Electronic Supplementary Information

A universal biosensor for multiplex DNA detection based on hairpin probe assisted cascade signal amplification

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1. Experiments

1.1. Apparatus. Biojet HM 3030 dispenser, the Guillotine cutting module ZQ 4200, and portable strip reader DT1030 were purchased from Shanghai Goldbio Tech. Co., LTD (Shanghai, China).

1.2. Chemicals and materials. Streptavidin, $HAuCl_4 \cdot 3H_2O$, trisodium citrate, Triton X-100, sodium dodecyl sulfate (SDS), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Klenow fragment exo- and Nt.BbvClwere purchased from New England Biolabs Inc., Anti-streptavidin was purchased from Abcam (San Francisco, CA, USA). Glass fiber (CFSP001700) and nitrocellulose membrane (HFB18004) were purchased from Millipore (Billerica, MA). Other common chemicals were analytical reagent grade and were used as received. All solutions were prepared with ultrapure water (18.2 MΩ/cm) from a Millipore Milli-Q water purification system (Billerica, MA). Oligonucleotides purified by HPLC were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and listed in Table S1.

Table S1 Sequences of the DNA used in this study

Name	Sequence (5'→3')
HP 1	Biotin-*TAC AGT TCA TCT* TGA CCC ACA AGC GCG CTG AGG*G ATG AAC TGT AC*
Primer 1	AGA ACA CTT CTA CAG TTC
CP 1	GAA GTG TTC TAA AAA AAA A-Biotin
HP2	Biotin-*TACCCC ACC CCC* AGG ACG CCC CTT TCG CTG AGG A*GGG GTG GGG TA*A
Primer2	GTT AGG TAG ATA CCC CAC
CP2	TCT ACC TAA CAA AAA AAA A-Biotin
HP3	Biotin-*CAACCC ACC CCC* AAG ACG CCC CTT TCG CTG AGG A*GGG GTG GGT TG*A
Primer3	CTA TTG CTT GCA ACC CAC
CP3	CAA GCA ATA GAA AAA AAA A-Biotin
DNA1	GCG CTT GTG GGT CAA GAT GAA CT
DNA2	GCG CTT GTG G**C**T CAA GAT GAA CT

DNA3	GC**C** CTT GTG G**C**T CAA GAT GAA CT
A1	GAA AGG GGC GTC **C**TG GGG GTG GG
A2	GAA AGG GGC GTC **T**TG GGG GTG GG

The italicized, the underlined and the red regions of the hairpin-probe denote stem, target recognition component and NEase recognition site, respectively. The underlined regions of the primers denote tag sequences. The underlined regions of the capture probes (CP1, CP2, and CP3) denote anti-tag sequences, the green boldfaced letters denote variant positions. CP 1, CP 2 and CP 3 were dispensed on the test zone of the LFB.

1.3. Preparation of AuNPs, and SA-AuNP conjugates. AuNPs with an average diameter of 15±3.5 nm were prepared according to the reported methods with slight modifications[1]. All glassware used in this preparation was thoroughly cleaned in aqua regia (three parts HCl and one part HNO_3), rinsed in double distilled water, and oven dried prior to use. In a 500 mL, round-bottom flask, 100 mL of 0.01% $HAuCl_4$ in double distilled water were brought to a boil with vigorous stirring followed by the addition of 4.5 mL of 1% trisodium citrate. The solution turned deep blue within 20 s, and the final color changed to wine-red 60 s later. Boiling was pursued for an additional 10 min; the heating source was removed; and the colloid solution was stirred for another 15 min. The resulting AuNP solution was stored in dark bottles at 4°C and was used to prepare the SA-AuNP conjugates. The resulting solution of AuNPs was characterized by an absorption maximum at 520 nm.

For the preparation of the SA-AuNP conjugates, 1 mL of AuNP solution was first treated with 8 μL of 0.1 M of K_2CO_3 in order to adjust the pH of the solution to the isoelectric point (pI = 6.0) of SA, then the resulting solution was mixed with a 10 μL of solution containing 5 g L^{-1} SA and 5 g L^{-1} BSA. After stirring for 30 min, the conjugates were stored at 4 °C for use.

1.4. Preparation of test zone and control zone on nitrocellulose membrane. Briefly, 15 μL of 100-μM biotinylated capture probe were mixed with 15 μL of 1 g L^{-1} SA in PBS (pH 7.4), and the mixture was incubated for 2 h at room temperature. The formed

DNA-biotin-SA conjugates were dispensed onto the nitrocellulose membrane to form the test zone. For the multiplex detection of DNA in our work, two test zones were prepared with CP2 and CP3 at different locations on the nitrocellulose membrane. The control zone was prepared by dispensing concentrations of 1.0 g L^{-1} anti-SA antibody solutions. The distance between each zone was 3 mm. The membrane was then dried at room temperature for 12 h and stored at 4 °C in low humidity (30%).

