Anal Bioanal Chem (2009) 393:1173–1182
DOI 10.1007/s00216-008-2555-1

ORIGINAL PAPER

Label-free detection of DNA mutations by SPR: application to the early detection of inherited breast cancer

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Received: 1 August 2008 / Revised: 24 November 2008 / Accepted: 28 November 2008 / Published online: 23 December 2008

Abstract A screening analysis of DNA hybridization and the presence of DNA mutations using an surface plasmon resonance (SPR) biosensor is shown. The influence of lateral and vertical spacers, as well as several hybridization conditions, was studied to optimize the differentiation between fully complementary and mismatched DNA strands. Our results demonstrated that SPR biosensors were able to detect mismatch sequences related to inherited breast cancer, with high specificity and sensitivity. Using PCR synthetic sequences as targets, mutant sequences were clearly discriminated from fully complementary ones, and detection limits below 50 nM were achieved.

Keywords SPR biosensor · DNA hybridization · DNA mutation · BRCA-1 · Breast cancer

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Introduction

Detection of DNA mutations is of crucial interest in molecular biology. Particularly, it is a relevant tool for the diagnosis of several diseases, like some types of cancer related to inherited mutations. Some of the current methods for the identification of mutations have large turn-around times; are complex, tedious, and expensive; or require the use of labels [1]. Other methodologies, such as microarray technology, still present many uncertainness, like assay reproducibility, sensitivity, correlation of signal to sample abundance, and reliability to predict disease conditions [2, 3]. Biosensor devices, such as surface plasmon resonance sensors (SPR), are an interesting alternative to conventional DNA-hybridization screening methods because, in a few minutes, they can analyze biomolecular interactions in real-time and in a label-free way.

SPR biosensing is a widespread technique, and several commercial devices based on this technique are available. The sensing mechanism is based on the refractive index changes at the interface of a gold sensor chip when biomolecules interact with immobilized ligands on the sensor surface. The changes in refractive index are detected via angle changes of a reflected laser beam and are directly related to the amount of sensor surface-bound biomolecules [4].

Besides that SPR methodology has been used for many different applications [5], DNA detection still has several limitations to be overcome, particularly, those regarding sensitivity and selectivity to clearly identify mutations [6–15]. Usually, DNA detection by conventional SPR instruments is performed in the micromolar range of target probe [6–9, 12–16], and only a few works have reached the nanomolar range [8, 10, 11]. There are few examples of label-free detection in the subnanomolar range for short DNA probes; however, when trying to analyze mismatched

probes, the reported detection limits are considerably higher [17].

In order to boost up SPR detection sensitivity, various efforts have been made to reach the subnanomolar range, or even femtomolar detection limits [18]. The strategies implied the use of: (1) labels or amplification methods like DNA-capped gold nanoparticles [11], samples tagged with particles like polymer microspheres in a sandwich assay format [19], or enzymes [20], or (2) the employment of nonconventional SPR detection schemes [18, 21, 22].

In the case of PCR samples, it is particularly difficult to achieve low detection limits using conventional SPR devices, and reported values, without using amplification strategies, are typically above 100 nM [10, 15]. Label-free approaches have the ability to provide simple assay procedures; therefore, it is relevant to optimize the SPR methodology for analyzing mutations without labeling in order to be competitive with routine analysis methods, especially in the case of PCR samples.

In order to reach such optimization for the analysis of mutations in PCR targets, we have followed three different strategies:

1. To control the immobilization of the oligonucleotide probe via thiol chemistry in order to reach high probe densities avoiding physisorption
2. To enhance the hybridization efficiency by improving target accessibility
3. To increase target selectivity to clearly differentiate between fully and nonfully complementary targets

Thiol chemistry is a common method for immobilizing biomolecules on gold surfaces. Ideally, the thiol-derivatized DNA molecules interact with the surface exclusively through the sulfur atom, but it is also possible that the nitrogen-containing nucleotide side chains interact directly with the surface in a "lying flat" physisorbed configuration if the immobilization density is not well controlled [23]. On the other hand, if the DNA monolayer is too compact, steric hindrance forces can prevent the target probes from accessing the monolayer in order to reach the complementary sequence. Studies of oligonucleotides attached to gold via thiol linkers suggest that an efficient hybridization occurs when the DNA probe strands are laterally spaced and when they have an upright orientation with respect to the surface [6, 23–25].

The use of molecules acting as lateral spacers such as mercaptohexanol (MCH) [23] or mercaptoundecanol [6] has been proven to help control DNA immobilization density. They avoid physisorption phenomena and increase target accessibility by favoring an upright orientation. These organic molecules have been frequently reported in the literature; however, the use of spacers of a different nature, such as DNA sequences, is less widespread. In this work, both MCH and DNA sequences have been tested as spacer elements that can enhance target accessibility and hybridization efficiency.

Another additional way to increase target accessibility is the insertion of a nucleotidic sequence in the immobilized probe, which acts as a vertical spacer. This spacer sequence is not involved in the hybridization event and its role is just to separate the specific sequence a distance away from the surface in order to favor hybridization from a steric point of view.

