A Zn^{2+}-specific turn-on fluorescent probe for ratiometric sensing of pyrophosphate in both water and blood serum

Jinghan Wen, [a] *Zhirong Geng,* [a] *Yuxin Yin,* [a] *Zhong Zhang,* [b] *Zhilin Wang**,[a]

[a]State Key Laboratory of Coordination Chemistry, Coordination Chemistry Institute, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P. R. China, and [b]School of Chemistry and Chemical Engineering, Guangxi Normal University, Guilin 541004, P. R. China

E-mail: wangzl@nju.edu.cn

Supplementary Material (ESI) for Dalton Transactions

Content

Supplementary Material (ESI) for Dalton Transactions

1. Experimental

General

All the starting materials were of reagent quality and were obtained from commercial sources without further purification. Fetal bovine serum was bought from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. Inorganic pyrophosphatase from *Escherichia coli* was bought from New England Biolabs. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were determined by a Bruker DRX-500 spectrometer at 25±1□. All the UV-vis spectra were recorded by a Shimadzu UV-3100 spectrophotometer. The emission spectra were obtained using a PerkinElmer LS 55 fluorescence spectrometer. The pH values of sample solutions were monitored by a PHS-3 system. The electrospray ionization mass spectra were determined by a LCQ Fleet ThermoFisher mass spectrometer. 1,4,7-Tritosyl-1,4,7-triazaheptane was prepared according to a reported procedure[1] in 62% yield after crystallization. 2,6-Bis(bromomethyl)pyridine and the mother ring 3,6,9,15-tetraazabicyclo[9.3.1] pentadeca-1(15),11,13-triene (**L**) were synthesized following the procedure described by the references[2] in the yield of 49% and 65%, respectively.

Synthesis of 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3-methyl naphthalene (1)

1-chloromethyl naphthalene (0.0884 g, 0.5 mmol) was added to a solution of **L** (0.75 g, 3.64 mmol) and triethylamine (0.0608 g, 0.6 mmol) in one portion in freshly distilled $CHCl_3$ (10 mL). The resulting solution was then gently refluxed for 15 h under dry N_2. After cooling to room temperature, the obtained solution was washed with 1 M NaOH solution (5 mL×3) to remove the unreacted **L** and then with water (5 mL×3). Then the organic layer was dried over Na_2SO_4 and concentrated by evaporation. The crude product was purified by silica gel column chromatography with CH_2Cl_2/EtOH/$NH_3\cdot H_2O$ (3:1:0.1 v:v:v; R_f=0.28), affording **1** as a light yellow oil (0.095 g, 55%). ^{1}H NMR (500MHz, $CDCl_3$): δH 8.21 (d, *J*=8Hz, 1H), 7.65 (t, *J*=8Hz, 2H), 7.54 (m, 2H), 7.37 (t, *J*=7.5Hz, 1H), 7.31(t, *J*=7.5Hz, 1H), 7.17 (t, *J*=7.5Hz, 1H), 6.65 (d, *J*=7.5Hz, 1H), 6.36 (d, *J*=7.5Hz, 1H), 4.40 (s, 2H), 3.81 (s, 2H), 3.77 (s,2H), 3.39 (br s, 2H), 3.29 (br s, 2H), 3.03 (br s, 3H), 2.86 (br s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δC 160.47, 159.16, 136.56, 134.35, 133.63 132.29, 128.39, 128.07, 127.43, 126.12, 125.61, 124.99, 124.78, 119.38, 118.82, 60.18, 57.95, 53.56, 52.60, 47.39, 47.15, 46.69 ppm; Mass: ES-MS (CH_3CN), *m/z* (%): 347.42(100) [MH^+].

Spectroscopic study

The emission and excitation spectra of **1** and **1**-Zn^{2+} (20 μM) were determined in HEPES buffer (10 mM HEPES, pH 7.4). The pH dependence of emission was determined in 0.15 M NaCl aqueous solution at different pH values adjusted by 1 M HCl and 1 M NaOH. The excitation wavelength was 280 nm.

Selective fluorescent response of 1 to different metal cations

The fluorescent response of **1** to different metal cations was examined in HEPES buffer (10 mM, pH 7.4) containing 20 μM **1**. Metal cation aqueous solution (30 μL, 10 mM) was added to 3 mL of this solution, and the fluorescence spectra were determined after complete mixing. The excitation wavelength was 280 nm.

