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High surface density immobilization of oligonucleotide on silicon

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Oligonucleotide (11-mer) molecules are immmobilized on silicon in high surface population using either a permanent thioether bond or a chemo-selectively reversible disulfide bond to the surface thiol functionality. Substrate hydroxy groups are first silanized with an 11 carbon trichlorosilane containing a terminal, protected thiol moiety. Oligonucleotide modified with a tether possessing a terminal thiol group is further derivatized with a water-soluble, halobenzylic bifunctional reagent, which allows the complete conjugate to be attached to the surface through a permanent thioether bond. Alternatively, the oligonucleotide–tether complex can be combined with a pyridyldisulfide compound, which, in turn, facilitates the formation of a reversible disulfide bond with surface thiol. The amount of immobilized oligonucleotide was determined by radiochemical labeling with ^{32}P. Additional verification of surface amounts was obtained from X-ray photoelectron spectroscopic analysis of substrates. The results of the immobilization protocols are compared with the oligonucleotide surface population achieved through the conventional silanizing agent, mercaptopropyltrimethoxysilane. Finally, a preliminary confirmation of duplex formation of a TTU-attached 25-mer with its complementary strand is outlined.

The detection of infectious species such as viruses and bacteria and mutations at the genetic molecular level is expanding rapidly. This is fueled, in part, by the results emanating from the Human Genome Project, which recently identified the location and sequence of the over 100 000 human genes. In order to achieve the early diagnoses of diseases, new technologies for gene isolation, purification, synthesis, amplification and sequencing are being developed to meet a variety of technical challenges. These emerging technologies invariably require improved protocols for the surface immobilization of single-strand oligonucleotide or DNA in several key fields such as solid-phase polymerase chain reaction (PCR), isolation of nucleic acid-binding proteins and drugs and combinatorial chemistry-produced nucleic acid microarrays (the so-called 'gene chip').[1]

Numerous protocols have been developed for the surface immobilization of proteins,[2] and many of these methods can be adapted to realize the attachment of nucleic acids.[3] Adsorption represents the simplest method, since no reagents or special nucleic acid modifications are required. In this case, non-covalent forces are employed to affix nucleic acids to such materials as nitrocellulose and various polymers.[4] The main drawbacks of this approach are possible desorption from the substrate under hybridization conditions and the unavailability of base moieties for duplex formation caused by nucleic acid multi-point attachment. Cross-linking or entrapment in polymeric films has been used to fabricate a more permanent nucleic acid surface,[5,6] but these procedures still suffer from the several-point attachment problem.

Avidin/streptavidin–biotin complexation has enjoyed considerable popularity in terms of the attachment of single-strand oligonucleotides to the surfaces of various biosensors.[7–11] These two large proteins (70 kDa) each contain four binding sites for the biotin moiety which attaches to the macromolecule with very high affinity ($K_d = 10^{-15}$ M).[12] In biosensor development, the protein is first adsorbed on the surface of the transducer and is then exposed to an aqueous solution of biotinylated nucleic acid. The intrinsic stability of the avidin–biotin system and the compatibility of the chemistry within a flow-injection context renders the attachment protocol easy to employ, but the presence of the underlying protein layer yields possible multiple sites for non-specific adsorption. Furthermore, not all biotin bonding sites are available which, apparently, results in a surface population of nucleic acid of 1 pmol cm^{-2}.[11]

In view of the current interest in thiol-based self-assembling monolayers on gold, it is not surprising that there have been attempts to attach nucleic acids to this metal through appropriately derivatized oligonucleotides and DNA.[13] The general idea, of course, is to mimic the monolayer assembly of long-chain alkanethiols,[14] but the stability of such films is questionable because of intermolecular repulsion between nucleic acid chains. This is undoubtedly responsible for wash-off phenomena at elevated temperature.[15] With respect to detection technology, immobilization of oligonucleotide layers using alkanethiols has been employed in surface plasmon resonance (SPR) studies of interfacial nucleic acid chemistry.[16]

