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Neutralized chimeric DNA probe for detection of single nucleotide polymorphism on surface plasmon resonance biosensor

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ABSTRACT

An implementation of neutralized chimeric DNA oligomer as a probe for sensitive detection of single nucleotide polymorphisms (SNPs) on a surface plasmon resonance imaging sensor is investigated. The chimeric DNA oligomer was synthesized in a conventional DNA synthesizer, containing neutral nucleotides with a methylated phosphate group. The secondary structures and melting points of the chimeric DNA fragment and its complexes with perfect-matched and single-mismatched complementary DNA molecules were examined by using circular dichroism and UV–vis spectroscopy in comparison with the native probe DNA counterpart. The results indicate that the chimeric DNA complexes can form a B-form structure and exhibit high thermostability. Moreover, the hybridization and discrimination efficiency of the chimeric probe DNA for the SNP genotyping were verified by using the SPRi sensor under different experimental conditions. The data reveal the effects of the ionic strength and operation temperature on the selectivity of the chimeric probe DNA for the SNP detection. The hybridization condition with a low ionic strength and high temperature allows the chimeric probe DNA distinguishing perfect-matched and single-mismatched target DNA molecules to the best extent, likely due to the reduced electrostatic repulsive force and presence of the additional methyl group on the backbone. Consequently, the direct and label-free detection with the SPR technique and neutralized chimeric probe DNA can be realized for the SNP genotyping by optimizing the operation condition and sequence design.

1. Introduction

Single nucleotide polymorphisms (SNPs) are frequently occurring genetic variation in the genome that is found in more than 1% of the population (Kypr et al., 2009). They have become important indicators to link sequence variations to phenotypic changes and to allow biologists in detecting genes related to common diseases. Therefore, over a decade a plenty of efforts have been devoted to developing rapid, sensitive, specific and affordable technologies for SNP analysis, which include differential hybridization strategies, single-base extension after amplification, mismatch endonuclease-based detection and DNA sequencing (Chakrabarti and Schwarz, 1999; Chang et al., 2017; Hur et al., 2015; Petrovykh et al., 2003; Piliarik et al., 2010, 2005; Schwarz et al., 1999; Zhao et al., 1999). However, most of them relay on approaches to amplify sample numbers and detection signals, which may lead to high cost, large noises, cumbersome operation and inefficiency in genotyping. In addition, the readouts from aforementioned techniques are not able to interrogate the kinetic and dynamic information from the target DNA fragments with mutant and wild-type reference DNA. Developing a better understanding of DNA hybridization will greatly facilitate the elucidation of molecular mechanisms involved in numerous biochemical processes and the sophisticated relation with diseases (Islam et al., 2017; Piliarik et al., 2010). Therefore, it is highly desirable to develop a new sensing technology for real-time SNP detections in a sensitive and specific way.

Surface plasmon resonance (SPR) biosensor is a popular optical analytical tool in which an analyzed liquid sample with target molecules is brought in contact with a metal sensor surface (Wu et al., 2017). The metal surface supports resonantly excited surface plasmons by coupling the incident light on the basis of fulfilling the phase matching condition. The capture of analyte molecules by the probe elements immobilized on the surface is sensitively detected upon induced refractive index changes at the interface. The SPR biosensors afford label-free and in-situ molecular detection with very high sensitivity for a wide spectrum of

