Supplementary Material (ESI) for Organic and Biomolecular Chemistry
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Supporting Information for

Versatile trifunctional chemosensor of rhodamine derivative for Zn^{2+}, Cu^{2+} and His/Cys in aqueous solution and living cell

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1. Materials and Apparatus

Unless otherwise mentioned, all the reagents were of analytic grade. ^{1}H-NMR and ^{13}C-NMR spectras were measured on a Bruker AM-400 spectrometer with chemical shifts reported as ppm (in $CDCl_3$, TMS as internal standard). Mass spectrometry were obtained with a HP 5989A spectrometer. All pH measurements were made with a Sartorius basic pH-Meter PB-20. Absorption spectra were determined on a Varian Cary 100 Spectrophotometer. Fluorescence spectra were determined on a Varian Cary Eclipse. HPLC strace was determined on a HP-1100 spectrometer. IR was obtained with a Nicolet-470 spectrometer.

2. Synthesis of chemosensor RP

Rhodamine B hydrozide (1): In this work, **1** was synthesized according to the reference[1]. To a 50 mL flask, rhodamine B (480 mg, 1.0 mmol) was dissolved in 12 mL ethanol. 1.2 mL (excess) hydrazine hydrate (85%) was then added. After the addition, the mixture was heated to reflux for 2 h. The solution changed from dark purple to light orange and became clear. Then the mixture was cooled and solvent was removed under redured pressure. 1 M HCl (about 20 mL) was added to the solid in the flask to generate a clear red solution. After that, 1 M NaOH (about 28 mL) was added slowly with stirring until the pH of the solution reached 9~10. The resulting precipatate was filtered and washed 3 times with 6 mL water, and then dired in air. The product was then chromatographed on silica gel using dichloromethane/methanol 30: 1 (v/v) as eluant to afford 365 mg (80%) **1** as pink solid.

RP: 1 (0.21 mmol, 100 mg) was dissolved in 2 mL absolute methanol, di-2-pyridyl ketone (0.42 mmol, 78 mg) were added, and then 2 drops of acetic acid were added to the mixture. The mixture was heated to reflux and stirred for 3 h. After that, the solvent was removed in vacuum to give a purple solid. The crude product was then chromatographed on silica gel using dichloromethane/methanol 20: 1 (v/v) as eluant to afford 88 mg (65%) **RP** as purple solid. Mp: 125.2 ^{o}C. IR (KBr), υ: 2965, 2354, 2327, 1704, 1615, 1502, 1454, 1430, 1301, 1218, 1116, 791, 745 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.20 (t, J = 7.2 Hz, 12H), 3.35-3.40 (m, 8H), 6.34 (dd, J_1 = 9.0 Hz, J_2 = 2.6 Hz, 2H), 6.43 (d, J = 2.4 Hz, 2H), 6.63 (d, J = 8.8 Hz, 2H), 7.17-7.23 (m, 3H), 7.34 (d, J = 7.6 Hz, 1H), 7.42 (t, J = 7.0 Hz, 1H), 7.48 (td, J_1 = 7.4 Hz, J_2 = 1.0 Hz, 1H), 7.58 (td, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 7.65 (td, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.78 (d, J = 7.2 Hz, 1H), 8.44 (d, J = 4.4, 1H), 8.62 (d, J = 4.4, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 12.66, 44.40, 67.74, 98.00, 106.99, 107.78,

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123.08, 123.31, 123.76, 123.98, 124.03, 124.09, 128.19, 130.41, 132.66, 135.37, 136.17, 148.43, 148.70, 148.99, 151.39, 153.82. 154.70, 156.10, 160.87, 165.47. HRMS (ES+): Calcd for $([M+H])^+$, 623.3134, found, 623.3138.