Avoidance of oligonucleotide multi-point interaction with surfaces has been attempted through attachment of linking agents to one of the ends of the nucleic acid chain. Using this method, covalent binding to a substrate can be effected with the possible retention of the tertiary structure of the nucleic acid. Recently, a carboxyl-modified surface of crystalline silicon served as a substrate for the attachment of thiol-modified DNA by means of an electrostatically adsorbed layer of polylysine and a heterobifunctional cross-linker.[17] Various other linking strategies have been used to react oligonucleotides to different surface materials, including the use of cyanic chloride[18] and hydrazide or Schiff's base chemistries.[19] Thus, 5′-aldehyde-modified DNA oligonucleotides have been attached to hydrazide functionalized Sephacryl beads (cross-linked dextran–acrylamide copolymer for gel filtration).[20] In a similar vein, various alkoxysilanes have figured prominently in terms of the derivatization of surfaces to provide covalent linking groups. These include aminopropyltriethoxysilane (APTES),[21] 3-mercaptopropyltrimethoxysilane (MPS),[22] bromoacetamidosilanes[23] and glycidoxypropyl-triethoxysilane (GOPS),[24] which hydrolyze on to a surface to form stable, cross-linked films. In

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the case of APTES, succinic anhydride is often used to modify the amino group to a carboxylic acid moeity, which is then attached to an amino-linked nucleic acid by carbodiimide coupling. MPS can be used to form disulfide linkages with thiol-containing biomolecules, whereas GOPS has been employed in schemes involving long polyether chains to provide a greater distance between the surface and nucleic acid. Despite their relatively widespread use in biomolecule immobilization, these short chain silanes possess several disadvantages, including the tendency to form multilayer structures, which can be hydrolytically removed from surfaces.[25] Furthermore, cross-linking reactions can lead to a lack of availability of interfacial functional groups.[26] It has also been reported that the immobilization of silanized nucleic acids, comprising a direct covalent conjugation of an active silyl moiety to oligonucleotides, has been performed.[27]

Trichlorosilanes, where the alkyl chain is 8–18 carbons in length, offer a number of distinct advantages including higher surface reactivity than trialkoxysilanes and the ability to form monolayer-like coatings through the self-assembly process.[28] The latter also presents the possibility of employing film diluents for the purpose of modifying the functional group population. Silanes containing only one surface reactive functional group produce poorly stable films.[29] Therefore, bifunctional silanes have been synthesized such that other molecules can be attached subsequent to the surface silanization process. For example, 1-thioacetato-16-(trichlorosilyl)hexadecane has been described as a potential linking agent for biomolecules.[30] In this paper, we discuss the attachment of 11-mer oligonucleotide to the surfaces of silicon wafers using the bifunctional trichlorosilane 1-thiotrifluoroacetato-11-(trichlorosilyl)undecane (TTU).[31–34] This molecule is used in conjunction with a tethering thiol group and linking agents that are capable of the permanent and reversible binding of nucleic acid to the substrate. Preliminary hybridization studies using an immobilized 25-mer oligonucleotide are also outlined.

Experimental

Materials and reagents

ω-Undecanoyl alcohol (98%), 6,6′-dithiodinicotinic acid, trifluoroacetic anhydride (99%), hydrogen hexachloroplatinate (IV) hydrate (99.99%), octyltrichlorosilane (99%) (C8), 3-mercaptopropyltrimethoxysilane (MPS), *N*-bromosuccinimide (NBS), 1,1′-azobis(cyclohexanecarbonitrile) (ACN) and dimethylformamide (DMF)–sulfur trioxide complex were obtained from Aldrich and used as received. Bis(maleimido)hexane (BMH) was obtained from Pierce (Rockford, IL, USA). Various common solvents and chemicals were obtained from BDH and used without further treatment except as follows. Dichloromethane and acetonitrile were distilled over P_2O_5, toluene was distilled over Na and pyridine was distilled over KOH. Benzene and DMF were dried over molecular sieves before use. Silicon wafers, obtained from International Wafer Service, were supplied approximately 0.4 mm thick and were polished on one side to a mirror finish. They were cut to a size of about 1 × 1 cm using a diamond-tipped pencil.

