SUPPORTING INFORMATION

Photoelectrochemical bioanalysis of microRNA on yolk-in-shell Au@CdS based on catalytic hairpin assembly-mediated CRISPR-Cas12a system

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EXPERIMENTAL SECTION

Material and Reagent. Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), tetraethyl orthosilicate (TEOS), (3-aminopropyl) triethoxysilane (APTES), hydrogen peroxide (H_2O_2), polyvinylpyrrolidone (PVP; MW = 10000), sodium hydroxide, ammonia ($NH_3 \cdot H_2O$, 28%),4-chloro-1-naphthol, potassium chloride, sodium chloride, sodium sulfate, sodium citrate, ascorbic acid, 4-(N-maleimidomethyl) cyclohexane-1-carboxylic acid 3-sulfo-N-hydroxysuccinimide ester sodium salt (Sulfo-SMCC), 3,3',5,5'-tetramethylbenzidine (TMB), tris(2-carboxyethy)phosphine hydrochloride (TCEP), and cadmium chloride were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Engen® Lba Cas12a and the corresponding 10 × NEBuffer 2.1 were purchased from New England Biolabs Inc. (Ipswich, MA).Horseradish peroxidase, all oligonucleotides used in this work were synthesized and high-performance liquid chromatography (HPLC) purified by Shanghai Sangon Biotechnology Co., Ltd., and the sequences (5'- 3') were as follows:

H1: TCAACATCAGTCTGATAAGCTATTTAGATCGTTACGCTAACTATGATAGCTTATCAGACT

H2:

TAAGCTATCATAGTTAGCGTAACGATCTAAATAGCTTATCAGACTTAGATCGTTACGCTAACTATG

crRNA: UAAUUUCUACUAAGUGUAGAUGAUCGUUACGCUAACUAUGA

ssDNA linker: Biotin-T_{100}-$(CH_2)_6$-SH

miRNA-21: UAGCUUAUCAGACUGAUGUUGA

miRNA-122: UGGAGUGUGACAAUGGUGUUUG

miRNA-155: UAA UGC UAA UCG UGA UAG GG

Synthesis of Gold Nanoparticles. Gold(III) chloroauric acid aqueous solution (0.1 M, 305 μL) was added to a boiling deionized water (30 mL), followed by the addition of trisodium citrate aqueous solution (5 mM, 6 mL). After vigorously stirring under reflux for 0.5 h, the reaction solution was cooled to room temperature. After that, PVP (0.235 mL, 12.8 g/L) was added and then the colloidal solution was stirred for 12 h. Finally, the colloidal solution was centrifuged and dispersed in 4 mL aqueous solution for further use.

Synthesis of Yolk-Shell Au@CdS. (1) core-shell Au@SiO_2: TEOS (3.5 mL) was added to a solution containing Au nanoparticles solution (0.55 mL), ammonia (3.5 mL) and isopropanol (20 mL). After stirring at room temperature for 4 h, the products were collected by centrifugation and washed with deionized water three times, and finally dried at 80 °C for 10 h. (2) core-shell

Au@SiO_2@CdS: the as-formed core-shell Au@SiO_2 nanoparticles (0.15 g) were dispersed in deionized water (50 mL) with the assistance of ultrasound. Then, sodium citrate aqueous solution (1 M, 1 mL) and cadmium chloride aqueous solution (1 M, 0.5 mL) were added into the above solution. After stirring for an additional 10 mins, ammonia (2 mL) and thiourea aqueous solution (1 M, 2 mL) were slowly added sequentially. Finally, the well-distributed solution was transferred and heated in an oil bath at 80 °C for 3 h. Afterward, the precipitate was cooled to room temperature and collected by centrifugation. Then it was rinsed with deionized water and ethanol several times to remove the impurities. After being dried in a vacuum drying oven at 60 °C for 12 h, the Au@SiO_2@CdS nanoparticles were obtained. (4) yolk-shell Au@CdS: the obtained Au@SiO_2@CdS were put into NaOH aqueous solution (1 M, 50 mL) at 90 °C for 3 h to remove the SiO_2 template. Finally, the synthetic yolk-shell Au@CdS were washed with deionized water and ethanol until the pH of the supernatant was 7 before being dried in a vacuum drying oven at 60 °C for 12 h.

