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# SPR enhanced DNA biosensor for sensitive detection of donkey meat adulteration

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ABSTRACT

Herein, a surface plasmon resonance (SPR) enhanced DNA biosensor has been developed for real-time detection of donkey meat marker using biotinylated reporter and streptavidin functionalized gold nanostars (Stre@GNSs). Compared to the direct detection assay, this sandwich format for the enhancement of the signal, resulted in 6-folds orders increase in the sensitivity. Target DNA could be detected with the lower limit of quantification (LLOQ) of 1.0 nM with a relative standard deviation (RSD, n = 3) of 0.85%. In addition, the fabricated SPR sensor showed good selectivity for the target analyte over full complementary, single-base mismatch, three base-mismatch and non-complementary oligonucleotides. Finally, the proposed sensor was successfully applied for detection of donkey meat adulteration with various percentages in homemade beef sausage, as a real sample. The results indicated that the proposed biosensor provides a high specificity, easy, good sensitivity and fast approach for identification of donkey meat adulteration in food samples.

## 1. Introduction

Since the level of awareness about food quality and safety has recently increased, food fraud has become a major global issue. Hence, the identification of meat products adulteration with unfavorable and inappropriate animal species is important from health, economic and religious points of views (Mousavi et al., 2015; von Bargen, Brockmeyer, & Humpf, 2014). In addition, the mixing of undeclared meat species in meat products affects the consumer rights and their tracing, particularly in the processed meat products from point of view food regulation is crucial to check the food stuff mislabeling (Mehdizadeh et al., 2014; von Bargen et al., 2014). Given all the above facts, the extension of approaches for species authentication is very important to supervise meat fraud and consumer protection. Currently, the protein-based techniques (e.g. electrophoresis, isoelectric focusing, ELISA and chromatography) have been utilized for meat adulteration. These methods are laborious, expensive, and sophisticated instrumentation with greatly technical proficiency (Calvo, Osta, & Zaragoza, 2002; von Bargen et al., 2014). The SPR technique as an optical device works based on measuring the refractive index changes on a gold sensor surface. When the target molecule is bound or absorbed to ligand-coated sensor surface, the refractive index of fluidic sample changes on the gold surface. The level of change in SPR curve and the obtained response units from the shift angles are related to the quantity of analyte and can be monitored in real time form (Scarano, Mascini, Turner, & Minunni, 2010).

SPR biosensors have been developed not only to investigate the interactions between protein-protein, nucleotides and drug-albumin but also to utilize in the probes of cell surface marker-antibody and studying cellular morphological changes induced by various factors (Fathi et al., 2018; Fathi, Rahbarghazi, & Rashidi, 2018; Pope, Soste, Eyford, Anderson, & Pearson, 2009). Moreover, some poison detections in foodstuffs have been performed such as paralytic shellfish poisoning or quinolones in fish, egg, and poultry meat via the SPR based biosensors (Fonfra et al., 2007).

The enhancement of plasmonic properties and refractive index

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using metal nanoparticles such as gold and magnetic nanoparticles have been developed in nanobiotechnology and nano-medicine for the ultrasensitive detections in SPR-based biosensors extensively (Fathi, Rashidi, & Omidi, 2018; Petryayeva & Krull, 2011). In all of the intensified SPR systems at a direct or sandwich assay, every nanoparticle is covalently attached with analyte molecules (e.g. protein or DNA molecules) which are able to bind with a specific captured probe on modified gold surface (Hossain & Maragos, 2018; Wang et al., 2019). The nanoparticle identifiers as secondary proteins and enhanced labels provide a much higher refractive index change at the evanescent filed of modified gold sensor surface (e.g. antibody or DNA) during biorecognition process (D'Agata, Palladino, & Spoto, 2017; Luo et al., 2016).

In the present study, a novel GNSs-enhanced SPR biosensor was developed to detect donkey meat adulteration in homemade sausage products for the first time. To this end, simple and sandwich hybridization systems were utilized. In the simple format, the related synthesized oligonucleotides were hybridized with the immobilized capture probe on the gold sensor surface. In the sandwich format, the assembled capture probe on the gold chip and reporter probe crammed target DNA to constitute sandwich sensing format. Stre@GNSs were synthesized via physicochemical adsorption of streptavidin on GNSs surface and characterized by ultraviolet and visible spectrophotometer (UV–Vis) and transmission electron microscope (TEM).

## 2. Materials and methods

### 2.1. Materials and reagents

The synthetic oligonucleotides were purchased from Exiqon Company (Denmark). Their sequences were illustrated in Table S1. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was obtained from Bio basic Inc (Markham, Ontario, Canada). Immobilization buffer (I-buffer) was $KH_2PO_4$ (1 M, pH 3.8). Hybridization buffer (H-buffer) was containing 150 mM NaCl, 20 mM $Na_2HPO_4$, 0.1 mM EDTA and 0.005% Tween 20 (pH 7.4).). Streptavidin (Streptomyces avidinii) was obtained from Sigma-Aldrich. Hydrogen tetrachloroaurate (III) (HAuCl4) was obtained from Alfa-Aesar (Ward Hill, MA, USA). Polyvinylpyrrolidone (PVP, MW = 10000), N, *N*-dimethylformamide (DMF), $Na_2HPO_4$, Sodium borohydride ($NaBH_4$), 6-Mercapto-1-hexanol (MCH) were from Merck (Darmstadt, Germany). *Alu* I (Thermo Scientific, Italy, 301054) and *MSP* I (Takara, Bio Inc., Japan, K3001AA) restriction enzymes were purchased. Pure gold chip was supplied from Bionavis Company (Finland). All other chemicals were prepared from Sigma-Aldrich (US). All solutions were prepared using Milli-Q water (18 MΩ·cm resistivity) from a Millipore system except oligonucleotides solutions that were produced using nuclease-free water (Thermo Scientific, 00253876). Sample of beef meat was provided from popular retail markets (Tabriz, located at the northwest of Iran). Muscle samples from donkey were obtained from Veterinary Hospital (Faculty of Veterinary Medicine, University of Tehran, Karaj, Iran). Also, the total sausage ingredients were prepared from a factory in Tabriz, Iran.

