Electronic Supplementary Material (ESI) for ChemComm.
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Electronic Supplementary Information

Cascade signal amplification using Hg^{2+}-induced oxidation of silver nanoparticles and cation exchange reaction for ICP-MS bioassay

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Experimental section

Reagents and Chemicals

Deionized water (DIW, 18.2 MΩ cm) was obtained from a water purification system (PCUJ-10, Sichuan Ultrapure Technology Co., Ltd, Chengdu, China). All oligonucleotides (Table S1) used in this work were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). High purity trisodium citrate, $NaBH_4$, $AgNO_3$, $CuCl_2$, Na_2S, starch and HNO_3 were obtained from Chron Chemicals (Chengdu, China). $Hg(NO_3)_2$ was ordered from Lixiang Mercury Industry Co., Ltd (Guizhou, China). Myoglobin (MYO) and mucin were acquired from Diamond (Shanghai, China). Hemoglobin (HEMO) was ordered from Sigma Aldrich (Shanghai, China). Immunoglobulin G (IgG), transferrin (TRF) and human serum albumin (HSA) were obtained from Bioss Biotechnology Co., Ltd (Beijing, China). CEA was purchased from Cell to Antibody & Antigen Biotech. Co., Ltd (Zhengzhou, China). Human serum samples were provided by the West China Hospital, Sichuan University (Chengdu, China). All working solutions were prepared with HEPES buffer (10 mM, pH 7.4).

Table S1 Sequences of oligonucleotides used in this work

Name	**Sequence (5'-3')**
Probe1-DNA	TTTTTTTACCACATCATCCATATAACTGAAAGCCAATTTTTT
Target DNA (HBV-DNA)	TTGGCTTTCAGTTATATGGATGATGTGGTA
Single-base mismatched	TTGGCTTTCAGTTATATTGATGATGTGGTA
Two-base mismatched	TTGGCTTTCTGTTATATGGATCATGTGGTA
Probe2-CEA	CTTTTTTATACCAGCTTATTCAATTTTTTTTG

Instruments

An iCAP Q inductively coupled plasma mass spectrometer (Thermo Fisher Scientific Inc., Germany) was used for detection of ^{63}Cu throughout this work, with the operational parameters listed in Table S2. Ultraviolet visible absorption spectra were recorded on a UH-5300 UV-vis spectrophotometer (Hitachi, Japan). TEM characterization of Ag NPs and CuS NPs was performed with a Tecnai G2 F20 S-TWIN transmission electron microscope at an accelerating voltage of 200 kV (FEI Co., USA). X-ray diffraction (XRD) patterns were recorded with an X-ray diffractometer (EMPYREAN, Panalytical Inc., Netherlands). The X-ray photoelectron spectra (XPS) was collected with an AXIS Ultra DLD spectrometer (Kratos, UK). Zeta potentials were determined using a Zetasizer Nano ZS (Malvern Instruments, UK). Ultracentrifuge filters (3 and 100 kDa cutoff, 0.5 mL) were purchased from Merck Millipore (Darmstadt, Germany). A H1750R-model centrifuge (Cence Instrument Co., Ltd, Changsha, China) was used for the pretreatment of serum samples. Nylon 66 and polyethersulfone filtration membranes (0.22 μm) were purchased from Tianjin Jinteng Experiment Equipment Co., Ltd (Tianjin, China).

Table S2 Operating parameters of ICP-MS

Parameters	Value
RF power	1548 W
Detector voltage	1520 V
Dwell time	0.01 s
Nebulizer gas flow	1.0 L min^{-1}
Auxiliary gas flow	0.8 L min^{-1}
Cool gas flow	14 L min^{-1}
Isotope monitored	^{63}Cu

Synthesis

Synthesis of Ag NPs. Ag NPs were prepared based on chemical reduction method according to the literature.[1] Briefly, 5 mL of 1.25 mM silver nitrate was added dropwise to the chilled mixture of 2.0 mM sodium borohydride (15 mL, used as a reducing agent) and 2.5 mM trisodium citrate (2 mL, used as a stabilizing agent) under vigorous stirring at room temperature. The solution turned from pale yellow to bright yellow as silver nitrate was dropped in. After stirring for 30 min, the synthesis was ceased and the prepared Ag NPs were isolated from the mixture by ultracentrifugation (3 kDa cutoff). The precipitate was redispersed in DIW and stored for use in this work.

Synthesis of CuS NPs. CuS NPs were prepared according to the literature with slight modification.[2] Briefly, 4 g of starch was added to 500 mL DIW which was heated to 70-80°C under stirring for 15 min, to prepare a transparent starch solution. Then, 1.344 g $CuCl_2$ and 0.78 g Na_2S were dissolved separately in 200 mL of hot starch solution. The resultant $CuCl_2$ and Na_2S solutions were maintained at 70°C under vigorous stirring for 5 min. Thereafter, the hot Na_2S-starch solution was added dropwise to the hot $CuCl_2$-starch solution under vigorous stirring. The mixture was allowed to react for 30 min at 70°C and then cooled to room temperature. The formed dark green product was collected by centrifugation, washed three times with DIW and dried at 60 °C for 7 h. Finally, the as-prepared CuS NPs were resuspended in DIW for further characterization and use in this work.

