A DNA Self-Assembled Monolayer for the Specific Attachment of Unmodified Double- or Single-Stranded DNA

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ABSTRACT   A novel method for DNA surface immobilization and a paradigm for the attachment of unmodified DNA of any length or sequence are described herein. The development of a DNA self-assembled monolayer (DNA-SAM) that incorporates a DNA-thiol into a monolayer of inert alkane thiolates is reported. This DNA-SAM specifically hybridized complementary oligonucleotides while resisting the nonspecific adsorption of noncomplementary DNA and irrelevant proteins. Duplex DNA, having a single-stranded "capture tail," specifically bound to the DNA-SAM if the sequence of the "tail" was complementary to DNA presented in the SAM. The sense strand of the hybridized duplex DNA could be covalently attached to the surface by an enzymatic ligation reaction (leaving the anti-sense strand dissociable). DNA-binding proteins specifically bound to these surfaces only if their cognate sites were present in the duplex DNA.

INTRODUCTION

Biotechnology has been hard pressed to develop DNA analytical tools to keep up with the demands of ambitious biomedical goals such as sequencing the human genome, DNA diagnostics, and gene expression monitoring. To be cost-effective, these tools should be as automated as possible and be conducive to massive parallel processing. It was thought that DNA attached to surfaces would offer improvements over solution-based studies where efficiency is limited by the one-tube-per-experiment rule. Spatially addressable DNA arrays enable the analysis of results to be performed in parallel by optical readers. Fodor and colleagues (1991) used a chip as a solid support for in situ photochemical DNA synthesis. For the presented oligonucleotides to be spatially addressable, the sequence of each species must be predetermined by a masking scheme. This means that each gene chip must be custom-fabricated. The length of DNA that can be presented by this method is severely limited by rates of error in photochemistry and DNA synthesis. Others developed methods for the attachment of DNA to gel layers or glass. These methods allow for the attachment of longer oligonucleotides. However, the nature of the attachment presents DNA in random orientation, which can complicate comparative analyses such as single base mismatch determination. Additionally, DNA immobilized on glass may produce a surface that is prone to nonspecific binding.

We have generated DNA-presenting surfaces by incorporating composite molecules, DNA-thiols, into self-assembled monolayers (SAMs). Alkyl thiolates self-assemble, by chemisorption, onto gold to form layers of single molecule thickness. Thiols have been functionalized with a variety of headgroups to form SAMs with desired properties (Pale-

Grosdemange et al., 1991). For example, thiols terminated with nitrilo triacetic acid (NTA) have been shown to self-assemble with ethylene glycol-terminated thiolates (Bamdad et al., 1997; Sigal et al., 1996) to yield a composite surface that specifically binds histidine-tagged proteins while resisting nonspecific adsorption.

SAMs were created that displayed oligonucleotides by exposing gold substrates to a solution containing an inert alkyl thiol and an alkyl thiol terminated with DNA. DNA was synthesized on a standard commercial instrument (see Methods). While the side chains were still protected, the 5′ hydroxyl was reacted with carbonyldiimidazole (CDI), then with an amine-terminated thiol. The DNA-thiol composite molecule was cleaved from the solid support and deprotected by standard protocols (Kumar and Advanti, 1992) (see Scheme 3). The DNA-functionalized thiols were mixed with triethylene glycol-terminated thiols (designed to resist nonspecific binding) and adsorbed onto gold to form a DNA-presenting monolayer.

Surface plasmon resonance (SPR) was used to verify that the DNA-thiol had been incorporated into the SAM by detecting the change in mass at the surface after hybridizing with a complementary oligo. SPR is an optical technique that is used to quantitate changes in mass at an interface. Since the DNA incorporated into the SAM was only a 10-base oligo, the technique was thought to be too insensitive to detect the minor change in mass induced by hybridization with a 10-base complementary oligo. Therefore, we hybridized a 50-basepair DNA duplex fragment via a 10-base single-stranded capture tail that was complementary to the surface oligo. SPR was again used to detect the interaction of DNA-binding proteins with their cognate sites present on the duplex DNA.

METHODS

Gal-4 (1–100) was prepared as described in Reece et al., 1993. LexA-B17 is the DNA-binding domain of LexA (1–87) fused to a short acidic peptide (Ma and Ptashne, 1987). The protein was prepared according to Brent and Ptashne (1984).

Triethylene glycol-functionalized thiolates, **3**, were prepared according to Pale-Grosdemange et al., 1991.

Preparation of 1, a disulfide amine: outlined in scheme 2

(a) 1-hydroxy-3,6,9-trioxaeicos-19-ene

This was prepared according to published reports (Pale-Grosdemange et al., 1991).

(b) 1-tosyloxy-3,6,9-trioxaeicos-19-ene

One gram (3.3 mM) of (**a**) dissolved in 10 ml pyridine was placed in a 100-ml round-bottomed flask equipped with a magnetic stirrer. To this solution was added 2 Eq (1.26 g) tosyl chloride and the resulting solution stirred at room temperature (RT). The progress of the reaction was monitored by thin layer chromatography (TLC) and was complete after 6 h. The reaction mixture was then placed in a separatory funnel to which 25 ml water was also added. The mixture was extracted with ether (3×50 ml) and the combined ether extracts washed with saturated ammonium sulfate. After drying over sodium sulfate, the ether solution was filtered and rotoevaporated to give the crude tosylate, which was used without purification.

