Supporting Information

Simultaneous detection and speciation of mono- & di-valent copper ions with a dual-channel fluorescent nanoprobe

An-Qi Zheng, Chen-Xi Zhao, Xiao-Juan Wang, Yang Shu* and Jian-Hua Wang*

Department of Chemistry, College of Sciences, Box 332, Northeastern University, Shenyang 110819, China

*Corresponding author.

E-mail: shuyang@mail.neu.edu.cn (Y. Shu); jianhuajrz@mail.neu.edu.cn (J.H. Wang)

Tel: +86-24-83688944

Experimental

Materials and chemicals

Rhodamine B (RhB), acetonitrile (CH_3CN), copper nitrate trihydrate, anhydrous citric acid, dichloromethane, bovine serum albumin, glutaraldehyde (50%, v/v) absolute ethanol, ethylenediaminetetraacetic acid (EDTA), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydro (EDC·HCl), N-Hydroxy succinimide (NHS) and BSA were the product of Sigma-Aldric Co. Ltd (Shanghai, China). The tetrakis (acetonitrile)copper (I) hexafluorophosphate as a Cu^+ source, BCS, NaCl, methanol, N, N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), hydrazine hydrate (85%, v/v) and uncoated CuNPs with initial nominal sizes of 80-100 nm (purity: 99.8%) were obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

Instrumentation

Fluorescence spectra were recorded on a F-7000 fluorescence spectrophotometer (Hitachi High Technologies, Japan) and a Fluormax 4 spectrophotometer (Horiba Scientific, France). UV-vis absorption spectra were obtained by a U-3900 UV-vis absorption spectrophotometer (Hitachi High Technologies, Japan). An Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS (Agilent Technologies, USA) equipped with an orthogonal ESI source was applied in the positive ionization mode for RBH identification. A GGX-200 graphite furnace atomic absorption spectrometer (GFAAS, Beijing Hai-Guang Instrument Co. Ltd, China) was used for the quantification of copper. SEM images were achieved by a scanning electron microscopy (SEM, Ultra Plus, Zeiss, Germany). Fourier transform infrared (FT-IR) spectra were obtained using a Nicolet 6700 spectrometer (Thermo Electron, USA). Zeta potentials and size distributions of the revalent materials were measured using a ZEN3600 Nano Zetasizer (Malvern, UK). Atomic force microscope (AFM) imaging was performed on a Dimension Icon ScanAsyst atomic force microscope (Bruker, Germany).

Preparation of BSA-CDs@RBH nanoprobe

CDs were prepared by following a previous procedure [1]. Shortly, 1.000 g of

anhydrous citric acid and 384 μL of ethylenediamine were dissolved in 10 mL deionized water. Then the mixture was heated for 2 min by microwave irradiation (700 w) followed by dialyzing against deionized water through a dialysis membrane (500-1000 WMCO) for 72 h. After removing water from the CDs solution by rotary evaporation, the CDs were dried under vacuum freeze drying.

Rhodamine hydrazide (RBH) was prepared with the following steps [2]. 1.2 g of RhB was dissolved into 30 mL of ethanol and the mixture was stirred vigorously at room temperature to form a homogeneous purple-red solution. 3.0 mL of hydrazine hydrate (85%, v/v) was dropwise slowly added into the solution under vigorous stirring. Then the mixture was refluxed for 5 h at 85℃. The solution changed from purple-red to light-orange and became transparent. After cooling down to room temperature, the solvents were partially removed by rotary evaporation to obtain the residual material. The white solid was then precipitated and washed with deionized water and dried under vacuum for overnight.

The preparation of CDs decorated BSA@RBH nanoprobe was conducted in the following. 0.2 g of BSA was added to 5 mL of Milli-Q water, followed by adding 20 mg mL^{-1} of RBH ethanol solution (8 mL) under ultrasonic treatment at 40 W. Subsequently, 5 μL of glutaraldehyde (50%, v/v) was added to the mixture which was continuously stirred for overnight. The large particles and microparticles were removed by 12000 rpm centrifugation for 10 min, and then the BSA encapsulated RBH nanoparticles (BSA@RBH NPs) were collected by 23000 rpm centrifugation for 30 min. The collected BSA@RBH NPs were washed three times with water and ethanol to remove any excessive BSA and RBH. Finally, the purified BSA@RBH nanoparticles were dispersed in 10 ml of deionized water. 20 mg of CDs were thereafter added to the above solution, followed by introduction of 83 mg EDC·HCl

and 124 mg of NHS. The mixture was magnetically stirred for 12 h. The CDs decorated BSA@RBH nanoprobe, shortly as BSA-CDs@RBH, were collected by centrifugation at 23000 rpm and washed three times with water and ethanol to rinse away any excessive EDC·HCl, NHS, CDs and RBH. Finally, the yellow powder was obtained under vacuum freeze drying.

