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Detection of DNA hybridisation in a diluted serum matrix by surface plasmon resonance and film bulk acoustic resonators

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ABSTRACT

Nanomolar quantities of single-stranded DNA products ~100 nucleotides long can be detected in diluted 1% serum by surface plasmon resonance (SPR) and film bulk acoustic resonators (FBARs). We have used a novel FBAR sensor in parallel with SPR and obtained promising results with both the acoustic and the optical device. Oligonucleotides and a repellent lipoamide, Lipa-DEA, were allowed to assemble on the sensor chip surfaces for only 15 minutes by dispensing. Lipa-DEA surrounds the analyte-binding probes on the surface and effectively reduces the non-specific binding of bovine serum albumin and non-complementary strands. In a highly diluted serum matrix, the non-specific binding is, however, a hindrance, and the background response must be reduced. Nanomolar concentrations of short complementary oligos could be detected in buffer, whereas the response was too low to be measured in serum. DNA strands that are approximately 100 base pairs long at concentrations as low as 1-nM could be detected both in buffer and in 1% serum by both SPR and the FBAR resonator.

Keywords: surface plasmon resonance, film bulk acoustic resonator, DNA hybridisation detection, serum, self-assembled monolayer, DNA sensor

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1. Introduction

The measurement of DNA hybridisation is interesting and critical in several areas of analysis such as molecular biology, genotyping, diagnostics and screening of genetically modified food ingredients [1,2]. There are several techniques available when developing methods for the detection of DNA hybridisation, but surface plasmon resonance (SPR) [3,4] has remained the label-free method of choice for real-time detection of DNA hybridisation in the nanomolar range. Thickness shear mode resonators (QCM sensors) have also been used frequently for the detection of oligonucleotide interactions, and recently, shear mode film bulk acoustic resonators (FBARs) have been developed for label-free detection of biomolecular interactions, including DNA hybridisation [5-8]. All of these techniques rely on detecting a change in surface mass when an interaction takes place. In SPR, the change in refractive index, which can be correlated to the changes of mass, is measured, whereas the QCM and FBAR techniques both rely on detecting a change in surface mass through a decrease in frequency. Apart from the mass, QCM frequency shifts are also dependent almost equally on both the elasticity and viscosity of the added layer, whereas for the FBAR, the frequency shift is, apart from mass, dependent also on viscosity [9, 10]. With the FBAR, the resonance frequency is in the gigahertz range, enabling much larger frequency shifts and a lower detection limit, compared to the QCM device, which operates in the 5-10 megahertz region [5]. FBAR is a promising technology and may be an option when constructing sensors for point-of-care applications because it can be easily miniaturised and integrated into small, low-cost hand-held devices.

Construction of a specific sensing surface with sufficient sensitivity and selectivity, however, remains very critical. The pioneering work of Herne and Tarlov on SPR

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characterisation of thiol-modified, single-stranded DNA (ssDNA) probes and mercaptohexanol self-assembled monolayers (SAMs) on gold surfaces has been widely cited and further elaborated [11]. These studies have revealed that the surface hybridisation properties of the target are not only affected by the surface probe length but also by factors such as probe density, surface orientation and target sequence [12-15]. It is common to use a procedure in which the oligos are allowed to assemble on the gold surface for hours. We have previously demonstrated that the assembly can also be performed *in situ* during 10-15 minutes with a good surface coverage of the probes [16-18].

To reduce the non-specific binding of proteins or DNA in the sample matrix, thiolated hydrophilic organic molecules like mercaptohexanol and polyethyleneglycol (PEG) -derived compounds have been embedded between the oligos on the surface [11, 15]. Apart from reducing the non-specific binding, the blocking molecules induce the DNA-oligos to take an upright position on the surface, thus maximising their hybridisation capacity [11, 15]. The *N*,*N*-bis(2-hydroxyethyl)-α-lipoamide, Lipa-DEA, (Fig. 1a) that we have used as a blocking agent rendered the surface more repellent than mercaptohexanol [17-19]. The hydrophilic groups of the blocking molecules reduce the non-specific binding caused by other specimens in the sample matrix. Non-specific binding is, however, still noticeable when label-free sensors are employed, and very few DNA studies have been performed, even in diluted serum. Reducing the non-specific binding is vitally important, especially with SPR and mass-sensors because any molecule binding non-specifically to the surface can be regarded as a potential interference, causing a false positive signal.

Most papers describe the hybridisation of short (20-40 nucleotides) complementary ssDNA strands, which very rarely occur *per se* in nature [11-17, 19]. Short DNA-oligos

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contain less specific information compared to strands of ~100 nucleotides. There are, however, only a few examples in the literature describing the measurement of long DNA amplicons or of synthesised ~100-base pair (bp) DNA strands with SPR. Moreover, most of the papers present results obtained in buffer [11-18, 20-23]. Some examples from the literature of the measurement of longer DNA strands with SPR can be found. For example, *Fusarium culmorum* (a fungal pathogen of cereals) has been analysed through 570-bp, unlabelled polymerase chain reaction (PCR) amplicons [21]. TP53 gene mutations have been used for early diagnosis of different cancers [22], and for apoptosis-associated genes, nanogram–levels of 400-540-bp PCR products have been detected by biotinylated probes coupled to avidin layers [23].

There are only a few papers describing DNA measurement from a serum matrix by SPR. Lee et al. have reported the detection of hybridisation of short complementary strands at quite high concentrations of 0.1 and 1-µM in 50% serum and of 1-µM in whole (100%) bovine serum [24, 25]. Gong et al. [26], however, reported the interference of serum proteins already in dilute serum (1%), and above serum concentrations of 15%, the specific responses were severely inhibited due to a high non-specific binding of serum proteins on the surface.

