

Aptamer-Functionalized Gold Nanoparticles as Probes in a Dry-Reagent Strip Biosensor for Protein Analysis

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The highly specific molecular recognition properties of aptamers are combined with the unique optical properties of gold nanoparticles for the development of a dry-reagent strip biosensor that enables qualitative (visual)/quantitative detection of protein within minutes. A model system comprising thrombin as an analyte and a pair of aptamer probes is used to demonstrate the proof-of-concept on the conventional lateral flow test strip. The assay avoids the multiple incubation and washing steps performed in most current aptamer-based protein analyses. Although qualitative tests are realized by observing the color change of the test zone, quantitative data are obtained by recording the optical responses of the test zone with a portable “strip reader”. The response of the biosensor is linear over the range of 5–100 nM of thrombin with a detection limit of 2.5 nM (S/N = 3). By comparing the analytical performances of the aptamer-based strip biosensor with the antibody-based strip sensor, we can demonstrate that aptamers are equivalent or superior to antibodies in terms of specificity and sensitivity, respectively. The sensor was used successfully for detection of thrombin in human plasma samples. It shows great promise for use of aptamer-functionalized gold nanoparticle probes in dry-reagent strip biosensors for point-of-care or in-field detection of proteins.

The detection and quantification of extremely low concentrations of proteins play pivotal roles in basic discovery research and clinical applications. Several commonly used techniques for protein detection have been developed, such as radioimmunoassay,¹ protein chips,² enzyme-linked immunosorbent assay,³ fluores-

cence,⁴ quartz crystal microbalance,⁵ and surface-enhanced raman spectroscopy.⁶ Although these conventional strategies provide accurate, sensitive detection of proteins, there are still some inconveniences that exist, such as the utilization of radioactive substances, time-consuming sample purification, incubation, washing steps before analysis and enzymatic reactions, and technical expertise as well as the specialized equipment. Recently, a lateral flow biosensor, also called a dry-reagent strip biosensor, using combined chromatography with conventional immunoassay has gained increasing attention in protein analysis and clinical diagnosis.^{7–9} Compared with the methods mentioned above, such a lateral flow biosensor has several advantages: user-friendly format, very short assay time (generally several minutes), less interference due to chromatographic separation, long-term stability over a wide range of climates, a relatively low cost, and no requirements for skilled technicians. This ideal technique is suitable for on-site testing by people who are untrained analysts.¹⁰ Today, most of these methods that lately have been extensively applied are based on the use of antibodies as affinity reagents. For example, Shim et al.¹¹ have used a colloidal gold-antibody probe for the detection of atrazine in water samples. Sithigorngul et al.¹² applied a simple and rapid method for detection of pathogenic isolates of *Vibrio harveyi*. However, the utilization of antibodies may encounter some drawbacks with their production, stability, and modification, and searching for other alternative candidates is ongoing. Aptamers, the artificial nucleic acid ligands, were first discovered by two groups in 1990.^{13,14} Since then, aptamers have been described toward a wide range of targets,

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such as metal ions,¹⁵ organic molecules,¹⁶ peptides,¹⁷ proteins,^{18–21} and whole cells²² due to their inherent characteristics, such as their high association constant with target proteins, easy production and labeling with signal moieties, cost-effectiveness,²³ and stability during long-time storage.²⁴ Aptamer-functionalized gold nanoparticles have attracted considerable interest^{25–29} and have been applied to the determination of proteins using quartz crystal microbalance,²⁵ electrochemical impedance spectroscopy,²⁶ electrochemiluminescence,²⁷ fluorescence spectroscopy,²⁸ and colorimetric determination.²⁹ However, most aptamer-based analysis involves long analysis time, multiple incubation steps, and separation steps, which prevent its wide application.³⁰

In the present work, we first report aptamer-functionalized gold nanoparticles as probes in a dry-reagent strip biosensor for protein analysis. A pair of aptamer probes and thrombin analyte (which plays an essential role in the coagulation system³¹) was used as the model system to demonstrate the proof-of-concept. Resulting protein detection characteristics of the aptamer-based dry-reagent strip biosensor were then compared to that of the antibody-based strip biosensor. The results indicate that aptamer-based dry-reagent strip biosensors exhibit similar sensitivity and are even superior with respect to specificity. The sensor was used successfully for detection of thrombin in human plasma samples. We could clearly show that aptamer-functionalized gold nanoparticles represent a suitable class of probes for the development of dry-reagent strip-type biosensors for point-of-care or in-field detection of proteins.