Detection of Cu^{+} and Cu^{2+}

The tetrakis(acetonitrile)copper hexafluorophosphate was chosen as the source of Cu^{+}. Nitrogen purging ensures the removal of O_2 in CH_3CN to prevent Cu^{+} from being oxidized. Then, a 0.1 mol L^{-1} of Cu^{+} stock solution in CH_3CN was prepared. A stock solution of Cu^{2+} at a same concentration was obtained by dissolving appropriate amount of copper nitrate trihydrate in deionized water.

For investigating the feasibility of fluorescence measurement of Cu^{2+} with the BSA-CDs@RBH nanoprobe, a series of Cu^{2+} solutions at various concentrations (0.5-20 μmol L^{-1}) were taken to mix with BSA-CDs@RBH dispersion at 0.2 mg mL^{-1}. The fluorescence emission spectra of the above mixture were then recorded at $\lambda_{ex}/\lambda_{em}$ 360/450 nm and 360/575 nm, respectively.

For demonstrating the fluorescence measurement of mono- and di-valent copper ions, Cu^{+} and Cu^{2+} solutions with a series of concentrations, i.e., 0.5-20 μmol L^{-1}, were taken to mix with BSA-CDs@RBH dispersion and BCS solutions to give final concentrations of 0.2 mg mL^{-1} for BSA-CDs@RBH and 40.0 μmol L^{-1} for BCS. The fluorescence emission spectra of the above mixture were then recorded at $\lambda_{ex}/\lambda_{em}$ 360/450 nm and 360/575 nm, respectively, after incubation for 40 min at room temperature.

Monitoring Cu^{+} and Cu^{2+} in the degradation of CuNPs

Appropriate amount of dry powder CuNPs were dispersed in ice water bath for 10 min to make a 3.2 mg/mL suspension. The suspension was then diluted for 100-fold in various media including deionized water and NaCl (10 mmol L^{-1}) solution. The aliquots of the above dispersions were allowed to stand for various time intervals within two periods, i.e., 0, 0.75, 1.5, 2.25, 3.0, 5.0 h and 24, 48, 96, 120, 144 h. The mixture were then centrifuged at 9000 rpm for 5 min to separate the released mono- and di-valent copper ions with residual CuNPs. The total content of copper ions, i.e., Cu^{2+} and Cu^{+}, in the supernatant was measured by following the above procedure.

References

1. F. K. Du, F. Zeng, Y. H. Ming and S. Z. Wu, *Microchim. Acta*, 2013, **180**, 453-460.

2. J. Y. Sun, H. Mei and F. Gao, *Biosens. Bioelectron.*, 2017, **91**, 70-75.

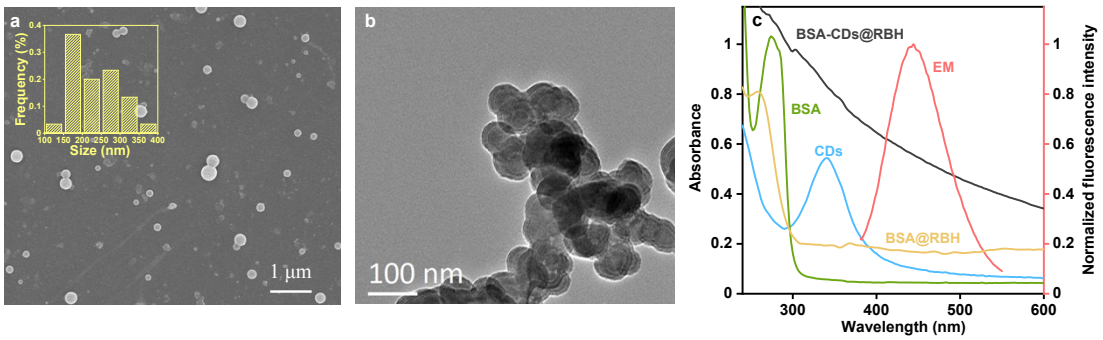


Fig. S1 (a) SEM image (Inset: particle size distribution) and (b) the TEM image of the BSA-CDs@RBH nanoprobe. (c) UV/vis absorption spectra of CDs, BSA, BSA@RBH and BSA-CDs@RBH, as well as fluorescence spectrum of BSA-CDs@RBH/BCS probe at λex 360 nm, with a maximum emission at 450 nm.

