

Aptamer Pseudoknot-Functionalized Electronic Sensor for Reagentless and Single-Step Detection of Immunoglobulin E in Human Serum

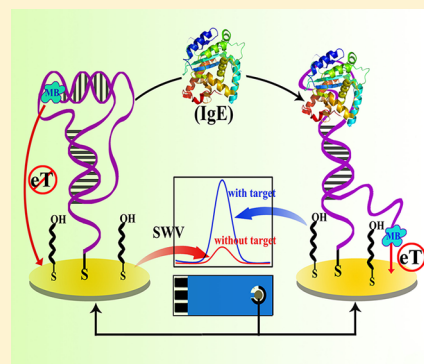
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S Supporting Information

ABSTRACT: The development of electronic sensors with minimized usage of reagents and washing steps in the sensing protocols will significantly facilitate the detection of biomolecules. In this work, by using a new pseudoknot design of the aptamer probes, the construction of an electronic sensor for reagentless and single-step detection of immunoglobulin E (IgE) in human serum is described. The pseudoknot aptamer probes are self-assembled on the disposable electrode surface. The association of IgE with the aptamer probes leads to conformational changes of the pseudoknot aptamer structures and brings the redox-tags in close proximity to the electrode, resulting in amplified current response for monitoring IgE. The effects of the pseudoknot structure and the immobilization concentration of the aptamer probes on the sensor performance are evaluated. Under optimal conditions, the detection limit for IgE is estimated to be 60 pM. The sensor is also selective and can be employed to detect IgE in human serum samples. The developed sensor can achieve reagentless, washing-free and low-cost (with the disposable electrode) electrochemical detection of proteins, making this device a convenient sensing platform for the monitoring of different biomarkers when coupled with the appropriate aptamer probes.



Immunoglobulin E (IgE), which has been found only in mammals, is the least abundant isotype of immunoglobulin in human serum.¹ Although present with low content in the human body, IgE plays a major role in allergic diseases. IgE can bind to basophils and mast cells to trigger the release of chemicals like histamine, leukotrienes, and certain interleukins that can cause symptoms associated with allergies such as asthma, dermatitis, and pollenosis.^{2,3} Elevated concentration of IgE has also been demonstrated to be related to other immune deficiency diseases.⁴ The monitoring of IgE is therefore important for the diagnosis of different allergy-mediated disorders.

Enzyme-linked immunosorbent assays (ELISAs) have been the standard methods for the detection of IgE in different sample media.⁵ Although sensitive and reliable, the ELISA approaches commonly involve enzyme labels and multiple washing steps, which increase the cost and complexity for IgE detection. Besides, the stability issue of the antibody recognition component and the requirement for highly trained personnel have further limited the wide application of these methods in routine clinical diagnosis. In the past two decades, there have been significant advancements in developing alternatives for protein detections by using the aptamer recognition probes, which were first reported by Szostak and Gold in 1990.^{6,7} Aptamers, selected from random nucleic acid

libraries by the SELEX technique, are synthetic oligonucleotides (DNA or RNA) that can bind to a variety of targets (proteins, cells, peptides, ions and small molecules, etc.) with high affinity and specificity.^{8–10} Because of their superior advantages in terms of cost, stability, ease of synthesis, and manipulation over antibodies, aptamers have been increasingly used in different types of bioassays. Since the first selection of the IgE aptamer by Wiegand and co-workers,¹¹ several optical,^{12,13} electrochemical,¹⁴ and mechanical¹⁵ biosensing schemes for IgE based on the aptamer probes have been reported. Among these detection methods, the electrochemical approaches are particularly suitable for point-of-care analysis of IgE due to the inherent advantages such as high sensitivity, low cost, simplicity, and portability of the electrochemical devices. Indeed, the development of the electronic aptamer sensors by the Plaxco group has significantly facilitated the detection of proteins.^{16–20} The sensing mechanism of these electronic aptamer sensors is simply based on protein-induced conformational changes of the redox-labeled aptamer probes, which alters the distance between the redox labels and the sensor

