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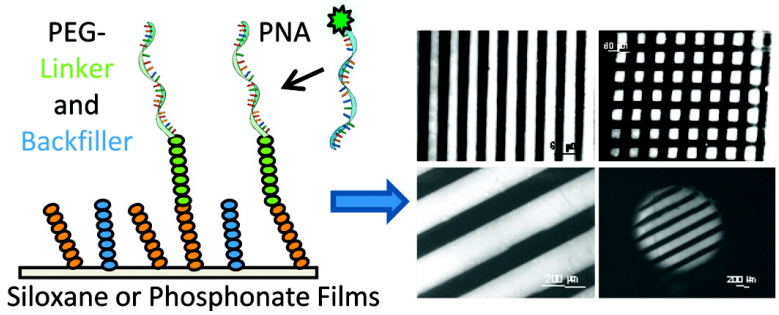

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PNA-PEG Modified Silicon Platforms as Functional Bio-Interfaces for Applications in DNA Microarrays and Biosensors

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The synthesis and characterization of two types of silicon-based biofunctional interfaces are reported; each interface bonds a dense layer of poly(ethylene glycol) (PEG_n) and peptide nucleic acid (PNA) probes. Phosphonate self-assembled monolayers were derivatized with PNA using a maleimido-terminated PEG_{45}. Similarly, siloxane monolayers were functionalized with PNA using a maleimido-terminated PEG_{45} spacer and were subsequently modified with a shorter methoxy-terminated PEG_{12} ("back-filling"). The long PEG_{45} spacer was used to distance the PNA probe from the surface and to minimize undesirable nonspecific adsorption of DNA analyte. The short PEG_{12} "back-filler" was used to provide additional passivation of the surface against nonspecific DNA adsorption. X-ray photoelectron spectroscopic (XPS) analysis near the C 1s and N 1s ionization edges was done to characterize chemical groups formed in the near-surface region, which confirmed binding of PEG and PNA to the phosphonate and silane films. XPS also indicated that additional PEG chains were tethered to the surface during the back-filling process. Fluorescence hybridization experiments were carried out with complementary and noncDNA strands; both phosphonate and siloxane biofunctional surfaces were effective for hybridization of cDNA strands and significantly reduced nonspecific adsorption of the analyte. Spatial patterns were prepared by polydimethylsiloxane (PDMS) micromolding on the PNA-functionalized surfaces; selective hybridization of fluorescently labeled DNA was shown at the PNA functionalized regions, and physisorption at the probe-less PEG-functionalized regions was dramatically reduced. These results show that PNA-PEG derivatized phosphonate monolayers hold promise for the smooth integration of device surface chemistry with semiconductor technology for the fabrication of DNA biosensors. In addition, our results confirm that PNA-PEG derivatized self-assembled carboxyalkylsiloxane films are promising substrates for DNA microarray applications.

Introduction

Biofunctional interfaces between organics and inorganics (for example, semiconductor materials) enjoy increasing interest in basic and applied science because of the many possible applications of these structures to fields including proteomics, microarray technology, and biosensors. It is expected that these interfaces will be able to perform specific functions, such as biorecognition in the context of an electrical measurement, better than either purely organic or inorganic systems. For example, surface-mediated hybridization, in which single-stranded DNA is tethered to a solid support and binds DNA analyte molecules from solution, underpins the modern microarray and biosensor biotechnologies[1,2] that are now widely used for genotyping, studying gene expression, and biological detection.[3–8] Microarrays, in which thousands of hybridization reactions are carried out in parallel, are commonly used to address fundamental questions of biology and for sample characterization, even on the whole-genome scale.

Biosensors are typically dedicated to specific tasks, such as detection of a small number of analytes, and data acquisition is often performed in real-time. For such applications single stranded DNA is commonly covalently immobilized via a linker onto the semiconductor surface, which has been modified with a thin organic film. Common approaches entail formation of thiol-modified DNA monolayers[9,10] or binding of DNA to siloxane layers via cross-coupling linker species[11–15] that often involve maleimido or *N*-hydroxysuccinimidyl groups. Approaches to surface immobilization of single-stranded nucleic acids require that the hybridization specificity of the unbound probe is preserved and that nonspecific interactions between surface and analyte are minimized; these remain important goals for improving performance of DNA microarray and biosensor applications. As described in a review by Levicky and Horgan,[1] the behavior of nucleic acids in solution with regard to hybridization between probe and complementary target DNA molecules can differ from analogous hybridization processes at the solid−liquid interface. For hybridization at a surface, nonspecific probe-surface interactions can be an important issue that influences the efficacy and capacity of DNA target hybridization devices. To reduce nonspecific adsorption of DNA