Zn^{2+} titration of 1 solution determined by fluorescence and UV

The fluorescent titration of **1** was investigated by adding aliquots of 1.2 μL of $ZnCl_2$ aqueous solution (1 mM) to 3 mL of **1** solution (40 μM, 10 mM HEPES, pH 7.4) in a cuvette. The spectra

Supplementary Material (ESI) for Dalton Transactions

were recorded immediately after mixing. The excitation wavelength was 280 nm. And the UV titration experiment resembled the fluorescent titration. Aliquots of 2 μL of $ZnCl_2$ solution (1 mM) were added to 1 mL of **1** solution (20 μM, 10 mM HEPES, pH 7.4).

Selective fluorescent response of 1-Zn^{2+} to different anions

The fluorescent response of **1**-Zn^{2+} to different anions was investigated by first adding 6 μL of $ZnCl_2$ aqueous solution (10 mM) to 3 mL of **1** solution (20 μM, 10 mM HEPES, pH 7.4) in a cuvette. Then the experiment was further carried out by adding 12 μL of anion aqueous solution (50 mM) to the obtained **1**-Zn^{2+} solution. The spectra were recorded immediately after mixing. The excitation wavelength was 280 nm.

PPi titration of 1-Zn^{2+} solution determined by fluorescence and UV

The fluorescent/UV titration was carried out by first adding $ZnCl_2$ aqueous solution (6 μL/2 μL, 10 mM) to 3 mL/1 mL HEPES buffer (10 mM, pH 7.4) containing 20 μM **1**. Then the fluorescent/UV titration was investigated by adding aliquots of 5 μL/2 μL of PPi aqueous solution (1 mM) to the obtained **1**-Zn^{2+} solution. All of the spectra were recorded after complete mixing.

Fluorescent measurements with 1-Zn^{2+} in serum

HPLC-grade CH_3CN (5 mL) was added into fetal bovine serum (2.5 mL). The suspension was vigorously shaken and then centrifuged at 12000 rpm for 5 min. The supernatant was collected and lyophilized. The residue was then dissolved in 2 mL HEPES buffer (10 mM, pH 7.4) to obtain reconstituted serum[3] and used as stock solution. Different volumes of the serum were first added to the cuvette, to which HEPES buffer (10 mM, pH 7.4) was then added and the final volume of the solution was maintained at 3 mL. Fluorescent titrations were followed by adding 6 μL of $ZnCl_2$ (10 mM) and 10 μL of **1** (6 mM) aqueous solution, and the final concentration of **1**-Zn^{2+} was 20 μM. After complete mixing, the fluorescent spectra were recorded.

Inorganic PPase assay

One unit of inorganic PPase we used in the assay is the amount of enzyme that generates 40 nmol of phosphate per minute from pyrophosphate under standard conditions (a 10 minute reaction at 75□ in 50 mM Tricine, pH 8.5, 1 mM $MgCl_2$, 0.32 mM PPi reaction volume of 0.5 mL). The solution for the assay we thereby prepared contained 50 mM Tricine (pH 8.5), 1 mM $MgCl_2$, 25 μM PPi, and 40 μM **1**-Zn^{2+}. The emission spectra were recorded immediately after complete mixing, and in the presence of each concentration of the enzyme, they were measured three times to ensure the accuracy.

References

1 H. Koyama and T. Yoshino, *Bull. Chem. Soc. Jpn.*, 1972, **45**, 481-484.

2 (a) X. M. Zhang, *WO Pat.*, 97/13763, 1997; (b) G. E. Kiefer, J. Simon and J. R. Garlich, *WO Pat.*, 94/26754, 1994.

3 A. J. Alpert and A. K. Shukla, *ABRF 2003: Translating biology using proteomics and functional genomics*, Poster n° P111-W, Denver, 2003.

