# Electronic Supplementary Information

# $Co_9S_8$ nanoparticles-functionalized CdS nanoflower for signal-off photoelectrochemical bioanalysis of carcinoembryonic antigen with hybridization chain reaction

Kangyao Zhang,*[a] Kejun Wang,[a] Jianhui Zhang,[a] Meirong Tan,[a] Mingdi Xu,*[b] Wenqiang Lai [c] and Dianping Tang [d]

[a] School of Advanced Manufacturing, Fuzhou University, Jinjiang 362200, People's Republic of China

[b] College of Ecological Environment and Urban Construction, Fujian University of Technology, Fuzhou 350108, People's Republic of China

[c] Key Laboratory of Modern Analytical Science and Separation Technology of Fujian Province, Key Laboratory of Pollution Monitoring and Control of Fujian Province, College of Chemistry, Chemical Engineering and Environment, Minnan Normal University, Zhangzhou 363000, People's Republic of China

[d] Key Laboratory of Analysis and Detection for Food Safety (Ministry of Education & Fujian Province), Institute of Nanomedicine and Nanobiosensing, Department of Chemistry, Fuzhou University, Fuzhou 350108, People's Republic of China

## EXPERIMENTAL SECTION

**Material and reagent.** Cadmium acetate dihydrate [$Cd(CH_3COO)_2 \cdot 2H_2O$], cobalt(II) acetate tetrahydrate [$Co(CH_3COO)_2 \cdot 4H_2O$], copper nitrate trihydrate [$Cu(NO_3)_2 \cdot 2H_2O$], gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) and ascorbic acid (AA) were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$), thiourea, diethylenetriamine (DETA), ethyl alcohol (EtOH), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), trisodium citrate, and thioglycolic acid (TGA) were purchased from Aladdin (Shanghai, China). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and 6-mercapto-1-hexanol (MCH) were obtained from Sigma-Aldrich (USA). All other chemicals used in this work were of analytical grade. Water was purified with Millipore water purification system (18.25 MΩ $cm^{-1}$, Milli-Q, Millipore) and used throughout the work. All oligonucleotides were synthesized by Sangon Biotech. Inc. (Shanghai, China), and the sequences are listed below.

**Table S1** The list of the oligonucleotide sequences used in this work

| Oligonucleotide | Sequence (5' to 3') |
|---|---|
| Capture aptamer (CA) | SH-AAAA<u>AGGGGGTGAAGGGATACCC</u> |
| Trigger aptamer (TA) | GACCCTAAGCATACATTGTACTTAAT<u>TTAACTTATTCGACC ATA</u> |
| Hairpin DNA1($H_1$) | ATTAAGTACA**ATGTATGCTTAGGGTC***GACTTCAATA***GACC CTAAGCATACAT** |
| Hairpin DNA2 ($H_2$) | $NH_2$-TTATTT**GACCCTAAGCATACAT***TGTACTTAAT* **ATGTATGCTTAGGGTC**TATTGAAGTC |

Note: The underlined sequences in the capture aptamer and trigger aptamer were two aptamers for different epitopes of target CEA, respectively. In the hairpin probes, the italicized and bold sequences were the loop and stem regions of the hairpins.

**Apparatus.** Scanning electron microscopy (SEM; SU8020, Hitachi Instruments, Japan), transmission electron microscopy (TEM; Tecnai G2 F20, FEI Co., USA), X-ray photoelectron spectroscopy (XPS; Scientific ESCALAB 250, USA), X-ray diffraction (XRD; PANalytical X'Pert spectrometer, Netherlands), and UV-visible diffuse reflectance spectrum (DRS; Cary

7000, Agilent Technologies, Inc) were used to characterize the as-prepared materials in this work. All photoelectrochemical measurement processes were performed on CHI430A electrochemical workstation (Chenhua Instruments, China).

