Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Colorimetric detection of serum doxycycline with D-histidine-functionalized gold nanoclusters as nanozymes

Yuying Song, [a,b] Juan Qiao, [a,c] Wei Liu [b] and Li Qi * [a,c]

[a] Beijing National Laboratory for Molecular Sciences; Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P.R. China

[b] School of Pharmacy, Xinxiang Medical University , Xinxiang 453003, P. R. China

[c] School of Chemical Sciences, University of Chinese Academy of Sciences, Beijing 100049, P. R. China

*** Correspondence author:**

qili@iccas.ac.cn

Experiments

Materials and chemicals

$HAuCl_4$ was obtained from Shenyang Jinke Reagent Factory (Shenyang, China). D-Histidine (D-His) and other L-amino acids (L-AAs) were bought from TCI Shanghai Co. Ltd. (Shanghai, China). Cupric sulfate ($CuSO_4$) was purchased from Tianjin Komeo Chemical Reagent Co. Ltd. (Tianjin, China). Ciprofloxacin, streptomycin, tetracycline, oxytetracycline and norfloxacin were bought from MedChemexpress Company (Shanghai, China). Sodium acetate (NaAc) was gotten from Beijing Yili Fine Chemicals Co., Ltd. (Beijing, China). Human serum albumin (HSA) was purchased from Sigma-Aldrich Corp. (St Louis, USA). Vitamin C, glucose, zinc sulfate ($ZnSO_4$) and manganese sulfate ($MnSO_4$) were obtained from Aladdin Chemistry Company (Shanghai, China). Hydrogen peroxide (H_2O_2, 30.0%, w/w), DC, 3, 3', 5, 5'-tetramethylbenzidine (TMB) and other chemicals were purchased from Beijing Innochem Technology Co. Ltd. (Beijing, China). The aqueous solutions were prepared with Milli-Q water (Millipore, Bedford, MA, USA).

Instruments

The ultraviolet-visible (UV-*vis*) absorption spectra were recorded using a TU-1900 UV-*vis* double-beam spectrometer (Purkinje General, China). A 1.0 mL capacity cuvette with a 1.0 cm path length was used for measuring the UV-*vis* absorbance. All of the fluorescence measurements were performed using an F-4600 fluorescence spectrophotometer (Hitachi, Japan). Fourier transform infrared (FT-IR) spectra were recorded by an FT-IR spectrophotometer (TENSOR-27, Germany). The zeta potential measurements were carried out with a Zetasizer laser particle analyser (Zetasizer Nano ZS ZEN3600, British). Transmission electron microscopy (TEM) images were obtained using a transmission electron microscope (JEM-2010, Japan electron optics laboratory, Japan) at a voltage of 200 kV. The morphology of the D-His@AuNCs was characterized by scanning electron microscopy (SEM) (S-4800, Hitachi, Japan).

Preparation of D-His@AuNCs

All of the glasswares were washed with aqua regia ($HCl:HNO_3$ volume ratio = 3:1) and rinsed with ultrapure water. The D-His@AuNCs was prepared with D-His as the reducing and capping agent. Simply, in a 10.0 mL-glass flask, 0.82 mL of $HAuCl_4$ (20.0 mM) and 6.0 mL of D-His (100.0 mM) aqueous solutions were added and mixed under gentle stirring at 25 ^oC for 2.0 h. Then, the D-His@AuNCs solution was centrifuged to remove the larger particles at 10,000

rpm for 10 min. Finally, the D-His@AuNCs supernatant was collected and stored at 4 ^{o}C for further use.

DC testing

DC stock solution (100.0 μM) was prepared and various concentrations were obtained by serial dilution of the stock solution.

For testing DC, 30.0 μL Cu^{2+} stock solution (0.20 mM) and a series of 30.0 μL of standard solutions of DC (0.50-1.25 mM) were added into 150.0 μL of D-His@AuNCs solution. Then, TMB (36.0 μL, 25.0 mM) and H_2O_2 (90.0 μL, 10.0 M) was mixed with acetate buffer solution (2.664 mL, 0.20 M, pH 3.0). The final mixture was incubated at 25 ^{o}C for 10 min before conducting the UV-*vis* absorption measurements.

Metabolic assay of DC in rat serum

Three male-Sprague-Dawley-rats (about 250 g) were obtained from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All experiments using rat samples were performed in accordance with the institutional animal care and use guidelines of China (GB/T 27416-2014) and approved by the ethics committee at Institute of Chemistry, Chinese Academy of Sciences.