To achieve a better target selectivity, since a mismatched duplex is not as stable as that formed by a fully paired one, differentiation between both could be possible by controlling experimental variables affecting the hybridization at the transducer–solution interface. High stringency conditions slightly destabilize duplexes, and it is a simple way to favor hybridization of perfectly matching sequences with respect to their imperfect counterparts, allowing a higher degree of differentiation between both. High stringency can be achieved using a hybridization buffer of low ionic strength as ions are required to stabilize DNA's negative charges. Another way is the introduction of denaturing agents such as formamide ($H–CO–NH_2$) in the hybridization buffer. Formamide competes with the nucleotide bases in the formation of hydrogen bonds, thereby destabilizing the duplex structure and increasing specificity and selectivity of the detection.

All the above factors (the effect of lateral and vertical spacers, the use of formamide and different stringency conditions) have been studied and applied to the detection of mutations at the BRCA1 gene, which is involved in the development of inherited breast cancer. The BRCA1 gene functions as a tumor suppressor gene encoding a protein that is involved in DNA repair. Women carrying mutations in this gene have a dramatically increased risk of developing breast and ovarian cancer during their lifetime. The identification of these mutations is of relevance because interventions such as early breast cancer screening can decrease morbidity and mortality for these patients [26, 27].

In our experiments, short probes, 12 and 25 mer long, were first used to optimize the immobilization and hybridization conditions, which are employed afterwards for PCR target detection. Synthetic PCR DNA sequences are used as models for PCR samples. This synthetic PCR samples have been designed to be identical to DNA sequences obtained after PCR of the BRCA1 gene from patients carrying the selected mutation. The mutations of the BRCA1 chosen for this work were 916delTT and R1443X. Both mutations are involved in the inheritance of a greater susceptibility for the development of breast cancer [28]. 916delTT implies a deletion of two thymines causing a frame shift mutation in BRCA1 gene in exon 11, at codon 266, leading to a stop codon at position 285 (E266fsX285). R1443X is a trans-

version of a cytosine into a thymine, leading to a nonsense mutation in BRCA1 gene (Arg1443ter) in exon 13, codon 1443.

For biosensing detection, a commercial SPR biosensor (SENSIA SL, Spain) has been employed. In addition, ex-situ methods like fluorescence and radiolabeling have been used as reference techniques for some experiments.

Experimental

DNA sequences

The DNA sequences used for the analysis are shown in Table 1. Sequences used for immobilization bear a thiol linker (C6-SH) at the 5′ end, whereas target sequences employed for testing conditions by ex-situ experiments (fluorescence or radiolabeling) are labeled with a CY3 fluorophore or ^{32}P. For detection of the mutations related to breast cancer by SPR, synthetic PCR sequences corresponding to the mutant and wild-type phenotype of 916delTT and R1443X mutations were employed.

As we are only interested in identification of the mutant phenotypes, our experiments are designed to have as the immobilized probe the one which carries the selected mutation. In this way, only the mutant target sequence will be fully complementary to the immobilized one, giving, for the optimized conditions, higher SPR signals than the non-mutation-carrying wild-type target. Wild-type and mutant targets are synthetic sequences that simulate the corresponding PCR products obtained from real patients. They are identical in the nucleotidic composition to the expected amplified DNA, bearing a 13–15-mer region, which is involved in the hybridization process, flanked by two extra DNA sequences at its 5′ and 3′ sides that do not participate in the hybridization. Experiments using these synthetic sequences ensure that our protocol will be valid for future tests with patient samples. Specifications of these sequences are shown in Table 2.

DNA immobilization and hybridization

Experiments were performed using a SPR biosensor and ex-situ methods. For the biosensor, immobilization of DNA probes was carried out in flux at a rate of 12 μl/min using a 1-μM sample prepared on a volume of 300 μl in 50 mM phosphate buffered (PB) solution with 0.5 M NaCl, pH 7. Hybridization was accomplished at room temperature using a sample target volume of 300 μl in buffers of different stringency or containing a percentage of formamide, at neutral pH. The tested conditions were: 30 mM PB, 1×-SSC (0.15 M in NaCl, 0.015 M in sodium citrate), 0.5×-SSC, 3×-SSC, and 5×-SSC, respectively. When a percentage of formamide was introduced, the employed buffer was 5×-SSC.

For ex-situ measurements, 1- and 10-μM samples used for immobilization and hybridization, respectively, were directly dropped onto the gold surface. The chip was kept under a humid atmosphere at 30 °C for 3 and 1 h, respectively, and then rinsed with the immobilization or hybridization buffer, whichever applies. Finally, the chip was rinsed with water and air-dried under nitrogen flux.

