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A signal conversion system using binding-induced strand displacement for disease biomarker assay

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ABSTRACT

Using the principle of binding-induced DNA strand displacement (BINSD), a DNAzyme-powered nanomachine biosensor for multiple biomarkers *via* magnetic beads-based signal conversion was designed. It can convert multiple biomarkers recognition into the release of predesigned output nucleic acids tagged with the streptavidin proteins (SA-DNA) for activation of DNA nanomachines. In general, we adopted the complementary base pairing rules and affinity ligand specific recognition, and three types of signal conversion systems were constructed, respectively. They realized the universal, sensitive, accurate and specific detection of multiple biomarkers. Taking the advantage of strong anti-interference capability of the magnetic separation, this strategy could be employed for the detection of various biomarkers in clinical practice.

Keywords: signal conversion, binding-induced strand displacement, fluorescence, disease biomarkers, DNA nanomachine

INTRODUCTION

Recent advances have shown that DNA nanotechnology-based assays have attractive advantages when they were used for sensing biomarkers.[1-4] Binding-induced DNA strand displacement (BINSD) is the technology in which two or more DNA probes can induce strand displacement in the presence of target due to their specific recognition with the targets. Therefore, BINSD method has been applied to develop different assays for proteins and nucleic acids.[5-9] For instance, with protein affinity ligand of DNA probe, BINSD is usually constructed in a way that the protein recognition initiates the strand displacement process, and the displaced output DNA was triggered for following use.[7, 10, 11] Thus, BINSD is capable to convert the signal of the target into the signal of DNA, which is easy to be combined with other amplification strategies.

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To facilitate the dynamic DNA assemblies or disassembles, the homogenized components are usually used for fabricating the amplified or repeated nanodevices.[12-17] Due to the feature of nucleic acids, a DNAzyme is often employed as the component for the integration of DNA nanotechnology-based structures.[18-20] Compared with traditional proteases, a DNAzyme has the advantage of easy construction for point-of-care and on-site assay because of its higher chemical and thermal stability.[21, 22] Due to its intrinsic nature, it is easy to be coupled with various DNA amplification technologies to improve the detection sensitivity.[23, 24] Regarding to our previously reported work, we had constructed the DNAzyme-powered nanomachines for detection of proteins by assembling the nucleic acids components onto the gold nanoparticles. [14, 15, 25] On the basis of them, we observed that the 8-17E DNAzyme for fabricating the DNAzyme-powered DNA nanomachine is capable to generate the better catalytic effect and response performance.

With the self-power property of the DNAzyme, these nanomachines can be automatically activated by the external stimuli, executing predesigned the function. However, there are some issues to be addressed. Firstly, the substrates of DNAzyme contained the ribonucleic acid located on the cleavage site, which is susceptible to the nuclease in the biological matrices. Secondly, the unspecific cleavage of substrates usually generated the background signal. Most DNA nanomachines are only applicable to the nucleic acids or proteins with dual-site binding manner of aptamer (e.g. thrombin). Thirdly, currently constructed DNAzyme-powered DNA nanomachines are difficult to operate in high concentrations of serum as a result of the instability of Au-S bond, and this hinders their application in clinical diagnosis.[17]

To expand the application of DNA nanomachines, we take advantage of a binding-induced DNA displacement platform, to incorporate with the downstream DNAzyme-powered DNA nanomachine. In this design, the magnetically biorecognition platform was fabricated by conjugating the nucleic acid components. Upon binding with the targets, DNA components tagged with streptavidin (SA-DNA) can be displaced from the platform to act as the downstream stimulus. Then, the streptavidin of SA-DNA stimulated the DNAzyme-powered DNA nanomachine to generate the amplified fluorescence signal for indicating the concentrations of the target **(Scheme 1)**. In this system, the biorecognition affinity ligand components (nucleic acids probes, aptamers, and antibodies) conjugated onto the platform captured the targets in serum firstly and then utilized the advantage of DNA nanomachine to detect the displaced SA-DNA translators quickly and accurately.

EXPERIMENTAL SECTION

Materials and reagents

All DNA oligonucleotides (the detailed sequences were listed in Table S1) in this work were synthesized and purified using HPLC by Integrated DNA Technologies (Coralville, IA, USA), after careful design and analysis by the IDT Oligo Analyzer. Stock solutions of the oligonucleotides were diluted using deionized water (>18.2 MΩ cm^{-1}), then stored at -20 °C for further use. Deionized water with conductivity of 18.2 MΩ cm^{-1} was from a water purification system (Millipore, Bedford, MA, USA) and used for preparation of all of the solutions in this work. The fluorescence assay was conducted by a multimode microplate reader (DX880, Beckman Coulter) with excitation at 485 nm and emission detected at 515 nm.