(c) 1-azido-3,6,9-trioxaeicos-19-ene

The crude tosylate from the previous reaction was dissolved in a 100-ml round-bottomed flask equipped with 12 ml N,N-dimethylformamide (DMF). To this was added 10 Eq sodium azide. The reaction was maintained between 60 and 80°C for 12 h and the progress monitored by TLC. When the reaction was complete, the DMF was rotoevaporated. The residue was dissolved in 100 ml ether and 50 ml water. The layers were separated and the water layer was extracted with ether. The combined ether layers were dried over sodium sulfate, filtered, and evaporated. The crude product was chromatographed on silica gel; the product was eluted with 40% ether/60% hexane. The ^{1}H-NMR (300 mega Hz, CDCl$_3$) was quite similar to that found for (**a**): 5.8 (M, 1H, vinylic); 4.9–5.0 (M, 2H, terminal olefinic); 3.4–3.8 (M, 14H); 2.1 (M, 2H, allylic); 1.2–1.6 (M, 14H).

(d) 1-amino-3,6,9-trioxaeicos-19-ene

Lithium aluminum hydride (950 ml; 25 mM) and 25 ml freshly distilled tetrahydrofuran (THF) were placed in a flame-dried 200-ml round-bottomed flask equipped with a magnetic stirrer and an addition funnel. To this stirring mixture was added (dropwise) 6.3 g (19 mM) of **3** dissolved in 25 ml THF. After the addition was complete the reaction mixture was refluxed for 2 h while the progress of the reaction was monitored by TLC. The reaction mixture was then cooled in an ice bath and water carefully added dropwise through the addition funnel until the solid material changed from a deep gray color to white. The clear solution was decanted into a separatory funnel and extracted with 100 ml 1 N HCl. The aqueous layer was separated and made basic by the addition of 25 ml 5 N NaOH (to pH 14). The resulting mixture was extracted with ethyl acetate. The combined extracts were dried over potassium carbonate, filtered, and evaporated to give 4.4 g of the crude amine product (**d**). The product was purified by chromatography on silica gel and eluted with ethyl acetate/methanol. The resulting primary amine was characterized by a positive Kaiser test and ^{1}H-NMR (300 mHz, CDCl$_3$). The spectrum differed from that of (**c**) in that the methylene adjacent to the NH$_2$ group was shifted apart from the other downfield methylenes: 5.8 (M, ^{1}H, vinylic), 4.9–5.0 (m, 2H, terminal olefinic), 3.4–3.8 (m, 14H), 2.9 (t, 2H, CH$_2$ adjacent to NH$_2$), 2.05 (m, 2H, allylic), 1.2–1.6 (m, 14H).

(e) 1-(N-t-butoxycarbonyl)amino-3,6,9-trioxaeicos-19-ene

In a 100-ml round-bottomed flask was placed 6.02 g (0.02 mM) of (**d**) in 30 ml 10% triethyl amine in DMF under nitrogen. To this was added 2 Eq

(9.2 ml) di-t-butyl dicarbonate (Aldrich, Milwaukee, WI). The resulting solution was stirred at 40–50°C for 0.5 h; TLC showed that the reaction was complete at that point. The solvent was then rotoevaporated and the product purified by chromatography on silica gel. The product was characterized by TLC and ^{1}H-NMR (300 mHz, CDCl$_3$): 5.75 (M, ^{1}H, vinylic), 4.85–4.95 (M, 2H, terminal olefinic), 3.4–3.6 (m, 12H), 3.3 (q, 2H, CH$_2$ adjacent to amide), 2.0 (m, 2H, allylic), 1.2–1.5 (m, 14H), d1.4 (s, 9H, t-butyl). The NMR indicated that the amino group was monoderivatized.

(f) 1-(N-t-butoxycarbonyl)amino-21-acetyl-3,6,9-trioxa-21-thiaheneicosane

Approximately 6 g of the protected amine and 8.3 ml thiolacetic acid were dissolved in 300 ml methanol, to which 100 mg azobisisobutyronitrile (AIBN) initiator was also added. The solution was placed in a photochemical apparatus, magnetically stirred, and irradiated with a medium pressure 450 W Hanovia lamp for 6 h. The solvent was then evaporated. ^{1}H-NMR of the crude material showed the absence of a double bond. Most notable new peaks were a triplet at 2.8 for the CH$_2$ adjacent to S and a singlet at 2.25 for the acetyl CH$_3$ group.

