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Aptamer–Au NPs conjugates-enhanced SPR sensing for the ultrasensitive sandwich immunoassay

Jianlong Wang, Ahsan Munir, Zhonghong Li, H. Susan Zhou*

Department of Chemical Engineering, Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA 01609, United States

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ABSTRACT

The goal of this work is to explore the amplification effect of aptamer–gold nanoparticles (Au NPs) conjugates for ultrasensitive detection of large biomolecules by surface plasmon resonance (SPR). A novel sandwich immunoassay is designed to demonstrate the amplification effect of aptamer–Au NPs conjugates by using human immunoglobulin E (IgE) as model analyte. Human IgE, captured by immobilized goat anti-human IgE on SPR gold film, is sensitively detected by SPR spectroscopy with a lowest detection limit of 1 ng/ml after anti-human IgE aptamer–Au NPs conjugates is used as amplification reagent. Meanwhile, the non-specific adsorption of aptamer–Au NPs conjugates on goat anti-human IgE is confirmed by SPR spectroscopy and then it is minimized by treating aptamer–Au NPs conjugates with 6-mercaptopentanol (MCH). These results confirm that aptamer–Au NPs conjugates is a powerful sandwich element and an excellent amplification reagent for SPR-based sandwich immunoassay.

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1. Introduction

Since screened through the systematic evolution of ligands by exponential enrichment (SELEX) process from random RNA or DNA libraries, considerable progress has been made in the development of nucleic acid (aptamer)-based bioseparation and detection (Goringer et al., 2003; Tombelli et al., 2005b). A large number of aptamers have been selected for all kinds of analytes in the past of a few years (Ellington and Szostak, 1990). These DNA or RNA molecules, with unprecedented advantages such as high selectivity and affinity toward their targets, chemical stability, synthetic convenience, easy modification by functional group have become increasingly important molecular tools for diagnostics and therapeutics (Willner et al., 2008; Willner and Zayats, 2007; Phillips et al., 2008). In particular, aptamer has been used to develop biosensors for detecting different types of analytes including small biomolecules (Nutiu and Li, 2003; Liu et al., 2006; Zou et al., 2007; Zayats et al., 2006), metal ions (Wilcox et al., 2008; Noeske et al., 2007; Radi and O'Sullivan, 2006), and proteins (Lai et al., 2007; Stadtherr et al., 2005; Kirby et al., 2004). Most of aptamer-based biosensors and bioassay methods are developed based on electrochemistry (Kim et al., 2008), fluorescence (Ho et al., 2008), colorimetric (Lu and Liu, 2007) and quartz crystal microbalance (QCM) (Liss et al., 2002). Surface plasmon resonance (SPR) spectroscopy is seldom combined with aptamer technique to develop

biosensor. SPR can in situ detect the concentrations of biomolecules during the binding process, being a powerful surface-sensitive characterization method. Furthermore, the kinetic data including the equilibrium constant, the association and dissociation parameters between biomolecules can also be obtained by simulating SPR kinetic curves (Campbell and Kim, 2007). Therefore, using SPR will be advantageous in understanding the interfacial binding characteristics of aptamer with its target molecules. It is expected that the application of aptamer technique in SPR field will provide a novel and simple method to design and develop biosensor with high selectivity and sensitivity.

There have been relatively few efforts made to construct SPR biosensor with aptamer technique (Lee et al., 2008a; Li et al., 2007; Tombelli et al., 2005a). This is due to the fact that the change in SPR signal resulting from the binding process of aptamer with its target molecules is not very large. In general, there are two changes that may lead to the change of SPR signal during the binding process of aptamer with its target molecules. One is the conformational change of aptamer. Usually, aptamer is stable with single-stranded DNAs form. However, they can fold into special three-dimensional structures through base pairing under the help of its target molecules (Wang et al., 2005). Obviously, this conformational change of aptamer is difficult to be observed by SPR because the molecular weight of aptamer is unchanged. The other change comes from the binding of target molecules with aptamer. When the target molecules are small molecules, there is only a trivial increase in the mass change of aptamer because of the binding of small molecules. Thus, SPR is still difficult to detect this binding process. When the target molecules are large biomolecules such as