We have previously described the measurement of breast cancer-relevant, 92-624-base long DNA amplicons at nanomolar concentrations in buffer [18]. In this study, we have now used synthetic, ~100-base long, breast-cancer-specific DNA strands mimicking single-stranded PCR amplicons (ss PCR) (Fig. 1b), and to demonstrate the hybridisation in a more complex matrix, we used diluted (1%) serum, which is also a more readily available matrix compared to the breast tissue biopsy samples hosting the breast-cancer-specific sequences. Monolayers of a disulphide-modified ssDNA and the lipoamide, Lipa-DEA, were constructed in this study by dispensing, whereas in the

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previous study, they were produced *in situ* [18]. Both short complementary oligos and synthetic ~100-nucleotide-long DNA strands were studied. Two different detection methods (SPR and FBAR) were used to determine the hybridisation both in buffer and in diluted serum. This paper reports detection of hybridisation of unlabelled DNA in the nanomolar range by SPR and FBAR in diluted serum.

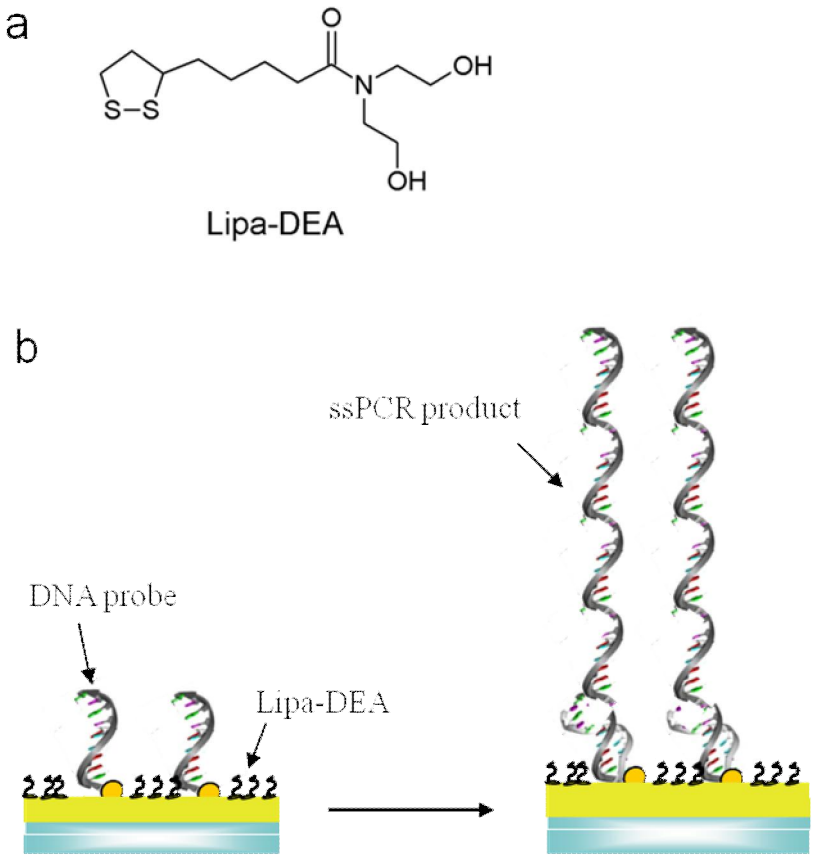

Fig. 1 a *The structure of the lipoamide, Lipa-DEA,* ***b*** *A schematic of the surface assembly of DNA probes and blocking molecules on the sensor/resonator surface and binding of a single-stranded PCR product.*

2. Experimental

2.1. Materials

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The DNA products were all breast-cancer-specific as described by Vikholm-Lundin et al. [18] and were ordered from Metabion (Martinsried, Germany) as HPLC purified. The disulphide-modified, ssDNA-probes that were assembled on the gold surfaces were: DMT-S-S-PTGS2-27 with a sequence of 5´-DMT-S-S-C6-CGA TTG TAT TCG GAT AGG ATT TTA TGG-3´ and DMT-S-S-CALCA-25: 5´-DMT-S-S-C6-GCT TCC GAT CAC ACT CAT TTA CAC A-3´. DMT-S-S- refers to a disulphide modification of the surface oligos. The sequences for the complementary ss-oligos were: PTGS2-27: 5´-CCA TAA AAT CCT ATC CGA ATA CAA TCG-3´ and CALCA-25: 5´-TGT GTA AAT GAG TGT GAT CGG AAG C-3´. These complementary ss-oligos as well as the longer ss-strands (PTGS2-123 and CALCA-92) were both used as analytes. The sequences for the long synthetic ss-strands as well as the hybridisation sites (underlined) for the complementary oligos attached on the surface are presented here: PTGS2-123: 5´-CTA TAT CCA ACC CCA CTC CTA ATA AAA CAA CCA AAA AAC AAA CTT ACG TAT TAA ACA ATT TTC TCC ATA AAA TCC TAT CCG AAT ACA ATC GCA CTT ATA CTA ATC AAA TCC CAC ACT CAT ACA-3´ and CALCA-92 respectively: 5´-TGG GTA TAT GTT GGG AGA TAG TAA TGG GTT TGG GTG TGT GTA AAT GAG TGT GAT CGG AAG CGA GTG TGA GTT TGA TTT AGG TAG GGA TTA TA-3´.

The DNA products were solubilised in MilliQ water and diluted in phosphate running buffer at the desired concentrations just prior to use. *N*,*N*-bis(2-hydroxyethyl)-α-lipoamide (Lipa-DEA) was prepared as previously described [27].

All of the chemicals used were of analytical grade. Hydrogen peroxide (30%) and Na_2HPO_4 were purchased from Merck KGaA, sodium chloride (NaCl) from J.T.Baker, sodium hydroxide (NaOH) from Akzo Nobel (Sweden) and EDTA (ethylenediaminetetraacetic acid) from Fluka AG (Buchs, Germany). Ammonium

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hydroxide (28-30% NH_3), bovine serum albumin (BSA, minimum 98% purity), sodium dodecyl sulphate (SDS) and human serum from male AB plasma (H-4522) were purchased from SIGMA (Mannheim, Germany). Human serum was diluted at a ratio of 1:100 (1% solution v/v) in running buffer (20-mM Na_2HPO_4, 0.3-M NaCl, 1-mM EDTA pH 7.4) and then filtered through a 0.45-µm syringe filter (PALL Corporation, Cornwall, UK) prior to use.

