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Supporting information

Molecular Beacon-Functionalized Gold Nanoparticles as Probes in Dry-Reagent Strip Biosensor for DNA Analysis

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Experimental Section

Instruments: Airjet AJQ 3000 dispenser, Biojet BJQ 3000 dispenser, Clamshell Laminator and the Guillotine cutting module CM 4000 were from Biodot LTD (Irvine, CA). Portable strip reader DT1030 was purchased from Shanghai Goldbio Tech. Co., LTD (Shanghai, China).

Reagents and Materials: Streptavidin from *Streptomyces avidinii*, $HAuCl_4$, sucrose, hydroxylamine, Tween 20, dithiothreitol (DTT), Triton X-100, trisodium citrate, bovine serum albumin (BSA) and sodium chloride-sodium citrate (SSC) Buffer 20× concentrate (pH 7.0), phosphate buffer saline (PBS, PH 7.4, 0.01 M) were purchased from Sigma-Aldrich. Glass fibers (GFCP000800), cellulose fiber sample pads (CFSP001700), laminated cards (HF000MC100) and nitrocellulose membranes (HFB18004 and HFB 24004) were purchased from Millipore (Billerica, MA). Human plasma samples were purchased from Golden West Biologicals (Temecula, CA). All chemicals used in this study were analytical reagent grade. All other solutions were prepared with ultrapure (>18 MΩ) water from a Millipore Milli-Q water purification system (Billerica, MA).

DNA oligonucleotides were obtained from Integrated DNA Technologies, Inc. (Coralville, IA) and had the following sequence (From 5′ to 3′):

MB: *(C6Thiol)-ACACGCTCATCATAACCTTCAGCAAGCTTT AAC TCATAGTGAGCGTGT-(biotin)*

Complementary target DNA: *ACGCTCACTATGAGTTAAA G C TTGCTGAAGGTTATGA*

Non-complementary DNA: *ATGGCATCGCTTAGCTGCCAGTA C ACTGATTGAAGACATCATAGTGCAGACAAGCATATC*

Control DNA probe *:(Biotin)-ACGCTCACTATGAGTTAAAGC*

MB-2*: (C6Thiol)-ACACGCTCATCAAGCTTTAA CTCATA GT GA GCGTGT-(biotin)*

Complementary target DNA for MB-2 (T2)*:ACGCTCACTATG AGTTAAAGCTTG*

One mismatch target DNA for MB-2 (T2-M): *ACGCT****G****ACTATG AGTTAAAG CTTG*

Noncomplementary DNA for MB-2: *ATGGCATCGCTTAGCTG CCA GTAC ACTGATTGAAGACATCATAG TGCA G ACAAGCA TATC*

Control DNA probe for MB-2:(Biotin)-*ACGCTCACTATGA GTTAAAG CT TGCTGAAG*

Preparation of Au-NP-MB-biotin probes Au-NPs with average diameter 15 nm ± 3.5 nm were prepared according to the reported method with slight modifications.[25]A molecular beacon modified with with a thiol at its 5′ end and a biotin at its 3′ end was used for conjugation with Au-NPs. Before conjugation reaction, the thiolated MB was activated by the following procedure: 98 μL of thiolated MB (1.0 OD) was mixed with 2 μL of triethylamine and 7.7 mg of DTT to react for 30 min at room temperature (RT), then the excess DTT was removed by four times extract with 400 μL of ethylacetate solution. Conjugation reactions were carried out by adding the activated MB to 1 mL of the 5 fold concentrated Au-NP solution. After standing at 4

^{o}C for 24 h, the solution was subjected to "aging" by the addition of NaCl up to a concentration of 150 mM, and certain quantity of 1 % SDS was added to reach a final concentration of 0.01 %. The solution was allowed to stand for another 24 h at 4 ^{o}C, and the excess of reagents was removed by centrifugation for 20 minutes at 12000 rpm. The supernatant was discarded, and the red pellet was redispersed in 1 mL of buffer containing 20 mM Na_3PO_4, 5% BSA, 0.25% Tween and 10% sucrose.