### 2.2. Apparatus

A multi-parameter SPR device (MP-SPR Navi 210A, BioNavis Ltd, Tampere-region, Finland) with gold chips (BioNavis Ltd, Finland) was utilized to study DNA-DNA interactions. All of the Au-sensor chips were made of 240 $mm^2$ BK7-glass that coated with a 50 nm gold layer and have a Cr layer underneath as the adhesive metal layer. The analysis was carried out in the fixed angle way at the operating temperature of 37 °C. Also, a wavelength of 785 nm was applied for a laser light source in the SPR instrument during the tests. The morphology of the GNSs and Stre@GNSs was assessed by TEM (LEO 906E from Carl Zeiss, Oberkochen, Germany, 100 KV). The UV–Vis absorption spectra were taken on the Cytation™ 5 cell imaging (BioTek, Winooski, USA).

### 2.3. Synthesis of gold nanostars (GNSs)

Gold nanostars were prepared via a two-step seed-mediated growth method. Firstly 2–3 nm Au seeds were synthesized. In detail, 22 μL of 0.1136 M $HAuCl_4$ aqueous solution was added to a 47.5 mL solution of 0.098 g Polyvinylpyrrolidone (PVP, MW10000) in DMF/$H_2O$ (18:1 v/v). Then 2.5 mL of 10 mM freshly prepared ice-cold $NaBH_4$ solution was added and stirred for 2 h at room temperature. The resultant solution (seed solution) was stored at room temperature overnight before use. In the second (growth) step, 42 μL of 50 mM $HAuCl_4$ was added to 15 mL of 4.6 mM PVP solution in DMF. Then 20 μL of seed solution was injected into the mixture rapidly and stirred for 2 h. It changed from colorless to blue, indicating the formation of GNSs. The resultant blue solution was centrifuged, supernatant removed, and the gold nanostars were re-dispersed in ultrapure water (Amjadi & Abolghasemi-Fakhri, 2018).

### 2.4. Modification of GNSs with streptavidin (Stre@ GNSs)

For the synthesis of streptavidin-modified GNSs, a simple method as described elsewhere, (He, Liu, He, & Cui, 2011; Li, Yu, Cui, Yang, & Bian, 2013) was used. Briefly, 1 mL GNSs (pH adjusted to 7.0 using 0.1 M NaOH) was added to 50 μL of 1 mg $mL^{-1}$ streptavidin and incubated at 37 °C for 30 min. After that, a 5% BSA solution was added to the mixture to provide a final concentration of 1% BSA, incubated at 37 °C for 5 min. After centrifugation at 12500 rpm, the final precipitate was dispersed in 250 μL of 0.1 M PBS (pH 7.4) and kept at 4°C until use.

### 2.5. Immobilization of capture probe on the surface of Au-SPR biosensors

Each Au-sensor chip was cleaned by immersion in a boiling solution of $NH_3$ (30%), $H_2O_2$ (30%) and milliQ water in ratio 1:1:5 for 10 min at 95 °C. Then, Au-sensors were thoroughly washed several times with Milli-Q water and dried by nitrogen stream for immobilization, instantly. All sensor chips were incubated with 80 μL of thiolated capture probe (1.35 μM) which was earlier activated by TCEP, in a closed container for 48 h (Milkani, Khaing, Morais, Lambert, & McGimpsey, 2011) at room temperature. The details about protocol of probe activation with TCEP was mentioned in supplementary. Following incubation, it was rinsed in PBS solution (0.01 M, pH 7.4) to remove excess capture probe and dried with nitrogen. Then the CP-modified Au-sensor chip was incubated with blocking solution of MCH (1 mM, 100 μL) for 1 h at room temperature (in the dark) for removal non-specific binding and washed with soaking in H-buffer 4 times. After blow-drying by nitrogen, to avoid any prism contamination in the direct contact of the glass chip and prism during the SPR analysis, the glass surface of prepared Au-sensor chip was precisely cleaned with ethanol and optical tissue. Finally, freshly sensor chip was docked into the sensor-chip-holder of SPR, prepared for the recording of initial scans before and after modification with CP and also for hybridization reactions as described in Sections 2.6 and 2.7.

### 2.6. Donkey marker detection based SPR bio-sensing (simple format)

The DNA target hybridization measurement was performed at a flow rate of 10 μL/min with a sensor temperature fixed at 37 °C in fixed angle mode. For this purpose, first for baseline operation, H-buffer was used. When the baseline was stable, various concentrations (2–200 nM) of synthetic oligonucleotides in H-buffer were injected on the capture probe-modified chip and monitored for 8 min. After hybridization reaction, the sensor chip was automatically washed with H-buffer to remove the non-hybridized DNA target. The analytical signal in sensograms, reported as Resonance Units (RU), was consequent from the difference between the observed value before the target injection (baseline) and after the hybridization reaction, once the sensor had been rinsed by H-buffer.

### 2.7. Donkey marker detection based Stre@GNSs SPR bio-sensing (sandwich format)

The flow rate and sensor temperature of the experiment for target injection were set the same as values mentioned above. First for baseline operation, H-buffer was used. When the baseline was stable, various concentrations (1–500 nM) of synthetic oligonucleotides in H-buffer were injected over CP-modified sensor surfaces. After reacting for 8 min, the sensor chip was automatically washed with H-buffer to remove the non-hybridized target. To obtain more sensitive results, the sandwich system was used. After the binding of DNA target to the sensor surface, 1 μM bio-reporter was injected for 5 min, and then it was rinsed by H-buffer solution for several minutes. Afterward, the Stre@GNSs solution with volume of 50 μL and OD = 0.177 was injected at the flow rate of 10 μL/min and reacted for 5 min followed by washing it by H-buffer solution. Subsequently, the SPR response values can be observed at the same time.