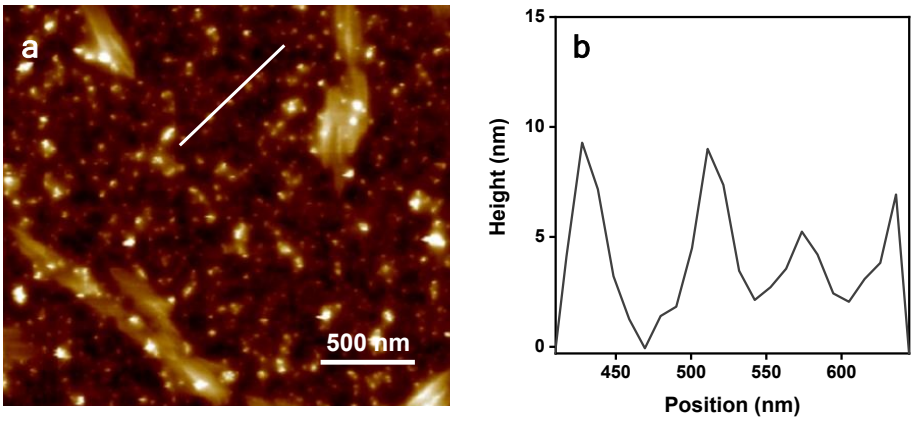


Fig. S2 AFM image and corresponding height distribution curve of the CDs.

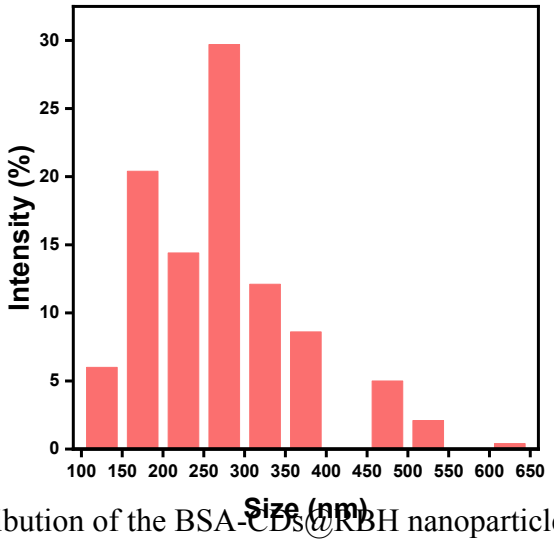


Fig. S3 The size distribution of the BSA-CDs@RBH nanoparticles measured by DLS.

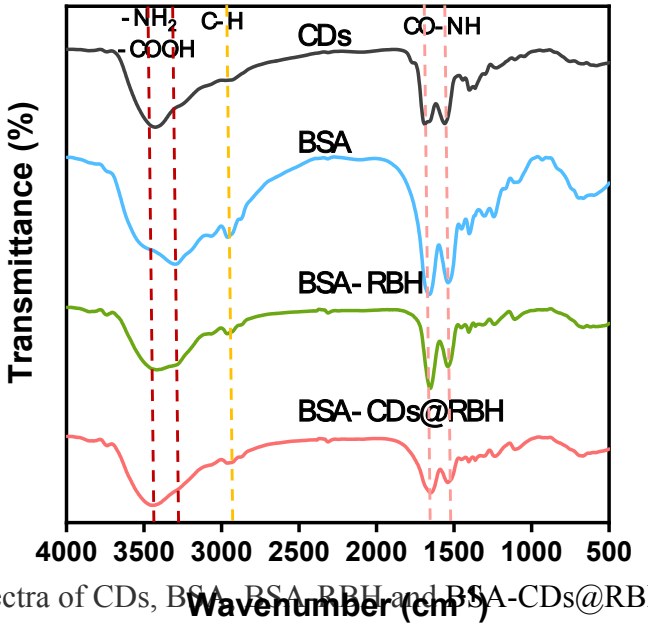


Fig. S4 FT-IR spectra of CDs, BSA, BSA-RBH and BSA-CDs@RBH.