3. The characterization data of chemosensor RP

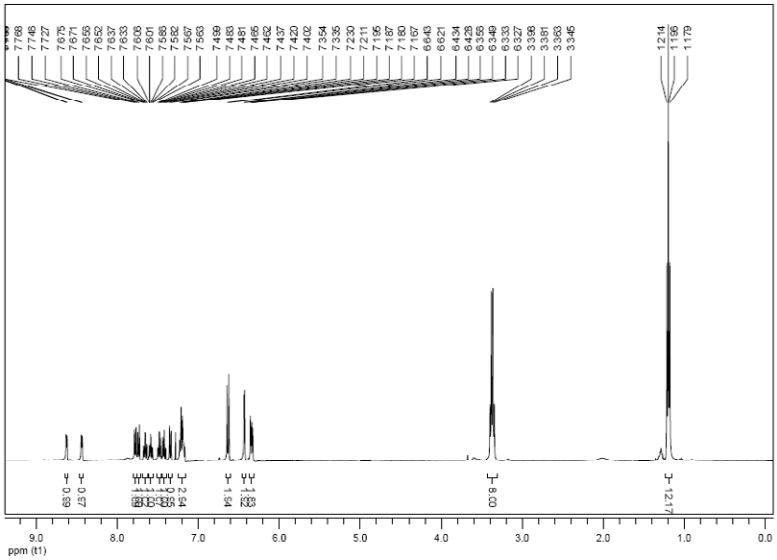

Fig. S1 (a) The ^{1}H NMR spectra of **RP**

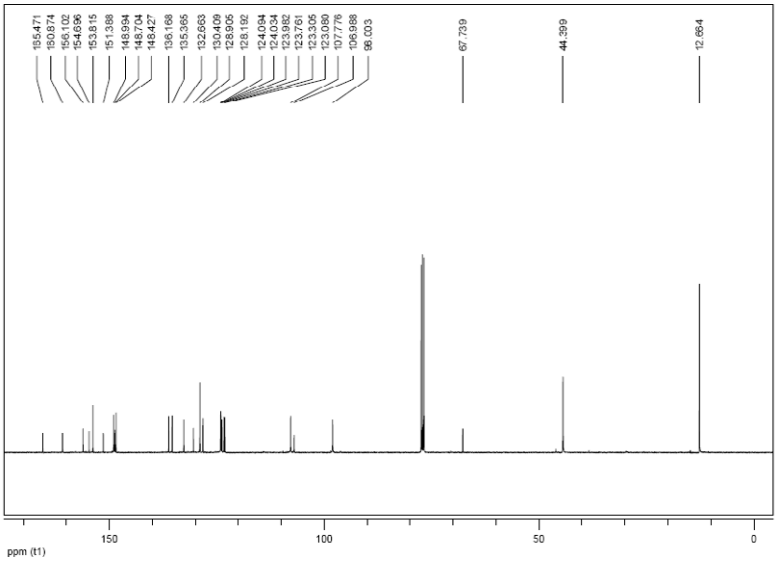

Fig. S1 (b) The ^{13}C NMR spectra of **RP**

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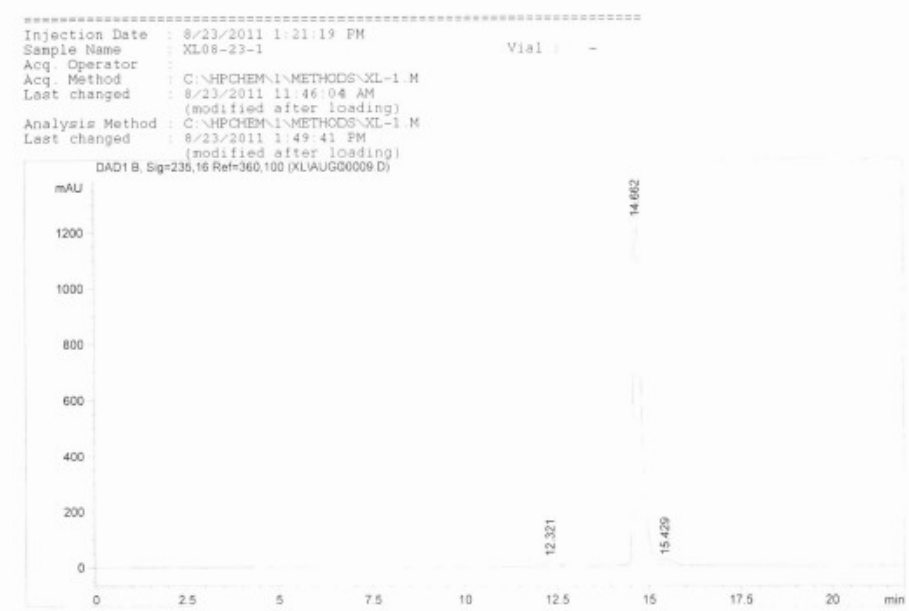