(g) 1,42-diamino-3,6,9,34,37,40-hexaoxa-21,22-dithiadotetracontane 1

The resulting crude product was dissolved in 150 ml 1 N HCl in methanol and refluxed for 6 h. After solvent evaporation, TLC retention times and a Kaiser test, on the crude product, indicated the presence of a free amine. The material was dissolved in methanol and O$_2$ was bubbled through for 6 h to form the disulfide. The solvent was evaporated and the product was purified by chromatography on silica gel equilibrated with 1% NH$_3$/99% MeOH. The disulfide amine, **g**, was eluted with 3% NH$_3$/97% MeOH. FAB-MS (glycerol matrix) of this material showed the (M + 1) ion at m/z 669 as the largest peak above m/z 200 amu. Additionally, major fragment ions at m/z 334 [loss of HS(CH$_2$)$_{11}$(OCH$_2$CH$_2$)O(CH$_2$)$_2$NH$_2$] and m/z 302 [loss of HSS(CH$_2$)$_{11}$(OCH$_2$CH$_2$)$_2$O(CH$_2$)$_2$NH$_2$] were observed. The ^{1}H-NMR (300 mHz, CDCl$_3$) was fully consistent with the structure of **g**: 3.4–3.7 (m, 24H, CH$_2$'s adjacent to O's; the upfield portion of this multiplet consists of two clean triplets for the two equivalents. CH$_2$'s adjacent to the CH$_2$NH$_2$'s and the two equiv. CH$_2$'s adjacent to the aliphatic CH$_2$'s, respectively), 2.85 (br. t, 4H, 2 equiv. CH$_2$'s adjacent to the NH$_2$'s; these are broadened by coupling to the NH$_2$'s as a function of concentration and sharpen when the solution is shaken with D$_2$O), 2.65 (t, 4H 2 equiv. CH$_2$'s adjacent to S), 1.1–1.7 [m, 40H, 18 aliphatic CH$_2$'s plus both NH$_2$'s; the latter were seen as "humps" (which disappeared upon shaking with D$_2$O) between 1.5 and 1.7].

Preparation of DNA-triethylene glycol disulfide 2: outlined in scheme 3

Using a 15 μM "T" column, the sequence 5'-GCTCGAGGCT-3' was synthesized by standard methods on an Expedite DNA Synthesis System (Perseptive Biosystems, Framingham, MA). While still attached to the resin and protected, the 5'-deprotected hydroxyl was reacted with an 80% saturated solution of CDI in dioxane to give the 5'-imidazolylacylated product. The column was then washed with anhydrous acetonitrile and removed from the synthesizer. Under anhydrous conditions, 280 mg of **1**, [NH$_2$CH$_2$CH$_2$(OCH$_2$CH$_2$)$_2$(CH$_2$)$_{11}$S]$_2$, dissolved in 1 ml acetonitrile, was syringe-injected into the column and rocked at 37° for 12 h. The column was washed extensively with acetonitrile to remove unreacted disulfide ligand. The product was cleaved from the resin and deprotected by standard ammonium hydroxide treatment with the exception that incubation at 65° was limited to 8 h to prevent hydrolysis of the carbamate linkage. The solvent was removed under reduced pressure. HPLC analysis showed the generation of a new species (50–75% pure) that eluted from the column much later than the underivatized 10-mer DNA and a little slower than the 10-mer DNA with the trityl group still attached to the 5' end. The elution

profile of the product is consistent with expected results for **2**: DNA derivatized with the alkyl thiol chain.

Preparation of DNA functionalized monolayers on gold substrates

Gold substrates were prepared according to Sigal et al. Solutions of 95–99% **3** (1 mM) and 1–5% **2** dissolved in acetonitrile were prepared. Gold substrates were incubated in the solution for 8–12 h at 45°, rinsed with acetonitrile, dried in a stream of argon, and glued onto stripped CM-5 SPR chip cassettes (Biacore AB, Uppsala, Sweden).

Surface plasmon resonance measurements

SPR experiments were carried out in a BIAcore instrument (Biacore AB, Uppsala, Sweden). Experiments were performed at RT in phosphate-buffered saline (PBS) at a constant flow rate of 5 ml/min. DNA hybridization to the DNA-SAM and subsequent protein binding to that DNA was accomplished by sequential injections of 25–35 ml of DNA, then protein-diluted in PBS. The adsorption of these macromolecules to the chip surface causes a shift in the resonance angle, which is reported in resonance units (RU; 1 RU = 1/10,000°). To enzymatically ligate DNA to the surfaces, DNA-SAMs were docked in a BIAcore instrument and equilibrated in PBS flowing at 5 ml/min for 10 min. Baseline RU measurements were recorded. The chips were removed from the instrument and bathed in a solution containing 5′-phosphorylated duplex DNA for 0.5 h at RT. Excess solution was aspirated off the chip, then the SAM was saturated with a solution of DNA T4 ligase dissolved in ligase buffer and incubated at RT for 1.5 h. The chips were rinsed and reinserted into the BIAcore device. The surfaces were equilibrated in PBS and RU measurements were recorded. Differences between RU recorded before and after ligation were attributed to the added mass of DNA adsorbed to the surface.