Syntheses

1-(Thiotrifluoroacetato)-11-(trichlorosilyl)undecane was synthesized and characterized as described previously.[32] The sodium salt of 2,5-bis(bromomethyl) benzenesulfonate (BMBS) was produced by bromomethylation of *p*-xylene followed by conversion to the sulfonate (sodium salt) with DMF–sulfur trioxide reagent and NaOH. The sodium salt of 6,6′-dithiodinicotinic acid (DNDS) was made by treatment of the parent compound with NaOH prior to purification by azeotropic distillation with benzene.

Oligonucleotide synthesis of the following sequences TAAAGCTCAAA (control-oligo) and TAAAGCTCAAA-C6-SH (thiol-oligo; C6 is the length of the carbon chain in the tether) was performed using standard CE phosphoramidite chemistry with conventional Applied Biosystems reagents. In order to produce the thiol-oligo an iodine solution was employed in conjunction with 3′-thiol modification cartridges (Glen Research). The oligonucleotides were purified using standard procedures with Poly-Pak cartridges purchased from Glen Research. The final products were checked for purity by HPLC and stored in 20% acetonitrile, in propylene vials.

Oligonucleotides of the sequences 5′-TATAAAAAGAGA-GAGAGATCGAGTC-3′, (F1), the corresponding thiol-containing oligo, 5′-TATAAAAAGAGAGAGAGATCGAGTC-C6-SH-3′, and the complementary oligo 5′-ATATTTTTCTCTCTCTCTAGCTCAG-3′ (F2) were synthesized and purified at the Centre for Applied Genomics, Hospital for Sick Children, Toronto, Ontario, Canada.

Equipment

HPLC measurement of oligonucleotide purity was effected with a Dionex DX500 ion-exchange system equipped with an AD20 absorbance detector and a Nuclepore PA-100 anion exchange column. Oligonucleotide synthesis was performed on an Applied Biosystems Model 392 automated synthesizer. X-ray photoelectron spectroscopic (XPS) analysis of the surfaces of silicon wafers was achieved with a Leybold MAX-200 spectrometer using an Mg Kα source run at 15 kV and 20 mA. The energy scale of the spectrometer was calibrated to the Ag $3d_{5/2}$ and Cu $2p_{3/2}$ peaks at 368.3 and 932.7 eV, respectively. The binding energy scale was calibrated to the main C 1s feature at 285.0 eV. For all samples, a survey run (pass energy = 192 eV, and from 0 to 1000 eV on the binding energy scale) was performed along with higher resolution scans of the most relevant regions. Each sample was analyzed at a 90° angle relative to the electron detector using an X-ray spot size of 4 × 7 mm. Satellite subtraction and data normalization were performed with software obtained from the manufacturer, while quantitative and peak-fitting work was done using the ESCA Tools program. Quantitative measurements of low resolution spectra were performed using empirically derived sensitivity factors: C 1s = 0.34, O 1s = 0.78, Si 2p = 0.4 and F 1s = 1.00.

In order to expose silanized wafers to a radiochemical labeling protocol, a special housing was fabricated that allowed leakage-free treatment of 12 substrates at one time.

Samples for radiochemical scintillation counting were examined with a Beckman LS 5000TD automated counter using a standard ^{32}P program.

Procedures

Prior to silanization, Si wafers were subjected to a careful cleaning and hydration procedure that allows the formation of an optimized population of surface hydroxyl functionalities.[34] Subsequent to detergent and chloroform washing, and sonication with H_2O_2, dried wafers were stored for several hours in a humidifying chamber. Substrates were silanized in a dry-box for 2 h with 2 ml of a 10^{-3} M solution in dry toluene of a mixture of 30% TTU and 70% C8. Following washing with toluene and chloroform and drying in a stream of nitrogen, the functional group chemistry introduced on wafer surfaces was confirmed by XPS. The TTU-coated wafers were treated with hydroxylamine in water (pH 8.5) for 2 h to effect deprotection of the thiol group. Removal of the TFA group was confirmed by

XPS. Silicon wafers, previously hydroxylated as described, were silanized with MPS in the same manner.