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 B, Sig=235,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.321	PB	0.2240	328.28760	21.75647	1.6215
2	14.662	BB	0.2297	1.93609e4	1315.40686	95.6295
3	15.429	BB	0.3587	556.55426	24.34593	2.7490
Totals :				2.02457e4	1361.50926	

Fig. S1 (c) HPLC trace of **RP**

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4. The pH-titration of free RP and [RP@Zn^{2+}]

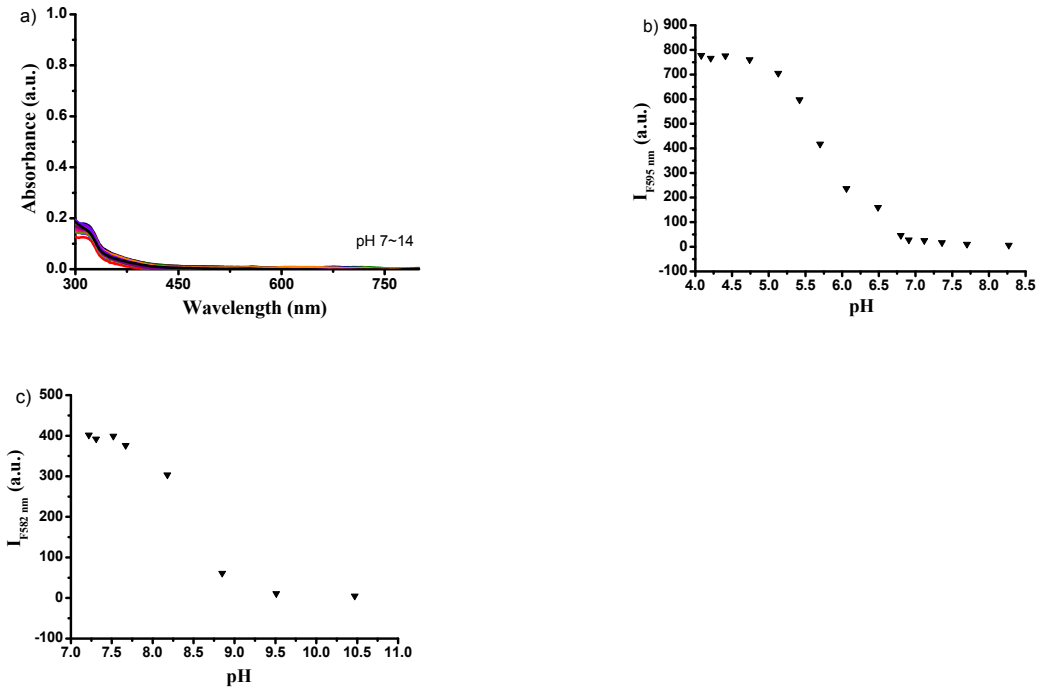

Fig. S2 The influence of pH on the UV-vis absorption and fluorescence of **RP** (10 μM) without Zn^{2+} (a and b) and with 10 μM Zn^{2+} (c) in MeCN/water solution (5:5, v/v), the pH of the solution was adjusted by adding 10% $HClO_4$ or 2 M NaOH. Excitation was performed at 562 nm.