1.5. Preparation of LFB. LFB was prepared according to the reported methods with slight modifications[1]. LFB consists of three components: sample pad, nitrocellulose membrane, and absorbent pad. The sample pad was made from glass fiber and saturated with a buffer (pH 8.0) containing 2% Triton X-100, 0.02 M Tris-HCl, and 1% BSA. Then, the pad was dried at room temperature. The prepared sample pad was stored in low humidity (30%) at room temperature.

At first, the nitrocellulose membrane, the prepared sample pad and the absorbent pad were attached along the long axis of a plastic adhesive backing after the protective sheet was peeled off, and each part overlapped 2-3 mm to ensure that the solution migrates through the strip during the assay. Then the capture probe and anti-SA antibody solutions were dispensed onto the nitrocellulose membrane using Biojet HM 3030 dispenser as 1.4 in ESI. At last, the plate assembled with sample and absorbent pads, as well as the modified nitrocellulose membrane was cut into 0.4-cm wide strips with cutting module ZQ 4200.

Visual detection of DNA was simply realized by observing the color intensity of the test zone of the LFB. The optical intensities of the test and the control zones were recorded simultaneously by using a strip reader, which is equipped with a digital camera and software. The software can search the red bands in a fixed reaction area automatically and then figure out parameters such as peak height and area integral.

1.6. NASDP amplified detection of nucleic acids. A 1 μM solution of the hairpin probe was prepared in the buffer [Tris-HCl (50-mM, pH 8.0) and 200 mM NaCl]. The

solution was incubated at 95 °C for 5 min and then allowed to slowly cool to room temperature over 30 min so that the probe correctly folded into a hairpin structure.

To detect nucleic acids using the LFB, different DNA samples (DNA1, DNA2, DNA3, A1 or A2) were added in the reaction solution [Tris-HCl (50 mM, pH 7.9), 50 nM probe, 50 nM primer, 2.5 U Klenow fragment *exo*$^-$, 5 U Nt.BbvC Ⅰ, 400 μM dNTPs, 0.1% BSA, 200 mM NaCl and 5 mM $MgCl_2$], and the final volume was 100 μL. The mixture was incubated at 37°C for 60 min. The resulting products were first mixed with SA-AuNPs and after 5 min applied on the sample pad of the biosensor along with 100-μL 4× SSC. 15 min later, the intensities of the red bands on the test and control zones were recorded by using the portable strip reader mentioned above.

2. Figures

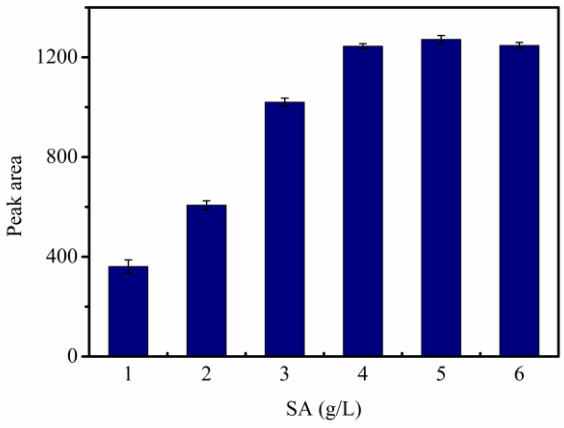

Figure S1. Effect of the amount of SA on the response of the LFB when 1 μM target DNA was used without NASDP amplification. Value represents the intensity mean ± s.d. of triplicate reactions at the indicated concentration of SA.

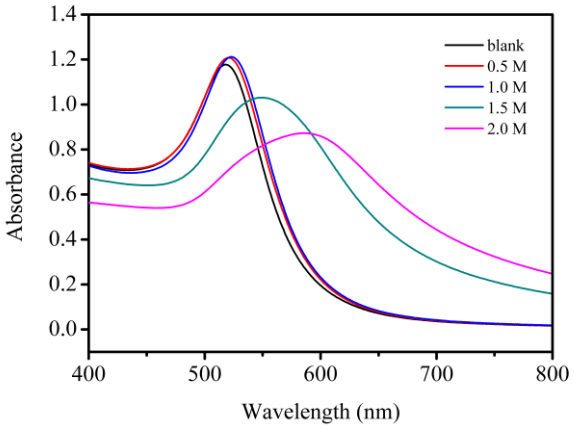

Figure S2. UV-vis absorption spectra of SA-AuNP conjugate upon treatment with various concentrations of Na^+.