**Synthesis of CdS nanoflower and $Co_9S_8$ nanoparticles decorated CdS nanoflower.** The hierarchical CdS nanoflower was prepared by a solvothermal treatment according to previous literature with some modifications.[1] Initially, 2 mmol of $Cd(CH_3COO)_2 \cdot 2H_2O$ and 10 mmol of thiourea were dissolved in 60 mL mixed solution of DETA and EtOH (note: the volume ratio of DETA and EtOH was 1:5). After being stirred for 60 min, the mixture was transferred to an autoclave and kept at 120 °C for 24 h, followed by a natural cooling process. Then, the obtained products were washed with distilled water and ethanol alternately, and finally dried in a vacuum oven at 50 °C.

Next, cobalt sulfide-decorated cadmium sulfide nanoflower ($Co_9S_8$@CdS) was obtained by a hydrothermal method.[2] Prior to synthesis, 100 mg of the as-prepared CdS nanoflower was dispersed in 30 mL of $H_2O$. Thereafter, 50 mg of $Co(CH_3COO)_2 \cdot 4H_2O$ was added to the above solution. After being stirred for 2 h, 242 mg of $Na_2S \cdot 9H_2O$ was added slowly into the above mixture under magnetic stirring. Then, the solution was continuously stirred for about 30 min and subsequently was transferred into a Teflon-lined autoclave and kept at 120 °C for 20 h. The obtained products were washed with distilled water and ethanol alternately, and finally dried in a vacuum oven at 50 °C. Besides, the bare $Co_9S_8$ was also synthesized without the addition of CdS.

**Preparation of CuS nanoparticles (NPs) and hairpin probe-CuS NPs conjugates.** TGA-stabilized CuS NPs were prepared according to the literature.[3] Briefly, 0.1 mmol of $Cu(NO_3)_2 \cdot 2H_2O$ was dissolved in 50 ml of distilled water. Then, 15 μL of TGA was added into the solution under constant stirring and the pH was adjusted to 9.0 by drop-wise addition of NaOH solution (0.5 M). After bubbled with $N_2$ for 30 min, $Na_2S \cdot 9H_2O$ solution (50 mL, 5.0 mM) was added dropwise to the mixture. The resulting solution was continued for 24 h under $N_2$ bubbled. The product was washed with ethanol and deionized water several times and dispersed in water to form CuS NPs solution.

The synthetic process of hairpin probe-CuS NPs conjugates was as follows.[4] Initially, 1.0 mL of CuS NPs solution was dispersed into 0.5 mL of imidazole solution (0.1 M, pH 6.8)

containing 3.0 mg NHS and 2.0 mg EDC. After shaking at room temperature for 2 h, the activated CuS NPs were collected by centrifugation and washing with PBS (10 mM, pH 7.4) for several times. Following that, the obtained CuS NPs were mixed with hairpin DNA2 solution (0.5 mL, 5.0 μM), and incubated at 4 °C for 12 h with slight shaking. Afterward, the non-immobilized oligonucleotides were removed by centrifugation and washing as before. Finally, the obtained bio-conjugates were suspended in 1.0 mL of PBS (10 mM, pH 7.4) and then stored at 4 °C for further use.

**Synthesis of gold nanoparticles.** Gold nanoparticles were prepared was as follows. Initially, $HAuCl_4$ solution (1.0 mL, 1.0 wt%) was mixed with ultrapure water (99 mL), and the mixture was heated until boiling. After that, trisodium citrate solution (2.5 mL, 1.0 wt%) was injected quickly into the above solution. The solution was heated and stirred until the solution turned a wine-red color. Finally, the resultant solution was stored at 4 °C.

**Preparation of working photoelectrode.** Prior to fabrication, the fluorine-doped tin oxide (FTO) glass electrode was ultrasonically cleaned in water, ethanol, and water in sequence, and then was dried at 60 °C. Next, 25 μL of $Co_9S_8$@CdS suspension (1.0 mg $mL^{-1}$, dispersed in water) was dropped onto the FTO with a fixed area of 0.28 $cm^2$, and naturally dried at 25 °C. Then, 20 μL of colloidal gold nanoparticles (Au NPs) was dropped on the surface of FTO, and dried at 25 °C. After that, 20 μL of CEA capture aptamer solution (3.0 μM, and the thiolated DNA sequences were previously activated by 10 μM TCEP in the dark for 60 min to reduce the disulfide bonded) was pipetted onto the electrode and incubated for 6 h at 25 °C, and then blocked with 1.0 mM MCH for 1 h to eliminate the possible active site. Finally, the resulting FTO was washed with PBS (10 mM, pH 7.4) and stored at 4 °C when not in use.