The carboxyl-terminated Fe_3O_4 magnetic beads (MBs, 1 μm, 10 mg mL^{-1}) were purchased from Thermal, Inc. (USA). A solution of 20 nm gold nanoparticle (AuNP) was obtained from Ted Pella (Redding, CA). 10×phosphate buffered saline (PBS) and trihydroxymethyl aminomethane (Tris) were provided by Sangon Biotech. Co., Ltd. (Shanghai, China). Magnesium chloride ($MgCl_2$), sodium chloride (NaCl), potassium chloride (KCl), bovine

serum albumin (BSA) and biotin were ordered from Sigma-Aldrich (St. Louis, MO, USA). RNase-free water, exonuclease I (Exo I) and Exo III were obtained from TaKaRa Bio Inc (Dalian, China). Sodium dodecyl sulfate (SDS), sodium azide (NaN_3), monoethanolamine, ethylenediaminetetraacetic acid disodium salt (EDTA), dithiothreitol (DTT), imidazole, sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), N-(3(Dimethylamino)propyl)-N′-ethylcarbodiimide hydrochloride (EDC) , Tween-20 and glycerol were purchased from Chengdu Chron Chemicals Co., Ltd. (Chengdu, China). Carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), biotin/anti-CEA, biotin/anti-AFP, platelet-derived growth factor BB (PDGF-BB), prostate-specific antigen (PSA), human serum albumin (HSA), transferrin (TRF), thrombin and carbohydrate antigen 19-9 (CA 19-9) were purchased from R&D Systems (USA). The buffers used were as follows: (A) washing buffer, saline-sodium citrate (SSC) buffer, 30 mM sodium citrate, 300 mM NaCl, pH = 7.0, (B) 10 mM sodium phosphate buffered saline (PBS), pH = 7.4, (C)100 mM MES buffer, pH = 6.0, (D) MBs store buffer, 0.1% (*w*/*v*) BSA in 100 mM PBS at pH = 7.4, (E) conjugation buffer, 100 mM EDC in 100 mM imidazole buffer at pH = 7.0, and (F) antibody exchange buffer, 55 mM PBS, 150 mM NaCl and 20 mM EDTA, pH = 7.2.

Preparation of DNA-based signal conversion system (DNscs), aptamer-based signal conversion system (Apscs) and antibody-based signal conversion system (ABscs)

The conjugation of the capture probe with carboxyl-terminated magnetic beads was performed by the EDC reaction. In a typical experiment, 1 mg of the carboxyl-terminated magnetic beads was transferred into a 1.5 mL centrifuge tube and washed three times with 200 μL of 100 mM imidazole solution (pH = 7.0). Then, 20 μg of 5'-terminated amino modified SA-DNA capture probe or aptamer was added into the tube. The carboxyl-terminated magnetic beads were activated by 100 mM fresh prepared EDC at 50 °C for 3 h with continuous shaking. After washing three times with 500 μL of washing buffer (0.5% SDS in SSC buffer) and 2 times with RNase-free water without at 65 °C, 400 μM SA-DNA was added to the solution. Another 3 h shaking incubation at room temperature, and then washed twice with 10 mM PBS, two systems were obtained (DNscs and APscs). The DNscs and APscs probes were resuspended in storage buffer (0.2% NaN_3 in 10 mM PBS) and stored at a concentration level of 5 mg mL^{-1} in the refrigerator at 4 °C for further use.

The procedure of modification of the antibody-MBs was as follows: First, 1 mL of carboxyl-modified MBs (100 mg mL^{-1}) was taken into the polystyrene tube and washed 2 times by using MES buffer solution. And then 10 mL of activation buffer was used to suspend the MBs by shaking. 100 mg EDC was added and stirring 15 min at room temperature. After 2 times washing by conjugation buffer, the MBs were resuspended in 5 mL conjugation buffer. Dissolving the antibodies in a 5 mL buffer and it was mixed with the MBs suspension. It was incubated at room temperature for 2-4 h with vortexing. The resultant solution was washed and resuspended in 10 mL of 40 mM ethanolamine, stirred gently for 30 min, then washed with a storage buffer and resuspended to the desired storage concentration of 10 mg mL^{-1} at 4 °C for further use.

Preparation of Antibody-DNA probe

The protocol was reported by previous works.[15] Briefly, 1 μL 10 mM sulfo-SMCC dissolved in DMSO was added to 20 μg antibodies and incubated at room temperature for 2 h. DNA (3 μL, 100 μM) was reduced with DTT (4 μL,100 mM) in 50 μL antibody exchange buffer. The solutions of sulfo-SMCC activated antibodies and reduced DNA were purified by G-50 spin column (Amersham Cat no 27-5330-02). The activated antibodies and DNA were mixed and dialyzed (Slide-A-Lyzer, Pierce Cat No. 69562) overnight with PBS buffer (pH = 7.2). The dialyzed solution was collected and stored at 4 °C for the following use.