2.2. Gold chips for SPR measurements

Thin glass slides were coated with a 50-nm-thin gold layer in-house by RF magnetron sputtering using a two-target sputter coater (Edwards E306A, BOC Edwards, Crawley, West Sussex, UK). A layer of indium oxide of approximately 10 nm in thickness was sputtered on the glass surface to improve adhesion and chemical resistance of the gold layer to the ammonia/peroxide treatment [28]. The gold slides were cleaned in a boiling solution of hydrogen peroxide and ammonia in water (1:1:5) and rinsed with water prior to the probe and lipoamide layer formation.

2.3. Formation of the self-assembled monolayers for SPR measurements

The self-assembled monolayers were constructed by manually dispensing the probe and lipoamide in a clean room environment. First a 7-µM thiolated oligo solution was dispensed on a pre-cleaned gold slide, then the probes were allowed to assemble for 15 min, and the surface was flushed with fresh MilliQ water. Then, a 700-µM solution of Lipa-DEA was dispensed on the surface, incubated for 15 min, and finally the surface was flushed with water to remove any loosely bound molecules. The manually dispensed surfaces were stored at 4ºC in a humid atmosphere for 3-6 days before SPR

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measurements due to the fact that storage of the layers has been found to improve the blocking behaviour [29].

2.4. SPR measurements

SPR measurements were performed with a Biacore 3000 SPR instrument (GE Healthcare) by mounting the gold slides in the Biacore plastic chip cassette with two-sided adhesive tape. After priming the system, the amount of non-specific binding was first assessed by injecting a 1.7-μM solution of BSA for 15 min. The surface was washed for 10 min with running buffer, and then the amount of non-specific DNA binding was measured by injecting likewise 0.5-μM of non-complementary (non-comp) DNA (non-pairing oligo) on the surface. All of the samples were diluted in running buffer, i.e., 20-mM Na_2HPO_4, 0.3-M NaCl, 1-mM EDTA with a of pH 7.4. The flow in the Biacore instrument was 20 μL/min.

The complementary oligos (PTGS2-27 or CALCA-25) or longer ss-DNA strands (PTGS2-123 or CALCA-92) to be analysed were run on the surface in a similar manner (15-min sample injection, 10-min wash and a regeneration cycle). The surface was regenerated by injecting a 10-mM NaOH-0.1% SDS solution twice for 2 min with a 1-min buffer rinse between injections. The following sample at a higher concentration was then injected over the regenerated surface. A sample of 0-serum was injected three to four times prior to running the samples diluted in serum to stabilise the surface with respect to the non-specific binding. The regeneration cycles were also performed in these cases. All of the measurements were performed at 25ºC. The sensing area of a channel in the SPR Biacore instrument (GEHealthcare) is 0.9 mm^2 [30].

The SPR response of 1000 RU was estimated to correspond to a surface coverage of 100 ng/cm^2 [30-32].

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2.5. FBAR resonators

The FBARs used in this study consisted of a 500-nm-thick piezoelectric layer of zinc oxide sandwiched between two electrodes and mounted on top of an acoustic mirror to acoustically separate the resonator from the substrate. A silicon dioxide (SiO_2) layer isolated the electrodes from the liquid. A thin gold layer on top of the isolation layer served as an interface to the biochemical functionalisation. Acoustic vibrations were excited when an alternating electric field was applied to the electrodes. The fundamental resonance frequency of the FBAR was around 800 MHz and decreased when additional mass was added on top of the resonator. The resonance frequency could be determined in real time using a network analyser (Agilent 8720ES). The resonator set-up and read-out methods are described in detail by Nirschl et al. [7, 8]. A frequency shift of 4.4 kHz corresponded to a surface mass of 1 ng/cm^2, and the sensing area of an FBAR pixel was 0.04 mm^2 [7, 8].

2.6. Layer formation and FBAR measurements

The FBAR gold surface was cleaned in oxygen plasma, and a binary solution of 7-µM of thiolated oligo and 700-µM of Lipa-DEA was brought onto the sensor using a piezo dispenser (sciFLEXARRAYER S5, Scienion AG, Germany) in an atmosphere of 15°C and 50% humidity and kept for at least 1 h in the same environment. The chips were stored for up to 3 days prior to the measurement. Non-complementary and complementary oligos were injected, and a baseline was recorded for at least 5 min. The cell was rinsed with buffer, and a minimum of 500 µL liquid was used to fully exchange the liquids in the flow cell. Regeneration of the surface was done as described for the

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SPR measurements. All measurements on the FBAR were performed at 25°C, and there was no continuous flow during the measurements.

3. Results and discussion

3.1 Hybridisation with short complementary oligos

Before performing the hybridisation measurements, the amount of non-specific binding (NSB) of BSA and non-complementary short oligos to the probe/Lipa-DEA monolayers was measured in buffer. The NSB for a 1.7-μM BSA solution was 20 ± 5 and 60 ± 20 RU and 30 ± 5 and 140 ± 15 RU for 0.5-μM of non-complementary DNA for the PTGS2/Lipa-DEA and CALCA/Lipa-DEA surfaces as measured with SPR (Table 1). This BSA concentration is commonly used in immunoassays for blocking the non-specific binding sites. Binding of BSA is low due to the Lipa-DEA, which is able to resist non-specific binding efficiently. Lipa-DEA has previously been shown to be superior to mercaptohexanol normally used against NSB on DNA surfaces [19]. The NSB of non-complementary DNA was very low except for CALCA/Lipa-DEA, in which case the higher NSB seems to be due to the chosen probe sequence. These results are in accordance with those reported for probe/lipoamide layers assembled *in situ*,in which a higher NSB binding was observed for CALCA than for PTGS2 [18]. The NSB of BSA and non-complementary strands as measured with the FBAR resonator was below 10 kHz or 2 ng/cm^2 (as 4.4 kHz corresponded to 1 ng/cm^2), if only mass changes and no contribution due to viscosity are taken into account [8, 33]. This result agrees quite well with the SPR results, as ten resonance units correspond to 1 ng/cm^2. It seems that the surfaces can be prepared by dispensing with a quite low NSB, especially for protein contaminants like BSA and non-complementary strands.