EXPERIMENTAL SECTION

Apparatus. The Airjet AJQ 3000 dispenser, Biojet BJQ 3000 dispenser, clamshell laminator and the guillotine cutting module CM 4000 were from Biodot LTD (Irvine, CA). The Portable strip reader, DT1030, was purchased from Shanghai Goldbio Tech. Co., LTD (Shanghai, China).

Reagents and Materials. Streptavidin from *Streptomyces avidinii*, dithiothreitol (DTT), triethylamine (TEA), ethyl acetate, Na₃PO₄·12H₂O, HAuCl₄, trisodium citrate, sucrose, Tween 20, Triton X-100, sodium chloride–sodium citrate (SSC) buffer (pH 7.0), phosphate buffer saline (PBS, pH 7.4, 0.01 M), bovine serum albumin (BSA), human serum albumin (HSA), casein

(from bovine milk), and thrombin (from human plasma) were purchased from Sigma-Aldrich. Glass fibers (GFCP000800), cellulose fiber sample pads (CFSP001700), laminated cards (HF000MC100) and nitrocellulose membranes (HFB24004) were provided by Millipore (Bedford, MA). Rabbit IgG and human IgM were purchased from Thermo Scientific. Human plasma samples were purchased from Golden West Biologicals (Temecula, CA). Sheep polyclonal antibody to thrombin was obtained from Abcam (Cambridge, MA). The aptamers and oligonucleotide probes used in this study were obtained from Integrated DNA Technologies, Inc. (Coralville, IA) and have the following sequences:³²

Primary aptamer: thiolated 15-mer aptamer with polyT(20) tail, 5'-SH-(CH₂)₆-TT TTT TTT TTT TTT TTT TTT GGT TGG TGT GGT TGG-3'.

Secondary aptamer: biotinylated 29-mer aptamer, 5'-biotin-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3'.

DNA oligonucleotide (control probe): 5'-AAA AAA AAA AAA AAA AAA AA-biotin-3'.

All other chemicals were of analytical reagent grade. All buffer solutions were prepared using ultrapure (>18 MΩ cm) water from a Millipore Milli-Q water purification system (Billerica, MA).

Preparation of Gold Nanoparticles (Gold NPs). Approximately 15 ± 3.5-nm-diameter colloidal gold NPs were synthesized according to the citrate reduction of HAuCl₄.^{33,34} All glassware used in this preparation was thoroughly cleaned in aqua regia (three parts HCl, one part HNO₃), rinsed with double distilled H₂O, and oven-dried prior to use. A 250 mL aqueous solution of 0.01% HAuCl₄ was heated to boiling and vigorously stirred in a 500 mL round-bottom flask; 4.5 mL of 1% trisodium citrate was added quickly to this solution. The solution turned deep blue within 20 s, and then the final color changed to wine-red after 60 s. Boiling was continued for an additional 10 min. The solution was cooled to room temperature with a continuous stirring for another 15 min. The colloids were stored in dark bottles at 4 °C.

Preparation of Gold NP–Aptamer Conjugates. The thiolated aptamer (primary aptamer) was used for conjugation with gold NPs. Before conjugation, the thiolated aptamer was activated following this procedure: 100 μL of thiolated aptamer (1.0 OD) was mixed with 2 μL of TEA and 7.7 mg of DTT to react for 1 h at room temperature, then the excess DTT was removed by extraction with 400 μL of ethylacetate solution four times. A 1 mL portion of 5-fold concentrated gold NP solution was added into the activated aptamer solution. After standing for 24 h, the conjugate was slowly aged with addition of PBS until a final concentration of 0.01 M. The solution was allowed to stand for another 24 h at 4 °C, followed by centrifugation for 20 min at 12 000 rpm to remove the excess reagents. After discarding the supernatant, the red pellets were washed, recentrifuged, and redispersed in 1 mL of an aqueous solution containing 20 mM Na₃PO₄·12H₂O, 5% BSA, 0.25% Tween 20, and 10% sucrose.