Preparation of DNA nanomachine

To prepare the DNA nanomachine, substrate, probe 1 and spacer DNA were mixed with AuNP at the molar ratio

of [substrate]:[probe 1]:[sapcer DNA]:[AuNP]=1000:100:100:1. Firstly, the substrate (6 μL, 100 μM) and probe 1 (6 μL, 100 μM) were added to the AuNPs solutions (20 nm, 500 μL), then the mixture containing 0.05% Tween 20 was incubated at room temperature for overnight in dark. 20 μL 3 M NaCl was utilized for salting DNA nanomachine with an interval of 40 min for 6 time to maximize the loading amount. After that, the mixture was incubated at room temperature for 24 h. To reduce the nonspecific interaction between the DNA oligomers and AuNPs, a certain concentration of DNA spacer was added to block the bare surface of AuNP. After incubation at room temperature for another 24 h, the mixed solution was washed 4 times using 500 μL of PBS buffer (pH = 7.4) containing 0.05% Tween 20 by centrifugation at 11, 000 g for 20 min. Finally, the functionalized AuNPs were resuspended in 250 μL 10 mM PBS (pH = 7.4) then stored at 4 °C prior to use.

Determination of probes loading on MBs

To determine the probe loading coverage, Exo I and Exo III were used to release the fluorescence labelled DNA probe from the MBs and to determine it with an external standard. Firstly, 5 μL 5 U μL^{-1} Exo I and Exo III were added to 0.5 mg functionalized MBs and placed in the dark. After 2 h incubation at 37 °C, the solution was isolated using an external magnet. Then, 95 μL of supernatant was transferred into a 96-well plate, which was then loaded onto a fluorescence microplate reader for fluorescence measurement.

Observing the DNA nanomachine operation

To evaluate the operation of the DNA nanomachine responded to streptavidin and SA-DNA, 0.23 nM AuNP track, 4 nM biotin-probe and various concentrations of streptavidin or SA-DNA were mixed in 100 μL of 50 mM Tris-HAc buffer (pH = 8.0) containing 100 mM NaCl and 10 mM $MgCl_2$. Solutions were transferred to a 96-well microplate. The fluorescence was measured for one hour an interval of 5 min at room temperature.

Using the signal conversion system for sensing miRNA, protein and antigens

For sensing miRNA and protein, miRNA-21 and PDGF-BB were chosen as the model targets. Firstly, 0.5 mg functionalized DNscs were washed two times with wash buffer. The reaction solution containing 100 mM NaCl, 50 mM $MgCl_2$, 100 pM miRNA-21 and 10 mM PBS were then added into the 200 μL PCR tube with the washed DNscs. Then, the solutions were mixed and shook at room temperature for 30 min. After magnetic separation, the solution was collected and transferred into the tube that contained 0.23 nM DNA nanomachine solutions. Finally, the mixtures were pipetted into the 96-well microplate for the following fluorescence measurement by a fluorescence microplate reader.

Similarly, AFP and CEA were chosen as the model targets for investigating the analytical performance using the signal conversion system. The washed 0.5 mg ABscs was added into the solutions consisting of 100 mM NaCl, 50 mM $MgCl_2$, and 10 mM PBS. Solutions of 5 nM P1 for AFP or 5 nM P1 for CEA were mixed with 100 pM AFP or 100 pM CEA to trigger the BINSD reaction at room temperature for 30 min by a mixing vibrator. The mixtures were pipetted into the 96-well microplate for the following fluorescence measurement by a fluorescence microplate reader.

Detection of liver cancer biomarker derived from clinical samples

Serum samples were collected from West China Hospital of Sichuan University and all experiments were performed with the approval of the Committee on Biomedical Ethical of the hospital. Before determination of the cancer biomarkers, the blood samples were treated by centrifugation. It was carried out at 3000 rpm for 5 min to obtain the serum samples. After that, 5 μL serum from clinical samples were mixed with the signal conversion system and the subsequent detection protocol was the same as mentioned above.