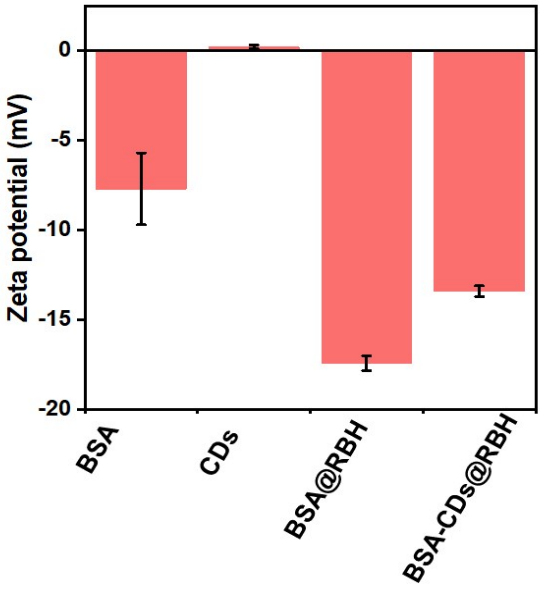


Fig. S5 Zeta potei RBH (8 mg mL^{-1}) and BSA-CDs@RBH (8 mg mL^{-1}).

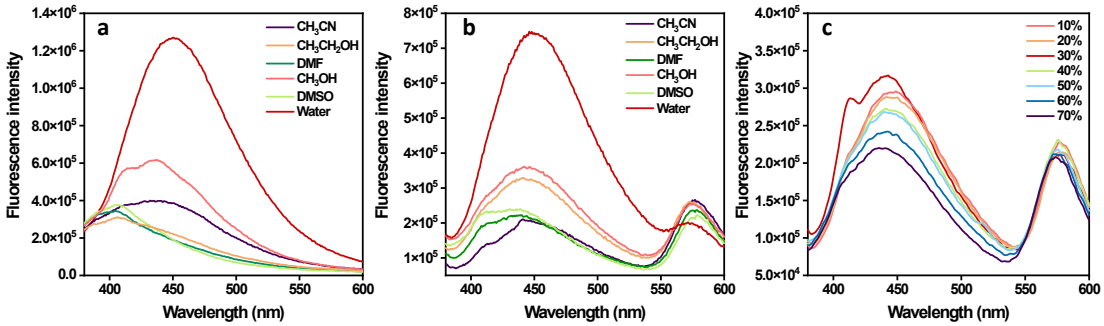


Fig. S6 (a) Fluorescence emission spectra of BSA-CDs@RBH/BCS (0.2 mg mL^{-1} BSA-CDs@RBH; 40.0 μmol L^{-1} BCS) (b) Fluorescence emission spectra of BSA-CDs@RBH/BCS (0.2 mg mL^{-1} BSA-CDs@RBH; 40.0 μmol L^{-1} BCS) with Cu^{2+} (20.0 μmol L^{-1}) in 40% solvent aqueous solution (v/v, DMF, DMSO, CH_3OH, CH_3CH_2OH, water and CH_3CN) and (c) CH_3CN-H_2O mixtures with different volume fractions.

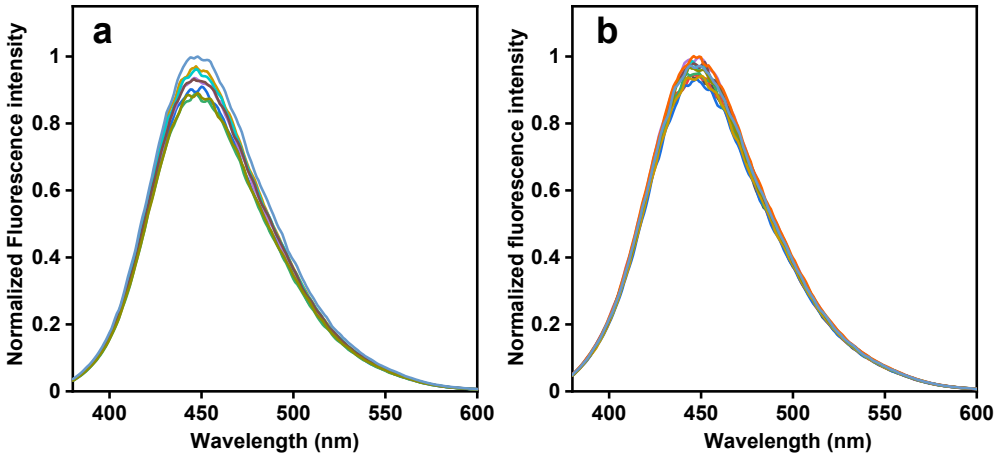


Fig. S7 The fluorescence response of CDs to (a) Cu^{+} and (b) Cu^{2+} at various concentrations, i.e., 0, 100, 150, 200, 250, 300, 500 μmol L^{-1}.