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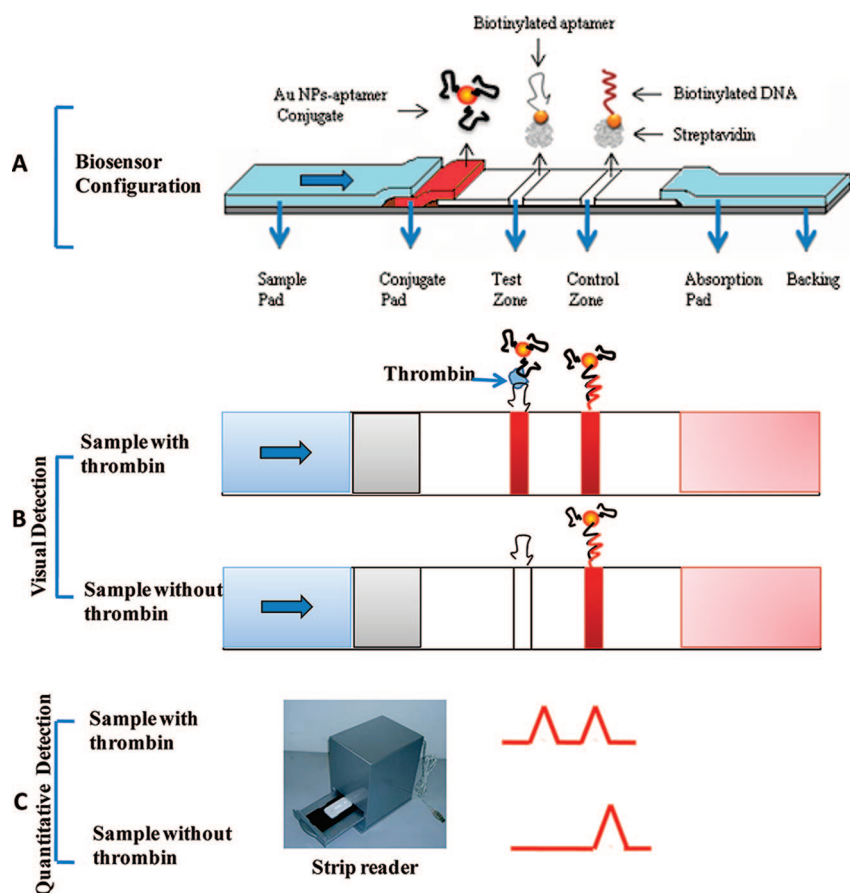


Figure 1. Schematic illustration of the configuration and measurement principle of the aptamer-based strip biosensor: (A) configuration of the biosensor; (B) the principle of visual detection in the presence and absence of thrombin; (C) quantitative detection with a portable strip reader.

Preparation of Aptamer-Based Dry-Reagent Strip Biosensor. The biosensor consists of the following components: sample application pad, conjugate pad, nitrocellulose membrane, and absorption pad. A schematic diagram of the biosensor is shown in Figure 1A. The sample application pad (17 mm × 30 cm) was made from cellulose fiber (CFSP001700, Millipore) and was soaked with a buffer (pH 8.0) containing 0.25% Triton X-100, 0.05 M Tris-HCl, and 0.15 mM NaCl. Then it was dried and stored in desiccators at room temperature. The conjugate pad was prepared by dispensing a desired volume of gold NP-aptamer conjugate solution onto the glass fiber pad (8 mm × 30 cm) using the Airjet AJQ 3000 dispenser. The pad was dried at room temperature and stored in a desiccator at 4 °C.