Synthesis of Yolk-in-Shell Au@CdS Nanostructures. (1) SiO_2 nanoparticles: TEOS (3.5 mL) was added to a solution containing ethanol (92 mL), deionized water (17.2 mL) and ammonia (4 mL). After stirring at room temperature for 4 h, the products were collected by centrifugation and washed with water three times. Then the SiO_2 nanoparticles were dispersed in solution containing isopropanol (30 mL) and APTES (0.4 mL). The colloidal solution was refluxed for 3 h and then cooled to room temperature, and the products were collected by centrifugation and washed with water three times, and finally dried at 60 °C for 12 h. (2) Au@SiO_2 Janus nanoparticles: APTES-treated SiO_2 nanoparticles (75 mg) were dispersed in deionized water (30 mL), and the colloidal solution was ultrasonicated for 2 min. Then Au nanoparticles solution (0.55 mL) was added and the colloidal solution was stirred for 2 h at room temperature. Finally, Au@SiO_2 Janus nanoparticles were collected by centrifugation and washed with water three times, and dried at 60 °C for 12 h. (3) Au@SiO_2@CdS nanoparticles: the as-formed Au@SiO_2 Janus nanoparticles (0.15 g) were dispersed in deionized water (50 mL) with the assistance of ultrasound. Then, sodium citrate aqueous solution (1 M, 1 mL) and cadmium chloride aqueous solution (1 M, 0.5 mL) were added into the above solution. After stirring for an additional 10 mins, ammonia (2 mL) and thiourea aqueous solution (1 M, 2 mL) were slowly added sequentially. Finally, the well-distributed solution was transferred and heated in an oil bath at 80 °C for 3 hours. Afterward, the precipitate was cooled to room temperature and collected by centrifugation. Then it was rinsed with deionized water and ethanol several times to

remove the impurities. After being dried in a vacuum drying oven at 60 °C for 12 h, the Au@SiO_2@CdS nanoparticles were obtained. (4) yolk-in-shell Au@CdS: the obtained Au@SiO_2@CdS nanoparticles were put into NaOH aqueous solution (1 M, 50 mL) at 90 °C for 3 h to remove the SiO_2 template. Finally, the synthetic yolk-in-shell Au@CdS nanoparticles were washed with deionized water and ethanol until the pH of the supernatant was 7 before being dried in a vacuum drying oven at 60 °C for 12 h.

Conjugation of Magnetic Bead-ssDNA-HRP Probes. In a typical procedure, disulfide-labeled ssDNA (100 μM, 200 μL) was reduced by TCEP (200 μM, 200 μL) for 1 h and then mixed with streptavidin-coated MBs (0.1 μg/mL, 20 μL) for 2.5 h to complete the biotin-streptavidin binding. MBs-ssDNA was obtained by removing free ssDNA after multiple magnetic separations. Then sulfo-SMCC was used as a linker to conjugate HRP and MBs-ssDNA. MBs-ssDNA (disperse in 500 μL PBS), HRP (400 μL, 1 mg/mL) and sulfo-SMCC (1 mg) were mixed and shaken for 2 hours, and then separated and purified to obtain MBs-ssDNA-HRP.

Target-Triggered CHA-Mediated CRISPR-Cas12a Trans-Cleaving MB-ssDNA-HRP. Prior to experiment, all hairpin probes were annealed at 95 °C for 4 min and gradually cooled to room temperature to form stem-loop structure. Then CHA reaction sample (20 μL) contained H1 (2 μM), H2 (2 μM) and different concentrations of miR-21 to conduct reaction at 37 °C for 2 h to generate H1/H2 duplex. Meanwhile, Cas12a-crRNA was pre-prepared by mixing Cas12a protein (3 μL, 20 μM) with crRNA (1 μL, 20 μM), 10 × NEBuffer (2 μL) and PBS buffer (14 μL, 10 mM). The above-mentioned Cas12a-crRNA solution (3 μL) was mixed with CHA reaction solution (1 μL) and MB-ssDNA-HRP probes (16 μL) for 1h to digest ssDNA linker. The supernatant was collected via magnetic separation and added solution containing 4-CN (1.5 mg/mL, 25 μL) and H_2O_2 (10 mM, 5 μL). The mixture was incubated at 37 °C for 30 min and dropped on the yolk-in-shell Au@CdS/FTO surface and air-dried at room temperature.

PEC Measurement. The cleaned FTO electrode was stuck by the waterproof transparent tape with a hole (r = 2.5 mm). Following that, the above-prepared yolk-in-shell Au@CdS aqueous suspension (25 μL, 4 mg/mL) was thrown on the active surface of FTO electrode and dried at room temperature (yolk-in-shell Au@CdS/FTO). Subsequently, the photocurrent was measured in Na_2SO_4 supporting electrolyte (0.1 M, containing 10 mM ascorbic acid) at an applied potential of 0 V on an electrochemical workstation (AutoLab, μAutIII.Fra2.v, Eco Chemie, Netherlands) using a 500 W Xe

lamp (NBET, Beijing, China) as excitation light source with a three-electrode system (i.e., the modified or target-treated FTO working electrode, a Pt wire counter electrode, and an Ag/AgCl reference electrode).

