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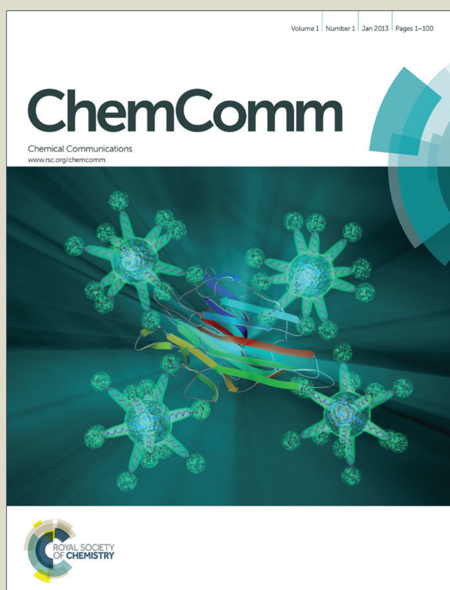

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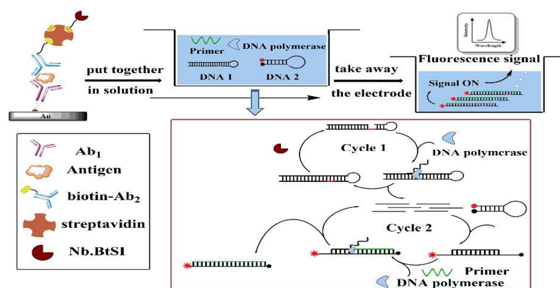

A convenient method is presented employing DNA machine for detection of biomarkers without corresponding aptamers with signal amplification.

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

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A convenient method is first presented which employed DNA machine for protein biomarker detection. The detection limit is 400 times lower compared with that without DNA machine. This study provided a promising method which could realize most of protein biomarkers detection without corresponding aptamers using DNA machine for signal amplification.

Serum cancer biomarker has been regarded as the best target for cancers due to its micro-trauma and high sensitivity, for example prostate specific antigen (PSA).[1] Popular conventional detecting method for above serum protein biomarker generally used nanomaterials for signal amplification to improve biosensing sensitivity.[2] Although nanomaterials such as gold nanoparticles, quantum dots (QDs), etc.[3-7] hold many excellent properties including larger surface area which could immobilize more enzyme for protein analysis, related problems appear during their application. For example, the synthesis process of these nanomaterials was complicated and showed instability among different batches which brought much difficulty in construction of biosensor. Therefore, other amplification methods for antigen detection are urgently needed.

To date, a means of amplified DNA detection methods based on DNA machine have attracted increasing interest and used for detection of various biomolecules.[8-10] Mechanical transformations occurred on a nucleic acid “track”, leading hybridization with the appropriate DNA and yielding a nucleic acid “product” that provided an amplified readout signal for the operation of the machine.[11] However, DNA machine is faced with some limiting factors which greatly restrict its wide application. For example, it was mainly used for analysis of DNA, RNA or some molecules which have corresponding aptamers. And it was impossible to establish its function for serum protein biomarkers which play very important roles in clinical diagnosis because they did not have appropriate DNA aptamer.

Here, we first presented a convenient method which employed DNA machine in detection of PSA as a model of serum protein biomarker. The designed method employed amplification technique of isothermal polymerization system and connected the target antigen concentration to the amount of Nb.BtSI which was modified on the surface of electrode. The effect of amplification is noticeable and the detection limit is estimated to be improved by 400 times during the PSA antigen analysis compared with that without employment of DNA machine. This study provided a promising method which could realize most of protein biomarkers detection without corresponding aptamers using DNA machine for signal amplification.

The principle of the strategy is shown in Scheme 1. One part is sandwich immunoreaction for a target protein and Nb.BtSI modification. The other part is the formation of DNA machine occurred in solution which relies on Nb.BtSI decorated on the surface of electrode. Particularly as described in the experimental section (supporting information), the gold electrode was modified with biotinylated Nb.BtSI through a sandwich immunocomplex and streptavidin. Then, the modified electrode was simply immersed into prepared reaction buffer containing a stem-loop structure DNA 1, a hairpin fluorescence probe DNA 2 possessing a fluorophore Cy5 and a quencher linked to the ends of the stem, a short primer, polymerase Klenow and dNTPs mixture for reaction at 37℃. The primer is complementary to the stem region of the probe. When the modified Nb.BtSI was introduced into the mixture, it recognized the nicking site of DNA1 and the first isothermal amplified reaction was initiated under proper conditions. In this cycle, DNA 1 was consumed and then regenerated, releasing a large amount of DNA fragments. These DNA fragments were complementary to the loop region of the probe DNA 2 and opened its hairpin structure. Then the primer annealed with the open stem and triggered polymerization reaction. During this process of primer extension, above DNA fragments were recycled with strand