Analytical procedure

For the detection of HBV DNA and CEA, 40 μL of 1 μM probe1-DNA (or probe2-CEA) was mixed with 48 μL of 5 μM Hg^{2+} in 200 μL of HEPES buffer (10 mM, pH 7.4) at room temperature for 1.5 h to form the T-Hg-T hairpin structure. Then, different concentrations of target HBV DNA or CEA (50 μL) as well as 32 μL of Ag NPs solution were added to the mixture, the reaction lasted for 45 min to accomplish the oxidation of Ag NPs. Then, 50 μL of CuS NPs solution was added to initiate the cation exchange reaction. After reacting for 15 min at room temperature, the mixture was passed through a Nylon 66 microfiltration membrane (0.22 μm) to separate CuS NPs and ionic Cu^{2+}, 50 μL of the filtrate was diluted to 5 mL with 2% (v/v) HNO_3 followed by ICP-MS detection of the Cu^{2+} ions.

Pretreatment of human serum samples

Human serum samples were donated by the West China Hospital of Sichuan University. 400 μL of the serum sample was firstly placed into an ultrafiltration tube (0.5 mL, 100 kDa cutoff) and then centrifuged at 8000 rpm for 10 min. The filtrate was quantitatively transferred to a clean centrifuge tube before a further 100-fold dilution with pure water.

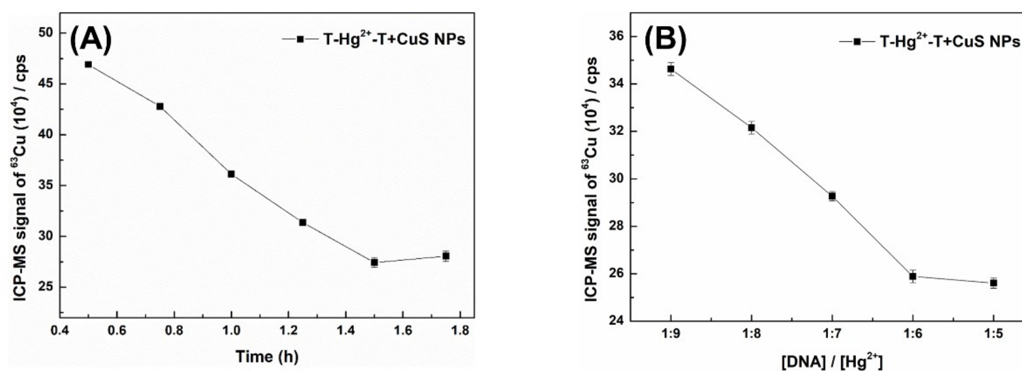

Fig. S1 Optimization of the formation of T-Hg^{2+}-T hairpin. Effect of (A) incubation time and (B) molar ratios of probe1-DNA and Hg^{2+}. CuS NPs were added to indicate the residual Hg^{2+} ions in solution.