## 3. Results and discussion

### 3.1. Characterization and optimization of attachment DNA probe on SPR sensor surface

Stre@GNSs labels attached to the biotinylated reporter probe through streptavidin–biotin affinity and efficiently increase the refractive index on the gold sensor surface. Therefore, the increasing of mass density near the gold sensor and plasmonic coupling of GNSs and gold film can provide excellent sensitivity improvement in SPR responses. The schematic illustration of the steps in preparation of enhanced SPR based biosensor was shown in Scheme 1.

The amount of DNA probe immobilized on the Au-sensor surface will greatly effect hybridization efficiency and the SPR biosensor response. As surface coverage of thiol-modified probe on the gold chip was a function of deposition time, therefore the probe immobilization time was optimized and characterized by the angle shift in the initial scan. In this way, the thiolated CP was incubated on the gold chip in a closed container for different times (2, 24 and 48 h) at RT. MCH was used to remove the non-specific binding and to facilitate target DNA hybridization by spacing the CP oligonucleotides (Gong et al., 2006). The resonance curve angle shift of bare gold sensor compared to the CP-immobilized sensor has been presented in Fig. 1A. The SPR curve displays the steepest slope fall of the peak during adsorption of a new layer on the sensor surface and shift of TIR (total internal reflection). Due to forming a new DNA layer the thickness of the absorbed layer is increased and the SPR curves shift to higher angles and become broader. As shown in Fig. 1A shift angle of the CP-modified gold sensor at 48 h is more than other chips. So, the optimal deposition time was selected to be 48 h. On the other hand, binding of 1 ng/mm$^2$ of the ligand on chip surface corresponds with the output of 100 m° (milidegree) SPR response, as described previously (De Mol, 2010). Accordingly, after 48 h deposition time of probe, almost 280 m° shift was observed. Therefore, the amount of ligand attached to the sensor surface was estimated to be ≈2.8 ng/mm$^2$.

Furthermore, the SPR modified Au-sensor chip was characterized through the SPR monitoring of target DNA hybridization to the immobilized capture probe. For this purpose, 25 nM DNA target was injected on the sensor surface, which had been incubated previously with a capture probe at different times and blocked by MCH blocking solution. Following injection, the SPR sensograms was monitored. 1.2 pg/mm$^2$ of surface-bound DNA (irrespective of sequence length) correlated with an increase of 1 RU response signal (Stenberg, Persson, Roos, & Urbaniczky 1991). According to sensograms exhibited in Fig. 1B, 48 h incubation time with CP leads to the greatest amount of hybridization, which confirms the greatest amount of probe immobilization and also the results of SPR curves.

### 3.2. Characterization of GNSs and Stre@GNSs

In the synthesis of GNSs, PVP reduced $HAuCl_4$ to deposit Au onto the seeds through a kinetically controlled process. PVP is responsible for shape-control by adsorbing and desorbing from the different crystal facets in a preferential sequence (Koczkur, Mourdikoudis, Polavarapu, & Skrabalak, 2015).

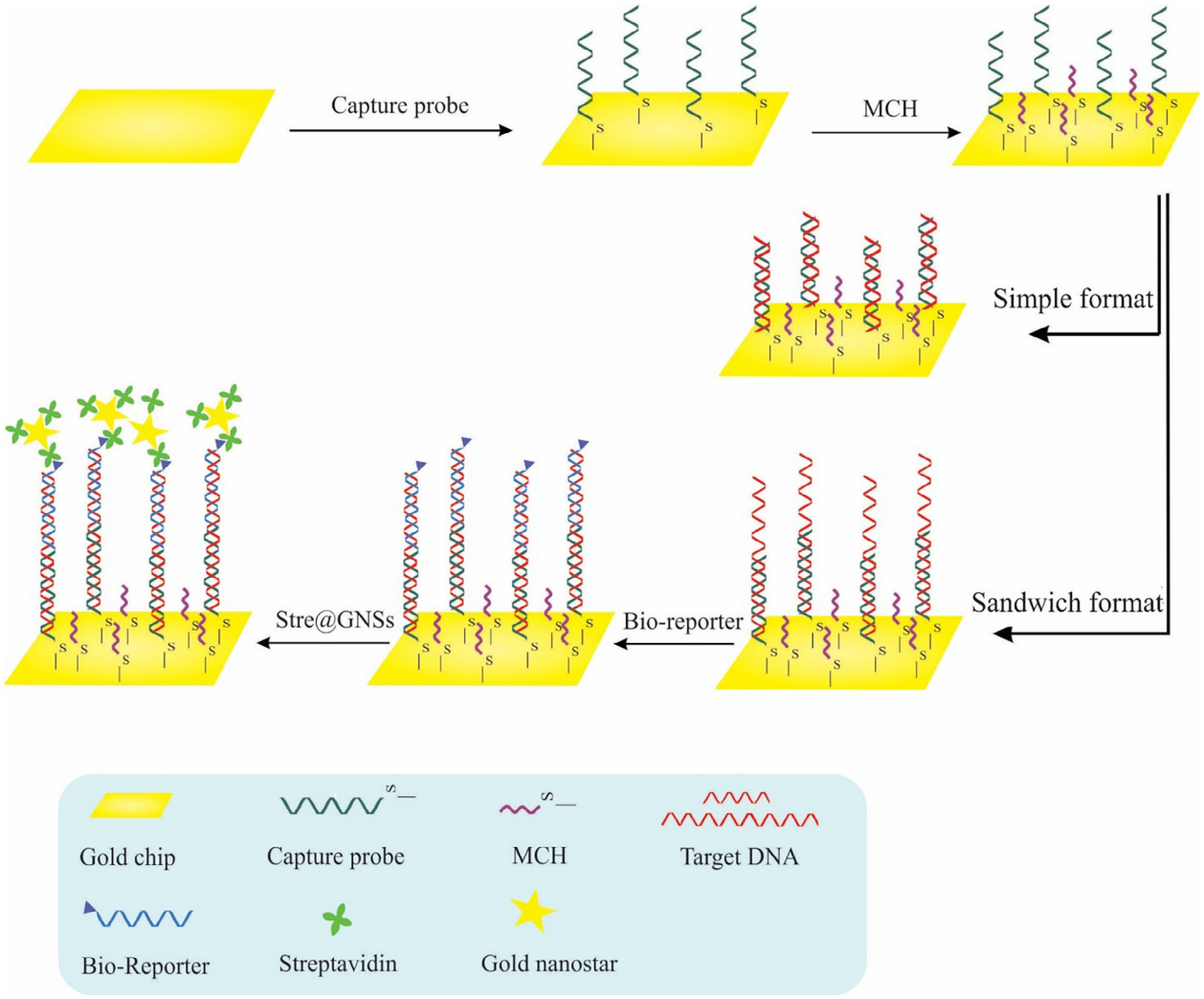
The particle size and morphology of the GNSs, were investigated by TEM image. As shown in Fig. 1C and D, GNSs comprise of multiple crystal domains which in term of their distribution and number on the structure have a mixed morphology with the average diameter of 40 ± 5 nm. The conjugation of the multi-domain GNSs structure with streptavidin can facilitate obscuring the tip of the nanoparticles and their longer diameter (arrows in Fig. 1C and D), indicats the successful bioconjugation of Stre@GNSs. In addition, successful bioconjugation further confirmed by UV–Vis absorption spectra. Although GNSs have a noticeable structural heterogeneity (Nehl, Liao, & Hafner, 2006), as seen in Fig. 1E, their UV–vis absorption spectra display broad peaks in the NIR (near IR) and visible. It has been recognized that elongated gold nanoparticles such as GNSs have a longitudinal plasmon resonance about 520 nm (related to the spherical gold nanoparticles) red shifts (Link & El-Sayed, 1999). The broad absorption band at 762.5 nm is related to the elongated tip of the nanoparticles and the transverse absorption band of the tips at 600 nm similar to that seen for gold nanorods, or related to the presence of slightly asymmetric or large spherical nanoparticles in the sample solution, is illustrated (Fig. 1E, blue curve). The formation of Stre@GNSs bioconjugation led to the change in the refractive index near the GNSs surface, so the long-wavelength band of Stre@GNSs was red-shifted about 5 nm compared to the pure GNSs (Fig. 1E, red curve), demonstrating that GNSs were successfully conjugated with streptavidin. The use of UV–vis spectroscopy is a common method for validation of the successful modification on the gold nanoparticle surfaces (Billingsley, Riley, & Day, 2017).