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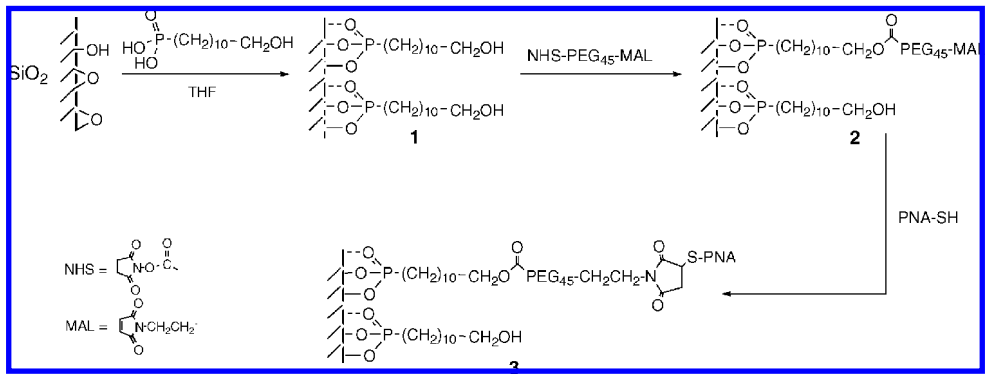
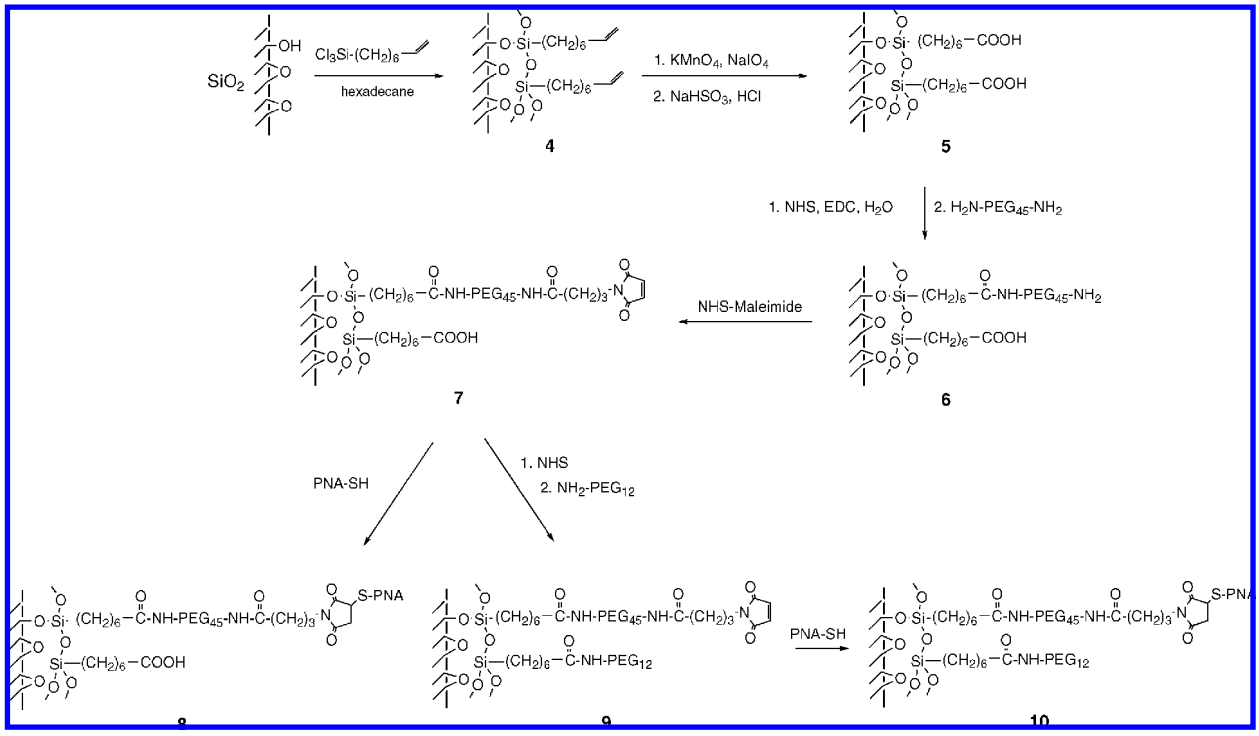
Scheme 1. Synthesis of PNA-PEG-Functionalized Undecyl Phosphonate Monolayers



Scheme 2. Synthesis of PNA-PEG-Functionalized Siloxane Self-Assembled Films



analyte, biocompatible spacers such as cellulose,[16] acrylamide,[17] and PEG[18] have been used to link the DNA probe strand to the surface.

These approaches have indeed proved useful; however, there remains room for improved chemical design. For example, all surface chemistry reactions should be as mild as possible to enable smooth integration with device fabrication. Synthesis in aqueous media under ambient conditions would be especially ideal for attaching biointerfaces to sensing units such as silicon nanowires or chips equipped with metal electrical circuitry for detection. Furthermore, the biologically passivating layer between the probe and the surface should be as dense as possible to effectively minimize analyte nonspecific adsorption. With these goals in mind, we have synthesized two types of PNA-PEG-functionalized monolayers based on alkylphosphonates and alkenylsiloxanes[19–21] on silicon oxide-coated silicon surfaces. PNA (peptide nucleic acid[22]) is a DNA mimic that involves a pseudopeptide backbone of repeating *N*-(2-aminoethyl)glycine units linked by peptide bonds and bearing nucleotide bases. PNA has attracted broad attention in the field of medicinal chemistry because of its chemical robustness and efficient and sequence-specific binding to both single stranded RNA and DNA. In one approach, a self-assembled monolayer of a hydroxyl-terminated alkylphosphonic acid was bonded under ambient conditions to a silicon oxide-coated silicon surface. The phosphonate monolayer was subsequently modified with PEG and PNA using *N*-hydroxysuccinimidyl and maleimido cross-coupling reagents. In another approach, a siloxane film formed from octenyltrichlorosilane was bonded to a silicon oxide-coated silicon surface and was further modified with PEG and PNA using related cross-coupling reagents. To increase the areal density of the PEG layer between probe and surface, these PNA-PEG-functionalized surfaces were further modified using shorter CH_3-terminated PEG, in a process we call "back-filling". All surfaces were characterized by XPS, which was carried out near the C 1s ionization edge to characterize chemical groups formed in the near-surface region. Fluorescence hybridization assays using complementary and noncDNA strands were performed to assess the effectiveness of the surface for reducing nonspecific adsorption of the analyte. Patterning experiments were also done to determine the efficacy of PEG in minimizing nonspecific adsorption of the DNA analyte and