2. ESI-MS, ^{1}H NMR and ^{13}C NMR spectra of 1

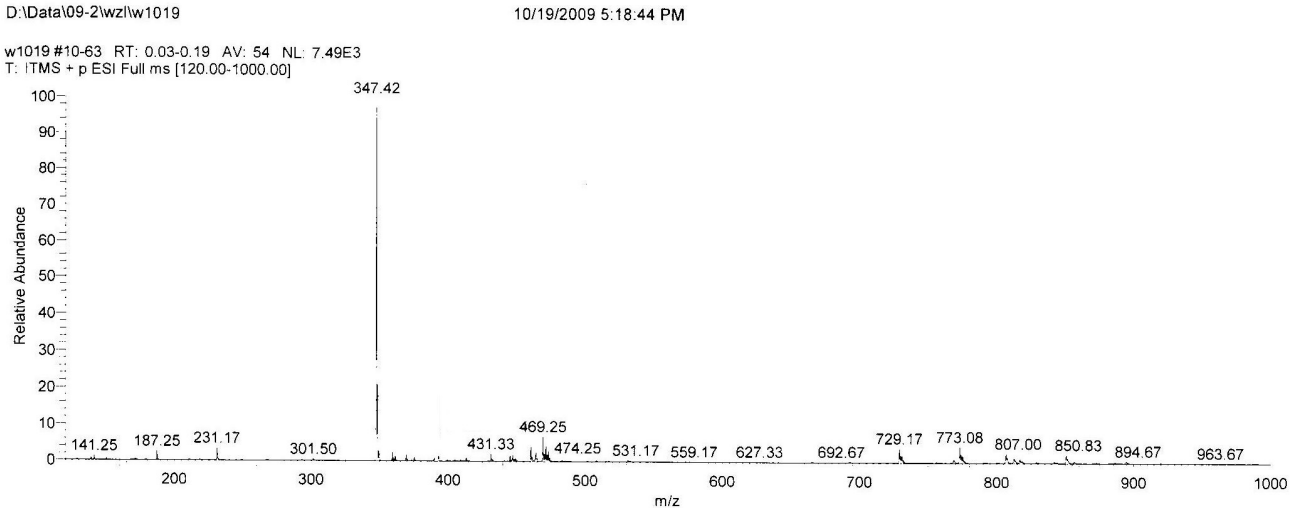

Fig. S1 ESI-MS spectrum of **1** in CH_3CN.

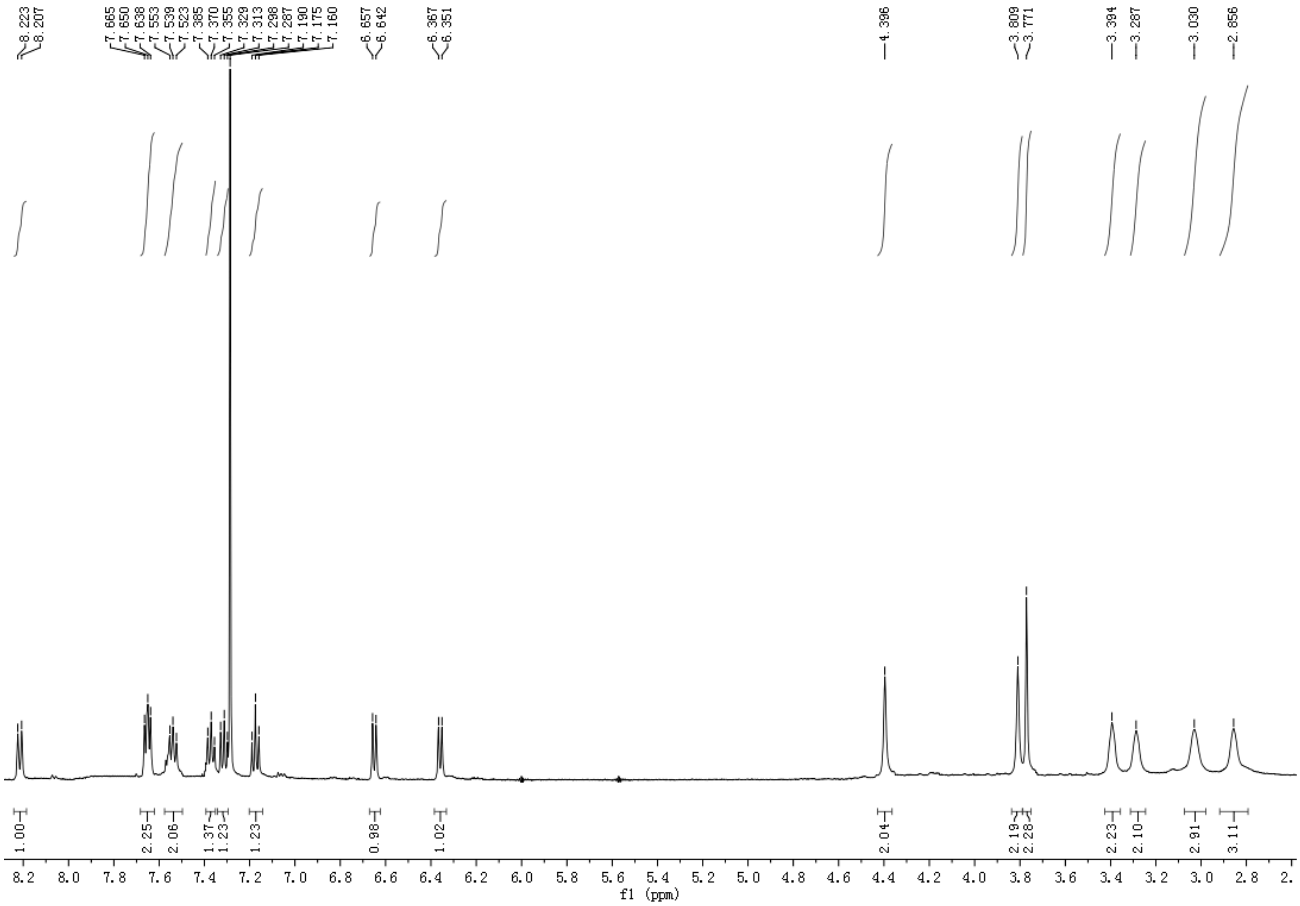

Fig. S2 ^{1}H NMR spectrum of **1** in $CDCl_3$ (500 MHz).