### 3.3. Selection of the best laser source wavelength in SPR instrument for enhancement

Due to having two different wavelengths of the laser source in a SPR instrument (670 and 785 nm), we examined the enhancement intensity of GNSs at these wavelengths to choose the best option. From the obtained experimental result (data not shown), GNSs with $\lambda_{max}$ 767.5 nm had a more response unit ($500 \times 10^{-4}$ RU) in laser source wavelength 785 nm compared with 670 nm ($100 \times 10^{-4}$ RU). So, we hypothesized that the extra intensifying effect by plasmonic coupling between the GNSs and the gold layer is found when the $\lambda_{max}$ of gold nanostars are close the wavelength of the excitation laser source in SPR instrument. Based on these data, in this work, we used a laser operating at 785 nm as the excitation laser source in SPR for detection DNA target molecules.

### 3.4. Analytical performance of the prepared SPR sensor

A simple hybridization assay with related oligonucleotides was performed on the prepared sensor surface. The reaction progress between the immobilized capture probe and target DNA was recorded by the SPR sensograms. As shown in Fig. 2A and C, the response of SPR toward the target DNA increased gradually with the increasing of target DNA concentrations. The calibration plot exhibited a linear relationship ($R^2$ = 0.956) between SPR response and the logarithm of marker concentration in the range of 2 to 200 nM with the equation of (Y = 14.071 log [target DNA] + 34.824) (Fig. 2B). Based on the results, LLOQ (the lower limit of quantification) of the SPR sensor for target DNA, which was the lowest concentration obtained in the laboratory in practice, was obtained as 2 nM. The relative standard deviation (RSD) of method was calculated to be 3.050% by 3 times repeated determination of 2 nM target DNA.




**Scheme 1.** Schematic illustration of the SPR based sensor fabrication steps.

GNSs amplify the SPR signal presumably via two mechanisms: (i) Great resonance angle shift at the sensor surface due to nanoparticle mass (Kim, Lee, Kim & Lee, 2017; Lyon et al., 1999) (ii) Coupling between the SPR incitement wavelength in the LSPR and the NIR and its combination with the first effect as if there is a substantial overlap (Kim et al., 2017). Accordingly, a sandwich hybridization assay was designed for signal amplification using related synthetic oligonucleotides as target DNA, bio-reporter and Stre@GNSs. Streptavidin is a homo-tetramer with an extraordinarily high affinity for biotin ($K_a$: $10^{15}$ mol/L), and streptavidin/biotin system is considered as one of the most powerful noncovalent biological interactions. Therefore, this system is used commonly as terminal functional groups in many biological studies to increase the selectivity of the interactions. Hydrogen bonding between the ureido ring of biotin molecules and the amino acid groups of streptavidin and also hydrophobic interactions are the major binding forces involved in the association of biotin and streptavidin molecules. Not only is the strength of biotin-streptavidin association similar to covalent bonds, but also the stability of this complex in variable range of pH and temperature conditions is very high (DeChancie & Houk, 2007). Fig. 3A and C illustrated that Stre@GNSs enhanced SPR signal toward the target concentration. As expected, the SPR response increased as the target concentrations increased. The calibration curve presented a proper linear relationship for SPR responses versus the logarithm of related target different concentrations from 1 to 500 nM with correlation coefficient of $R^2 = 0.987$ (Fig. 3B). The obtained LLOQ of the enhanced sandwich assay for donkey marker detection was 1 nM. The RSD was achieved to be 0.853% for 3 replicate determinations of 1 nM target analyte. Comparing the two calibration plots (slope of plots) (Fig. 2B and B) reveals that the sensitivity of GNSs enhanced sandwich hybridization assay was about 6 times greater than using simple hybridization assay. Hence, we deduce that in sandwich assay the original interaction signal amplifies notably and increase the capability of the assay to distinguish between two very close concentrations of analyte which in turn increases the reliability of the method. In addition, the RSD resulting sandwich assay (0.853%, n = 3) for 1 nM target analyte, followed by the introduction of Stre@GNSs compare with that obtained from simple assay (3.050%, n = 3) for 2 nM target analyte, had a proper result. In Table 1, some of the methods used for the quantification of species origin in meat products reported in literatures have been summarized.

