Electronic supplementary information

Label-free fluorescent immunoassay for Cu^{2+} ions detection based on UV degradation of immunocomplex and metal ion chelates

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Materials and instruments

$CuSO_4$, $FeSO_4$, $MgSO_4$, $AgNO_3$, $CoCO_3$, $MnCl_2$, $PbCl_2$, NaCl, $CrCl_3$, NH_4Cl, $CdCl_2$, $HgCl_2$, $AlCl_3$, $FeCl_3$, $ZnCl_2$, CuCl, EDTA, NaOH, HNO_3 and $HClO_4$ were all purchased from Chengdu Kelong Chemical Reagent (China). CdSe/ZnS QDs were products of Shanghai Xingzi Material Co., Ltd. (China). Bovine serum albumin (BSA) was purchased from Gibco (USA). Cu^{2+}-EDTA-BSA conjugate and mouse anti Cu^{2+}-EDTA McAb were purchased from Wuxi Determine Bio-Tech Co., Ltd. (China). Horseradish peroxidase (HRP) was supplied by Beijing Biosynthesis Biotechnology Co., Ltd. (China). Mouse anti Cu^{2+}-EDTA McAb-HRP conjugate was home-prepared by a reported glutaraldehyde protocol[1]. *Ganoderma* and *American ginseng* were purchased from local pharmacy. Lake water was collected from Gongqingtuan Lake in Southwest University campus. SuperBlock® T20 used for microplate blocking was provided by Thermo Fisher Scientific Inc. (USA). Carbonate buffer saline (0.05 M, pH 9.0) was used as the coating buffer for immobilizing antigen and antibody on microplate. PBS at 0.01 M and pH 7.4 containing 4.0 mM EDTA was used to dilute and chelate Cu^{2+} ions. CdSe/ZnS QDs were diluted by 0.01 M PBS at pH 9.5. PBS (0.01 M, pH 7.4) containing 0.05% Tween-20 was adopted as washing buffer. The digestion solution for *Ganoderma* and *American ginseng* was a mixture of concentrated HNO_3 and concentrated $HClO_4$ (v/v = 4:1). All other reagents were analytical reagent grade and used without further purification. Polystyrene high-affinity 96-well microplate was obtained from Greiner Bio-One (Germany).

An UV camera obscura (ZF-20D) provided by Shanghai Guanghao Instrument Co.,

Ltd. (China) was used to provide UV radiation with a power of 40 W and a wavelength of 254 nm. An ELGA PURELAB classic system (France) was adopted to prepare ultra-pure water (18.2MΩ) used in the whole work. A TAS-990G atomic absorption spectrometer (Beijing Purkinje General Instrument Co., Ltd., China) was used to provide reference values for Cu^{2+} ions detection. An Infinite 200 PRO multifunctional microplate reader (Tecan, Austria) was adopted to detect FL signals.

Procedure of Cu^{2+} ions detection using label-free FIA

The microplate was coated with 10 μg/mL mouse anti Cu^{2+}-EDTA McAb dissolved in the coating buffer (100 μL/well) overnight at 4 °C. Next it was washed thrice with 300 μL of the washing buffer and blocked using 150 μL of the blocking buffer for 90 min at 37 °C. After thrice washing, 100 μL of sample solution containing Cu^{2+}-EDTA chelates was injected into the microplate and allowed to react for 90 min at 37°C. After thrice washing for removing unbound Cu^{2+}-EDTA chelates, the microplate was exposed to UV radiation for 2 h to release free Cu^{2+} ions. Then 100 μL of CdSe/ZnS QDs at 5 μg/mL was injected into the microplate. After 10 min, the FL signal was collected to quantify the concentration of Cu^{2+} ions.

ELISA tests for sixteen cations chelates

The microplate was coated with 10 μg/mL Cu^{2+}-EDTA-BSA conjugate dissolved in the coating buffer (100 μL/well) overnight at 4 °C. Next it was washed thrice with 300 μL of the washing buffer and blocked using 150 μL of the blocking buffer for 90 min at 37 °C. After thrice washing, the microplate was filled with 50 μL of mouse anti

Cu^{2+}-EDTA McAb-HRP conjugate and the same volume of cations chelates solution (cations concentration 250 ng/mL). After 90-min incubation at 37 °C, the microplate was washed thrice to remove the unbound reactants. Lastly a standard TMB color reaction was conducted to measure the absorbance value.

