



MutS protein-based fiber optic particle plasmon resonance biosensor for detecting single nucleotide polymorphisms

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Abstract

A new biosensing method is presented to detect gene mutation by integrating the MutS protein from bacteria with a fiber optic particle plasmon resonance (FOPPR) sensing system. In this method, the MutS protein is conjugated with gold nanoparticles (AuNPs) deposited on an optical fiber core surface. The target double-stranded DNA containing an A and C mismatched base pair in a sample can be captured by the MutS protein, causing increased absorption of green light launching into the fiber and hence a decrease in transmitted light intensity through the fiber. As the signal change is enhanced through consecutive total internal reflections along the fiber, the limit of detection for an AC mismatch heteroduplex DNA can be as low as 0.49 nM. Because a microfluidic chip is used to contain the optical fiber, the narrow channel width allows an analysis time as short as 15 min. Furthermore, the label-free and real-time nature of the FOPPR sensing system enables determination of binding affinity and kinetics between MutS and single-base mismatched DNA. The method has been validated using a heterozygous PCR sample from a patient to determine the allelic fraction. The obtained allelic fraction of 0.474 reasonably agrees with the expected allelic fraction of 0.5. Therefore, the MutS-functionalized FOPPR sensor may potentially provide a convenient quantitative tool to detect single nucleotide polymorphisms in biological samples with a short analysis time at the point-of-care sites.

Keywords Biosensor · Fiber optic particle plasmon resonance · Gold nanoparticle · Single nucleotide polymorphism · MutS protein

Introduction

Single nucleotide polymorphism (SNP) is the most common form of genetic mutation and occurs at a specific position in a genome with a change in single nucleotide base (A, C, G, or T). Most types of SNPs do not have detrimental effects on

health, but some certain types have been known to increase the risk of developing pathological conditions, including cancers such as bladder cancer [1] and hereditary nonpolyposis colon cancer [2], sickle cell anemia [3], coronary heart disease [4], and autoimmune diseases [5]. Therefore, accurate detection of SNPs plays an essential role in prophylaxis, early diagnosis, and determination of proneness of thousands of single gene disorders, and then choice of a proper therapy.

SNP detection technologies in general fall into two categories: SNP discovery and SNP screening. SNP discovery includes SNPs that are not yet known. Most methods to discover and genotype SNPs rely on sequencing which includes early sequencing methods and next-generation sequencing methods such as pyrosequencing, bridge amplification, ligase-mediated sequencing, and real-time single-molecule sequencing [6, 7]. These methods are reliable but suffer from shortcomings such as complicated procedures, time-consuming, and requiring expensive equipment and professional operator. Therefore, it is important to develop fast, simple, and inexpensive SNP screening methods. SNP screening pertains to known SNPs and requires prior knowledge of the sequence.

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In terms of an important application, the noninvasive prenatal testing (NIPT) is a less invasive screening approach to detect prenatal chromosomal disorders. Considering that chorionic villus sampling and amniotic fluid testing may result in miscarriage, NIPT is an important option for pregnant women who are at increased risk for trisomy 21, 18, and 13. There are two main methods of NIPT: the massively parallel sequencing method and the selective amplification of only a specific region of the target chromosome and then performing next-generation sequencing (NGS) [8]. However, there is still insufficient data to detect fetal monogenic point mutation diseases especially when the mother is a heterozygous carrier. Relative haplotype dosage (RHDO) analysis should be used to distinguish and quantify haplotypes linked to the mutation site of a gene. However, polymerase chain reaction (PCR)-based NGS has significant fault in RHDO [9]. In this research, focusing of direct quantitative detection of SNP could contribute the accuracy in RHDO of NIPT.

Currently, the reported SNP screening methods can be mainly classified into two groups: hybridization-based methods and enzyme-assisted methods. For hybridization-based methods, the discrimination step relies on the difference in hybridization free energy between the mutant and wild type, which is typically small for perfectly matched and single-mismatched duplex. Enzyme-assisted discrimination in general is more selective and often further enables DNA amplification. Among the many enzyme-assisted methods, PCR remains to be the gold standard. However, PCR requires high-precision thermal cycling, limiting its use in rapid SNP analysis. Moreover, PCR is also susceptible to contamination and amplification bias, limiting its accuracy in quantitative analysis. Other enzyme-assisted methods based on primer extension reaction [10], nucleases [11, 12], DNazymes [13], ligases [14], and DNA mismatch-binding protein [15, 16] have been reported. Although many of these methods offer a high level of SNP discrimination, those in the form of the homogeneous assay are not suitable for routine clinical laboratory while the other heterogeneous assays either display insufficient sensitivity and/or require a sophisticated setup or time-consuming amplification procedure.

The high specificity of the biological interaction between a DNA mismatch-binding protein, MutS protein, and a mismatch-containing heteroduplex DNA has been exploited for the detection of SNPs [17, 18]. In *Escherichia coli*, DNA mismatch repair is initiated by the binding of the MutS protein to base-pair mismatched DNA [19]. It has been suggested that MutS is capable of inducing DNA bending upon mismatch recognition in the presence of adenosine and subsequently undergoes conformational transitions that promote its interaction with MutL to signal repair [20]. The affinity of mismatch binding may depend on the MutS origin, mismatch type, and the sequence context [21]. It has been found that the order of affinity of MutS for all possible DNA mismatches was $G-T > G-G > A-A \approx T-T \approx C-T > C-A >$

$G-A > C-C > G-C$ [22]. Hence, MutS proteins have been considered a promising probe to detect mismatched double-stranded DNA (dsDNA). Therefore, the integration of the MutS protein with a label-free and real-time biosensor could create a simple and rapid method to detect SNPs. Recently, many biosensors for SNPs based on MutS protein have been developed, including fluorescence biosensor [23], voltammetric biosensor [24–26], electrochemical impedance (EIS) biosensor [27–29], field-effect transistor (FET) biosensor [30], quartz-crystal microbalance (QCM) biosensor [31], surface plasmon resonance (SPR) biosensor [15, 32], and nanoplasmonic biosensor [16, 33]. However, it is challenging to realize a compact and low-cost biosensor system that can detect SNPs in a label-free and real-time mode. Furthermore, these reports have not provided the details about biochemical information including equilibrium binding constants and binding kinetic constants between a mismatched heteroduplex DNA and the MutS protein.