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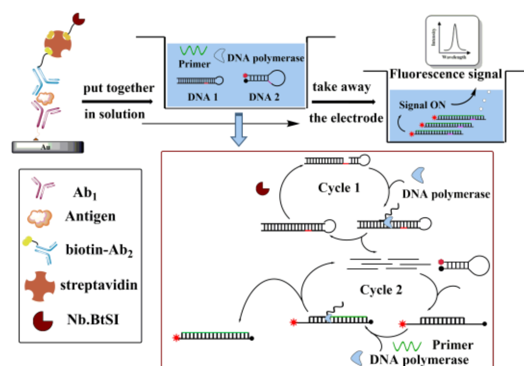
displacement reaction. At the same time, obvious fluorescence could appear as the conformational change of DNA2 with the separation of fluorophore Cy5 and the quencher. While without target antigen, Nb.BtSI could not be modified on the electrode, the following cleavage polymerization reaction could not occur.

From the whole process, we could found that the greatly amplified final signal is influenced by the amount of target antigen (PSA) because it reflects the amount of Nb.BtSI who plays a key role in the mentioned DNA machine. Then, even minute amounts of elevated concentration of target PSA could lead to an increased amount of Nb.BtSI and further produce obvious fluorescence enhancement.



Scheme 1 The mechanism of isothermal amplified detection of PSA based on strand-displacement polymerization reaction.

First the feasibility of this strategy was verified and showed in Fig.1A. Hairpin DNA2 in solution showed no obvious fluorescence signal due to the quencher which was located close to the fluorophore (curve a). However, we could find that under the conditions mentioned in Scheme 1 with PSA, an obvious fluorescence response appeared around 670 nm (curve d). This indicated the process of isothermal polymerization had successfully performed which opened the hairpin structure of fluorescent probe. While in the absence of target PSA (curve b), or klenow (curve c), similar fluorescence signals were obtained as blank curve a. That further verified the great enhanced fluorescence response was due to the designed DNA machine and meanwhile showed the extra-low background which is very important to the biosensor. Electrochemical impedance spectroscopy (EIS) was also used to confirm the successful modification of Nb.BtSI (Fig. S1).

As a biosensing method for antigen, the selectivity is also a key parameter for the evaluation of the performance of this presented biosensor. Potential interferents which also could be found in serum were used in the following experiment including bovine serum albumin (BSA), carcino-embryonic antigen (CEA) and α-fetoprotein (AFP) to find its potential influence to the target PSA proteins respectively. As shown in Fig. 1B, the biosensor had good specificity toward target PSA while BSA, AFP and CEA were also present in detecting solution. These tests denoted that the developed strategy could be used to identify target protein with high specificity.

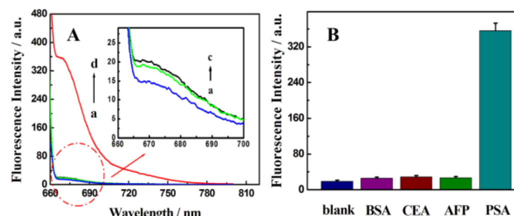


Fig. 1 (A) Fluorescence spectra of reaction solution of different conditions. (a) blank, (b) without target PSA, (c) without klenow polymerase, and (d) with 100 ng mL^{-1} PSA in the presence of klenow polymerase and dNTPs; (B) Specificity of the biosensor for PSA detection compared with solution containing 100 ng mL^{-1} of BSA, CEA and AFP. Blank is fluorescence response of blank PBS with no target antigen or interference protein.