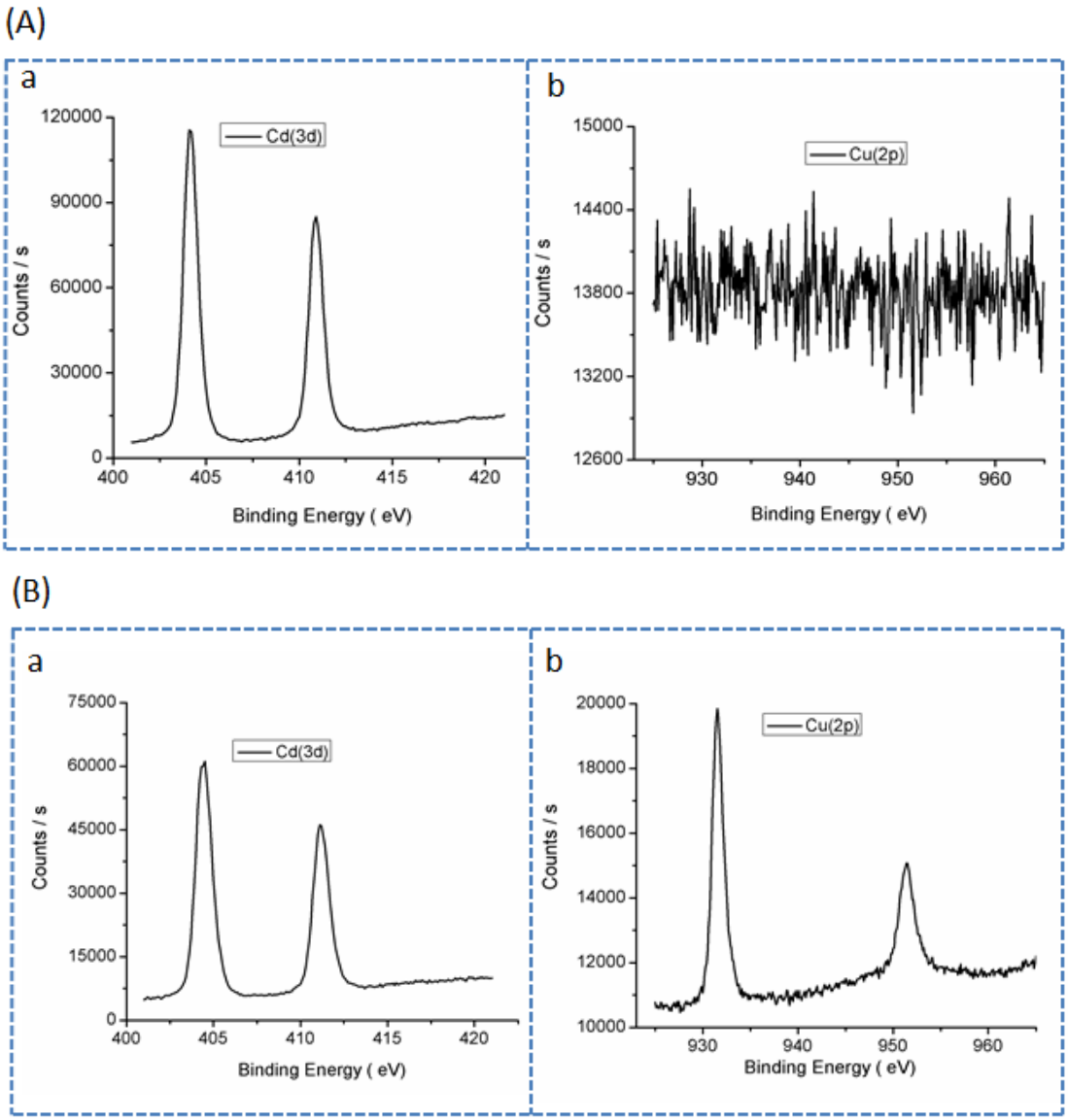

Fig. S1 (A) XPS spectra of Cd (a) and Cu (b) of untreated QDs. (B) XPS spectra of Cd (a) and Cu (b) of QDs reacted with Cu^{2+} ions.

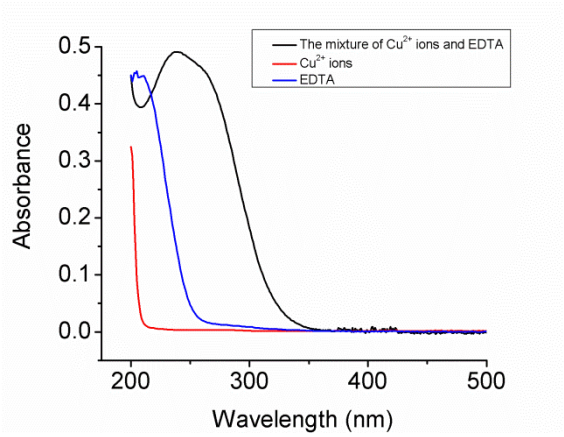

Fig. S2 The UV absorbance spectra of Cu^{2+} ions, EDTA and the mixture of Cu^{2+} ions and EDTA.