RESULTS

We generated self-assembled monolayers that present DNA: DNA-SAMs. Two species of thiol were incorporated into the SAMs: **2** is a DNA-terminated thiol and **3** is a triethylene glycol-terminated thiol. The inert thiols, **3**, were used as spacer molecules because they had been shown to resist the nonspecific binding of proteins and they were small enough to fill in gaps in the monolayer that might form around the bulky DNA-terminated thiols. As the number of bases in the DNA "headgroup" increases, monolayer formation is inhibited because the energetic advantage of packing the carbons of the lipid-like portion into the SAM must be greater than the entropic cost of ordering the attached bases. Therefore, our strategy was to form a SAM that presented relatively short strands of DNA (10–15 bases); then, if necessary, to enzymatically attach longer strands of DNA to the preassembled surface (Scheme 1).
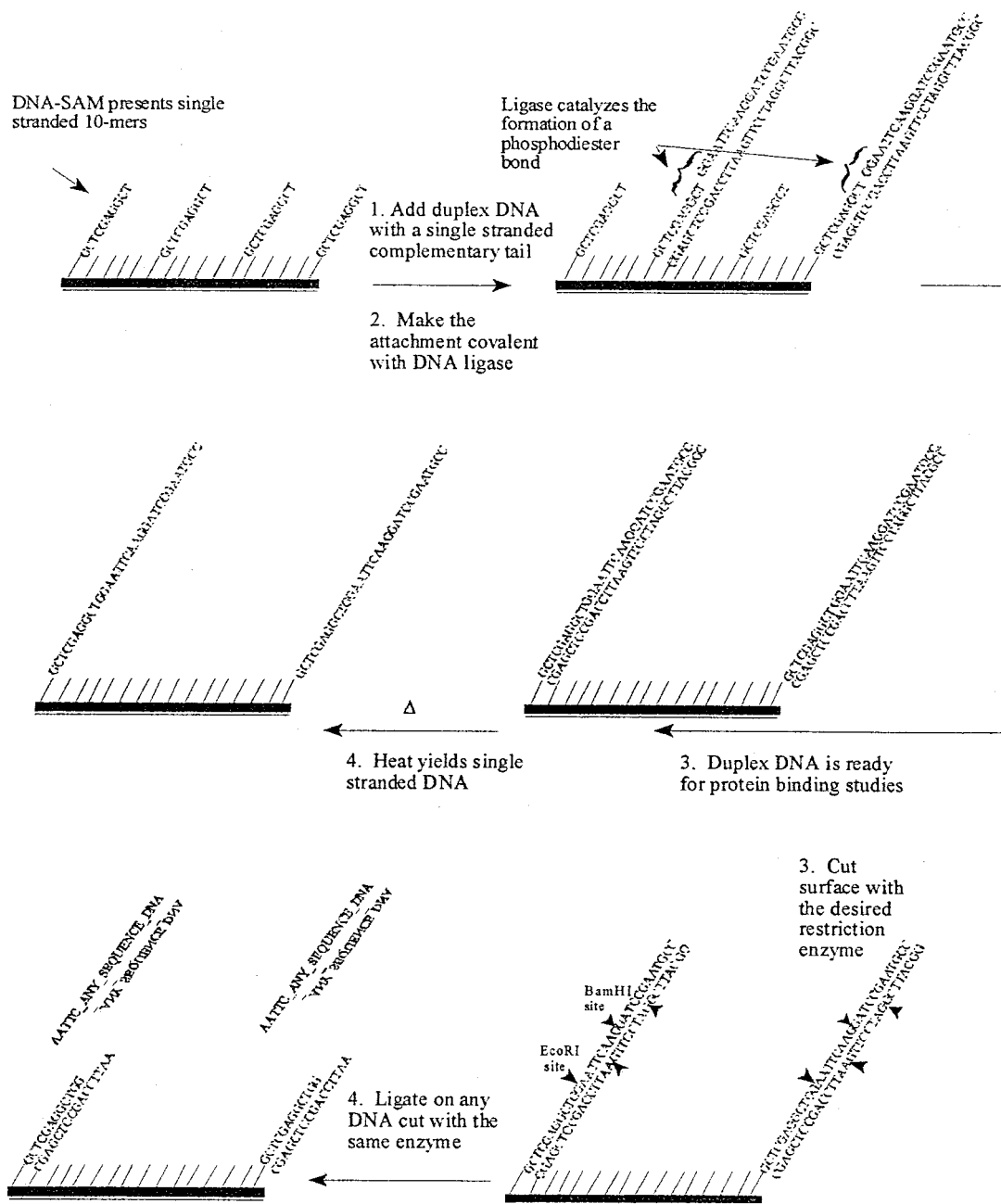
The composite DNA-thiol was prepared as follows. A thiol functionalized with a reactive amine, **1**, was prepared according to Scheme 2. It was prepared as a disulfide so that the sulfur would not be available in subsequent reactive steps. A 10-base oligonucleotide was derivatized with this disulfide as the last step in a DNA synthesis, according to Scheme 3. Stock solutions of **2** and **3**, dissolved in either acetonitrile or ethanol, were prepared. Mixed-species SAMs were self-assembled onto gold substrates.

SAMs so functionalized with single-stranded DNA (ssDNA) were characterized by surface plasmon resonance in a BIAcore instrument to assess their ability to 1) hybridize duplex DNA having a single-stranded complementary "tail"; 2) bind proteins to their cognate sites on the duplex DNA hybridized to the DNA-SAM; 3) resist nonspecific binding of irrelevant DNA and proteins; and 4) provide a suitable scaffold for the specific binding of DNA modifying enzymes.