Herein, a label-free and real-time biosensor was developed for the detection of SNPs using a fiber optic particle plasmon resonance (FOPPR) sensing system [34] based on MutS-conjugated gold nanoparticles (AuNPs) that have been immobilized on an unclad section of an optical fiber. The principle of our method for detection of SNPs is based on nanoplasmonic absorption by AuNPs via fiber optic evanescent wave excitation, by which the evanescent field at the fiber core surface excites the particle plasmon resonance (PPR) of immobilized AuNPs. When the MutS protein on the AuNP surface interacts with a mismatched heteroduplex DNA, the absorption coefficient of the AuNP increases because the local refractive index (RI) at the AuNP surface increases, leading to a decrease of light intensity exiting the optical fiber [35, 36]. Because the light propagates in the fiber core by virtue of consecutive total internal reflection (TIR), the multiple TIRs increase the optical path length and the excitation of guided modes in TIR greatly enhances light/matter interaction [37], resulting in significant increase in sensing sensitivity [34, 38]. Such an intensity change has been demonstrated to have a linearity relationship with the RI change. Thus, the increase of local RI due to biomolecular binding events can also be interrogated by this biosensing scheme. The FOPPR sensing technique offers the opportunity to measure biomolecular interaction in real-time with high sensitivity and without the need of labeling.

In this method, MutS was conjugated with AuNPs on the fiber core surface of an optical fiber as a sensor fiber for testing single-base mismatched DNA. A sample solution consisting of single-base mismatched double-strand DNA (dsDNA) was produced by hybridization between a single-strand DNA (ssDNA) detection probe (ssDNA^d) and a single-base mismatched single-strand DNA target (ssDNA^t). A reference solution consisting of the perfectly matched dsDNA was formed by hybridization between ssDNA^d and the perfectly matched ssDNA (ssDNA^c). When the sample or reference solution was

injected into a sensor chip containing the sensor fiber, the interaction between MutS with the single-base mismatched dsDNA or perfectly matched dsDNA, respectively, was recorded in form of a sensorgram. Such a real-time sensorgram also facilitates the determination of equilibrium binding constant and binding kinetic constant between the MutS protein and a single-base mismatched dsDNA.

β -Thalassemia is an inherited blood disorder that reduces the production of hemoglobin and is caused by a point mutation of human hemoglobin subunit beta gene (*HBB* gene). It is highly prevalent, with 1.5% of the global population reported to be carriers across the world [39]. β -Thalassemia is caused by a single point mutation possibly not only in codon-26 (Glu→Lys; GAG→AAG) but also in IVS-II-654 (C→T), codon-41/42 (-TCTT), codon-28 (A→G), codon-17 (A→T), and many others [40]. As MutS binds to AC mismatch heteroduplex DNA with relatively low affinity [22, 41], we chose codon-26 as a model in this study to ensure that the method is applicable to other kinds of mismatches. By applying this method to a series of ssDNA^t standards to construct a calibration graph, rapid and quantitative detection of SNPs is achieved.

Materials and methods

Materials and reagents

All reagents were used as received. (3-Mercaptopropyl)-methyl-dimethoxy silane (MPDMS) was purchased from Tokyo Chemical Industry. Hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄·3H₂O) and trisodium citrate were purchased from Alfa Aesar. 11-Mercaptoundecanoic acid (MUA), 6-mercapto-1-hexanol (MCH), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), 2-(N-morpholino)ethanesulfonic acid (MES), magnesium chloride (MgCl₂), and dithiothreitol (C₄H₁₀O₂S₂) (DTT) were purchased from Sigma-Aldrich. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from ACROS. Ethanolamine (C₂H₇NO), and trisodium citrate, 2-amino-2-(hydroxymethyl)propane-1,3-diol (C₄H₁₁NO₃) (Tris) was purchased from J.T.Baker. Ethanol (C₂H₅OH) and toluene (C₆H₅CH₃) were purchased from Honeywell Burdick & Jackson. MutS protein from *Thermus thermophilus* was obtained from Excellgen (EG-198). This protein (12 µg/µL) was supplied

in a storage buffer (20 mM HEPES, pH 7.4, 250 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol). A reaction buffer containing 20 mM Tris-HCl, pH 8.0, 10 mM NaCl, 1 mM DTT, 0.1 mM EDTA, and 2 mM MgCl₂ was prepared for MutS in subsequent experiments. Ultrapure water (18.2 MΩ·cm) was purified by Milli-Q water system (Millipore) and used to prepare all aqueous solutions. The RI of the solutions was measured by a refractometer (RA-620, Kyoto Electronics).

Three oligonucleotides were purchased from GENEWIZ with sequences of the oligonucleotides as summarized in Table 1. The single-strand DNA detection probe (ssDNA^d) was used to form homoduplex with the single-strand target DNA having a complementary sequence (ssDNA^{t,c}) and heteroduplex with the single-strand target DNA having a single mismatched base (ssDNA^{t,m}).

Gold nanoparticle synthesis

In total, 20 mL of hydrogen tetrachloroauric (III) trihydrate acid solution with a concentration of 0.88 mM in a two-neck round-bottom flask was heated to boiling with vigorous stirring for 20 min. Then, 2.4 mL of a fresh 1% trisodium citrate solution was rapidly added in the boiling solution. The resulting solution was kept boiling for 20 min and the color changed from yellow to burgundy red. The solution was then allowed to cool down to room temperature while kept stirring. The spectrum of the AuNP solution was obtained by a double beam UV-visible spectrometer (Cintra202, GBC). The peak wavelength of the AuNP solution was at about 519 nm. The images of the AuNPs were obtained by a transmission electron microscope (TEM, JEOL JEM-2010). As shown in Fig. S1 of Supplementary Information (ESM), the AuNPs are spherical in shape, and the particle size of the gold nanoparticles calculated from the TEM image is 13.1 ± 1.0 nm. The average hydrodynamic diameter of the AuNPs was estimated to be 16.6 nm by using dynamic light scattering (DLS), as shown in Fig. S2 of ESM.