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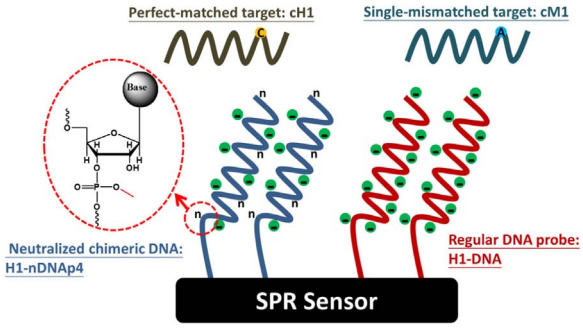
applications, including DNA hybridization events (Homola, 2003; Piliarik and Homola, 2008; Piliarik et al., 2009, 2005; Shushama et al., 2017). Real-time detection of DNA manipulation such as hybridization kinetics, and enzymatic modifications using SPR biosensors was initially reported by Nygren et al. (Nilsson et al., 1995). They demonstrated a methodology to discriminate between probes that are either fully matched to the target sequence or contain single nucleotide mismatches by analyzing hybridization kinetics at different temperatures (Persson et al., 1997). The advances have greatly improved the process for the DNA analysis using traditional approaches and, however, a direct detection for single nucleotide mutation was still required for fast and high-throughput screening. Nakatani et al. introduced synthetic ligands that specifically bind with high affinity to the guanine (G)–guanine mismatch, one of four types of SNP (Nakatani et al., 2001). A series of small specific ligands for targeting mismatched base pairs in DNA duplexes were immobilized on a SPR sensor and used to successfully and directly detect the G-G, G-A and C-C mismatches in hetroduplexes or trinucleotide repeats (Hagihara et al., 2004; Kobori et al., 2004; Nakatani et al., 2001).

Recently, considerable efforts have been contributed to design of DNA analogues with novel properties unfound in nature to improve stability, functionality and binding characteristics. These have been utilized to develop innovative therapeutic agents and new probes for diagnostics. Several non-natural nucleosides have been implemented, such as locked nucleic acid (LNA), peptide nucleic acid (PNA), phosphoramidates morpholino (MORF), and hexitol nucleic acid (HNA) oligomers, which have proven advantages over regular nucleic acids in terms of biostability in body fluids without compromising the specific interaction with complementary target nucleic acids (Briones and Moreno, 2012). PNA is composed of the electrically neutral peptidomimetic backbone, and it retains the ability of forming duplexes with complementary DNA (cDNA) with improved hybridizing affinity. This is attributed to the lack of electrostatic repulsion occurring between DNA strands. In previous works, PNA probes were used for detection of single-mismatched DNA targets with high specificity and sensitivity in adequate buffers and temperatures (Burgener et al., 2000; Lao et al., 2009), and in standard PBS buffer (Ananthanawat et al., 2010). Coordinately, applications of LNA benefit from the conformational restricted sugar motifs and the possibility of synthesizing chimeric molecules, containing both LNA nucleotides and DNA or RNA nucleotides. LNA has been showed also to form the strongest duplexes with complementary RNA, particular microRNA, according to Watson–Crick rules (Obika et al., 1998; Petersen and Wengel, 2003). In addition to PNAs and LNAs, the other uncharged DNA analogues, phosphorodiamidate morpholino oligomers (PMO) and methyl-phosphonate oligonucleotides (MPO), are synthesized for therapeutic applications. The uncharged character of DNA analogues enables to avoid the interferences in a binding reaction from background electric charges in the field-effect transistors (FET) measurements for sensitive DNA detection (Gao et al., 2007). Therefore, on the basis of the current successful examples, the DNA sensors incorporated with artificial probes possess considerable applicability in a growing number of the future analysis of genomics and diagnosis.

We currently described the fully electrically neutral DNA (nDNA) with the “RO-P-O” backbone (wherein R can be methyl, ethyl, aryl, or alkyl group) to evaluate the difference in DNA detection performance on FET and SPR imaging biosensors (Chen et al., 2013). The circular dichroism (CD) spectroscopy analysis indicated that the nDNA holds the capability to specifically hybridize with its cDNA and to form the identical secondary structures as the DNA/cDNA duplex. Furthermore, it was demonstrated that using the nDNA probe in the FET measurement leads to significant improvement in the detection sensitivity due to the electrically neutral property of nDNA to avoid the interference of charge noise from recognition elements and to afford high surface density of probe molecules on sensor chips.