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		SPR [RU]	FBAR [kHz]	SPR [RU]	FBAR [kHz]
		PTGS2		CALCA	
BSA		20 ± 5	< 10	60 ± 20	< 10
Non-comp DNA		30 ± 5	< 10	140 ± 15	< 10
Conc [nM]		In buffer			
Short strand	1	190 ± 10	50*	130 ± 5*	130*
	1000	330 ± 10	140	280 ± 10	160
Long strand	1	130 ± 5	30*	160 ± 10	30
	1000	900 ± 10	290	740 ± 10	330
Conc [nM]		In serum			
Long strand	1	80 ± 10	75	60 ± 10	65
	1000	720 ± 10	310	380 ± 10	280

*10-nM

FBAR standard deviation was approximately 30%

Table 1 *The SPR and FBAR responses for hybridisation of short and long DNA strands with probe/Lipa-DEA layers with samples diluted either in buffer or in a 1% serum matrix.*

Hybridisation with the short complementary oligos in a concentration range of 0.001 – 1000-nM diluted in buffer was very fast, especially for the higher concentrations (Fig. 2 and 3a). Saturation was reached within a few minutes. Some dissociation of the oligos or changes in the refractive index due to the solution could be observed from the binding curves (Fig. 3a). The responses were steadily increasing in accordance with the increasing DNA concentration. PTGS2-27 at concentrations below 1-nM were giving clearly lower surface densities (6 and 9 ± 1 ng/cm^2 at 0.01 and 0.1-nM, respectively as

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shown in Fig. 5a, insert) compared to 1-nM (19 ± 1 ng/cm^2, Fig. 5a, insert), suggesting that the detection limit in buffer for short complementary oligos lies in the range of 0.1 – 1-nM for PTGS2-27 as measured by SPR. With the FBAR, the most prominent response when measuring complementary CALCA-25 can be seen between concentrations of 6 and 60-nM (Fig. 2), which agrees with that observed by SPR. But, even the lowest measured concentration of 2-nM shows a decrease in resonance frequency. Thus, it seems that concentrations below 10-nM of short DNA strands in buffer can be detected with the FBAR. The surfaces can be regenerated quite efficiently and can be reused as shown in Fig. 2. A somewhat higher density was obtained for CALCA-25 as measured with the FBAR than with SPR (30 and 13 ± 1 ng/cm^2 at 10-nM and 36 and 28 ± 1 ng/cm^2 at 1000-nM, respectively). Apart from a mass contribution, the resonance frequency change of FBARs is due to a change in the viscosity of the layer [33]. This most probably explains the higher surface density obtained with the FBAR when the frequency change is calculated taking into account mass only. Up to 60% and 20% of the frequency change depending on the DNA strand concentration (10 and 1000-nM) might be due to the viscosity, assuming that the same surface density for target binding has to be obtained with the FBAR and SPR. The surface densities as measured with the FBAR (32 ng/cm^2 at 1000-nM) agree with that of SPR for PTGS2-27, but at 10-nM, the surface density was even lower with the FBAR. The lack of a viscosity effect cannot be explained for PTGS2-27 but might be related to the layer formation of the probes on the surface.

The response can be regarded as specific because the non-complementary binding of a much higher concentration of 0.5 -μM gave a surface density of 3 ± 0.5 ng/cm^2. In the case of CALCA-25, the much higher NSB of non-complementary DNA (14 ± 2 ng/cm^2) was obtained as discussed earlier, and due to this effect, a specific binding in buffer

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could not be observed below a CALCA-25 concentration of 100-nM (13 and 26 ± 1 ng/cm^2 for 10 and 100-nM, respectively; Fig. 5b, inset). It is quite clear that the hybridisation efficiency relies on the probe/ target sequence [13].

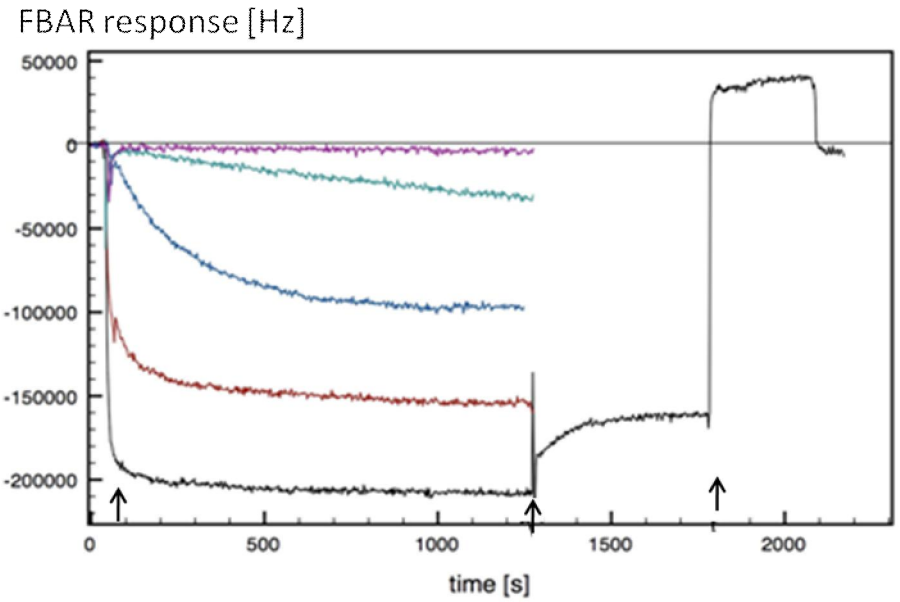

Fig. 2 *Kinetic curves showing the hybridisation of CALCA-25 to layers of DMT-S-S-CALCA-25 and Lipa-DEA as measured by FBAR. The concentrations of the CALCA-25 samples in buffer starting with the uppermost curve were 2, 6, 60, 600-nM and 6-µM (the lowest curve). The first arrow shows injection of the sample, the second arrow rinsing with buffer and the third dehybridisation performed with 0.1% SDS-10-mM NaOH.*