Fabrication of sensor fibers

The optical fibers used as the FOPPR sensor fibers are multi-mode plastic-clad silica fibers (model F-MBC, Newport) with core and cladding diameters of 400 and 430 µm, respectively. The total length of each optical fiber was 70 mm with a

Table 1 DNA sequences used in the experiments

Type	DNA sequence
ssDNA detection probe (ssDNA ^d)	5'-NH ₂ -AAAA AAA AAA TGCC CAG GGC CTC ACC ACC AAC TTC-3'
Mismatched target ssDNA (ssDNA ^{t,m})	5'-GAA GTT GGT GGT <u>A</u> AG GCC CTG GGCA-3
Perfectly matched target ssDNA (ssDNA ^{t,c})	5'-GAA GTT GGT GGT <u>G</u> AG GCC CTG GGCA-3'

segment of 20 mm of the coating and cladding in the middle section of the fiber removed by using a CO₂ laser processing system (V-460, Universal Laser Systems Inc.) to form a sensor zone. The fiber end faces were polished to an optically smooth surface to facilitate light coupling.

The partially unclad fibers after cleaning by an oxygen plasma cleaner (Harrick Plasma, PDC-001) were immersed in a mixture of 2% MPDMS in toluene for 4 h to functionalize the fiber surfaces with a thiol group. Subsequently, the modified fibers were immersed in a AuNP solution for 6 min to allow the AuNPs to self-assemble on the sensor zone of the optical fibers. The AuNP surfaces on the fibers were then modified with a mixed self-assembled monolayer (SAM) of MUA/MCH by immersing the fibers in an ethanolic solution of MUA (0.4 mM) and MCH (1.6 mM) overnight at ambient temperature. Subsequently, the carboxyl group of the mixed SAM was activated by immersing the fibers in an aqueous solution of EDC (0.1 M) and NHS (0.025 M) in MES buffer (50 mM, pH = 6.2) for 1 h. Then, a solution of MutS protein (1 µg/mL) in 20 mM HEPES buffer (pH 7.8) was allowed to react with the activated carboxyl group on the AuNP surfaces for 2 h. Finally, the unreacted carboxyl group was deactivated by immersing the fibers in ethanolamine (1 M) for 30 min. These partially unclad fibers after modification with AuNPs were then used as the sensor fibers.

Preparation of standards and samples

Stock solutions of ssDNA^d, ssDNA^{t,c}, and ssDNA^{t,m} were prepared in a reaction buffer with a concentration of 10⁻⁶ M. Series dilution of the stock solutions of ssDNA^{t,c} and ssDNA^{t,m} was carried out to prepare ssDNA^{t,c} standard solutions and ssDNA^{t,m} standard solutions, respectively, with a concentration ranging from 5 × 10⁻⁷ to 5 × 10⁻¹⁰ M. Then, 100 µL each of ssDNA^{t,c} and ssDNA^{t,m} standard solution was mixed with 100 µL of ssDNA^d solution (10⁻⁶ M) for hybridization and allowed to stand at 4 °C for at least 3 h to form GC homoduplex and AC heteroduplex, respectively.

All the specimens in this study have passed the institutional review boards of National Cheng Kung University Hospital, and the patients signed the consent forms. The specimens were 20 mL of whole blood from pregnant women in the hospital. The whole blood samples were first centrifuged at 1850×g at 4 °C for 10 min, and separated into plasma, white blood cells, and red blood cells from top to bottom. In this study, we only keep the white blood cells for DNA extraction. The DNA was extracted by the QIAamp DNA blood maxi kit (Qiagen, 51192). The white blood cells were collected in a 50.0-mL tube and mixed with 500 µL protease K and 6 mL AL buffer. The tube was flipped upside-down 15 times and vortexed for another 1 min, then incubated for 10 min at 70 °C. After incubation, the sample was mixed with 5 mL ethanol (> 96%) and the tube was flipped upside-down 10

times, then all of the solution was transferred to a QIAamp Maxi column. Afterwards, 5 mL AW1 buffer and 5 mL AW2 buffer were added sequentially and the solution was centrifuged for 1 min and 15 min, respectively, at 4500×g to wash the samples. In the end, 1 mL AE buffer was added and the solution was allowed to stand for 5 min and then centrifuged for 2 min at 4500×g for DNA elution.

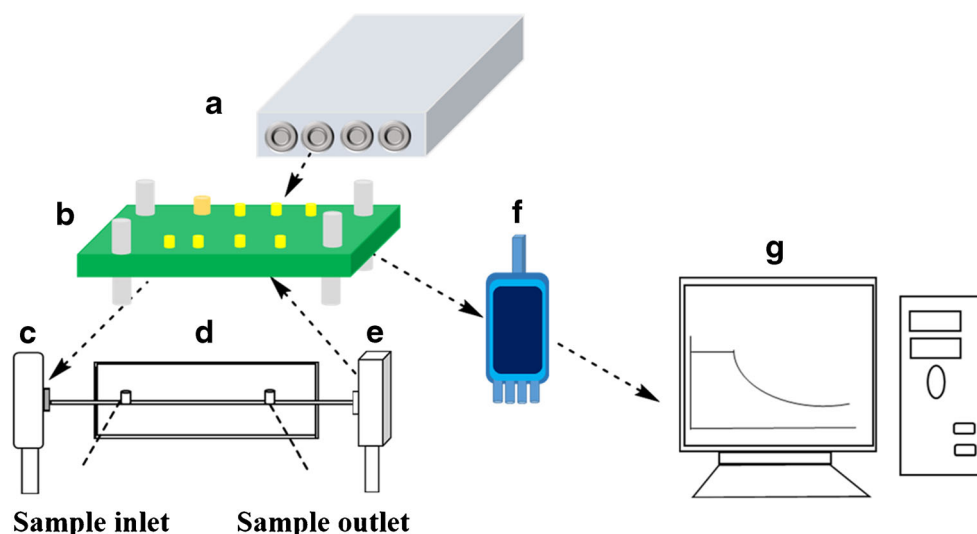
The eluted DNA then underwent PCR amplification using the PyroMark PCR master mix (Qiagen 978703) kit. The total volume of each PCR procedure was 25.0 µL, containing 12.5 µL Pyromark PCR master mix, 0.5 µL codon 17–26 forward primer (10 mM), 0.5 µL codon 17–26 reverse primer (10 mM), extracted DNA from white blood cell (0.2–20.0 ng), and then adjusted with nuclease-free water to 25.0 µL. The primers used were as follows: forward = 5' GGA GAA GTC TGC CGT TAC TGC 3'; reverse = 5' GCC TAT CAG AAA CCC AAG AGT C 3'. The PCR procedure was started with 15 min denature at 95 °C, then amplification was achieved by thermal cycling for 40 cycles with denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Final extension was performed at 72 °C for 10 min and then cooled to 12 °C. The PCR product was 161 bp in length, quantified by Qubit 2.0 fluorometer with Qubit dsDNA BR assay kit (Invitrogen), and was then stored at -20 °C until use.