In order to evaluate the selectivity of the prepared SPR sensor for target detection, full complementary, single-base mismatch, three base-mismatch, and non-complementary oligonucleotides at a concentration of 10 nM were used. As seen in Fig. 4A and B, the SPR responses resulting from single-base mismatch, three base-mismatch, and non-complementary oligonucleotides hybridization were very low around that of the negative control or free target (RU < 20), while the SPR response of full complementary oligonucleotide was significantly higher than the others. These results suggested that the prepared SPR sensor was highly specific to discriminate full complementary and mismatched oligonucleotides.

Also to evaluate the precision of the sensors, the reproducibility of

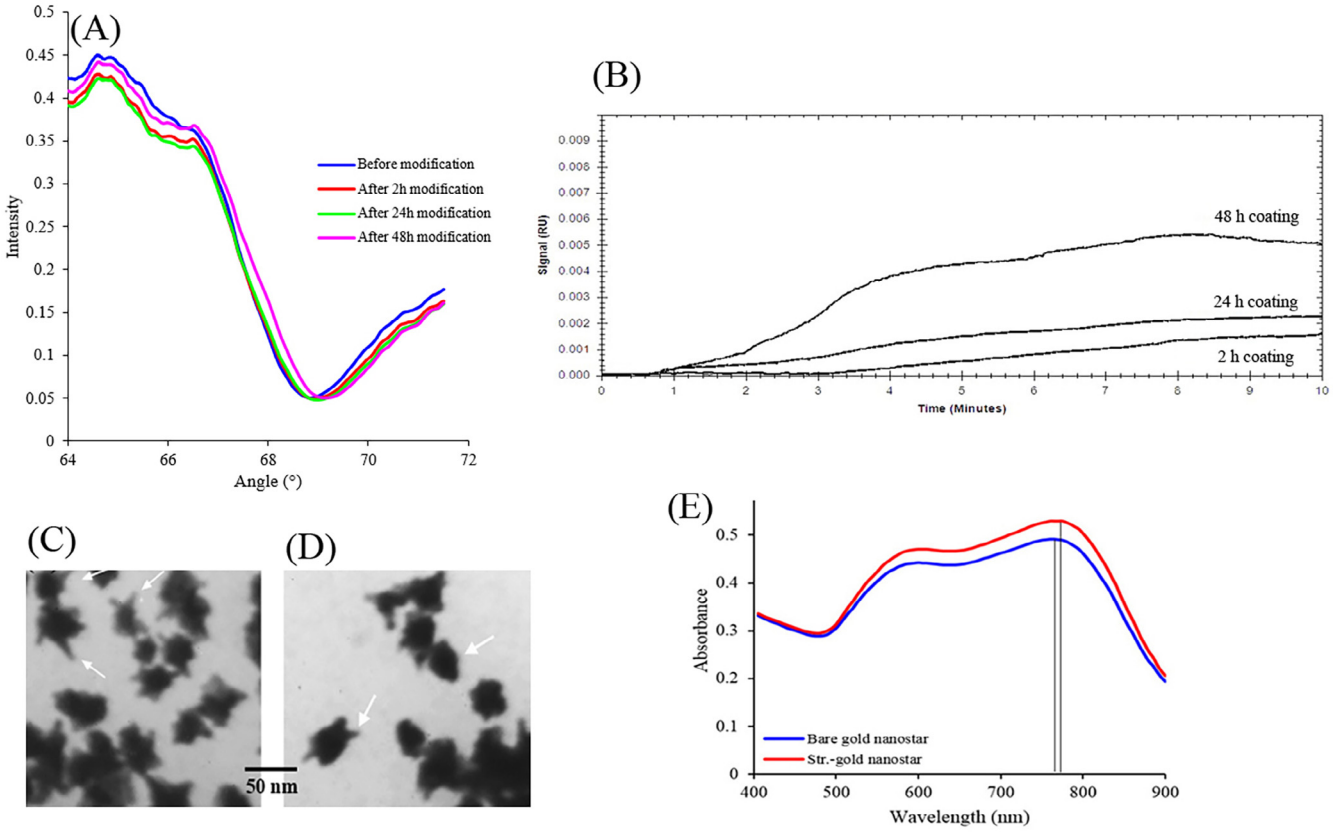



**Fig. 1.** A) SPR curve of angle shift in gold chips before and after modification with capture probe at 2, 24 and 48 h. B) The SPR sensograms obtained from hybridization of 25 nM target DNA with immobilized CP at different times. C) Illustrative TEM image of GNSs and D) TEM image of Stre@GNSs; E) An Uv–vis spectra of GNSs (blue curve) and Stre@GNSs (red curve). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the proposed biosensor was examined. The reproducibility as an inter-laboratory test was investigated in two different days for 2 and 50 nM concentrations of the target probe and the desirable RSDs were calculated as 5.40% and 4.74%.