* Corresponding author. Tel.: +1 508 831 5275; fax: +1 508 831 5936.
E-mail address: szhou@wpi.edu (H.S. Zhou).

protein, SPR can observe easily the binding process. Although there are a few studies on the binding process of aptamer with proteins by SPR and some SPR-based aptasensor have also been developed (Tang et al., 2007; Lee et al., 2008b), the sensitivity of these SPR-based aptasensors is still limited. Therefore, methods to amplify the SPR signal resulting from the binding process between aptamer and its target molecules is very critical for designing and developing sensitive SPR-based aptamer biosensor.

Recent work on the colorimetric bioassay based on aptamer–Au NPs conjugates demonstrated that aptamer technique can be easily combined with nanomaterials (Liu and Lu, 2004, 2006; Li et al., 2008) to amplify signal strength. Interestingly, one of the advantages of Au NPs is that it can greatly enhance the sensitivity of SPR-based sensor through the electronic coupling interaction between the localized surface plasmon of the NPs and the surface plasmon wave associated with the SPR gold film (Lyon et al., 1999; Link and El-Sayad, 1999). Thus, it is desirable to increase the sensitivity of SPR sensor for the selective detection of biomolecules using aptamer–Au NPs conjugates. In the conjugates, aptamer promises the specific binding with target molecules, and Au NPs enable the enhancement of SPR signal.

In our previous work, we combined the amplifying characteristic of Au NPs with the advantages of aptamer techniques to develop a method for detecting small molecules by SPR spectroscopy (Wang and Zhou, 2008). Here, we focus strongly on exploring the ability of aptamer–Au NPs conjugates in increasing the sensitivity of SPR for the detection of large biomolecules. For this purpose, we designed a novel sandwich immunoassay to evaluate the amplifying characteristic of aptamer–Au NPs conjugates by using human IgE as a model large biomolecule. We select human IgE as model molecules because human IgE plays a key role in allergic reactions. Furthermore, the concentration of human IgE in human serum is so low (~ 1 nM) that it was difficult to be detected directly (Maehashi et al., 2007). Our results confirmed that the lowest detection limit of human IgE after the amplification of aptamer–Au NPs conjugates can reach $0.001 \mu\text{g/ml}$, which is much higher than that of results obtained in general by SPR detection ($\sim 1 \mu\text{g/ml}$).

2. Experimental

2.1. Materials

Trisodium citrate, hydrogen tetrachloroaurate(III) (HAuCl_4), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), ethanolamine, albumin from bovine serum (BSA), 3-mercaptopropionic acid (MPA) and 6-mercaptohexan-1-ol (MCH) were purchased from Sigma and used as received. Sodium hydrogen phosphate heptahydrate, potassium dihydrogen phosphate, sodium chloride and 2-mercaptoethanol (MCE) were ordered from Alfa Aesar. Human IgE and goat anti-human IgE were obtained from Fitzgerald Industries International (USA). Human immunoglobulin M (IgM) and human immunoglobulin A (IgA) were ordered from Thermo Scientific (USA). The 5'-thiol-modified aptamer of human IgE were obtained from Integrated DNA Technologies (IDT). The sequence was 5'-SH-C6-ATC TAC GGG GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG CCC C-3'. The thiol-aptamer solutions were prepared by dissolving thiol-aptamer in 50 mM pH 8.0 Tris–HCl buffer including 138 mM NaCl and 5 mM MgCl_2 . Protein solutions were prepared by dissolving different proteins in pH 7.4 PBS (10 mM). All glassware used in the experiment was cleaned in a bath of freshly prepared 3:1 HCl: HNO_3 (aqua regia) and rinsed thoroughly in H_2O prior to use (caution: aqua regia solution is dangerous and should be handled with care).