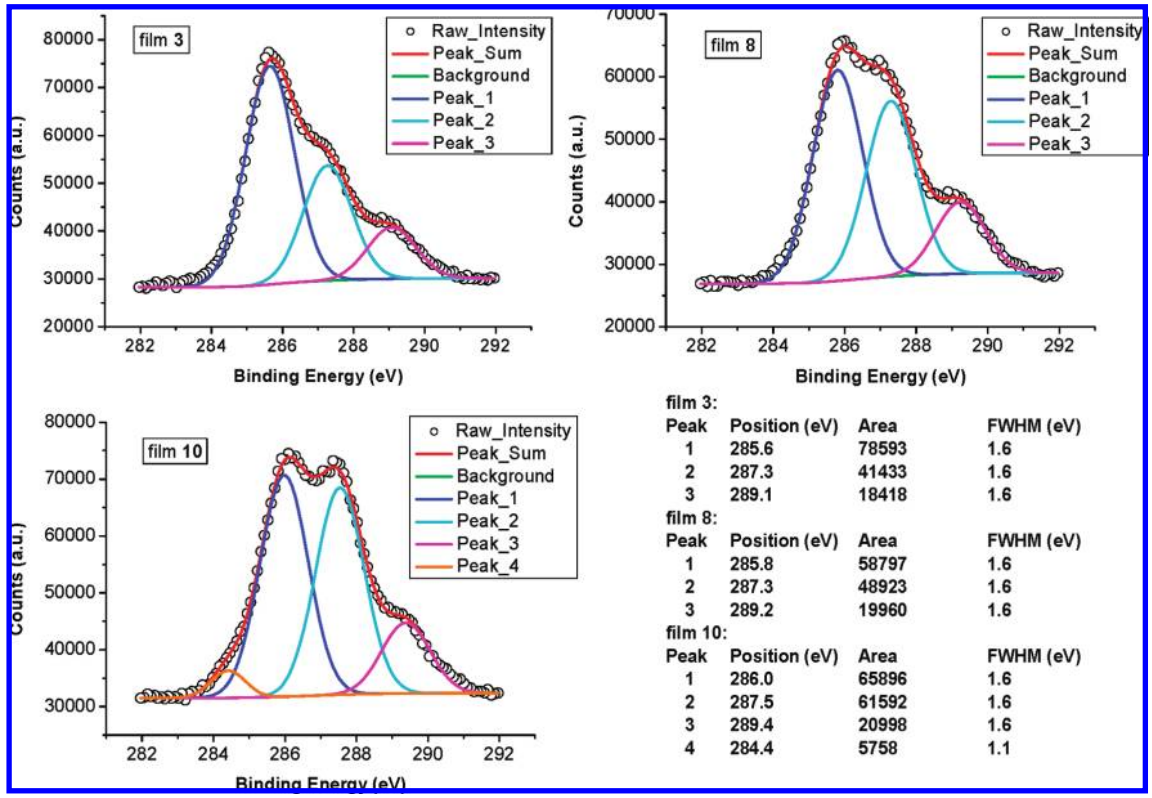


Figure 1. XPS scans of the C 1s regions and fitting results of PNA-PEG functionalized phosphonate monolayer **3**, PNA-PEG functionalized silane film **8**, and backfilled PNA-PEG functionalized silane film **10**.

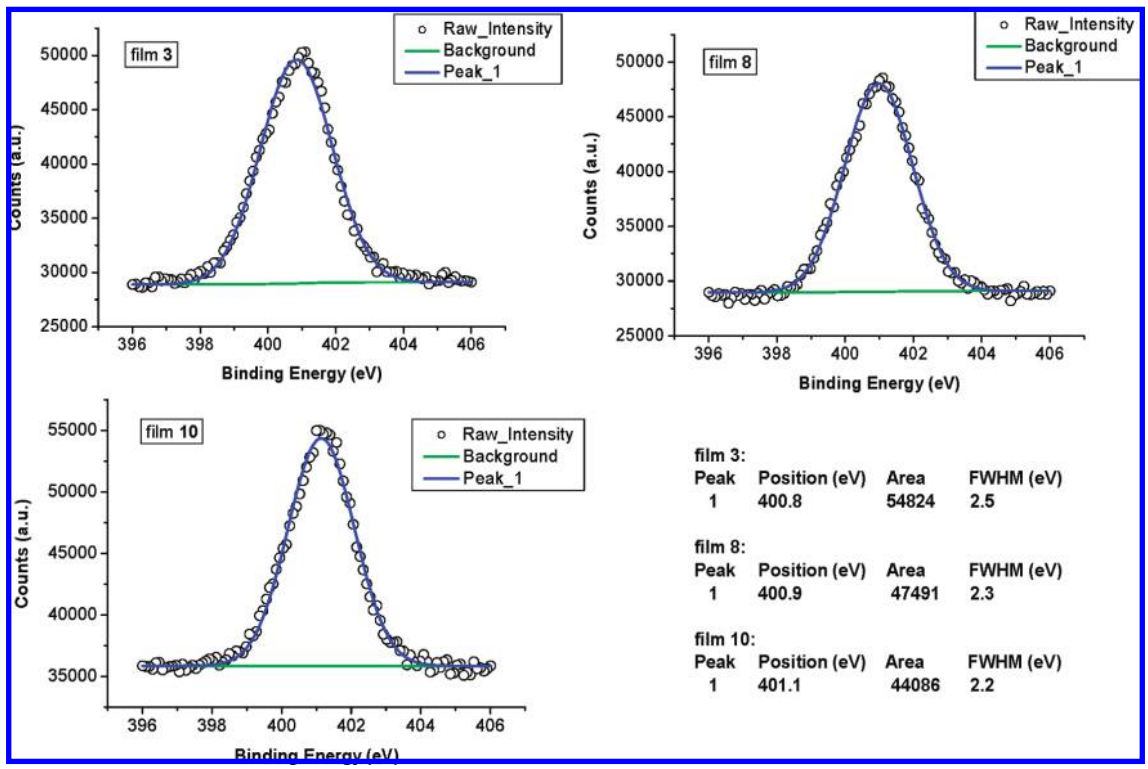


Figure 2. XPS scans of the N 1s edges and fitting results for PNA-PEG functionalized phosphonate monolayer **3**, PNA-PEG functionalized silane film **8**, and backfilled PNA-PEG functionalized silane film **10**.

to evaluate these surfaces as substrates for DNA microarray and biosensor applications.

