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Selective adsorption of oligonucleotides on switchable self-assembled monolayers

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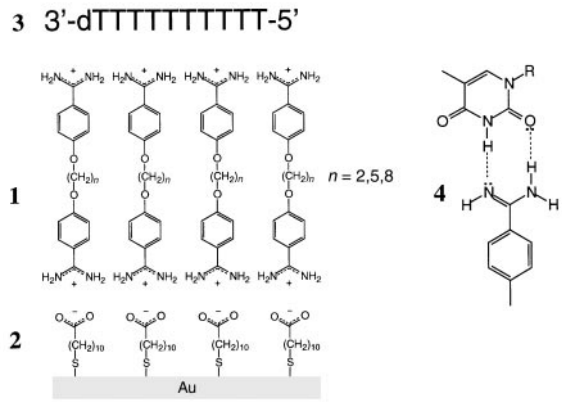
Short oligonucleotides adsorb rapidly and selectively on pH-switchable self-assembled layers of bisbenzamidines on modified gold substrates.

Today's methods of gene analysis require rapid detection of specific DNA or RNA base sequences present in small amounts in complex mixtures.[1] These commonly make use of pre-synthesized probe oligonucleotides or oligonucleotide analogues[2] capable of hybridizing specifically to the sequence of interest.[3] Different techniques for detection of the hybridization reaction are available. These can rely on either the use of labeled probes,[3] *e.g.* radioactive, fluorescent and chemiluminescent, or the use of unlabeled probes where the probe usually is covalently bound to a surface and the hybridization reaction is detected microgravimetrically,[2,4] optically[5,6] or electrochemically.[7] The chemical modification of the probe required in these techniques may cause problems such as *e.g.* slow or nonspecific hybridization reactions and, in the case of sensors, may prevent their reuse.

Techniques for reversible attachment of the probe to the surface would be attractive in this regard. We report here a system for base-selective and reversible adsorption of nucleic acids to a surface reflected in layer thickness changes that can be detected optically.

The system is based on α,ω-bis(4-amidinophenoxy)alkanes **1**



that we previously showed form stable ordered pH-switchable layers on gold or modified gold substrates.[8,9] In view of the DNA binding properties of the bisbenzamidines[10,11] we decided to investigate whether the amidine layers exhibit selectivity for oligonucleotides enriched in one particular base or whether the system can be used to detect DNA hybridization. First we compared the adsorption of a 10-mer of deoxythymidylic acid (T10) **3** using three bisbenzamidines with different alkyl chain lengths (Table 1). The bisbenzamidines gave rapid increases in layer thickness where, for the longer amidines, the final thickness approached the molecular length corresponding to an extended conformation of the amphiphile.[9] A marked increase in the layer thickness was thereafter produced upon addition of the oligonucleotide. The change in thickness increased with the molecular length of the bisbenzamidine. It is known that bisbenzamidines interact with and may precipitate nucleic acids,[12] suggesting that the film formation is related to the different solubilities of the amidine–nucleic acid pairs. However, no visible precipitation was observed in the experiments and the changes in film thickness observed with SiO_2 surfaces under otherwise identical conditions were negligible. This was also the case when performing the experiment on the bare MUA SAM, leaving out the amidine. A preadsorbed amidine layer is thus necessary for the additional DNA layer buildup.

It is seen in Fig. 1 that the bands characteristic for the amidines are present in the IR absorption spectra of the SAM modified with **1** (n = 5). The position and intensities of the bands corresponding to N–H and O–H stretching vibrations (at *ca*. 3159 and 3355 cm^{-1}) show that the amidine groups are involved in hydrogen bonding, presumably with the SAM carboxylic acid head groups.[9] A comparison of the intensities of the bands corresponding to the C–H out-of-plane bending vibration (840 cm^{-1}, weak) and the ring C=C stretching vibration (1611 cm^{-1}, strong) further shows that this amidine prefers an orientation with the phenyl groups oriented nearly perpendicular to the surface.[9] After adsorption of the oligonucleotide additional bands appeared. The main change was the appearance of bands assigned to the phosphate groups at 1000–1270 cm^{-1}, the increase in intensity and broadening of the band corresponding to the N–H and O–H stretching to lower frequencies and the reduced intensity of the ring C=C stretching vibration parallel to an increase in intensity of the C–H out-of-plane vibration. These observations indicate further extensive hydrogen bonding in the adsorbed DNA layer as well as a