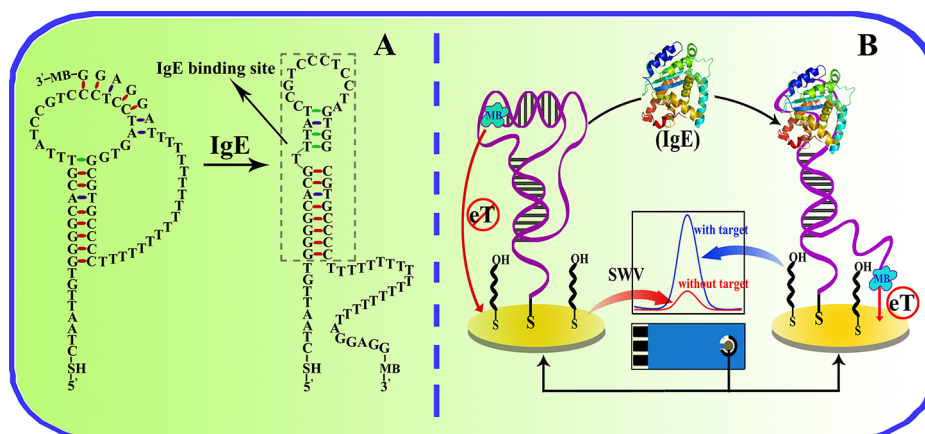
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Scheme 1. (A) Conformational Change of the Pseudoknot Structure of the SH-IgE-BA Aptamer upon binding to IgE. (B) Illustration of the Reagentless and Single-Step Electronic Detection of IgE on the SPCE Sensor



electrode surface and generates decreased/increased current responses for the detection of the corresponding proteins. Although these sensors can offer convenient detection of proteins, the sensitivities are compromised by the high background currents and limited signal gains.

In response to the limitations, we report here the development of an electronic sensor for the detection of IgE in human serum by using the pseudoknot-structured aptamer probes. With this pseudoknot design, the background current can be minimized due to the relatively rigid structure of the probes, which potentially reduces the chance for the collisions between the redox-labels and the electrode surface. Besides, the sensitivity can be enhanced because of the “signal-on” sensing format of the sensor with improved signal gain. Moreover, the employment of the screen printed carbon electrode (SPCE) enables the detection of IgE in a disposable, reagentless and washing-free fashion. By coupling the pseudoknot design of the aptamer probes with the SPCE, we can thus achieve sensitive, simple, and convenient detection of IgE in human serum samples.

EXPERIMENTAL SECTION

Chemicals and Materials. Tris–HCl, 6-mercapto-1-hexanol (MCH), and HAuCl_4 were purchased from Sigma (St. Louis, MO, USA). The disposable SPCE, composed of a carbon working electrode (3 mm in diameter), a carbon counter electrode, and a silver pseudo reference electrode, was obtained from Zensor R&D Co., Ltd. (Taichung, Taiwan). IgE purified from human plasma was purchased from Meridian Life Science, Inc. (Saco, ME, USA) and other reagents were bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The pseudoknot IgE binding aptamer (IgE-BA) was designed and evaluated by using the Mfold web server developed by Zuker.²¹ The thiol (5′) and methylene blue (MB, 3′)-modified IgE binding aptamer (SH-IgE-BA) with the following sequence was synthesized and HPLC purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). SH-IgE-BA: 5′-SH-(CH_2)₆-CTA ATT GTG GGG CAC GTT TAT CCG TCC CTC CTA GTG GCG TGC CCC TTTTTTTTTTTTTTTT TAG GAG G-(CH_2)₇-MB-3′. Other reagents were of analytical grade and used as received. Aqueous solutions were prepared using ultrapure water (specific resistance of 18 $\text{M}\Omega\cdot\text{cm}$).