Fabrication of sensor chips

The sensor chips were fabricated according to the method previously described [42]. Briefly, the sensor chips were composed of two polycarbonate plates, a cover and a bottom plate, with dimensions of 2.5 cm (width) × 5.0 cm (length) × 0.2 cm (thickness) and fabricated by an injection molding machine. The bottom plate contained a microchannel with a depth of 800 µm and a width of 800 µm to accommodate a sensor fiber. The cover plate contained two small access holes as an inlet and an outlet for sample introduction. The cover and the bottom plates were glued by a 3M sticker to form a sensor chip. Teflon tubing was then attached to the chip through both the inlet and outlet. The free volume of the microchannel was estimated to be 10.3 µL.

The biosensor system employed was similar to that in our previous research [43]. As shown in Fig. 1, it consists of a light-emitting diode (LED, model IF-E93, Industrial Fiber Optic, Inc.) with a peak wavelength of 530 nm, a LED driver circuit to drive the LED with 1-kHz frequency modulation (homemade), a sensor module, a photodiode (S1336-18BK, Hamamatsu), a photoreceiver amplification circuit (homemade), a power supply (PMT-D1V100W1AA, Delta Electronics), a signal acquisition module (NI-9234, National Instruments), and a graphical user interface programmed by LabVIEW® (National Instruments). The sensor module consists of a chip holder to load a sensor chip and a sample

Fig. 1 Schematic representation of the experimental setup used for the FOPPR sensing system and its working principle. The setup consists of (A) power supply, (B) LED driver and photoreceiver amplification circuit, (C) LED, (D) sensor chip, (E) photodiode, (F) signal acquisition module, and (G) computer



injection loop (Rheodyne 7725i) to load the sample into the sensor chip.

Quantitative analysis is performed by comparing the normalized transmitted light intensity through the sensor fiber (I/I_0) in the form of a molecular binding kinetic curve [44], where I_0 is the light intensity exiting the sensor fiber which is immersed in a blank and I is the real-time light intensity exiting the same sensor fiber when immersed in a sample. The system monitors the real-time light intensity signal level changes on a second-by-second basis. A calibration curve is set up by plotting the sensor response, $\Delta I/I_0$, where $\Delta I = I_0 - I_s$ and I_s is the steady-state light intensity in the sample, versus log concentration of the analyte. I_s is calculated as an average of 100 steady-state data points in the signal calculation window prior to the next step of sample injection. In case a significant long-term baseline drift of the system due to environmental effects such as dramatic room temperature change and power supply instability is observed, a home-written baseline correction algorithm programmed by LabVIEW® (National Instruments) would be used to correct the signal from the baseline drift [45]. The real-time signals I were also used to calculate the equilibrium affinity constant and kinetic rate constants [44].

Results and discussion

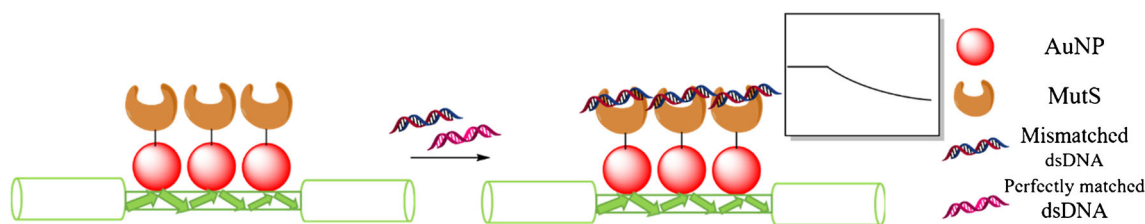
Scheme 1 shows a schematic illustration of the label-free FOPPR sensor for the MutS protein-mediated mismatched dsDNA recognition. The surface of immobilized AuNPs on the fiber core surface was first treated with a binary mixed-thiol mixture consisting of carboxyl (-COOH) terminated thiol and hydroxyl (-OH) terminated thiol. This architecture allows bioconjugation of MutS protein on the AuNP surface using the -COOH group and utilization of the -OH moiety on the

AuNP surface to cover the space under the MutS molecules to minimize nonspecific adsorption of perfectly matched dsDNA and other matrix molecules.

Selectivity of the sensor

Before testing the ability of MutS to bind heteroduplexes containing single-base mismatches, we first tested whether this molecule was capable of binding homoduplexes. In diagnostic applications, a specific analyte must be detected in the presence of a relatively high amount of nonspecific species. As MutS binds to AC mismatch heteroduplex DNA with relatively low affinity [22, 41], we employed codon-26 with an AC mismatch as a model and a GC homoduplex as a nonspecific species in this study.

As shown in Fig. 2, the sensor response due to nonspecific binding in the presence of 10^{-6} M ssDNA^d plus 10^{-6} M ssDNA^{tc} is indistinguishable from the blank signal I_0 . This indicates that there is insignificant interference from high concentration of the homoduplex DNA. However, in the presence of 10^{-8} M ssDNA^{t,m} plus 10^{-8} M ssDNA^d, the sensor response due to the binding of MutS with the heteroduplex DNA had well detectable change ($\Delta I/I_0 = 0.215\%$). As the optical fiber is placed in the microfluidic channel of a sensor chip, the narrow channel width reduces the molecular transport time to the sensor surface and thus shortens the response time [46]. The response time of this specific biointeraction, which is defined as the time required to reach 90% of the signal change, is 900 s. Please note that all the refractive indices of the blank, AC heteroduplex DNA solution, and GC homoduplex DNA solution as measured by a refractometer were the same at 1.3330, indicating that the change in sensor response is not due to bulk RI difference between the blank and the analyte solutions but rather due to the molecular binding at the AuNP surface to cause local RI change. Therefore,



Scheme 1 Schematic illustration of the surface architecture and the biosensing strategy using MutS protein-conjugated AuNPs that have been immobilized on a fiber core surface for interaction with dsDNA having a single-base mismatch

we can confirm that the measured signal change is caused by the specific binding between the MutS protein and the mismatched dsDNA formed by hybridization between ssDNA^{t,m} and ssDNA^d.