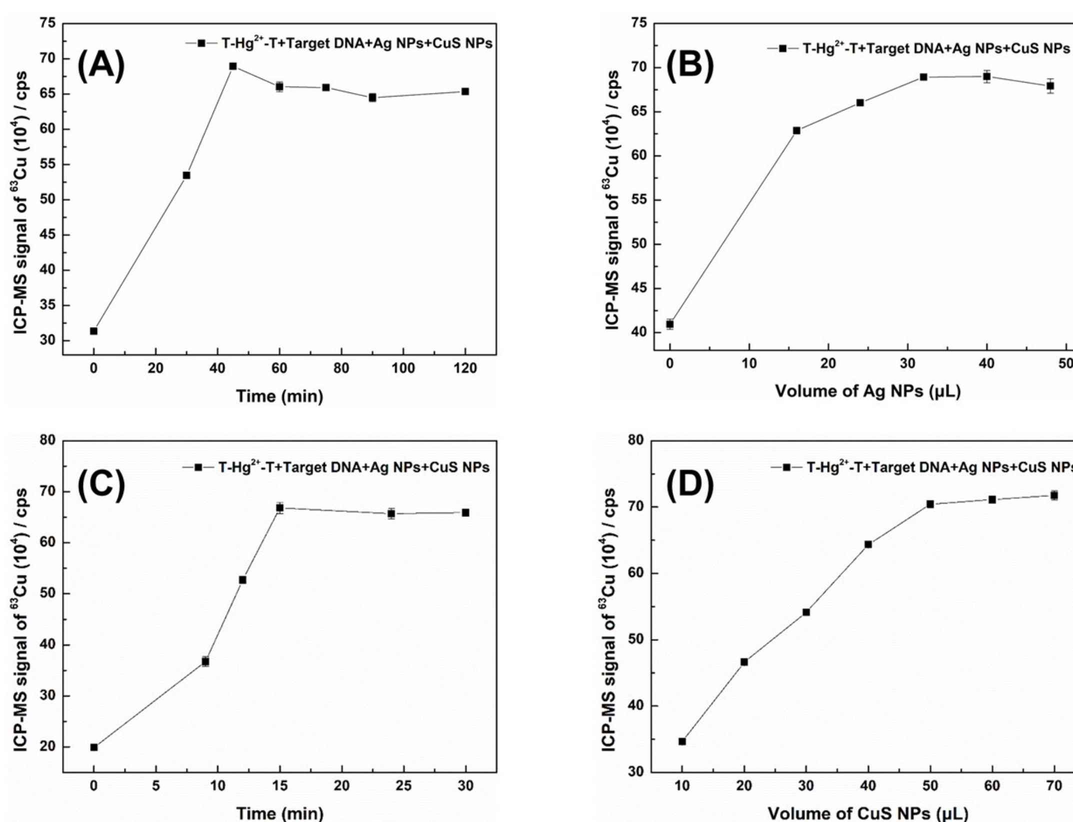

Fig. S2 Optimization of the cascade reaction conditions. Effect of (A) incubation time and (B) dosage of Ag NPs on the reaction between Hg^{2+} and Ag NPs. Effect of (C) incubation time and (D) dosage of CuS NPs on the reaction between Ag^{+} and CuS NPs. The concentration of HBV DNA was remained at 10 pM and the total volume of the reaction solution was fixed at 370 μL so that the optimization of the dosage of Ag NPs and CuS NPs was carried out by adding different volume of our prepared Ag NPs and CuS NPs solutions.

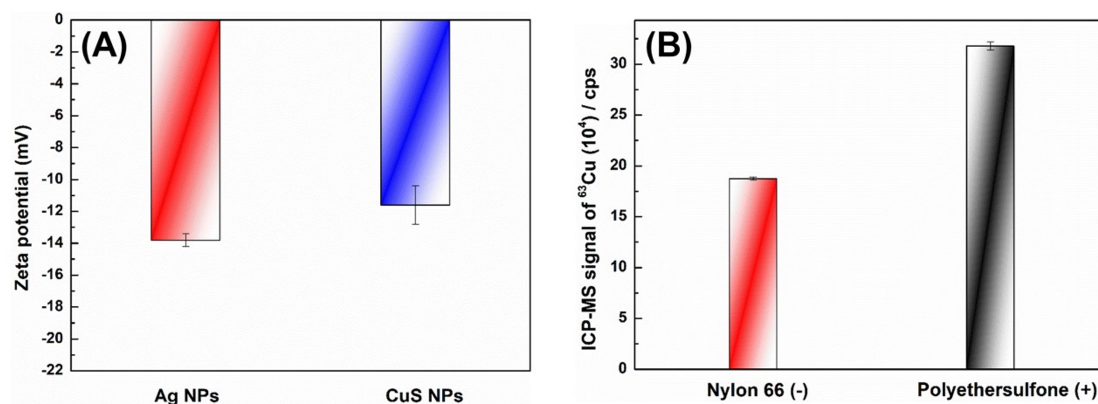

Fig. S3 (A) Zeta-potentials of the prepared Ag NPs and CuS NPs. (B) ICP-MS signal of CuS NPs filtered by different filtration membranes.

Table S3 Comparison of LODs with other methods for the detection of nucleic acids

Method	Signal amplification strategy	LOD	Reference
Colorimetric	Rolling circle amplification (RCA)	3.3 pM	3
Fluorescent	Entropy-driven catalysis (EDC)+ catalytic hairpin assembly (CHA)	15.6 fM	4
Fluorescent	Exonuclease III-assisted signal amplification	15 pM	5
Fluorescent	Strand displacement reaction (SDR)+ cation exchange reaction (CER)	25 fM	6
SP-ICPMS	Hybridization chain reaction (HCR)	3 fM	7
ICP-MS	Double-strand DNA templated Cu NPs	4 pM	8
ICP-MS	MNAzyme amplification	11-20 pM	9
ICP-MS	Silver nanoparticles and cation exchange reaction cascade signal amplification	16 fM	This work

Table S4 Comparison of LODs with other methods for the detection of CEA

Method	Assay strategy	LOD	Reference
Colorimetric	Salt-induced AuNPs aggregation	3 ng mL^{-1}	10
Fluorescent	Upconversion fluorescence resonance energy transfer	10.7 pg mL^{-1}	11
Electrochemiluminescence	Ratiometric ECL based on single luminophor	34.58 fg mL^{-1}	12
SP-ICPMS	$RecJ_f$ exonuclease-assisted target recycling amplification+HCR+DNA-templated Au nanoclusters	0.5 pg mL^{-1}	13
SP-ICPMS	ZnSe quantum dots as labels	0.006 ng mL^{-1}	14
ICP-MS	Lanthanide nanoparticles as labels	9.2 pg mL^{-1}	15
ICP-MS	Silver nanoparticles and cation exchange reaction cascade signal amplification	26 fg mL^{-1}	This work

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