PARTIAL RESULTS AND DISCUSSION

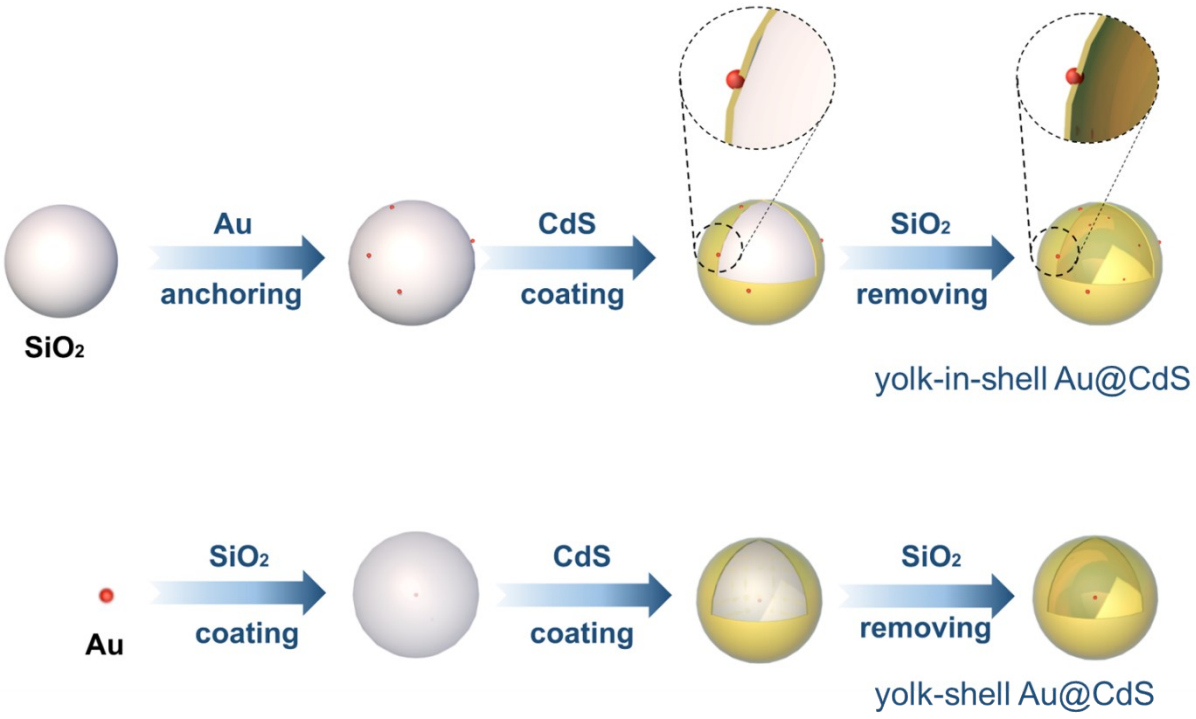

Fig. S1. Schematic illustration of the formation of yolk-in-shell Au@CdS and yolk-shell Au@CdS.

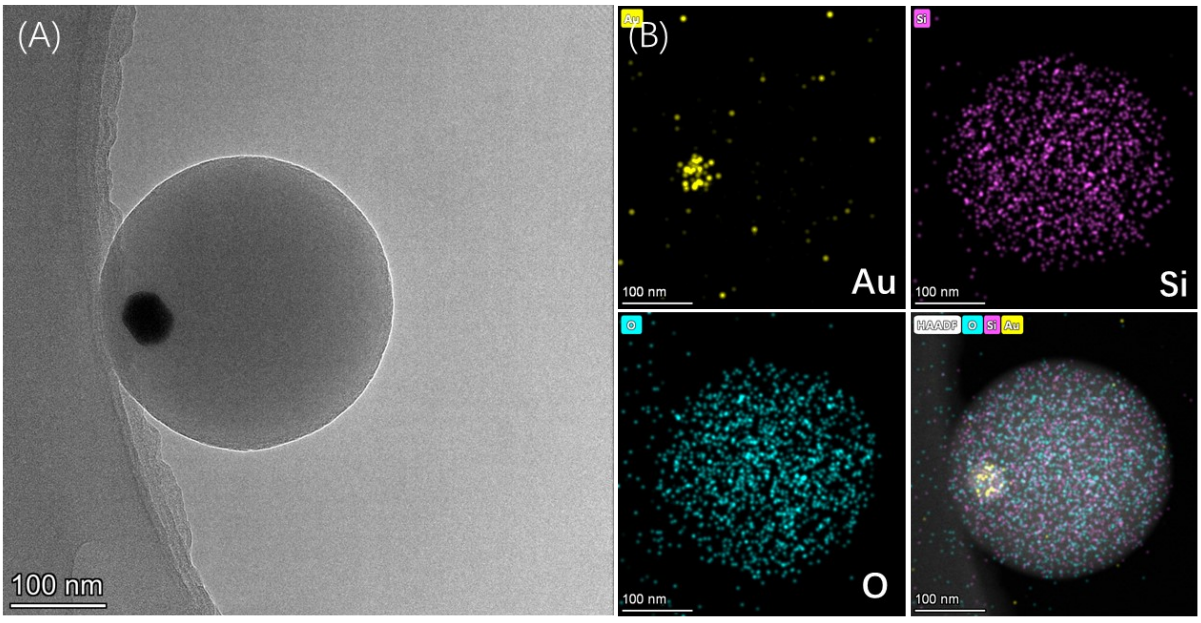

Fig. S2. (A) TEM image and (B) element mapping of Au@SiO_2 Janus nanoparticles.