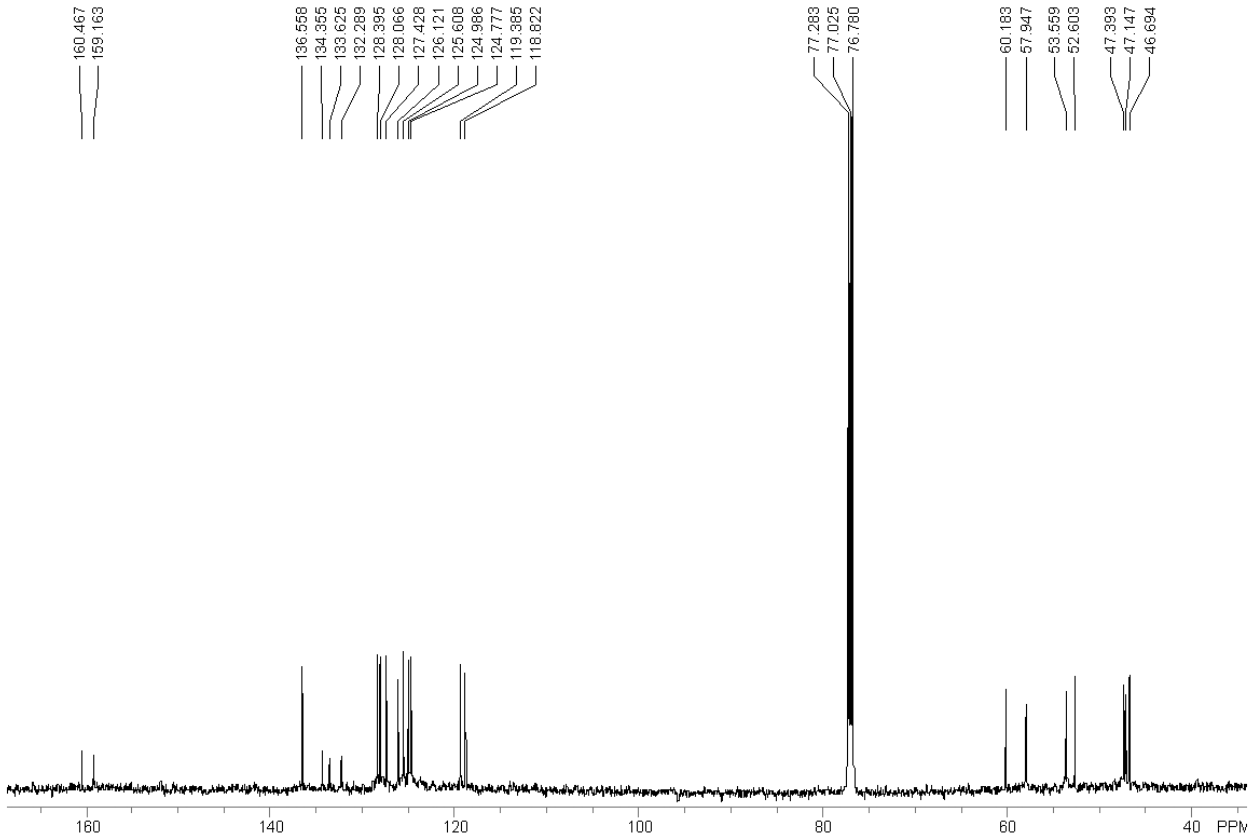

Fig. S3 ^{13}C NMR spectrum of **1** in $CDCl_3$ (125 MHz).