Experimental Section

Materials. All chemicals and solvents were purchased from Sigma-Aldrich and were used without further purification. Functionalized poly(ethylene glycols), such as NH_2-$(CH_2CH_2O)_{44}CH_2CH_2$-NH_2 (NH_2-PEG_{45}-NH_2), NH_2-$(CH_2CH_2O)_{11}CH_2CH_2$−OCH_3 (NH_2-PEG_{12}), NHS-$(CH_2CH_2O)_{44}CH_2CH_2$-MAL (NHS-PEG_{45}-MAL; NHS = *N*-hydroxysuccinimidyl; MAL = maleimido), and NHS-$(CH_2CH_2O)_{11}CH_2CH_2$-OCH_3 (NHS-PEG_{12}) were purchased from Rapp Polymere and were used without further purification. Cy3-fluorescently labeled comple-

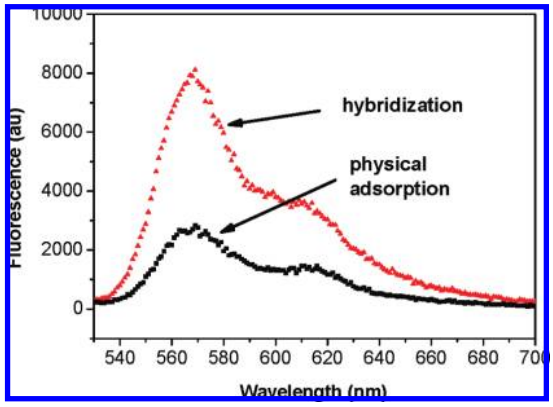


Figure 3. Fluorescence spectra of hybridization in the presence of complementary and noncomplementary Cy3-labeled DNA for PEG-less PNA functionalized phosphonate monolayers.

mentary 12mer DNA (5′-TGCTTCCGACTA-3′) and noncomplementary 12mer DNA (5′-ACTAATTCTTAT-3′) were purchased from IBA GmbH (Göttingen, Germany). Thiol- and amino-terminated 12mer PNA (5′-Cys-TAGTCGGAAGCA-Lys-3′) were purchased from Eurogentec. 11-Hydroxyundecylphosphonic acid was synthesized as previously reported.[16–18] Silicon wafers (both native oxide coated and 100 nm thermally oxidized Si (001) wafers, cut into 20 × 15 mm^2 sample pieces prior to functionalization) were purchased from Holm Silicium (Germany). The silicone elastomer kit, Sylgard 184 (manufacturer Dow Corning), was purchased from Sasco Holz GmbH (Munich, Germany).

Preparation of Phosphonate Self-Assembled Monolayers (1). Wafers were sonicated in acetone for 10 min and then rinsed with isopropanol and water. The samples were immersed in a solution of water/H_2O_2/NH_3 (5/1/1 by volume) at about 80 °C for 10 min. They were then sonicated in water for 10 min, dried under nitrogen flux and immediately used for the preparation of phosphonate monolayers. In this process the freshly cleaned wafers were placed vertically in a 25 μM solution of 11-hydroxyundecylphosphonic acid in dry THF. The solvent was allowed to evaporate slowly for about 2 h so that the meniscus slowly traversed the surface of the wafer, transferring the phosphonic acid to the SiO_2/Si.

The solution reservoir was large enough to ensure that no appreciable change in the concentration occurred during the process. Coated wafers were then gently removed from the vertical holders and put in the oven at 120 °C for 18−20 h to set the film as a phosphonate monolayer. Hydrogen bonding among phosphonic acid head-groups and hydroxyl tail groups of the starting 11-hydroxyundecylphosphonic acid can give rise to surface multilayers. For this reason all the samples were sonicated twice in methanol for 10 min and then once in a solution of water/THF/triethylamine (10/3/1 by volume) for 5 min. Finally they were sonicated in water for 10 min and dried under nitrogen flux. To obtain complete coverage of the silicon surface a second functionalization process was usually performed by repeating the deposition step.

Preparation of PEG-Maleimide Functionalized Phosphonate Monolayer (2). 11-Hydroxyundecylphosphonate monolayers were treated overnight with a 19 mM solution of NHS-PEG-MAL in dry acetonitrile at room temperature under argon. They were then sonicated sequentially in dry acetonitrile for 5 min and in THF for 5 min. The samples were dried under nitrogen flux, stored under vacuum, and briefly washed with water just before any further coupling reactions were done with PNA.

Preparation of PNA-Functionalized Phosphonate Monolayer (3). 12mer PNA-oligonucleotide (5′-Cys-TAGTCGGAAGCA-Lys-3′) was diluted with degassed water (pH 7.0) to a final concentration of 25 μM. Small drops of the solution were deposited manually on the surface of freshly prepared samples of **2**. The chips were incubated overnight at room temperature in a humidified chamber (to prevent drying of the droplets), then washed carefully with sonication in water (5 to 10 min), and finally blown dry with nitrogen gas.

Preparation of Alkenylsiloxane Films (4). Wafers were cleaned in a three-step procedure: (i) immersion in a solution of $NH_4OH/H_2O_2/H_2O$ (1/1/4 by volume) at 80 °C for 10 min and washing with deionized water, (ii) immersion in a $HCl/H_2O_2/H_2O$ (1/1/4 by volume) at 80 °C for 10 min and washing with deionized water, (iii) immersion in a solution of $NH_4OH/H_2O_2/H_2O$ (1/1/4 by volume) at 80 °C for 10 min and thorough washing with deionized water. The wafer pieces were then flushed with nitrogen and dried in a vacuum oven at 120 °C for 5 min; they were used within half an hour of cleaning. Films from octenyltrichlorosilane (OCT) were prepared according to procedures described in the literature.[23] In a typical reaction, a flask was charged with three silicon wafers, anhydrous hexadecane (15 mL), and octenyltrichlorosilane (0.1% wt). The mixture was allowed to react under nitrogen for 24 h. After silanization the wafers were rinsed with anhydrous toluene and sonicated in $CHCl_3$ for a few minutes to remove organic residuals. Finally, they were blown dry in a nitrogen flow and stored at room temperature under vacuum until used.

Oxidation of Alkenylsiloxane Films (5). The terminal alkenyl group of the octenylsiloxane film was cleaved to give the terminal carboxylic acid by permanganate-periodate oxidation.[24] Stock solutions of $KMnO_4$ (5 mM), $NaIO_4$ (195 mM), and K_2CO_3 (18 mM) were prepared. Immediately prior to reaction, 1 mL from each solution was combined with distilled water to make the oxidation reagent at pH = 7.5 and was 10-fold diluted with respect to each component, that is, 7 mL of distilled water was added to 1 mL $KMnO_4$, 1 mL $NaIO_4$, and 1 mL K_2CO_3. The octenylsiloxane films were exposed to this solution for 24 h to ensure quantitative oxidation of the surface alkenyl groups. The samples were then rinsed sequentially in 20 mL of $NaHSO_3$ (0.3 mM), water, HCl (0.1 M), water, and ethanol. The samples were finally dried in a flow of nitrogen and stored under vacuum until further use.

