Electronic supplementary information for

Coupling a novel spiro rhodamine B lactam derivative to Fe$_3$O$_4$ nanoparticles for

visual detection of free copper ion with high sensitivity and specificity

Hongyan Zhang,[ab] Xiaoxue Zeng,[a] Danlong Chen,[a] Ying Guo,[a] Wenjing Jiang,[a]

Liangjun Xu,[a] FengFu Fu[a*]

[a]Key Lab of Analysis and Detection for Food Safety of Ministry of Education, Fujian

Provincial Key Lab of Analysis and Detection for Food Safety, College of Chemistry,

Fuzhou University, Fuzhou, Fujian, 350116, China

[b]College of Pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou,

Fujian, 350122, China

1. Reagents and Chemicals

Unless otherwise mentioned, all reagents were of analytical grade and were used without further purification. Rhodamine B (95 %), iron (III) acetylacetonate (98 %), dimethyl sulfoxide (DMSO, HPLC grade) and oleylamine were purchased from J&K Scientific Ltd. (Beijing, China). Hydrazine hydrate (100 %) and benzyl ether (99 %) were obtained from Acros Co., Ltd. (Beijing, China). Glyoxal (39 %), 3,4-dihydroxybenzhydrazide (97 %), and n-hexane were purchased from Aladdin Co., Ltd. (Shanghai, China). Dichloromethane and sodium acetate trihydrate were provided by Xilong Chemical Co., Ltd. (Guangdong, China). Methanol, absolute ethanol and silica gel (200 meshs) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water (18.2 MΩ/ cm) was obtained from a Millipore Milli-Q water purification system.
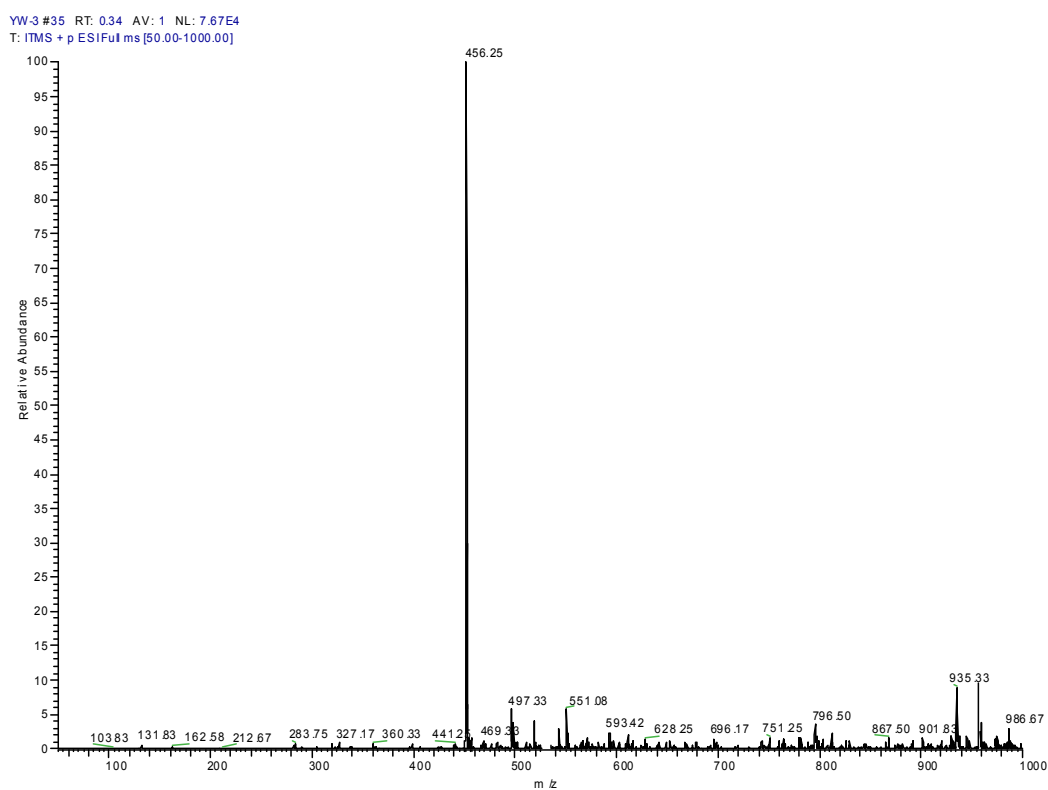
2. Apparatus

UV-visible spectra were recorded with a TU-1950 spectrophotometer (Persee, China). ESI-MS (electrospray ionization mass spectrometry) data was obtained with a LCQ Fleet 100452 liquid chromatography-mass spectrometer (LC-MS, Thermo Fisher, USA). ^{1}H-NMR (nuclear magnetic resonance) spectra was recorded by a Bruker AVANCE III NMR spectrometer (Germany) with chemical shifts reported as ppm (TMS as internal standard). The transmission electron microscopic images of magnetic nanoparticles were obtained by using a Tecnai F30 Field Emission Gun Transmission Electron Microscope (TEM, Philips-FEI, Netherlands) operating at an acceleration voltage of 300 kV. Fourier transform infrared (FT-IR) spectra were recorded by a Nicolet 6700 infrared spectrometer (Thermo Fisher, USA) in the range of 350-7800 cm^{-1} with a resolution of 4 cm$_{-1}$.