3. Zn^{2+} fluorescent response and binding behavior of 1

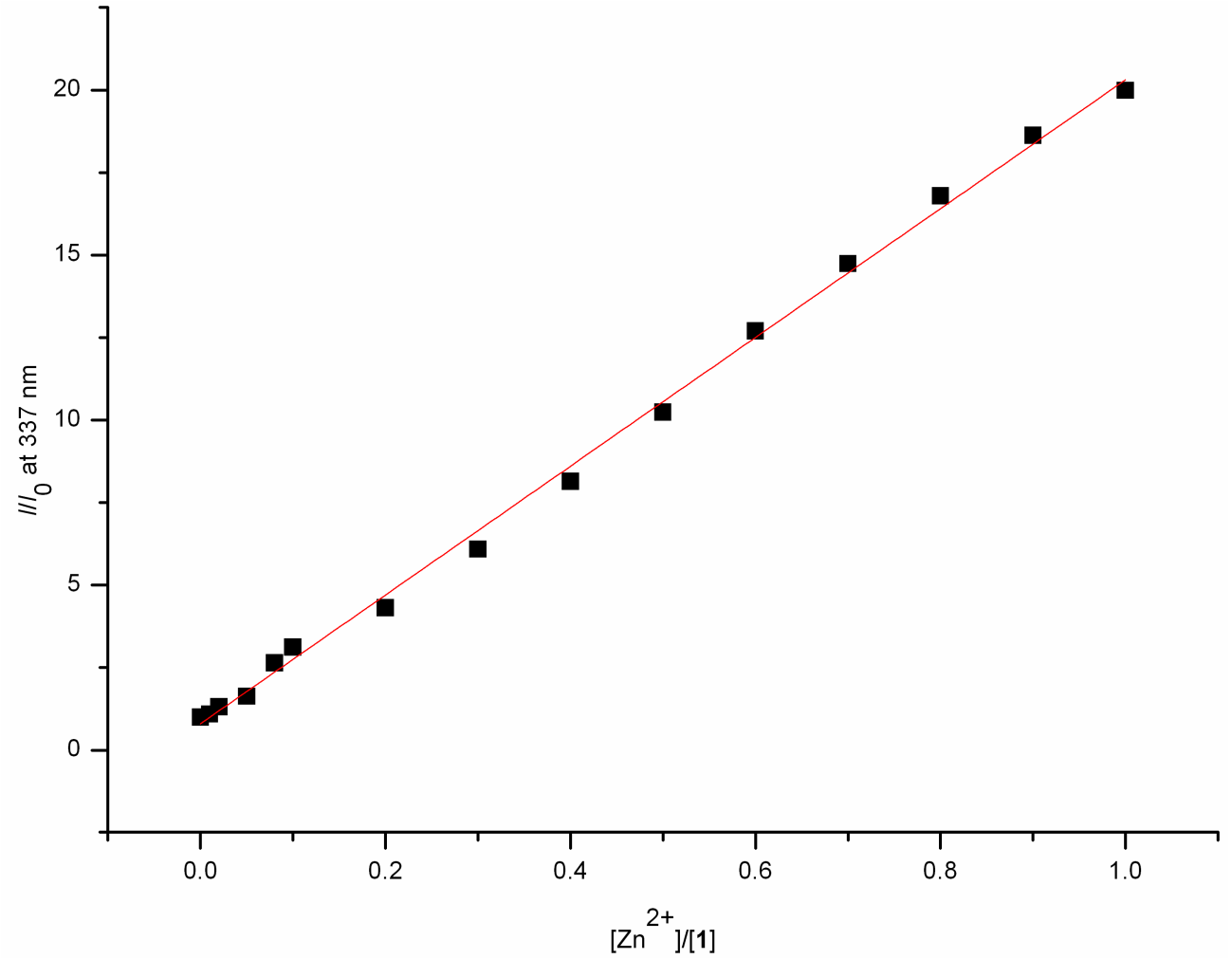

Fig. S4 The linear increasing range of the emission spectra of 40 μM **1** vs. the concentration of Zn^{2+} in HEPES buffer (10 mM, pH 7.4).

