Supporting Informations

Human serum biomarker detection based on cascade signal amplification strategy by DNA molecule machine

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Experimental Section

Reagents.

Thioglycolic acid (TGA), Bovine serum albumin (BSA), imidazole, N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), (+)-Biotin N-hydroxysuccinimide were purchased from Sigma-Aldrich. EDC and NHS were dissolved in water immediately before use. Human prostate-specific antigen (PSA), two mouse antihuman total PSA monoclonal antibodies (primary capture antibody, Ab_1 and biotin linked secondary detection antibody, Biotin-Ab_2) were received from Shanghai Linc-Bio Science Co., Ltd. New England Biolabs (USA) provided Nb.BtSI and NEBuffer, while Klenow exo minus was bought from Thermo Fisher Scientific (USA). Streptavidin was from AMRESCO All other reagents were of analytical reagent grade. DNA oligonucleotides labeled with Cy5 and BHQ, and a dNTP mix stock solution were obtained from TaKaRa Biotechnology (Dalian) Co., Ltd. The sequences of employed oligonucleotides are as Table 1:

Table S1. The oligonucleotide sequences used in this study

DNA	Sequence
DNA1	5'-ATCCAGATGGTCGATAACCTGCAGTGAGATCCGTAGTTTTTTCTACGGATCT↓**CACTGC**AGGTTATCGACCATCTGGAT-3'
DNA2	5'-(Cy5)AGACTCGAGCGGATCCAGATGGTCGATAACCTCAGCCGCTCGAGTCT(BHQ)-3'
Primer	5'-CTCGAGCG-3'
Comparison DNA3	5'-(Cy5)-CCACCACATTCAAATTCACAACTA↓**CACTGC**AGGAAGAGATGTTACGAGTTCGTGGTGG(BHQ)-3'
Comparison DNA4	5'-TTTTTCCTGCAGTGTAGTTTTT-3'

[a] The underlined bold letters of DNA1 are the recognition sequence of Nb.BtSI, and the arrow indicates the nicking position.

Sandwich Immunoreaction on gold electrode.

Gold disk electrodes (diameter of 2.0 mm) were polished with 0.05 μm alumina powder prior to use and then cleaned ultrasonically in acetone and water for 3 min. They were then electrochemically cleaned by a series of oxidation and reduction cycles in 0.5M H_2SO_4.

For antibody immobilization, electrodes were first subjected to 10 mM TGA in water for 90 min to obtain –COOH on the surface of gold electrode. Then the –COOH modified electrode was immersed in 1 mL freshly prepared Imidazole-HCl buffer containing 20 mg EDC and 10 mg NHS for 30 min at room temperature. Then, 10 μL of 10 μg mL^{-1} Ab_1 was dropped onto the electrode surface, allowing it to react for 16 h to get the Ab_1-immobilized gold electrode.

The washed Ab_1-gold electrode was incubated in 5% BSA for 30 min at 37 °C to block the excess active groups on the surface, followed by washing, and used for PSA detection. 10 μL of various concentrations of target PSA was dropped onto the surface of the Ab_1-gold electrode and incubated for 90 min at 37 °C. Then the reaction solution was removed and the electrode was rinsed with pure water and dried again with nitrogen. Following this, 10 μL of biotin-Ab_2 was dropped on the electrode and incubated at 37 °C for another 90 min. The modified electrode was then washed thrice and used for the following operation.

Immobilization of Nb.BtSI and operation of the DNA machine.

After the sandwich immunoreaction, the electrodes were incubated with 10 μL of 5 μg mL^{-1} streptavidin solution at 37 °C for 60 min. After washing with PBS, 10 μL of 5μg mL^{-1} (+)-biotin N-hydroxysuccinimide was added to each electrode and the electrodes were incubated at 37 °C for 40 min. Finally, Nb.BtSI was introduced to the surface of electrode by incubation 2 μL Nb.BtSI (10 U μL^{-1}) for 60 min.