SPR experimental set up and sensor chip preparation

The SPR sensor system (SENSIA S.L., Spain) used for the experiments works with the Kretschmann configuration and monitors the binding events in real time by carrying out

Table 1 Oligonucleotide sequences and modifications

Name	Oligonucleotide
Ex-situ experiments	
I-12T0	SH-$(CH_2)_6$-3′-AACGACGGCCAG-5′
I-12T5	SH-$(CH_2)_6$-3′-C6-TTTTTAACGACGGCCAG-5′
I-12T10	SH-$(CH_2)_6$-3′-TTTTTTTTTTAACGACGGCCAG-5′
I-12T15	SH-$(CH_2)_6$-3′-TTTTTTTTTTTTTTTAACGACGGCCAG-5′
I-12T20	SH-$(CH_2)_6$-3′-TTTTTTTTTTTTTTTTTTTTAACGACGGCCAG-5′
Comp12mer-Cy3-P32	5′-^{32}P -CTGGCCGTCGTT-Cy3-3′
SPR experiments (12- and 25-mer probes)	
I-12mer	SH-$(CH_2)_6$-5′-GACCGGCAGCAA -3′
I-12T15mer	SH-$(CH_2)_6$-5′-TTTTTTTTTTTTTTTGACCGGCAGCAA -3′
Comp12mer	5′- TTGCTGCCGGTC -3′
Cont12mer	5′-AACGACGGCCAG-3′
I-25mer	SH-$(CH_2)_6$-5′-GACGTTGTAAAACGACGGCCAG-3′
Comp25mer	5′-CTGGCCGTCGTTTTACAACGTC-3′
EMM25mer	5′-**G**TGGCCGTC GTTTTACAACGTCGTG-3′
IMM25mer	5′-CTGGCCGTCG TTTTA**G**AACGTCGTG-3′
Cont25mer	5′-CACGACGTTGTAAAA CGACGGCCAG-3′

Table 2 Sequences employed for the detection of breast cancer related mutations

916delTT (Del 2 T)
SH-M-916delTT: Immobilized sequence (mutant phenotype)
SH-(CH2)6-5′-TTTTTTTTTTTTTTT**GTTCTGTCAAACT**-3′
DNA-L1Spacer: DNA lateral spacer
SH-(CH2)6-5′-TTTTTTTTTTTTTTTAAATCTTAGTGTCC -3′
M-916delTT: Fully complementary sequence (mutant phenotype)
5′-TGCCACATGGCTCCACATGCA**AGTTTGACAGAAC**TACCCTGATACTTTTCTGGATGCC-3′
WT-916delTT: Mismatched complementary sequence (wild-type phenotype)
5′-TGCCACATGGCTCCACATGCA**AGTTTGAAACAGAAC**TACCCTGATACTTTTCTGGATGCC-3′
Negative control
5′-TGCCACATGGCTCCACATGCA**TCAAACTGTCTTG**TACCCTGATACTTTTCTGGATGCC-3′
R1443X (C->T)
SH-M-R1443X: Immobilized sequence (mutant phenotype)
SH-(CH2)6-5′-TTTTTTTTTTTTTTT**GACCTGTGAAAT**-3′
DNA-L2Spacer: DNA lateral spacer
SH-(CH2)6-5′-TTTTTTTTTTTTTTTAGAATCCCCAG-3′
M-R1443X: Fully complementary sequence (mutant phenotype)
5′-TTTTCTGATGTGCTTTGTTCTGG**ATTTCACAGGTC**CTCAAGGGCAGAAGAGTCACTTATG-3′
WT-R1443X: Mismatched complementary sequence (wild-type phenotype)
5′-TTTTCTGATGTGCTTTGTTCTGG**ATTTCGCAGGTC**CTCAAGGGCAGAAGAGTCACTTATG-3′
Negative control
5′-TTTTCTGATGTGCTTTGTTCTGG**TAAAGTGTCCAG**CTCAAGGGCAGAAGAGTCACTTATG-3′

detection at a fixed angle of incidence. The device has two flow cells (300 nL each) and a sensing area of 3 mm^2. The sensor chip is a microscope cover glass slide (10×10× 0.15 mm) coated with 2 nm of chromium and 45 nm of gold. Prior to the experiments, the sensor chip surface is cleaned using organic solvents (trichloroethylene, acetone, and ethanol), rinsed with water, and finally treated with piranha solution (70% H_2SO_4–30% H_2O_2) for a few seconds, then rinsed again with water and dried under nitrogen flux.

The liquid handling system is fully automated and incorporated into the SPR platform. A continuous buffer-flow is delivered onto the sensor surface at a constant speed of 12 μL/min by using a peristaltic pump. Injection of the samples is controlled by diaphragm pumps that load precise volumes of 250 μL into each flow cell via its corresponding injection valve. Samples are then pumped into the flow cell in order to reach the sensor chip at a constant speed of 12 μL/min.