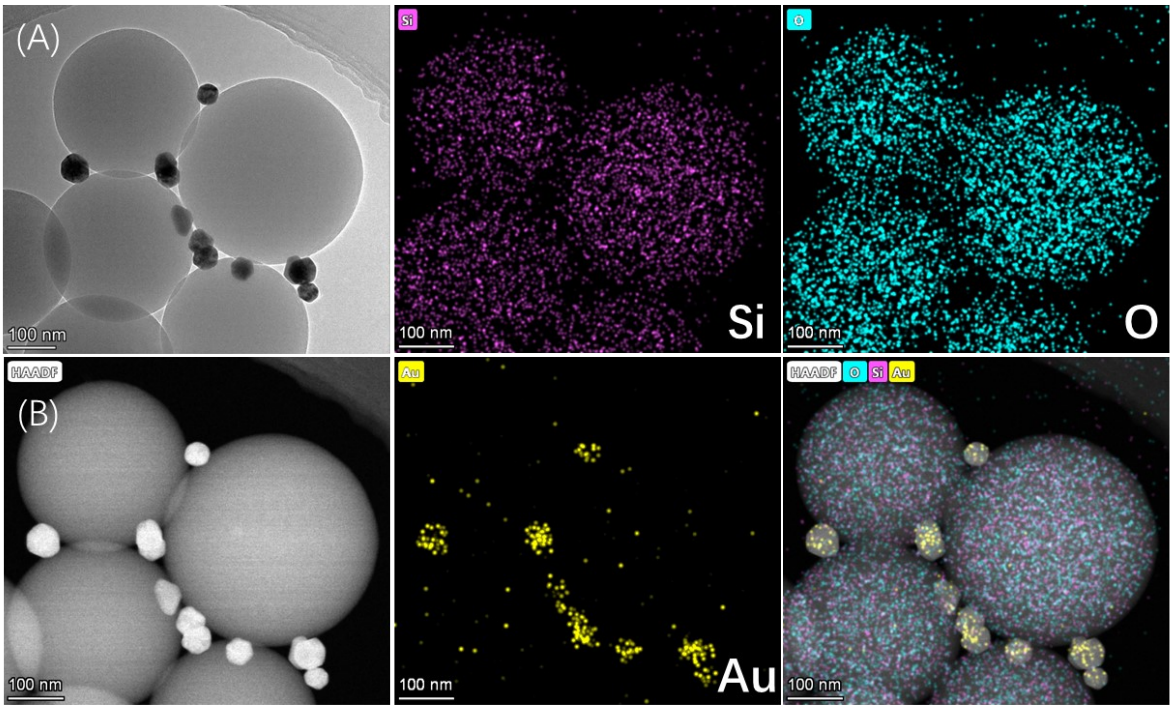

Fig. S3. (A) TEM image, (B) HAADF-STEM image and element mapping of Au@SiO_2 Janus nanoparticles.

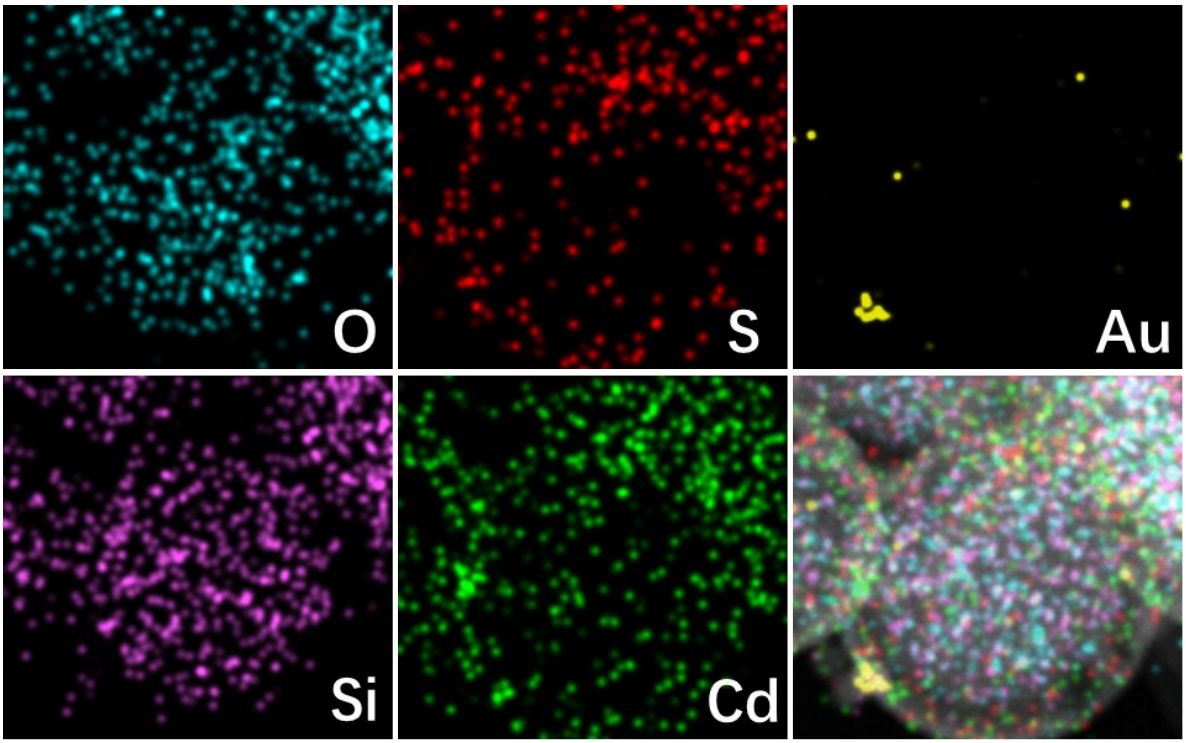

Fig. S4. Element mapping of Au@SiO_2@CdS nanoparticles.