In this work, we demonstrate the feasibility of the chimeric DNA molecule, composed of native and methylated neutral nucleotides, as a probe for sensitive and specific SNP genotyping on label-free real-time SPR imaging biosensor (SPRi) (Scheme 1). The target fragment, cH1, is hemagglutinin 1 DNA in influenza virus strains, which mutates frequently over time and is strongly associated with diverse genetic diseases. The SNP detection for cH1 is critical in infectious disease surveillance and management for reducing the morbidity and mortality of illness. In this study, the methylated nucleotides allow DNA synthesis with native nucleotides in conventional DNA synthesizers to afford ease of implementation in industrial, and versatility in design of sequence and duplex structure. The formation and secondary structure of the duplex of the chimeric DNA probe with target cH1 were verified using CD spectroscopy. The dissociation melting points of the duplexes with and without mismatched point were determined by UV–vis spectrometer with a temperature control module. For the preparation of sensor chips, the chimeric DNA probes were immobilized via aldehyde-amine conjugation chemistry, which was confirmed by X-ray photoelectron spectroscopy (XPS) measurements. Importantly, the SPRi biosensor was employed to investigate the hybridization efficiency of selectively neutralized DNA probe and target cH1. The effects of the ionic strength and hybridization temperature were put into account with a purpose to discriminate from the single base mismatched DNA for potential use in the SNP genotyping. Consequently, given the rise in advanced sensing technologies, we envision that the analytical technology combined with potent biorecognition element and label-free optical sensor could serve as a promising tool in SNP validation in a rapid and sensitive fashion.



Scheme 1. Schematic illustration of probe and target design for SNP detection.

2. Materials and methods

2.1. Chemical reagents

PBS solution contains 10 mM phosphate buffer, 137 mM sodium chloride, and 2.7 mM potassium chloride, adjusted to pH7.4. Orthophosphoric acid, sodium cyanoborohydride, tris(hydroxymethyl) aminomethane (Tris), trimethylamine (TEA), glycerol, 11-amino-1-undecanethiol hydrochloride (MUAM) and 6-mercapto-1-hexanol (MCH) were bought from Sigma-Aldrich (USA). Glutaraldehyde (25%) was purchased from Fluka (USA). Sodium chloride (NaCl), sodium phosphate tribasic, absolute ethanol (99.5%), and ethanolamine (C_2H_7NO) were acquired from Acros Organics (USA). All other chemicals used in this study were reagent grade.

2.2. Regular and neutral sequences

The sequences of regular DNA fragments and their chimeric counterparts were all the same and the difference between these two kinds of DNAs was found in their structure (Table 1). Chimeric DNA was an oligonucleotide analogue and was supplied by Helios Bioelectronics Inc. (Hsinchu, Taiwan) and all oligomers were synthesized by MDBio Inc. (Taiwan). The DNA analogue with the “RO-P-O” backbone (wherein R is methyl group, Scheme 1) used in this study was synthesized by using Fmoc-protected phosphoramidites. The neutralized probe H1-nDNAp4 has four methylated nucleotides in the backbone as noted by the super-

Table 1
The sequence of natural probe DNA, neutralized chimeric probe DNA and their complementary target DNA molecules.

DNA	Identifier	Sequence (5′-3′)
Probe	H1-DNA	NH_2-C_6-CACAC TCTGT CAACC TAC
	H1-nDNAp4	NH_2-C_6-C^nACA^{n}C TC^{n}TGT^n CAACC TAC
Target	cH1	CCATT GTGAC TGTCC TCAAG TAGGT TGACA GAGTG TG
	cM1	CCATT GTGAC TGTCC TCAAG TAGGT TGAAA GAGTG TG

Note: the nucleotides with superscripts "n" in the probe H1-nDNAp4 are the neutralized nucleotides with methylated phosphate groups.

scripts "n". A regular probe DNA (H1-DNA) has a phosphate-deoxyribose backbone and charged phosphate groups on the DNA that are negative in a normal physiological environment (pH 7.4). Moreover, two perfect-matched (cH1) and single-mismatched (cM1) target DNA fragments were synthesized for the hybridization experiments with the probe DNA molecules. The experimental methods for the circular dichroism spectroscopy and melting point analysis for DNA fragments are shown in Supplemental Content.