Preparation of Diamino-PEG Functionalized Siloxane Film (6). The carboxylate-terminated films were reacted with a water solution of 75 mM 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimidehydrochloride (EDC) and 15 mM *N*-hydroxysuccinimide (NHS) for one hour. They were then thoroughly rinsed with water and placed in a solution of NH_2-PEG_{45}-NH_2 (10 mg/mL) in carbonate buffer (0.1 M) at pH = 8.5. The reaction was run overnight; the wafers were then sonicated in $CHCl_3$ for a few minutes and dried under nitrogen flow. Samples were finally dried in vacuo at room temperature for several hours.

Preparation of PEG-Maleimide Functionalized Siloxane Film (7). Films of **6** were kept in a solution of 3-maleimidopropionic-acid-NHS (20 mg/mL) in dry acetonitrile at room temperature under nitrogen overnight. They were then sonicated first in dry acetonitrile for 5 min and then in THF for 5 min. The samples were finally dried under nitrogen flow, stored under vacuum, and briefly washed with water just before coupling reaction with PNA.

Preparation of PNA-Functionalized Silane Film (8). 12mer PNA (5′-Cys-TAGTCGGAAGCA-Lys-3′) was diluted with degassed water (pH 7.0) to a final concentration of 25 μM. Small drops of the solution were deposited manually on the surface of **7** that had been freshly reacted with 3-maleimidopropionic-acid-NHS. The chips were incubated overnight at room temperature in a humidified chamber (to prevent drying of the droplets), then washed carefully by sonication in water (5−10 min), and finally blown dry with nitrogen gas.

Preparation of Back-Filled Functionalized Siloxane Film (9). Samples of **7** were reacted with NHS as described above and then exposed to a solution of NH_2-PEG_{12} (20 mg/mL) in carbonate buffer (0.1 M) at pH = 8.5. The reaction was allowed to run overnight; the wafers were then sonicated in $CHCl_3$ for a few minutes and dried under a nitrogen flow. Samples were finally dried under vacuum at room temperature for several hours and stored under vacuum until further use.

Preparation of PNA-Functionalized Back-Filled Siloxane Film (10). Samples of **9** were reacted with 12mer PNA (5′-Cys-TAGTCGGAAGCA-Lys-3′) as described above.

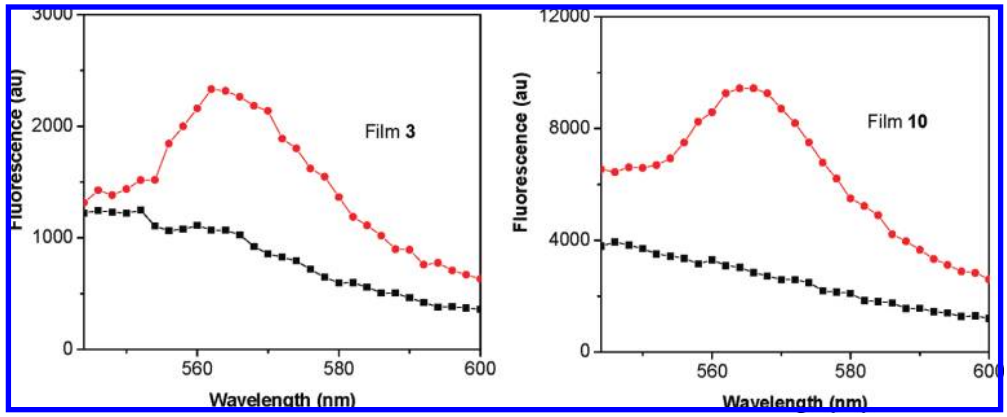


Figure 4. Fluorescence emission peak at 570 nm in the presence of complementary (red curves) and noncomplementary (black curves) Cy3-labeled DNA: (left) PNA-PEG functionalized phosphonate monolayer **3** and (right) backfilled PNA-PEG functionalized silane film **10**. The slight increase in the background signal is due to the filter configuration in the experimental setup.

Table 1. XPS Binding Energies near the Carbon Ionization Edge

bond	binding energy (eV)
C−C (alkane)	286
CH_2−O−CH_2 (PEG)	287
O=C−O	289.2

Preparation of PEG-less PNA-Functionalized Reference Surfaces. Reference surfaces (without PEG) were prepared by reaction of the phosphonate monolayer with thiol-terminated 12mer PNA (5′-Cys-TAGTCGGAAGCA-Lys-3′) using 3-maleimidopropionic-acid-NHS as a cross-linker and by reaction of the carboxylated siloxane film with amino-terminated 12mer PNA (5′-Cys-TAGTCGGAAGCA-Lys-3′) using an NHS-based cross-linker. Reactions were carried out according to the experimental conditions described above.

DNA Hybridization Assays. 12mer DNA (5′-TGCTTCCGACTA-3′) labeled at the 5′- terminus with a fluorophore (Cy3) was diluted to a final concentration of 1 μM in hybridization buffer consisting of 2 mM 3-morpholinopropanesulfonic acid (MOPS) and 3 mM NaCl (pH = 6.8). The chips were incubated in the hybridization solution for 25 min at room temperature and then washed carefully three times with buffer, then with water (without sonication), and dried with nitrogen. For noncomplementary experiments, 12mer DNA (5′-ACTAATTCT-TAT-3′) was used.

Micropatterning. Samples were patterned by micromolding.[25–27] Poly(dimethyl siloxane) (PDMS) molds were prepared by casting a 10/1 w/w mixture of silicone prepolymer and curing agent onto photolithographically microstructured silicon masters or nickel TEM grids (two grids staked together on top of a magnet), followed by baking at 80° for 45 min. After cooling to room temperature, solidified PDMS was carefully cut off and gently removed from the master. The molds thus obtained were sonicated first in water for 15 min, then in ethanol for 15 min, and finally in water for 5 min. They were then treated with oxygen plasma for 2 min to render the surface hydrophilic and were used within an hour of the plasma treatment. Hybridization line patterns were obtained by micromolding of PNA onto maleimide-functionalized PEGylated films followed by 1 h exposure of the wafer to a solution of Cy3-labeled cDNA (1 μM in PBS buffer). Checkered hybridization patterns were obtained by micromolding of PNA onto MAL-functionalized PEGylated films followed by micromolding of Cy3-labeled cDNA onto this pattern at a 90° angle. The residence time of Cy3-labeled DNA inside the mold channels was 1 h. Finally the wafers were washed with DI water and imaged by fluorescence microscopy.