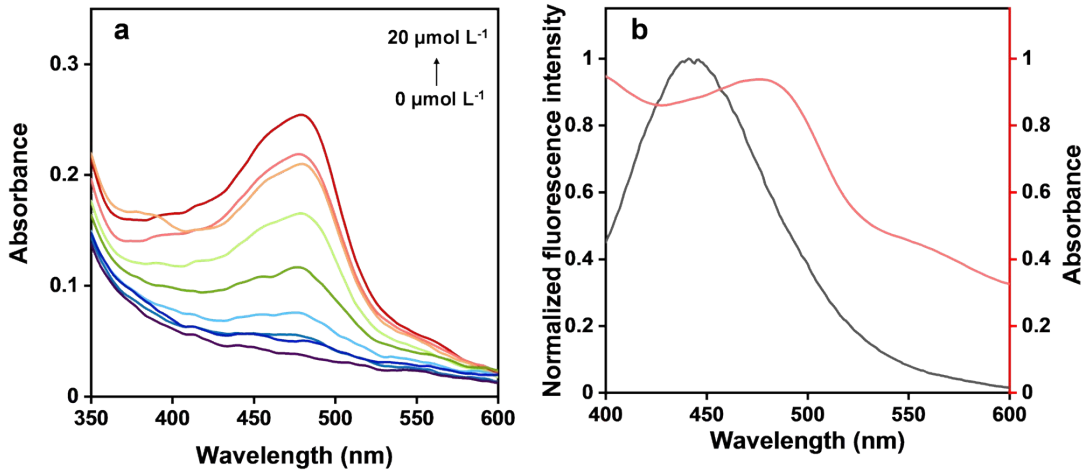


Fig. S8 (a) UV-vis absorption spectra of BSA-CDs@RBH/BCS (0.2 mg mL^{-1} BSA-CDs@RBH; 40.0 μmol L^{-1} BCS) probing system in the presence of various amounts of Cu^{+}, i.e., 0, 0.5, 1, 2, 4, 8, 12, 16, 20 μmol L^{-1}. (b) The fluorescence emission spectrum of the BSA-CDs@RBH nanoprobe (0.2 mg mL^{-1}) and the UV-vis absorption spectrum of BSA-CDs@RBH/BCS (0.2 mg mL^{-1} BSA-CDs@RBH; 40 μmol L^{-1} BCS) in the presence of 20 μmol L^{-1} Cu^{+}.

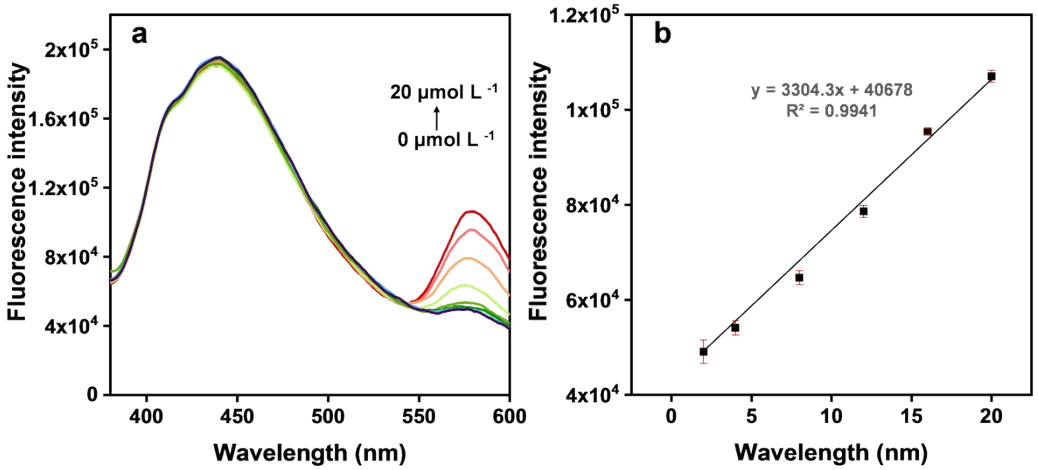


Fig. S9 (a) Fluorescence emission spectra of BSA-CDs@RBH nanoprobe (0.2 mg mL^{-1}) in the presence of various amounts of Cu^{2+} (0, 0.5, 1, 2, 4, 8, 12, 16, 20 μmol L^{-1}). (b) The variation of fluorescence intensity of BSA-CDs@RBH nanoprobe with the concentration of Cu^{2+}, illustrating a linear relationship between the fluorescence intensity and the concentration of Cu^{2+} (2, 4, 8, 12, 16, 20 μmol L^{-1}).