Sensitivity of the sensor

The sensitivity of the sensor was determined by employing a MutS-functionalized sensor fiber in response to solutions of AC heteroduplex DNA of increasing concentration in a sensor chip. The microchannel in the sensor chip was firstly filled with a reaction buffer as a blank. Then, solutions of AC heteroduplex DNA of increasing concentration (5×10^{-10} M– 5×10^{-7} M) were sequentially introduced into the microfluidic channel. For each injection, the introduced fluid was kept in the microchannel of the sensor chip for 15 min in static mode. As shown by a representative sensorgram in Fig. 3A, the steady-state signal intensity (I_s) for each injection decreases with increasing concentration of AC heteroduplex DNA. In detail, the inset of Fig. 3A shows a zoomed-in view of the sensorgram to indicate that after the steady state of molecular binding has been reached, I_s in the signal calculation window as indicated by the solid-line box is calculated. Then, the valve of the sample injection loop was

turned on; this action may induce a release of liquid pressure in the microchannel and causes a very small increase in light intensity signal. Subsequently, the sample in a syringe was injected into the sensor chip via the sample injection loop and the duration between turning on the valve and sample injection could vary. Molecular binding at the AuNP surface is then revealed by the molecular binding kinetic curve. Similar calculation applies to I_0 . Using the values of I_s and the corresponding concentrations, a standard calibration curve ($n = 5$) as shown in Fig. 3B can be constructed. The linear regression equation of the plot is $y = 0.01899 + 0.00201x$, and the correlation coefficient (r) is 0.9981. The linear dynamic range is about three orders. For this

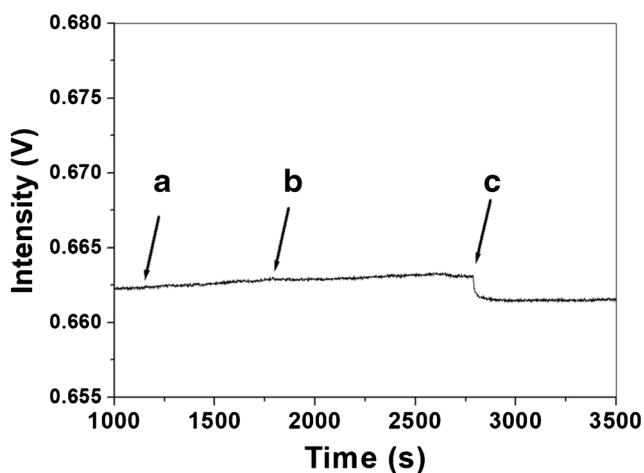


Fig. 2 A real-time sensorgram showing the responses of a sensor fiber in a reaction buffer (a) followed by injection of a solution containing a perfectly matched DNA formed by 10^{-6} M ssDNA^d (b) and 10^{-6} M ssDNA^{t,c} and then (c) a solution containing a mismatched DNA formed by 10^{-8} M ssDNA^d mixed with 10^{-8} M ssDNA^{t,m}

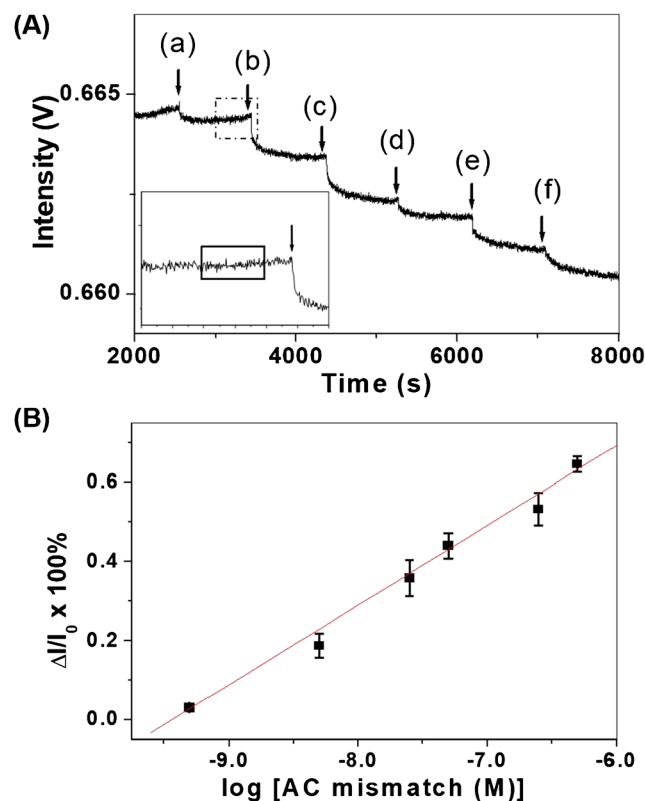


Fig. 3 (A) A sensorgram in response to solutions of different concentrations of AC heteroduplex DNA. (a) to (f) represent the heteroduplex DNA concentration of (a) 5×10^{-10} M, (b) 5×10^{-9} M, (c) 2.5×10^{-8} M, (d) 5×10^{-8} M, (e) 2.5×10^{-7} M, and (f) 5×10^{-7} M. Inset: a zoomed-in view of the dashed-line box where the data calculation window is indicated by the solid-line box. (B) The corresponding calibration curve ($n = 5$)

biosensing system, the root-mean-square background noise is 0.0053% per 200 s when normalized to I_0 . From the calibration curve, a limit of detection (LOD) at the definition of signal-to-noise ratio of 3 for the mismatched dsDNA is calculated to be 4.9×10^{-10} M. This LOD is superior or comparable with other kinds of label-free MutS-based biosensors including QCM biosensor [31], EIS biosensor [28, 29], and FET biosensor [30]. Recently, we have demonstrated that the sensitivity can be further enhanced by several orders using a nanogold-linked biosorbent assay in the FOPPR biosensor [43, 47].