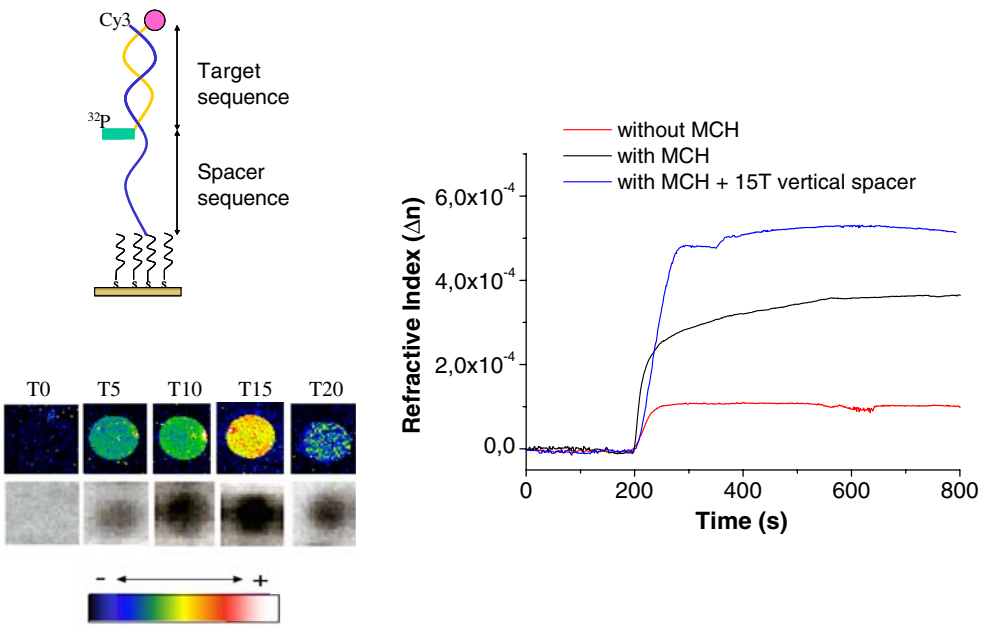

Fig. 1 Simultaneous fluorescent and radiolabeled detection of 12 mer targets upon a 1 μM DNA monolayer using different vertical spacer lengths and example of the effect of lateral MCH and the selected T15 vertical spacer on hybridization followed by SPR

The experimental detection limit was defined as a value in the hybridization $\geq 7 \cdot 10^{-5}$ (3× SD of baseline) and at least three times higher than the standard deviation of the noncomplementary target signal. It has also been established that mismatched targets must present a signal at least three times lower than the standard deviation of the complementary one to be statistically different. The ability to discriminate a point mutation is determined by the following expression (Eq. 1):

Ability to discriminate a mutation(%):

$$100 - \left(\frac{\text{Index}_{\text{Mismatch}}}{\text{Index}_{\text{Complementary}}} \right) \times 100 \quad (1)$$

Ex-situ measurements using fluorescence and radiolabeling based methods

Experiments were carried out on microscope slides coated with 5 nm of chromium and 450 nm of gold purchased from EMF (USA). These experiments were done to find the optimal length of the receptor probes, giving a maximum hybridization signal. The hybridization is detected either by fluorescence using a Scan Array 4000 (GSI Lumonics) for fluorescent probes or by radiography for radiolabeled probes.

Results

The effect of vertical and lateral spacers was firstly evaluated using 12 mer probes immobilized at 1 μM. Immobilization and hybridization were carried out in 0.5 M NaCl–50 mM PB and 1 M NaCl–50 mM PB respectively (Fig. 1). The aim of vertical spacers is to separate the specific sequence from the surface, making it more sterically available for hybridization. This effect was evaluated ex-situ using a dually fluorescent and radiolabeled 10 μM target probe. A thymine based spacer sequence was chosen due to the low affinity of thymine bases towards gold surfaces, contributing to keep the DNA immobilized strands in an upright configuration [25]. Different vertical spacers, ranging from 5 to 20 thymidines, were employed. Figure 1 shows that when the length of the spacers increases, the accessibility is enhanced. The optimal hybridization signal, either by fluorescence or radiolabeling measurements, was achieved with the 15 thymidine spacer. Longer spacers gave rise to lower surface coverage, reducing the availability of DNA probes in agreement with other authors [29]. Hybridization in the SPR sensor using a 500 nM target in a 15-T modified DNA monolayer, showed an equivalent result, demonstrating that this strategy successfully enhanced the hybridization signal (Fig. 1).

To evaluate the effect of introducing a lateral spacer among immobilized probes, 1 mM MCH was added after DNA immobilization. Figure 1 shows this effect using a 500-nM target. MCH introduction into the monolayer considerably enhanced the hybridization signal in agreement with previous results [23, 24, 29–31], clearly indicating that this treatment also favors target accessibility.

Both factors, the use of MCH and the 15-T spacer, were evaluated on larger probes of 25 mer, where an analysis of target mutations detection was carried out. Hybridization was done in the same buffer conditions as those established for 12-mer probes. These conditions allowed good detection limits for complementary targets in the range of 10 nM (Fig. 2); however, discrimination among mismatches was extremely hard to achieve. Neither internal nor external mismatches were clearly distinguishable from the complementary ones (data not shown). Therefore, different stringency conditions were evaluated, by controlling ionic strength during hybridization. Figure 3 shows that as stringency is increased by lowering the ionic strength of the hybridization