Serum concentrations above 10% are not recommended to avoid clogging of the micro-channels of the Biacore 3000 instrument [30]. Because of this effect and due to reports on high non-specific binding from very diluted serum, the complementary oligos were spiked in 1% serum. Serum injections were performed three times prior to

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measurement of the DNA samples spiked in 1% serum. The serum response gradually decreased with injections, settling down to 200 ± 50 RU after the third injection. Thus, for the zero sample, a NSB higher than that of BSA or non-complementary DNA was obtained. This response was, however, not surprisingly high as even a highly diluted serum matrix contains large amounts of proteins that could bind to the surface and, without Lipa-DEA, the NSB would have been even 10-fold. Gong et al. have reported severe non-specific binding of serum proteins from diluted matrices [26]. However, as a response of 200 RU (20 ng/cm^2) was in the range of that obtained for complementary oligos in buffer, sub-nanomolar concentrations of complementary DNA were not detectable (Fig. 5a and 5b; insets). This effect can also be observed from the lower set of curves in Fig. 3b. Serum proteins apart from non-specifically adsorbing on the surface, were also causing a shift in the SPR resonance due to changes in the refractive index of the solution. Because the response for hybridisation of the short complementary oligos in buffer at 1-µM was only 105 ± 35 RU higher than the NSB of serum proteins, no remarkable response could be expected in serum. The response for the highest concentrations of 0.1–1-µM was still observable despite the resonance shift caused by the serum. Others have also reported markedly reduced hybridisation responses at or below complementary strand concentrations of 1-µM in diluted serum solutions [24-26]. A lower NSB should, however, be needed to detect low concentrations of short complementary oligos. The hybridisation of short complementary oligos in 1% serum was not measured by FBAR because of the relatively low responses obtained by SPR.

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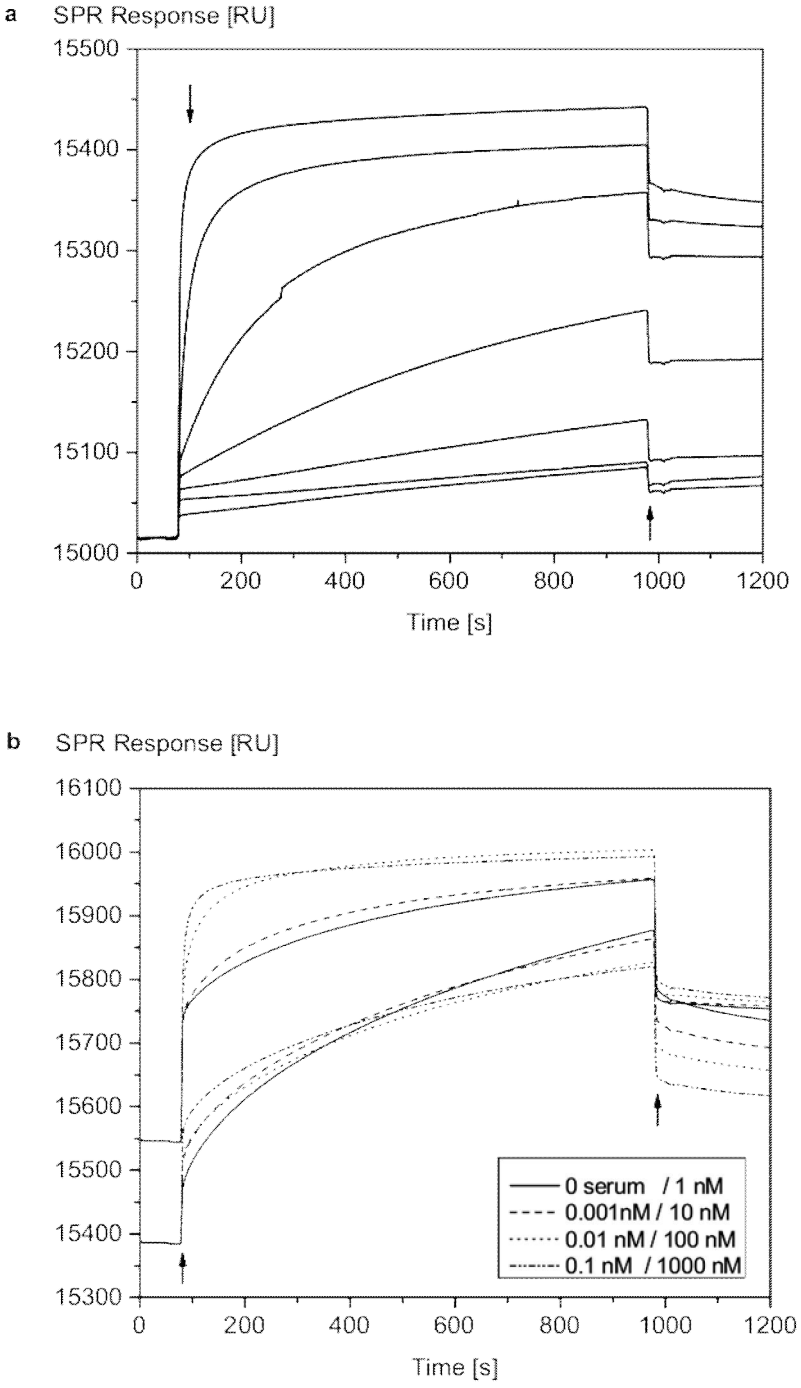

Fig. 3 *Hybridisation of complementary PTGS2-27 to layers of DMT-S-S-PTGS2 and Lipa-DEA in* ***a*** *buffer and* ***b*** *1% serum. In Fig. 3a, the curves are shown according to concentration, the lowest curve representing responses measured with a 0.001-nM solution and the uppermost curve representing a concentration of 1000-nM. In Fig. 3b, the lower set of curves represents solutions in the range of 0-serum–0.1-nM and the*

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upper set of curves, solutions in the range of 1–1000-nM. The sample injection and initiation of the buffer wash cycle are indicated by arrows in all figures.