Using the afore-mentioned cell, silanized wafers were exposed to radiolabeled oligonucleotide in order to assess the efficiency of nucleic acid–linker–silane coupling. The protocol employed was as follows. Both control- and thiol-oligos from three product vials (see above) were dissolved in 200 μl of phosphate-buffered saline (PBS) buffer solution containing 10 mM $MgSO_4$ (pH 7.5). Thiol-oligo solution (198 μl), polynucleotide kinase solution (Pharmacia) (9 μl of 200 u diluted with 20 μl of PBS buffer) and 125 μl of [γ-^{32}P]ATP (special order from Amersham containing no β-mercaptoethanol) solution (2 mCi ml^{-1}) were mixed and incubated at 37 °C for 16 h. An analogous experiment was conducted for the control-oligo sample. Both oligo solutions were extracted twice with 200 μl of chloroform before 70 μl of the thiol-oligo aqueous phase was removed to be reacted with either 5 mg of DNDS or BMBS, both at room temperature for 1 h. Control-oligo solution (also 70 μl) was allowed to remain at room temperature for 1 h. Two NAP-5 desalting columns (Pharmacia) were equilibrated with PBS buffer (pH 7.5) , and a third column was treated with PBS buffer adjusted to pH 3.0. The control- and thiol-oligo/DNDS-reacted solutions were loaded on to separate columns followed by elution with PBS buffer at pH 7.5. A similar procedure was adopted for the BMBS-reacted case except that the buffer solution used had a pH of 3.0. A sample (200 μl) of each of control-oligo and linker-treated thiol-oligo solutions were introduced to TTU- and MPS-silanized wafers clamped in the cell mentioned above (in duplicate). After overnight reaction, the wafers (12) were washed copiously with buffer and water before treatment with 20 ml of aqueous scintillation solution (Amersham) and final counting. At various stages of the overall protocol the concentration of oligonucleotide was monitored by absorption spectroscopy.

After six weeks a specific thiol-oligo/BMBS-treated wafer was analyzed by XPS to effect a direct comparison of radiolabeling measurement with surface analysis.

For the preliminary hybridization experiments, the deprotected TTU substrates were placed in solutions of 1 mM BMH, a homobifunctional linker with maleimide reactive groups, in dimethyl sulfoxide (DMSO). The linker-modified substrate was then placed in a 20 μM solution of the 25-mer thiolated oligonucleotide in DMSO. This experiment was repeated with a non-thiolated oligonucleotide. Additionally, immobilization was performed *via* direct disulfide formation between the thiolated oligonucleotide and TTU. A 20 μM solution of the complimentary strand, in TRIS buffer (10 mM TRIS–HCl, 1.5 M NaCl, 1 mM EDTA) spiked with a ^{32}P-labeled oligo, was then exposed to the immobilized oligo. Hybridization was performed for 1.5 h at room temperature, after which substrates were rinsed with high-salt buffer, then water. The immobilization experiments were replicated using ^{32}P-labeled oligos, to determine the percentage of hybridized species.

Results and discussion

Strategy for the surface immobilization of oligonucleotide

Before evaluating the results of the experiments described above, we outline the criteria employed in a plan for the surface attachment of nucleic acid under very mild conditions. In overall terms, the goal is to achieve a stable, high population of nucleic acid molecules per unit area in a controllable fashion using a minimum number of chemical steps and aqueous solution chemistry. Furthermore, an additional consideration is the possibility for attachment of oligonucleotide in either permanent or reversible chemistries. With respect to oligonucleotide synthesis, conventional protocols result in concentrations of nucleic acid which are at best 1 mM. Accordingly, it is desirable to effect the aqueous conjugation reactions at relatively low concentrations (neutral pH) at room temperature. Although oligonucleotides contain intrinsic reactive functionalities such as the free phosphate hydroxyl group, the exocyclic amines, enolizeable carbonyl groups on the bases, cleavable glycosidic bonds and ribose OH (RNA), we chose to employ disulfide and thioether linkages. The fast (10 M^{-1} min) S_N2 interchange reaction and thioether formation from two thiol groups, involving reactive leaving groups, occurs in an aqueous environment resulting in stable bonds. These chemistries, which are particularly compatible with the bifunctional silane species mentioned above, are depicted in terms of the approach used here in Fig. 1.