The controlled blank serum samples and five different serum samples (at 0.5 h, 1.0 h, 2.0 h, 4.0 h, 8.0 h) were collected after 10.0 mg/kg DC dissolved in physiological saline solution was injected into the abdominal cavity of rats. The rat serum samples were pre-treated to eliminate the interference from proteins. Simply, 0.1 mL of the fresh rat serum samples was diluted by 0.1 mL of water, which was heated in a water-bath to boil for 20 min. Consequently, the samples were centrifuged at 10,000 rpm for 10 min and the supernatant was collected and stored at 4 ^{o}C for further analysis.

The proposed colorimetric D-His@AuNCs-TMB-H_2O_2 system was applied to determination of DC in the rat serum samples. 30.0 μL Cu^{2+} solution (0.20 mM) and 30.0 μL rat serums were mixed. Then, D-His@AuNCs solution (150.0 μL), TMB (36.0 μL, 25.0 mM), H_2O_2 (90.0 μL, 10.0 M) and acetate buffer (2.664 mL, 0.20 M, pH 3.0) were added. After the mixture was mixed and incubated at 25 °C for 10 min, the UV-*vis* absorption measurements were conducted.

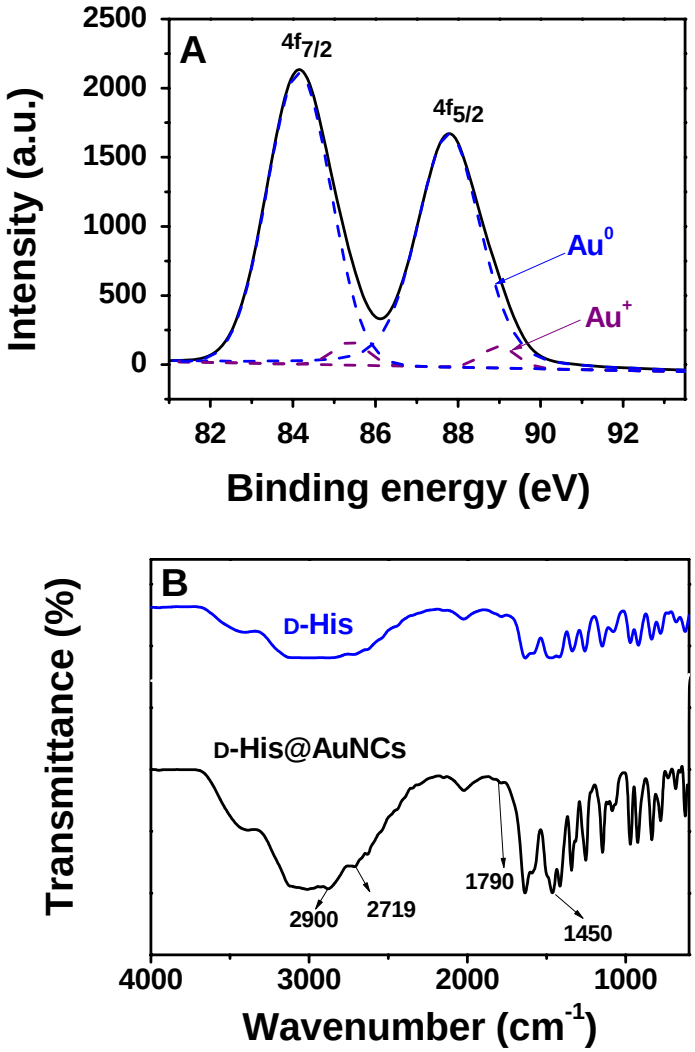

Fig. S1. (A) XPS spectra of Au 4f orbitals of D-His@AuNCs; (B) FT-IR spectra of D-His and D-His@AuNC.

