Electronic Supplementary Information (ESI)

Enzyme-free amplified DNA assay: five order of linearity provided by metal stable isotope detection

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Experimental section

Reagents and materials. Streptavidin, bovine serum albumin (BSA) and DNA oligonucleotides were purchased from Sangon Inc. (Shanghai, China). All the DNA sequences are listed in Tab.S1. All oligonucleotides were dissolved in deionized water to obtain stock solutions of 100 μM and stored at -20 ℃. Carboxyl-terminated magnetic beads (Affimag SLC 3222, 2-3μm, 10 mg/mL, SiO_2 matrix) were purchased from Baseline Chromtech Research Centre (Tianjin, China). The magnetic separator was bought from Invitrogen Co. Chloroauric acid hydrate ($HAuCl_4 \cdot 4H_2O$) and Tris-(hydroxymethyl) methyl amino methane were purchased from Aladdin Bio-Chem Technology Co. Ltd. (Shanghai, China). 1-(3-Dimethylaminopropyl)-3-Ethylcarbodiimide hydrochloride (EDC) and imidazole was purchased from Beijing Solarbio Science and Technology Co. Ltd. (Beijing, China). Hydrochloric acid, nitric acid, and trisodium citrate were purchased from Changzheng Chemical Reagent Co. Ltd. (Chengdu, China). Deionized water (DIW) was used throughout this study, and all reagents were at least analytical grade. Hybridization buffer: 20 mM Tris-HCl, pH=8.0, 0.5 M NaCl. Washing buffer: 7 mM Tris-HCl, pH=8.0, 0.17 M NaCl, 0.05% Tween 20.

Table S1. Sequences of the oligonucleotide used in this work

Name	Sequences (3'−5')
Capture DNA	NH_2- A_{20} TGACGATCTCTAAAAGGTGTGACT
Target DNA	AGGGAGTCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCA
Aux1	biotin- TTTTT GATTTTCCCAGACTCCCTCCGATTAGACGTTAGCCG TTTTT -biotin
Aux2	biotin- TTTTT AGGGAGTCTGGGAAAATCCGGCTAACGTCTAATCGG TTTTT -biotin
M1	AGGGAGTCTGGGAAAATCAGTCACACCTTTTAGAGATCATCA
M2	AGGGAGTCTGGGAAAATCAGTCACACCTTTTATAGATCATCA

NC	ACCCTCCGATTCGGGCAACACGAGTTTGGGCAGCTTGGATGC

The underlined letters represent the target recognition sequences; the letters in the same color represent the complementary sequences; the orange represent the mismatched base sequence.

For an HCR amplified DNA quantitative assay, 3'-end amine-functionalized capture DNA was designed to capture target DNA in the analytical sample, when it was immobilized on the MBs. Aux1 and Aux2 was the biotinylated amplifying DNA probes, using for HCR amplification and connecting with SA-AuNPs. M1, M2, NC were designed for specific investigation, they are one based mismatched, two based mismatched and completely mismatched DNA sequences respectively compared to target DNA.

An ICPMS (ELAN DRC-e, Perkin Elmer, Inc., Shelton, CT, USA) equipped with alumina sample injector tube and quartz torch was used to quantify 197A, the operating parameters as shown in Tab. S2. AMS-100 thermostatic mixer (Allsheng Instrument Co. Ltd, Hangzhou, China) was used for the hybridization of DNA. A TGL-16S high-speed freezing centrifuge (Sichuan Shuke Instrument Co. Ltd, Sichuan, China) was used for AuNPs separation.

Table S2. ICP-MS instrumental operating parameters

Parameters	**Values**
RF power	1175 W
Auxiliary gas flow	15 L min^{-1}
Auxiliary gas flow	1.2 L min^{-1}
Resolution	0.7 amu
Dwell time	30 ms
Dead time	50 ns
Isotope monitored	^{197}Au

Preparation of AuNPs. All glassware used in this experimentation was dipped in aqua regia for 24 h, thoroughly cleaned with distilled H_2O and oven-dried prior to use. The solution of $HAuCl_4$ and trisodium citrate were filtered through a 0.45 μm filter membrane prior to use. 100 mL 0.01% $HAuCl_4 \cdot 4H_2O$ was heated to boiling and then 1 mL 1% (m/v) trisodium citrate was added in to the solution quickly. After boiled for 30 minutes, the colloidal suspension was cooled down and stored at 4 ^oC.

Preparation of AuNPs-SA. AuNPs-SA was prepared according to literatures[1] with slight modification. 1 mL of pH-adjusted AuNPs suspension (pH=6.4, adjusted by 0.1 M K_2CO_3) was added to 20 μL of 1 mg /mL streptavidin, and then incubated at room temperature for 30 min, the solution was further added to 10% BSA to give a final concentration of 1% BSA, followed by incubated for 5 min. The resulted solution was centrifuged at 13000 rpm for 30 min. The red soft sediment was dispersed with hybridization buffer containing 2% BSA, and stored at 4 ^oC.

Preparation of capture DNA labeled MBs. The capture DNA was combined with MBs according to the protocol reported by Miao et al.[2]. 48 μL of 10 mg mL^{-1} carboxyl-modified MBs was transferred into a 2 mL centrifuge tube. The MBs were washed three times with 100 μL of 0.1 M imidazole-HCl buffer (pH=7.0), suspended to a final volume of 500 μL in the same buffer solution containing 0.1 M EDC, and incubated at 37 °C for 30 min. Then 400 pmol of capture DNA was added and incubated for 2 h. The MBs-captured probe was washed three times with washing buffer, and incubated with 10% BSA for 1 h to minimize the nonspecific adsorption. The resulting capture DNA labeled MBs were magnetically separated, washed, resuspended in the hybridization buffer, and stored at 4 °C for further use.