Determination of binding affinity and kinetics

The availability of analytical techniques that can detect native biomolecular interactions without prior knowledge of the sequence and interferences from labels like fluorescent and radioactive probes is desirable for studying of SNPs. The real-time FOPPR kinetic data provide opportunities to examine the nature of biomolecular interactions without labels and determine molecular binding affinities and kinetics of the binding events [34, 42, 44]. Using an approach as previously described [44], the association rate constant (k_a) and dissociation rate constant (k_d) for the binding between the immobilized MutS protein and AC heteroduplex DNA are $(4.39 \pm 0.64) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $(5.95 \pm 3.03) \times 10^{-3} \text{ s}^{-1}$, respectively. The ratio between k_d and k_a reveals the dissociation equilibrium constant (K_D) of MutS to AC heteroduplex DNA, which was found to be $14.2 \pm 8.9 \text{ nM}$. These calculated values of k_a , k_d , and K_D agree reasonably well with previously reported values by fluorescence ($k_a = 6 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, $k_d = 0.05 \text{ s}^{-1}$, $K_D = 10\text{--}20 \text{ nM}$) [48] and single gold-bridged nanoprobe ($k_a = 2.97 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, $K_D = 4.46 \text{ nM}$) [33].

Method validation with clinical specimens

To demonstrate the accuracy and precision of the method for SNP detection using human genomic DNA, we used templates isolated from white blood cells in whole blood samples. One genomic DNA is heterozygous from a patient and another genomic DNA is homozygous from a healthy person. As shown in Fig. 4A, the sensor response ($\Delta I/I_0$) of a MutS-functionalized sensor fiber in a sample solution of AC heteroduplex from a patient shows a value of 0.15%. Using the calibration curve as shown in Fig. 3B, the average concentration ($n = 3$) of the AC heteroduplex before dilution is estimated to be $82.3 \pm 1.7 \text{ nM}$, with a coefficient of variation (CV) of 2.1%. This indicates that specific binding reaction occurs between the AC heteroduplex and the MutS protein. On the other hand, Fig. 4B shows that in the presence of a sample solution of a healthy person (GC pair), the signal is indistinguishable from a blank. This further confirms the specificity of the biosensor for real samples.

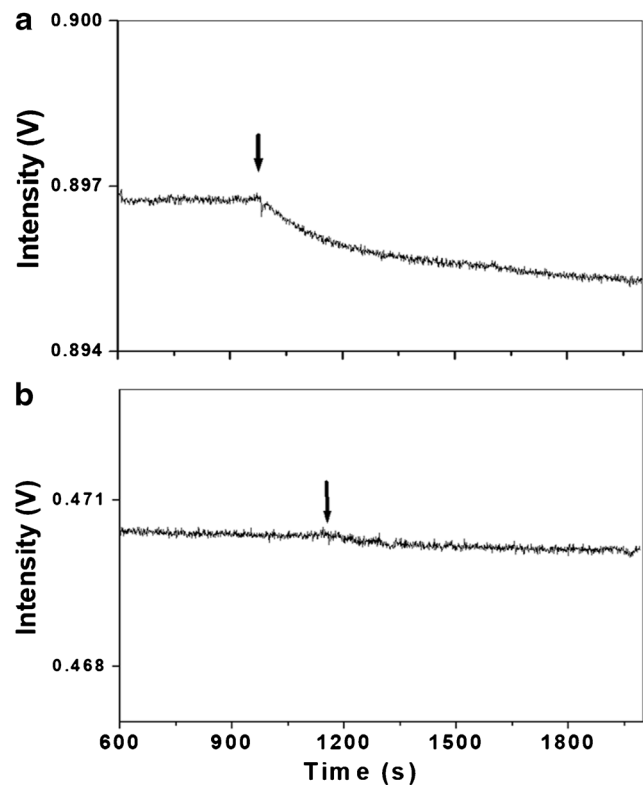


Fig. 4 (A) A sensorgram obtained with a heterozygous PCR sample from a patient. (B) A sensorgram obtained with a homozygous PCR sample from a healthy person. The arrows indicate the injection time. Each interval in the y-axes is 3 mV

To evaluate the accuracy and precision of the method for clinical specimens, the concentration of the AC heteroduplex in the PCR sample from the patient was compared to the total DNA concentration in the PCR sample, which was found to be $17.3 \pm 2.2 \mu\text{g}/\mu\text{L}$. As such, the heterozygous sample gave allelic fraction of 0.474 ± 0.010 , which agrees reasonably well with the expected allelic fraction of 0.5. This indicates the good accuracy and precision of the method.

Conclusions

We have demonstrated the feasibility of using MutS-functionalized FOPPR sensor for label-free and real-time detection of single-base mismatched dsDNA in the PCR sample. The biosensing method is rapid, highly sensitive, and easy to operate, and requires small sample volume. Thanks to the advances in photonics and electronics, LED and photodiode are quite cheap nowadays, while the signal modulation and demodulation hardware are not difficult to be implemented by a single electronic board. Thus, the development of commercial low-cost instrumentation for this biosensing method is very possible.

The LOD for AC heteroduplex DNA obtained by this method is 0.49 nM and the analysis time excluding sample

preparation is short (≤ 15 min). As MutS binds to AC mismatch heteroduplex DNA with relatively low affinity, it is expected the LOD would be even lower for G-T, G-G, A-A, T-T, and C-T heteroduplex DNAs because the order of affinity of MutS for all possible DNA mismatches was $G-T > G-G > A-A \approx T-T \approx C-T > C-A > G-A > C-C > G-C$ [22, 41]. Moreover, validation of the method using a heterozygous PCR sample from a patient exhibits results reasonably agree with the expected allelic fraction of 0.5. Therefore, the FOPPR sensor may potentially be used quantitatively to detect SNPs in biological samples with a short analysis time at the point-of-care sites.

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Data availability All data generated and analyses during this study are included in this published article and its supplementary material file.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study was reviewed and approved by the Institutional Review Board (IRB) of National Cheng Kung University Hospital. The patients/participants provided their written informed consent to participate in this study.

Source of biological material National Cheng Kung University Hospital.