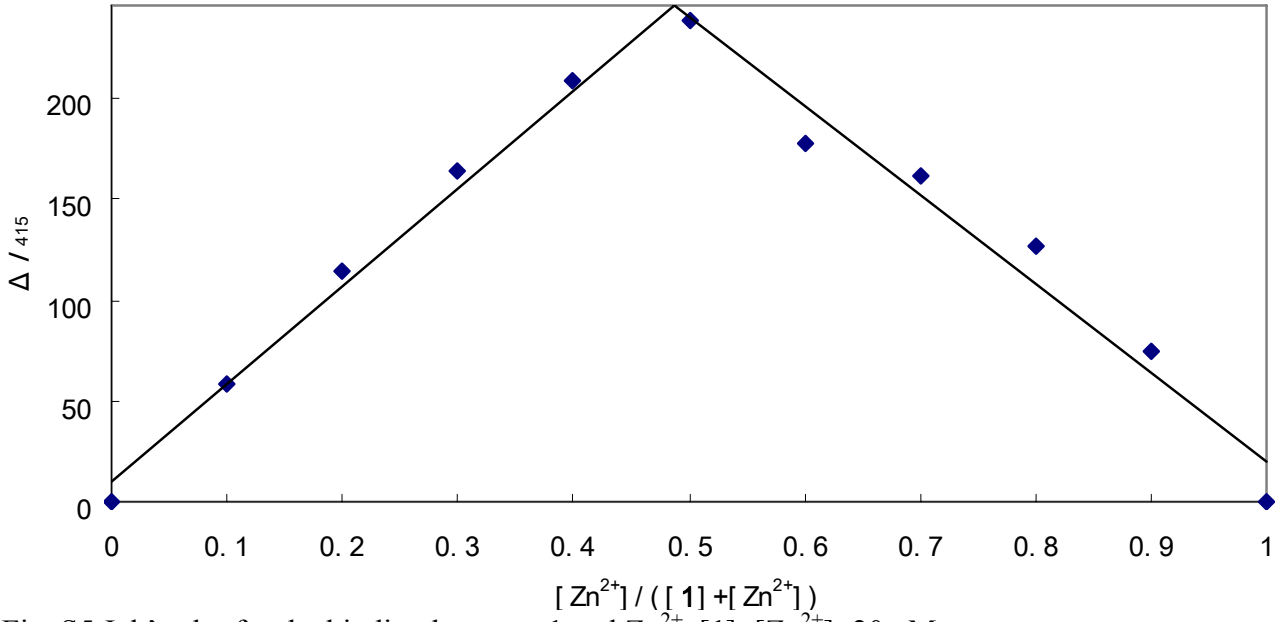

Fig. S5 Job's plot for the binding between **1** and Zn^{2+}. [**1**]+[Zn^{2+}]=20 μM.