2.2. Synthesis of Au NPs with the diameter of ~ 13 nm

Au NPs stabilized with citrate was synthesized according to the literature procedure (Storhoff et al., 1998). Briefly, 100 ml of 1 mM HAuCl_4 (4 ml 1% (w/w) HAuCl_4 solution dissolved in 96 ml H_2O) was brought to a reflux while stirring and then 10 ml of a 38.8 mM trisodium citrate (10 ml 1.14% (w/w) trisodium citrate) solution was added quickly, which resulted in a color change of the solution from pale yellow to deep red. After the color change, the solution was refluxed for an additional 15 min and left to cool to room temperature. The average size of prepared Au NPs was 13.9 nm, confirmed by our previous work (Wang et al., 2009b). The concentration of the prepared Au NPs was ~ 9.1 nM, calculated by the quantity of starting material (HAuCl_4) and the size of Au NPs at the wavelength of 519 nm. This calculation was based on the method reported by Mucic et al. (1998). Unless specially mentioned, this concentration was used for all experiments.

2.3. Human IgE aptamer–Au NPs preparation

Human IgE aptamer–Au NPs conjugates were prepared according to our previous work with minor modification (Wang and Zhou, 2008). Briefly, transfer 10 ml of the already prepared Au NPs to the NaOH-treated glass vials and then add $138.83 \mu\text{l}$ of 5'-thiol-modified aptamer of human IgE (15.1 μM) with magnetic stirring to facilitate the reaction for 16 h. During this process, 5'-thiol-modified aptamer of human IgE was loaded onto the surface of Au NPs by gold–sulfur affinity. Centrifuge the modified Au NPs at $14,000 \times g$ at room temperature for 25 min twice to remove the free thiol-aptamer. At last, disperse the Au NPs in 10 ml of buffer containing 27.6 mM NaCl, 10 mM Tris–HCl, 1 mM MgCl_2 , pH 8.0. It should be pointed out that no Au NPs aggregation was observed in our experiment. This is because the surface of Au NPs was loaded by aptamer. The negative charges on aptamer due to phosphate groups create an electrostatic repulsion between the adjacent Au NPs aptamer conjugates. This prevents Au NPs aptamer conjugates to come close to each other. Furthermore, the low ionic strength buffer could also contribute to the dispersion of Au NPs.

2.4. MCH treatment

The prepared aptamer–Au NPs conjugates were treated with MCH according to Zhao et al.'s report with minor modification (Zhao et al., 2008). Briefly, MCH was added to the prepared aptamer–Au NPs conjugates solution to give a final MCH concentration of $\sim 10 \mu\text{M}$. The MCH treatment was performed at room temperature for 0.5 h and stored at 4°C for 8 h. After that, the reaction was quenched by two washes with equal volumes of ethylacetate, which removed excess MCH from solution. A similar centrifugal procedure was used to collect the aptamer–Au NPs conjugates. At last, the aptamer–Au NPs conjugates were dispersed in 10 ml buffer containing 27.6 mM NaCl, 10 mM Tris–HCl, 1 mM MgCl_2 , pH 8.0, again. To determine the final number of aptamers loaded on each Au NPs, the MCE solution (1.0 M, $10 \mu\text{l}$) was used to displace aptamers from the surface of Au NPs (9.1 nM, $990 \mu\text{l}$). The amount of displaced aptamer in the supernatant solution was detected by UV spectrophotometric method, which was used to calculate its quantity on each Au NP by Wang et al. (2009a) and Huang et al. (2005). The number of aptamer on each Au NPs was about ~ 34 . This value was smaller than that of previous reports (Wang et al., 2009a; Huang et al., 2005). The reason was that some of the non-specifically adsorbed and chemically attached aptamers were removed from the Au NPs surface by MCH treatment.