Characterization

XPS was carried out under ultrahigh vacuum conditions (5×10^{-10} mbar). The XPS system was equipped with a Mg KR X-ray source (1253.6 eV; X-ray current: 20 mA; X-ray Voltage: 10 kV) and a Escalab MK II analyzer. The peak areas for the determination of the surface atom composition were calculated by the integration of a Voigt fit (80% Gaussian and 20% Lorentzian).

Fluorescence measurements were carried out in an experimental laser setup. Fluorescence measurements were conducted by positioning an optical fiber mount over the chip region of interest. Light from an Ar^+ laser (λ = 514 nm) was guided onto the surface at an angle of about 45°, whereas fluorescence from Cy3 dyes (emission peak at 570 nm typical for Cy3 conjugates) was collected by a second fiber oriented normal to the surface plane. Cy3 fluorescence was measured by coupling light from the detection fiber into a monochromator equipped with a single photon counting module. The same setup was successfully used in the past for investigating the structural properties of oligonucleotides monolayers on gold surfaces.[28]

Fluorescence microscopy was carried out using a motorized inverted microscope (Axiovert100M, Zeiss) equipped with a mounting frame with temperature controls for the microscope stage and controlled by SimplePCI (Compix). Illumination was generated using a mercury light source (HB 100) and filter block (Chroma Technology Corp.) provided with band-pass filter of 450−490 nm for excitation, FT510 beam splitter and emission long pass filter of 510−565 nm.

Results and Discussion

One major requirement for a device to bind a DNA analyte specifically is to promote its hybridization onto a surface-tethered cDNA probe while minimizing its nonspecific adsorption. In this work we present the synthesis and characterization of phosphonate and siloxane films modified with PEG and PNA as selective probes for cDNA hybridization. PNA is a peptide analogue of DNA and is used as a DNA hybridization partner due to its chemical robustness and stability. The role of the PEG is to minimize nonspecific adsorption of DNA analyte. The chemical synthesis of PNA-PEG-modified phosphonate and siloxane films is shown in Schemes 1 and 2, respectively. Hydroxyl-terminated phosphonate monolayers can be prepared easily under ambient conditions via reaction of an hydroxyl-terminated alkylphosphonic acid and the native or thermally grown silicon oxide-coated silicon surface. Such self-assembled monolayers are very appealing for biotechnological applications, due not only to the mild conditions of their preparation, but also to the ability to perform chemical amplification of their termini with a variety of biomolecules via *N*-hydroxysuccini-

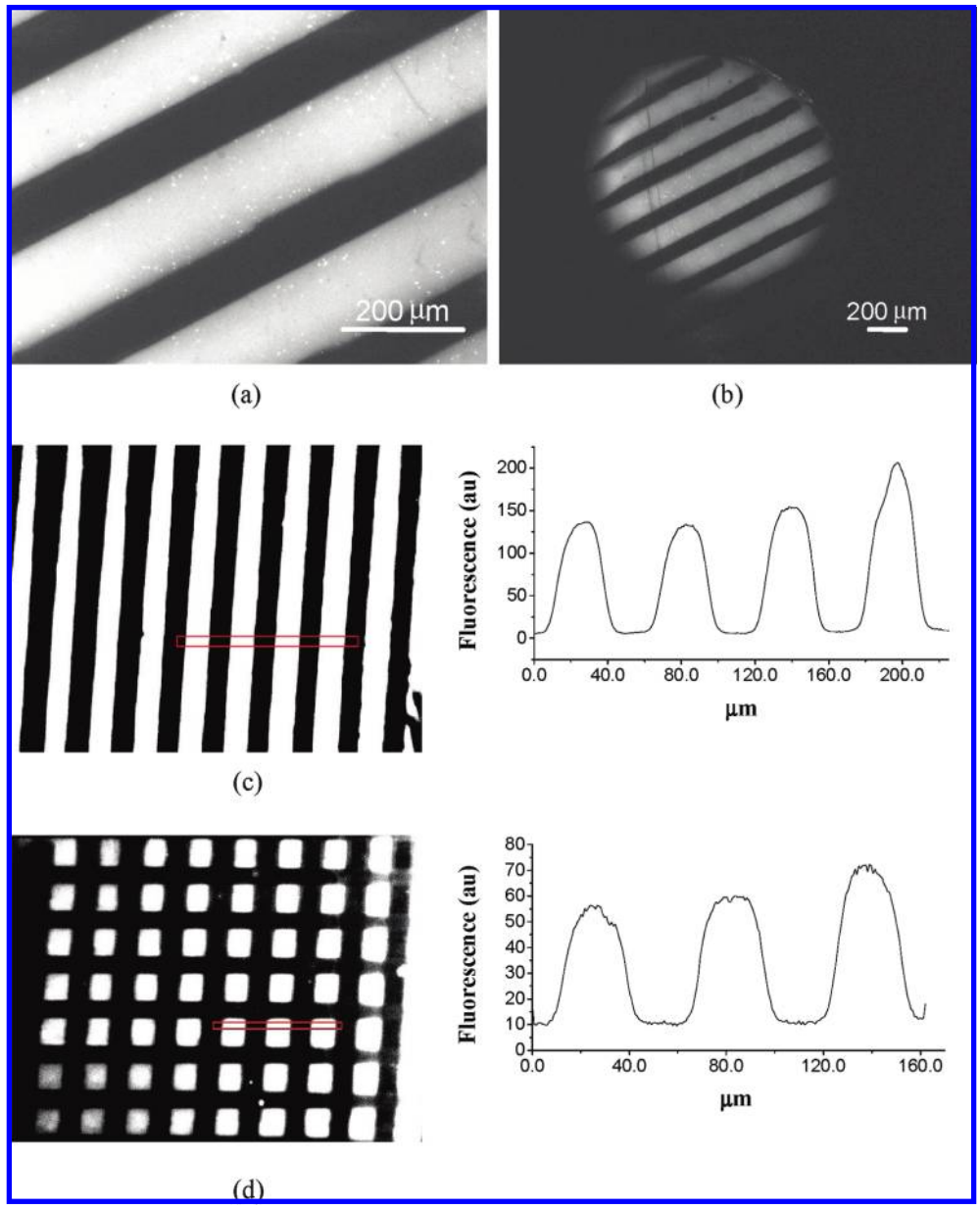


Figure 5. Representative fluorescence images showing spatial arrays of complementary Cy3-labeled DNA hybridized onto PNA. (a−c) Array obtained by exposing a PEG-PNA pattern to solution of complementary Cy3-DNA. (d) Checkered pattern obtained by micromolding of complementary Cy3-DNA onto a preformed PEG-PNA line pattern.

