Supporting Information

Perovskite-gold Shell Composite Nanoprobe for Trimodal Lateral Flow Immunoassay Detection of SARS-CoV-2 Antibodies

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Experimental Methods

Materials

Chloroauric acid ($HAuCl_4 \cdot 3H_2O$, 99%), silver nitrate ($AgNO_3$, 99%), sodium acetate (99%), ethanol (99%), sodium borohydride ($NaBH_4$, 99%), Tween-20 (chemically pure) were purchased from Sinopharm Chemical Reagent Co., Ltd.; Oleic acid (OA, 90%), oleyl amine (OAm, 80-90%), lead bromide ($PbBr_2$, 99%), N,N-dimethylformamide (DMF, 99.8%), polyethyleneimine (PEI, 99%) were purchased from Beijing Energy Technology Co., Ltd.; Trisodium citrate (TSC, 99%) was purchased from Shanghai Hushi Chemical Reagent Co., Ltd.; Cesium bromide (CsBr, analytical grade) was purchased from 3A Chemicals Ltd.; Tetramethyl orthosilicate (TMOS, 98%) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd.; Bovine serum albumin (BSA, ≥98%), polyvinylpyrrolidone (PVP) were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. All chemical reagents were of analytical grade, and ultrapure water (resistivity 18.25 MΩ·cm) was used for experiments.

SARS-CoV-2 N protein was provided by Jiangnan University; Mouse anti-SARS-CoV-2 N protein IgG antibody, goat anti-human IgG antibody, recombinant SARS-CoV-2 N protein IgG antibody were purchased from Sino Biological Inc.; COVID19-PS-Mab15 mouse anti-human N protein antibody was purchased from Fapon Biotech Inc.

Characterizations

Transmission electron microscope (JEM-1400, JEOL Ltd.), UV-Vis spectrophotometer (UV-2450, Shimadzu Corporation), fluorescence spectrophotometer (F-7000, Hitachi High-Tech Corporation), high-speed centrifuge (TG16-WS, Hunan Xiangyi Laboratory Instrument Development Co., Ltd.), dispenser/cutter for lateral flow assays (HGS510, Hangzhou Feng Hang Technology Co., Ltd.), infrared thermal imager (FOTRIC 226s, FOTRIC Smart Technology (Shanghai) Co., Ltd.), and 808 nm near-infrared laser (FU808AD2000-F34, Shenzhen Fulei Technology Co., Ltd.), etc.

Synthesis of Au_{shell}

First, silver nanoparticles (Ag NPs) were prepared as templates. In a 100 mL three-neck flask containing 75 mL ultrapure water and 20 mL of 1% TSC aqueous solution, the mixture was heated in a 70 °C water bath with stirring at 800 rpm for 15 min. Then, 1.7 mL of 1% $AgNO_3$ solution was added, followed by the rapid injection of 2 mL of freshly prepared 0.1% $NaBH_4$ solution in ice water. The reaction was allowed to proceed for 1 h to obtain the Ag seed solution. Then, 20 mL of this seed solution was added to a 250 mL three-neck flask containing 150 mL ultrapure water and 4 mL of 1% TSC. Under boiling conditions, 3.4 mL of 1% $AgNO_3$ solution was added sequentially. This process was repeated three times with a 1 h interval each time, ultimately obtaining Ag NPs colloid. The colloid was aged under 254 nm UV light for 30 min before use.

5 mL of the above Ag NPs colloid was centrifuged at 8000 rpm for 15 min and redispersed in 5 mL ultrapure water. It was mixed with 45 mL of 1 mg/mL PVP solution and heated to boiling under reflux. Then, 1 mM $HAuCl_4$ solution was slowly

added at a rate of 1 mL/min. When 4 mL of $HAuCl_4$ had been added, the system color turned blue, and the absorption peak red-shifted to 720 nm. The reaction was allowed to proceed for 12 min, then immediately cooled in an ice bath to terminate the reaction. The mixture was centrifuged at 12000 rpm for 15 min, washed once, and finally redispersed in 50 mL ultrapure water. The product was stored at 4 °C.