A final brief comment concerns the use of C8 as a diluent of the reactive silanes. The reason for the surface incorporation of this non-bifunctional silane lay in the observation that increased proximity of thiol groups can lead to undesirable cross-linking of these reactive functionalities through surface oxidation reactions. Second, we have found that control of the surface population of thiol groups by C8 leads to facile optimization of attachment of nucleic acid on the silicon substrate.

Surface population of oligonucleotide from ^{32}P labeling

The control sample of oligonucleotide was employed to determine the level of surface binding of nucleic acid caused by non-specific binding forces such as hydrophobic interactions and hydrogen bonding. Both 30% TTU–70% C8 and 100% MPS-treated substrates were compared with respect to the yield of surface immobilization using three ^{32}P-labeled oligonucleotides; these were control-oligo and thiol-oligos linked to DNDS (DNDS-oligo) and BMBS (BMBS-oligo). In this fashion, both silanized surfaces (0.2 cm^2) were exposed to identical solutions of labeled oligonucleotide at the same time. Concentration and activity values for the three solutions are given in Table 1.

The raw activity data for the various wafers, with and without background correction, and calculations of final surface amounts of oligonucleotide are presented in Tables 2 and 3, respectively. Two noteworthy features of these results are the indications of non-specific adsorption of control oligonucleo-

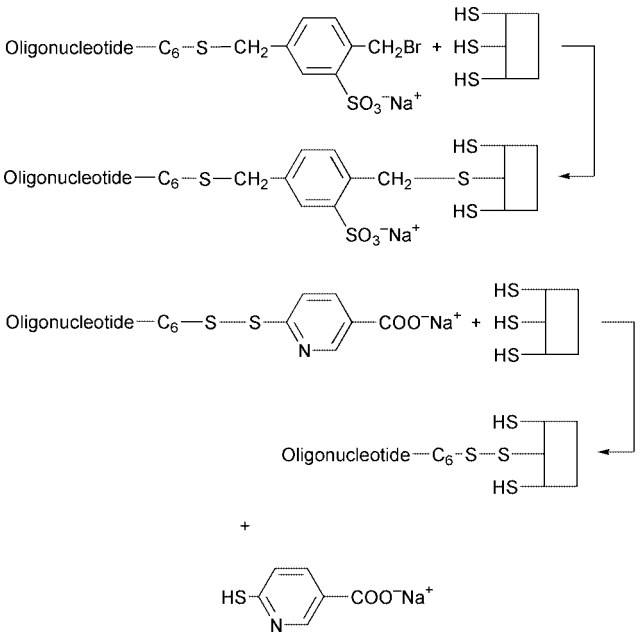

Fig. 1 Surface attachment of BMBS and DNDS derivatives of 11-mer oligonucleotide through the formation of thioether and disulfide bonds, respectively.

tide on both TTU and MPS surfaces, although the level exhibited by the latter is three times that for the TTU surface, and the tendency to greater variability in amounts of nucleic acid on MPS surfaces. The deprotected mixed C8/TTU surface displays considerable hydrophobic character (*e.g.*, water contact angle $>80°$); accordingly, interaction of the immobilized silane with the nucleic acid bases can be expected in aqueous solution. A rough measure of the molecular coverage caused by this non-specific effect can be gleaned from consideration of the surface area of wafer exposed to the control-oligo solution and the surface concentration. Assuming that island structures are not formed, the 2 pmol cm^{-2} value yields a surface population of about one molecule per 10^4 Å^2. This value is comparable with the coverage yielded by the avidin–biotin system, where surface attachment is governed by the size of the protein molecule (100 Å diameter) and the available biotin binding sites. Although there are four binding sites for biotinylated moieties, only one is available for protein bound at an interface. The variability of nucleic acid attached to MPS-treated surfaces is undoubtedly connected to the structure formed by the silane on hydroxylated surfaces. Using pentafluorinated probes and XPS, we have shown that this trimethoxysilane yields poorly defined multilayers on silicon surfaces, which are caused by cross-linking processes.[33] Furthermore, such polymerization results in both a significant diminution in the surface population of thiol groups (compared with TTU) and exposure of polar moieties at the substrate–liquid interface.