Preparation of DSNAB The DSNAB consists of four components: sample pad, conjugate pad, nitrocellulose membrane and absorbent pad. All of the components were mounted on a common backing layer (typically an inert plastic, e.g., polyester) using the Clamshell Laminator (Biodot, CA, USA). The sample pad (17 mm × 30 cm) was made from glass fiber (CFSP001700, Millipore) and saturated with a buffer (pH 8.0) containing 0.25% Triton X-100, 0.05 M Tris-HCl and 0.15 M NaCl. Then it was dried and stored in a desiccator at RT. The conjugate pad (8 mm× 30 cm) was prepared by dispensing a desired volume of Au-NP-MB-Biotin conjugate solution onto the glass fiber pad with the dispenser Airjet AJQ 3000, and then drying it at RT. The pad was stored in a desiccator at 4 °C. Nitrocellulose membrane (25 mm × 30 cm) was used to immobilize streptavidin and control DNA probes at different zones to form test zone and control zone, respectively. To facilitate the immobilization of control DNA probes on the nitrocellulose membrane, streptavidin was used to react with the biotinylated control DNA probes to form the streptavidin-biotin DNA conjugates. Briefly, 60 μL of 1 mM biotinylated DNA probes and 140 μL of PBS were added to 300 μL of 1.67 mg/mL streptavidin solution, and the mixture was incubated 1 h at RT. The excess DNA probes were removed by centrifugation for 20 min with a centrifugal filter (cut off 30000, Millipore) at 6000 rpm. The conjugates were washed three times with 500 μL of PBS in the same centrifugal filter. Finally, 500 μL of PBS was added into the

remaining solution in the filter. The conjugates were then dispensed on the nitrocellulose membrane with the dispenser Biojet BJQ 3000. The distance between the test zone and control zone is around 2 mm. The membrane was then dried at RT for 1 h and stored at 4 °C in a dry state. Finally**, t**he sample pad, conjugate pad, nitrocellulose membrane, absorption pad were assembled on a plastic adhesive backing (60 mm × 30 cm) using the clamshell laminator. Each part overlaps 2 mm to ensure the solution migrating through the strip during the assay. Strips with a 4-mm width were cut by using the Guillotin cutting module CM 4000.

Sample test procedure The procedure of DSNAB test is following: One hundred twenty microliters of sample solution containing a desired concentration of target DNA in 4×SSC (1 % BSA) buffer was applied to the sample application zone. After waiting for a desired time (for example, 6 min), another 50 μL 4×SSC (1 % BSA) buffer was applied to wash the biosensor. The bands were visualized within 6 min. For quantitative measurements, the strip was inserted into the strip reader DT1030, the optical intensity of the test line and control line could be recorded simultaneously. In the case of the detection of DNA in plasma sample, 5 μL of human plasma spiked with DNA was first applied to the biosensor, and then the biosensor was dipped into a centrifuge tube containing 175 μL of 4×SSC (1 % BSA) buffer. After 15 min, the strip was read with the strip reader.

Table. 1 Analytical performance comparisons with other methods

Method/Ref	Probe	LOD	Assay time	Differentiating Mismatches	Detector
*LFNA/ 12	Au-NP-DNA	1 nM	~30 min	No	Scanner
LFNA/13	Au NP-DNA	500 pM	~30 min	Yes, require PCR	Scanner
Colorimetric/11	Au NP-DNA	0.5 nM	10 min	Yes,Temperature control	UV-Vis
ECL/4	Au NP-MB	50 aM	> 1 h	No	ECL
FL /5	Au NP-MB	0.2 nM	30 min	Yes	FL
FL/16	MB probe	10 nM	16 h	no	FL
DSNAB	Au NP-MB	50 pM	15 min	Yes,direct test	Portable reader

*LFNA: Lateral flow nucleic acid test; ECL: Electrochemical-luminescence; FL: Fluorescence