The DNA-SAMs were mounted in plastic chip cassettes and inserted into a BIAcore SPR instrument. This device measures, in real time, optical changes at a surface/solution interface that can be correlated to changes in mass occurring at the interface. The BIAcore device has an integrated flow circuit that defines discrete flow cells on a homogeneous chip. Two 50-bp double-stranded (ds) DNA samples were sequentially injected over the same flow cell of the DNA-SAM. The samples were identical except that one had a single-stranded, 10-base "tail" whose sequence was complementary to the DNA on the surface, while the other had a 10-base noncomplementary tail. Fig. 1 shows that only DNA bearing a single-stranded "tail" whose sequence was complementary to the DNA incorporated into the SAM bound to the surface. As a negative control, the same DNA without a single-stranded capture tail was incubated with the DNA-SAM but did not bind (Fig. 2). DNA having a scrambled sequence tail also did not bind (data not shown).

DNA-SAMs prepared from ethanol solutions bound high levels of nonspecific DNA and protein and did not hybridize complementary single-stranded DNA (data not shown). Since SAMs composed of the inert thiol, **3**, resist nonspecific adsorption (Pale-Grosdemange, 1991) we concluded that highly ordered monolayers had not formed in the ethanol-based solutions.

We next tried to enzymatically attach the incoming duplex DNA to the DNA-SAM. DNA ligase catalyzes the formation of a phosphodiester bond between a juxtaposed 5′ phosphate group and a 3′ terminal hydroxyl in duplex DNA. If the enzyme were able to function at the surface, the nick in the coding strand, between the 5′ end of the incoming double stranded dsDNA and the 3′ end of the ssDNA on the SAM would be covalently joined. This reaction is dependent on the presence of a 5′ phosphate group and synthetic DNA is not normally 5′ phosphorylated. Our strategy was to hybridize three samples of duplex DNA bearing a complementary single-stranded "tail" to the DNA-SAM, then incubate with DNA T4 ligase under suitable buffer conditions. As a negative control, one sample was not 5′ phosphorylated and therefore could not be ligated to the surface. For a second negative control, T4 ligase was not added to one reaction mixture. Following ligation, the surfaces were rinsed in PBS (running buffer) and inserted in the BIAcore instrument under constant flow conditions. The baseline for the two negative control DNA-SAMs decreased with time, consistent with DNA slowly leaching off the surface. When the ligation procedure was done using 5′ phosphorylated DNA and T4 ligase, a stable baseline resulted that survived

DNA-SAM presents single
stranded 10-mers

Ligase catalyzes the
formation of a
phosphodiester
bond

1. Add duplex DNA
with a single stranded
complementary tail

2. Make the
attachment covalent
with DNA ligase

Δ

4. Heat yields single
stranded DNA

3. Duplex DNA is ready
for protein binding studies

3. Cut
surface with
the desired
restriction
enzyme

BamHI
site

EcoRI
site

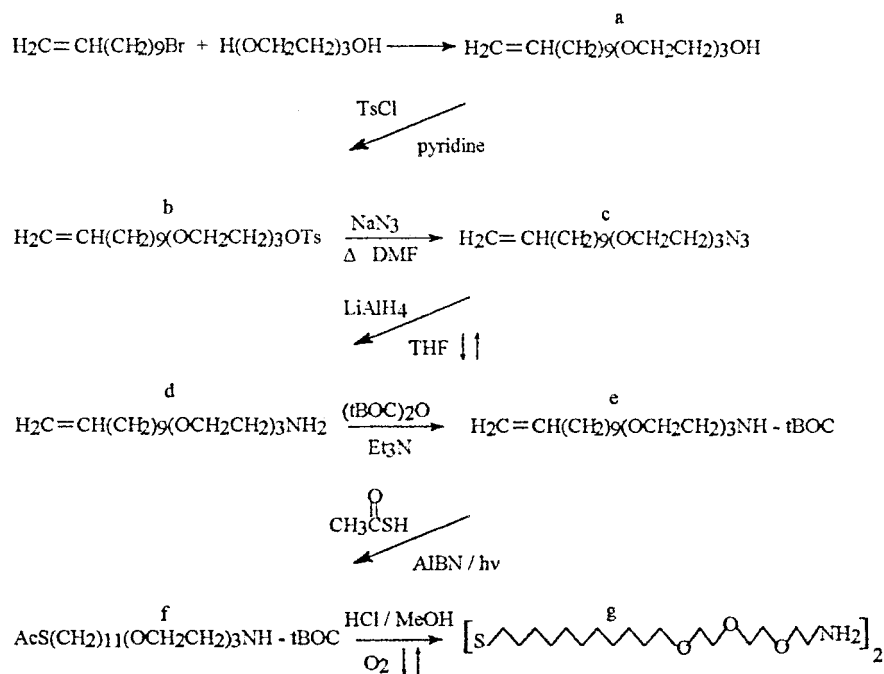4. Ligate on any
DNA cut with the
same enzyme

Scheme 1

several washing steps, which is consistent with DNA co-valently attached to the DNA-SAMs.

We next sought to demonstrate binding to specific DNA protein binding domains presented on DNA-SAMs. dsDNA containing two Gal-4 binding sites was hybridized, then enzymatically ligated to DNA-SAMs. These surfaces were then mounted in a BIAcore device and Gal-4 protein, or an irrelevant protein (LexA-B17), were incubated with the surfaces. The sensorgram shown in Fig. 3 demonstrates that DNA-SAMs modified with DNA bearing Gal-4 binding sites specifically bound Gal-4 protein and did not bind an unrelated protein.