The most interesting result depicted in Table 3 is the level of nucleic acid attachment achieved using the TTU-BMBS-oligo system (over 50 pmol cm^{-2}). This value is about 25 times the amount caused by non-specific adsorption. We believe that the latter effect will be significantly diminished for the experiments conducted with the thiol-containing nucleic acid because of the surface area occupied by covalently bound oligo (a close-packed monolayer of perpendicularly oriented single-strand oligonucleotide would yield approximately 100 pmol cm^{-2} as governed by geometric constraints). Comparing the two linkers, with TTU DNDS yields approximately one third the amount of nucleic acid per unit area bound by the benzylbromine derivative. We attribute this result to differences in the effectiveness of the two surface-to-reagent reactions in producing the immobilized nucleic acid. The benzylbromine reaction involves the formation of a thioether bond through nucleophilic attack of the surface –SH functionality on the labile carbon–bromine bond. In contrast, the DNDS method operates *via* an S_N2 thiol–disulfide exchange process to yield a new disulfide bond. The discrepancy in amount of attached, tethered oligo is undoubtedly connected to leaving group capabilities of the bromide anion as compared with the nicotinic acid derivative. Finally, we note that both the BMBS and DNDS protocols immobilize only about twice the amount of nucleic acid as attached by non-specific forces to the 100% MPS-treated substrate. This observation is clearly associated with the combined effects of the tendency for high non-specific binding and lack of available thiol functionalities for this silanized surface. Indeed, we have established that multilayer MPS films generate almost four times less available surface –SH groups than even highly diluted TTU-based surfaces.

Table 1 ^{32}P activity of oligonucleotide derivatives

Oligo	Concentration/ pmol μl^{-1}	Activity[a] (vol.)/ cpm μl^{-1}	Activity[a] (mole)/ 10^{15} cpm mol^{-1}
Control	0.43	92276 ± 1.55%	2.15 ± 2.45%
DNDS	0.34	45687 ± 1.78%	1.33 ± 2.95%
BMBS	0.39	25711 ± 2.04%	0.67 ± 3.08%

[a] Counts per minute with relative percentage counting error.

Table 2 Raw counting data for surface-immobilised oligonucleotide derivatives

Surface–oligo[a]	Activity/cpm	Activity with correction[b]/cpm	Relative error[c] (%)
TTU–control	1122	1109	6.2
TTU–control	755	742	7.6
TTU–DNDS	4237	4224	3.1
TTU–DNDS	3358	3345	3.5
TTU–BMBS	7725	7712	2.3
TTU–BMBS	6546	6533	2.5
MPS–control	2496	2483	4.1
MPS–control	3281	3268	3.5
MPS–DNDS	3204	3191	3.6
MPS–DNDS	4932	4919	2.9
MPS–BMBS	1117	1104	6.2
MPS–BMBS	2501	2488	4.1

[a] Duplicate analysis. [b] Background count rate was 13.3 cpm. [c] Relative counting error.

Table 3 Density of oligonucleotide immobilised on surfaces treated with TTU and MPS

Surface–oligo	Amount/ pmol	Amount per unit area/ pmol cm^{-2}	Average/ pmol cm^{-2}	Ratio[a]
TTU–control	0.52	2.6		
TTU–control	0.34	1.7	2.2	1
TTU–DNDS	3.2	16.2		
TTU–DNDS	2.5	12.9	14.5	7
TTU–BMBS	11.4	57.9		
TTU–BMBS	9.8	50.1	54.0	25
MPS–control	1.1	5.9		
MPS–control	1.5	7.8	6.8	1
MPS–DNDS	2.4	12.2		
MPS–DNDS	3.7	18.9	15.6	2
MPS–BMBS	1.7	8.5		
MPS–BMBS	3.7	19.1	13.8	2

[a] Normalised to control experiment for each silanized surface.