The test zone and control zone on the nitrocellulose membrane (25 mm × 30 mm) were prepared by dispensing the secondary aptamer and biotinylated DNA (control probe, complementary with the primary aptamer) solutions, respectively. The distance between two zones was around 0.2 cm. To facilitate the immobilization of the probes, streptavidin was used to react with the biotinylated aptamer (secondary aptamer) and biotinylated DNA. Briefly, 42 μ L of 10 OD biotinylated aptamer was mixed with 250 μ L of 2 mg/mL streptavidin. After incubating 1 h at room temperature, 508 μ L of PBS was added to the mixture. The excess aptamer was removed by centrifugation for 30 min with a centrifugal filter (cutoff 30 000, Millipore) at 6000 rpm. The conjugate was washed twice with 500 μ L of PBS in the same centrifugal filter. The remaining solution in the filter was collected,

and the solution was diluted to 500 μ L by adding PBS. Following the same procedure, the biotinylated DNA with a concentration of 10 OD was used to prepare the streptavidin-biotinylated DNA conjugate. The streptavidin-biotinylated aptamer and streptavidin-biotinylated DNA were dispensed on the nitrocellulose membrane to form the test zone and control zone, respectively. The membrane was dried at room temperature for 1 h and stored at 4 °C. Finally, all of the parts were assembled on a plastic adhesive backing layer (typically, an inert plastic, e.g., polyester) using the clamshell laminator (Biodot, CA). Each part overlapped 2 mm to ensure the solution migrating through the biosensor during the assay (Figure 1A). Biosensors with a 4.7 mm width were cut by using the guillotine cutting module CM 4000.

Preparation of Antibody-Based Dry-Reagent Strip Biosensor. The antibody-based dry-reagent strip biosensor was prepared using a protocol similar to that described above. Polyclonal antibody (against thrombin) and the secondary antibody (against polyclonal antibody) were used to prepare the test zone and control zone on the nitrocellulose membrane. Polyclonal antibody was also conjugated with gold NPs for the preparation of antibody-gold NP conjugates. Briefly, 0.05 mg of polyclonal antibody was added in 1 mL of 5-fold concentrated gold NP solution (pH 9.0). Following 1 h of incubation at room temperature, 110 μ L of 10% BSA solution was added into the mixture. After 30 min, the solution was centrifuged at 12 000 rpm for 18 min, and the supernatant was removed. Then the nanoparticles were washed with 1 mL of PBS (1% BSA) and separated as above. The

resulting ruby sediments were dispensed in 1 mL of an aqueous solution containing 20 mM $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$, 5% BSA, 0.25% Tween 20 and 10% sucrose. The resulting solution was dispensed onto the glass fiber to obtain the conjugate pad. The pretreatment of the sample pad, assembling of different membranes on the back material, and strip cutting followed the same procedure as described in the preparation of the aptamer-based dry-reagent strip biosensor.

Assay Procedure. A 120 μL portion of sample solution containing a desired concentration of thrombin in running buffer ($15 \times \text{SSC}$ containing 1% BSA buffer for the aptamer-based biosensor, 0.01 M PBS containing 1% BSA for the antibody-based biosensor) was added onto the sample application pad, and the solution migrated toward the absorption pad. The test zone and control zone were evaluated visually within 10 min. For quantitative measurements, the optical intensity of the red bands was read using the portable “strip reader” instrument combined with “GoldBio strip reader” software.

For detection of thrombin in complex biological matrixes, such as plasma, 1 μL of plasma spiked with different quantities of thrombin was applied onto the biosensor, and then 120 μL of $15 \times \text{SSC}$ (1% BSA) buffer was applied. The results were obtained by recording the optical responses with the strip reader after 10 min.

RESULTS AND DISCUSSION

Figure 1 schematically illustrates the configuration and measuring principle of the aptamer-based dry-reagent strip biosensor. The biosensor consists of four components: sample application pad, conjugate pad, nitrocellulose membrane, and absorbent pad (Figure 1A). All of the components were mounted on a common backing layer (typically, an inert plastic, e.g., polyester). A model target thrombin and a pair of aptamer probes, which bind thrombin in two different sites, were used to demonstrate the proof-of-concept. Typically, the sample solution containing thrombin was added onto the sample application pad. Subsequently, the solution migrated by capillary action and rehydrated the gold NP–primary aptamer conjugates. Then the binding between the gold NP–primary aptamer and thrombin occurred, and the formed thrombin–aptamer–gold NP complexes continued to migrate along the strip. When they reached the test zone, the complexes were captured by the secondary aptamer immobilized on the test zone via interaction between the secondary aptamer and thrombin. A characteristic red band could be observed because of the accumulation of gold NPs on the test zone (Figure 1B). The capillary action caused the liquid sample to migrate further. Once the solution passed through the control zone, the excess gold NP–primary aptamer conjugates were captured on the control zone via the hybridization events between the control DNA probes (preimmobilized on the control zone) and the primary aptamer, thus forming a second red band (Figure 1B, top). In the absence of thrombin, only the red band is observed in the control zone, and no red band is observed in the test zone (Figure 1B, bottom). In this case, the red band in the control zone (control line) shows that the biosensor is working properly. Qualitative analysis is simply performed by observing the color change of the test zone (Figure 1B), and quantitative analysis is realized by reading the optical intensities of the red bands with a portable strip reader