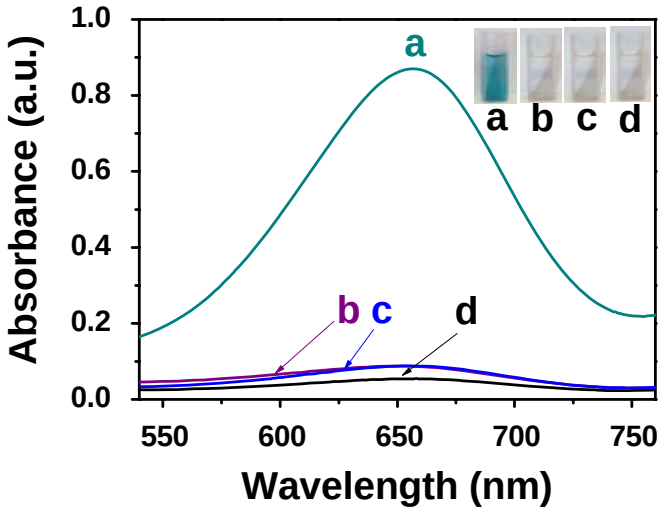

Fig. S2. The UV-*vis* absorption spectra and photos of different systems: (a) D-His@AuNC-TMB-H_2O_2, (b) TMB-H_2O_2, (c) D-His@AuNC-TMB and (d) D-His-TMB-H_2O_2.

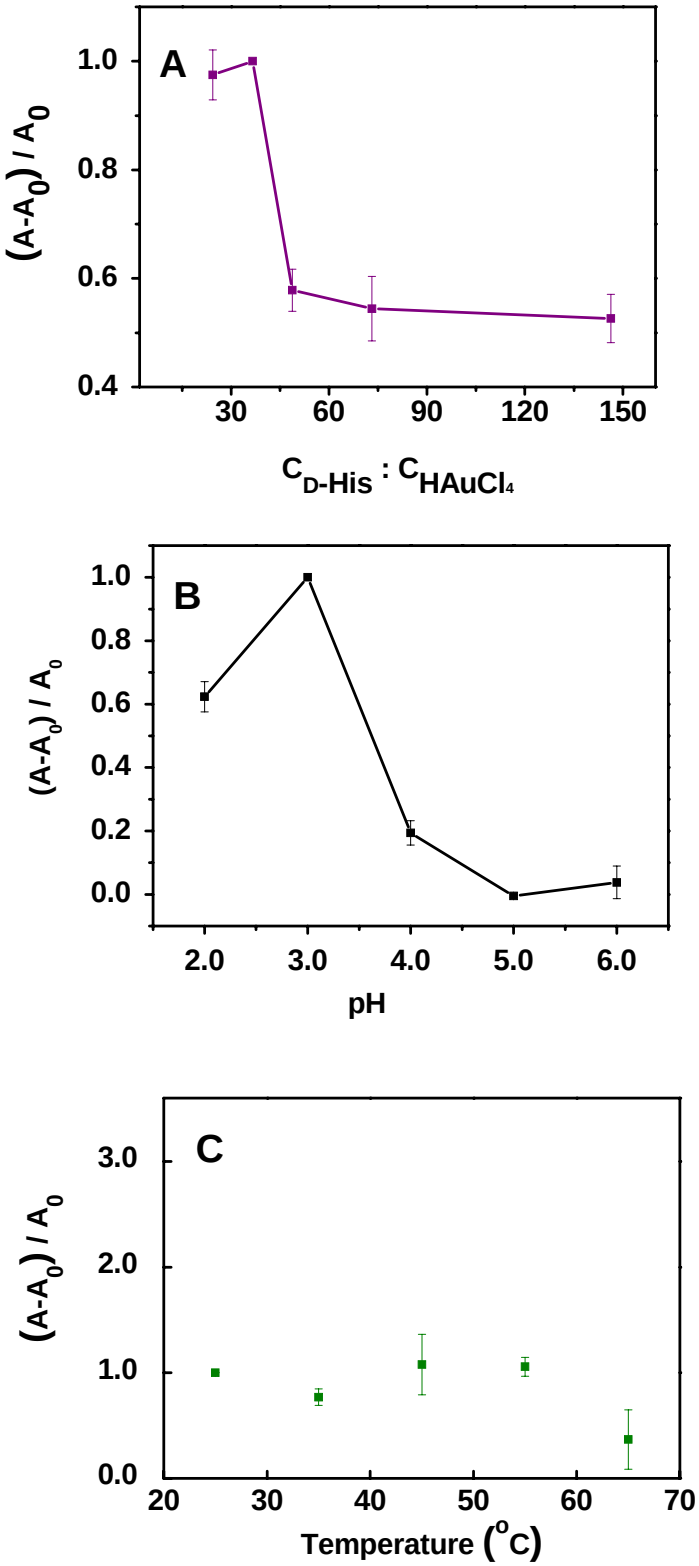

Fig. S3. Dependence of the peroxidase-like activity of D-His@AuNCs on (A) concentration ratio of D-His to $HAuCl_4$; (B) buffer pH; (C) temperature.