Synthesis of $CsPbBr_3@SiO_2$

$CsPbBr_3@SiO_2$ was synthesized using a one-pot method. 10 mL DMF, 0.6 mL OAm (melted at 80 °C) and 1.8 mL OA were added and mixed evenly, followed by preheating in a 90 °C oil bath. Then, 0.1468 g $PbBr_2$ and 0.0851 g CsBr were quickly added and stirred at 90 °C, 300 rpm for 1.5 h to obtain the precursor solution. Then, 2 mL of this precursor solution was taken, mixed with 40 μL of 2.8% ammonia water, and set aside as solution A.

Into a 60 mL glass bottle, 20 mL toluene and 10 μL TMOS were added, and preheated in a 50 °C oil bath. Under stirring at 1500 rpm, 0.4 mL of solution A was quickly added. The solution immediately turned bright yellow. After 10 s, the speed was reduced to 150 rpm and the reaction was allowed to proceed for 2 h until yellow precipitate was observed. The product was collected by centrifugation at 9000 rpm for 5 min. It was washed twice with anhydrous ethanol (9000 rpm, 5 min), then washed once with ultrapure water (12000 rpm, 15 min). Finally, the product was dispersed in 40 mL ultrapure water.

Preparation of $CsPbBr_3@SiO_2\text{-}Au_{shell}$ Composite Material

6 mL of $CsPbBr_3@SiO_2$ aqueous solution was placed in a 50 mL centrifuge tube,

mixed with 10 mL ultrapure water, and subjected to vigorous sonication for 3 min. Then, 3 mL of 1 mg/mL PEI aqueous solution was added, and sonication was continued for 20 min to allow PEI coating on the particle surface for charge reversal. The mixture was centrifuged at 12500 rpm for 20 min, and the supernatant was discarded. Centrifugal washing with ultrapure water was repeated three times to remove excess PEI, and the product was finally redispersed in 10 mL ultrapure water. This product was labeled as $CsPbBr_3@SiO_2@PEI$.

To 10 mL of $CsPbBr_3@SiO_2@PEI$ solution, 4.5 mL of the above-prepared Au_{shell} colloid (volume ratio $V(Au_{shell}) : V(CsPbBr_3@SiO_2) = 0.75: 1$) was added, followed by vigorous sonication for 45 min to achieve uniform loading of Au_{shell}. The mixture was centrifuged at 12000 rpm for 15 min, washed twice, and redispersed in 2 mL ultrapure water to obtain the $CsPbBr_3@SiO_2$-Au_{shell} composite material.

Preparation of the Trimodal Probe

2 mL of the $CsPbBr_3@SiO_2$-Au_{shell} composite material was taken, and its pH was adjusted to 8.5 using 0.1 mol/L potassium carbonate solution, followed by sonication for 5 min for mixing. SARS-CoV-2 N protein was added to a final concentration of 30 μg/mL, and the mixture was incubated at 25 °C, 220 rpm in a shaker for 40 min to complete coupling. The mixture was centrifuged at 10000 rpm for 15 min, the supernatant was discarded, and the precipitate was resuspended in PBS buffer (0.01 mol/L, pH 7.4) containing 5% BSA. Blocking was performed at 25 °C, 150 rpm for 15 min, followed by blocking overnight at 4 °C. After another centrifugation step, the probe was resuspended in 1 mL of resuspension buffer (0.01 mol/L PBS, pH 7.4,

containing 1% BSA) and stored for later use.

Preparation of the Immunochromatographic Test Strips

The test strip consisted of a sample pad, conjugate pad, nitrocellulose (NC) membrane, absorbent pad, and PVC backing plate. The sample pad was immersed in treatment buffer (20 mmol/L Tris-HCl, pH 8.0, containing 2% BSA, 0.5% Tween-20, 1% PVP 8000) for 10 min, then dried under vacuum at 37 °C for 2 h before use. Using the dispenser, 1 mg/mL goat anti-human IgG antibody (test line, T line) and 1 mg/mL mouse anti-N protein antibody COVID19-PS-Mab15 (control line, C line) were sprayed onto the NC membrane at a rate of 1 μL/cm. The membrane was dried overnight at 37 °C. Then, the sample pad, conjugate pad, NC membrane, and absorbent pad were sequentially overlapped and pasted onto the PVC backing plate with a 2 mm overlap between components. Finally, the strips were cut into 3 mm width using a cutter and stored at room temperature in a desiccator.