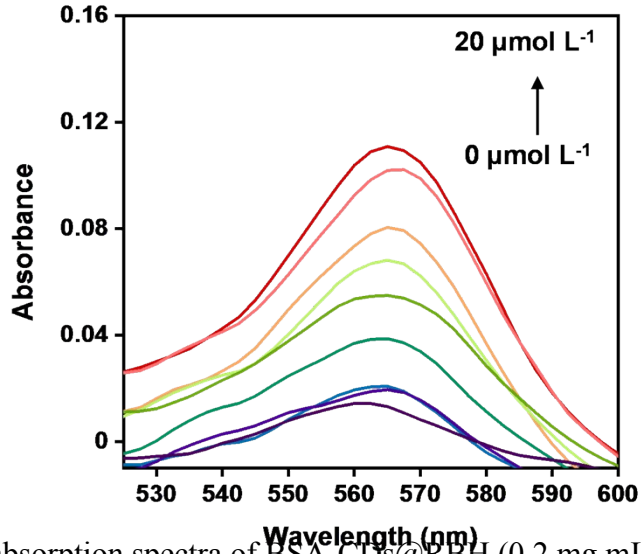


Fig. S10 UV-vis absorption spectra of BSA-CDs@RBH (0.2 mg mL^{-1}) in the presence of various amounts of Cu^{2+} (0, 0.5, 1, 2, 4, 8, 12, 16, 20).

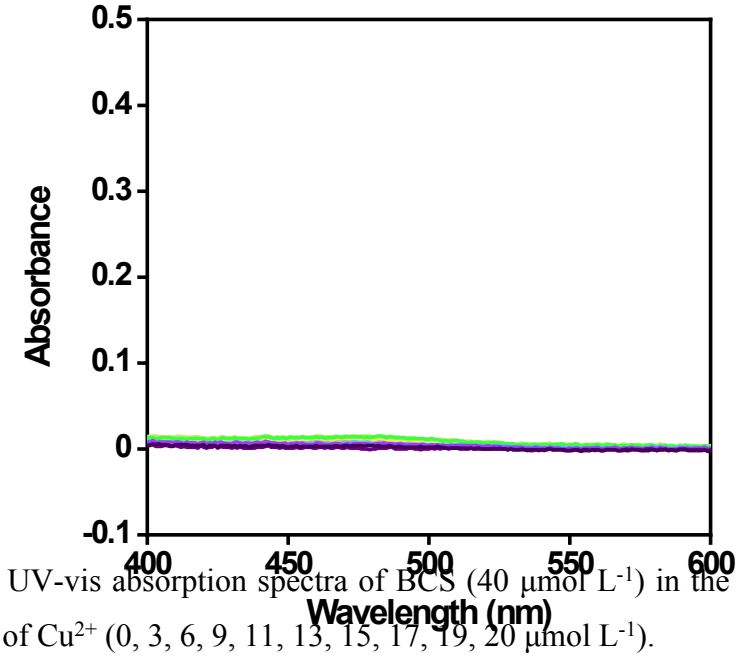
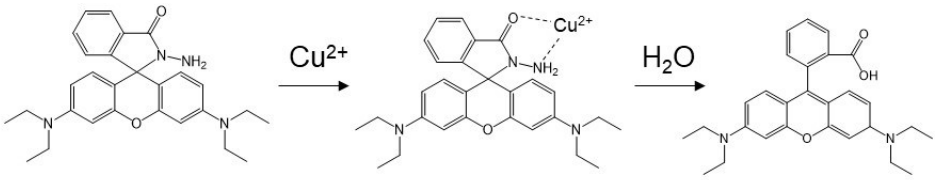


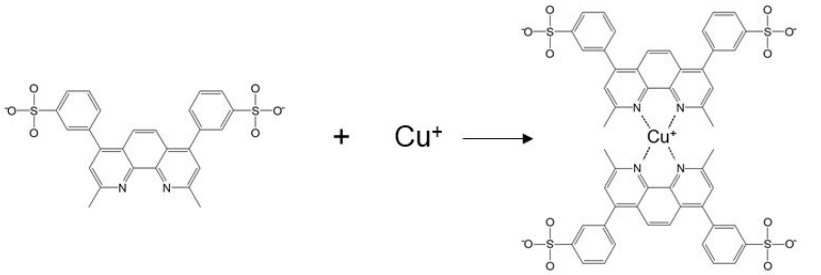
Fig. S11 UV-vis absorption spectra of BCS (40 μmol L^{-1}) in the presence of various amounts of Cu^{2+} (0, 3, 6, 9, 11, 13, 15, 17, 19, 20 μmol L^{-1}).

(1) 

(2) BSA + Cu^{2+} ⟶ Cu^{+}

Sche

CDs

(3) 

cata

proc

Cu+ chelate.