Table S1. Kinetic parameters (K_m and V_{max}) of the nanozymes and HRP

Catalyst	Substrate	K_m (mM)	V_{max} (10^{-8} M·s^{-1})	Ref.
D-His@AuNCs	TMB	0.41	7.69	This work
	H_2O_2	72.0	5.55	
HRP	TMB	0.43	10.0	L.Z. Gao, et al., *Nat. Nanotechnol.* 2007, *2*, 577
	H_2O_2	3.70	8.17	

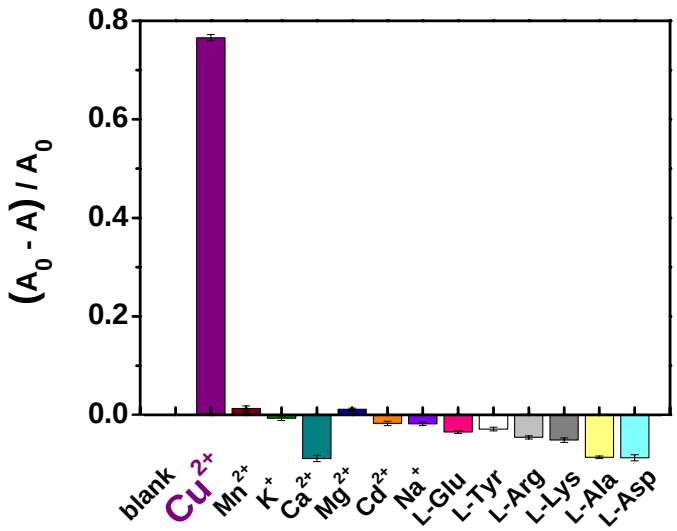

Fig. S4. Influence of different metal cations (2.0 μM) and L-amino acids (2.0 μM) on the peroxidase-like activity of D-His@AuNCs. Concentration of Cu^{2+} was 2.0 μM.

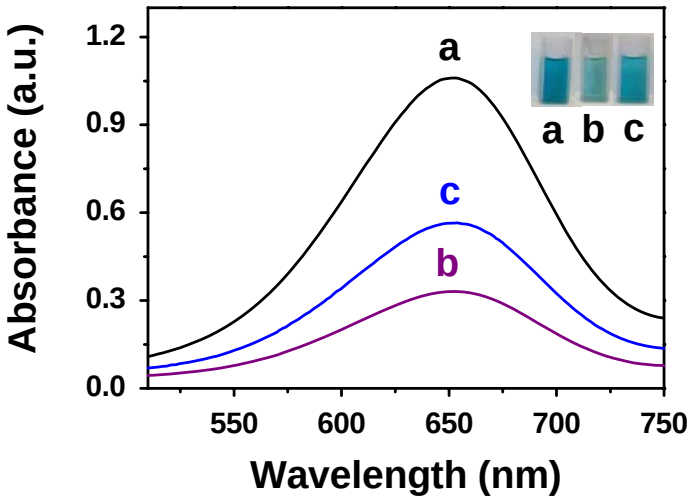

Fig. S5. The UV-*vis* absorption spectra and photos under daylight of (a) D-His@AuNCs-TMB-H_2O_2, (b) D-His@AuNCs-TMB-H_2O_2-Cu^{2+}, (c) D-His@AuNCs-TMB-H_2O_2-Cu^{2+}-DC.

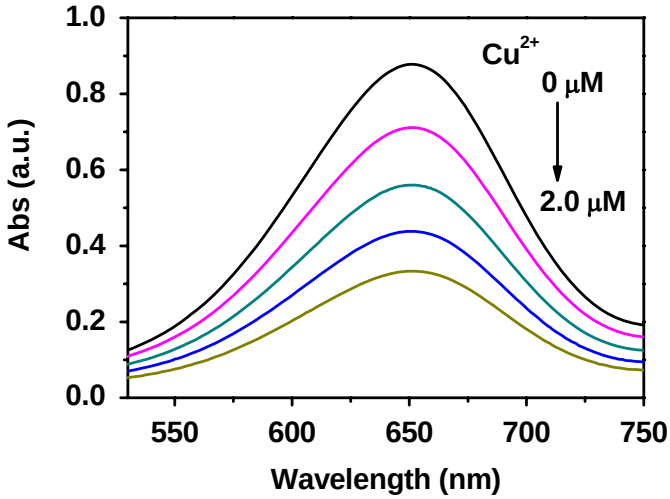

Fig. S6. Dependence of the peroxidase-like activity of D-His@AuNCs on concentrations of Cu^{2+}.