2.5. *In situ* SPR measurement

The SPR experiments were done using Eco Chemie Autolab SPR systems (Brinkmann Instruments, New York, USA). It works with a laser diode fixed at a wavelength of 670 nm, using a vibrating mirror to modulate the angle of incidence of the p-polarized light beam on the SPR substrate. The instrument was equipped with a cuvette. Gold sensor disk (25 mm in diameter) was mounted on the hemicylindrical lens (with index-matching oil) to form the base of the cuvette. The cuvette could contain the sample with adjustable volume from 10 to 1000 μl . An O-ring (3 mm inner diameter) between the cuvette and disk prevents leakage. An auto-sampler (Eco Chemie) with controllable aspirating–dispensing–mixing pipette was used to add samples into the cuvette and provide constant mixture by aspiration and dispensing during measurements. This experimental arrangement maintains a homogenous solution and reproducible hydrodynamic conditions. The inject rate and mixing rate for all samples were 10 and 40 $\mu\text{l/s}$ respectively with the total volume for all samples dispensed in SPR cell equal to 40 μl . This setup allows us to measure the SPR angle shift in millidegrees (m°) as a response unit to quantify the binding amount of macromolecules to the sensor surface.

For detailed experiment, the SPR gold film was initially immersed into the MPA solution for 12 h in order to assemble the monolayer of MPA by the interaction between Au and thiol group in MPA. Then the MPA modified gold film was thoroughly rinsed with 10 mM PBS buffer and water to remove the weakly adsorbed MPA. The MPA modified SPR gold film was immersed by a pH 7.4 PBS buffer including 150 mM EDC and 50 mM NHS for 0.5 h to activate the carboxyl of MPA. After that, this gold film was immersed into 0.1 mg/ml goat anti-human IgE solution at 4 $^\circ\text{C}$ for overnight. And then, goat anti-human IgE modified SPR gold film was immersed into 1 M ethanolamine for 1 h to block the unreacted esters and prevent electrostatic binding of proteins or other small molecules. The resulting chip was then rinsed thoroughly with water and used as a sensing surface for detecting the concentration of human IgE. After goat anti-human IgE was immobilized on SPR gold film, the human IgE solution with different concentrations were first added

to SPR cell and reacted for 10 min followed by washing SPR cell with buffer. Lastly, the aptamer–Au NPs conjugates was added to the SPR cell and SPR angle–time curves were recorded. In order to reduce the disturbance of protein denaturation resulted from the regenerating process for the detecting results, a new substrate was used after each detection. The modification of every gold substrate was done under the same experimental condition. To confirm the reproducibility of the detection result for human IgE, different concentrations of the human IgE were repeatedly detected for three times.

3. Results and discussion

3.1. The detection principle of SPR sandwich immunoassay

The principle of SPR biosensor for detecting human IgE based on aptamer is shown in Fig. 1. Goat anti-human IgE are immobilized on SPR gold film as sensor surface, followed by injection of human IgE into SPR cell for 10 min, and then aptamer–Au NPs conjugates work as amplification reagent to enhance the SPR signal for the detection of IgE. The major difference of the SPR sandwich immunoassay developed in this work with general sandwich immunoassay which require secondary antibody labeled by enzyme or NPs for amplification and detection of protein is that we use aptamer–Au NPs conjugates as amplification reagent to enhance the SPR signal. Although the amplification effect resulted by antibody labeled by enzyme or NPs is very good, one inevitable disadvantage is that antibodies labeled by enzyme or NPs are sensitive to temperature and can be denaturated easily upon contact with surfaces, leading to limited shelf-lives (Luzi et al., 2003; Tombelli et al., 2005b). Compared with antibodies labeled by enzyme or NP, aptamer–Au NPs conjugates can provide long-term storage and undergoes reversible thermodynamic denaturation because both aptamer and Au NPs possess high stability (Maehashi et al., 2007). At the same time, by using aptamer–Au NPs conjugates as amplification reagent, the present immunoassay design nicely combines the specificity of antibody (immobilized) and aptamer (added after human IgE reacted with the immobilized antibody) with