According to Fig. 3a (and the insets in Fig. 5a and 5b), hybridisation of the surfaces in buffer with 1-µM of complementary PTGS2-27 and CALCA-25 resulted in a response of 330 and 280 ± 10 RU (33 and 28 ± 1 ng/cm^2), respectively. When considering also the base pair length, the hybridised target density was almost the same, corresponding to 2.4 and 2.2 ± 0.1 x 10^{12} molecules/cm^2 for PTGS2-27 and CALCA-25, respectively. These target densities are in accordance with the study of Gong et al [26], who have reported of a target density of 2.2 ± 0.8 x 10^{12} molecules/cm^2 for a 1-µM, 20-mer, SH-ssDNA surface-assembled for 5 h and back-filled with 10-µM of mercaptoundecanol for 18 h.

However, the hybridisation response for the *in situ*-produced PTGS2-27 and CALCA-25 layers was much higher (470 and 550 ± 15 RU) corresponding to target densities of 3.5 and 4.2 ± 0.2 x 10^{12} molecules/cm^2, respectively [18]. The response was thus reduced by 25% for PTGS2-27 when the layer was made by dispensing, whereas in the case of CALCA-25, it was reduced by half. This is most probably due to differences in the arrangement of the layer when the probes and lipoamide are assembled *in situ* [18] or by dispensing. Consequently, it seems likely that DMT-S-S-PTGS2-27 assembles on the surface in a more similar way despite being assembled *in situ* or by dispensing, whereas the DMT-S-S-CALCA-25 probes were assembled differently on the surface. DMT-S-S-CALCA-25 and DMT-S-S-PTGS2-27, apart from a small difference in base pair length, also have differences in the sequence of the bases. The surface densities of the surface probes produced *in situ* were 3-fold that of the complementary strand and ranged from 1.0 ± 0.1 x 10^{13} to 1.5 ± 0.2 x 10^{13} for DMT-S-S-PTGS2-27 and DMT-S-S-CALCA-25,

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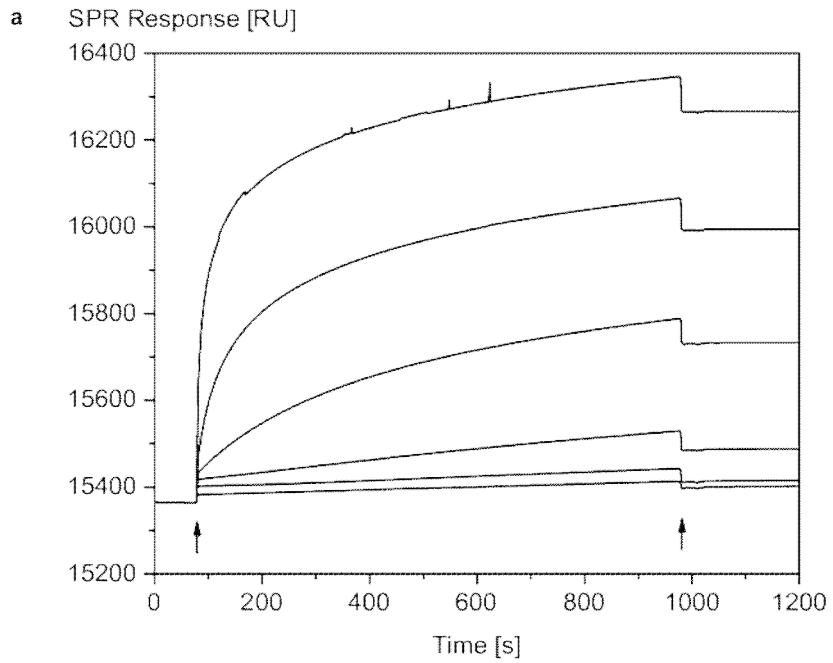
respectively [18]. The surface density of the manually dispensed layer might be lower, but irrespectively, the hybridisation ratio can be roughly regarded to be in the range of 15-20%. The surface coverages of the probes could not be calculated because the layers were produced by dispensing.

3.2. *Hybridisation of the long ssDNA strands*

To amplify the response and thus improve the sensitivity of the analysis, the same measurements were also performed with the long PTGS2-123 and CALCA-92 single-stranded DNA products. SPR binding curves gained with the long single stranded DNA products in a concentration range of 0.01-1000 nM in buffer and in 1% serum are shown in Fig. 4a and 4b, respectively.