2.3. SPR imaging sensor

The SPR sensor platform in this study is an SPR imaging system with six flow chambers, which was developed by Homola et al. (Prague, Czech Republic) (Piliarik et al., 2010; Piliarik and Homola, 2008). A narrow-band light source is used and linearly p-polarized by a polarizer. The light beam at 750 nm impinges on a glass substrate and excites SPs at the metal-dielectric interface. The SPR platform with a self-referencing function is able to detect the smallest signal corresponding to the change in the refractive index unit (RIU) better than 10^{-6} (Piliarik and Homola, 2008). It also can be illustrated in terms of protein surface coverage that the sensor can detect the changes are as small as 0.2 pg/mm^2. The SPR chips were prepared by using clean BK7 glass substrates. The glass substrates were pre-coated with an adhesion layer of chromium (thickness approx. 2 nm), and an SP-supporting gold layer with a thickness of 48 nm was subsequently deposited via vapor deposition process at a pressure below 1×10^{-6} Torr. A flow rate of 50 μl/min was controlled in all experiments, and experiments were performed at various temperatures of 27 and 30 °C. For the probe conjugation and XPS characterization, the detailed experimental methods are present in Supplemental Content.

3. Results and discussion

3.1. Detections of regular and neutralized DNA duplex formations

CD is a phenomenon arising from interactions of chiral molecules with circularly polarized light with wavelengths from 200 to 300 nm, where bases of DNA absorb light (Paramasivan et al., 2007). DNA samples, including single-stranded H1-DNA, H1-nDNAp4, cH1, double-stranded H1-DNA/cH1 and H1-nDNAp4/cH1 were prepared at 5 μM in phosphate buffer solutions with ionic strength of 150 mM and 10 mM in Fig. 1. From the experimental data in Fig. 1a, the CD spectra of single- and double-stranded oligomers were very similar in band magnitudes and positions at high ionic strength, i.e. 150 mM. All spectra exhibited positive long wavelength bands at about 260–280 nm and negative bands around 245 nm, which are characteristic signals of the B-form structure. Therefore, the H1-DNA/cH1 and H1-nDNAp4/cH1 duplexes with and without methyl phosphonate backbone appear no significant difference in the secondary structures in agreement with previous works with fully neutral DNA oligomers (Chen et al., 2013; Clark et al., 1997). Moreover, the single-stranded DNA fragments adopted the B-form structure, indicating the presence of self-complementary oligonucleotides (Pearson et al., 2005, 2002). The intramolecular interaction is strongly associated with the oligonucleotide primary structure and experimental environment. DNA fragments consisting of GC-rich trinucleotide repeats and self-complementary oligonucleotides can adopt both a B-form duplex and a hairpin as observed in our experiments in solutions at high ionic strength.

The conformation of DNA can be affected by the environmental conditions, such as pH, concentration of DNA and electrolytes. To emphasize the role of the molecular charge in the backbone of DNA, we particularly paid our attention on the ionic strength of the buffer. At a high ionic strength, complementary two DNA nucleotides associate easily due to the screening of negatively charged phosphate groups in the backbones to reduce the electrostatic repulsion. At the low ionic strength, the conformation and affinity to complementary DNA could be altered. Therefore, we dissolved DNA samples in a buffer solution with a low ionic strength of 10 mM for the CD measurements. In Fig. 1b, the position and shape of the CD spectra are similar to that at high ionic strength, showing the formation of the B-form structures in all samples. Moreover, the amplitudes for the H1-DNA/cH1 and H1-nDNAp4/cH1 duplexes are higher than that for the single-stranded fragments. The difference can be ascribed to the amount of the B-form structure in the samples. We suggest that the single-stranded nucleotides likely form a hairpin structure with a double-helical stem. Because of the single-stranded loop, the duplex to hairpin transition is usually accompanied by reduction of the CD amplitude (Kypr et al., 2009). Herein, the effect of the ionic strength on the formation of duplex structures for methylated and regular DNA was not obvious. Nevertheless, the CD experiments confirmed that the methylation to the phosphate-deoxyribose backbone and ionic strength does not cause any significant influence on the secondary structure of the formed duplex.

T_m is an intrinsic physical parameter of nucleic acid complex, which is an index of the thermal stability of a nucleic acid. T_m is strongly dependent on the base number, base sequence, nucleic acid concentration, solvent conditions, presence of mismatch, nucleic acid analogue structure and so on (Hur et al., 2015). Therefore, the melting points of the H1-DNA/cH1 and H1-nDNAp4/cH1 duplexes were examined to compare their thermostability. As shown in Fig. 2, in the solution with low ionic strength of 10 mM, the melting point of the H1-nDNAp4/cH1 (T_m = 37.3 °C) was higher than that of H1-DNA/cH1 (T_m = 35.6 °C). The finding indicates that H1-nDNAp4 enables the formation of a stable helix structure with its complementary target DNA, likely due to the reduced electrostatic repulsion between two phosphonate backbones. The increments in melting points were observed in the neutral DNA analogues, PNA and LNA, revealing the important role of the backbone charge and conformation in stabilizing complex structure and high hybridization efficiency (Chakrabarti and Schwarz, 1999; Hur et al., 2015; Schwarz et al., 1999).