midyl[29,30] (NHS) and maleimido[31,32] (MAL) cross-linkers. As shown in Scheme 1, a hydroxyl-terminated alkylphosphonic acid was reacted with the native silicon oxide-coated silicon surface in anhydrous THF to obtain the corresponding hydroxyl-terminated phosphonate monolayer (**1**). The monolayer was then chemically amplified via reaction with α-NHS-ω-MAL-terminated PEG_{45} in anhydrous acetonitrile to give **2**. Subsequently, **2** was reacted with SH-terminated PNA in water to give **3**.

As shown in Scheme 2, octenylsiloxane film **4** was oxidized by permanganate-periodate, which cleaves the terminal vinyl group to give carboxylic acid **5**. The carboxylated siloxane film was then reacted with *N*-hydroxysuccinimide (NHS) to form active ester groups capable of rapid reaction with amines to give amide bonds that are stable toward hydrolytic scission. The NHS modified surface was treated with an aqueous solution of diamino-terminated PEG_{45} to yield **6**. Surface **6** was treated with (3-maleidoiminopropionyloxy)succinimide in dry acetonitrile, which gives **7**, and **7** was then functionalized with SH-terminated PNA in water to give PNA-PEG-functionalized **8**. Unreacted COOH groups of **7** were coupled with a shorter amino-terminated PEG_{12} via NHS chemistry to increase the surface density of grafted PEG, thus providing additional passivation of the surface against nonspecific DNA adsorption. The lower molecular weight of PEG_{12} (MW = 550 Daltons) compared to PEG_{45} (MW = 2000 Daltons) ensured reactivity of PEG_{12} at the residual COOH surface groups because of entropic reasons. In fact, the yield of reaction of "grafting to" processes is mostly governed by entropy which is dependent on the size of the macromolecules reacting at the surface active sites.[35] In previous work[33] we showed that implementing the PEG back-filling process on a lipid-PEG-terminated siloxane film increased the surface density of grafted PEG thus causing lipid substituents to stick up above the PEG layer. Back-filling gave **9**, which was functionalized with SH-terminated PNA in water leading to PNA-PEG-functionalized surface **10**. Back-filling was also attempted using NHS-terminated PEG_{12} to react with any residual OH groups of PNA-PEG-functionalized

phosphonates, but no further introduction of PEG was noted, which may be due to low reactivity of these OH groups at the surface of the highly organized film. We typically note that hydroxyphosphonate monolayers show sluggish reactivity at their surface OH groups, which we attribute to their being in a hydrogen bonded network on the film surface.[31]

X-ray photoelectron spectroscopic (XPS) analysis was done at the C 1s and N 1s ionization edges to characterize the functional groups formed in the near surface region at each stage of the overall synthesis procedure: The different bonding environments for each species causes the C1s electrons to have different binding energies, which are discernible by XPS. Table 1 shows the expected binding energies for the relevant carbon bonds.[34]

XPS scans of the C 1s and N 1s regions of surfaces **3**, **8**, and **10** are shown in Figures 1 and 2; the associated curve fitting results were obtained after background subtraction. All C 1s spectra show peaks close to the expected binding energies of 286, 287, and 289.2 eV, which are assigned as shown in Table 1. [33,35] All N 1s spectra showed a major peak at 401 eV indicative of the nitrogen in the PNA backbone.

The XPS peak areas listed in Figures 1 and 2 are related to the three different chemical moieties present on the surface: the phosphonate or siloxane films themselves, the PEG spacers, and the PNA. The last two species were covalently linked in two separate synthetic steps with yields <100%, as is reasonable for the comparative steric sizes of the surface-bound alkyl chains and the cross-linking reagents and also the low intrinsic reactivity of the surface film terminal groups (especially the OH groups of the phosphonate monolayers). Nonetheless, some important information can be obtained from the XPS analysis. First it should be noted that the peak intensity ratio, C-C/C-O, in the C 1s spectrum is greater for PNA-PEG-functionalized phosphonate monolayer **3** than it is for PNA-PEG-functionalized siloxane **8**. We attribute this to the fact that the C_{11} alkylphosphonate chains of **3** are longer than the C_7 carboxyalkylsiloxane ones of **8** and that the phosphonates likely form a more highly ordered, densely packed, self-assembled monolayer. This ratio decreases going from **8** to **10**, consistent with our contention that additional PEG chains have been introduced at the surface by the back-filling process. Based on the ratio of the areas calculated for the CH_2-alkyl and the CH_2-O signals it is also clear that grafting yields of the PEG-linkers to the phosphonate and siloxane films are modest. This is confirmed by X-ray reflectivity measurements (see Supporting Information), which reveal a rather diffuse layer about 8.5 ± 1 nm thick for the phosphonate-PEG film **2** and 6.0 ± 1 nm thick for the silane-PEG film **7**. Both films are probably quasi-porous in the sense that the molecular density decreases continuously with film thickness. PEG chains grafted at interfaces have been shown to adopt various conformations depending on PEG surface density and chain length.[36] Our results suggest that both PEG linkers used in this work form PEG chain brushes with lengths around 6−7 nm.