### *3.5. Application of the prepared biosensor for analysis of donkey adulteration in cooked sausages*

In order to evaluate the reliability of the proposed SPR biosensor to analyze the donkey adulteration in real samples, at first, the homemade sausages were prepared from donkey-beef dual mixtures containing 1%, 5% and 25% values of donkey meat and also pure beef (100% beef). Then the DNA extraction from the samples was performed with "DNeasy mericon food kit" (QIAGEN, Cat. 69514) according to the manufacturer's protocol. The extracted DNA was re-suspended in nuclease free water and its purity and concentration were determined using nanodrop spectrophotometer (ND-1000, USA). Followed by the digestion for 16 h using *Alu* I and *MSP* I restriction enzymes and at that time, inactivation of digestion, stopping of nonspecific digestion and strand separation was accomplished by heating in a thermal cycler (Applied Biosystems, US). The sandwich hybridization assay for the digested DNA (volume; 80 μL) was used similar to what described for oligonucleotides. The SPR responses (RU) of these samples were illustrated in Figs. S1A and B. The donkey specific SPR-based biosensor noticeably detected 1% of donkey meat in homemade sausage produced from donkey-beef dual mixtures. Thus, this substantially returns the more sensitivity and specificity of the developed biosensor to recognize the target DNA in processed food products by heat and severe pressure, which, occasionally leads to degrading DNA (Brodmann & Moor, 2003; Haider, Nabulsi, & Al-Safadi, 2012; Hird et al., 2006; Kesmen, Gulluce, Sahin, & Yetim, 2009). The produced sausage of pure beef demonstrated the SPR response that was nearly similar to the baseline response of the non-hybridized capture probe (Figs. S1A and B.). The results showed that the developed SPR based biosensor could detect donkey adulteration in real samples.

The precision was examined as an important feature of the proposed sensor for extracted DNA from real samples. In this regard, we test the reproducibility of the proposed sensor. The reproducibility as an Inter-laboratory test was checked in two different days for homemade beef sausages containing 1% and 5% values of donkey meat and the acceptable RSDs were calculated as 6.37% and 5.84%.

Recently, DNA-based techniques, in particular, polymerase chain reaction (PCR) based approaches have been proposed including restriction fragment length polymorphism (PCR-RFLP) (Doosti, Ghasemi Dehkordi, & Rahimi, 2011), single or multiplex PCR (Kitpipit, Sittichan, & Thanakiatkrai, 2014), Taqman or SYBR GREEN Real-time PCR (Soares, Amaral, Oliveira, & Mafra, 2013) and randomly amplified polymorphic DNA (RAPD–PCR) (Saez, Sanz, & Toldrá, 2004). The sensitivity of recently published PCR methods to identify species in raw products is excellent. While in the case of processed foods, DNA degradation (Musto, Faraone, Cellini, & Musto, 2014) may lead to false negative results, thereby decreasing the sensitivity and reliability of the PCR techniques in species identification.

Although the PCR-based approaches have been a necessary step to assess genomic DNA, there are some sensing approaches without PCR analyses, such as surface plasmon resonance (SPR) (Nelson, Grimsrud, Liles, Goodman, & Corn, 2001) , electrochemical (Dong et al., 2015; Pakchin, Nakhjavani, Saber, Ghanbari, & Omidi, 2017; Samadi Pakchin, Ghanbari, Saber, & Omidi, 2018), fluorescent microarrays (Sueda, Yuan, & Matsumoto, 2002), and nanoparticle-based biosensors (Huang,

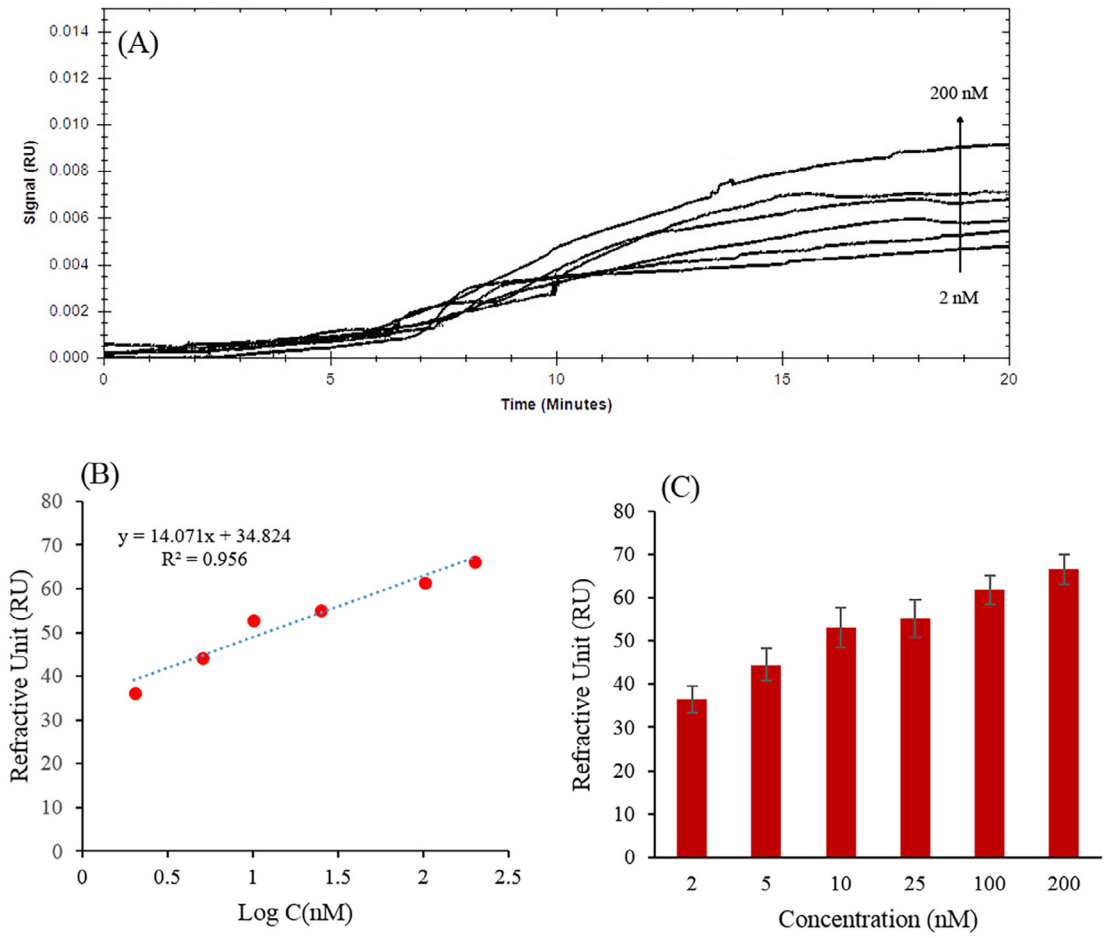



**Fig. 2.** A) The SPR response at different target concentrations in simple format (2, 5, 10, 25, 100 and 200 nM, from bottom to up). B) The calibration curve between the refractive unit and the logarithm of the target concentration. C) The bar graphs of SPR responses versus different target concentrations in simple format. Error bars indicate the standard deviations for three replicates.