3. Synthesis and ESI-MS characterization of compound 1 and compound 2

About 1.20 g of rhodamine B (2.5 mmol) was dissolved in 30 mL absolute ethanol in a 100 mL flask, then 4.0 mL of hydrazine hydrate (64%) was dropwise added under vigorous stirring at room temperature. After the addition of hydrazine hydrate, the stirred mixture was sequentially refluxed for 2 h by heating the whole. The solution became clear and its colour changed from dark purple to light orange, then the mixture was evaporated to obtain solid by using rotary evaporation under reduced pressure. The solid in the flask was dissolved with 80 mL of 1 M HCl, then 1
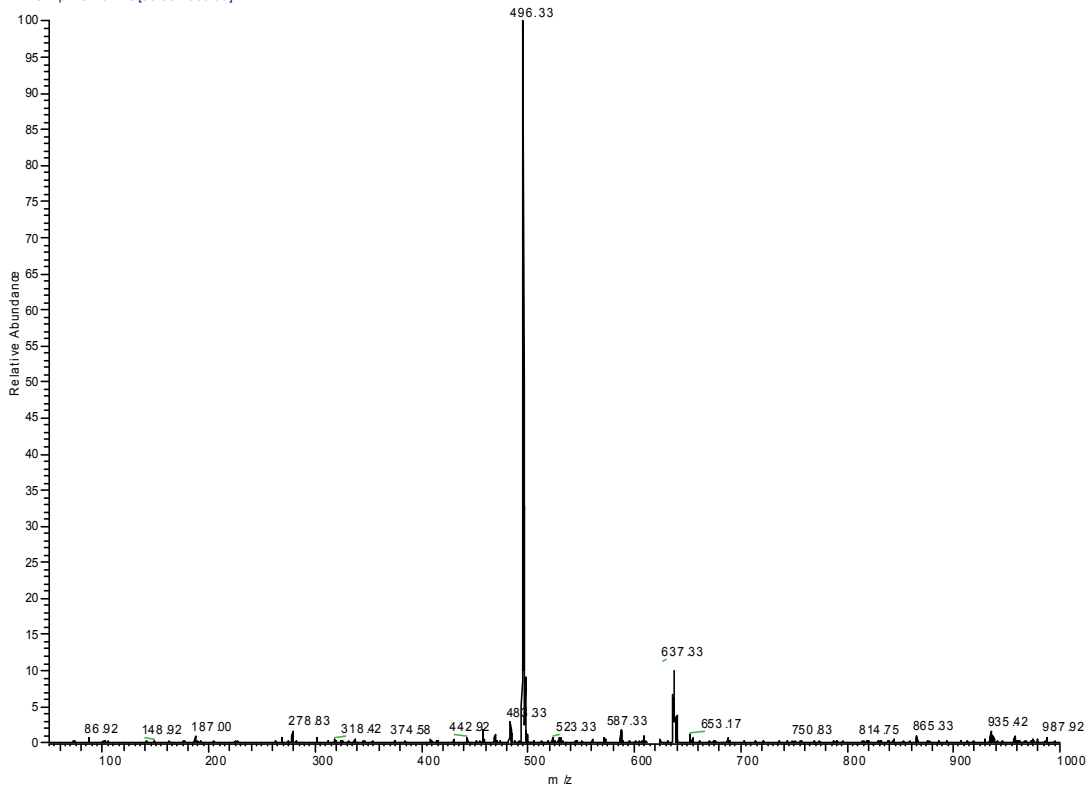
M NaOH solution (about 85 mL) was slowly added with stirring to adjust the pH of the solution to 9-10. The resulting precipitate was filtered and washed 3 times with 15 mL distilled water, and was dried under vacuum oven to afford compound 1 as pink solid (0.86 g, 75%).

Secondly, above 0.5 g of compound 1 (1.1 mmol) was dissolved in absolute ethanol (13 mL), and an excess of glyoxal (2.0 mL, 40 %) was added. The mixture was stirred rapidly for 8 h under the conditions of protection from light at room temperature. The resulting mixture was then added into 60 mL of saturated NaCl solution and the light red precipitate was observed immediately. The resulting precipitate was filtered and washed 3 times with cold ethanol (about 15 mL), and was dried under vacuum oven to afford compound 2 as yellow solid (0.46 g, 76%).