XPS of immobilized nucleic acid

The relatively short half-life of ^{32}P provides a unique opportunity to correlate signals obtained from XPS analysis of surfaces with specific amounts of attached nucleic acid. Semi-quantitative analysis by XPS can be extremely useful when the analysis of a large number of substrate surfaces is required or when the monitoring of the effectiveness of a particular surface reaction is desirable. A survey spectrum of a silicon wafer with ^{32}P-labeled oligo attached to it through BMBS (50 pmol cm^{-2}), which was stored in a clean environment for 6 weeks subsequent to counting, revealed the key signals for N 1s and P 2p. This spectrum also revealed a small peak assigned to F 1s. We attribute the presence of fluorine to incomplete deprotection of the trifluorothioester group from the TTU-treated surface. Based on previous work we assess this signal as originating from $<5\%$ of available surface-bound silane. The high-resolution spectrum depicted in Fig. 2 shows that both the exocyclic amine and intracyclic nitrogen functionalities found in the various nucleic acid bases are resolved. The range of functional groups expected from carbon is shown in the high-resolution C 1s spectrum also given in Fig. 2. A compendium of assigned XPS signals and measured values for relative atomic percentages is given in Table 4. We note that the peak for sulfur is not considered owing to severe interference from signals originating from substrate silicon and that the carbon peak will

have contributions from TTU, BMBS and adventitious carbon. Significant attenuation of C 1s photoelectrons from the linker is expected owing to the length of the nucleic acid and tethering chains. A feature of Table 4 is the ratio of relative atomic percentages for N to P (1.2 : 1). The analytical value for phosphorus is clearly higher than that expected for the oligo–C6 tether (4.1 : 1) and is ascribed to contamination from phosphate originating from the PBS buffer employed in the immobilization procedures. Previous work has shown that it is difficult to prevent or remove chemisorption of this species from surfaces. Despite the semi-quantitative nature of these results, correlation of photoelectron counts with specific surface populations obtained from radiochemical labeling has proven to be invaluable in our studies of surface nucleic acid chemistry.

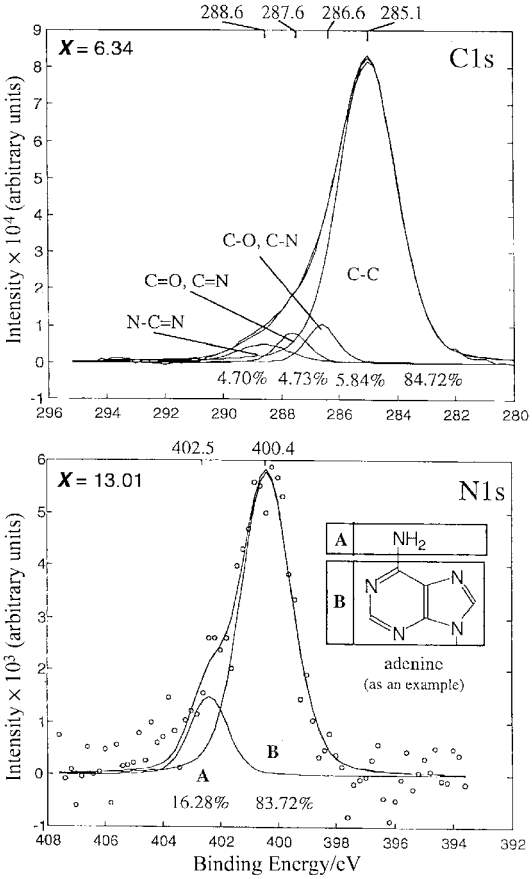

Fig. 2 Peak-fitted high-resolution XP spectra in the C 1s and N 1s regions for oligonucleotide attached to silicon *via* BMBS linker.