RESULTS AND DISCUSSION

The principle of signal conversion system

As shown in **Scheme 1,** on the basis of binding-induced DNA strand displacement (BINSD), the magnetic biorecongnition platform was responsible for converting diverse biomarkers into nucleic acids tagged with the streptavidin proteins (SA-DNA). For operation of the DNA nanomachine, gold nanoparticle (AuNP) was employed as a scaffold to build the moving track attached with the FAM fluorophore-labelled substrate. For responding to SA-DNA , biotin- labelled probe 1 was anchored on the surface of the AuNP through Au-S bonding. Probe 2 was designed to contain the DNAzyme sequence domain near to the 3′ end and a biotin molecule was modified at 5′ end. At first, the fluorescence was quenched due to a resonant energy transfer to AuNP. When SA-DNA was incubated with the DNA nanomachine, the biotin-labelled nucleic acid probe (probe 1 and probe 2) would bind to the same SA-DNA through streptavidin-biotin interaction, resulting in recruiting the probe 2 onto the surface of the DNA nanomachine. Due to the proximity and localized concentrations, the DNAzyme could hybridize with and cleave the substrates, resulting in fluorescence enhancement. Subsequently, enzymatic cleavage drove the DNAzyme to move autonomously along the surface of the AuNP and produce the amplified fluorescence signal. For sensing the targets in complex matrix, we were able to exchange the biological matrices into the simple buffer solutions via magnetic separation, avoiding the false positive signal resulting from the biotiols in biological samples.

Verification of principle feasibility

To illustrate feasibility of the principle, we firstly verified that the signal generation efficiency of DNAzyme-powered DNA nanomachines induced by SA-DNA. Compared with the free streptavidin(SA), the nucleic acids bound streptavidin(SA-DNA) do not compromise the activity of the DNAzyme-powered DNA nanomachine. Because of the biotin-labeled DNA probe on DNA nanomachine, SA or SA-DNA can be specifically captured by biotin-streptavidin interaction. It suggests that there should be almost no difference about SA and DA-DNA when they were used to trigger the DNA nanomachine to operate, facilating using the upstream generated SA-DNA to streamline the downstream DNA nanomachines **(Fig. S1).** Besides, the sensitivity was evaluated by varying concentrations of SA-DNA, the DNAzyme-powered DNA nanomachine can determine as low as 0.5 pM SA-DNA **(Fig. S2).**

In general, we constructed the magnetic biorecognition platform for target binding and SA-DNA releasing. The DNA probe payload on the MBs is an important factor of this signal conversion system. Coverage of biorecognition probe was chacterized by an external calibration method and the detailed procedure was outlined in the experimental section. The coverage of biorecognition probe on one magnetic bead was estimated to be 45000 hDNA and 38000 aptamers **(Fig. S3)**. From the high coverage related to the magnetic biorecognition platform, it proved that we had successfully prepared the platform ready for use.

Application of DNA-based signal conversion system to miRNA detection

By choosing the miRNA-21 as the model target, we attempted to demonstrate the feasibility of such a signal conversion system. The scheme of DNAzyme-powered DNA nanomachines for the analysis of miRNAs coupled with magnetic biorecognition platform was illustrated in **Fig.1a**. The dsDNA consisting of hDNA and SA-DNA was assembled onto magnetic beads, forming the magnetic biorecognition platform for miRNA-21. The miRNA was fully recognized by the hDNA, and the SA-DNA was displaced and released. Then, the free SA-DNA was

transferred to trigger the DNAzyme-powered DNA nanomachine after magnetic separation.

As shown in **Fig. 1b**, the fluorescence signal intensity is correlated with the target concentrations, which suggested that the upstream miRNA-21 biorecognition should be coupled with the downstream DNAzyme-powered DNA nanomachine successfully. It also had a good liner correlation and it could detect as low as 0.5 pM miRNA-21 **(Fig 1c)**.

We also tested the specificity of the signal conversion system by the variants of miRNA-21. miRNA-21, miRNA-21 with single mutation (s-miRNA-21), miRNA-21 with double-site mutations (d-miRNA-21) and random sequence (r-sequence) were used to incubate with the signal conversion system. It showed that there was no significant fluorescence enhancement when the interference sequences were introduced. In contrast, the signal of wild type DNA sequence was significantly increased **(Fig. S4).**

Given DNA strand displacement reaction occurring at the surface of MBs, we optimized the target recognition time (**Fig. S5**). When the capture time was 10 min, the capture of the target was incomplete, both the background and signal intensities were low at this point. Approaching to 30 min, the free miRNA-21 fully reacted with hDNA, while SA-DNA was released from the magnetic platform to activate the downstream DNA nanomachines, resulting in a higher signal background ratio.