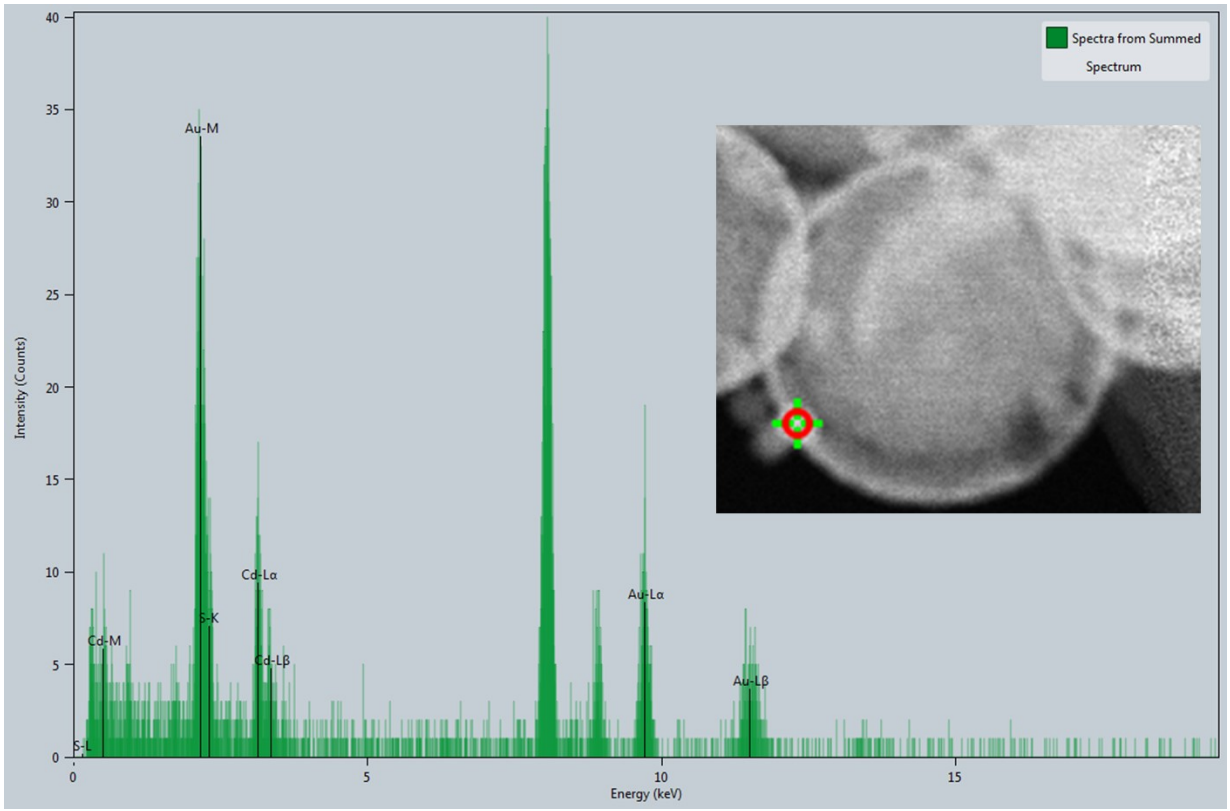

Fig. S5. EDS of Au@SiO_2@CdS nanoparticles.