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5. The selective and competitive experiments

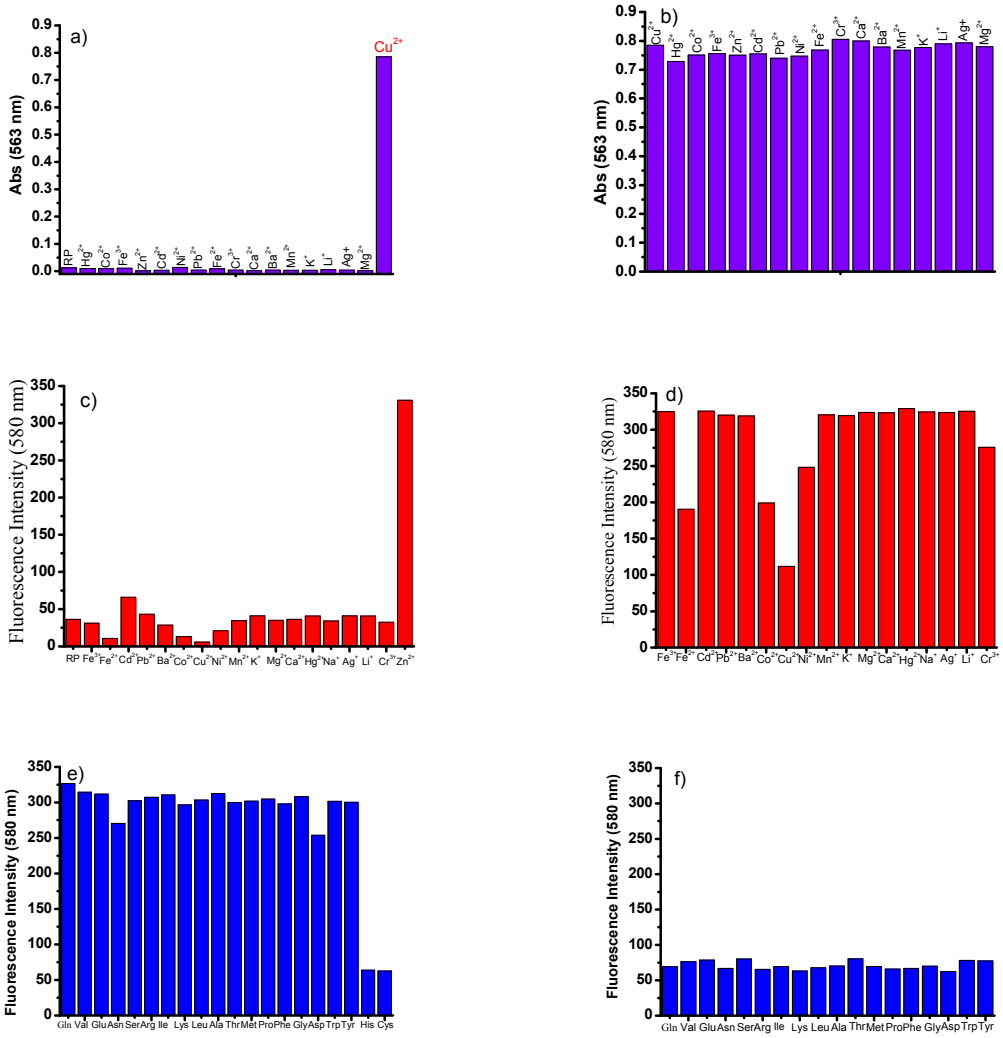

Fig. S3 Left figures were the selective experiments and right figures were the competitive experiments. Figures (a, b) all metal ions were 20 μM; figures (c, d) all metal ions were 10 μM; figure (e) all amino acids were 100 μM; figure (f) the competitive experiments of His (all amino acids were 100 μM). Excitation was performed at 530 nm.