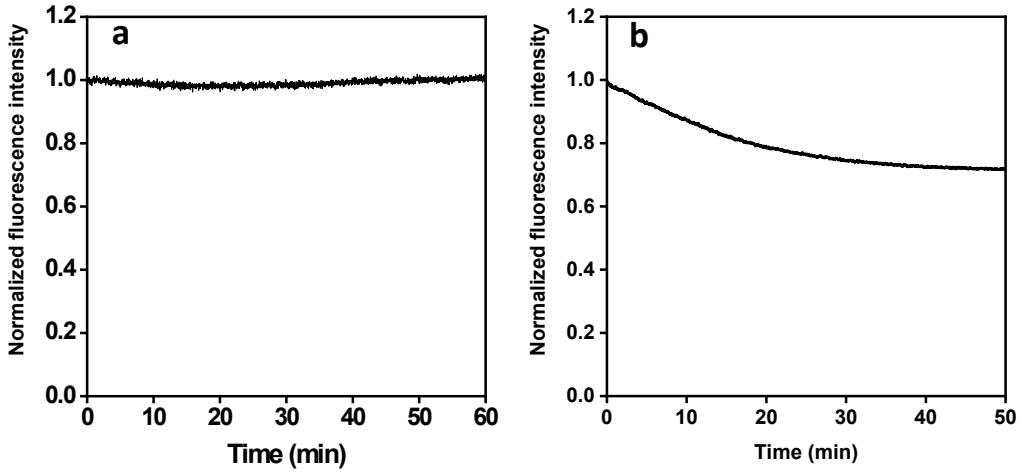


Fig. S12 (a) The dependence of fluorescence ($\lambda_{ex}/\lambda_{em}$ 360/450 nm) on the irradiation time for the BSA-CDs@RBH/BCS nanoprobe (0.2 mg mL^{-1} BSA-CDs@RBH; 40 μmol L^{-1} BCS). (b) The time-dependent variation of fluorescence intensity at $\lambda_{ex}/\lambda_{em}$=360 nm/450 nm for the BSA-CDs@RBH/BCS nanoprobe (0.2 mg mL^{-1} BSA-CDs@RBH; 40 μmol L^{-1} BCS) with 20.0 μmol L^{-1} Cu^{2+}.

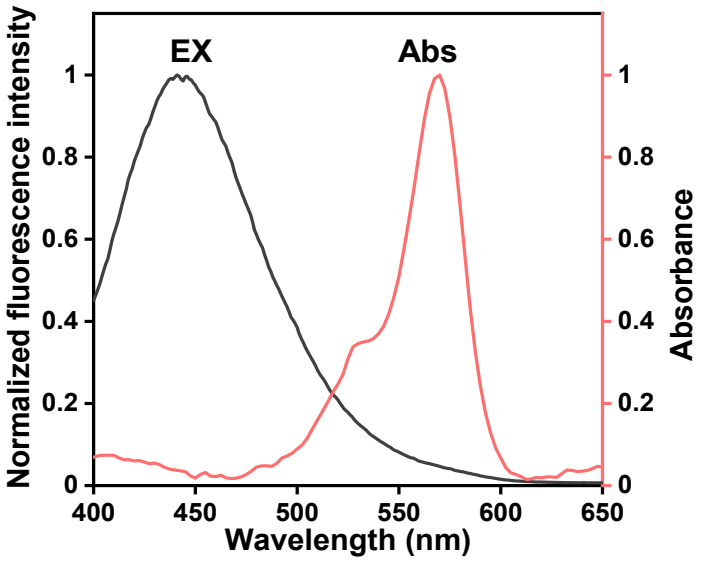


Fig. S13 The overlap of the fluorescence emission spectrum of the BSA-CDs@RBH/BCS nanoprobe and the UV-vis absorbance spectrum of RHO.