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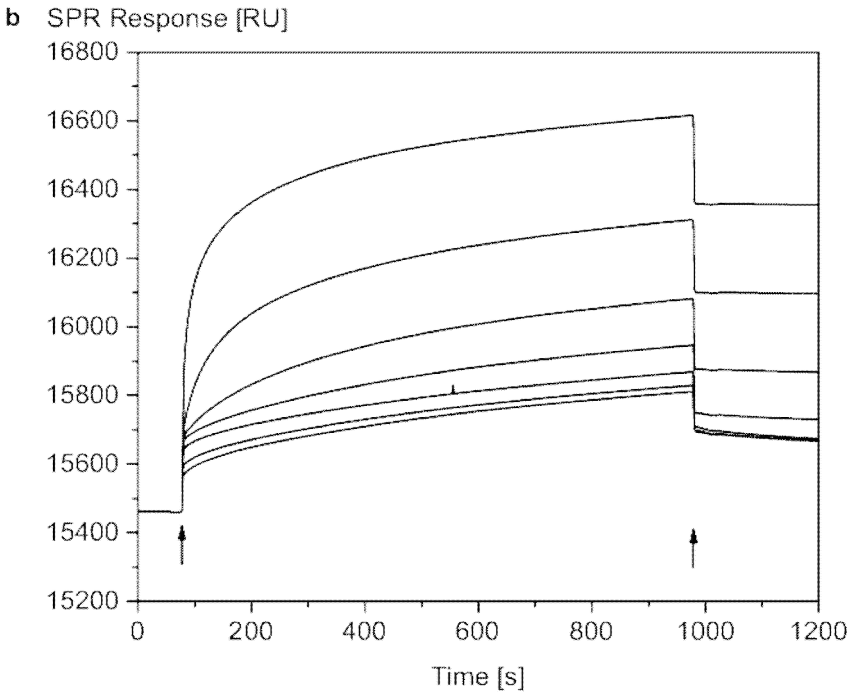

Fig. 4 *Hybridisation of long PTGS2-123 DNA strands* ***a*** *in buffer and* ***b*** *in 1% serum to manually dispensed layers of DMT-S-S-PTGS2-27 and Lipa-DEA. The order of curves is given according to concentration, the lowest curve representing 0.01-nM (Fig. 4a) or 0-serum (Fig. 4b), and then the following, a concentration ten times higher. The uppermost curve in both figures represents a 1000-nM DNA sample. The hybridisation response for the lowest detectable and for the highest measured concentrations are given in Table 1.*

Fig. 4a shows that the 0.1-nM and 1-nM PTGS2-123 ssDNA strands in buffer (the second lowest concentration in Fig. 4a) resulted in a response of 60 and 130 ± 5 RU, respectively. The FBAR resonator showed a response of 30 ± 20 kHz for both PTGS2-123 and CALCA-92 at 1-nM (Table 1). This corresponds to a target surface density of about 7 ± 5 ng/cm^2. The surface density is only half of that obtained by SPR (13 and 16 ± 0.5 ng/cm^2 for PTGS2-123 and CALCA-92, respectively). The NSB of BSA and the

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non-complementary DNA for PTGS2-123 were 30 and 20 ± 5 RU for SPR and below 10 kHz for FBAR (Table 1), suggesting that the detection limits for both SPR and the FBAR were in the nanomolar range.

Hybridisation with long PTGS2-123 and CALCA-92 DNA products in buffer at the highest concentration (1-μM) measured showed a response of 900 and 740 ± 10 RU and 290 and 330 ± 20 kHz as measured with SPR and the FBAR, respectively (Table 1). This gives a surface density of 90 ± 1 ng/cm^2 for PTGS2-123 as measured with SPR and a slightly lower value (66 ± 5 ng/cm^2) for the FBAR (Fig. 5a). Hybridisation with CALCA-92 gave the same surface density for both methods (74 ± 1 and 75 ± 5 ng/cm^2 for SPR and the FBAR, respectively; Fig. 5b). The standard curves, however, show that the surface densities obtained for all but the PTGS2-123 are in the same range for SPR and the FBAR, if the frequency change is assumed to be due to the mass changes only. If there were a viscosity contribution, a higher change in resonance frequency would be expected. DNA strands with 92 and 123 base pairs would have a length of approximately 30 and 40 nm, as the nucleotide length is 0.33 nm [34]. The thickness of the hybridised layer is thus significantly above the FBAR penetration depth. The penetration depth of an FBAR operating at a frequency of 800 MHz is around 20 nm [33]. This might be the reason for the low resonance frequency observed.

Another explanation for the low frequency change obtained might be that the surface coverage of the FBAR was less than that of SPR. In fact, the difference in the results of the PTGS2-123 might be due to a difference in assembly of the DMT-S-S-PTGS2-27 probe on the FBAR surface. Assembly on the SPR sensor chips gave a similar surface density for the DMT-S-S-PTGS2-27 probes despite being assembled *in situ* or by dispensing, whereas the response decreased by half for the DMT-S-S-CALCA-25 probe

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as earlier discussed in section 3.1. The dispensing on the small-sized pixels of the FBAR might also have been more difficult for the DMT-S-S-PTGS2-27 probe.

The binding curves for the same samples spiked in serum as measured with SPR are shown in Fig. 4b and with both SPR and the FBAR in Fig. 5c-d. The responses caused by the serum corresponded to 200 ± 50 RU or 20 ± 5 ng/cm^2 with SPR and 160 ± 20 kHz or 36 ± 5 ng/cm^2 with FBAR, respectively. The FBAR response was about 40% higher than that of the SPR and could be attributed to a viscosity effect. The same accounts for the NSB of BSA that also gave a 40% higher response with the FBAR (Table 1). The values obtained for serum were subtracted from the values of the DNA-spiked samples. The response for the lowest PTGS2-123 ssDNA concentrations (0.01–0.1-nM) did not differ much from that of the serum. A 1-nM PTGS2-123 ssDNA concentration gave a response of 75 kHz and 80 ± 10 RU, corresponding to 17 and 8 ± 1 ng/cm^2 as measured with the FBAR and SPR, respectively (Table 1, Fig. 5c). In the case of CALCA-92, the corresponding responses after the serum baseline subtraction were 65 kHz and 60 ± 10 RU, that is 15 and 6 ± 1 ng/cm^2, respectively, for the FBAR and SPR at a 1-nM CALCA-92 concentration (Table 1, Fig. 5d). Fig. 5d shows that only minor differences in the surface densities as measured with SPR and the FBAR over the whole concentration range studied could be observed.