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6. Job's plot of RP and Cu^{2+}, Zn^{2+}

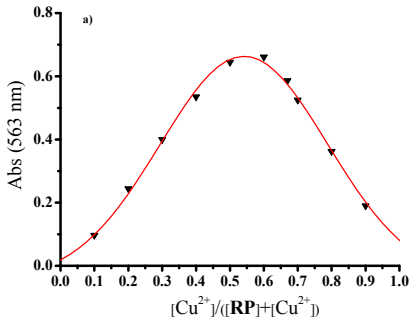

Fig. S4 (a) Job's plot of **RP** and Cu^{2+} ([**RP**]+[Cu^{2+}]=20 μM) in DMSO-H_2O (19:1, v/v).

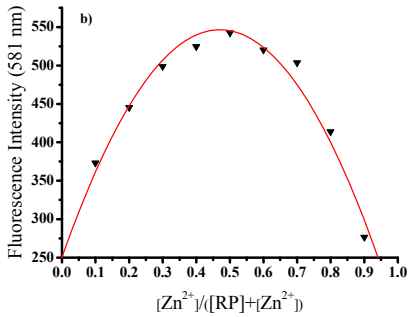

Fig. S4 (b) Job's plot of **RP** and Zn^{2+} ([**RP**]+[Zn^{2+}]=20 μM) in CH_3CH_2OH-H_2O (40:60, v/v, 10 mM Tris-$HClO_4$, pH 7.40). Excitation was performed at 562 nm.

7. ESI-MS of RP in the presence of Cu^{2+} or Zn^{2+}

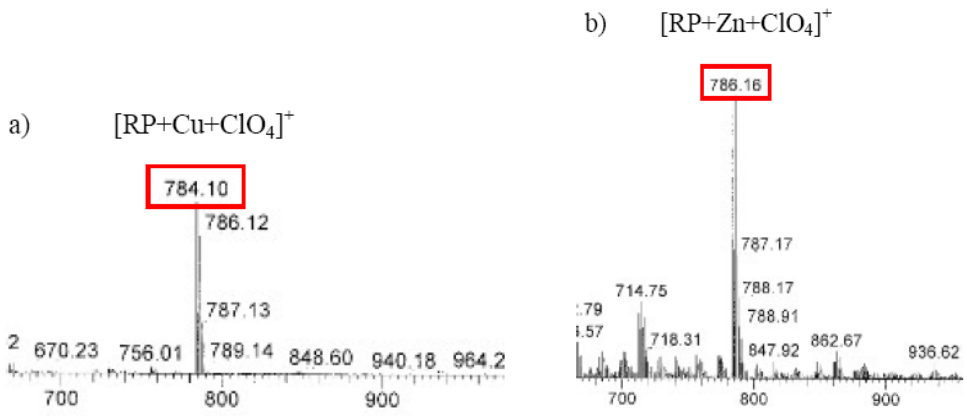

Fig. S5 (a) ESI-MS of **RP** in the presence of Cu^{2+} in DMSO.
(b) ESI-MS of **RP** in the presence of Zn^{2+} in CH_3CH_2OH.