Fabrication of the Sensor. The working electrode of the SPCE was first preconditioned in 20 mM Tris–HCl buffer (pH

7.4) by cycling the potential between -0.6 and $+0.6$ V at a scan rate of 0.5 V s^{-1} . Gold nanoparticles (AuNPs) were then electrochemically deposited on the working electrode of the SPCE to immobilize the SH-IgE-BA probes. Electrochemical deposition of AuNPs was performed by controlling the deposition potential at -0.66 V for 30 s in 1% HAuCl_4 solution to obtain the AuNP-deposited SPCE (AuNP/SPCE).^{22,23} Subsequently, the AuNP/SPCE was rinsed with water, dried with nitrogen, and immediately used for SH-IgE-BA probe immobilization.

Prior to electrode functionalization, the SH-IgE-BA probe (10 μM) was mixed with 5 mM TCEP in a microcentrifuge tube and the solution was incubated for 60 min at room temperature. Then, the mixture was diluted with 20 mM Tris–HCl buffer (140 mM NaCl, 1 mM MgCl_2 , and 5 mM KCl, pH 7.0) to a final concentration of 0.2 μM . Next, 10 μL of the diluted mixture was dropped on the working electrode (AuNP/SPCE) and the mixture was incubated overnight at room temperature in the dark. After careful rinsing with Tris–HCl buffer, the SH-IgE-BA/AuNP/SPCE was further incubated with 1 mM MCH for 2 h to block the sensing surface, followed by rinsing with Tris–HCl buffer to obtain the SH-IgE-BA/MCH/AuNP/SPCE sensor interface for IgE detection.

Electrochemical Detection of IgE. Serial aliquots of Tris–HCl buffer (50 μL) containing various concentrations of IgE were casted onto the SPCEs to cover all three electrodes and incubated for 1 h at room temperature. The electrodes were then subjected to square wave voltammetric (SWV) measurements on a CHI 621D workstation (CH Instruments Inc., Shanghai, China) by scanning the potential from -0.50 to -0.10 V with a step potential of 4 mV, a frequency of 25 Hz, and an amplitude of 25 mV. Data processing was made by using the “linear baseline correction” function of the CHI 621D software.

RESULTS AND DISCUSSION

The structure design of the pseudoknot SH-IgE-BA probes and the employment of these probes for reagentless and single-step electronic detection of IgE are illustrated in Scheme 1A,B, respectively. The pseudoknot SH-IgE-BA contains two stem-loop structures in which a portion of one loop is hybridized to form the stem of the other (Scheme 1A). The 5′-terminus of the probe is modified with thiol so that it can be immobilized on the AuNP/SPCE through the formation of Au–S bond, and

the 3'-terminus is labeled with a redox tag, MB, to generate the signal output for IgE detection. Meanwhile, a segment of ployT (T_{17}) is inserted at the 3'-terminus to assist the formation of a stable pseudoknot structure. When IgE binds to the aptamer region of the pseudoknot probe, it switches the pseudoknot form of the probe to a one-stem-loop structure, in which the stem at the 3'-terminus is displaced. By elucidating the structure switching capability of the pseudoknot SH-IgE-BA upon binding to IgE, the sensor is constructed by self-assembling the SH-IgE-BA on the surface of the AuNP/SPCE, followed by further blocking with MCH. In the absence of the IgE target, the SH-IgE-BA probes remain in the pseudoknot structures and the redox MB labels are positioned far away from the sensor surface (Scheme 1B). In this case, the electron transfer from the MB labels to the electrode is inhibited and small background current is expected. On the contrary, when IgE is incubated with the sensor (Scheme 1B), the IgE then associates with the SH-IgE-BA probes, causes conformational changes of the pseudoknot structures and releases the MB-labeled 3'-termini of the probes as indicated in Scheme 1A, which significantly increases the collisions between the MB labels and the electrode and leads to enhanced current response for monitoring IgE.