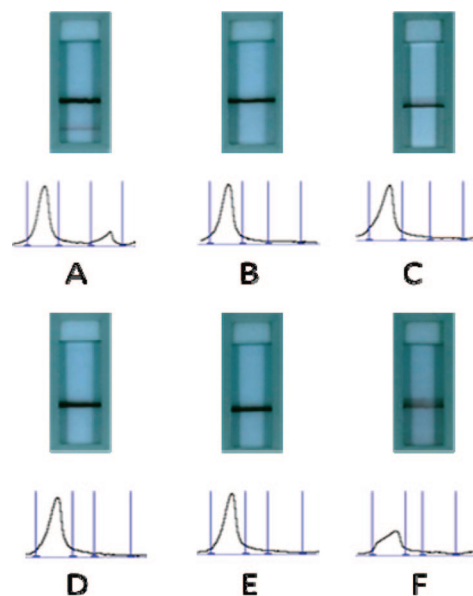


Figure 2. Typical photo images and recorded responses of the aptamer-based strip biosensor in the presence of (A) 50 nM thrombin, (B) 0 nM thrombin, (C) 500 nM HSA, (D) 500 nM IgG, (E) 500 nM IgM, and (F) 500 nM casein. Assay time, 10 min; sample solution was prepared with $15 \times \text{SSC}$ + 1% BSA; dispensing times of gold NP aptamer conjugate solution, 2; concentration of biotinylated aptamer for the preparation of test zone, 24 OD/mL.

(Figure 1C). The peak area is proportional to the amount of the captured gold NPs in the test zone, which is proportional to the concentration of thrombin in the sample solution.

Figure 2 displays typical photo images and the corresponding responses of 50 nM thrombin (A), 0 nM thrombin (B), 500 nM nonspecific protein, HSA (C), IgG (D), IgM (E), and casein (F). Two red bands occurred in the presence of thrombin, and only one red band (control zone) could be seen in the absence of thrombin or in the presence of the excess of unspecific proteins, such as HSA, IgG, casein, and IgM. The presence of unspecific proteins did not contribute the signal of the background. Well-defined peaks were observed, and the intensities were recorded by the strip reader shown on the bottom of Figure 2.

Optimization of Parameters. The amount of gold NP–aptamer conjugates loaded on the conjugate pad affects the intensities of both test and control zones greatly. It was determined by the dispensing volume of the conjugate solution. The histogram of the signal-to-noise (S/N) ratio of the biosensor for 50 nM of thrombin is shown in Figure 3A. The S/N ratio was found to be the highest for dispensing the gold NP–aptamer conjugates two times, one after another on the same conjugate pad. The decrease in the S/N at more dispensing times is ascribed to the increased background signal. Therefore, dispensing twice was selected as the optimal dispensing time in the following experiments.

The response of the biosensor is also relevant to the amount of secondary aptamer immobilized on the test zone. One can see from the Figure 3B that the S/N ratio of the biosensor increased with an increase in the secondary aptamer concentration from 4 to 24 OD/mL. It seems that a higher concentration of secondary aptamer dispensed on the test zone would lead to higher intensities of aptamer on the test zone, which would result in much