In contrast coupling of the PNA to the PEG-linkers seems to be quite effective. In film **3** the PEG-linker is characterized by 90 CH_2-O carbons (binding energy expected at around 287 eV) and only three carboxyl groups. The PNA moiety contains primarily carboxyl groups from the peptide backbone (binding energy expected at around 289 eV) and other carbons in the 12 bases with similar binding energies. Taking into account the number of atoms contributing to the different signals, we can estimate a yield of about 80% for film **3** from the ratio of the areas calculated for the CH_2O and the C═O edges. This yield is further substantiated by measuring the ratio between the area calculated for the nitrogen edge (divided by a factor of 2, due to the more sensitive measurement conditions), characteristic of the PNA moiety, with the area calculated for the CH_2-O signals characteristic of the PEG-linkers. For film **8**, similar yields can be estimated from the areas calculated from XPS spectral analysis. However, calculations here are difficult due to the contribution of the carboxyl groups of the siloxane chains to the overall C═O signal.

It should also be mentioned that C 1s and N 1s XPS spectra were taken on PEG-less PNA-modified phosphonate and siloxane reference surfaces; these showed that the extent of PNA functionalization was comparable for both PNA-PEG- and PNA-functionalized surfaces (data not shown), which shows that that the presence of PEG at the surface does not lessen the extent of surface functionalization with PNA probes.

Fluorescence hybridization experiments were carried out on PNA-PEG-modified surfaces and on PNA-modified reference surfaces that were not PEGylated to determine the efficacy of PEG for minimizing nonspecific adsorption of DNA. Cy3-labeled complementary and noncDNA were chosen as probes in order to discriminate between hybridization and mere physical adsorption. Two different areas of one sample surface were exposed to 1 μM solutions of Cy3-labeled complementary and noncDNA, respectively, for 25 min. Indeed, fluorescence spectra of samples lacking PEG (Figure 3) showed a remarkable Cy3 fluorescence emission peak at 570 nm for both regions, confirming that nonspecific adsorption of DNA plays an important role on biofunctionalized monolayers. On the other hand, the introduction of PEG units on the surface inhibited such nonspecific adsorption of the oligonucleotides. Fluorescence spectra of a PNA-PEG-functionalized phosphonate monolayer and a backfilled PNA-PEG-functionalized siloxane film are shown in Figure 4. Remarkably, while the red curves show a fluorescence peak at 570 nm (due to hybridized cDNA), the black curves do not show any significant peak, indicating that almost no adsorption of noncDNA took place. These results show that PEG is pivotal both for reduction of DNA nonspecific adsorption and for selective analyte detection. It should be pointed out that fluorescence intensities from different regions (complementary and noncomplementary) on a single sample can be compared quantitatively in our experimental setup; however, due to slight variations in optical alignment on sample exchange, we must refrain from comparing absolute intensities from different samples. (In this sense, we cannot *quantitatively* compare hybridization efficiency of PNA-PEG-functionalized phosphonate or siloxane films).

Patterning experiments involving spatial arrays were carried out to test the efficacy of PNA-PEG functionalized surfaces for preventing nonspecific adsorption of DNA analyte. For this purpose surfaces bearing PEG-MAL substituents (**2**, **7**, and **9**) were functionalized with PNA in spatially controlled fashion in two ways using soft-lithographic micromolding techniques, giving patterned surfaces **3**, **8**, and **10**, respectively. In the first experiment, a microstructured multichannel PDMS mold was used to deliver SH-terminated PNA onto the MAL-terminated surfaces. After removing the mold, the surfaces were exposed to a 1 μM solution of Cy3-labeled cDNA and incubated for 60 min. Representative fluorescence images are shown in Figure 5a−c, which clearly indicates that, while cDNA hybridized onto the PNA patterned lines, it did not adsorb nonspecifically onto the PEGylated spacing lines. For the second experiment, a microstructured multichannel PDMS mold was also used to deliver SH-terminated PNA onto the MAL-terminated surfaces.

After removing this mold, another mold was placed on the sample at a 90° angle with respect to the patterned PNA lines to directly deliver Cy3-labeled cDNA. The substrate was incubated for 60 min inside a humidity-controlled chamber to ensure that no evaporation occurred inside the channels. The representative fluorescence image shown in Figure 5d for the PNA-PEG-functionalized siloxane sample (**10**) clearly indicate that, while cDNA hybridized at PNA regions, it did not adsorb nonspecifically at PEGylated ones.

Conclusions

Alkylphosphonate monolayers and carboxyalkylsiloxane films functionalized with dense layers of poly(ethylene glycol) (PEG) and peptide nucleic acid (PNA) probes were prepared and evaluated as biofunctional interfaces with potential applications in DNA microdevices, such as microarrays and biosensors. PEG and PNA were covalently attached to the surfaces of the phosphonate monolayers and the siloxane films via standard *N*-hydroxysuccinimidyl and maleimido reagent-based conjugation chemistries. XPS analysis near the C 1s and N 1s ionization edges confirmed binding of PEG and PNA to these surfaces. Fluorescence hybridization experiments carried out in the presence of complementary and noncDNA showed that PNA-PEG functionalized surfaces were effective for hybridization of cDNA and minimized nonspecific adsorption of the analyte. These surfaces proved to be especially effective for minimizing analyte nonspecific adsorption by comparison with unPEGylated reference PNA-functionalized surfaces. Spatial patterns prepared by PDMS micromolding showed selective hybridization of fluorescently labeled DNA at PNA-PEG functionalized regions with a dramatic reduction in adsorption to probeless PEG-functionalized regions.

In summary, we have shown PNA-PEG functionalized phosphonate monolayers and siloxane films to be efficient substrates for the hybridization of target DNA with minimal nonspecific adsorption of analyte, which demonstrates their promise as biointerfaces for application in DNA microdevices. The functionalized phosphonate monolayers are especially well-suited for biosensing applications due to their mild preparation conditions, as well as to their facility for chemical amplification using simple, water-based conjugation chemistry; they are particularly indicated where very mild conditions are demanded for functionalization of sensitive detection units, such as silicon nanowires that are integrated in an electrical circuitry on a chip.[32] In contrast, the functionalized siloxane films seem suitable for microarray applications only where more robust chemistries, both in terms of film preparation and subsequent chemical amplification, can be tolerated.

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Supporting Information Available. X-ray reflectivity analysis of films **2** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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