Table 1 Layer thicknesses upon consecutive addition of bisbenzamidines and a 10-mer DNA oligonucleotide to a self-assembled monolayer (SAM) of mercaptoundecanoic acid (MUA) on gold[a]

	Layer thickness/Å			
DNA oligo-nucleotide[b]	**1** (n = 5)		**1** (n = 8)	
	amidine[c]	DNA[d]	amidine[c]	DNA[d]
T10[e]	13 ± 1	14 ± 2	23 ± 1	33 ± 3
C10	12 ± 0	2 ± 1	24 ± 2	3 ± 3
G10	14 ± 1	11 ± 1	24 ± 3	2 ± 1
A10	14 ± 1	1 ± 1	25 ± 5	12 ± 1

[a] The substrates were then immersed in a quartz ellipsometric cuvette containing 5 ml sodium borate buffer (0.01 M, pH 8.7, prepared from boric acid) thermostatted to 25 °C and equipped with a small magnetic stirrer and a pH electrode. Adsorption of compounds were then monitored *in situ* by null ellipsometry (ref. 8,9) assuming a film refractive index of 1.45. The reported values were determined when the change in the polariser angle had levelled off. After one experiment the surfaces were restored by adjusting the pH to 2–3 with 0.1 M HCl followed by rinsing with water. [b] 10-mer DNA oligonucleotides of deoxythymidylic acid (T10), deoxycytidylic acid (C10), deoxyguanylic acid (G10) and deoxyadenylic acid (A10). [c] Increase in layer thickness *ca*. 200 s after addition of **1** (n = 5) or **1** (n = 8) (100 μl of a 2.5 mM stock solution). [d] Increase in layer thickness 200–300 s after addition of the oligonucleotide (5 μl of a 0.5 mg ml^{-1} stock solution) to the solution described in note *c*. The spread of the thickness values from duplicate runs has been indicated. [e] Using **1** (n = 2) the layer thickness of **1** was 11 ±1 Å and of T10 was 5 ±1 Å.

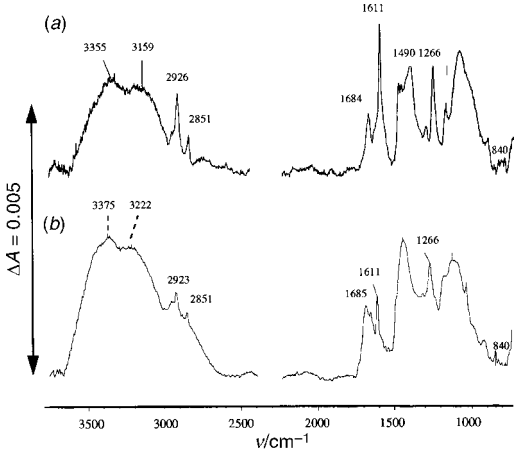


Fig. 1 Baseline corrected IR reflection absorption spectra of SAMs of MUA modified with (*a*) **1** (*n* = 5) and (*b*) **1** (*n* = 5) and a 15-mer random sequence DNA oligonucleotide.

change in the average tilt angle of the phenyl groups, the latter effect possibly due to adsorption of additional randomly ordered amidine molecules or an induced disorder of the SAM.

Table 1 shows the estimated final layer thicknesses upon addition of **1** (*n* = 5) or **1** (*n* = 8) in a first step and a 10-mer oligonucleotide in a second step. A striking difference in DNA base selectivity is seen when comparing the two amidines. Compound **1** (*n* = 5) binds preferentially T10 and G10 whereas **1** (*n* = 8) shows affinity for T10 and A10. Thus exchanging **1** (*n* = 5) with **1** (*n* = 8) in the second layer changes the base preference from G10 to A10. It should be noted that Brønsted basic head groups in close-packed monolayers, due to electrostatic effects, may be significantly less basic than the corresponding base in solution.[13,14] At the pH of the experiment, a proportion of the amidine groups may thus be uncharged. These groups may offer complementary binding sites for thymine resulting in the binding motif **4** similar to the one proposed to occur in a Langmuir–Blodgett film of guanidine and thymine functionalized amphiphiles at the air water interface,[15] or between thymine and amidine in complexes between ds-DNA and bisbenzamidines.[10]