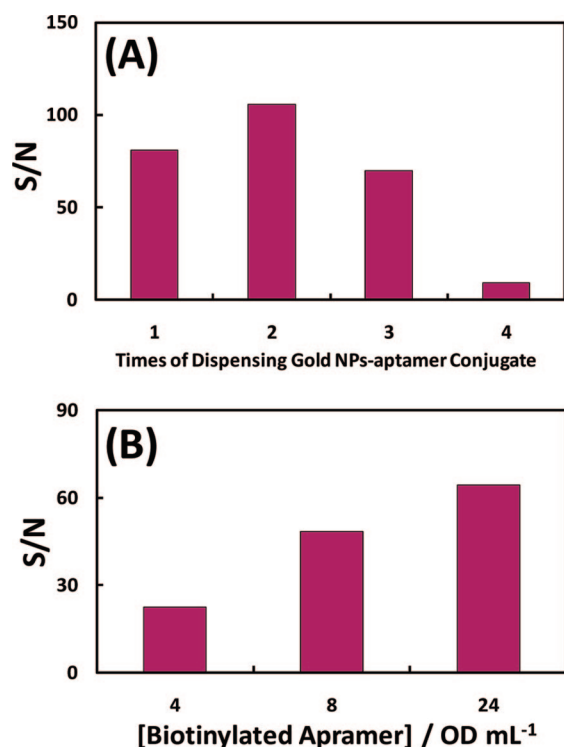


Figure 3. (A) Effect of dispensing times of gold NP-aptamer conjugate on the signal-to-noise ratio of the biosensor for 50 nM thrombin. Assay time, 10 min; sample solution was prepared with $15 \times \text{SSC} + 1\% \text{BSA}$. (B) The effect of biotinylated aptamer concentration (immobilized on the test zone) on the signal-to-noise ratio of the biosensor for 50 nM thrombin. Assay time, 10 min; sample solution was prepared with $15 \times \text{SSC} + 1\% \text{BSA}$; times of dispensing gold NP-aptamer conjugate, 2.

more gold NPs being captured through the sandwich reactions. An aptamer concentration of 24 OD/mL was used to prepare the test zone.

Another factor taken into account for the assay optimization is the use of various buffers. During the fabrication of the biosensor and assay, four kinds of buffers were used: (1) A 0.05 M Tris-HCl buffer containing 0.25% Triton X-100 and 0.15 M NaCl (pH 8.0) was used to saturate the sample pad. This treatment would facilitate the transportation of thrombin and reduce entrapment of thrombin in the sample pad. (2) A buffer containing 20 mM Na₃PO₄, 5% BSA, 0.25% Tween, and 10% sucrose was used to disperse the gold NP-aptamer conjugate pellets. During the preparation of the gold NP-aptamer conjugate, the addition of BSA, Tween 20, and sucrose could stabilize the nanoparticles and facilitate the release of the conjugate from the conjugate pad. Moreover, this buffer could reduce the nonspecific adsorption of the gold NP-aptamer conjugate on the nitrocellulose membrane. After rehydrating the conjugate, the components (BSA, Tween 20, and sucrose) that were dispersed in the running buffer migrated along the strip and then blocked the nitrocellulose membrane naturally without additional block steps. (3) PBS (0.01 M, pH 7.4) was used to prepare streptavidin-biotinylated aptamer solution. This buffer was employed to construct the test zone and control zone. (4) The composition of the running buffer has a significant effect on the performance of the biosensor. Several buffers, including Tris-HCl, PBS + 0.1% Tween, PBS, PBS + 1% BSA, $15 \times \text{SSC}$

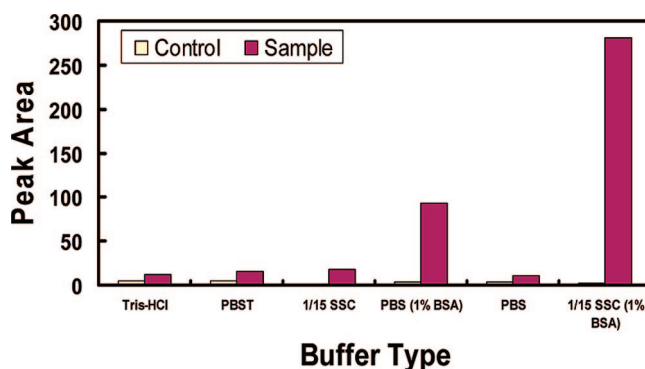


Figure 4. Effect of running buffer components on the response of 50 nM thrombin. Assay time, 10 min; dispensing time of gold NP aptamer conjugate on the conjugate pad, 2; concentration of biotinylated aptamer for the preparation of test zone, 24 OD/mL.