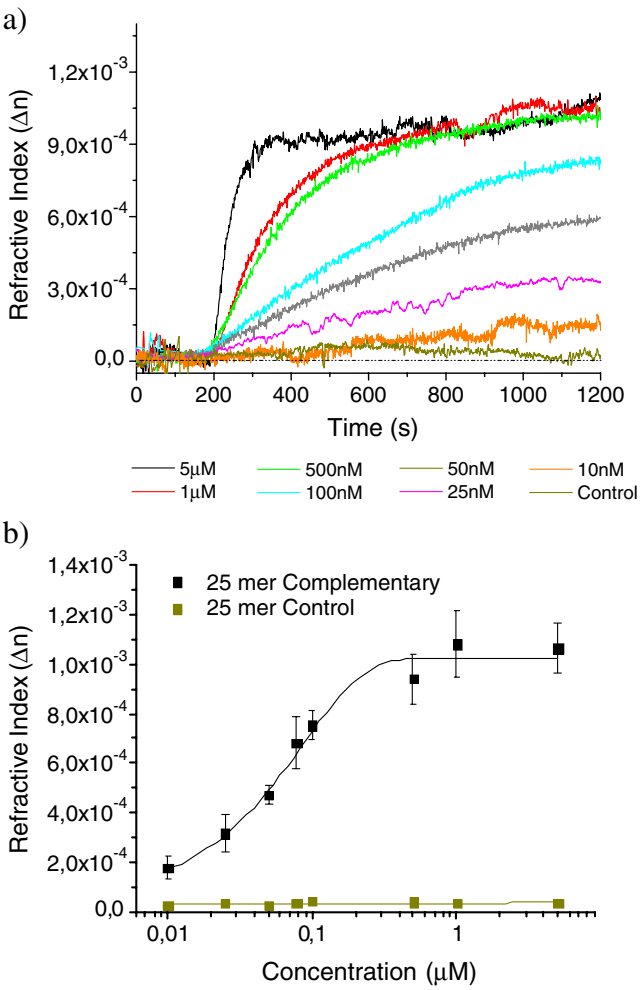

Fig. 2 **a** 25-mer hybridization signals in PB 50 mM–NaCl and **b** calibration curve

buffer, the hybridization signal is reduced, due to the duplex destabilization. Besides this decrease of hybridization signal, increased stringency yields a better level of selectivity between fully matched and mismatched targets. Among the evaluated buffers, PB buffers, even at high stringency conditions such 30 mM PB, did not give enough selectivity to discriminate between targets; this was only possible on sodium saline based ones. At SSC-1× and 0.5×, differences between fully and not fully paired targets were clearly distinguishable; however, hybridization signal at SSC-0.5× was dramatically low, and therefore, SSC-1× was chosen as the ideal condition for the identification of mutations. In such ionic strength conditions, a 100-nM limit of detection was found. Mismatched targets were clearly distinguishable from the fully paired ones only at target concentrations of 500 nM and higher (Fig. 3). For this concentration range, the abilities to discriminate point mutations were 13–36% and 21–35% for external and internal mismatches, respectively. Differentiation between internal and external mismatches, from a statistical point of view, was not possible to achieve in these conditions.

The above hybridization conditions were employed as a starting point for the analysis of mutations related to breast cancer gene when using the synthetic PCR targets carrying the 916delTT mutation. The total length of this sequence was 56 mer; however, the region involved in hybridization