Application of aptamer-based signal conversion system for protein probing

On the basis of aptamer, we engineered an aptamer-based signal conversion system (APscs) into the specific biosensing system for platelet-derived growth factor BB (PDGF-BB) by simply changing the recognition domain into PDGF-BB aptamers. The working principle was similar with the nucleic acids sensing, PDGF-BB bound to aptamer and induced conformational change. As a result, the corresponding SA-DNA was displaced into the bulk solution and participated in the downstream amplified sensing via DNAzyme-powerd DNA nanomachine **(Fig. 2a).** As shown in **Fig. 2b** and Fig. **2c**, such a protein responsive signal conversion system was available for responding to varying concentrations of PDGF-BB. Using such a novel and simple design, we were able to detect as low as 0.05 pM PDFG-BB protein. We also investigated the anti-interference capability of this signal conversion system using varying concentrations of human serum spiked with the PDGF-BB proteins. It was capable of translating the target in 100% human serum and producing the significant amplified fluorescence signal **(Fig. 2d).** When there was 80% serum mixed with the biorecognition platform, it still showed a good linearity in the dynamic range of 10 - 2000 pM (**Fig.S6**).

Antibody-based signal conversion system response to AFP and CEA.

Although aptamer is an extraordinary affinity ligand for proteins recognition, the available types of aptamers are limited by the screening technology (e.g. SELEX). To broaden the application of conversion system, another two types of protein biomarkers, AFP and CEA were employed as the model targets. By changing the aptamer into the antibody-DNA probe, the signal conversion system was adapted as the AFP and CEA biosensors. As shown in **Fig. 3a**, the specific polyclonal antibodies were firstly coupled on magnetic beads to capture target antigens. Target mediated BINSD was initiated by introducing the antibodies modified DNA probe into the magnetic biorecognition platform, generating the SA-DNA translators for triggering the downstream amplified sensing via DNAzyme-powered DNA nanomachine. The sensitivity of antibody-based signal conversion system (ABscs) was evaluated by varying concentrations of AFP and CEA, respectively (**Fig.3b** and **Fig.3e**). The linear relationship was obtained with a range from 0.05 pM to 2 nM. The results demonstrated that the heterogeneous BINSD design coupled with the signal conversion system had an excellent extensible features and advantageous capability for

protein sensing (**Fig.3c** and **Fig.3f**) with a low detection limit of 0.05 pM.

Progress towards medical field applications will require a more stable detection system that is capable to determine diverse proteins in biological matrices. Compared with the simple buffer, the abundant proteins in serum would probably influence the specific interaction between affinity ligand and target, resulting in the background. In this study, such an ABscs showed a good compatibility and stability in varied concentrations of human serum. Even in 100% human serum, it still made a differentiation between signal and background. (**Fig.3d**). At high concentrations of human serum condition, both signal conversion systems of AFP and CEA showed excellent linear response with varied concentrations of targets, respectively (**Fig. S6**).

To evaluate the selectivity of antibody or aptamer-based signal conversion system, we chose carbohydrate antigen 19-9 (CA19-9), thrombin, PSA, transferrin (TRF) and human serum albumin (HSA) as the interfering proteins for selectivity assays. PDGF-BB, AFP and CEA were selected as the targets, respectively. We observed that it was no significant fluorescence signal enhancement when the different types of signal conversion systems were incubated with the nonspecific proteins. On the other hand, the target molecules with a concentration (100 pM) generated a significant fluorescence increase (**Fig.4**), suggesting a good selectivity in these novel signal conversion system. In addition, the detection limit of this signal conversion system was also lower than or at least comparable to previously reported detection methods (**Table S2**).

Antibody-based signal conversion system for clinical diagnosis

In order to verify the analytical performance of antibody-based signal conversion system in clinical diagnosis, we employed it for biomarker detection in healthy people or liver tumour patients. As shown in **Table 1**, the levels of CEA and AFP from the liver tumour patients were apparently higher than those of the healthy people, and these results were consistent with those detected by enzyme-linked immunosorbent assay (ELISA). It suggested that the proposed signal conversion system could be manipulated and effective in complex matrice (e.g. human serum).

CONCLUSIONS

In this study, a novel signal conversion system was constructed by cooperating principle of binding-induced DNA strand displacement (BINSD) with DNAzyme-powered DNA nanomachine. By simply changing the biorecognition components, such a signal conversion system was easy to release the output nucleic acids tagged with the streptavidin (SA-DNA). The downstream DNAzyme-powered DNA nanomachine was activated by the released SA-DNA, achieving the quantitation of nucleic acids, proteins and antigens, respectively. Moreover, the signal conversion system showed the robustness and an interference-resistant capability when it was employed as the biosensors for targets in biological matrices. In addition, due to the application of magnetic separation, the signal conversion system constructed in this work had a strong anti-interference capability. They could still operate well even in 100% human serum. For clinical diagnosis, antibody-based signal conversion system had been successfully applied to discriminate healthy serum samples and liver tumour serum samples, which is corresponded with the results that detected by a traditional ELISA method. It is believed that such an intermediated translator streamlined the upstream biorecognition and downstream DNA nanomachine to fabricate signal conversion systems could be used for clinical diagnosis.