Next, we sought to show a correlation between the number of DNA protein-binding domains presented on the SAM and the amount of protein that could be bound. We generated variable-density DNA-SAMs by adjusting the ratio of inert thiol to DNA-thiol in acetonitrile solutions used for SAM deposition. The DNA-SAMs were mounted onto BIAcore cassettes. The surfaces were characterized by SPR, removed from the instrument, and DNA containing Gal-4 binding sites was ligated onto the SAMs. The SAMs were then reinserted into the BIAcore device. The amount of DNA attached to the SAMs (bearing protein binding sites) was determined by quantitating the change in mass at the
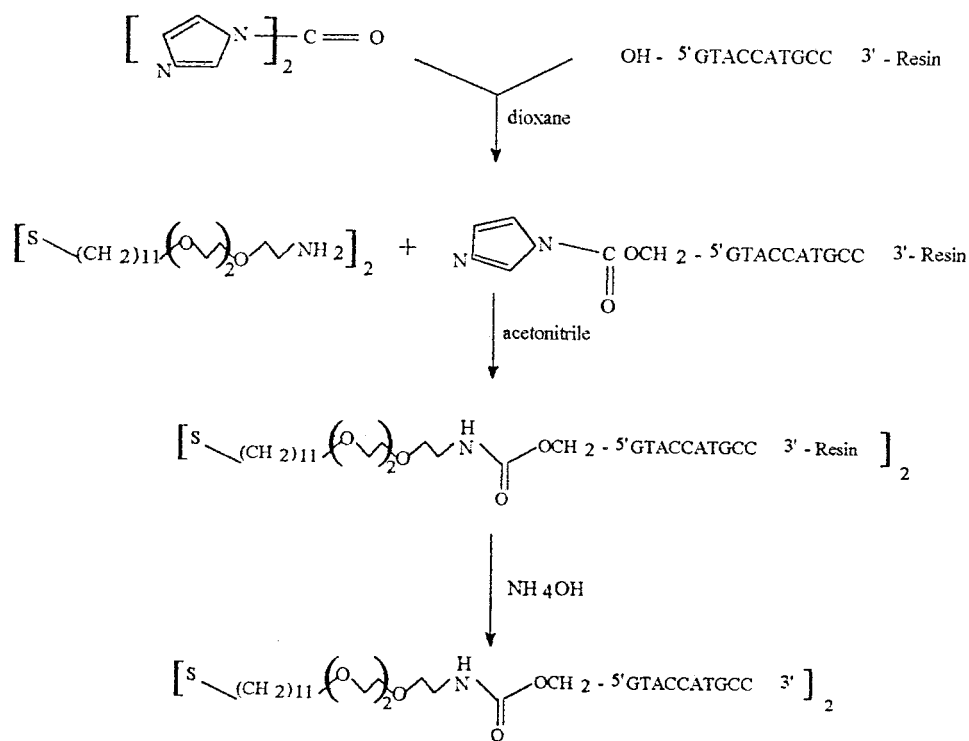
Scheme 2

a
$H_2C=CH(CH_2)_9Br + H(OCH_2CH_2)_3OH \longrightarrow H_2C=CH(CH_2)_9(OCH_2CH_2)_3OH$

TsCl / pyridine

b
$H_2C=CH(CH_2)_9(OCH_2CH_2)_3OTs \xrightarrow[\Delta \ \ DMF]{NaN_3} H_2C=CH(CH_2)_9(OCH_2CH_2)_3N_3$ c

LiAlH$_4$ / THF

d
$H_2C=CH(CH_2)_9(OCH_2CH_2)_3NH_2 \xrightarrow[Et_3N]{(tBOC)_2O} H_2C=CH(CH_2)_9(OCH_2CH_2)_3NH-tBOC$ e

$CH_3\overset{O}{\overset{\|}{C}}SH$ / AIBN / hv

f
$AcS(CH_2)_{11}(OCH_2CH_2)_3NH-tBOC \xrightarrow[O_2]{HCl/MeOH} [S \diagup\!\!\!\diagdown\!\!\!\diagup O \diagup\!\!\!\diagup O \diagup\!\!\!\diagup NH_2]_2$ g
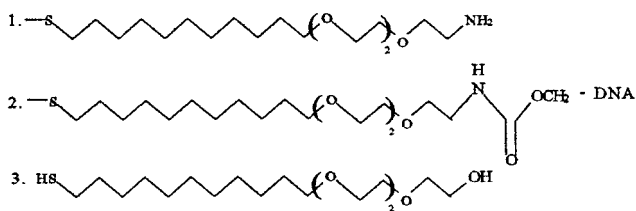
surface-solution interface. Gal-4 protein, which should bind to the presented DNA, or a negative control protein, LexA-B17, were then incubated with the surfaces in the BIAcore device. SPR measurements presented in Table 1 show that higher-density DNA-SAMs bound more of the recognition protein than lower-density DNA-SAMs. The control protein, LexA-B17, did not significantly bind to the surfaces. Table 2 shows that hybridized DNA bearing LexA binding sites preferentially bound LexA-B17 over Gal-4.