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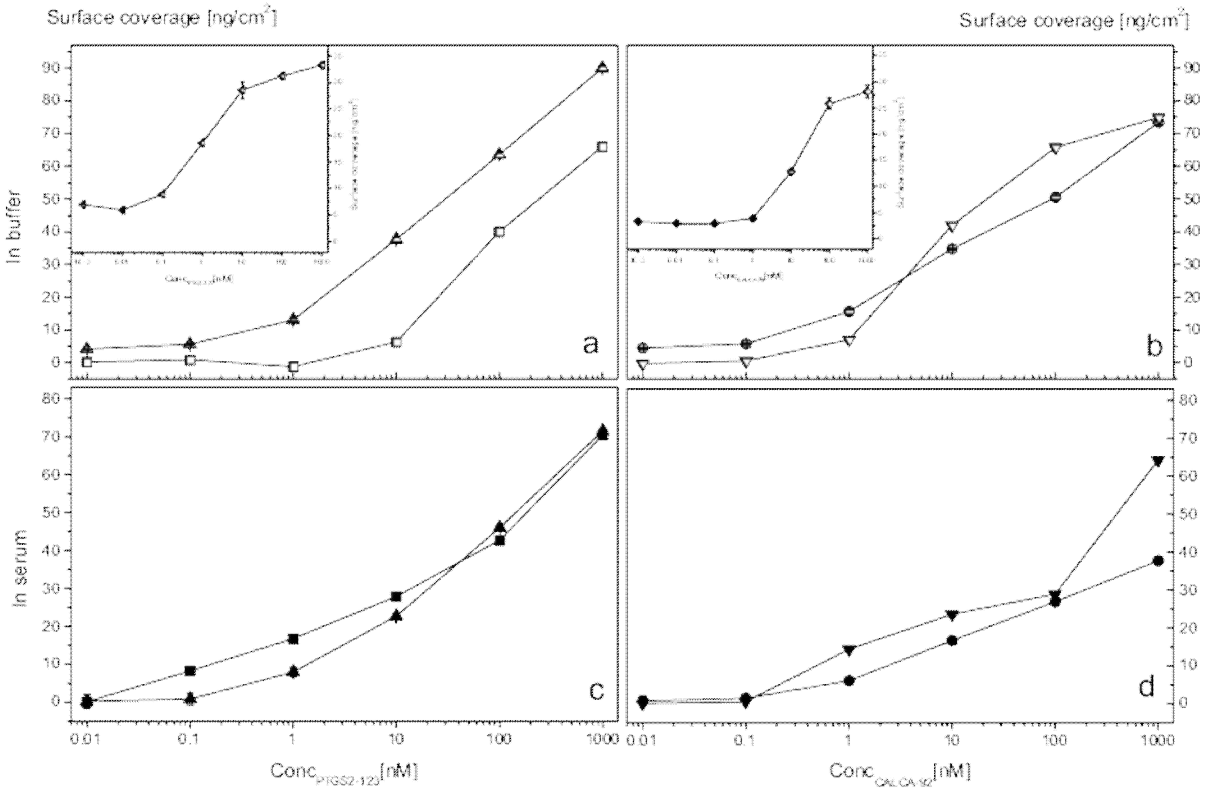

Fig. 5 *Hybridisation of long ssDNA strands to layers of Lipa-DEA and A, C) DMT-S-S-PTGS2-27 or B, D) DMT-S-S-CALCA-25 as measured with SPR (Δ,○) and the FBAR resonator (□, ▽), respectively. The samples were diluted either in buffer (Figures A and B, open symbols) or in serum (Figures C and D, filled symbols). The insets show the SPR curves for hybridisation with short complementary A) PTGS2-27 and B) CALCA-25 strands in buffer.*

The surface densities for a 1000-nM PTGS2-123 solution diluted in 1% serum were 70 and 72 ± 1 ng/cm^2 as measured by FBAR and by SPR (Table 1 and Fig 5c) and for a 1-µM CALCA-92, 64 and 38 ± 1 ng/cm^2 (Table 1 and Fig 5d), respectively. Though, for a 100-nM CALCA-92 solution diluted in serum, the surface densities were 28 ± 1 ng/cm^2 with both methods. The target densities in serum at 1-µM concentration

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correspond to $1.4 \pm 0.01 \times 10^{12}$ targets/cm^2 for PTGS2-123 (720 ± 10 RU, Table1) and $1.5 \pm 0.01 \times 10^{12}$ targets/cm^2 for CALCA-92 (380 ± 10 RU, Table 1). The target density curves are more or less alike in serum (Fig. 5c-d), indicating that the FBAR resonator can be used for detection of PCR-amplified samples from serum when coated with layers of probes and lipoamide. It is known that FBARs might be sensitive to the water content and viscoelastic properties of the layer [35-36]. However, due to the similar responses obtained here for the long DNA strands by SPR and the FBAR, nothing can be said about the viscosity contribution.

4. Conclusions

We have demonstrated that surface plasmon resonance (SPR) and film bulk acoustic resonators (FBAR) can be used for hybridisation of long, non-labelled DNA products diluted in 1% serum with nanomolar sensitivity. Oligonucleotides were allowed to assemble on the sensor surfaces for 10-15 min, whereafter the surfaces were post-treated with a repellent lipoamide, Lipa-DEA. The background noise from the serum was effectively minimised through use of Lipa-DEA. No contribution from a viscosity change of the layer could be observed because the surface densities obtained were in the same range and even higher for the long strands as measured with SPR. SPR can be used for monitoring short strands in buffer, but in serum, the response is too low to be distinguished from the non-specific binding of serum proteins.

The detection limit seems to be in the range of 1-nM for both FBAR and SPR for samples measured in buffer as well as for samples diluted in serum. Thus, long ssDNA strands spiked in serum down to a concentration of 1-nM can be detected. This concentration is very low, taking into account that no labelling is needed. When the length of the pairing strand is increased and the non-fouling properties of the surface

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improved, the response caused by the hybridisation can be distinguished nicely, even for the samples spiked in diluted serum.

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