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SUPPORTING INFORMATION

Table S1 and Fig. S1, S2, S3, S4, S5, S6, these materials are available in another document.

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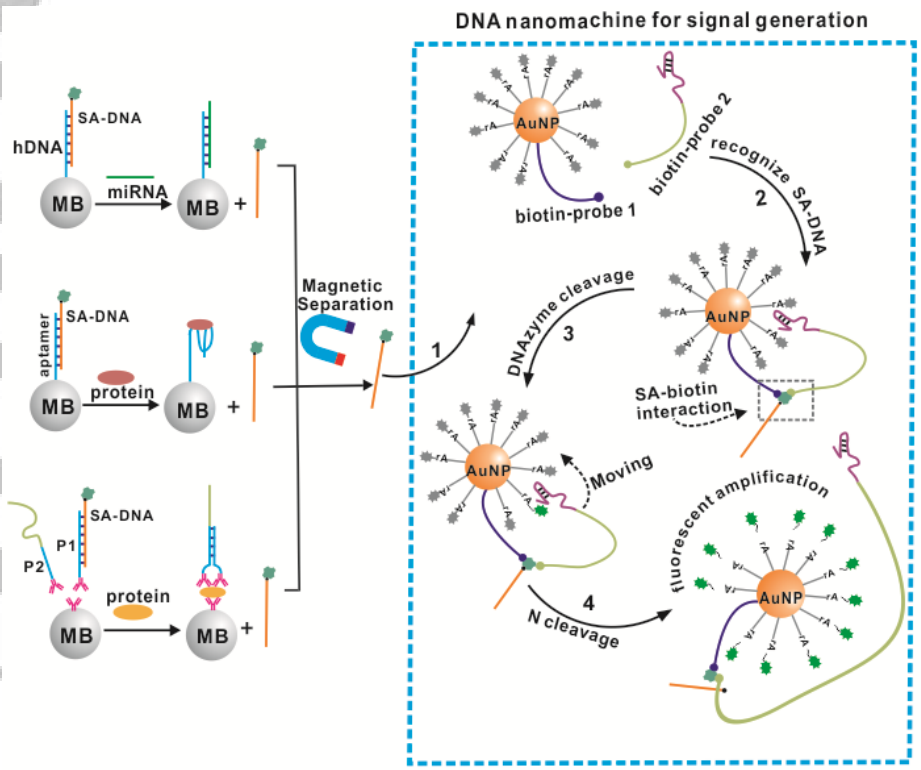
Table 1. Comparison of liver cancer biomarker results in healthy people and liver tumor patients

Test group	AFP (ng mL^{-1})	CEA (ng mL^{-1})
Antibody-based signal conversion system		
healthy people (n = 10)	5.26±2.14	9.22±2.39
liver tumor patients (n = 10)	89±22.7*	269.2±48.9*
ELISA Method		
healthy people (n = 10)	7.28±2.99	5.39±2.36
liver tumor patients (n = 10)	92.3±33.1*	297.2±62.3*

*Significance effect at the 0.05 level ($p < 0.05$). n means the number of samples derived from the independent individuals.



Scheme 1. Schematic illustration of signal conversion system based on binding-induced strand displacement for biomarkers assay. Multiple biomarkers recognition events are converted into the release of predesigned output nucleic acids tagged with the streptavidin proteins (SA-DNA). The downstream DNA nanomachine was activated by the SA-DNA, yielding the amplified signal.

