Supporting Information

Lateral Flow Nucleic Acid Biosensor for Cu^{2+} Detection in Aqueous Solution with High Sensitivity and Selectivity

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Experimental

Preparation of Au-nanoparticles (Au-NPs)-DNA probe 1 conjugates

Au-NPs with an average diameter of 15 nm ± 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 (3 parts HCl and 1 part HNO_3), rinsed in doubly distilled water, and oven-dried prior to use. In a 500-mL, round-bottom flask, 100 mL of 0.01% $HAuCl_4$ in doubly distilled water were brought to 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 after 60 s. 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 Au-NP solution was stored in a dark bottle at 4 °C and was used to prepare the Au-NP-DNA probe 1 conjugate.

Preparation of Au-NPs-DNA probe 1 conjugate

Briefly, 100 μL of ultra-pure water (18.2 MΩ/cm, Millipore, Billerica, MA, USA) containing 1 OD of thiolated DNA probe 1 (SH-C_6-GTGATAGCTGGTAAG, Shanghai Sangon Biological Engineering Technology, Shanghai, China) was added to 1 mL of Au-NP solution (4 times concentrated). After standing at 4 ^oC for 24 h, the solution was subjected to "aging" by the addition of NaCl up to a concentration of 150 mM, and a 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 ^oC, and the excess of reagent was removed by centrifugation for 20 min at 12000 rpm. The supernatant was discarded, and the red pellet was redispersed in 1 mL of buffer (pH 7.4) containing 20 mM Na_3PO_4, 5% BSA, 0.25% Tween, and 10% sucrose. The conjugate solution was dispensed onto a conjugate pad (Millipore) and dried at room temperature; then, it was stored in a refrigerator at 4 ^oC in low humidity (30%) until use.

Immobilization of DNA probe 2 and DNA probe 3 on nitrocellulose membrane

Biotinylated DNA probes (probes 2 and 3) were purchased from Shanghai Sangon Biological Engineering Technology. Briefly, 15 μL of 100 μM biotylated 15-mer DNA probe were mixed with 15 μL of 1 mg/mL streptavidin(SA) in PBS (pH 7.4), and the mixture was incubated for 2 h at room temperature. The formed DNA-biotin-streptavdin complexes were dispensed onto the nitrocellulose membrane (Shantou ealon membrane, 25 mm×30 cm, Capillary rate: 140±40 s, thickness: 145±20 μm) to form test and control zones. The distance

between the test zone and the control zone was 3 mm. The membrane was then dried at room temperature for 12 h and stored at 4 °C in low humidity (30%). The probe on the test line (5'-biotin-C_3-TCTCAACTCGTAGCA) hybridized specifically to the 3'-end of the cleaved substrate. The probe on the control line (5'-biotin-C_3-CTTACCAGCTATCAC) was complementary to the DNA probe 1 on Au-NPs.

Construction of lateral flow nucleic acid biosensors (LFNAB)

The sample pad was made from glass fiber microglass paper (Millipore) pre-soaked in pre-treatment buffer (pH 9.0, 4% Triton, 1% BSA, 2% glucose, 2% PEG-4000, 100 mM boric acid, 0.1% SDS, 50 nM of competitive DNA probe (AGCTACGAGTTGAGA), and 0.5 μg/mL of salmon sperm DNA), and dried at room temperature. The prepared sample pad was stored in low humidity (30%) at room temperature.

The nitrocellulose membrane, sample pad, conjugate pad, and absorbent pad were attached along the long axis of the plate after the protective sheet was peeled off. The overlap for each pad was 2~3 mm. All pads were attached to a plastic adhesive backing and cut into 0.4-cm-wide strips with a paper cutter.

Preparation of working solutions

DNAzyme and excess substrate were mixed at mole ratio of 1:5 in 4× saline-sodium citrate (4×SSC) , and then annealed by heating at 70 ˚C for 2 min and slowly cooling to room temperature for 1 h. For each test, 10 μl of DNAzyme-substrate solution were diluted into 90 μl 4×SSC with a final concentration of 100 nM substrate and 500 nM DNAzyme as working solution.