Bai, Dong, Guo, & Liu, 2015). Also, nano-biosensors via nanoparticles have been developed to detect and quantify the pork-specific sequences in meat and mixed biological samples (Ali, Hashim, Mustafa, Che Man, & Islam, 2012; Ali et al., 2011; Kuswandi, Gani, Kristiningrum, & Ahmad, 2017). To detect the pork fraud in the processed burger, a nano-biosensor has been designed via labeled single-stranded probe (Ali, Mustafa, Hashim, Che Man, & Foo, 2012). The detection methods of the mentioned biosensors were based on the fluorescence spectroscopy and colorimetric analyses which require sophisticated equipment and expert personnel.

The proposed biosensor is the first SPR DNA-based biosensor for detection of donkey meat adulteration, especially in cooked sausages. Taking into account the ability of SPR as a real time and label free method for some important investigations such as ligand-protein binding studies, and quantitative calculation of kinetic constants, this biosensor could be of great value. In addition, reusability of the sensor and consumption of minimal amount of samples are another advantages of the proposed SPR biosensors.

## 4. Conclusion

In this study, a novel SPR-based method was successfully developed to detect the donkey meat adulteration using bio-reporter and Stre@GNSs with enhanced SPR signals. The evaluation comprised a simple hybridization and GNSs enhanced sandwich hybridization assays. UV–vis and TEM were applied to characterize GNSs and Stre@GNSs. It revealed that the introduction of bio-reporter and Stre@GNSs to the sensor surface resulted in a considerably SPR signal enhancement and LLOQ of 1.0 nM with RSD (n = 3) of 0.853% obtained. In addition, the proposed biosensor was well applied for the detection of donkey meat adulteration with various percentages in homemade beef sausage, as a real sample, for the first time. Based on data, the prepared biosensor provides a high specificity, simple, reliable, proper sensitivity and rapid approach for the identification in food analysis suggesting a novel strategy for the production of SPR biosensors by rectifying the corresponding capture probes.

## CRediT authorship contribution statement

**Maryam Mansouri:** Investigation, Writing - original draft, Writing - review & editing. **Farzaneh Fathi:** Resources, Writing - original draft. **Roghayeh Jalili:** Investigation, Visualization. **Shahram Shoeibie:** Project administration, Funding acquisition. **Siavoush Dastmalchi:** Formal analysis. **Alireza Khataee:** Data curation. **Mohammad-Reza Rashidi:** Conceptualization, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