**Photoelectrochemical (PEC) measurement.** The PEC measurements were carried out in 0.1 M $Na_2SO_4$ containing 4 mM AA under the irradiation of 500 W Xe lamp (NBET, Beijing, China) on an electrochemical workstation with a classical three-electrode system including a modified FTO working electrode, a Pt-wire counter electrode, and a saturated calomel reference (SCE) electrode. The constant potential was set at 0 V, and current-time (i-t) curve method was used in the whole PEC experiments.

## ADDITIONAL RESULTS AND DISCUSSION

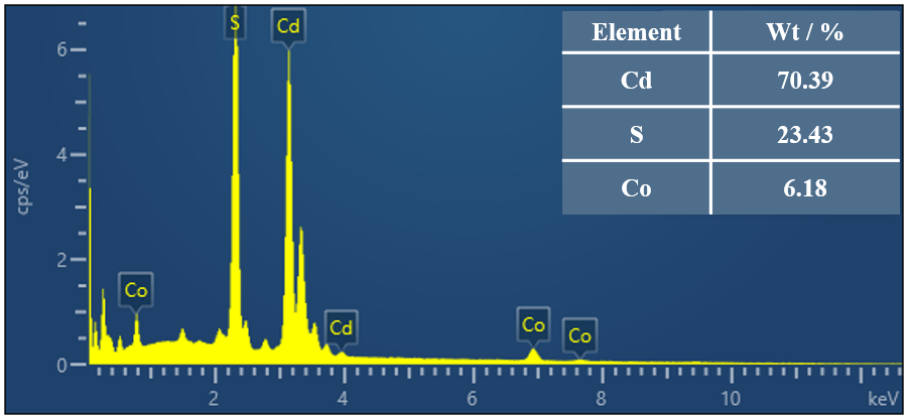


**Fig. S1** EDS spectrum of $Co_9S_8$@CdS nanohybrids.

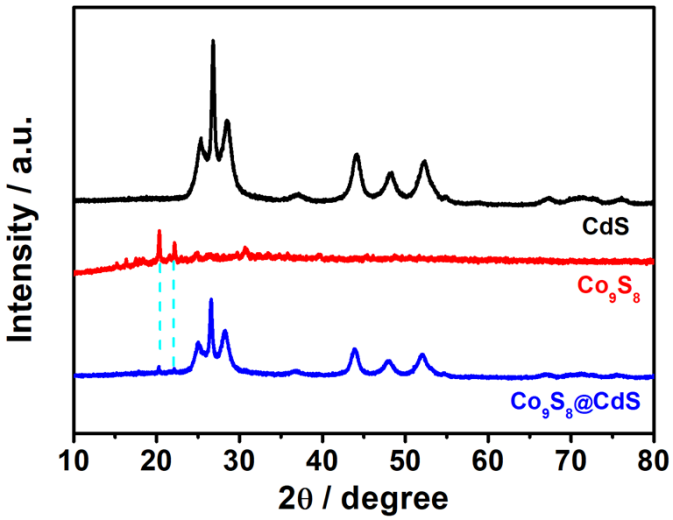


**Fig. S2** XRD patterns of as-prepared semiconductors.

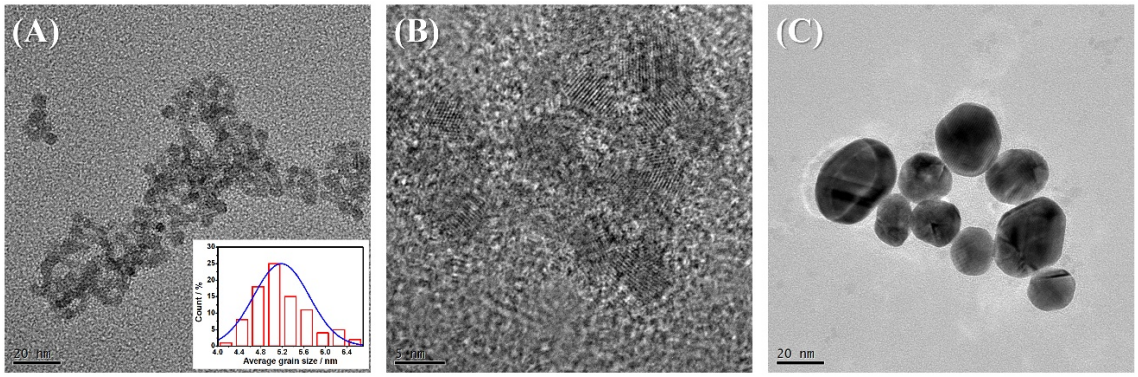


**Fig. S3** TEM images of (A, B) CuS NPs (inset: grain size distribution measured from Fig.S3A by Nano measurer 1.2) and (C) Au NPs.

UV-visible DRS was used to investigate the optical absorption capacities and corresponding bandgap energy values of CdS, $Co_9S_8$, and $Co_9S_8$@CdS nanohybrids. As displayed in Fig. S4A,

pure CdS nanoflower exhibited an absorption edge at the position of about 525 nm, while pristine $Co_9S_8$ nanoparticles showed a broad absorption in the range of 250-800 nm, suggesting that $Co_9S_8$ had a strong light harvesting capacity from the ultraviolet region to the