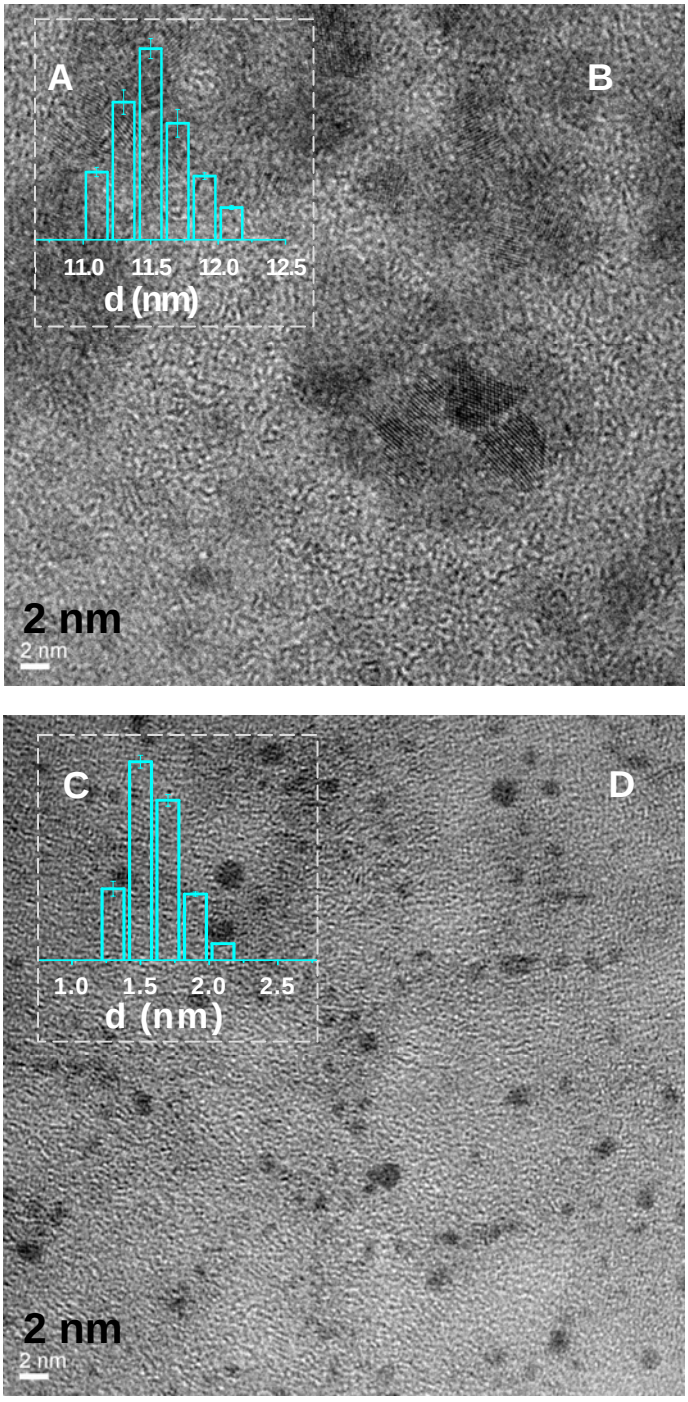

Fig. S7. (A) Size distribution of D-His@AuNC-Cu^{2+}; (B) TEM micrograph of D-His@AuNC-Cu^{2+}; (C) Size distribution of D-His@AuNC-Cu^{2+}-DC; (D) TEM micrograph of D-His@AuNC-Cu^{2+}-DC.