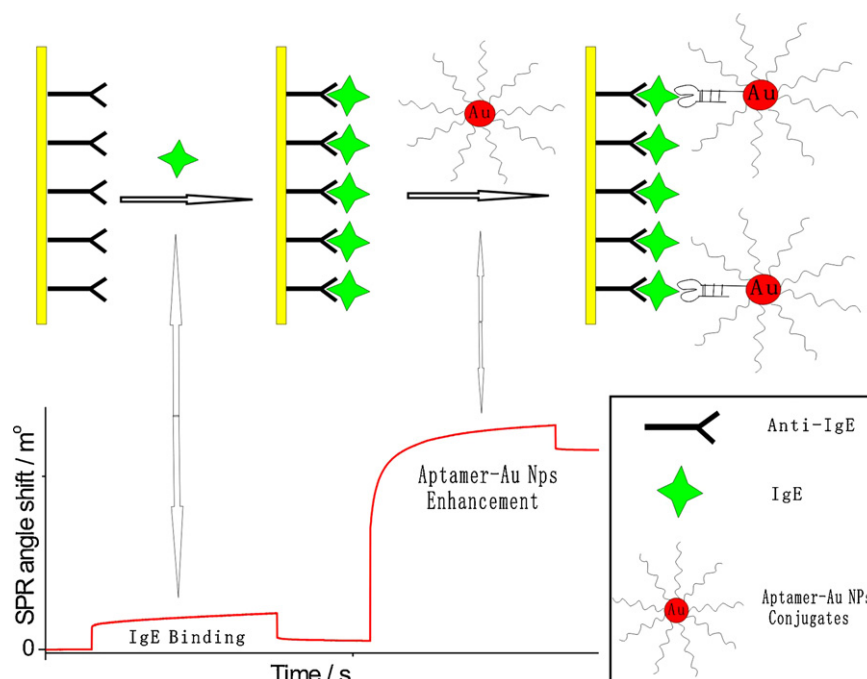


Fig. 1. A schematic representation of the SPR sandwich immunoassay for the ultrasensitive detection of human IgE that utilizes Au NPs–aptamer conjugates as amplification reagent.

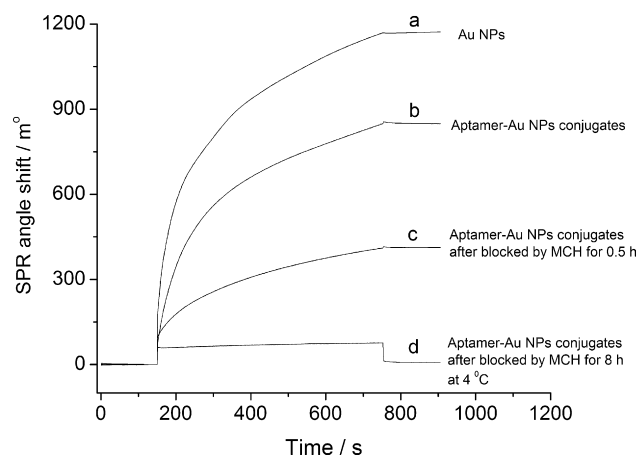


Fig. 2. SPR angle–time curves for in situ observing the non-specific adsorption of different kinds of Au NPs on goat anti-human IgE modified SPR gold film. (a) Au NPs; (b) aptamer–Au NPs conjugates; (c) aptamer–Au NPs conjugates after blocked by MCH for 0.5 h; (d) aptamer–Au NPs conjugates after blocked by MCH for 8 h at 4 °C.

the amplifying effect of nanomaterial. The nanomaterial will contribute to the increase of the detection sensitivity and the aptamer has the function of extending the type of sandwich immunoassay from antibody–antigen–antibody based sandwich immunoassay to antibody–antigen–aptamer-based sandwich immunoassay.