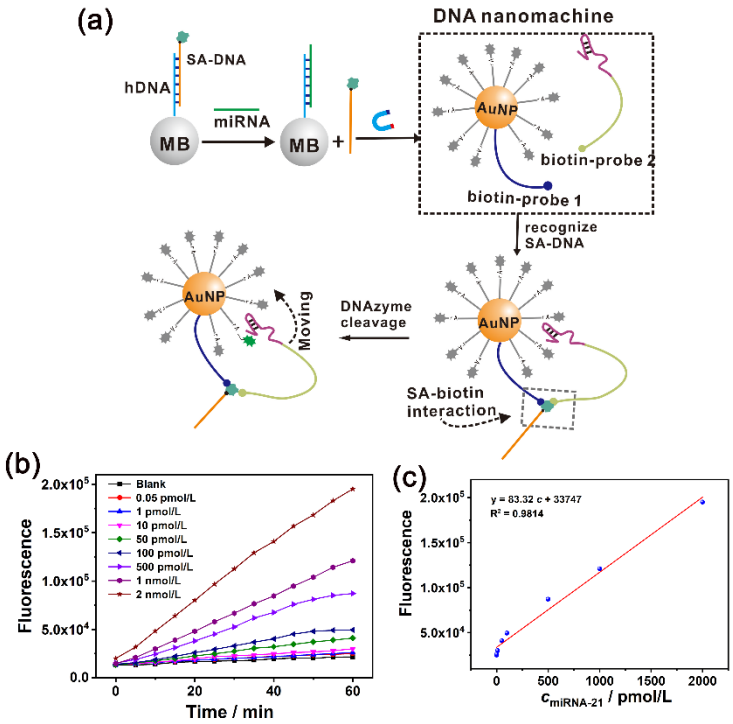


Fig.1 (a) Principle of the operation of DNA-based signal conversion system (DNscs). (b) Response to varying concentrations of miRNA-21. The target concentration from bottom to top was 0.5 pM, 1 pM, 5 pM,10 pM, 50 pM, 100 pM, 1 nM, 2 nM. (c) Linear relationship between the fluorescence intensity and the concentration of miRNA-21.

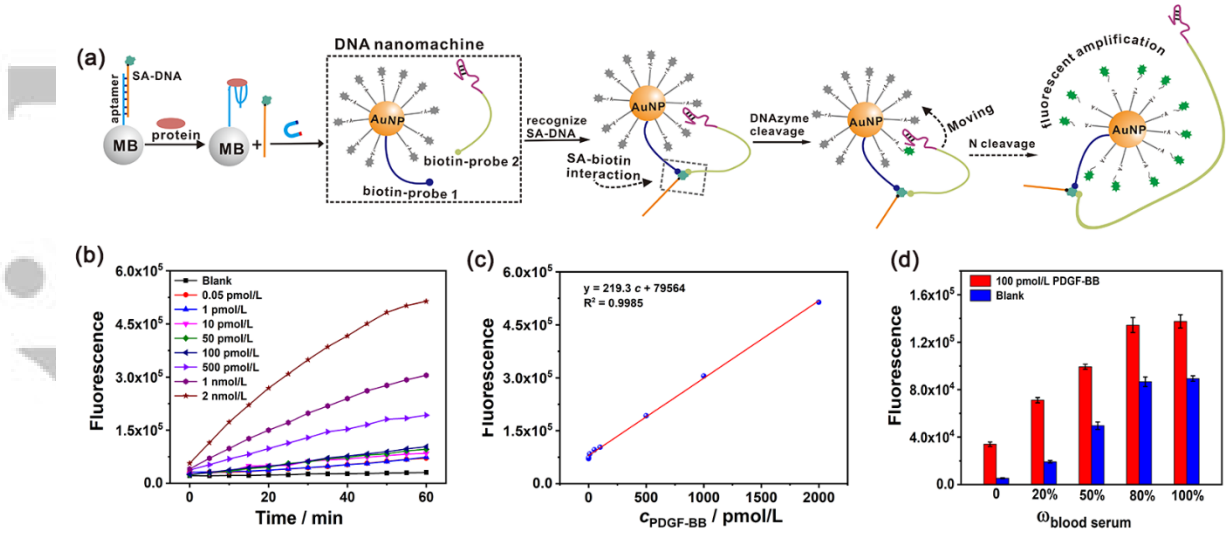


Fig.2 (a) The operation mechanism of aptamer-based signal conversion system (APscs). The aptamer consisted of a partial complementary sequence that hybridized with the SA-DNA was modified on magnetic beads. When the target protein is present, aptamer folds to form a tertiary structure that binds to the target protein, releasing SA-DNA. The following steps are similar to those of DNscs. (b) Response of the conversion system to varying concentrations of PDGF-BB, and (c) the linear relationship between the fluorescence intensity and the concentration. (d) Characterization of APscs in different concentrations of serum. Each error bar represents one standard deviation from triplicated analyses.