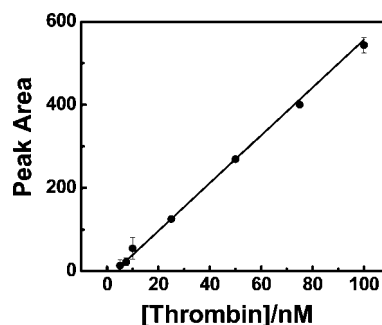


Figure 5. Calibration curve of the biosensor with different concentrations of thrombin in $15 \times \text{SSC}$ (1% BSA) buffer. Assay time, 10 min, sample volume, 120 μL .

and $15 \times \text{SSC} + 1\% \text{BSA}$ were tested, and the results are shown in Figure 4. Comparing the signals of the test zones, the best performance was obtained with the $15 \times \text{SSC}$ (1% BSA) buffer. Thereby, a $15 \times \text{SSC} + 1\% \text{BSA}$ buffer was selected for the experiments.

Analytical Performances. To investigate whether the biosensor could provide quantitative detection of thrombin, the intensities of the test zones were estimated and plotted as a function of different concentrations of thrombin. It was observed that well-defined peaks occurred, and the peak areas increased with an increase in the thrombin concentration. The useful analytical range extended from 5 to 100 nM of thrombin and was suitable for quantitative work (Figure 5). A plateau was observed at 500 nM thrombin. Since quantitative analysis relies on the stability of the analyte signal and reproducibility of the assay, and to determine the reproducibility of the biosensor, samples of 50 nM thrombin were loaded on six different biosensors that gave reproducible signals with a relative standard deviation (RSD) of 8.6%. The detection limit was 2.5 nM (based on $S/N = 3$) in connection with a 10 min assay time. This detection limit corresponds to 300 fmol in the 120 μL sample solution, which is comparable to that of the electrochemical assays^{33,35,36} and quartz crystal microbalance measurement.²⁵ The detection limit of the present method is not as low as the amplified electrochemical impedimetric aptasensor reported by Li and co-workers,²⁶ and

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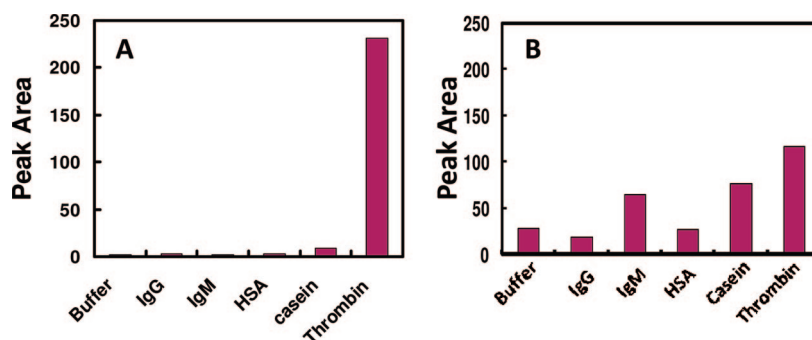


Figure 6. Response histograms of the aptamer-based strip biosensor (A) and antibody-based strip biosensor (B) in the presence of 50 nM thrombin and 500 nM casein, HSA, IgG, and IgM. Assay time, 10 min; running buffers, 15 × SSC + 1% BSA buffer for the aptamer-based strip biosensor, PBS +1% BSA for the antibody-based strip biosensor; sample volume, 120 μ L.

Table 1. Analytical Performances of Aptamer- and Antibody-Based Biosensors

strip biosensor	analytical performances		
	linear range (nM)	detection limit (nM)	RSD ($n = 6$)
aptamer-based	~5–100	2.5	8.6%
antibody-based	~7.5–250	5	7.8%

further improvements in the detection limit are under investigation by using dye-doped polystyrene nanosphere (400 nm) labels.

The shelf life of the biosensor was tested by storing the biosensors at room temperature. It was found that their responses did not change significantly after two months' storage at room temperature. The RSD of the biosensor for 20 nM of thrombin was less than 7% compared with that obtained with the newly prepared biosensors, indicating the biosensor has a good shelf life.