3.2. Minimize the non-specific adsorption of aptamer–Au NPs conjugates on goat anti-human IgE modified SPR gold film

Before detecting human IgE by this sandwich immunoassay, we first add aptamer–Au NPs conjugates into SPR cuvette in the absence of human IgE. An obvious SPR signal shift is observed (curve b in Fig. 2), which means there is a strong non-specific adsorption between goat anti-human IgE and aptamer–Au NPs conjugates. In order to find the reason for this non-specific adsorption, we tested the non-specific adsorption of Au NPs on goat anti-human IgE modified SPR gold film (curve a in Fig. 2). Comparing curve a and curve b in Fig. 2, we can see that the non-specific adsorption of Au NPs on goat anti-human IgE modified SPR gold film is much stronger than that of aptamer–Au NPs conjugates. The only difference between Au NPs and aptamer–Au NPs conjugates is that parts of citrate ions on the surface of Au NPs are substituted by thiol–aptamer. Thus, the hydrophobic and ionic interactions between goat anti-human IgE and citrate ions located on the surface of Au NPs might be the reason for the non-specific adsorption of aptamer–Au NPs conjugates on goat anti-human IgE (Zhang et al., 2008). Although this non-specific adsorption has been used to prepare Au–protein conjugates for the amplification detection of protein by SPR (Lyon et al., 1998; Gu et al., 1998), non-specific adsorption in general is adverse in biosensors because it reduces the sensitivity and specificity for target molecules. Therefore, it is very important to minimize the non-specific adsorption of aptamer–Au NPs conjugates on goat anti-human IgE modified SPR gold film for further sensitive detection of human IgE. Many researchers have confirmed that the negative citrate ions are easily substituted by other more Au-philic molecules such as thiol (Badia et al., 1997; Boal and Rotello, 2000; Chen, 2000; Goren et al., 2006). Thus, it is reasonable to reduce the non-specific adsorption by substituting citrate ions on the surface of aptamer–Au NPs conjugates by functional groups that possess high resistance ability for protein. In our experiment, we use MCH to substitute citrate ions on the surface of aptamer–Au NPs conjugates because of the high resistance ability of hydroxyl group in MCH for protein. Furthermore, MCH treatment can also remove non-specifically adsorbed aptamer on the Au NPs surface, which

helps to improve subsequent biomolecular recognition efficiency. MCH treatment is performed as explained in the experimental section. Curve c in Fig. 2 is the non-specific adsorption of aptamer–Au NPs conjugates treated by this method on goat anti-human IgE. We can clearly see that the SPR signal decreases from 848.9 to 412.78 m° after MCH treatment. However, the non-specific adsorption of 412.78 m° is still quite significant for the detection of human IgE. Thus, we prolong the time of MCH treatment to 8 h. Curve d in Fig. 2 is the non-specific adsorption curve of aptamer–Au NPs conjugates after treatment with MCH for 8 h. We can see the non-specific adsorption of aptamer–Au NPs conjugates gives a negligible SPR angle shift. Thus, the non-specific adsorption of aptamer–Au NPs conjugates on goat anti-human IgE modified SPR gold film is successfully minimized by MCH treatment.

3.3. The ultrasensitive detection of human IgE by SPR spectroscopy with aptamer–Au NPs conjugates as an amplification reagent

First, we utilize SPR angle–time curve to observe the binding process between human IgE and goat anti-human IgE. This is shown in the phase a of Fig. 3. The concentrations of human IgE are increased in the concentration range of 0, 0.001, 0.01, 0.1, 1, 2, 5 and 10 µg/ml. For concentration of human IgE larger than 1 µg/ml, we can clearly observe the SPR angle shift from ~2.8 to ~27.6 m° with the concentration of human IgE concentration increasing from 1 to 10 µg/ml. However, when the concentration of human IgE concentration is lower than 1 µg/ml, it is very difficult to discriminate the concentration of human IgE concentration by SPR spectroscopy. It indicates that the detection limit of SPR for human IgE is 1 µg/ml. This value is consistent with other SPR-based biosensor for human IgE (Wang et al., 2008). However, this detection limit is not enough for the detection of human IgE because human IgE is found at very low levels in human serum (~1 nM) (Maehashi et al., 2007). Thus, we further amplify the detecting signal of the aforesaid IgE sensor by using aptamer–Au NPs conjugates as an amplification reagent. The availability of this sandwich amplification is based on the fact that the binding site between goat anti-human IgE/IgE and aptamer/IgE is different (Wang et al., 2008). The two different binding sites give another advantage that human IgE binding with goat anti-human IgE will not dissociate from SPR gold film when aptamer–Au NPs conjugates is added. The amplification results of aptamer–Au NPs conjugates for SPR signal are shown in phase b of Fig. 3. The figure shows that SPR angle shifts obviously increase with the concentration of human IgE concentration increasing from 1 to 10 µg/ml, which is much larger than that of angle shift resulted by the binding of human