Moreover, single mismatched target DNA (cM1) formed complexes with probes of H1-DNA and H1-nDNAp4, followed by thermal analysis in the solution with low ionic strength (Fig. 2). The results reveal that the T_m values of H1-DNA/cM1and H1-nDNAp4/cM1 were 33.1 and 33.3 °C, respectively. The temperatures of natural and chimeric DNA fragments with the mismatched target were compatible. However, the difference in the melting temperatures of H1-nDNAp4 between perfect match and mismatch (ΔT_m = 4 °C) was significantly larger than that of H1-DNA (ΔT_m = 2.5 °C). This indicates that H1-nDNAp4 exists favorable tendency to hybridization, making a large difference in the melting temperature between specific hybridization and unpaired hybridization. It is believed that the ΔT_m of the chimeric DNA can be further elevated by careful design of the position of the methylated nucleotides in DNA sequence.

3.2. Immobilization of probe DNA on sensor chips

The modifications were confirmed using XPS to verify elemental

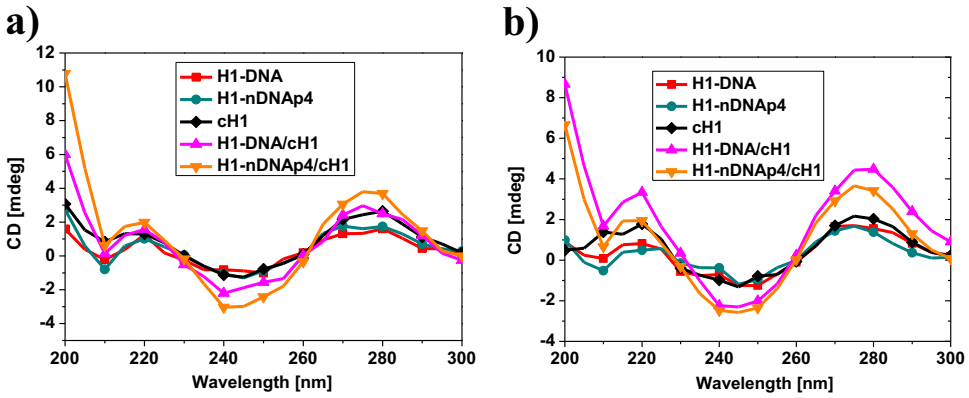


Fig. 1. CD spectra of single- and double-stranded DNA in phosphate buffer solutions with ionic strengths of 150 mM (a) and 10 mM (b). The wavelength of the polarized light ranges from 200 to 300 nm and the DNA samples were prepared at 5 μM.

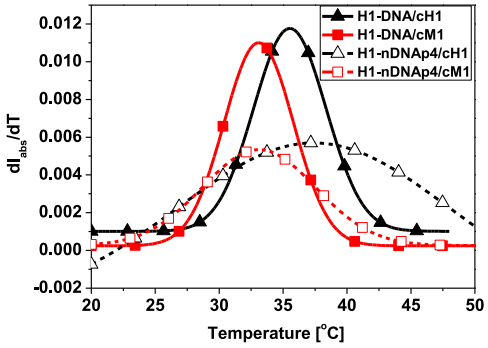


Fig. 2. Melting points of the perfect match H1-DNA/cH1 and H1-nDNAp4/cH, and single-mismatch H1-DNA/cM1 and H1-nDNAp4/cM1 in buffer solution with ionic strength of 10 mM.