Detection Procedure and Signal Readout

For detection, 10 μL of resuspension buffer (0.01 mol/L PBS, pH 7.4, containing 0.4% Tween-20, 3% BSA), 20 μL of serum sample, and 20 μL of the prepared composite probe solution were sequentially added into a microplate well. The microplate was incubated in a shaker (25 °C, 600 rpm) for 15 min. Then, the sample pad end of the test strip was inserted into the well, allowing the solution to chromatograph by capillary action for 15 min. After completion, the results were read via three modes: Colorimetric detection: The color change on the T line was directly observed under natural light. Fluorescence detection: The green fluorescence intensity on the T line was observed under a 365 nm UV lamp. Photothermal detection: The T line area was irradiated with an 808 nm near-infrared laser at a power density of 2.2 W/cm^2 for 120 s. The temperature-time curve was recorded using an infrared thermal imager, and quantitative detection was performed by analyzing the temperature change (ΔT).

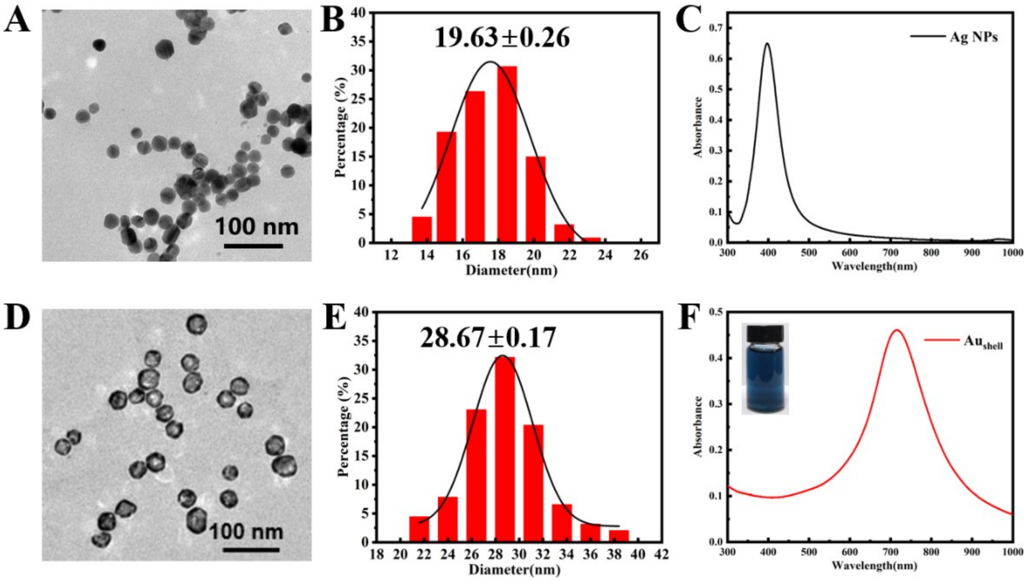

Fig. S1 TEM image (A), particle size distribution histogram (B) and UV-Vis absorption spectrum of Ag NPs (C); TEM image (D), particle size distribution histogram (E) and UV-Vis absorption spectrum of Au$_{shell}$ (inset: photographs of Au$_{shell}$ under daylight illumination) (F).