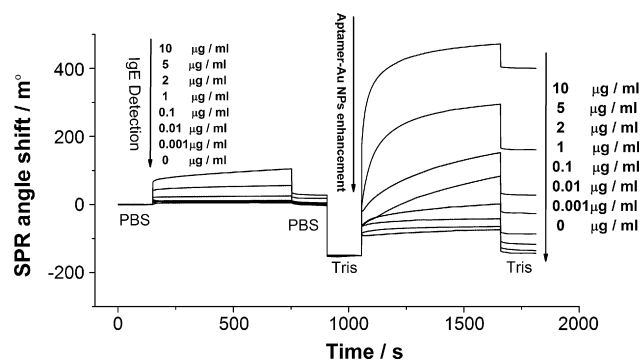


Fig. 3. Representative overlaid sensorgrams illustrating the real-time immunoassay of human IgE at different concentrations (0, 0.001, 0.01, 0.1, 1, 2, 5 and 100 µg/ml, from bottom to top). Profiles between time, 0 < t < 900 s indicate the adsorption of human IgE on goat anti-human IgE modified SPR gold film and profiles after t > 900 s show aptamer–Au NPs conjugates enhancement.

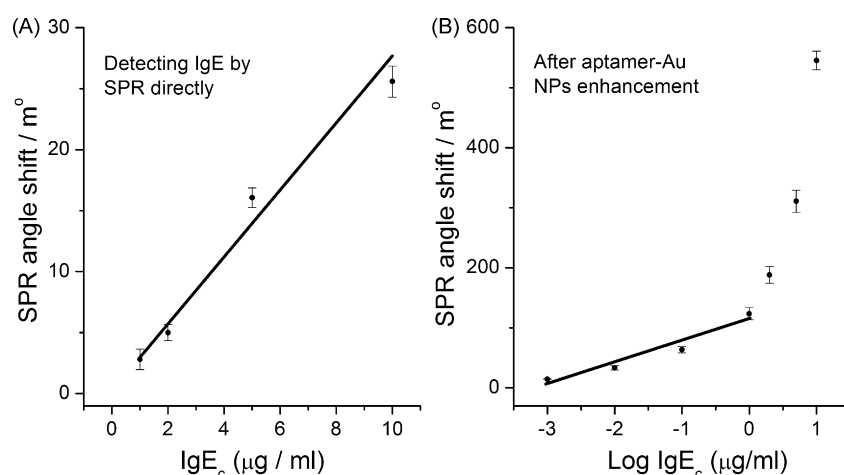


Fig. 4. Calibration graphs for human IgE concentration. (A) The linear relation between human IgE concentration and SPR angle shift resulted by the adsorption of human IgE on goat anti-human IgE modified SPR gold film; (B) the linear relationship between the logarithms of human IgE concentrations and the SPR angle shift resulted by the adsorption of aptamer–Au NPs conjugates. The error bars represent average standard errors for three measurements.

IgE with goat anti-human IgE. More importantly, after the amplification of aptamer–Au NPs conjugates, we can easily discriminate the concentration of human IgE from 0.001 to 0.1 $\mu\text{g/ml}$ by observing the change of SPR curves. By analyzing the change of SPR angle shift resulted by the adsorption of aptamer–Au NPs conjugates with the concentrations of human IgE, we obtain a linear relationship between the logarithms of human IgE concentrations and the SPR angle shift over a range of 0.001–1 $\mu\text{g/ml}$ (shown in Fig. 4B). The detection limit of 0.001 $\mu\text{g/ml}$ reached for analyzing the concentration of human IgE is much lower compared to the detection results obtained by general SPR sensor or other sensors, such as 1 $\mu\text{g/ml}$ with SPR sensor (Wang et al., 2008), 0.1 $\mu\text{g/ml}$ with QCM sensor (Liss et al., 2002), 0.02 $\mu\text{g/ml}$ with electrochemical sensor (Xu et al., 2005). Although the linear relation between the logarithms of human IgE concentrations and the SPR angle shift resulted by the adsorption of aptamer–Au NPs conjugates is lost when the concentration of human IgE is larger than 1 $\mu\text{g/ml}$, the detection of human IgE with high concentration (≥ 1 $\mu\text{g/ml}$) can be accomplished by the first step of this sandwich immunoassay, which utilizes goat anti-human IgE to detect human IgE, directly. Fig. 4A shows the calibration curves between SPR angle shift resulted by the adsorption of human IgE and human IgE concentration, an obvious linear relation is observed over a human IgE range 1–10 $\mu\text{g/ml}$. Therefore, we achieve a total detecting range for human IgE from 0.001 to 10 $\mu\text{g/ml}$ using the current sandwich immunoassay construction.