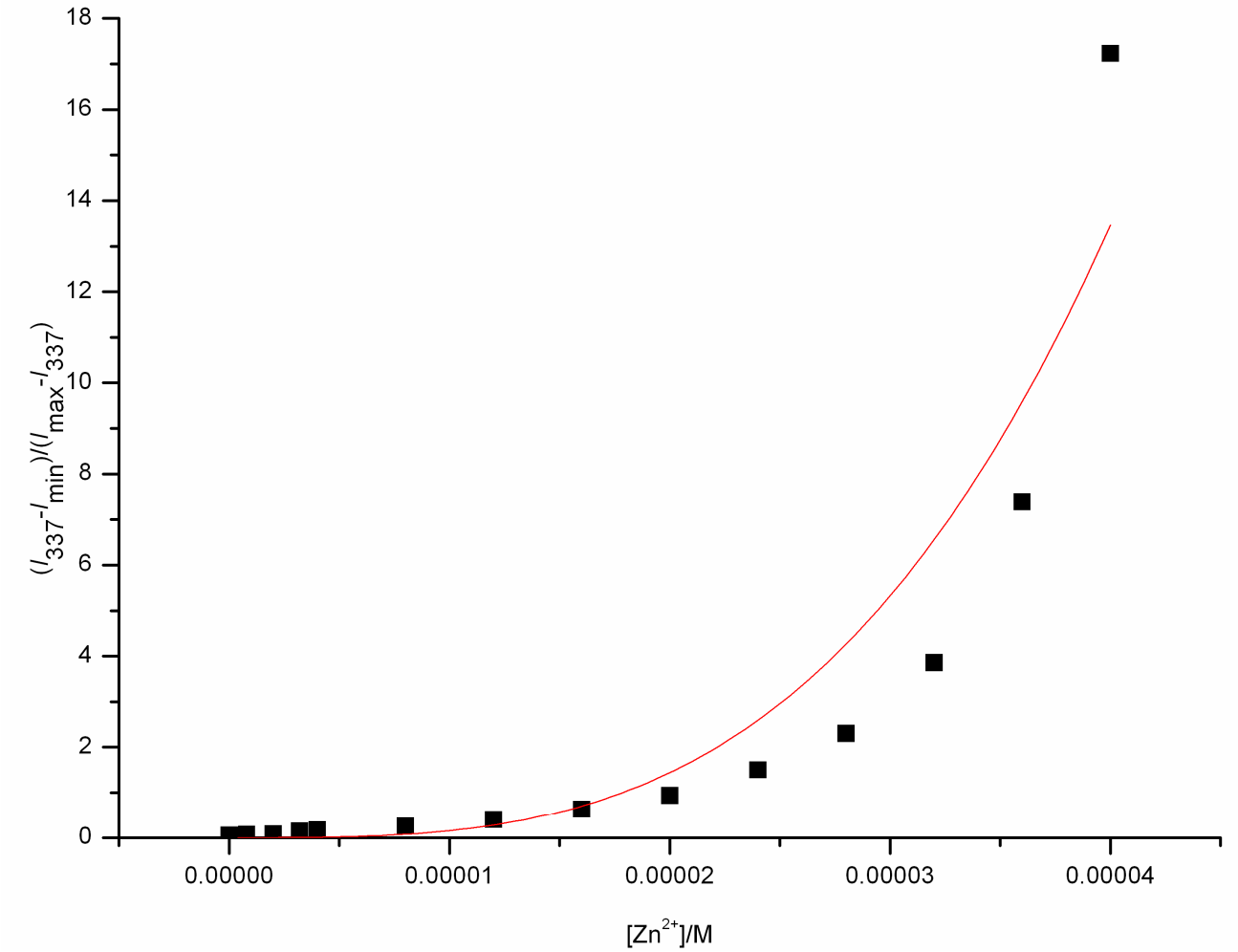

Fig. S6 The emission change of **1** induced by Zn^{2+} titration. The binding constant was obtained by nonlinear fitting to the data, and the red line is the resulted fitting line.

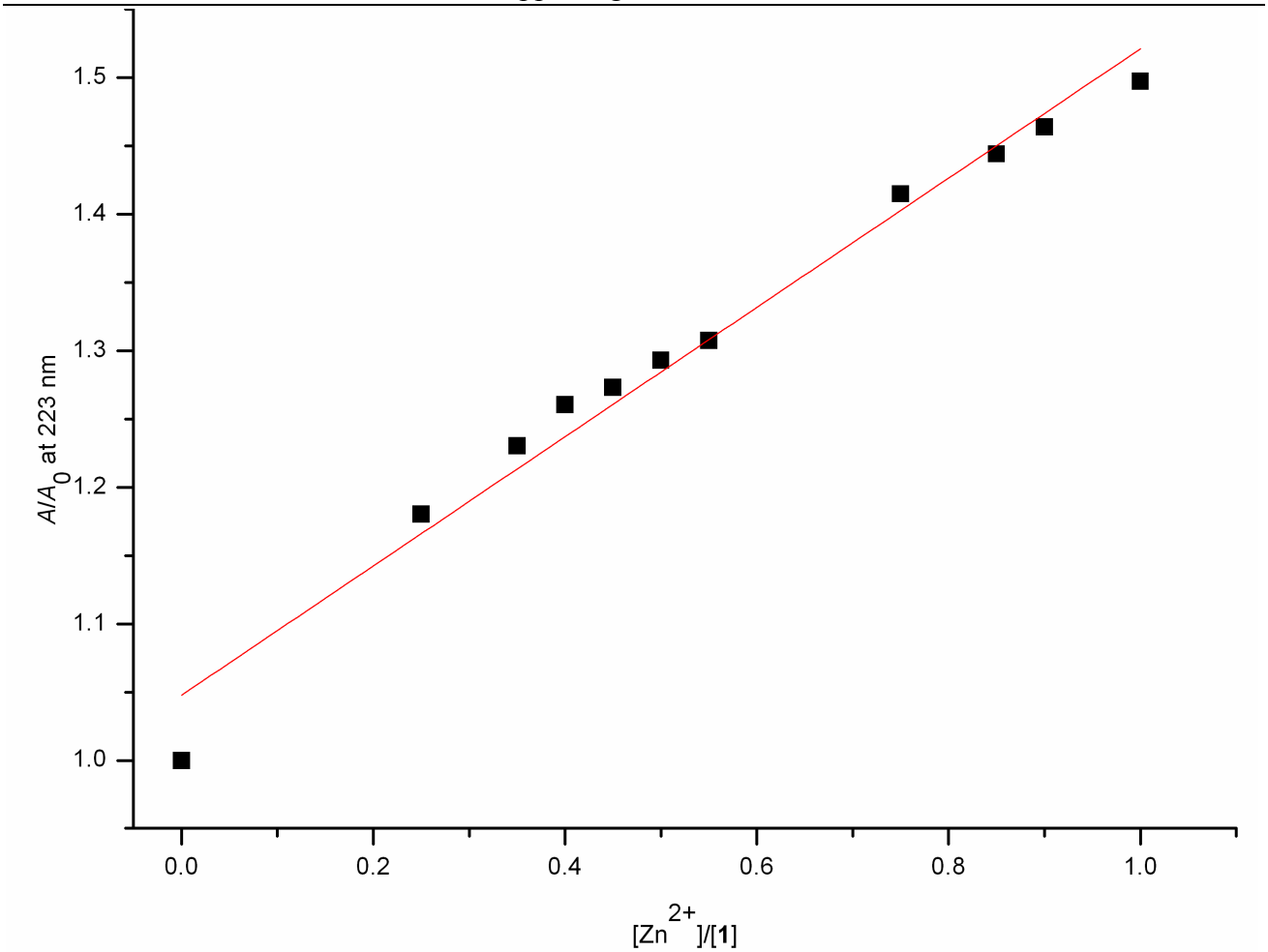

Fig. S7 The linear increasing range of the absorption spectra of 20 μM **1** vs. the concentration of Zn^{2+} in HEPES buffer (10 mM, pH 7.4).

