ratio, $r_{sp} = A_o/A_{sp}$, where A_{sp} is the set-point amplitude and A_o is the amplitude of free cantilever oscillation, on DNA-functionalized and cleaned diamond surface regions (see squares in Figure 3a). Figure 4 summarizes the results. Within the experimental parameter, the phase contrast between diamond and DNA is positive and approaches zero for set-point ratio approaching 1, that is, for increasing tip−surface distance. For the phase contrast near zero, which corresponds to minimal tip−surface interaction, the DNA layer thickness reaches 76 Å. Our experiments show that OM-AFM would lead to a significant underestimation of the DNA thickness if the set-point ratio was not optimized with respect to the tip−sample interactions. The height of 76 Å is, however, still significantly lower than the expected height of about 130 Å.

The topographic surface profile of DNA double helix molecules bonded on diamond is shown in Figure 4b. It reveals broad undulations due to collective interaction of several DNA oligomers with the tip. The height is modulated with a periodicity of about 30−50 nm and an amplitude of ±5 Å.

No pinholes can be detected in the layer. Obviously, a closed DNA film has been synthesized on diamond. The discrepancy between expected height of 130 Å and the real height of 76 Å originates from a tilted arrangement of DNA molecules on diamond. Using triangular geometry, the tilt angle is about 36°, which is similar to results of DNA bonding on gold surfaces.[10] Further experiments are currently being performed to characterize the double helix axis orientation as a function of applied potentials.

In conclusion, we presented the first characterization of DNA double helix bonding on single-crystalline diamond using contact-mode and oscillating-mode AFM experiments in buffer solutions. The layer is compact with a thickness of 76 Å. The height shows some undulations due to tip and DNA interactions, which modulates with ±5 Å. From these data, we conclude that the double helix axis is oriented at about 36° with respect to the diamond surface. Contact-mode AFM scratching experiments show that relatively high forces of >45 nN are required to remove the DNA molecules, which is significantly higher than that detected on other substrates, such as gold. It is attributed to a high and chemically stable bonding strength of DNA to diamond, which makes diamond a promising candidate for bioapplications.

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