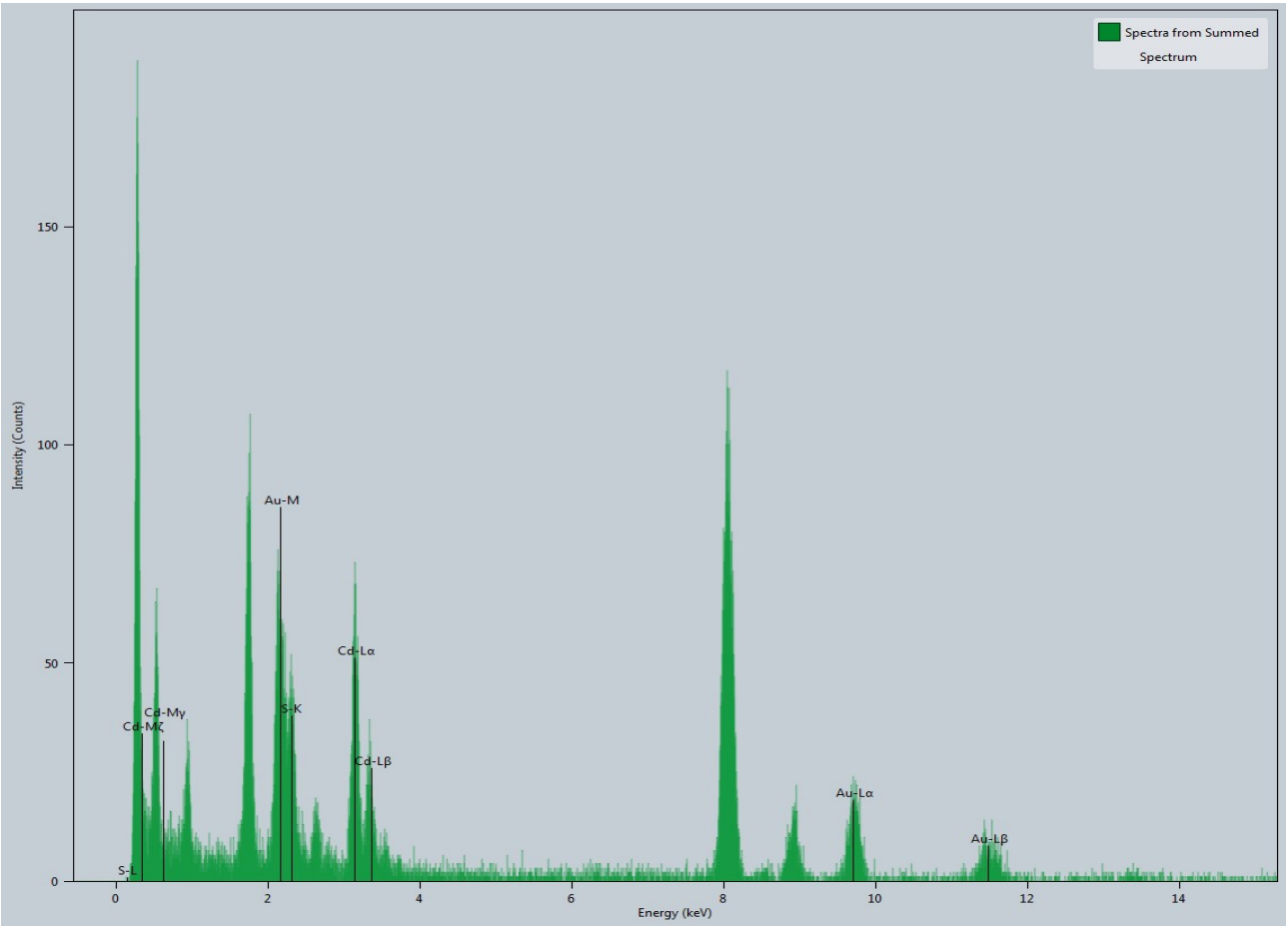

Fig. S6. EDS of yolk-in-shell Au@CdS.