Table 4 Peak-fitted XPS results for BMBS-oligo immobilized on TTU surface

Element	Binding energy/eV	Assignment	% of signal	Total relative atom %
F 1s	685.6	TFA	100	1.4
O1s	532.6	SiO_2, + organic	100	30.1
N 1s	400.4	Intracyclic N	83.7	1.7
	402.5	Exocyclic NH_2	16.3	
C 1s	285.1	C–C	84.7	35.3
	286.6	C–O, C–N	5.9	
	287.6	C=O, C=N	4.7	
	288.6	N–C=N	4.7	
P $2p_{3/2}$	133.1	Phosphate	66.7	1.4
P $2p_{1/2}$	133.9	Phosphate	33.3	
Si 2p	99.1	Si^0	64.4	30.1
	102.7	SiO_2	35.6	

Hybridization of TTU-attached oligonucleotide: a preliminary study

For work with biosensors and DNA microarrays we are studying the TTU immobilization of longer nucleic probes (25-mers) on appropriate substrate surfaces. Experiments have been conducted with the 25-mer 5′-TATAAAAAGAGAGA-GAGATCGAGTC-**C6**-3′ (F1) in terms of its attachment to silicon *via* the 3′-end thiolated version, and hybridization with its complementary strand (F2). Table 5 depicts the results of these early studies, which involved the measurement of surface amounts by radiolabeling as discussed above. The results indicate that less primary binding is generated for the longer chain moiety, which is very likely caused by steric effects. The use of DMSO, under certain circumstances has been found to reduce levels of non-specific adsorption. Importantly, the data confirm that F2 forms a duplex with F1, although it is noteworthy that this is not quantitative in the sense that approximately only about 20% of the surface-attached species hybridize. The levels of non-specific adsorption observed for F2 on the F1-attached surfaces would not be expected to be as high in the analogous F1 on F1 case, since there will be a blocking of surfaces sites connected to the formation of genuine duplex material between F2 and F1.

Conclusions

The results of this work demonstrate that the use of a surface thioether bond in conjunction with a self-assembling silane, rather than a multilayer-forming agent, can produce a high molecular density of oligonucleotide on a silicon surface. The surface immobilization protocol described here offers considerable flexibility in terms of specific applications. For example, the tethering hydrocarbon chain length and that in the diluent can be varied easily between 2 and 20 carbons. The diluting silane can terminate in $–NH_2$, –OH and –COOH groups, resulting in the capabilities for both changes in surface free energy and further reaction of the surface. In addition, the chemistry can be employed with a wide range of substrates such as quartz, ceramics, metal oxides and polymers. The basic requirement is that the surface be capable of forming a layer of hydroxyl functionalities. We have shown, for example, that the method operates very well on chromium-based electrodes and on glass surfaces used in DNA microarray technology. With respect to surface type, it is interesting to note that the new linker, BMBS, can also be used to immobilize oligonucleotides to substrates without the use of the intervening silane. The benzylbromine group will react with a wide variety of nucleophiles present on functionalized polymers such as polystyrene, polyacrylamide, dextrose, polyethylene and polytetrafluoroethylene. Finally, the use of DNDS introduces the

Table 5 Immobilization and hybridization of Si surface-attached 25-mer oligonucleotide

System[a]	Amount per area/ pmol cm^{-2}
TTU/**SH-F1 in DMSO**	26.1
TTU/**F1 in DMSO**	5.1
TTU/SH–F1 in DMSO/**F2 in TRIS**	3.1
TTU/SH–F1 in DMSO/**F1 in TRIS**	1.3
TTU/BMH/**SH-F1 in DMSO**	30.3
TTU/BMH/**F1 in DMSO**	8.1
TTU/BMH/SH-F1 in DMSO/**F2 in TRIS**	7.2
TTU/BMH/SH-F1 in DMSO/**F1 in TRIS**	4.6

[a] TRIS, buffer solution at pH 8.2; bold specifies measured amount per unit surface area for reaction of the specified nucleic acid.

possibility of employing a nucleophile to remove any bound nucleic acid through the capability of the pyridyl moiety to act as a leaving group.

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