References

- Andrew AS, Gui J, Sanderson AC, Mason RA, Morlock EV, Schned AR, et al. Bladder cancer SNP panel predicts susceptibility and survival. *Hum Genet.* 2009;125:527–39. <https://doi.org/10.1007/s00439-009-0645-6>.
- Yu L, Yin B, Qu K, Li J, Jin Q, Liu L, et al. Screening for susceptibility genes in hereditary non-polyposis colorectal cancer. *Oncol Lett.* 2018;15:9413–9. <https://doi.org/10.3892/ol.2018.8504>.
- Fertrin KY, Costa FF. Genomic polymorphisms in sickle cell disease: implications for clinical diversity and treatment. *Expert Rev Hematol.* 2010;3:443–58. <https://doi.org/10.1586/ehm.10.44>.
- Bare LA, Morrison AC, Rowland CM, Shiffman D, Luke MM, Iakoubova OA, et al. Five common gene variants identify elevated genetic risk for coronary heart disease. *Gen Med.* 2007;9:682–9. <https://doi.org/10.1097/GIM.0b013e318156fb62>.
- Jonkers IH, Wijmenga C. Context-specific effects of genetic variants associated with autoimmune disease. *Hum Mol Genet.* 2017;26(R2):R185–92. <https://doi.org/10.1093/hmg/ddx254>.
- Mardis ER. Next-generation DNA sequencing methods. *Annu Rev Genom Hum Genet.* 2008;9:387–402. <https://doi.org/10.1146/annurev.genom.9.081307.164359>.
- Shendure J, Balasubramanian S, Church GM, Gilbert W, Rogers J, Schloss JA, et al. DNA sequencing at 40: past, present and future. *Nature.* 2017;550:345–53. <https://doi.org/10.1038/nature24286>.
- Samura O. Update on noninvasive prenatal testing: a review based on current worldwide research. *J Obstet Gynaecol Res.* 2020;46:1246–54. <https://doi.org/10.1111/jog.14268>.
- Zhang J, Li J, Saucier JB, Feng Y, Jiang Y, Sinson J, et al. Non-invasive prenatal sequencing for multiple Mendelian monogenic disorders using circulating cell-free fetal DNA. *Nat Med.* 2019;25:439–47. <https://doi.org/10.1038/s41591-018-0334-x>.
- Fahy E, Nazarbachi R, Zomorodi M, Hermsstadt C, Parker WD, Davis RE, et al. Multiplex fluorescence-based primer extension method for quantitative mutation analysis of mitochondrial DNA and its diagnostic application for Alzheimer's disease. *Nucleic Acids Res.* 1997;25:3102–9. <https://doi.org/10.1093/nar/25.15.3102>.
- Chen YT, Hsu CL, Hou SY. Detection of single-nucleotide polymorphisms using gold nanoparticles and single-strand-specific nucleases. *Anal Biochem.* 2008;375:299–305. <https://doi.org/10.1016/j.ab.2007.12.036>.
- Xu W, Xue X, Li T, Zeng H, Liu X. Ultrasensitive and selective colorimetric DNA detection by nicking endonuclease assisted nanoparticle amplification. *Angew Chem Int Ed.* 2009;48:6849–52. <https://doi.org/10.1002/anie.200901772>.
- Deng M, Zhang D, Zhou Y, Zhou X. Highly effective colorimetric and visual detection of nucleic acids using an asymmetrically split peroxidase DNase. *J Am Chem Soc.* 2008;130:13095–102. <https://doi.org/10.1021/ja803507d>.
- Lapitan LDS Jr, Xu Y, Guo Y, Zhou D. Combining magnetic nanoparticle capture and poly-enzyme nanobead amplification for ultrasensitive detection and discrimination of DNA single nucleotide polymorphisms. *Nanoscale.* 2019;11:1195–204. <https://doi.org/10.1039/C8NR07641C>.
- Gotoh M, Hasebe M, Ohira T, Hasegawa Y, Shinohara Y, Sota H, et al. Rapid method for detection of point mutations using mismatch binding protein (MutS) and an optical biosensor. *Gen Anal Biomol Eng.* 1997;14:47–50. [https://doi.org/10.1016/S1050-3862\(97\)00009-0](https://doi.org/10.1016/S1050-3862(97)00009-0).
- Ma X, Truong PL, Ahn NH, Sim SJ. Single gold nanoplasmonic sensor for clinical cancer diagnosis based on specific interaction between nucleic acids and protein. *Biosens Bioelectron.* 2015;67:59–65. <https://doi.org/10.1016/j.bios.2014.06.038>.
- Lishanski A, Ostrander EA, Rine J. Mutation detection by mismatch binding protein, MutS, in amplified DNA: application to the cystic fibrosis gene. *Proc Natl Acad Sci U S A.* 1994;91:2674–8. <https://doi.org/10.1073/pnas.91.7.2674>.
- Wagner R, Debbie P, Radman M. Mutation detection using immobilized mismatch binding protein (MutS). *Nucleic Acids Res.* 1995;23:3944–8. <https://doi.org/10.1093/nar/23.19.3944>.
- Su SS, Lahue RS, Au KG, Modrich P. Mismatch specificity of methyl-directed DNA mismatch correction *in vitro*. *J Biol Chem.* 1988;263:6829–35. [https://doi.org/10.1016/S0021-9258\(18\)68718-6](https://doi.org/10.1016/S0021-9258(18)68718-6).
- Wang H, Yang Y, Schofield MJ, Du C, Fridman Y, Lee SD, et al. DNA bending and unbending by MutS govern mismatch recognition and specificity. *Proc Natl Acad Sci U S A.* 2003;100:14822–7. <https://doi.org/10.1073/pnas.2433654100>.