3.4. The selectivity of SPR sandwich immunoassay for the detection of human IgE

The selectivity of a sensing system is also important for the detection of human IgE. An excellent biosensor should not only possess good sensitivity, but should also have good selectivity. In order to detect the selectivity of the present biosensor, we choose three kinds of proteins (BSA, human IgM and human IgA) as controls and compare their SPR signal change with that of human IgE. The results are shown in Fig. 5. The black columns in Fig. 5 are the SPR angle shift resulted by the adsorption of different proteins on goat anti-human IgE modified SPR gold film. These columns show that the adsorptions of BSA, human IgA and human IgM are all very small even their concentrations are at a much higher level of 100 $\mu\text{g/ml}$. By contrast, the adsorption of 10 $\mu\text{g/ml}$ human IgE results in a 25.6 $^\circ$ of SPR angle shift. The grey columns in Fig. 5 are the SPR angle shift resulted by the adsorption of aptamer–Au NPs conjugates at

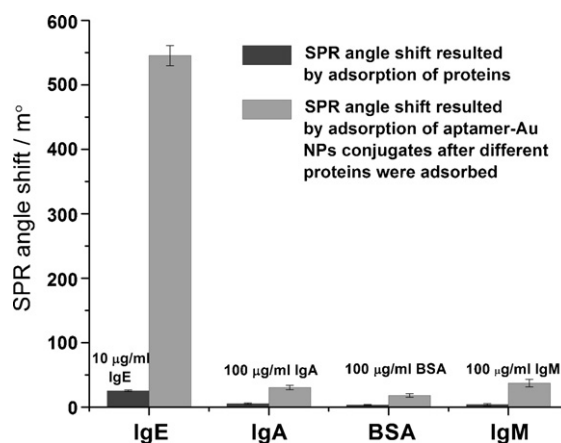


Fig. 5. Specificity analysis of the fabricated SPR immunosensor; (black columns) SPR angle shift resulted by the adsorption of different proteins on goat anti-human IgE modified SPR gold film; (grey columns) SPR angle shift resulted by the adsorption of different proteins on goat anti-human IgE modified SPR gold after aptamer–Au NPs conjugates enhancement. The concentration of human IgE was 10 $\mu\text{g/ml}$, and the concentration of each of BSA, IgA, and IgM was 100 $\mu\text{g/ml}$. The error bars represent average standard errors for three measurements.

different proteins modified SPR gold film. Clearly, the amplification effect of aptamer–Au NPs conjugates for IgE is much stronger than for the three control proteins. These results confirm that the present biosensor has a sufficient specificity and human IgE can be identified with high selectivity.

4. Conclusion

In conclusions, a modified methodology for the ultrasensitive detection of protein by SPR in sandwich manner has been developed, utilizing antibody and aptamer in aptamer–Au NPs conjugates as the sandwich elements and Au NPs in aptamer–Au NPs conjugates as amplification label. The human IgE was used as the model analyte to demonstrate the feasibility of the proposed methodology as a proof of concept. The non-specific adsorption of aptamer–Au NPs conjugates on goat anti-human IgE was demonstrated by SPR spectroscopy, and then was minimized by treating aptamer–Au NPs conjugates with MCH. Human IgE was successfully detected with high sensitivity and selectivity by combining both the specificity of antibody and aptamer and the amplification effect of

Au NPs. These results confirmed that the proposed method can provide an alternative tool for the ultrasensitive detection of various protein targets by SPR spectroscopy.

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