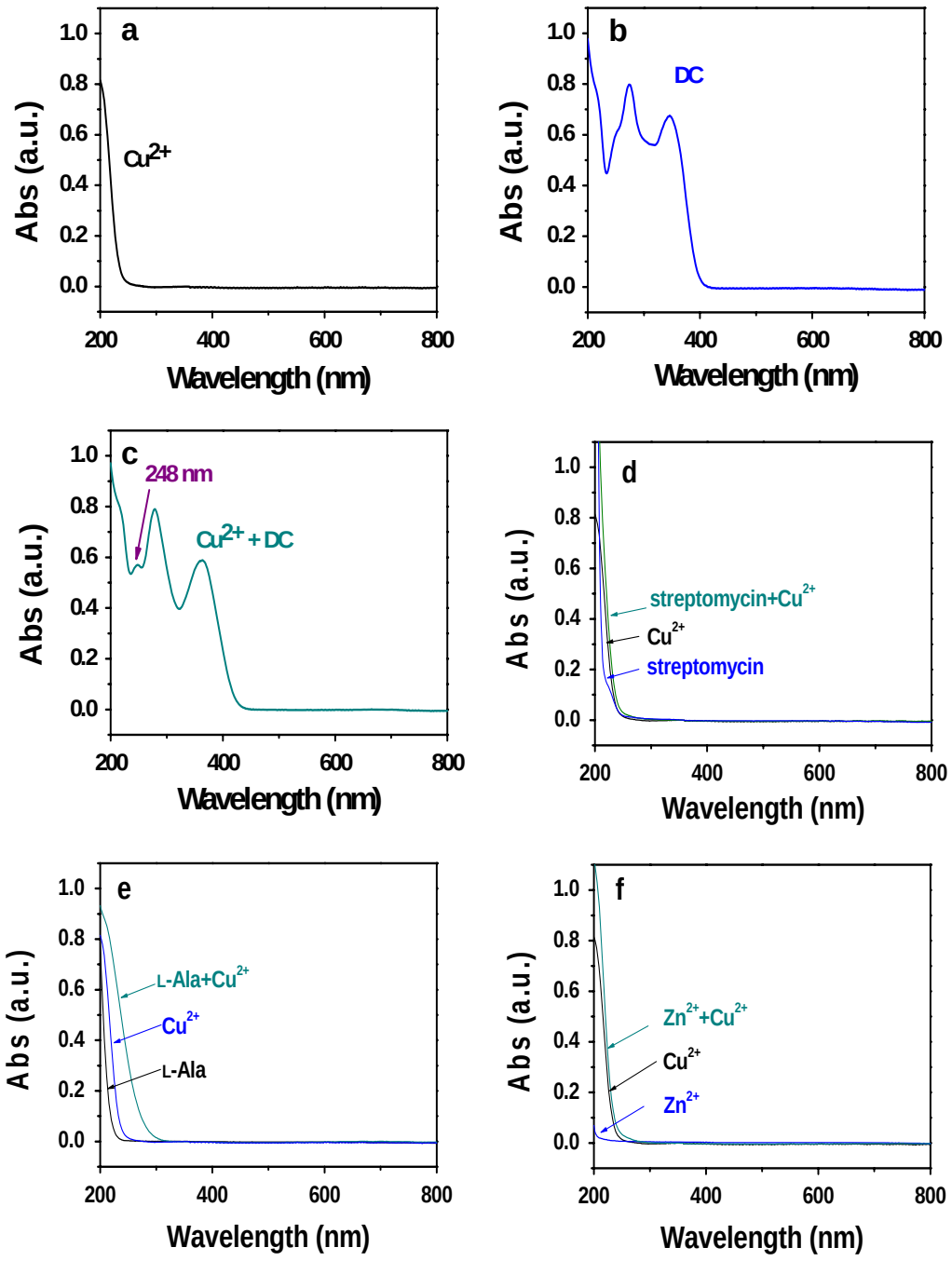

Fig. S8. (a) UV−vis spectra of Cu^{2+}; (b) DC; (c) Cu^{2+} + DC; (d) Cu^{2+} + streptorrycin; (e) Cu^{2+} + L-Ala and (f) Cu^{2+} + Zn^{2+} in aqueous solution.

Table S2. Recovery of the proposed assay*

Serums	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
1	5.0	4.6	91.4	1.3
	10.0	10.8	108.1	1.5
	12.5	13.7	109.6	4.7
2	5.0	5.4	108.7	1.7
	10.0	9.6	96.1	4.5
	12.5	13.6	108.7	2.7
3	5.0	5.1	101.4	2.6
	10.0	9.3	93.2	4.6
	12.5	12.3	98.5	1.0

* Blank controlled rat serums were used for recovery study (n=3).