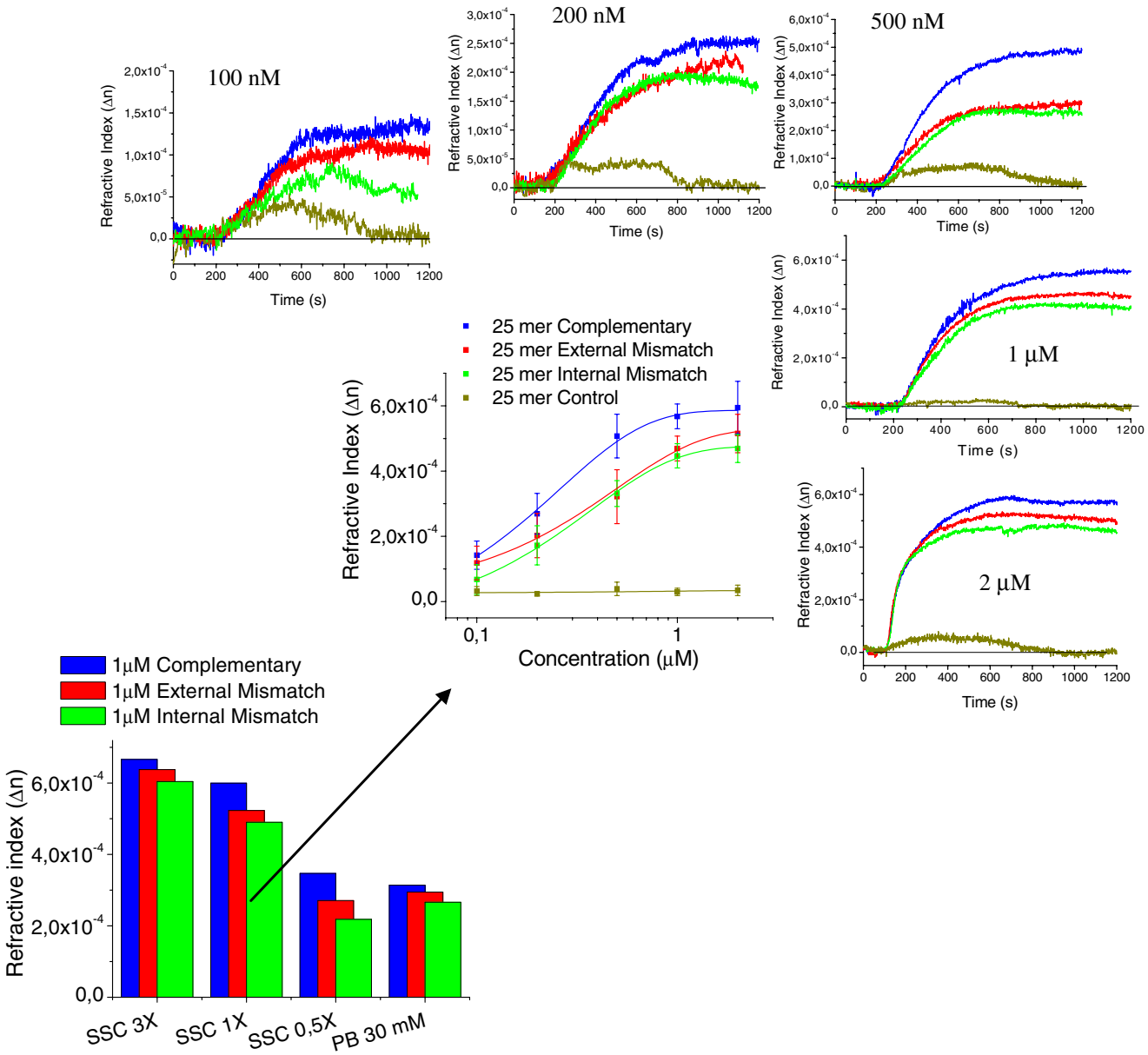

Fig. 3 Test of hybridization under different buffer conditions to discriminate between 1 µM fully complementary and mismatch sequences. Calibration curve for SSC 1× condition is also shown

was only 13 mer. This length was chosen because the optimal size of an oligonucleotide probe for effectively detecting the presence of a single mismatched base pair is between 11 and 15 nucleotides [32]. The hybridization reaction was done using immobilization probes bearing the 15-T spacer at two conditions, with and without MCH lateral spacer, as shown in Fig. 4. For both cases, results showed a 100-nM detection limit, similar to that obtained for 25-mer targets, which is lower than expected as PCR targets have higher molecular weight than the 25-mer ones. Conversely, selectivity was dramatically enhanced and the wild phenotype was strongly distinguishable from the mutant one. The ability to discriminate mutations was in the range of 35% for 500 nM 25-mer targets, whereas an ability of about 80% was found for PCR targets of the same concentration. These results could be explained in terms of the higher sterical hindrance of PCR targets when compared to shorter probes, like 25-mer ones. In the PCR target, only the internal region of 13 bases is involved in hybridization, while the extra DNA sequence at both sides would hinder hybridization from a steric hindrance point of view. This sterical factor could decrease the hybridization efficiency, although it might help to destabilize the not perfectly matched duplex more dramatically than the perfect one, thereby favoring selectivity.

The signals obtained for the MCH-treated and nontreated monolayers presented some differences. Both conditions gave the same detection limit, but the non-MCH-treated one showed a slightly better selectivity towards fully and nonfully matched targets. This could be due to the removal effect of the DNA receptor probes after MCH treatment, which contributes to an increase in accessibility, but on the other hand, it decreases the number of available receptors. As PCR targets are more inefficient to hybridize than short targets, the decrease in the number of immobilized probes would be more relevant than the increase in accessibility, lowering the hybridization efficiency when MCH is used. For that reason, the employment of MCH is not convenient for PCR target detection, and other strategies should be addressed. Among available ones, it has been suggested that longer lateral spacers might act more efficiently than the shorter ones (like MCH) [6]. Usually, organic spacers are employed, and to our knowledge, the use of other kinds of molecules, as DNA sequences, has only been employed by Demmers et al. [33]. They used DNA spacers in gold nanoparticles showing improved results in the hybridization efficiency. We have tried this latter strategy using a DNA sequence as lateral spacer with the 916delTT probe (carrying the 15T vertical spacer) through a coimmobilization procedure in order to reach lower detection limits.

Receptor and lateral spacing DNA sequences were immobilized simultaneously in flux onto the sensor chip

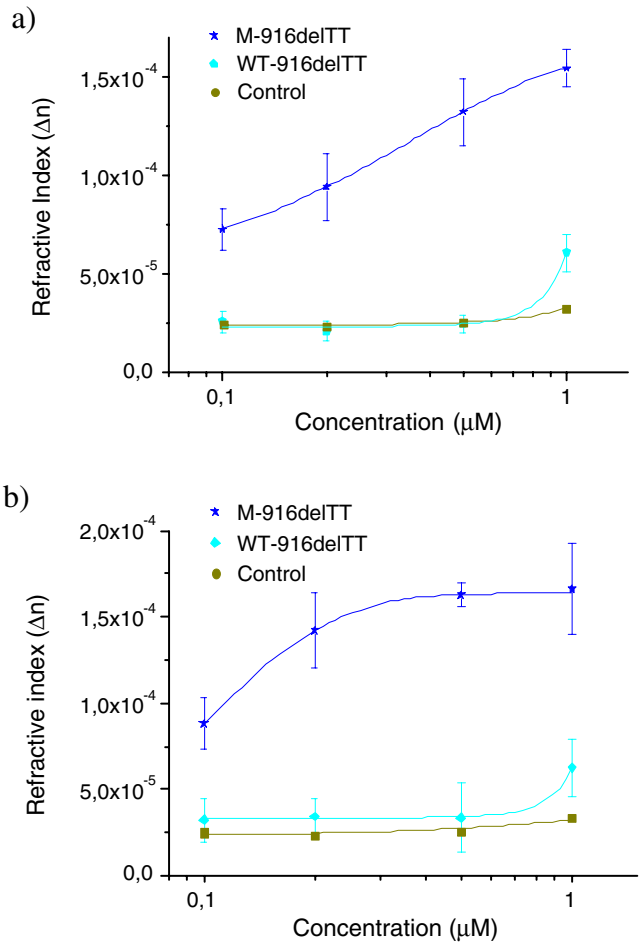

Fig. 4 Hybridization curves for 916delTT targets corresponding to the mutant (*dark blue*) or wild-type (*light blue*) phenotypes at two different conditions **a** using MCH and **b** without MCH treatment