Comparing the Analytical Performances of the Aptamer-Based Strip Biosensor with the Antibody-Based Strip Biosensor. We compared the performances of the aptamer-based dry-reagent strip biosensor with the antibody-based strip biosensor. First, we studied the specificities of the two types of biosensors. Figure 6 presents the response histograms of aptamer- (A) and antibody- (B) based strip biosensors in the presence of an excess of nonspecific proteins (HSA, IgG, IgM, and casein), which are present in high concentrations in a biological matrix. As shown in Figure 6A, a high response was observed when 50 nM thrombin was tested, whereas negligible signals were obtained in the absence of thrombin and presence of 500 nM of HSA, IgG, IgM, or casein. Because the concentration of BSA in the running buffer was nearly in a 3000-fold excess with respect to 50 nM thrombin, the presence of a high concentration of BSA did not affect the biosensor performance. In contrast, the antibody-based strip biosensor suffered interferences in the presence of these nonspecific proteins at high concentration levels (Figure 6B). From the above results, one can see the aptamer-based strip biosensor has a higher specificity than the antibody-based strip biosensor. The analytical ranges, detection limits, and RSDs of the two types of biosensors are summarized in Table 1. One can see the aptamer-based strip sensor exhibits the lower detection limit and a similar linear range and reproducibility.

Determination of Thrombin in Human Plasma. To test the practicality of the biosensor, experiments were performed by detection of thrombin in a complex sample matrix, such as plasma.

Plasma contains coagulation proteins, such as prothrombin, fibrin and fibrinogen, and coagulation cofactors. Thrombin is not present in plasma of a healthy subject when coagulation is not happening. The sample solutions were prepared by spiking different quantities of standard thrombin solution into the plasma. The addition of plasma would lead to the matrix effect, so the volume of plasma was optimized before the experiment. Different volumes of plasma (1 to 15 μ L) spiked with thrombin was added to the sample application pad, and then the biosensor was washed with running buffer. The signal was recorded with the strip reader after 10 min. A better performance in terms of higher signal-to-noise ratio was obtained when 1 μ L of plasma was added, so 1 μ L of plasma was applied in the following experiment. The plasma samples spiked with different quantities of thrombin were prepared to obtain the calibration curve in a complex biological matrix. The resulting plot of the peak area versus thrombin quantity is linear over the 1.2–60 pmol range with a detection limit of 0.6 pmol (based on S/N = 3, results not shown). The performance of the biosensor for detection of thrombin in plasma demonstrates the promise of using the proposed biosensor for developing diagnostic medical systems in clinical applications.

CONCLUSIONS

We have developed a dry-reagent strip biosensor based on aptamer-functionalized gold nanoparticles for protein analysis. A model system comprising a thrombin analyte and a pair of aptamer probes was used to demonstrate the proof-of-concept. Under optimal conditions, a linear relationship between the peak area and the thrombin concentration was observed in the range of 5–100 nM with a detection limit of 2.5 nM. The proposed biosensor is capable of detecting thrombin in human plasma samples at a detection limit of 0.6 pmol. Moreover, the presence of other interfering proteins, such as HSA, casein, IgG, and IgM showed no effect on the biosensor response, illustrating the good selectivity. By comparing the analytical performances of the aptamer-based strip biosensor with that of the antibody-based strip biosensor, the aptamer-based strip sensors were equivalent or superior to the antibody-based strip sensor in terms of specificity and sensitivity. It shows great promise for the development of aptamer-based dry-reagent strip biosensors for point-of-care or in-field detection of proteins. Specific applications for the determination of other proteins would require proper attention to the selection of a pair of aptamer probes, which can be realized by screening through the systematic evolution of ligands by expo-

nential enrichment process from random RNA and DNA libraries.³⁷ For the proteins binding with a single aptamer probe could be determined by using a competitive assay. Further improvements in the sensitivity of the aptamer-based dry-reagent strip biosensor could be achieved by using dye-doped polystyrene nanospheres or the development of dry-reagent strip biosensors based on quantum dot labels and fluorescence detectors.³⁸

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