We first characterized the construction of the sensor with a step-by-step approach by using cyclic voltammetry with the presence of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ electrochemical probes in KCl solution (0.1 M). As displayed in Figure 1, a couple of

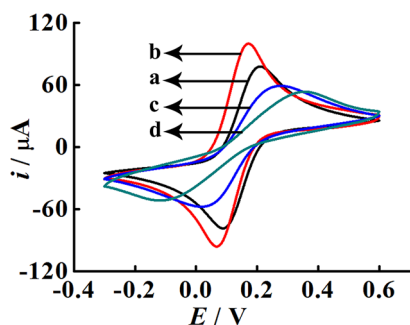


Figure 1. Typical cyclic voltammograms of the sensor at different construction stages: (a) bare SPCE, (b) AuNP/SPCE, (c) SH-IgE-BA/MCH/AuNP/SPCE, and (d) IgE/SH-IgE-BA/MCH/AuNP/SPCE. The voltammograms were recorded in 0.1 M KCl solution containing 1 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at a scan rate of 50 mV s^{-1} .

reversible, well-defined redox peaks of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (curve a) can be observed on the pretreated bare SPCE electrode because of the fast electron transfer ability of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ to the bare SPCE electrode. Subsequent electrochemical deposition of AuNPs on the SPCE leads to apparent increase in both current peaks (curve b). Such increases can be primarily attributed to the excellent electrical conductivity of AuNPs,²⁴ which promotes electron transfer from $[\text{Fe}(\text{CN})_6]^{3-/4-}$ to the electrode surface. However, after the formation of the self-assembled monolayer of SH-IgE-BA/MCH on the AuNP/SPCE, significant current drops and peak separation are observed (curve c), owing to two facts (note: to avoid current interference, the SH-IgE-BA used here is free of the MB labels). First, the negative charges of the phosphate backbones of SH-IgE-BA electrostatically repel $[\text{Fe}(\text{CN})_6]^{3-/4-}$ from approaching the electrode. Second, the steric hindrance of the pseudoknot structure of SH-IgE-BA can also partially prevent $[\text{Fe}(\text{CN})_6]^{3-/4-}$ from accessing the electrode. The incubation

of the sensor with IgE results in further current drops and peak separation (curve d) because of the increase in the steric hindrance effect of the IgE proteins. The results shown here thus reveal the successful preparation of the sensing surface and the association of IgE with the surface-bound SH-IgE-BA.

To further verify the feasibility of the pseudoknot aptamer-functionalized sensors for the detection of IgE, SWV responses of the sensors in the absence and presence of the IgE protein (60 nM) were compared. As shown in Figure 2, no peak

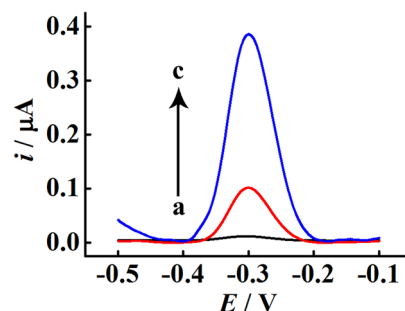


Figure 2. Typical SWV responses of (a) AuNP/SPCE and SH-IgE-BA/MCH/AuNP/SPCE incubated (b) without and (c) with the presence of IgE (60 nM). The immobilization concentration of SH-IgE-BA was 0.4 μM and the length of the stem at the 3' terminus was 8 bp. SWV measurements were performed in 20 mM Tris-HCl buffer (140 mM NaCl, 1 mM MgCl_2 , and 5 mM KCl, pH 7.0) by scanning the potential from -0.50 to -0.1 V with a step potential of 4 mV, a frequency of 25 Hz, and an amplitude of 25 mV.

current (curve a) is observed for the AuNP/SPCE electrode due to the lack of electroactive species in the buffer. After the preparation of the sensor with self-assembled SH-IgE-BA and MCH, a small background current peak (curve b) at about -0.3 V corresponding to the electro-oxidation of MB is observed. Such peak is possibly due to the electron tunneling from the MB labels to the sensor surface during the potential scan. Importantly, the incubation of the sensor with IgE (60 nM) leads to substantial increase in the current peak (curve c), which verifies that the bindings between IgE and SH-IgE-BA switch the structure of the probes and bring the MB labels in close proximity to the sensor surface. Such comparison indicates the potential of the sensor for the detection of IgE.