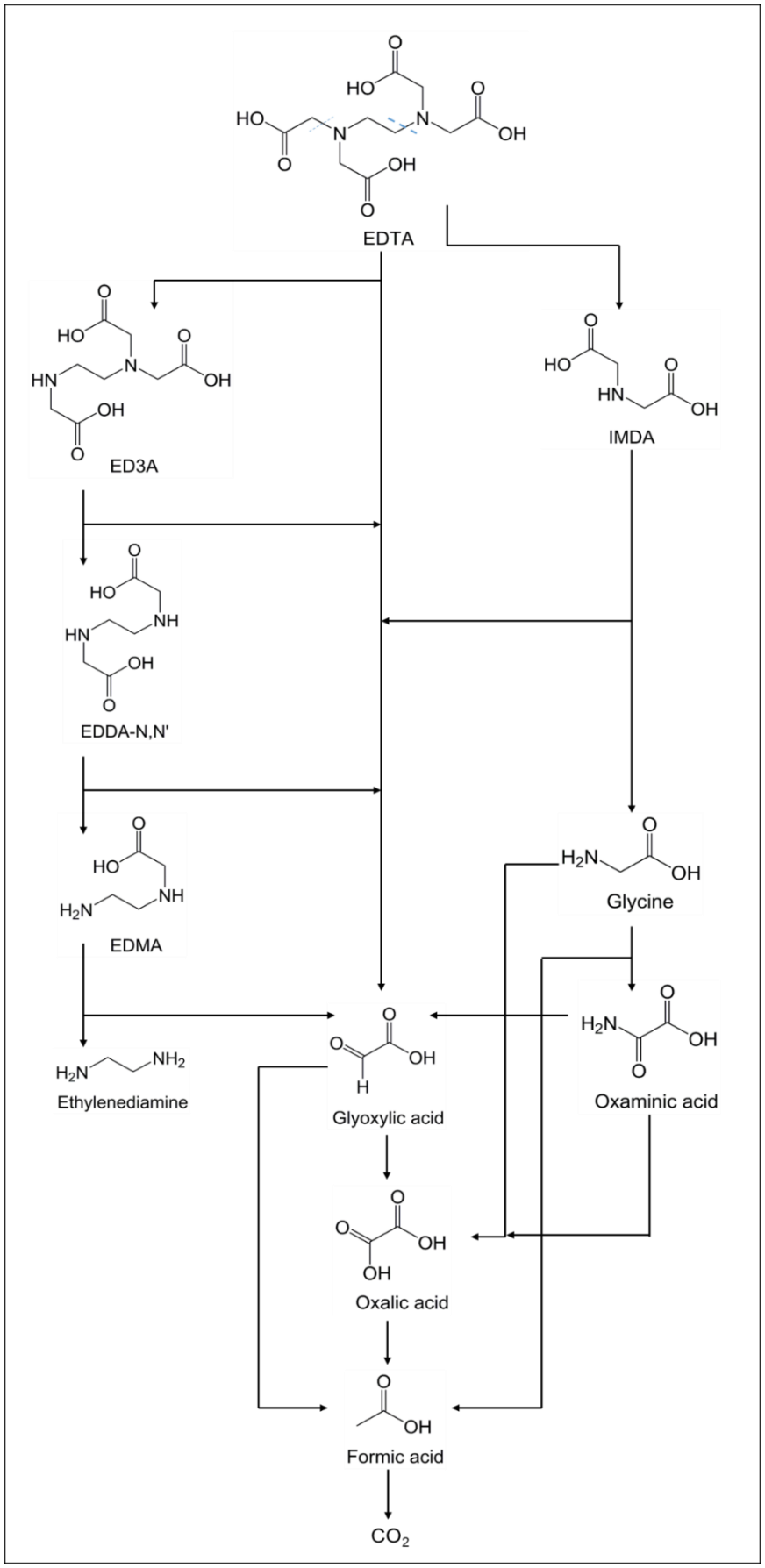

Fig. S3 The degradation mechanism of EDTA under UV exposure.

Table S1. Comparation of the proposed method and some typical reported methods.

Techniques	Sophisticated instrument	Specificity	Throughput	References
Atomic absorption spectrometry	Required	High	Low	4
Inductively coupled plasma mass spectroscopy	Required	High	Low	5
Inductively coupled plasma atomic emission spectrometry	Required	High	Low	6
X-ray fluorescence spectrometry	Required	High	Low	7
Electrochemical sensor	Not required	medium	High	9
Label-free fluorescent immunoassay	Not required	High	High	Proposed method

Notes and references

1 X. M. Wang, F. Q. Liu, Q. Shao, Z. R. Yin, L. M. Wang, Z. F. Fu, *Anal. Methods*, 2017, **9**, 2403.