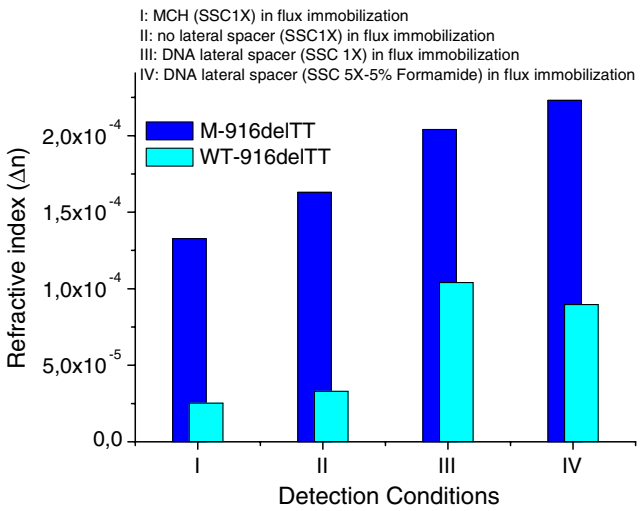

Fig. 5 In flux SPR hybridization signals of 500 nM 916delTT target using a lateral DNA spacer, MCH or no lateral spacer. It is also shown that the use of formamide increased selectivity between mutant and wild-type phenotype

at a concentration ratio of 2:2 μM, and detection was performed in SSC 1×. As it is shown in Fig. 5, for a 500-nM target, the use of a DNA spacer sequence gave a higher hybridization value than when MCH or no lateral spacer was used. Besides this increase in sensitivity, the wild-type-related signal was found to be higher with this coimmobilization strategy, which lowered its ability to discriminate between mutant and wild-type phenotypes. This lower differentiation could be explained by the immobilization in flux, which implies shorter times of immobilization; as a consequence, the two DNA sequences would need higher immobilization times to properly organize and achieve a configuration that favors hybridization while hindering physisorption phenomena.

The selectivity to differentiate phenotypes when using DNA lateral spacers could be improved using : (1) higher stringency conditions introducing agents like formamide and (2) longer immobilization times, which are demonstrated to benefit DNA immobilization through organization of the monolayer [34].

For the first approach, different formamide percentages were evaluated (data not shown), being 5% in nonionic stringent conditions, like SSC-5×, the one which gave the best hybridization signal. This condition produced slightly higher SPR signals and a higher level of selectivity to differentiate between complementary and mismatched targets than those found when performing hybridization in SSC 1× (Fig. 5). However, the best results were found when the immobilization is accomplished ex-situ for 3 h and hybridization is achieved in SSC 5×–5% formamide. In these conditions, hybridization was greatly improved, by one order of magnitude, and, in addition, selectivity was very high as well. Target concentrations of 50 nM were clearly detected, giving a refractive index signal of $1.93 \cdot 10^{-4} \pm$