The DNA machine reaction was initiated by immersing the modified electrodes into 100 μL of 50 mM Tris-HC1 reaction buffer containing DNA 1, DNA 2, primer, 0.1 U μL^{-1} polymerase Klenow and 2 mM dNTPs mixture at 37 °C. The fluorescence intensities of a series of target PSA antigen at different concentrations during above sandwich immunoreaction on gold electrode were recorded.

Fluorescence measurement.

All fluorescence measurements were carried out on a F4600 fluorometer (Hitachi, Japan). Excitation and emission wavelengths were set at 653 nm and 670 nm, respectively, with 5.0 nm bandwidths. The emission spectra were obtained by exciting the samples at 653 nm and scanning the emission from 600 to 800 nm. All samples were incubated at 37℃. The fluorescence intensity was recorded simultaneously when the fluorescence intensity became steady.

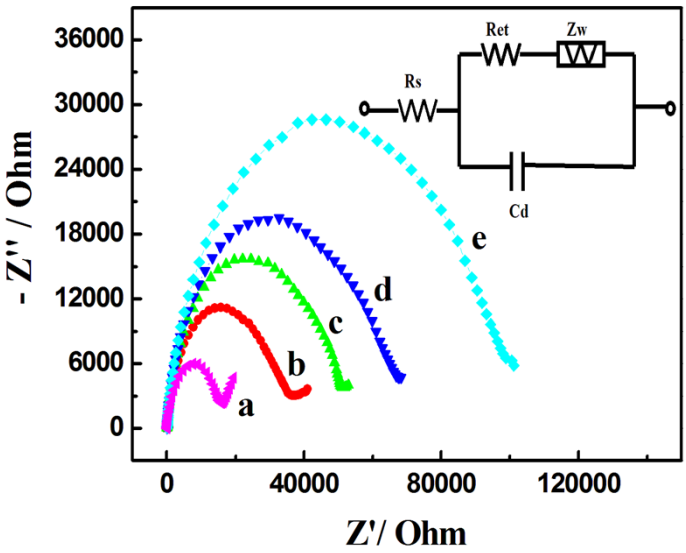


Fig. S1 The Nyquist plots obtained for electrode which was immobilized by Ab_1 (a), Ab_1/PSA (b), Ab_1/PSA/Ab_2 (c), Ab_1/PSA/Ab_2/ streptavidin (d), Ab_1/PSA/Ab_2/ streptavidin/biotin-Nb.BtSI (e) in 5 mM $[Fe(CN)_6^{3-/4-}]$ and 0.1M KCl solution.

Electrochemical impedance spectroscopy (EIS) was also used to confirm the successful modification of Nb.BtSI (Fig. S1). It can be observed that Ret increased when Ab_1, PSA antigen, Ab_2, streptavidin and Nb.BtSI were modified on the surface of electrode step by step. The reason is that the diffusion of $[Fe(CN)_6^{3-/4-}]$ toward the electrode surface was largely repelled by the continuously introduced biomolecules. Therefore, the repellence of the electrode to the negatively charged redox probes became weaker. This phenomenon further verified the modification of target antigen and Nb.BtSI which play important roles in the biosensing process.

Optimization of the experiment conditions.

In order to obtain an optimal sensing response, some experimental parameters including the concentration of the klenow polymerase or dNTPs, and the incubation time for isothermal reaction were studied. From Fig. S2A, we could find that when the concentration of polymerase increased, the fluorescence signal was enhanced until the used concentration of polymerase was up to 0.1U μL^{-1}. The similar trend was found in observation of different concentration of dNTPs and incubation time for isothermal reaction (Fig. S2B and Fig. S2C). Then 0.1U μL^{-1} klenow polymerase, 2.0 mM dNTPs, and 90 min of incubation time were chosen as optimal incubation conditions, respectively.