compositions. The surface chemistries were confirmed by XPS spectra of two principal elements, N1s and P2p, as the best indicator of adsorbed DNA in Table S1 in Supplemental Content (Zhao et al., 1999). Therefore, the elemental ratio of the as-prepared SAM (MUAM), glutaraldehyde-activated SAM (MUAM + glutaraldehyde) and DNA-immobilized surfaces were analyzed. The XPS signals for nitrogen can appear in all samples since the immobilization was carried out by using glutaraldehyde as a crosslinker to react with amine groups on surfaces and in the terminal group of probe DNA samples. Moreover, the nucleobases present abundant amine groups. Therefore, the atomic ratios of nitrogen in the DNA conjugated samples were obviously higher than the unmodified ones in Table S1. More prominently, a single of the P2p at 133.6 eV was observed in H1-DNA and H1-nDNAp4 samples, which is common to all nucleotides (Petrovykh et al., 2003). As a result, the XPS data demonstrated the successful immobilization of probe H1-DNA and H1-nDNAp4 oligomers by the amine-aldehyde reaction for the subsequent use in SPR.

3.3. Effect of ionic strength on hybridization efficiency

The SPRi sensing technique is advantageous in providing real-time high-throughput information of biomolecular interactions (Piliarik et al., 2010, 2009; Piliarik and Homola, 2008). Therefore, we attached the probe nucleotides of H1-DNA and H1-nDNAp4 onto the sensor chips for capturing of the target DNA, cH1, to quantitatively compare the hybridization efficiency. The experiments were carried out in a PBS solution with a ionic strength of 150 mM at 27 °C, and the concentrations of H1 were prepared from 1 to 20 μM. In addition, the assay for four experimental conditions was individually and simultaneously performed in different flow channels on the same chip. The experiments were conducted with three replicates. After stabilizing the baseline, the target cH1 solutions were introduced to react with the probe nucleotides for about 20 min, followed by rinsing with PBS. The sensorgrams were shown in Fig. 3a, b. The curves were fitted with Langmuir isotherm model to obtain the association constants were ka = 1.15×10^6, and 1.19×10^6 M^{-1} S^{-1} for capturing of cH1 with H1-DNA and H1-nDNAp4, respectively. It seems no obvious difference in the hybridization kinetics for two probes. The optical response was converted to the surface mass as the adsorption level according to the previous publication (Piliarik et al., 2010). Therefore, the quantitative calibration curves for the detection of cH1 with H1-DNA and H1-nDNAp4 were presented in Fig. 3c. Herein, we revealed a negligible difference in sensor responses to the cH1 with natural and chimeric DNA probes in the buffer solution with ionic strength of 150 mM at 27 °C. Nevertheless, we for the first time demonstrate the feasibility of the chimeric DNA with methylated neutral nucleotides to be used as a capture molecule to form a complex structure with the complementary target DNA.

In the solution with high ionic strength, cations enable screening the negatively charged phosphates leading to less electrostatic repulsion during the hybridization process and facilitating the formation of hydrogen bonds. On the contrary, the neutralized probes could insensitive to the ionic concentration, which occurs to the PNA, neutral DNA analogue (Ou et al., 2013). In this study, the hybridization efficiency of H1-nDNAp4 to cH1 was comparable with that of H1-DNA in the buffer with high ionic strength, which supports the charge screening effect on natural DNA. As a result, under the stringent conditions, i.e. low ionic strength, the large difference in the hybridization efficiency between natural and chimeric DNA probes as well as the discrimination to the single mismatch in target DNA could be anticipated.

The direct and simple SNP genotyping is expected to be an indispensable technique for realizing precision medicine. Herein, we synthesized a target DNA oligomer, cM1, to form duplexes containing a G-A mismatch with the probe H1-DNA and H1-nDNAp4. The SPRi biosensor was applied to distinguish the single mismatch and explore a suitable operation condition for detection with a high signal-to-noise ratio. In order to emphasize the strength of neutralized chimeric DNA molecules in the SNP detection, we prepared a buffer solution with low ionic strength (10 mM) to reduce the charge screening with counterions in DNA hybridization. Therefore, the 10 μM of the target cH1 and single-mismatched cM1 samples were separately prepared in the buffers with ionic strengths of 10 and 150 mM at a room temperature of 27 °C. The solutions were flowed over the SPRi chips with immobilized probe H1-DNA and H1-nDNAp4 oligomers. The quantitative adsorption levels from triplicate experiments were present in Fig. 4a. For better comparison in the effectiveness of the SNP detection, we define a discrimination factor (δ), which is a ratio of surface masses of cH1 and cM1 oligomers on the given probe molecules. In the 150 mM buffer, theδof H1-DNA and H1-nDNAp4 were 1.58 and 1.12, respectively. The low δ represents the poor