The assay procedures of detecting target DNA. The HCR process was carried out according to literature with slight modifications [3, 4]. First, a desired amount of target DNA (100 μL) was added into centrifuge tubes and incubated for 1 h at 37 °C; Second, the hybridized conjugates were washed three times with washing buffer, added by 100 μL of 0.5 μM Aux1 and incubated for 1 h; Third, 100 μL solution of 1 μM Aux1 and 1 μM Aux2 in hybridization buffer solution was added, and incubated for 120 min at 37 °C; Fourth, the modified beads were washed thoroughly with washing buffer to remove unhybridized oligonucleotides, followed by adding AuNPs-SA and incubating at 37 °C for 40 min; Fifth, the bioconjugates were washed and resuspended in 100 μL water, heated to 95 °C for 20 min, and then magnetically separated. The supernatants were transferred into a 2 mL centrifuge tube. Subsequently, 100 μL aqua regia was added in to the supernatants to dissolve the AuNPs. Finally, the signal of ^{197}Au isotope was recorded by ICPMS for target DNA quantification.

Supporting Figures

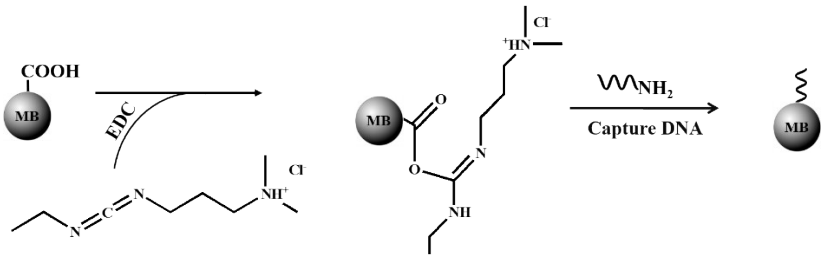

Fig. S1 The bioconjugation of MBs and capture DNA. The carboxyl-modified magnetic beads were activated by EDC firstly, then coupled with amine-functionalized DNA capture probe.

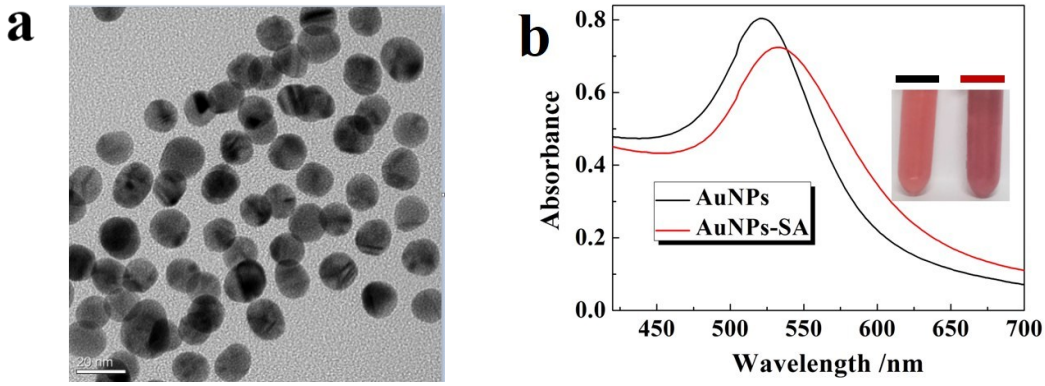

Fig. S2 Characterization of the AuNPs-SA. (a) TEM image of AuNPs-SA, and (b) UV absorption spectra and photo (inset) of AuNPs before and after binding with streptavidin.

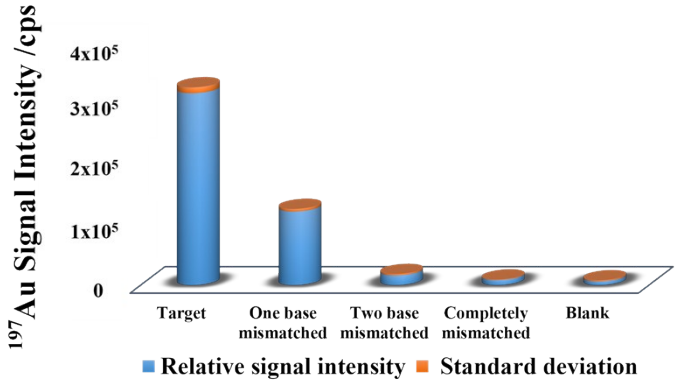

Fig. S3 The specificity toward mismatches sequences. The concentration of each sequence is 10 pM. (n=3)

References

1. G. H. Yao, R. P. Liang, X. D. Yu, C. F. Huang, L. Zhang and J. D. Qiu, *Anal. Chem.*, 2015, **87**, 929-936.
2. J. Miao, Z. Cao, Y. Zhou, A. Choiwan Lau and J. Lu, *Anal. Chem.*, 2008, **80**, 1606-1613.
3. Z. Xu, H. Yin, Y. Han, Y. Zhou and S. Ai, *Anal. Chim. Acta*, 2014, **829**, 9-14.
4. X. Wang, L. Ge, Y. Yu, S. Dong and F. Li, *Sens. Actuat.B-Chem*, 2015, **220**, 942-948.