Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information

An autonomous T-rich DNA machine based lateral flow biosensor for amplified visual detection of mercury ions

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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.BbvC I were 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')
DNA machine	TGT ACA TAC GAT GAT CGC TGG CTG AGG CCC CAG ATT CTT TCT TCC CTT GTT TGT TTC TGG GGC
CP1	5'-SH-*TTTTTTTTTT*- TGTACATACG
CP2	ATGATCGCTG -*TTTTTTTTTT*-Biotin-3'
CP3	5'-Biotin-*TTTTTTTTTT*-CGTATGTACA

The blue-colored, the red-colored and the underlined regions of the DNA machine denote the domain 1, 2, and 3, respectively. The italicized regions of CP1, CP2, and CP3 denote DNA spacer for eliminating the steric hinderence. CP 1 was used for the modification of gold nanoparticles, CP 2 and CP 3 were dispensed on the LFB to from test and control zones, respectively.

1.3. Preparation of AuNPs, and CP1-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 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 CP1-AuNP conjugates, 2 OD of 5'-thiolated CP1 was added to 1 mL of 4-fold concentrated AuNP solution and the resulting mixture was shaken gently at 4°C for 24 h. The CP1-coated AuNPs were subjected to "aging" by adding 150 µL of 1 M NaCl, then kept at 4°C overnight. The particles were centrifuged (10,000 rpm, 25 min) and rinsed three times with rinsing buffer (20 mM Na_3PO_4, 5% BSA, 0.25% Tween-20, 10% sucrose, and 0.1% NaN_3) to remove any unbound DNA. The red pellet was re-suspended in 1 mL of rinsing buffer and then stored in a refrigerator at 4 °C until use.

1.4. Preparation of test zone and control zone on nitrocellulose membrane. Briefly, 15 µL of 100 µM biotinylated CP2 and CP3 were mixed respectively with 15 µL of 1 g L^{-1} SA in PBS (pH 7.4), and the two mixtures were incubated for 2 h at room temperature. The formed CP2-biotin-SA and CP3-biotin-SA conjugates were dispensed onto the nitrocellulose membrane to form the test zone and control zone, respectively. 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 four components: sample pad, conjugated 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. The conjugate pad was made from glass fiber and dispensed with the conjugate solution (1.3 of ESI). Then the treated pad was dried at room temperature. The prepared conjugate pad was stored in low humidity (30%) at room temperature until use.

At first, the nitrocellulose membrane, the sample pad, conjugate 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 CP2-biotin-SA and CP3-biotin-SA conjugates were dispensed onto the nitrocellulose membrane using Biojet HM 3030 dispenser. 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 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. Visual amplified detection of Hg^{2+}. For each test, sample solution was incubated with DNA machine (50 nM) in a solution (NaAc (50 mM), $Mg(Ac)_2$ (10 mM), dNTPs (0.4 mM), Tris-acetate (20 mM), pH 7.9). To eliminate possible hybridization between probes, the resulting solution was heated at 95°C for 1 min and then cooled down to room temperature before adding enzymes. Next, the enzyme mixture of klenow fragment polymerase (8 units), Nt.BbvCⅠ(10 units) was added into the above solution to a final volume of 50 μL and incubate at 42°C for 1 h. After incubation, the resulting mixture was mixed well with 10 μL of 4× SSC, and then applied to the

sample pad of the LFB. 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 and Tables

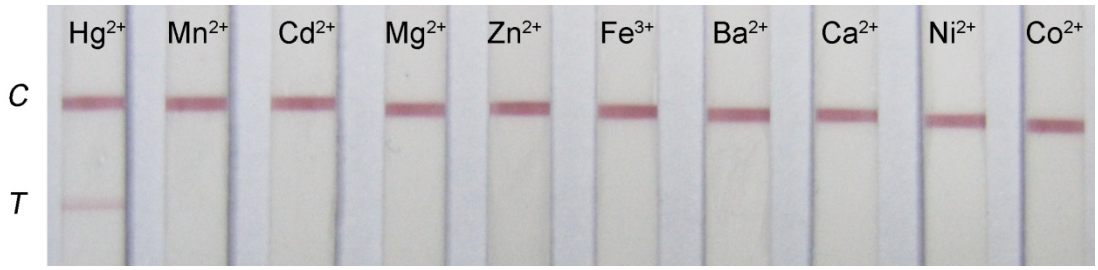


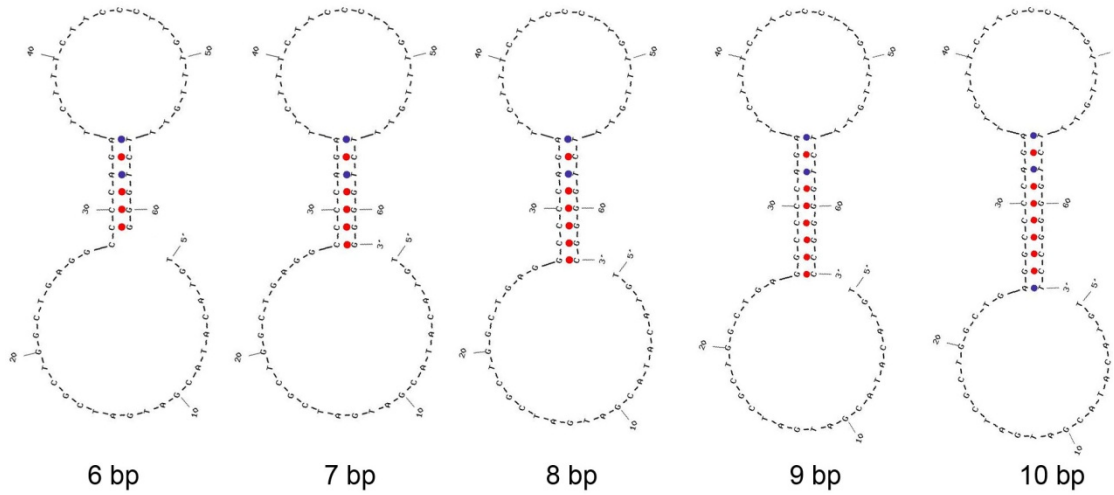
Figure S1. Photo images of the LFBs for specificity analysis using different cations of 2 μM, the concentration of Hg^{2+} was 30 nM.

Table S2 Measurements of Hg^{2+} spiked in river water

River water	Hg^{2+} spiked (nM)	Hg^{2+} measured (nM)[a]	Recovery (%)
1	10	9.13 ± 0.72	91.3%
2	30	32.8 ± 0.59	109.3%
3	50	55.4 ± 0.32	110.8%

[a]Average of five measurements±standard deviation.

3. The primed-template structures investigated in this work



4. References

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