Supporting Information

SERS-based immunoassay using core-shell nanotags and magnetic separation for rapid and sensitive detection of cTnI

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Experimental

Screening of cTnI Antibody Pairs

The high sensitivity of SERS immunoassay depends on the specific binding between antigen and antibody. Therefore, Colloidal gold immunochromatography was used to screen capture and tracer antibodies. Briefly, captured antibodies were immobilized on the detection line (T-line) of NC film by a desktop dot film gold spraying machine, and 5 μg tracer antibodies were labeled on gold nanoparticles by electrostatic adsorption. 60 μL 10 ng/mL cTnI bovine serum sample was mixed with 5 μL gold-labeled antibody and dripped on the sample pad on one side of the test strip. The strength of T-line was observed by naked eyes. As shown in Table S1, except for the capture antibody (Hytest 20C6) and the tracer antibody (Hytest 7B9) pairs of antibodies, the other antibody pairs did not have an immune response, and on the immunochromatographic strip, the detection line did not show or all showed positive.So Hytest 20C6 was chosen as capture antibody and Hytest 7B9 as tracer antibody in subsequent experiments.

Preparation of streptavidin (SA) magnetic beads

Preparation of γ-Fe₂0₃ magnetic nanoparticles. Ferric urea complexes were prepared as precursors, and $\gamma\text{-Fe}_2O_3$ was obtained by thermal decomposition.The specific steps are as follows: Ferric urea complexes were prepared by the reaction of $Fe(NO_3)_3 \cdot 9H_2O$ with urea at a molar ratio of 1:6.2. Firstly, under magnetic stirring, appropriate amount of $Fe(NO_3)_3 \cdot 9H_2O$ was dissolved in proper amount of absolute ethanol, then urea (CON_2H_4) was added and stirred for 2.5 h. The obtained light green powder was washed with anhydrous ethanol and dried in an oven to obtain iron urea. $\gamma\text{-Fe}_2O_3$ magnetic nanoparticles were prepared by heating iron urea to 200°C for 1 h.

Preparation of γ-Fe203@SiO2. Core-shell structured $\gamma\text{-Fe}_2O_3@SiO_2$ magnetic nanomaterials are prepared by reverse microemulsion system. Briefly, cyclohexane, n-hexanol and TritonX-100 were mixed evenly at a ratio of 5:2:1, and appropriate amount of $\gamma\text{-Fe}_2O_3$ nanoparticles were added, which were dispersed into the microemulsion system by ultrasound, then the upper liquid was poured out and stirred for 30 min. Then 1 mL of concentrated NH3 H2O and 3 mL of ethyl orthosilicate were added and stirred at 40°C for 3 h. Separate with magnet and wash with absolute ethanol three times, and finally calcine at high temperature (400~700°C) for 3 h, and $\gamma\text{-Fe}_2O_3@SiO_2$ magnetic nanoparticles were successfully synthesized.

Preparation of SA magnetic beads. Firstly, surface functionalization of magnetic particles was carried out: appropriate amount of prepared $\gamma\text{-Fe}_2O_3@SiO_2$ was added to a certain amount of mixture of methanol and glycerol, ultrasonic for 30~60 min, then appropriate amount of N-aminoethyl-γ-aminopropyl trimethoxysilane was added, and ultrasonic treatment was continued for 10~20 min, so that the solution was mixed evenly, reacted at 90°C for 2 h, washed with methanol several times, and the particles were collected after vacuum drying. Next, the functionalized