visible region. We observed that the $Co_9S_8$@CdS nanohybrids displayed a stronger light absorption than pristine CdS in visible light region, indicating that the introduction of $Co_9S_8$ could effectively enhance the visible-light absorption of CdS. Besides, the band gabs of these as-prepared materials could be estimated from the equation $(\alpha h\nu)^2 = A (h\nu - E_g)$, where α, h, ν, A, and $E_g$ are the absorption coefficient, Planck constant, light frequency, the constant, and band gap energy, respectively.[5] The $E_g$ values of CdS and $Co_9S_8$ were calculated to be 2.53 and 1.39 eV, respectively (Fig. S4B). Furthermore, the types and flat-band positions of CdS and $Co_9S_8$ were studied through Mott-Schottky (M-S) experiments. As shown in Fig. S4C-D, the corresponding slopes in M-S plots were positive, indicating that they were both n-type semiconductors. Generally, the flat band potential of n-type semiconductor is considered to be approximately equal to its conduction band (CB). Thus, CB of CdS and $Co_9S_8$ were located at around -0.43 and -0.72 V *vs* normal hydrogen electrode (NHE), respectively. Combined with the band gaps obtained from DRS data, the valence band (VB) of the two materials were estimated to be 2.10 and 0.67 V *vs* NHE, respectively. Detailed band structure and calculation results of nanohybrids were described in Fig. S4E.