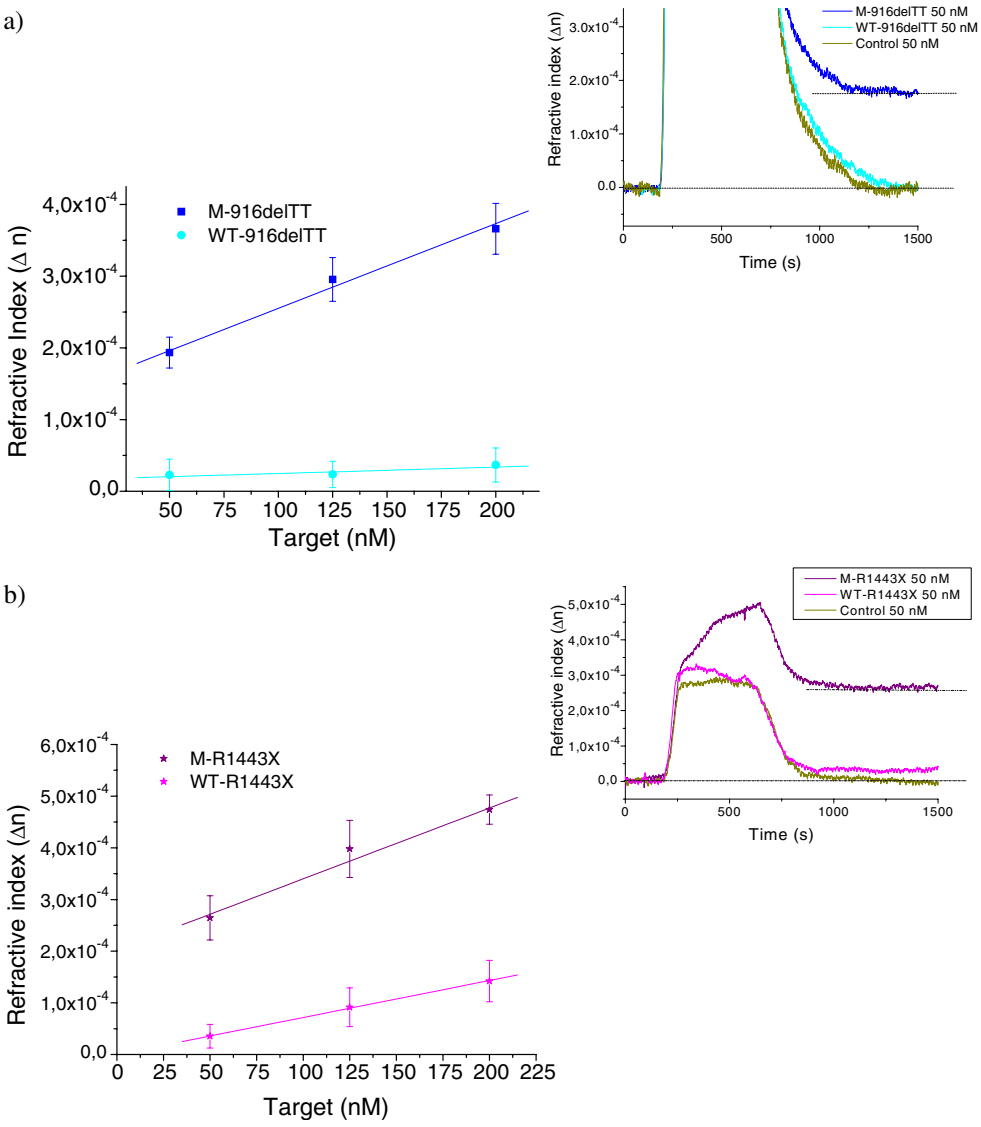

Fig. 6 SPR signals for different target concentrations of 916delTT and R1443X mutant and wild-type targets, respectively. An example of 50-nM detection is also displayed. Immobilization was done ex-situ using a lateral DNA spacer and hybridization was carried out using a 5% of formamide in the hybridization buffer

$2.2 \cdot 10^{-5}$, while an almost negligible signal of the mismatched target was obtained ($2.29 \cdot 10^{-5} \pm 2.2 \cdot 10^{-5}$) (Fig. 6a), indicating that detection limits below 50 nM could be achieved. The mismatch discrimination degree of this approach is around 88–92% for the evaluated concentration range. This value is considerably high when considering values from the literature, as those from Jiang et al., who reported an ability to discriminate mutations of only 34% for detection of 150 nM PCR targets [10].

Our results indicate that a sufficient immobilization time and the use of DNA sequences as lateral and vertical spacers to control the accessibility of target probes constitute an excellent strategy to improve both sensitivity and selectivity for the detection of PCR targets. The same methodology was tested for another breast cancer-related mutation, R1443X. As this mutation implies a change in only one sequence base, a lower selectivity could be expected. For this sequence, the best ratio R1443X-lateral DNA spacer was experimentally found to be 1:0.5 μM (data not shown). Similar results to that of 916delTT were found, with an ability to discriminate mutations of 70–86% for the evaluated concentration range, indicating that these conditions could be extrapolated to the detection of mutations of different nature (Fig. 6b).

Results obtained for our synthetic PCR sequences showed that DNA detection and screening of mutations can be easily achieved by conventional SPR biosensors, without using labels or target signal amplification schemes, allowing excellent levels of sensitivity and selectivity to discriminate between mutant and wild-type phenotypes. To our knowledge, the excellent selectivity and sensitivity levels found for PCR targets with the optimized conditions described in this paper have not been previously reported, placing this methodology as a very suitable platform for label-free early detection of genetic-related diseases, as it is the case of inherited breast cancer.

Conclusions

A new methodology for achieving direct and label-free DNA detection and screening of mutations has been demonstrated. Different strategies, like the use of spacers, either lateral or vertical, were employed. The use of MCH as a lateral spacer was found to increase sensitivity with short DNA target sequences; however, with longer ones, such as PCR targets, this spacer was not suitable. In this latter case, the use of DNA sequences as lateral spacers was found to be the best option to greatly improve hybridization. The use of a vertical spacer of 15 thymidines was also found to be critical in enhancing hybridization, either for short and PCR sequences. Detection limits of 10 nM were obtained for the detection of 25-mer targets, while for the identification of mutations, the limit of detection was 100 nM.

It has been demonstrated that controlling the stringency of the hybridization buffer allowed increasing the selectivity and the differentiation between mutant and wild-type phenotypes. Among different conditions, SSC 1× and SSC 5×–5% formamide were found to be the best ones; however, selectivity and sensitivity was dramatically improved with ex-situ immobilizations using DNA lateral spacer sequences. The optimized methodology to detect hybridization on BRCA1-related mutations has shown a high sensitivity with detection levels below 50 nM, while presenting an ability to discriminate between perfect and mismatched probes in the range of 80%. These results allocate conventional SPR biosensor methodology as a competitive and complementary tool for DNA analysis.

Acknowledgements The authors gratefully acknowledge the financial support from Fundación M. Botín.

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