magnetic particles were encapsulated with SA: phosphate buffer (0.1 mol/L) was prepared with deribonuclease water, 5 mg of functionalized magnetic particles were added to 1 mL phosphate solution, dispersed by ultrasound for 5 min, then 100 μL SA solution was added, shaking reaction at room temperature for 24 h, then 1 mL glutaraldehyde was added, and cultured for 2 h. Wash 4~5 times with sterilized phosphate buffer, using 1 mL each time, and finally disperse the particles in 1 mL phosphate solution, and store at 4°C.
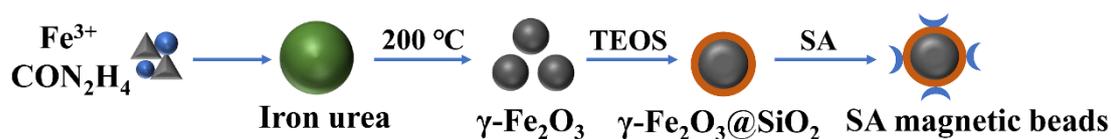
Supplementary data
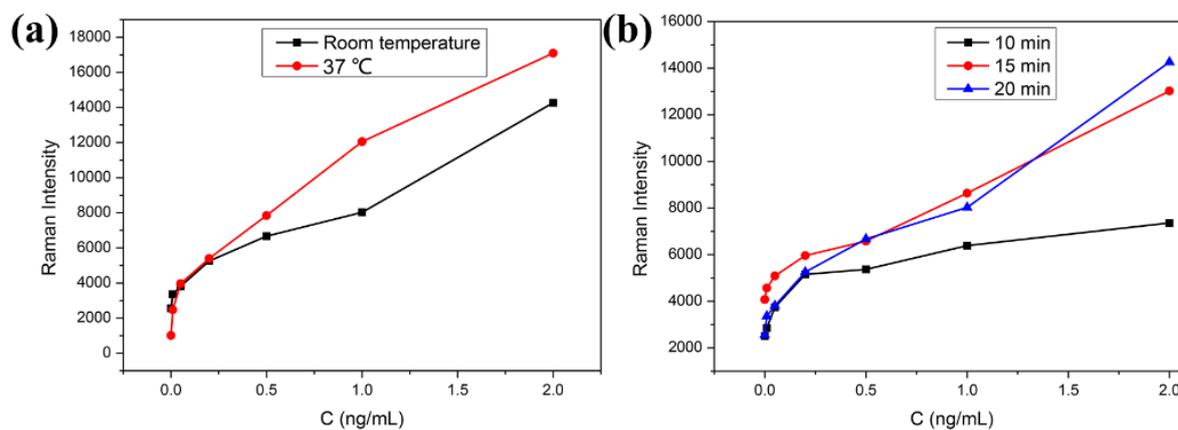

Fig.S1 Preparation process of SA magnetic beads

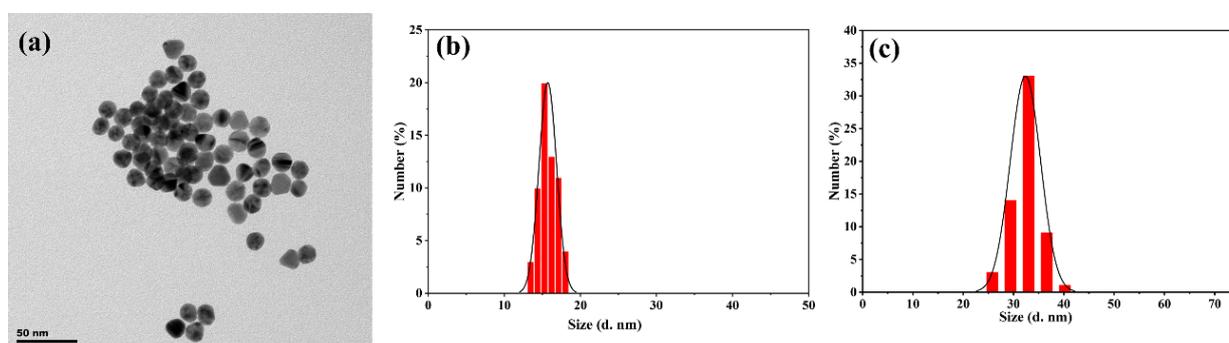Fig.S2 Raman intensity at different incubation (a) temperature and (b) time