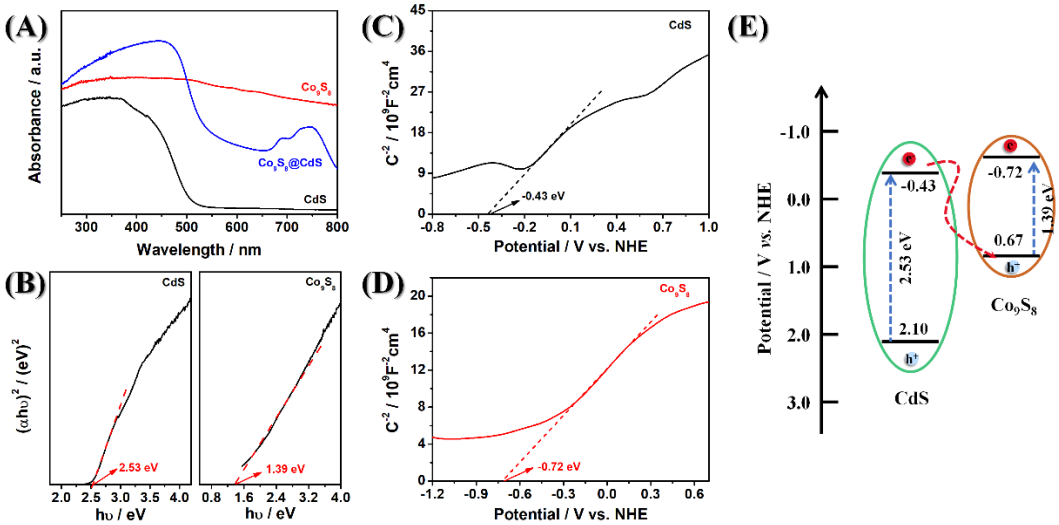


**Fig. S4** (A) UV-vis DRS spectrum of CdS, $Co_9S_8$, and $Co_9S_8$@CdS samples, (B) Plots of $(\alpha h\nu)^2$ versus hν for CdS and $Co_9S_8$, Mott-Schottky plots of (C) CdS and (D) $Co_9S_8$, (E) Schematic illustration of the energy band structure of CdS and $Co_9S_8$.

Fig. S5 displays the secondary structure of DNA sequences predicted by *Nucleic Acid Package* (NUPACK, provided by NUPACK webserver). Fig. S5A and Fig. S5B show the conformation of $H_1$ and $H_2$, respectively. After mixing $H_1$ and $H_2$, the hairpin structure of the two oligonucleotides remains unchanged according to the calculation result of NUPACK (Fig. S5C). When $H_1$, $H_2$, and CA exist simultaneously (Fig. S5D), no significant configuration changes in $H_1$ and $H_2$ were observed, suggesting that the introduction of CA cannot open the hairpin structure of $H_1$ and $H_2$. As expected, after adding TA in $H_1$ and $H_2$, the hairpin structures of $H_1$ and $H_2$ were opened smoothly (Fig. S5E).