The length of the stem at the 3'-terminus significantly affects the stability of the pseudoknot structure of the aptamer probes,²⁵ which in turn leads to different current responses for the sensors. In addition, the immobilization concentration of the SH-IgE-BA probes can also potentially affect the current signal output. To achieve optimal sensor performance, the effects of these two factors on the signal-to-noise ratio (i/i_0 , i , and i_0 , respectively, correspond to the SWV peak currents with the presence and absence of the target IgE) of the sensor were evaluated. The length of the stem at the 3'-terminus for the pseudoknot aptamer probe was varied at 6 bp (3'-MB-(CH_2)₇-GAG GAT), 7 bp (3'-MB-(CH_2)₇-GGA GGA T), and 8 bp (3'-MB-(CH_2)₇-GGG AGG AT) to monitor i/i_0 with the presence and absence of IgE (60 nM). As displayed in Figure 3A, although the pseudoknot aptamer probe with 6 bp stem at the 3'-terminus exhibits the highest current response for IgE, it has the smallest value of i/i_0 due to the relatively high background current, which is probably due to the less stability of the pseudoknot structure with only 6 bp stem at the 3'-terminus, increasing the collisions between the MB labels with

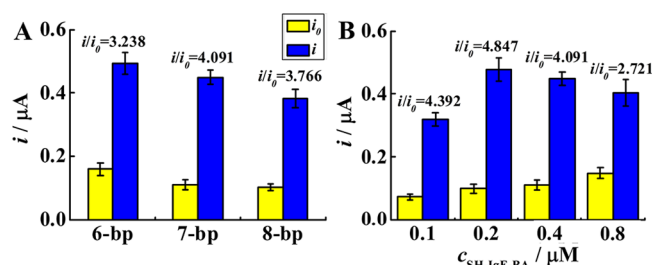


Figure 3. Effects of (A) the length of the stem at the 3'-terminus of the pseudoknot SH-IgE-BA (with the immobilization concentration of 0.4 μM) and (B) the immobilization concentration of SH-IgE-BA (with the length of the stem of 7 bp) on the signal-to-noise ratio (i/i_0 , i , and i_0 , respectively, correspond to the SWV peak currents with the presence and absence of the target IgE at 60 nM) of the sensor. Error bars: SD, $n = 3$. Other conditions, as in Figure 2.

the electrode surface in the absence of the IgE target. The pseudoknot aptamer probe with 8 bp stem shows similar background current response to that of the one with 7 bp stem, whereas the current response with the presence of IgE is slightly lower, assumably due to the enhanced stability of the pseudoknot structure with longer stem (8 bp vs 7 bp) that partially inhibits the displacement of the MB-labeled 3'-terminus of the probe. Based on the results, the pseudoknot aptamer probe with 7 bp stem at the 3'-terminus is selected for subsequent experiments. The effect of the immobilization concentration of SH-IgE-BA from 0.1 to 0.8 μM was also examined. According to Figure 3B, the increase in the concentration of SH-IgE-BA leads to gradual increase in the background current, owing to the increased amount of SH-IgE-BA immobilized on the sensor surface. However, the immobilization concentration of SH-IgE-BA at 0.2 μM shows the highest current response for the presence of IgE, giving the best i/i_0 value. This is because a lower concentration of SH-IgE-BA (0.1 μM) decreases the binding chance between IgE and the surface-immobilized SH-IgE-BA, while higher concentrations (0.4 and 0.8 μM) partially inhibit the bindings due to the steric hindrance of the SH-IgE-BA on the sensor with relatively higher surface densities.^{26,27} Both scenarios can result in decreased current response for the presence of IgE and correspondingly the i/i_0 value. The immobilization concentration of SH-IgE-BA at 0.2 μM with the surface density determined to be 2.51×10^{12} molecules per cm^2 (Supporting Information) is therefore used for subsequent experiments.