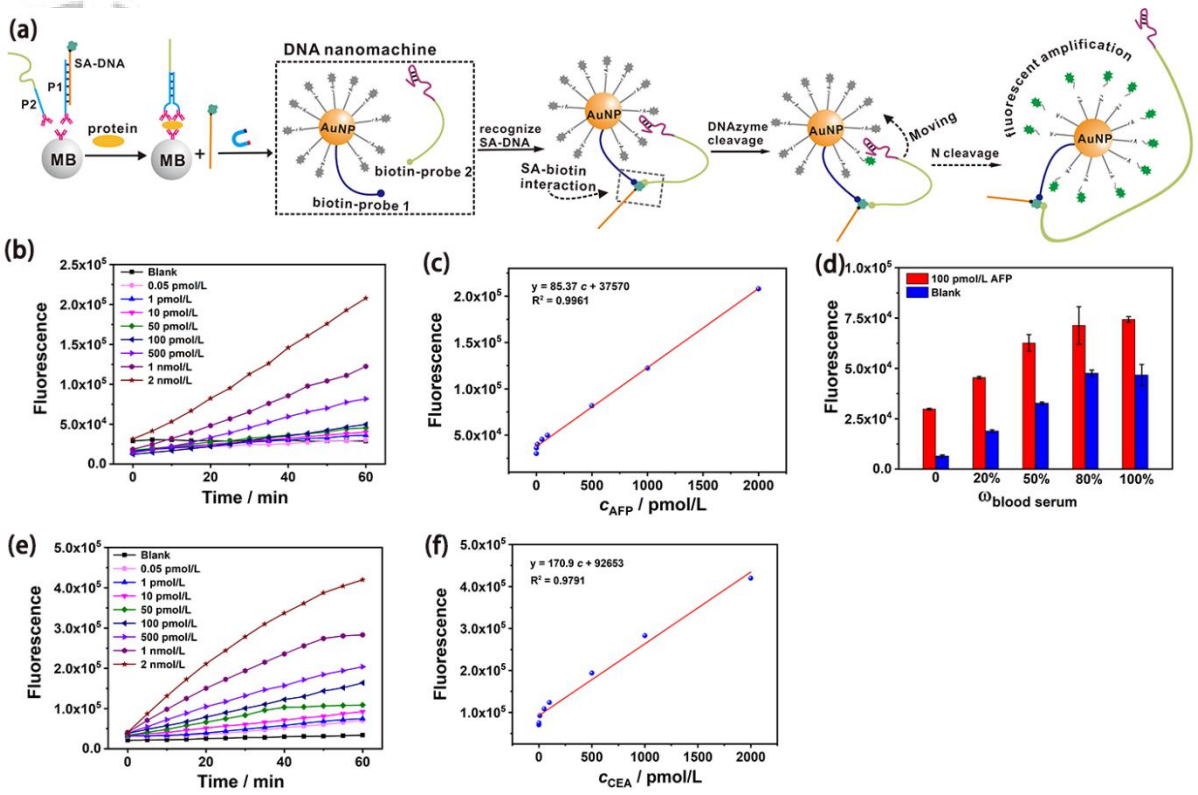


Fig.3 (a) The operation mechanism of antibody-based signal conversion system (ABscs). Response of the ABscs to varying concentrations of b) AFP, e) CEA. The target concentration from bottom to top was 0.5 pM, 1 M, 5 pM,10 pM, 50 pM, 100 pM, 1 nM, 2 nM. Linear relationship between the fluorescence intensity and the concentration of c) AFP, f) CEA. (d) Characterization of ABscs in different concentrations of serum. Each error bar represents one standard deviation from triplicated analyses.

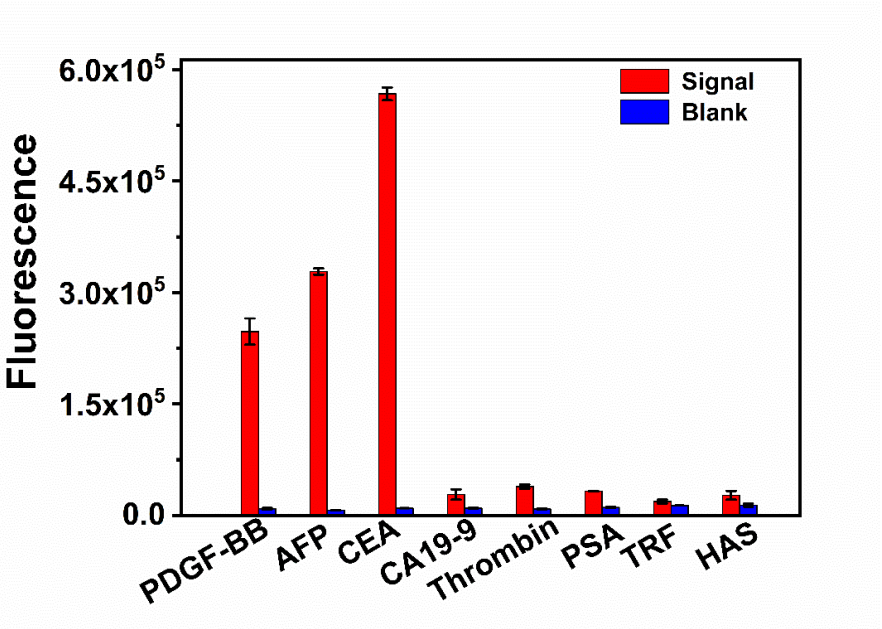


Fig.4 Investigation of specificity of ABscs. The concentration of interfering proteins was 1 nM and the concentration of target proteins was 100 pM. Each error bar represents one standard deviation from triplicated analyses.