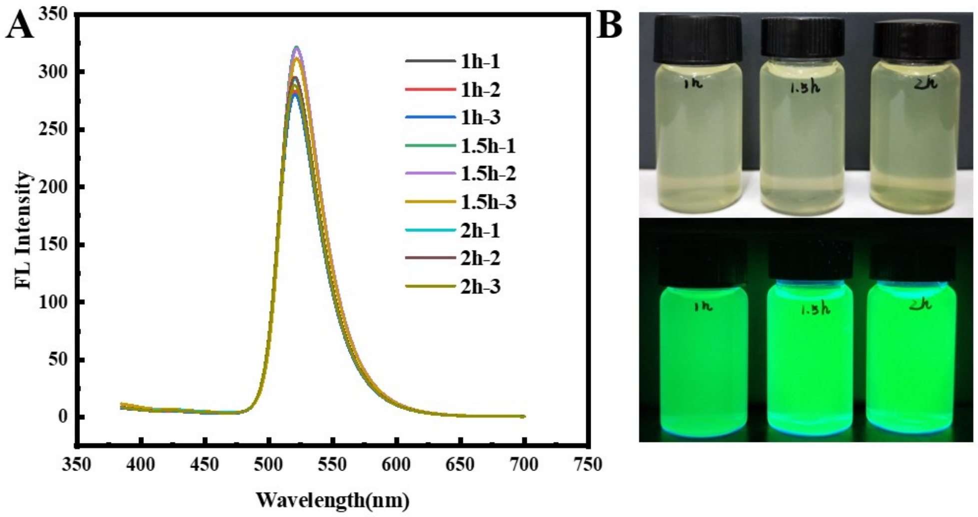

Fig. S2 Comparison of fluorescence intensity of $CsPbBr_3@SiO_2$ synthesized with different precursor reaction times (A); Photographs of $CsPbBr_3@SiO_2$ dispersions in water under daylight and 365 nm excitation for different reaction times (B).

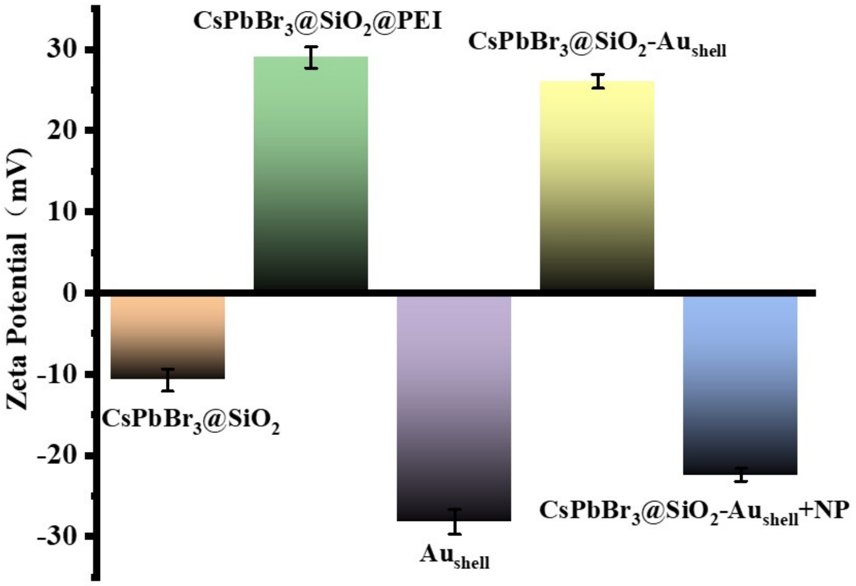

Fig. S3 Zeta potential changes during material assembly and probe preparation.