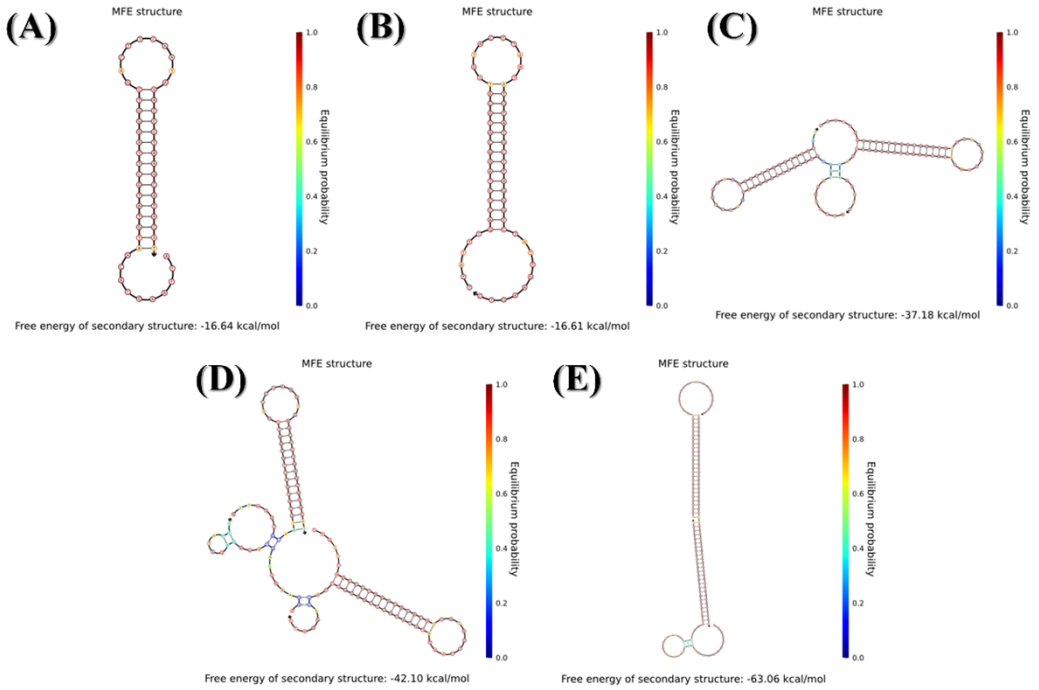


**Fig. S5** The analysis of secondary structure and its corresponding free energy about the used DNA sequences for CEA detection: (A) $H_1$, (B) $H_2$, (C) $H_1$ + $H_2$, (D) $H_1$ + $H_2$ + CA, (E) $H_1$ + $H_2$ + TA (note: the arrow in the figure represents the 3' end of oligonucleotide).

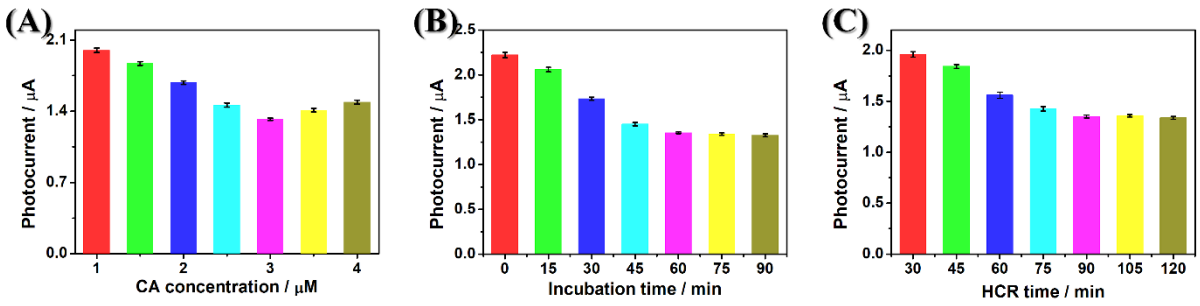


**Fig. S6.** Influence of (A) CA concentration, (B) incubation time for the aptamer/CEA/aptamer reaction, and (C) HCR reaction time on the photocurrent of PEC aptasensor (1.0 ng $mL^{-1}$ CEA used in all cases).

**Table S2** Comparison of analytical properties of differently methods toward target CEA

| Method | Materials | Linear range | LOD | Reference |
|---|---|---|---|---|
| Electrochemistry | $ZnMn_2O_4$@rGO | 0.01 - 50 ng $mL^{-1}$ | 1.93 pg $mL^{-1}$ | 6 |
| Electrochemistry and colorimetry | $Cu_2O$/Pt NPs | 0.0001 - 80 ng $mL^{-1}$; 0.1 - 80 ng $mL^{-1}$ | 0.03 pg $mL^{-1}$0; 0.026 ng $mL^{-1}$ | 7 |
| Colorimetry | Ni/C@$SiO_2$, CPS@PANI@Au | 0.006 - 12.00 ng $mL^{-1}$ | 1.56 pg $mL^{-1}$ | 8 |
| Fluorescence | None | 0.25 - 30 ng $mL^{-1}$ | 0.08 ng $mL^{-1}$ | 9 |
| Fluorescence | Cu/UiO-66 | 0.01 - 0.3 ng $mL^{-1}$ | 0.01 ng $mL^{-1}$ | 10 |
| Pressure | Ag/PDMS | 0.05 - 132 ng $mL^{-1}$ | 0.016 ng $mL^{-1}$ | 11 |
| Chemiluminescence | Ag@ZIF-67 | 0.05 - 500 ng $mL^{-1}$ | 4.53 pg $mL^{-1}$ | 12 |
| PEC | 3D Hollow CdS@Au | 0.015 - 2.4 ng $mL^{-1}$ | 3.5 pg $mL^{-1}$ | 13 |
| PEC | $Bi_2O_2S$ | 0.02 - 50 ng $mL^{-1}$ | 11.2 pg $mL^{-1}$ | 14 |
| PEC | $Co_9S_8$@CdS | 0.02 - 40 ng $mL^{-1}$ | 7.4 pg $mL^{-1}$ | This work |

## References


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