In order to investigate whether the consecutive addition protocol could be used to detect DNA hybridizations, we used a 10-mer probe[4] with a sequence complementary to the *Eco*R1 binding site of single stranded M13 phage DNAs (7249 base pairs). In order to test the selectivity, four 10-mer oligonucleotides with a varying number of mismatches were used. As seen in Fig. 2 the adsorption of the probe sequence (DNA 1) gave an

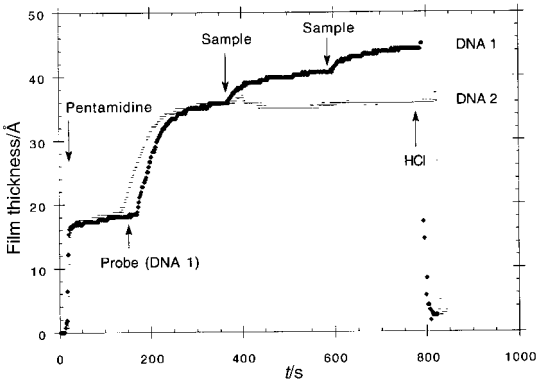


Fig. 2 Film thickness, estimated by *in situ* ellipsometry, *versus* time after the consecutive addition of **1** (*n* = 5), a 10-mer DNA probe (5 μl of a 0.5 mg ml^{-1} stock solution) and a sample oligonucleotide (2 × 5 μl of a 0.5 mg ml^{-1} stock solution) to a SAM of MUA on gold, as described in Table 1. The following DNA sequences were used with the number of antiparallel mismatches given in parantheses. Probe sequence: DNA 1: 3′-dTGCTTAAGGG-5′ (4); sample sequences: DNA 2: 3′-dCCCTTAAGCA-5′ (0); DNA 3: 3′-dCCCTGAAGCA-5′ (1); DNA 4: 3′-dAGCCGTACCC-5′ (7). The changes in layer thickness caused by the sample oligonucleotides were as follows: DNA 1: +9 Å; DNA 2: 0 Å; DNA 3: +3 Å; DNA 4: +9 Å. The spread of the thickness values from duplicate runs was less than 2 Å.

increase in film thickness of *ca.* 17 Å. Subsequent additions of the probe, as sample sequence, resulted in an additional 9 Å increase in film thickness. Adding instead a fully complementary sample sequence (DNA 2) resulted in no change or a slight decrease in layer thickness. A more pronounced decrease in the layer thickness was observed when adding a larger amount of DNA 2. Thus adding five times more DNA 2 resulted in a decrease in layer thickness of about 5 Å. The larger the number of mismatches, the larger the increase in film thickness caused by the sample sequence.

Additional control experiments were performed. The probe layer thickness depended on the sequences of the probe. For instance whereas sequences 1 and 4 (in Fig. 2) gave an increase in thickness of about 16 Å, sequences 2 and 3 resulted in an increase of about 10 Å. However, in the hybridization test, probe sequences giving similar thicknesses responded in accordance with the number of mismatches in the chain. Thus when adding sequence 1 to a surface saturated with DNA 2, 3 or 4, an additional increase in thickness of 1, 5 and 7 Å respectively was observed. Finally, regardless of the order of addition of the oligonucleotides the same end thickness was observed. This indicates a reversible adsorption process with a rapid exchange of adsorbates. As in the case of the adsorption of homo-oligonucleotides the molecular basis for the observed thickness changes remains obscure. The behaviour contrasts with what is commonly observed in hybridzation reactions using probes covalently bound to the surface.[2,4,6] In these cases, hybridization results in an increase in layer thickness. It is likely that rigid rod like double stranded DNA adsorbs more weakly or in a more compact conformation to the surface than more flexible single stranded DNA. The latter may more easily adjust its conformation to maximize the attractive interactions with the surface amidine groups.

The above results show that self-assembled monolayers of bisbenzamidines can be used for base selective binding of nucleic acids. Our future studies will show whether the system can be extended to polynucleotides and complex PCR products. Alternatively the protocol may be useful in studies of drug–DNA or protein–DNA interactions.

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