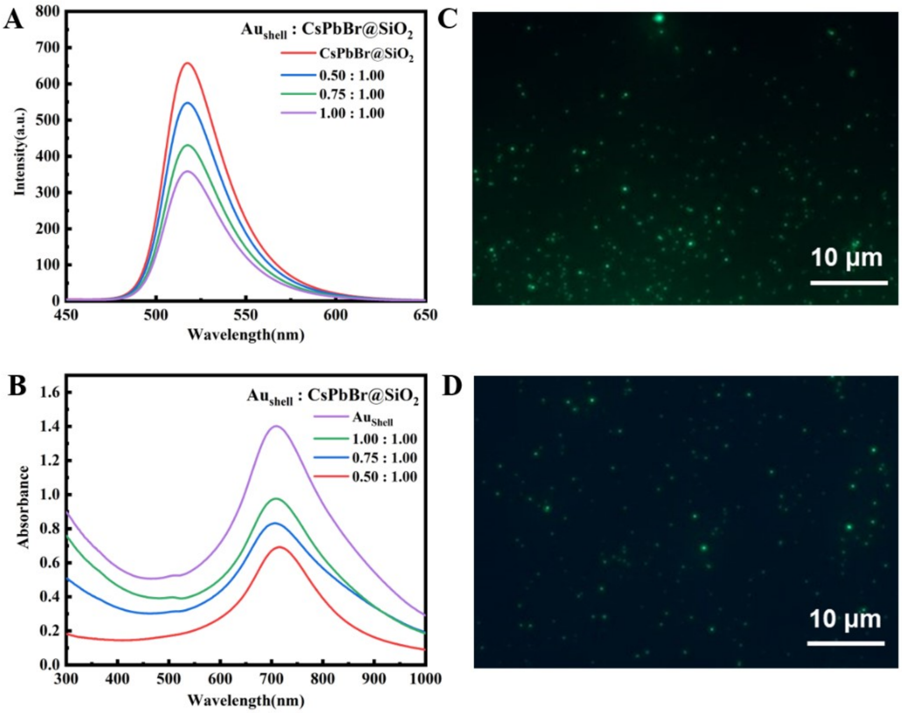

Fig. S4 Comparison of fluorescence emission peaks (A) and UV-Vis absorption (B) of $CsPbBr_3@SiO_2$-Au_{shell} prepared with different volume ratios $V(Au_{shell})$:$V(CsPbBr_3@SiO_2)$; Fluorescence microscopy images of $CsPbBr_3@SiO_2$ (C) and $CsPbBr_3@SiO_2$-Au_{shell} (D).

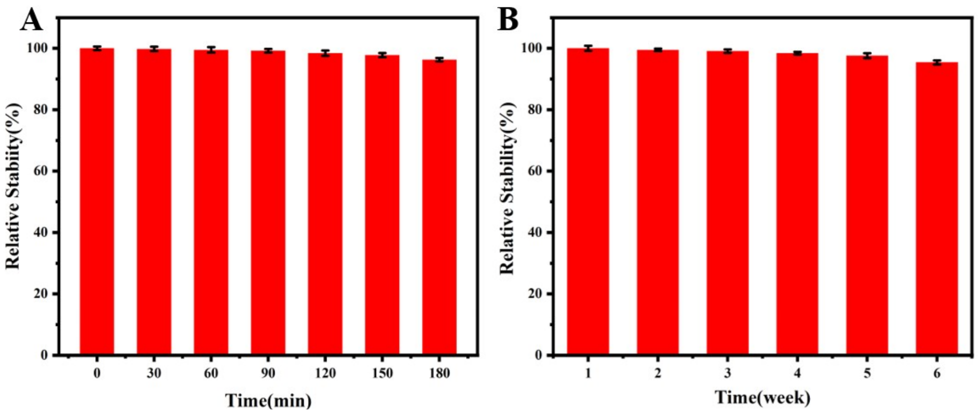

Fig. S5 Photostability of the $CsPbBr_3@SiO_2\text{-}Au_{shell}$ under continuous 365 nm UV irradiation over 180 min (A). Storage stability of the $CsPbBr_3@SiO_2\text{-}Au_{shell}$ at 4 ℃ for 6 weeks (B).

Table S1 Comparison of analytical performance of different LFIA systems for SARS-CoV-2 antibody detection.

Sample	Detection mode	Number	LODs (ng/mL)	Ref.
$ReSe_2$	Photothermal	1	0.86	1
AuNP-PEG-Cy3	Fluorescent	1	1	2
RBD/AuNPs	Colorimetric	1	45.1	3
PEI-Ru/$dSiO_2$	Electrochemiluminescence	1	0.52	4
Microspheres	Colorimetric	1	0.27	5
Au-Ag HNSs	Colorimetric	3	200	6
	Photothermal		20	
	SERS		20	
Fe_3O_4@MoS_2@Pt	Colorimetric	3	80	7
	Catalytic-colorimetric		20	
	Photothermal		10/8	
SQF@ZIF-8/Pt	Colorimetric	3	2.5	8
	Fluorometric		0.5	
	Catalytic		0.1	
$CsPbBr_3$@SiO_2-Au_{shell}	Colorimetric	3	40	This work
	Fluorescent		4	
	Photothermal		0.4	

Reference

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