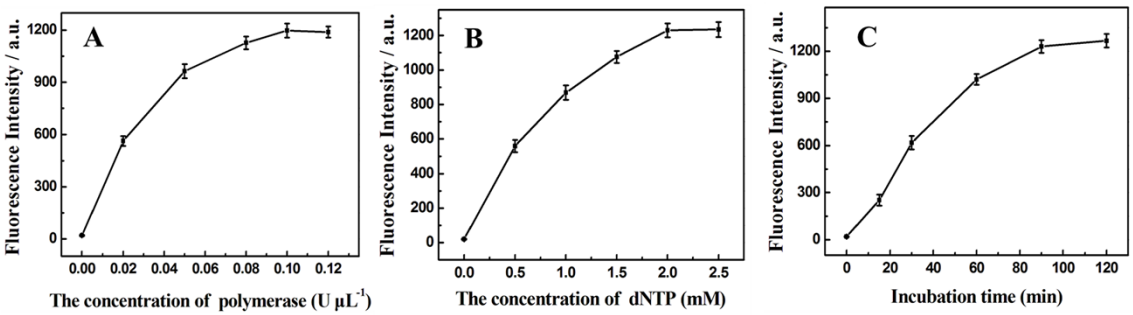


Fig. S2 Dependence of fluorescence intensity on (A) the concentration of polymerase, (B) the concentration of dNTP, and (C) incubation time. The concentration of PSA antigen was 10 μg mL^{-1} and the reaction temperature was 37 ℃.

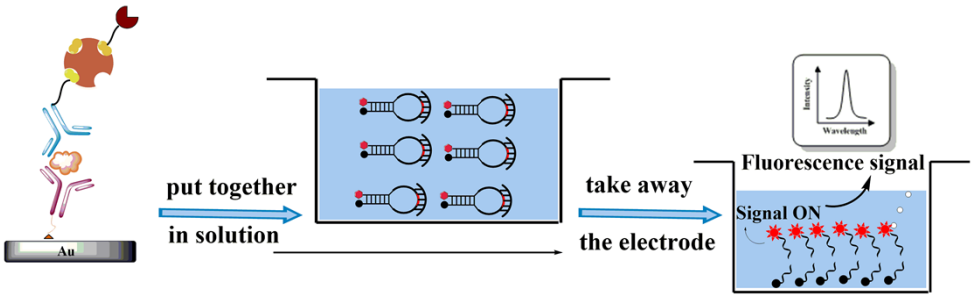
In order to find whether the proposed biosensing approach has potential clinical applications, three human serum samples with addition of 80 ng mL^{-1}, 8 ng mL^{-1}, and 2.5 ng mL^{-1} target PSA which were be located in the linearity range of the presented biosensor were detected. The results and recovery percentage were demonstrated in Table S2. The results showed that a higher signal could be observed in the sample containing more target PSA. We further calculated the value of the concentration of the PSA based on the above obtained linear regression equation. Also the testing value was compared with the addition amount of the antigen. From Table S2 we could find that the recovery percentage were in the range from 95% ~ 105% which means the obtained testing values of PSA concentration in human serum sample have good accuracy. Due to the presented linear range covers the danger zone of the PSA concentration in clinical diagnosis, the designed biosensor is competent in detection of PSA antigen with simple and convenient approach and therefore shows potential application prospect.

Table S2. Recovery experiments for PSA detection in human serum sample based on the presented method.

Serum sample	Addition of PSA (ng mL^{-1})	Testing value (ng mL^{-1})	Recovery percentage (%)
1	80	83.9	104
2	8.0	8.14	102
3	2.5	2.38	95.2

Another hairpin DNA and its partly- complementary DNA (named Comparison DNA3 and DNA4 respectively, which sequences are listed in Table S1) were employed in the comparison experiment. The process of electrode modification is just the same as former design using DNA machine (described in Scheme 1).The difference of comparison experiment without DNA machine is substance contained in the reacting solution. In the comparison experiment, DNA4 could not open the hairpin structure of DNA3 because it only partly complement with its loop domain. While in the presence of Nb.BtSI, it could recognize the nicking site of DNA3, breaking the

DNA skeleton of hairpin. Due to the instability of the broken DNA, the quencher linked to the ends of the stem was expected to a fluorophore Cy5. And then obvious fluorescent signal could appear. From this, we could find that the comparison experiment did not include the isothermal cyclical steps, and what impact the changes will bring on the sensitivity of the designed biosensor.



Scheme S1. Graphic illustration of comparison experiment without DNA machine.