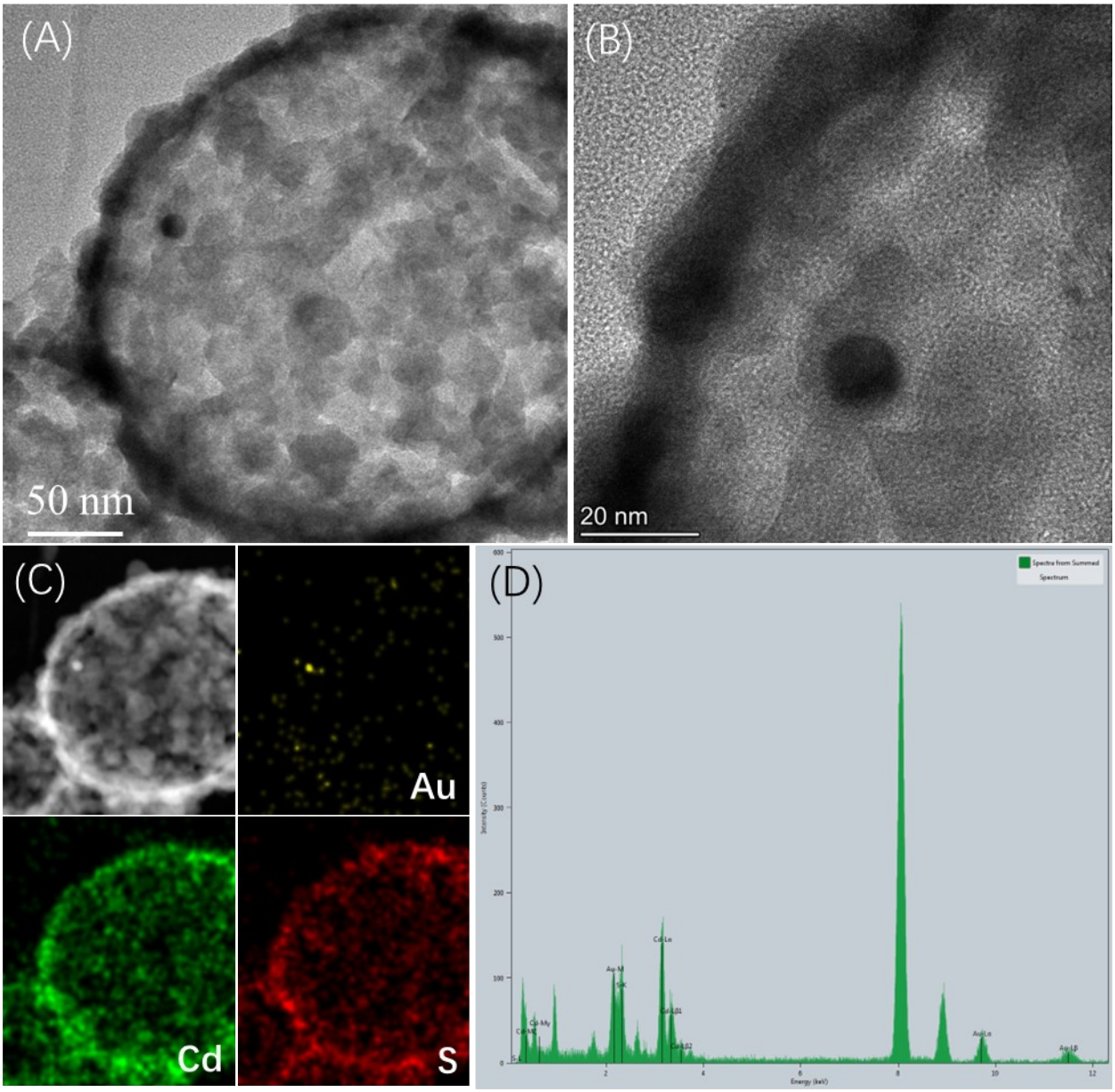

Fig. S7. (A, B) TEM images, (C) HAADF-STEM image and element mapping and (D) EDS of yolk-shell Au@CdS;

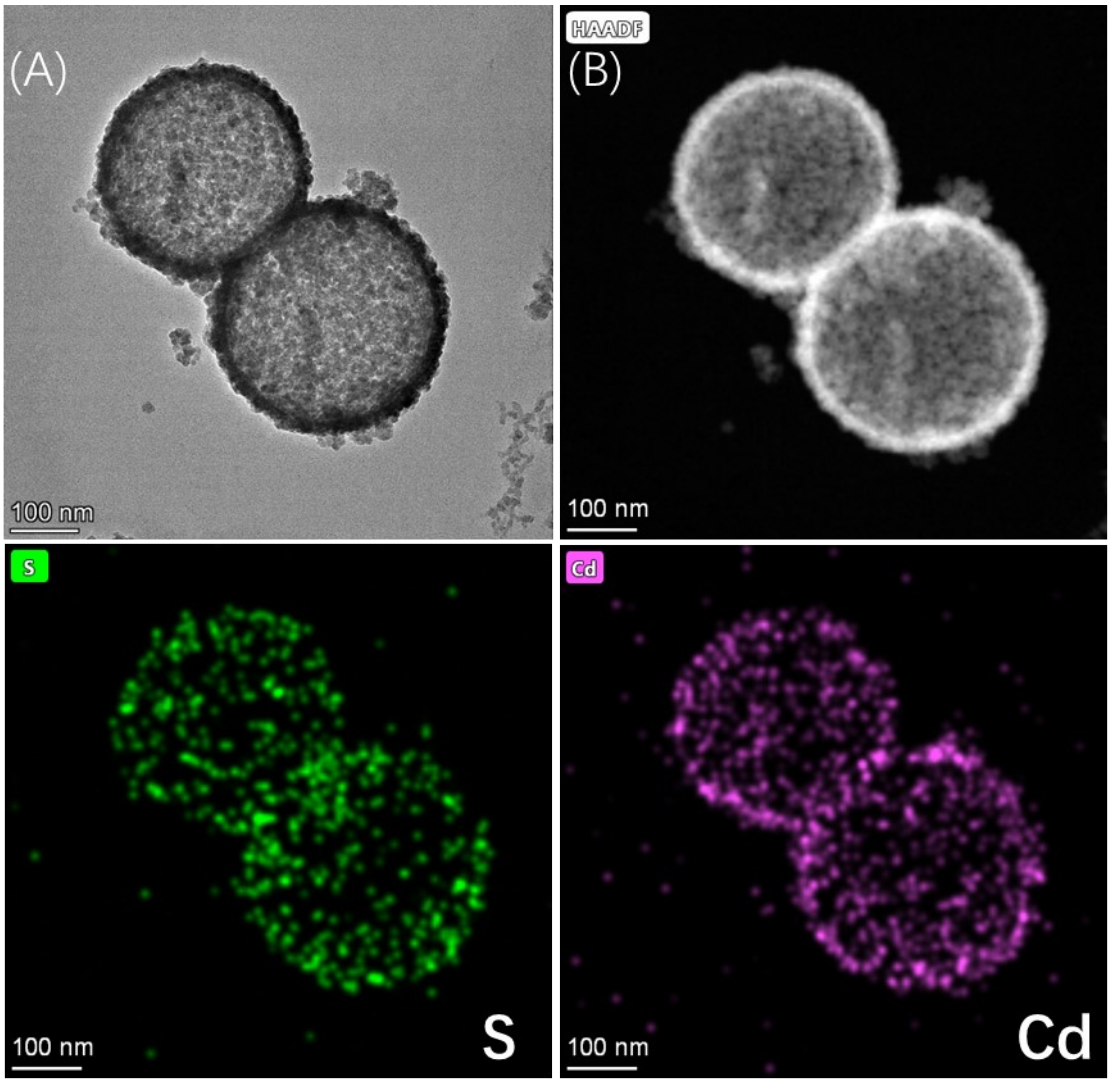

Fig. S8. (A) TEM image, (B) HAADF-STEM image and element mapping of hollow CdS.