Then the sensitivity and quantitative range for the presented amplified sensing method for antigen were studied. The optimized experimental conditions including the concentration of the klenow polymerase or dNTPs, and the incubation time for isothermal reaction were studied and showed in Fig. S2. Under the optimized conditions, results from PSA as a model target antigen with different antigen concentrations in the developed protocol were described in Fig. 2. Based on the recognition reaction between antigen and corresponding antibody, and affinity interaction between streptavidin and biotin, Nb.BtSI was introduced into the electrodes surface and triggered the DNA machine in solution, resulting obviously enhanced fluorescence signal. The fluorescence response was speculated to be influenced by the concentration of PSA antigen and then the fluorescence peak value at about 670 nm was recorded in the quantitative detection of PSA. From Fig. 2A, the fluorescence response was found to be enhanced when the antigen concentration increased from 0 ng mL^{-1} to 100 ng mL^{-1} in the sample solution (Fig. 2A (a)-(h)). Data in this range also demonstrated good linear relationships between fluorescence intensity and the logarithm of antigens concentration which was shown in Fig. 2B.

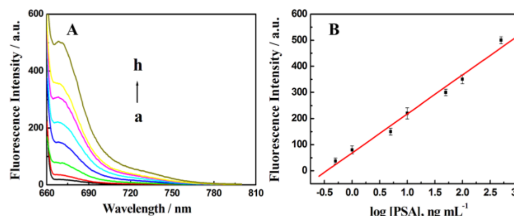


Fig. 2 (A) Fluorescence response of the amplified biosensor in the presence of different concentrations of PSA (from a to h: 0, 0.5, 1.0, 5.0, 10, 50, 100, 500 ng mL^{-1}, respectively); (B) The linear relationship between the fluorescence intensity and logarithm of PSA concentration (n=3).

A linear dependence between the peak currents and the logarithm of PSA concentration was obtained in a range from 0.5 ng mL^{-1} to 500 ng mL^{-1}. The linear regression equation was I (a.u.) =68.35+149.1 log c (PSA, ng mL^{-1}). The limit of detection (LOD, 0.5 ng mL^{-1}) is lower enough to meet the needs of clinical applications for the assay of PSA antigen for prostate diseases which is 1-4 ng mL^{-1} in normal range and 4-10 ng mL^{-1} in danger range.

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In order to find whether the proposed biosensing approach has potential clinical applications, three human serum samples with addition of standard PSA which was be located in the linearity range of the presented biosensor were detected. The results and recovery percentage were demonstrated in Table S2 which showed that the testing method has 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 shows potential application prospect.

Finally, the effect of amplification of the novel strategy which combined DNA machine for PSA antigen detection was studied compared with that without DNA machine described in Scheme S1. Here the reacting solution only contained a hairpin DNA and its partly- complementary sequence. The detailed difference is mentioned in the supporting information. Fig. 3 showed the results of the fluorescence response without DNA machine in the presence of different concentrations of PSA. From the results, we could find that the fluorescence signal was greatly weakened compared with that of amplified strategy even through using higher concentration (Fig. 3 A). Data in the range from 0 μg mL^{-1} to 10 μg mL^{-1} demonstrated a good linear relationship between fluorescence intensity and the logarithm of antigens concentration (showed in Fig. 3B) with a linear regression equation I (a.u.) = -32.78+42.47 log c (PSA, ng mL^{-1}). And the limit of detection is 200 ng mL^{-1}. From the comparison results, the effect of amplification is noticeable as using DNA machine in the detection and the detection limit is estimated to be improved by 400 times during the PSA antigen analysis.

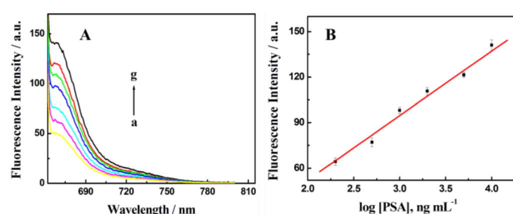

Fig. 3 (A) Fluorescence response of the comparison biosensor in the presence of different concentrations of PSA (from a to g: 0, 0.2, 0.5, 1, 2, 5, 10 μg mL^{-1}, respectively); (B) The linear relationship between the fluorescence intensity and logarithm of PSA concentration (n=3).

In summary, DNA molecule machine based on isothermal cycle reaction was first introduced to protein antigen detection and develop a simple but convenient sensing method for model PSA antigen. The proposed biosensing approach avoided the complicated synthesis and instability of nanomaterial but also showed great amplification effect. The method also could be applied for the detection of other antigens in human serum by simply changing the respective antibodies and provide a smart sensing strategy in human serum protein detection during precise and micro-trauma clinical diagnostics.

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