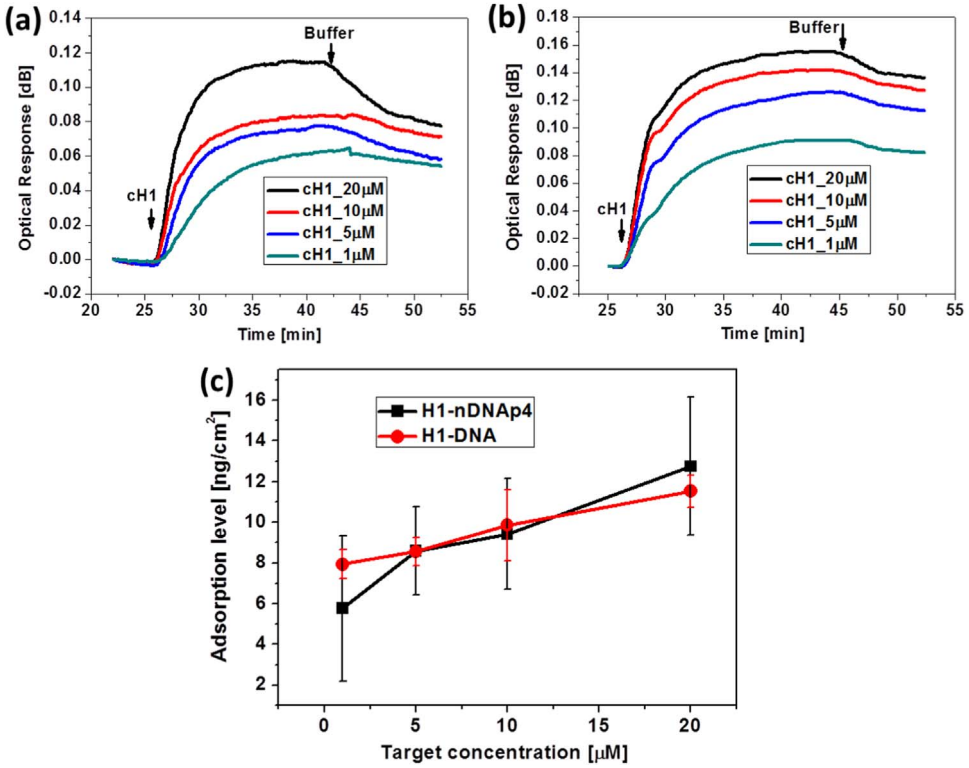


Fig. 3. SPRi sensorgrams for the detection of cH1 target molecules at concentrations of 1, 5, 10 and 20 μM with probes of H1-DNA (a) and H1-nDNAp4 (b). The calculated adsorption levels of cH1 on the SPRi biosensor in a buffer with the ionic strength of 150 mM at 27 °C (c).

ability to the SNP detection. It appears that the natural DNA probe exhibits better sensing capability than the chimeric probe in the 150 mM buffer. However, when the ionic strength of the buffer was prepared at 10 mM, the δ values become 1.13 and 4.59 for H1-DNA on H1-nDNAp4, respectively. Because of the strong electrostatic repulsive force between two fully negatively charged DNA oligomers, the formation of hydrogen bonds between H1-DNA and cH1 becomes energetically difficult in the buffer with the low ionic strength. On the contrary, the partially neutralized chimeric DNA probe remains comparable hybridization capability to the complementary cH1 target molecule. Therefore, the results prominently revealed the importance of the ionic strength in the hybridization efficiency and selectivity of the SNP detection. Moreover, we suspect that the additional methyl groups in the phosphate groups could interfere the formation of the hydrogen bonds between H1-nDNAp4 and cM1 even through their repulsive electric force was greatly reduced as found in previous study with fully neutral DNA (Chen et al., 2013).