CONCLUSION

We have generated DNA-SAMs that can present single- or double-stranded oligonucleotides above an inert background that resists the nonspecific adsorption of irrelevant DNA and proteins. The integration of molecular biology techniques into SAM technology vastly increases the versatility of these surfaces. It has also been demonstrated that the surface can capture long stretches of unmodified duplex

Scheme 3

$[\text{(pyrrole)}N-]_2-C=O$ OH - 5'GTACCATGCC   3' - Resin

dioxane

$[S\diagup(CH_2)_{11}(O \diagdown\!\!\!\diagup)_2 O \diagup NH_2]_2$ + $N-(\text{pyrrole})N-C \ OCH_2 - 5'GTACCATGCC$ 3'- Resin
                                                                          $\overset{\|}{O}$

acetonitrile

$[S\diagup(CH_2)_{11}(O\diagdown\!\!\!\diagup)_2 O \diagup \overset{H}{N} \diagdown\!\!\!\diagup OCH_2 - 5'GTACCATGCC \ \ 3' - Resin]_2$
                                                          $\overset{\|}{O}$

NH$_4$OH

$[S\diagup(CH_2)_{11}(O\diagdown\!\!\!\diagup)_2 O \diagup \overset{H}{N} \diagdown\!\!\!\diagup OCH_2 - 5'GTACCATGCC \ \ 3']_2$
                                                          $\overset{\|}{O}$

DNA as long as they have a complementary single-stranded "tail." DNA ligase, which catalyzes the formation of a phosphodiester bond between a juxtaposed 5′ phosphate group and a 3′ terminal hydroxyl in duplex DNA, covalently attaches the incoming DNA to the DNA incorporated into the SAM. Since the anti-sense strand is not covalently attached to the SAM, it can be dissociated by heat or chemical treatment, leaving ssDNA presented on the surface for hybridization studies. Alternatively, the dsDNA that is ligated to the DNA-SAM can be a polylinker that contains several restriction enzyme sites. A user could then digest the surface with a restriction enzyme to create a new surface that will only accept duplex DNA that has a complementary end (generated by cutting it with the same restriction enzyme). This creates a universal DNA acceptor surface ready for the ligation of similarly digested bacterially produced DNA of any sequence or length. This technique for the immobilization of unmodified DNA can be scaled up for the simultaneous presentation of hundreds of

FIGURE 2   DNA lacking a single-stranded tail does not hybridize to the DNA-SAM; 40-bp duplex DNA [500 pM/μl] containing two Gal-4 sites but lacking a single-stranded tail does not bind to the DNA-SAM even at 10 times the concentration used in Fig. 1.

thousands of DNA species. Islands of DNA-SAMS can be patterned on chips by photolithography (Clark, 1994) or microstamping (Singhvi et al., 1994) techniques. Each address on the chip would undergo the same processing until the last step, when distinct DNA-containing sample solutions cut with the appropriate restriction endonuclease would be applied to the proper chip addresses and enzymatically ligated. Existing technologies (ink jet) can deliver
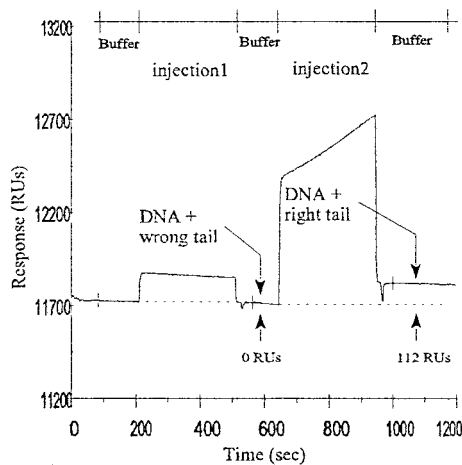

FIGURE 1   Duplex DNA having a single-stranded tail, complementary to the DNA incorporated into the SAM, hybridizes to the surface; DNA with a noncomplementary tail does not. DNA-SAMs, glued onto CM-5 chip cassettes, were docked into a BIAcore device. DNA sample-containing "plugs" interrupted the constant flow of buffer (PBS). The amount of DNA that bound to the surface is measured by the *net* increase in baseline resonance units (RU, where 1000 RU ≡ 1 ng protein/mm^2) after sample injection. This change in baseline corresponds to altered optical properties at the DNA-SAM/solution interface. Two duplex DNA samples [50 pM/μl] were injected over the DNA presenting SAM. The DNA samples were identical except for the sequence of a 10-base single-stranded "tail." The "tail" was either exactly complementary to the DNA presented on the surface, 5′-AGCCTCGAGC-3′, or noncomplementary, 5′-GACT-TCTAGC-3′. DNA bearing an exactly complementary tail (injection 2) hybridized to the DNA-SAM while the noncomplementary DNA did not.
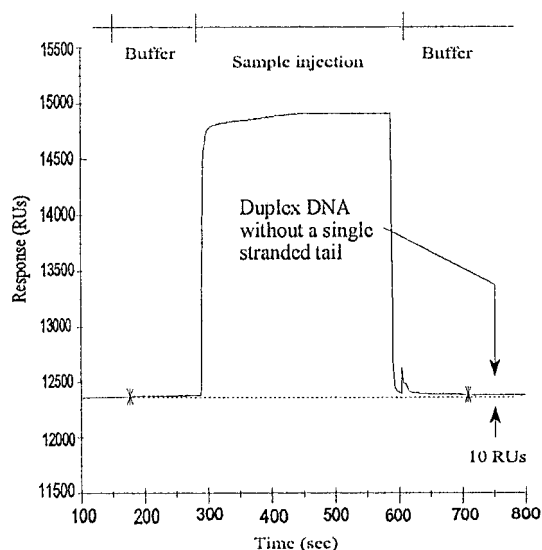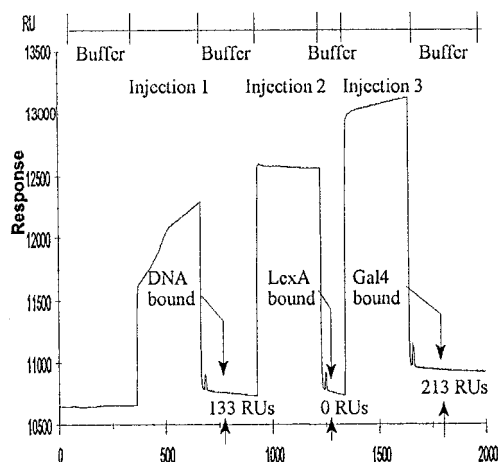