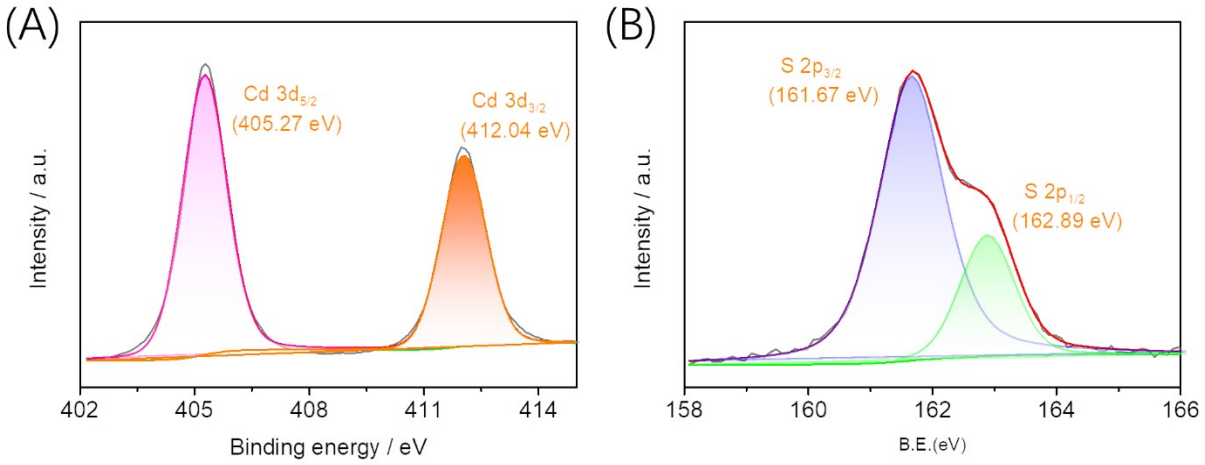

Fig. S9. High-resolution XPS spectra of (A) Cd 3d and (B) S 2p.

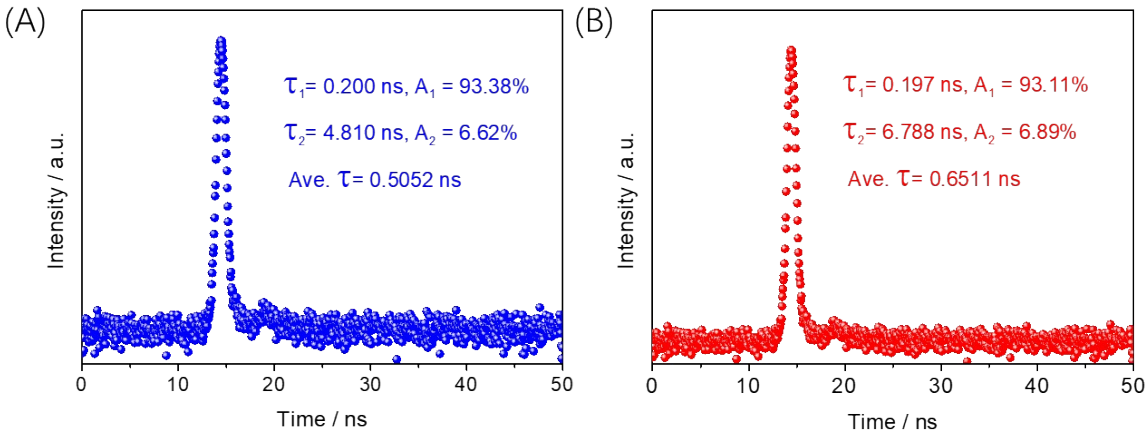

Fig. S10. TRPL decay of (A) yolk-shell Au@CdS and (B) yolk-in-shell Au@CdS.

Table S1 Comparison of this work and another assay for miR-21 determination.

Transduced signal	Linear range	Detection limit	Ref.
Duplex-specific nuclease-assisted CRISPR-Cas12a strategy	10 pM-10 nM	2.4 pM	1
CRISPR/Cas13a-based magnetic relaxation switching	1 pM-50 nM	0.22 pM	2
DNA nanocage-based CRISPR-Cas12a fluorescent biosensor	12.5 pM-0.4 nM	10 pM	3
CRISPR/Cas12a-based photoelectrochemical and fluorescence biosensor	0.01 pM-100 nM	5.8 fM/3.5 fM	4
CRISPR/Cas13a-based electrochemical assay	0.01 pM-10 nM	2.6 fM	5
Nucleic acid-functionalized MB@UIO electrochemical biosensor	0.02 pM-10 pM	8.2 fM	6
Colorimetric detection based on Ag/Pt nanoclusters	1.0-700 pM	0.6 pM	7
CHA-mediated CRISPR-Cas12a photoelectrochemical biosensor	0.01 pM -10 nM	4.2 fM	This work

References

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