Fig.S3 (a,b) TEM images and particle size distribution of AuNPs. (c)Particle size distribution of Au-4MBA@Ag
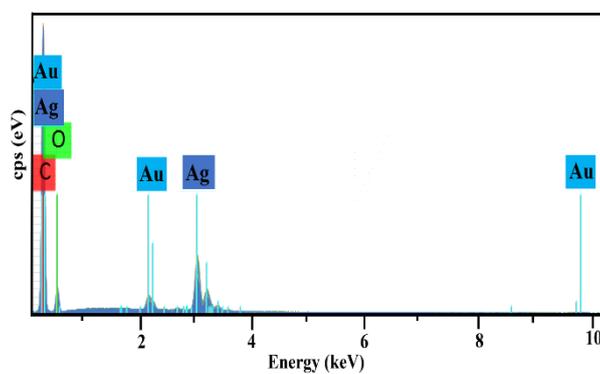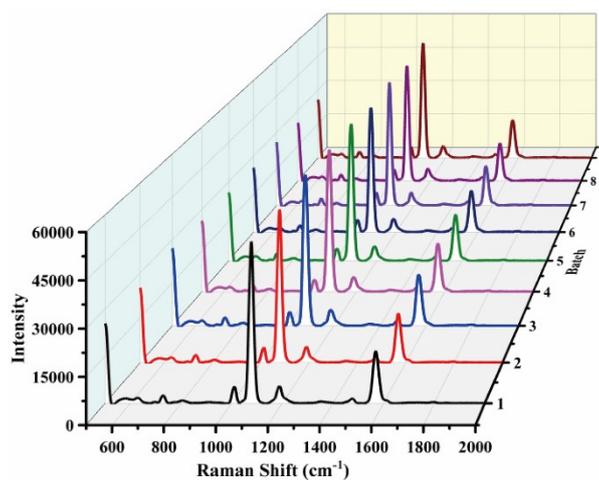

Fig.S4 EDS spectrum of Au-4MBA@Ag.
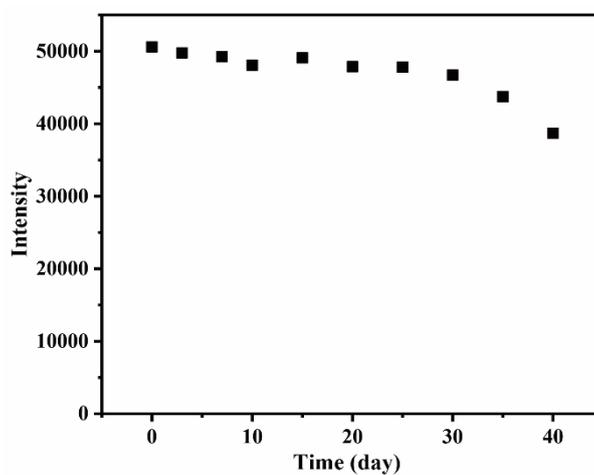
Fig.S5 Reproducibility of Au-4MBA@Ag core shell nanotags.

Fig.S6 Stability of Au-4MBA@Ag core shell nanotags.

Table S1 Screening of antibody pairs

Capture antibody	Tracer antibody	Result
Medix 7E2	Hytest 7B9	No
	Medix 2C7	No
	Hytest 19C7	No
Hytest 20C6	Hytest 7B9	Yes
	Medix 7E2	No
	Hytest 19C7	No
Hytest 19C7	Hytest 7B9	No
	Medix 7E2	No
	Medix 2C7	No
Medix 2C7	Hytest 7B9	No
	Medix 7E2	No
	Hytest 19C7	No

Table S2 Spike recovery experiment (n= 3)

	Theoretical value (ng/mL)	Raman Intensity	C of detection (ng/mL)	Recovery rate (%)
C 1	1.09	9177±318	1.0066±0.0589	92.35
C 2	0.91	7947±628	0.7910±0.1052	87.02

Table S3 Interference experiment (n= 3)

Interference	C (ng/mL)	Raman Intensity	C of detection (ng/mL)	Recovery rate(%)
Bilirubin	0.05	1946±498	0.0537±0.0319	102.60
	0.50	5259±243	0.3877±0.0317	85.42
	2.00	14084±2525	2.0892±0.6388	103.11
Hemoglobin	0.05	1689±178	0.0466±0.0115	93.20
	0.50	5923±595	0.5867±0.0647	117.34
	2.00	13618±1434	1.9579±0.3439	97.89
Triglyceride	0.05	1962±408	0.0606±0.0100	121.60
	0.50	5531±505	0.6242±0.0668	84.92
	2.00	12558±981	1.7057±0.2234	85.28
Heparin sodium	0.05	1912±277	0.0498±0.0180	99.60
	0.50	5838±460	0.4662±0.0648	93.24
	2.00	12752±2984	1.7830±0.7169	89.15
EDTA	0.05	1836±221	0.0446±0.0140	89.20
	0.50	5917±278	0.4766±0.0389	95.32
	2.00	12504±1552	1.6995±0.3411	84.98
Trisodium citrate	0.05	1841±236	0.0489±0.0081	97.80
	0.50	5732±188	0.4508±0.0258	90.16
	2.00	13142±1984	1.8527±0.4623	92.46