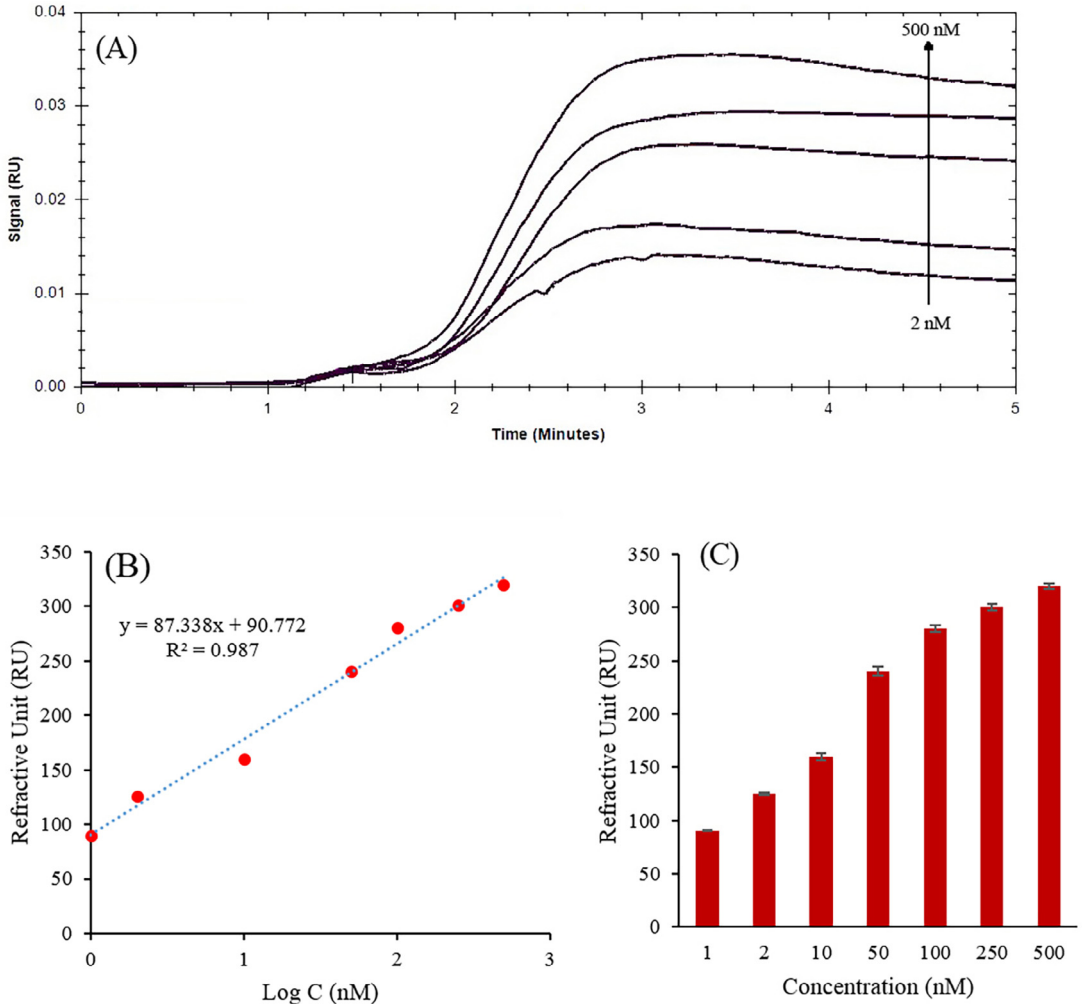



**Fig. 3.** A) The SPR response after Stre@GNSs injection in enhanced sandwich format at different target concentrations (1 nM, not shown, 2, 10, 50, 100, 250 and 500 nM, from bottom to up). B) The calibration curve between the refractive unit and the logarithm of the target concentration. C) The bar graphs of SPR responses versus different target concentrations in Stre@GNSs enhanced sandwich format. Error bars indicate the standard deviations for three replicates.

**Table 1**
Methods used for quantification of species origin in meat products.

| Technique | Species [a] | Quantification level | References |
|---|---|---|---|
| **Protein-based** Chromatography (HPLC) | P, h | 0.24% w/w marker peptides (raw) | (von Bargen et al., 2014) |
| **DNA-based** | | | |
| a) PCR based | | | |
| - PCR gel Electrophoresis | p | 0.1% w/w (raw) | (Calvo et al., 2002) |
| - Real time PCR TaqMan | mu, g | 1% w/w (heated) | (Rodriguez, Garcia, Gonzalez, Asensio, & Hernandez, 2004) |
| - Real time PCR SYBR Green | p, wa | 0.04 pg DNA (raw) | (Lopez et al., 2006) |
| | c, h | 0.4 pg DNA (raw) | (Lopez, Garrido, & Puyet, 2003) |
| b) Hybridization | | | |
| - Spectrofluorimetry | p | 1% w/w (raw and cooked) | (Ali et al., 2014) |
| - [b]NPs-enhanced SPR-based | d | 1% w/w (cooked); **1 nM** (LLOQ) | **This work** |

[a] c, cattle; d, donkey; g, goose; h, horse; mu, mule duck; p, pork; wa, wallaroo.
[b] NPs: Nanoparticles.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127163.

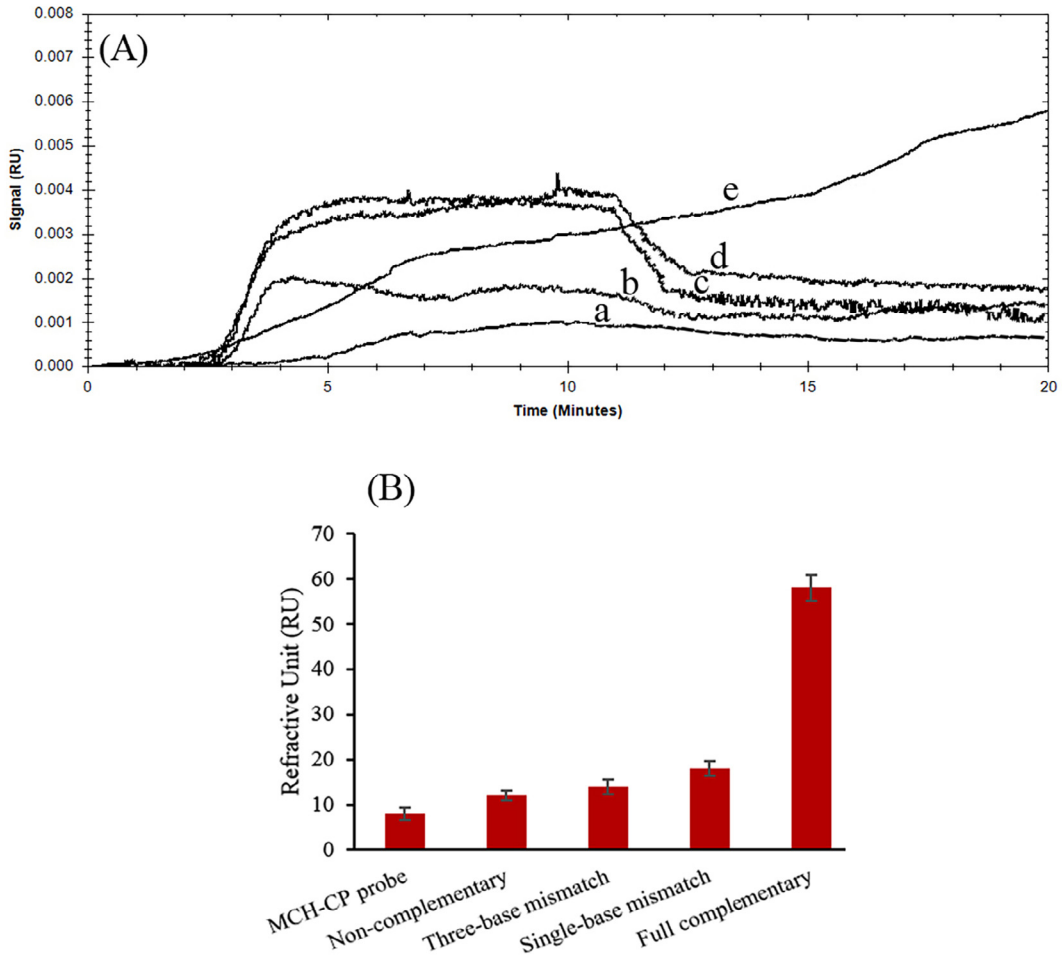


**Fig. 4.** A) The SPR response toward different sequences including full complementary, single-base mismatch, three base-mismatch, non-complementary (at a concentration of 10 nM) and free target (MCH-CP probe) from up to bottom (e-a). B) The bar graphs of SPR responses versus different sequences and free target.

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