3.4. Effect of temperature on SNP detection

In Fig. 2, the H1-nDNAp4/cH1 complex exhibits the higher melting point with respect to the H1-DNA/cH1, and larger the difference in the melting temperatures between perfect match and mismatch. Therefore, the environmental temperature could dramatically affect the formation of hydrogen bonds in hybridization and structural stability of DNA duplex, which can facilitate the SNP typing with chimeric neutralized DNA. As a result, we elevated the operation temperature to 30 °C in the SPRi biosensor for the hybridization of cH1 and cM1 target DNA oligomers with H1-DNA and H1-nDNAp4 probe oligomers in a solution with an ionic concentration of 10 mM. The adsorption levels of target molecules were present in Fig. 4b. The amounts of the captured cH1 and cM1 by H1-DNA were comparable as indicated by δ = 1.15, whereas the adsorption level of perfect-matched cH1 on H1-nDNAp4 was significantly higher than that of mismatched cM1,

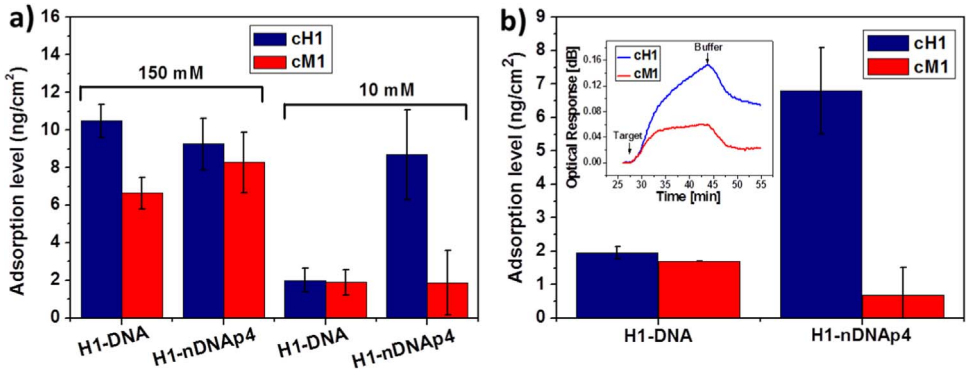


Fig. 4. The adsorption levels of the target cH1 and cM1 molecules on the SPRi biosensor. The probe oligomers were natural H1-DNA and chimeric H1-nDNAp4 immobilized on sensor chips. The hybridization experiments were carried out in buffers with ionic strengths of 150 and 10 mM at 27 °C (a). For the effect of temperature on SNP detection, the operation temperature at 30 °C in a solution with an ionic concentration of 10 mM was applied for the hybridization of cH1 and cM1 targets with H1-DNA and H1-nDNAp4 probe oligomers (b). The inserted SPRi sensorgrams is the detection of cH1 and cM1 targets using H1-nDNAp4 at 30 °C and an ionic concentration of 10 mM.

resulting in high δ = 9.86. Nevertheless, together with methylated nucleic acid, low ionic strength and high operation temperature, the SNP typing on the SPRi biosensor is applicable for rapid, sensitive and reliable detection. In addition, we envision that the improvement for the specificity in genotyping can be conducted by sophisticated design of the position of methylated oligonucleotide in the probe DNA or construction of heterodimers for high destabilizing effect of based mismatches. The detection strategy can also be adaptable to other sensor platforms and devices for enormous biomedical applications.

4. Conclusions

Novel neutralized chimeric DNA analogue was applied as a probe molecule on the SPRi biosensor for rapid and selective SNP genotyping. The sequence of the chimeric DNA can be customized by editing natural and methylated nucleotides in conventional DNA synthesizers to afford wide versatility and implementation. In this work, the operation condition was finely optimized to leverage the unique characteristics of neutralized DNA. Our results indicate that the low ionic strength and high operation temperature enable large response to the target perfect-matched DNA molecule and also the high discrimination to the single-mismatched DNA. This should be ascribed to the reduced electrostatic repulsive force and additional methyl groups on the backbone of oligomers. It is expected that the further improvement can be conducted by sophisticated design of the sequence of neutralized chimeric DNA or construction of heterodimers. Consequently, the neutralized DNA offers a promising hybridization probe for future developments of a high-sensitivity DNA sensor and medical applications.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2017.07.052.

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