FIGURE 3   A DNA-binding protein is specifically bound to its cognate sites on DNA hybridized to the DNA-SAM; 40-bp duplex DNA [50 pM/μl] bearing two Gal-4 recognition sites and having a single-stranded tail complementary to the DNA presented on the surface was injected over the DNA-SAM in a BIAcore instrument; 133 RU of sample DNA hybridized to the SAM. As a negative control, a DNA-binding protein, LexA-B17 [125 μg/ml], whose cognate sites were not present in the duplex DNA, was injected but did not bind. Gal-4 protein (213 RU) [125 μg/ml] bound to recognition sites on the hybridized DNA.

TABLE 1 DNA bearing protein recognition sites, when hybridized to variable density DNA-SAMs, specifically binds proportionate amounts of that protein

dsDNA Bearing 2 Gal-4 Sites (RU)	Gal-4 Bound (RU)	LexA-B17 Bound (RU)
52	164	ND*
74	213	0
441	365	ND
1378	1079	96

DNA-SAMs were prepared from serial dilutions of DNA-functionalized thiols to generate a panel of SAMs displaying decreasing concentrations of single-stranded DNA. The DNA-SAMs were docked in a BIAcore SPR instrument and baseline resonance unit (10^3 RU ≡ 1 ng protein/mm^2) measurements were recorded. The SAMs were then removed from the device and incubated at RT for 12 min in solutions containing a 40-base oligo [50 pM/μl] bearing two Gal-4 binding sites and having a single-stranded tail complementary to the DNA on the SAM. The surfaces were washed to remove excess DNA. The hybridized DNA was then covalently attached to the SAM by the addition of DNA ligase (in solution of ligase buffer for 15 min at RT), which catalyzes formation of a bond between the 5′ end of the incoming DNA and the 3′ end of the DNA displayed by the SAM. The duplex DNA-derivatized SAMs were washed and re-inserted into the BIAcore. Net changes in RU values corresponding to the addition of duplex DNA were measured. Equimolar solutions containing Gal-4 [125 μg/ml] or another DNA binding protein, LexA-B17 [125 μg/ml], were injected over the derivatized SAMs. The mass of each species that bound to the SAMs was detected by the SPR device and expressed in RU. *ND, not determined.

precise aliquots of liquid samples to spatially distinct locations of small dimensions with great accuracy.

This paradigm for the immobilization of DNA on surfaces is an improvement over existing technologies in that it enables the attachment of long pieces of unmodified DNA as single- or double-stranded. Surfaces can be modified by an end user to present customer-provided DNA, allowing experiments to be altered on the fly. The cost of producing DNA-derivatized thiols for molecular self-assembly onto surfaces is a fraction of the cost of photochemical reagents used to synthesize DNA in situ. In addition, bacterially produced DNA can be enzymatically attached to DNA-SAMs at a negligible cost.

TABLE 2 A homogeneous DNA-SAM bearing LexA recognition motifs preferentially binds LexA-B17 protein over Gal-4

Flow Cell	Gal-4 Bound (RU)	LexA-B17 Bound (RU)
1	ND	363
2	136	ND*
3	ND	345

DNA containing two LexA binding sites was hybridized, then ligated to a DNA-SAM, as described in the legend to Table 1. Individual flow cells within the DNA-SAM were created by the BIAcore instrument when it was pressure-sealed by an integrated flow circuit. Protein samples were separately injected over three flow cells. LexA-B17 bound to its cognate DNA presented by the SAM better than another DNA binding protein, Gal-4 (1–100). *ND, not determined.

The DNA surfaces described herein are compatible with diverse sensing technologies. They can be used with SPR devices to detect hybridization, protein-protein, or protein-DNA interactions. Fluorescently tagged oligos could be used to detect hybridization events on DNA-SAMs. SAMs are formed on a metal substrate and so are compatible with electronic sensing techniques such as those based on electron transfer (Meade and Kayyem, 1995). Arrays of discrete DNA-SAMs that each present a different species of DNA and are electronically addressable would enable the parallel processing of multiple DNA experiments. The marriage of this technology with microelectronics would allow for the development of logical chips for point-of-care medical diagnostics. Electronic sensing of DNA interactions using a chip that can also support DNA modifying enzyme reactions might also provide a critical interface between electronic and DNA computers (Adleman, 1994).

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