8. Partial ^{1}H NMR spectra of RP and Zn^{2+}

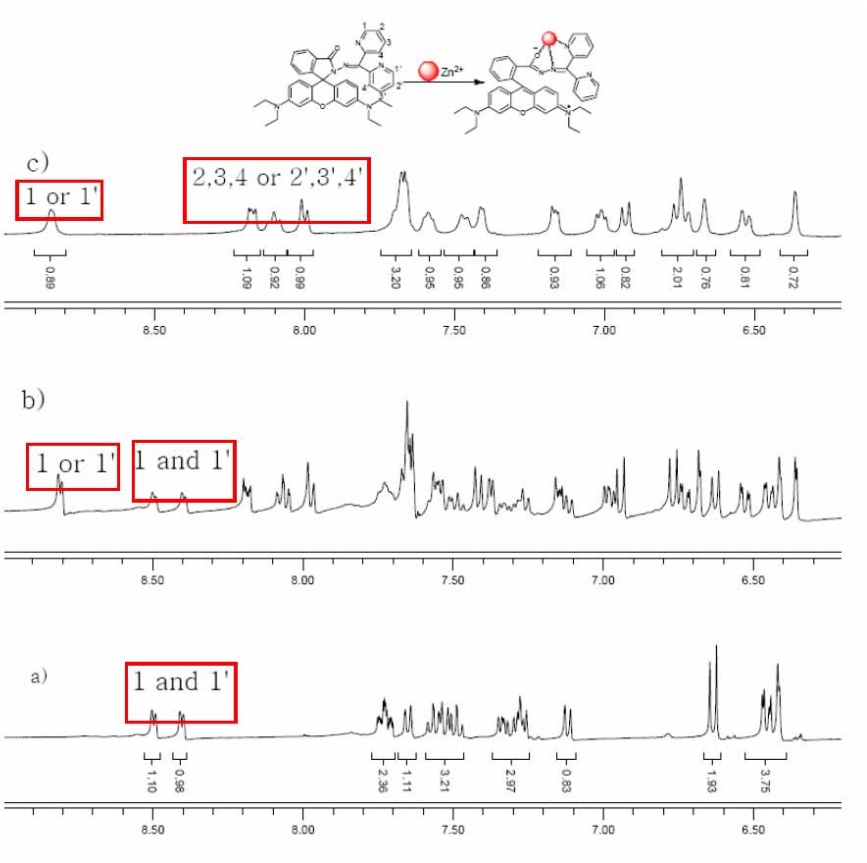

Fig. S6 ^{1}H-NMR spectra of (a) free sensor **RP**, (b) sensor **RP** + Zn^{2+} (0.3 eq), and (c) sensor **RP** + Zn^{2+} (0.5 eq) in CD_3CN. Inset: proposed binding mode of sensor **RP** with Zn^{2+}.

9. Partial ^{1}H NMR spectra of RP and Cu^{2+}

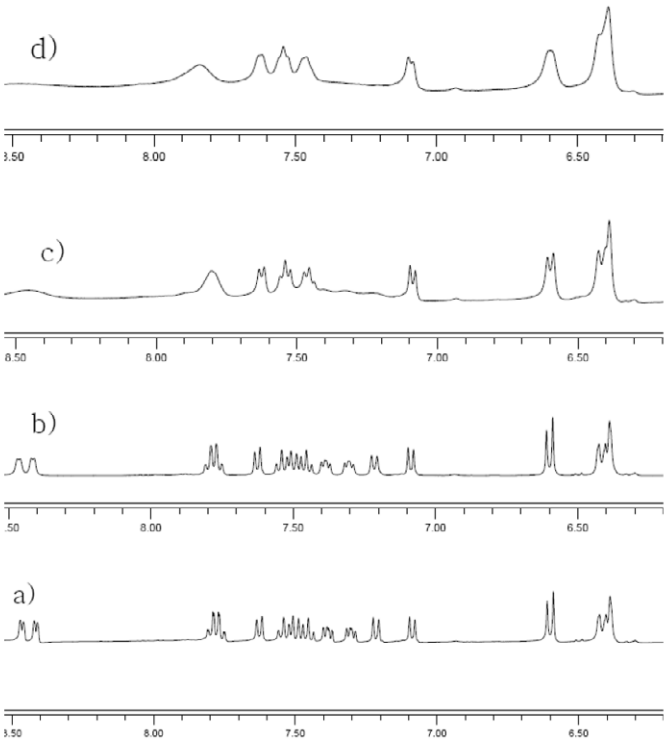

Fig. S7 ^{1}H-NMR spectra of (a) free sensor **RP**, (b) sensor **RP** + Cu^{2+} (0.01 eq), (c) sensor **RP** + Cu^{2+} (0.03 eq) and (d) sensor **RP** + Cu^{2+} (0.09 eq) in DMSO-d_6.

10. Reference

[1] Xiang, Y.; Tong, A.; Jin, P.; Ju, Y. *Org. Lett.*, 2006, **8**, 2863-2866.