After the optimization of the experimental conditions, the sensors were incubated with different concentrations of IgE to examine the dependence of the peak current upon the concentration of IgE. From Figure 4A, we can see that the SWV responses of the sensors increase accordingly with increasing concentration of IgE in the range from 0.1 to 100 nM. Figure 4B displays the calibration plot for quantitative analysis of IgE, in which the SWV peak current response shows a strong linear dependence upon the concentration of IgE from 0.1 to 100 nM, and the linear regression equation is determined to be $i = 0.1583 + 0.0058 c$ with a correlation coefficient (R^2) of 0.9908. The detection limit is estimated to be 60 pM (with 3 times the standard deviation of the blank). Such detection limit is comparable with or even slightly more sensitive than some other (Table 1) methods for IgE detection. Furthermore, six repetitive measurements of IgE at 60 nM yielded a relative standard deviation of 7.1%, indicating good reproducibility of the sensor.

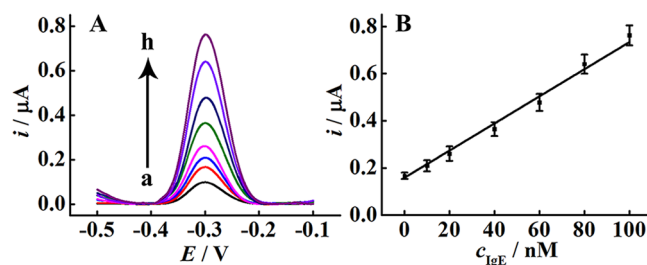


Figure 4. (A) Typical SWV responses of the sensors to different concentrations of IgE (a) 0 nM, (b) 0.1 nM, (c) 10 nM, (d) 20 nM, (e) 40 nM, (f) 60 nM, (g) 80 nM, and (h) 100 nM. (B) The resulting calibration plot of the concentration of IgE vs the peak current. Error bars: SD, $n = 3$. Other conditions, as in Figure 2.

Table 1. Comparison of Different Methods for IgE Detection

technique	linear range	detection limit	references
electrochemical	54 pM–36 nM	36 pM	14
electrochemical	1.0 nM–1.0 μM	1.0 nM	28
electrochemical	2.5–100 nM	0.1 nM	29
fluorescence	100 pM–140 nM	350 pM	13
fluorescence	100 pM–5.0 nM	100 pM	30
SPR	8.4–84 nM	2.0 nM	31
electrochemical	0.1–100 nM	60 pM	this work

To evaluate the specificity of our proposed sensor for IgE detection, other nontarget control proteins, including thrombin, CEA, and IgG, were tested. According to the results displayed in Figure 5, the incubation of the control proteins

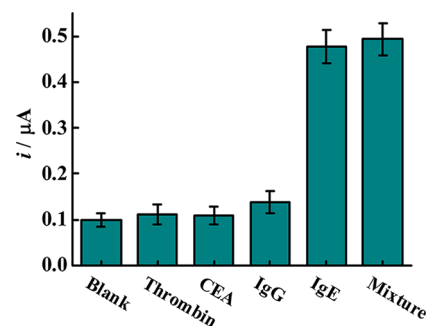


Figure 5. Specificity evaluation of the sensor for the detection of the IgE (60 nM) target against other control proteins of thrombin (0.6 μM), CEA (0.6 μM), IgG (0.6 μM), and the mixture of IgE and the control proteins. Error bars: SD, $n = 3$. Other conditions, as in Figure 2.

(0.6 μM , at 10-fold excess over the concentration of IgE), respectively, with the sensor generates insignificant changes in current peak when compared to that of the blank test (with the absence of IgE), whereas the presence of an even lower concentration of IgE (60 nM) leads to a drastic increase in the current peak. Besides, the mixture of IgE and other control proteins exhibits close current response to that of IgE, indicating the high specificity of the sensor that is basically associated with the high selectivity of the IgE binding aptamers.