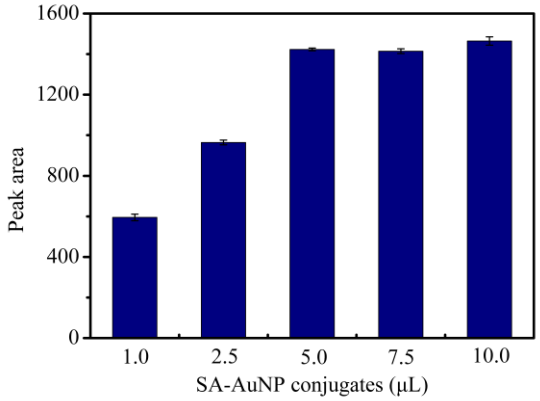

Figure S3. Effect of the amount of SA-AuNP conjugates on the response of the LFB when 1 μM target DNA was used without NASDP amplification. Value represents the intensity mean ± s.d. of triplicate reactions at the indicated amount of SA-AuNPs.

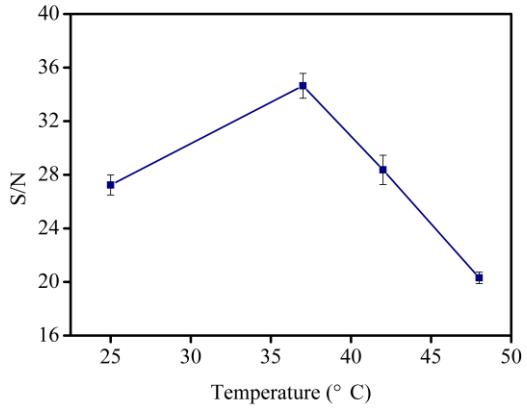

Figure S4. Effect of different NASDP temperatures on the LFB S/N ratio when 1 nM target DNA was used. Value represents the S/N mean ± s.d. of triplicate reactions at the indicated temperatures.

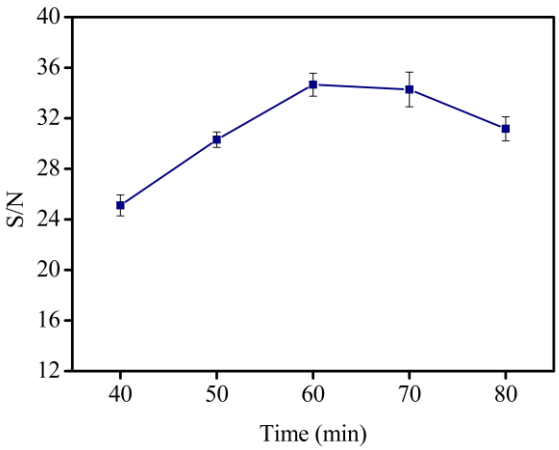

Figure S5. Effect of NASDP time on the LFB S/N ratio when 1 nM target DNA was used. NASDP was stopped by adding EDTA to a final concentration of 50 mM. Value represents the S/N mean ± s.d. of triplicate reactions at the indicated time intervals.

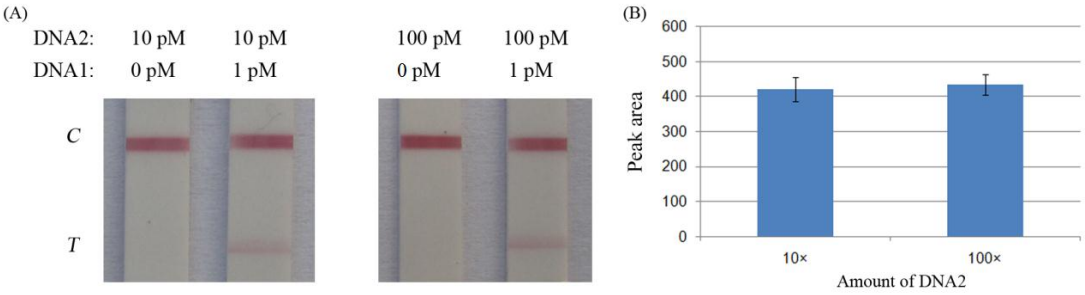

Figure S6. Performance of the developed LFB analyzing the mixed DNA samples in which mismatched DNA2 is in excess of matched DNA1. (A) Photo images of the LFBs for mixed DNA samples with different quantities of matched DNA1 and mismatched DNA2. (B) LFB responses (peak area) when 1 pM of target DNA was mixed with 10 × and 100× excess of the mismatched DNA2. Value represents the S/N mean ± s.d. of triplicate reactions at the indicated time intervals.

3. References

1 X. Mao, Y. Ma, A. Zhang, L. Zhang, L. Zeng and G. Liu, *Anal. Chem.*, 2009, **81**, 1660.