The ESI-MS analysis of compound 1

The ESI-MS analysis of compound 2

4. The ^{1}H-NMR characterization of RhBLA

^{1}H NMR (400 MHz, DMSO) δ 7.92 (d, J = 7.3 Hz, 1H), 7.19 (d, J = 8.3 Hz, 1H), 7.06 (d, J = 7.4 Hz, 1H), 6.80 (d, J = 8.2 Hz, 1H), 1.09 (t, J = 6.8 Hz, 10H).

Figure S1: The ^{1}H-NMR analysis of RhBLA. ^{1}H-NMR (400 MHz, C$_2$D$_6$OS, δ ppm)

5. The ^{13}C-NMR characterization of RhBLA

^{13}C NMR (400 MHz, DMSO) δ 40.06 (dp, J = 42.1, 21.0 Hz).

Figure S2: The ^{13}C-NMR analysis of RhBLA. ^{13}C-NMR (400 MHz, C$_2$D$_6$OS, δ ppm)

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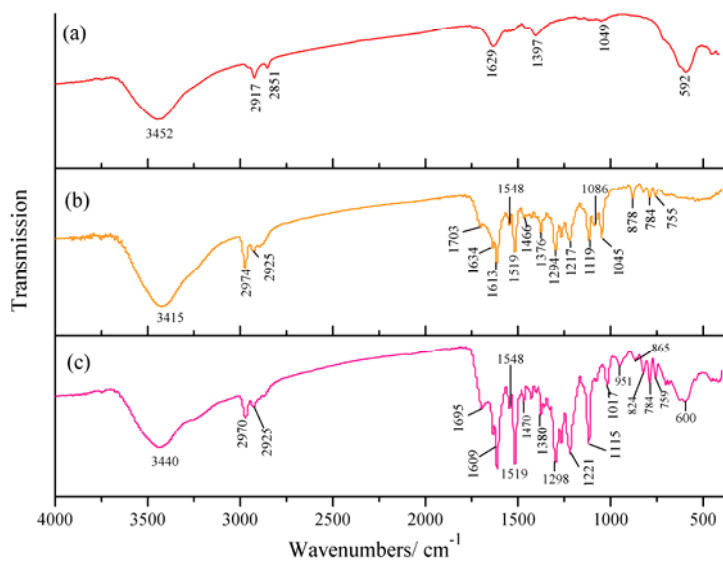## 6. The characterization of RhBLA-functionalized magnetic Fe₃O₄ NPs

Figure S3: FT-IR analytical results. (a): pure RhBLA; (b): Fe_3O_4 NPs; (c): RhBLA-functionalized Fe_3O_4 NPs.

7. Optimization of the chelating conditions between RhBLA and Cu^{2+}
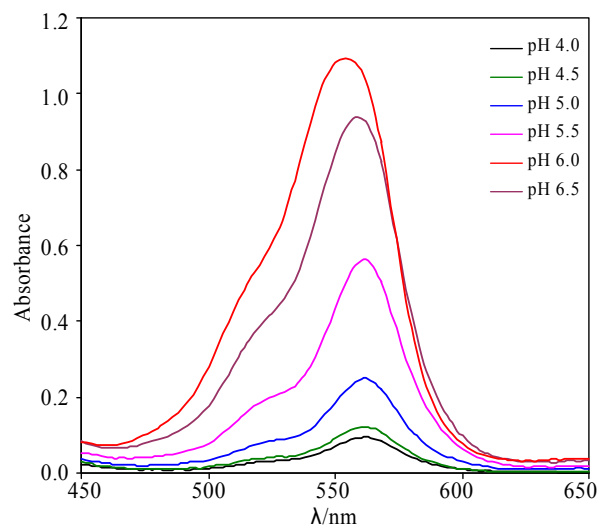

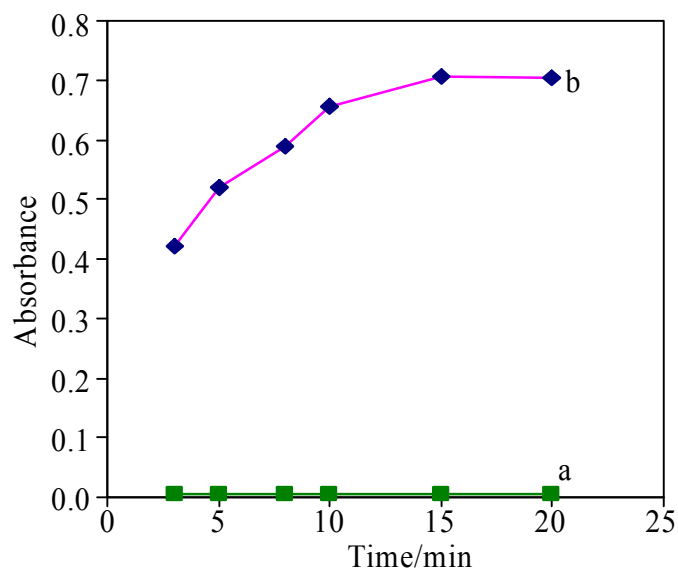Figure S4: The effect of pH on chelation of RhBLA and Cu^{2+}.