The applicability of the sensor to real samples is one of the major goals in sensor development. To check the feasibility of the proposed electronic sensor for the monitoring of IgE levels in human serums, various concentrations of IgE were added to human serum samples from healthy volunteers. The serum

samples were diluted 10 times with Tris-HCl buffer, followed by determining the concentration of IgE using the standard addition method with our developed sensing protocol. As shown in Table 2, the concentration of IgE in human serums

Table 2. Recovery Tests for IgE in Human Serum Samples Using the Proposed Method ($n = 6$)

sample	added (nM)	found (nM)	rate of recovery (%)	RSD (%)
1	0	0.108		4.7
2	0.1	0.212	99.5–103.3	4.2
3	1.0	1.098	98.1–104.5	5.5
4	5.0	5.107	98.4–103.7	4.9

from healthy individuals is about 1.0 nM measured by our method. This result is in agreement with previous reports.^{32,33} The recovery rate for IgE added to the human serums is in the range of 98.1–104.5% with relative standard deviations between 4.2% and 5.7%, suggesting that the developed sensor can be employed to detect IgE in real samples.

CONCLUSIONS

In conclusion, we have demonstrated that IgE can be monitored sensitively in a reagentless and single-step approach with the employment of the pseudoknot aptamer probes. The IgE sensing mechanism is simply based on target-induced conformational change of the aptamer probes, generating enhanced current response for sensitive detection of IgE down to 60 pM. The developed sensor is also selective, and importantly, can be applied to monitor IgE in human serum samples. Our IgE sensing approach eliminates the label conjugation and multiple washing steps, and has the advantages of speed, simplicity, high sensitivity, and ease of operation compared with other IgE detection techniques. With the successful demonstration for IgE detection, the developed sensor can be extended to detect other proteins or small molecules with careful design of the aptamer probes, potentially making the sensor a robust and universal platform for convenient monitoring of different targets.

ASSOCIATED CONTENT

Supporting Information

Material for the determination of the optimized surface density of the pseudoknot aptamer probes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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Supporting Information

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Determination of the optimized surface density of the pseudoknot aptamer probes

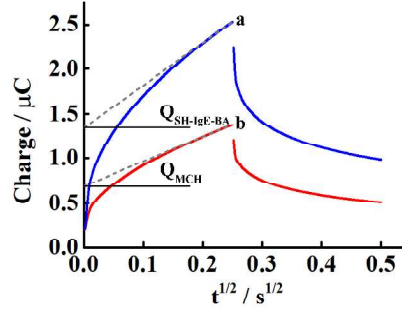


Figure S1. Chronocoulometric curves for AuNP/SPCE electrodes modified with (a) SH-IgE-BA/MCH and (b) MCH in 10 mM Tris-HCl buffer (pH 7.4) with the presence of 50 μ M RuHex. Redox charges of RuHex confined near the electrode surface were obtained from chronocoulometric intercepts at $t = 0$.

The chronocoulometric method was used to measure the density of SH-IgE-BA on the AuNP/SPCE electrode surface and the result was shown in Figure S1. By quoting the Cottrell's equations listed below,^{1,2} the surface coverage of the SH-IgE-BA on the AuNP/SPCE electrode was estimated to be 2.51×10^{12} molecules per cm^2 .

$$\Gamma_{Ru} = \frac{Q_{ds} - Q_{dl}}{nFA} \quad (1)$$

$$\Gamma_{DNA} = \Gamma_{Ru}(z/m)N_A \quad (2)$$

Where Γ_{Ru} is the amount of the redox marker confined near the electrode surface (mol/cm^2), $Q_{SH-IgE-BA}$ and Q_{MCH} are the surface charges (C), n is the number of electrons in the reaction, F is the Faraday constant (C/equiv), A is the area of the working AuNP/SPCE (cm^2), Γ_{DNA} is the surface density of DNA, z is the charge of the redox marker, m is the numbers of the bases in DNA, and N_A is Avogadro's number.

References:

- (1) Steel, A. B.; Herne, T. M.; Tarlov, M. J. Electrochemical Quantitation of DNA Immobilized on Gold Anal. Chem. **1998**, 70, 4670–4677.
- (2) Zhang, J.; Song, S. P.; Wang, L. H.; Pan, D.; Fan, C. H. A Gold Nanoparticle-Based Chronocoulometric DNA Sensor for Amplified Detection of DNA Nat. Protoc. **2007**, 2, 2888–2895.