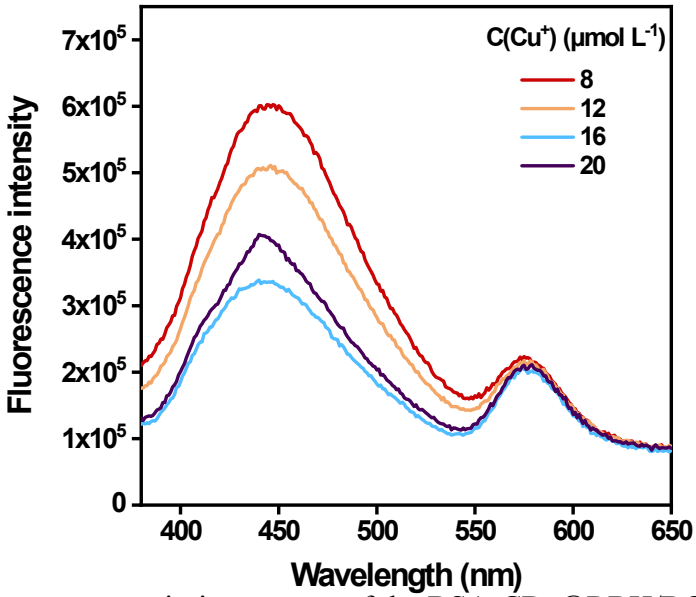


Fig. S14 Fluorescence emission spectra of the BSA-CDs@RBH/BCS dispersion (0.2 mg mL^{-1} BSA-CDs@RBH; 80 μmol L^{-1} BCS) with the addition of Cu^{+} at 8, 12, 16, 20 μmol L^{-1} in the presence of 20 μmol L^{-1} of Cu^{2+}.

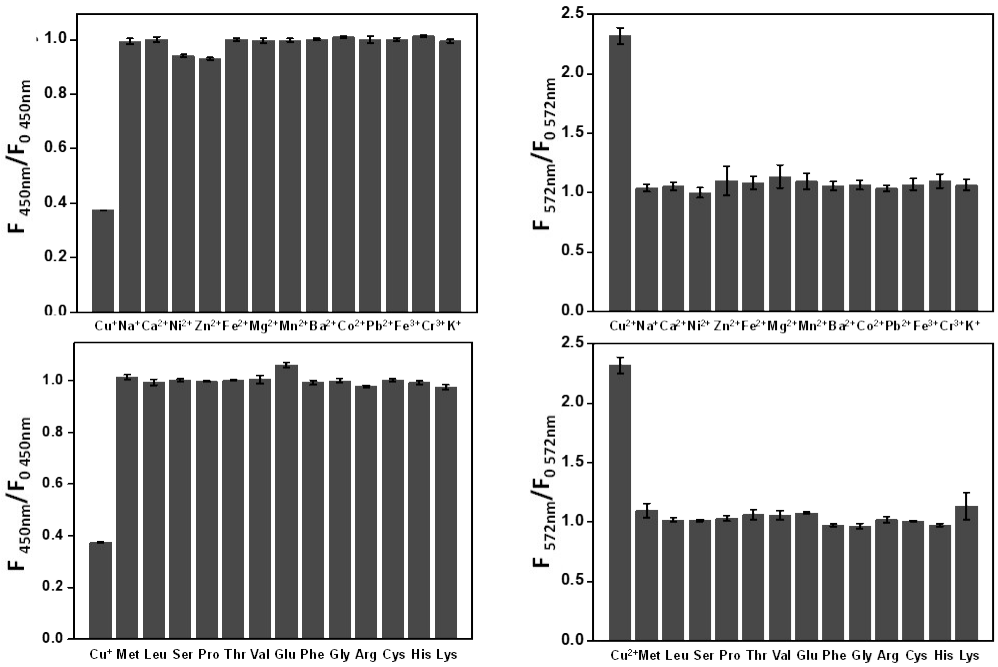


Fig. S15 The variation of fluorescence intensities of the BSA-CDs@RBH/BCS dispersion (0.2 mg mL^{-1} BSA-CDs@RBH; 40 μmol L^{-1} BCS) in the presence of 20 μmol L^{-1} of various cationic species and amino acids.

Table S1. The quantification and speciation of mono- and di-valent copper ions (μmol L^{-1}) by the BSA-CDs@RBH/BCS probe at λex/λem=360/450 nm and 360/575 nm with comparison to the results obtained by graphite furnace atomic absorption spectrometry (GFAAS).

Cu^{+} spiked	Cu^{2+} spiked	The present procedure			GFAAS
		Total Cu	Cu^{2+}	Cu^{+}	
13.0	3.0	16.2±0.4	13.0±0.7	3.2±0.4	16.5±1.1
6.0	11.0	17.5±0.5	7.0±1.3	10.5±0.8	18.1±1.2
3.0	15.0	18.7±0.2	3.5±1.2	15.3±1.1	18.6±0.7

Table S2. DLS data of CuNPs (80-100 nm in diameter, 32 μg mL^{-1}) in water and 10 mmol L^{-1} NaCl at various time.

Medium	Water		10 mmol L^{-1} NaCl	
Time (h)	Diameter (nm)	PDI	Diameter (nm)	PDI
0	504.0	0.541	922.1	0.798
0.75	565.3	0.607	793.6	0.790
1.5	541.3	0.598	1026.0	0.836
2.25	515.5	0.558	1170.0	0.847
3	610.0	0.544	1159.0	0.829
5	700.7	0.541	1809.0	0.867