Sample measurement

Copper ions with various concentrations were dissolved in ultra-pure water (18.2 MΩ/cm) from a Milli-Q water purification system. For its promotion on the cleavage, ascorbate was added to each reaction at a final concentration of 50 μM in the system.[2] The working solution was mixed with ion solution at a volume ratio of 1:1, providing the final substrate and DNAzyme concentration of 50 nM and 250 nM, respectively. The reaction mixture was applied to the sample pad after a 30-min incubation. The LFNABs were scanned when all liquid migrated to the absorption pad after 20 min. All tests were performed at room temperature. The metal salts used included $MgCl_2$, $CaCl_2$, $Mn(OAc)_2$, $FeCl_2$, $FeCl_3$ $CuCl_2$, $ZnCl_2$, $Cd(ClO_4)_2$, $Pb(NO_3)_2$, $Hg(NO_3)_2$, $Co(NO_3)_2$ and $Ni(NO_3)_2$.

For quantitative detection, a portable strip reader (Shanghai Goldbio Technology) was used to record the intensities of the test and control zones of the LFNABs.

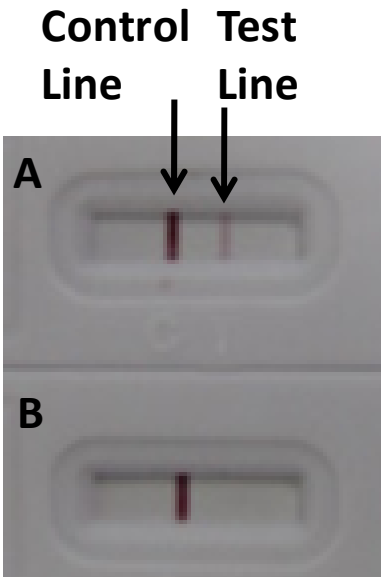

Fig. S1. Typical photo images of the LFNAB in the absence (A) and presence of 50 nM competitive DNA (B)on the sample pad. Sample solution: 0 μM Cu^{2+} (control); working solution: 4× SSC.

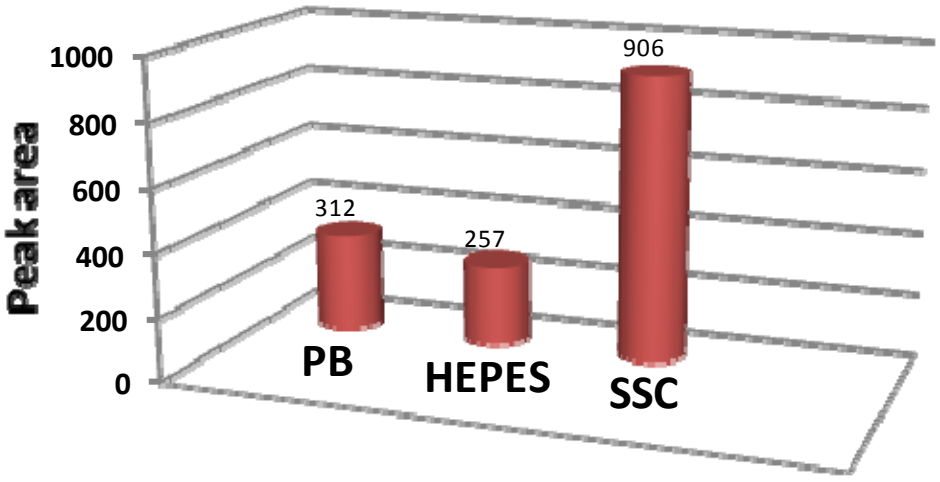

Fig. S2. Responses of 20 μM of Cu^{2+} on the LFNAB with different working buffers. PB: phosphate buffer (0.01M, pH 7.4); HEPES buffer (0.1M, pH 7.4), and SSC: saline-sodium citrate (4×, pH 7.4). The peak area was recorded with a portable strip reader.

References

1. X. Mao, Y. Ma, A. Zhang, L. Zhang, L., Zeng, G. Liu, *Anal. Chem*., 2009, **81**, 1660-1668.

2. J. Liu, Y. Lu, *J. Am. Chem. Soc.*, 2007, **129**(32), 9838-9839.