Figure S5: The effect of reaction time on chelation of RhBLA and Cu^{2+}. (a): reagent blank; (b):
RhBLA-Cu^{2+} complex.

8. *Color response of pure RhBLA to various metallic ions and different concentrations of Cu^{2+}.*

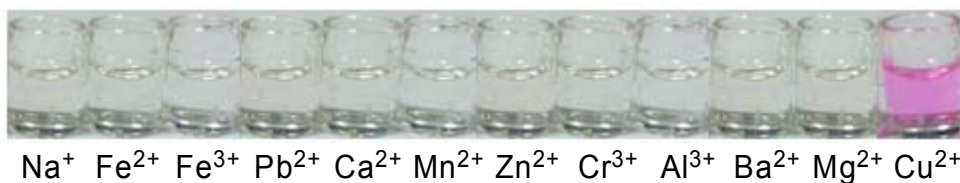Na⁺ Fe²⁺ Fe³⁺ Pb²⁺ Ca²⁺ Mn²⁺ Zn²⁺ Cr³⁺ Al³⁺ Ba²⁺ Mg²⁺ Cu²⁺

Figure S6: The photograph of pure RhBLA solution (in HAc-NaAc/methanol buffer, v/v=1/1, 50 mM, pH=6.0) in the presence of 35 μM Cu^{2+} or 50 μM of other metal ions.

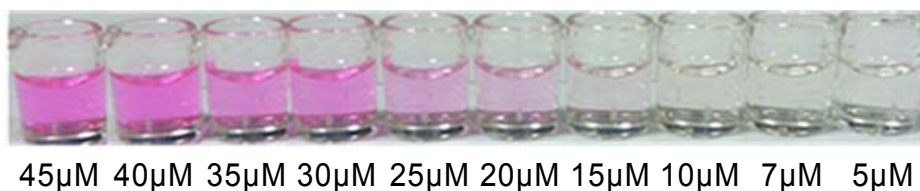45μM 40μM 35μM 30μM 25μM 20μM 15μM 10μM 7μM 5μM

Figure S7: The photographs of pure RhBLA solution (in HAc-NaAc/methanol buffer, v/v=1/1, 50 mM, pH=6.0) in the presence of different concentrations of Cu^{2+}.

Table S1: Comparison on the sensitivity among previous visual methods for detecting Cu^{2+} and this study.

Assay method	Visual detection limit	Ref.
Colorimetric recognition of Cu(II) by (2-dimethylaminoethyl)amino appended anthracene-9,10-diones	About 5 μM	[1]
Visual method based on azide- and alkyne-functionalized polydiacetylene vesicles	5 μM	[2]
Visual Detection of Copper(II) by Azide- and Alkyne-Functionalized Gold Nanoparticles Using Click Chemistry	50 μM	[3]
Colorimetric Cu^{2+} detection with a ligation DNAzyme and nanoparticles	10 μM	[4]
Visual detection based on phosphatidylserine-functionalized AuNPs	30 μM	[5]
Method by combined Fe_3O_4 NPs-Based Solid Phase Extraction with a Functionalized Gold Nanoparticle Probe	About 3.2 nM by using 200 mL sample	[6]
Method based on spiro rhodamine B lactam derivative (RhBLA)-functionalized Fe_3O_4 NPs	50 nM by using 100 mL sample	This study

References:

[1] N. Kaur and S. Kumar, *Dalton transactions* 2006, 3766.

[2] Q.-L. Xu, K. M. Lee, F. Wang and J. Yoon, *J. Mater. Chem.* 2011, 21, 15214.

[3] Y. Zhou, S.-X. Wang, K. Zhang and X.-Y. Jiang, *Angew. Chem. Int. Ed.* 2008, 120, 7564.

[4] J.-W. Liu and Yi Lu, *Chem. Commun.* 2007, 4872.

[5] W.-J. Yang, Y. He, L.-J. Xu, D.-L. Chen, M.-X. Li, H.-Y. Zhang and F.-F. Fu, *J. Mater. Chem. B* 2014, 2, 7765.

[6] Z.-Q. Tan, J.-F. Liu and G.-B. Jiang, *Nanoscale* 2012, 4, 6735.