21. Stanislawska-Sachadyn A, Sachadyn P. MutS as a tool for mutation detection. *Acta Biochim Polon*. 2005;52:575–83. https://doi.org/10.18388/abp.2005_3417.
22. Brown J, Brown T, Fox KR. Affinity of mismatch-binding protein MutS for heteroduplexes containing different mismatches. *Biochem J*. 2001;354:627–33. <https://doi.org/10.1042/0264-6021:3540627>.
23. Behrendorf HA, Pignot M, Windhab N, Kappel A. Rapid parallel mutation scanning of gene fragments using a microelectronic protein-DNA chip format. *Nucleic Acids Res*. 2002;30:e64. <https://doi.org/10.1093/nar/gnf063>.
24. Palecek E, Masarik M, Kizek R, Kuhlmeier D, Hassmann J, Schuelein J. Sensitive electrochemical determination of unlabeled MutS protein and detection of point mutations in DNA. *Anal Chem*. 2004;76:5930–6. <https://doi.org/10.1021/ac049474x>.
25. Han A, Takarada T, Shibata T, Nakayama M, Maeda M. A MutS protein-immobilized Au electrode for detecting single-base mismatch of DNA. *Anal Sci*. 2006;22:663–6. <https://doi.org/10.2116/analsci.22.663>.
26. Chen H, Liu XJ, Liu YL, Jiang JH, Shen GL, Yu RQ. Electrochemical scanning of DNA point mutations via MutS protein-mediated mismatch recognition. *Biosens Bioelectron*. 2009;24:1955–61. <https://doi.org/10.1016/j.bios.2008.09.029>.
27. Li CZ, Long YT, Lee JS, Kraatz HB. Protein-DNA interaction: impedance study of MutS binding to a DNA mismatch. *Chem Commun*. 2004:574–5. <https://doi.org/10.1039/B314642A>.
28. Gong H, Zhong T, Gao L, Li X, Bi L, Kraatz HB. Unlabeled hairpin DNA probe for electrochemical detection of single-nucleotide mismatches based on MutS–DNA interactions. *Anal Chem*. 2009;81:8639–43. <https://doi.org/10.1021/ac901371n>.
29. Cho M, Lee S, Han SY, Park JY, Rahman MA, Shim YB, et al. Electrochemical detection of mismatched DNA using a MutS probe. *Nucleic Acids Res*. 2006;34:e75. <https://doi.org/10.1093/nar/gkl364>.
30. Kim S, Kim TG, Byon HR, Shin HJ, Ban C, Choi HC. Recognition of single mismatched DNA using MutS-immobilized carbon nanotube field effect transistor devices. *J Phys Chem B*. 2009;113:12164–8. <https://doi.org/10.1021/jp9063559>.
31. Su X, Robelek R, Wu Y, Wang G, Knoll W. Detection of point mutation and insertion mutations in DNA using a quartz crystal microbalance and MutS, a mismatch binding protein. *Anal Chem*. 2004;76:489–94. <https://doi.org/10.1021/ac035175g>.
32. Wilson PK, Jiang T, Minunni ME, Turner AFP, Mascini M. A novel optical biosensor format for the detection of clinically relevant TP53 mutations. *Biosens Bioelectron*. 2005;20:2310–3. <https://doi.org/10.1016/j.bios.2004.11.020>.
33. Ma X, Song S, Kim S, Kwon MS, Lee H, Park W, et al. Single gold-bridged nanoprobe for identification of single point DNA mutations. *Nat Commun*. 2019;10:836. <https://doi.org/10.1038/s41467-019-08769-y>.
34. Cheng SF, Chau LK. Colloidal gold-modified optical fiber for chemical and biochemical sensing. *Anal Chem*. 2003;75:16–21. <https://doi.org/10.1021/ac020310v>.
35. Chiang CY, Hsieh ML, Huang KW, Chau LK, Chang CM, Lyu SR. Fiber optic particle plasmon resonance sensor for detection of interleukin-1 β in synovial fluids. *Biosens Bioelectron*. 2010;26:1036–42. <https://doi.org/10.1016/j.bios.2010.08.047>.
36. Wu CW, Chiang CY, Chen CH, Chiang CS, Wang CT, Chau LK. Self-referencing fiber optic particle plasmon resonance sensing system for real-time biological monitoring. *Talanta*. 2016;146:291–8. <https://doi.org/10.1016/j.talanta.2015.08.047>.
37. Wu WT, Chen CH, Chiang CY, Chau LK. Effect of surface coverage of gold nanoparticles on the refractive index sensitivity in fiber-optic nanoplasmonic sensing. *Sensors*. 2018;18:1759. <https://doi.org/10.3390/s18061759>.
38. Chau LK, Lin YF, Cheng SF, Lin TJ. Fiber-optic chemical and biochemical probes based on localized surface plasmon resonance. *Sens Actuat B*. 2006;113:100–5. <https://doi.org/10.1016/j.snb.2005.02.034>.
39. Origa R. β -Thalassemia. *Genet Med*. 2017;19:609–19. <https://doi.org/10.1038/gim.2016.173>.
40. Wang HC, Hsieh LL, Liu YC, Hsiao HH, Lin SK, Tsai WC, et al. The epidemiologic transition of thalassemia and associated hemoglobinopathies in southern Taiwan. *Ann Hematol*. 2017;96:183–8. <https://doi.org/10.1007/s00277-016-2868-7>.
41. Babic I, Andrew SE, Jirik FR. MutS interaction with mismatch and alkylated base containing DNA molecules detected by optical biosensor. *Mutat Res*. 1996;372:87–96. [https://doi.org/10.1016/S0027-5107\(96\)00170-4](https://doi.org/10.1016/S0027-5107(96)00170-4).
42. Tseng YT, Li WY, Yu YW, Chiang CY, Liu SQ, Chau LK, et al. Fiber optic particle plasmon resonance biosensor for label-free detection of nucleic acids and its application to HLA-B27 mRNA detection in patients with ankylosing spondylitis. *Sensors*. 2020;20:3137. <https://doi.org/10.3390/s20113137>.
43. Chaudhari PP, Chau LK, Tseng YT, Huang CJ, Chen YL. A fiber optic nanoplasmonic biosensor for the sensitive detection of ampicillin and its analogs. *Microchim Acta*. 2020;187:396. <https://doi.org/10.1007/s00604-020-04381-w>.
44. Chang TC, Wu CC, Wang SC, Chau LK, Hsieh WH. Using a fiber optic particle plasmon resonance biosensor to determine kinetic constants of antigen-antibody binding reaction. *Anal Chem*. 2013;85:245–50. <https://doi.org/10.1021/ac302590n>.
45. Yu SN, Tsai TH, Chau LK. Optical biosensing system and method thereof. US Patent Appl Pub: US 2021/0011011 A1.
46. Hsu WT, Hsieh WH, Cheng SF, Jen CP, Wu CC, Li CH, et al. Integration of fiber optic-particle plasmon resonance biosensor with microfluidic chip. *Anal Chim Acta*. 2011;697:75–82. <https://doi.org/10.1016/j.aca.2011.04.023>.
47. Chiang CY, Huang TT, Wang CH, Huang CJ, Tsai TH, Yu SN, et al. Fiber optic nanogold-linked immunosorbent assay for rapid detection of procalcitonin at femtomolar concentration level. *Biosens Bioelectron*. 2020;151:111871. <https://doi.org/10.1016/j.bios.2019.111871>.
48. Sharma A, Doucette C, Biro FN, Hingorani MM. Slow conformational changes in MutS and DNA direct ordered transitions between mismatch search, recognition and signaling of DNA repair. *J Mol Biol*. 2013;425:4192–205. <https://doi.org/10.1016/j.jmb.2013.08.011>.

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