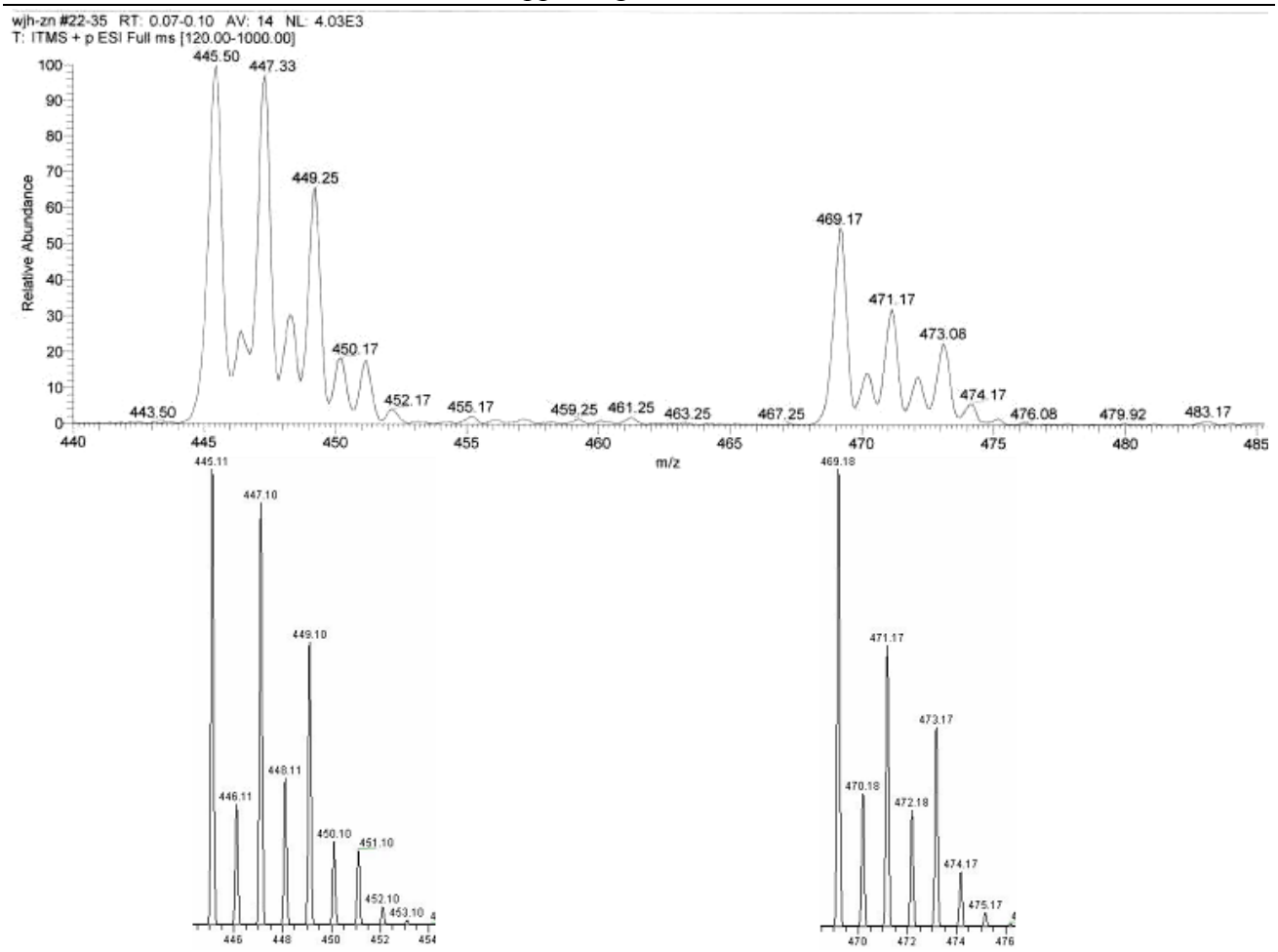

Fig. S8 Observed electrospray ionization(EI) mass spectrum and calculated isotope patterns for **1**-Zn^{2+}.

4. Fluorescent response and binding pattern of 1-Zn^{2+} with PPi

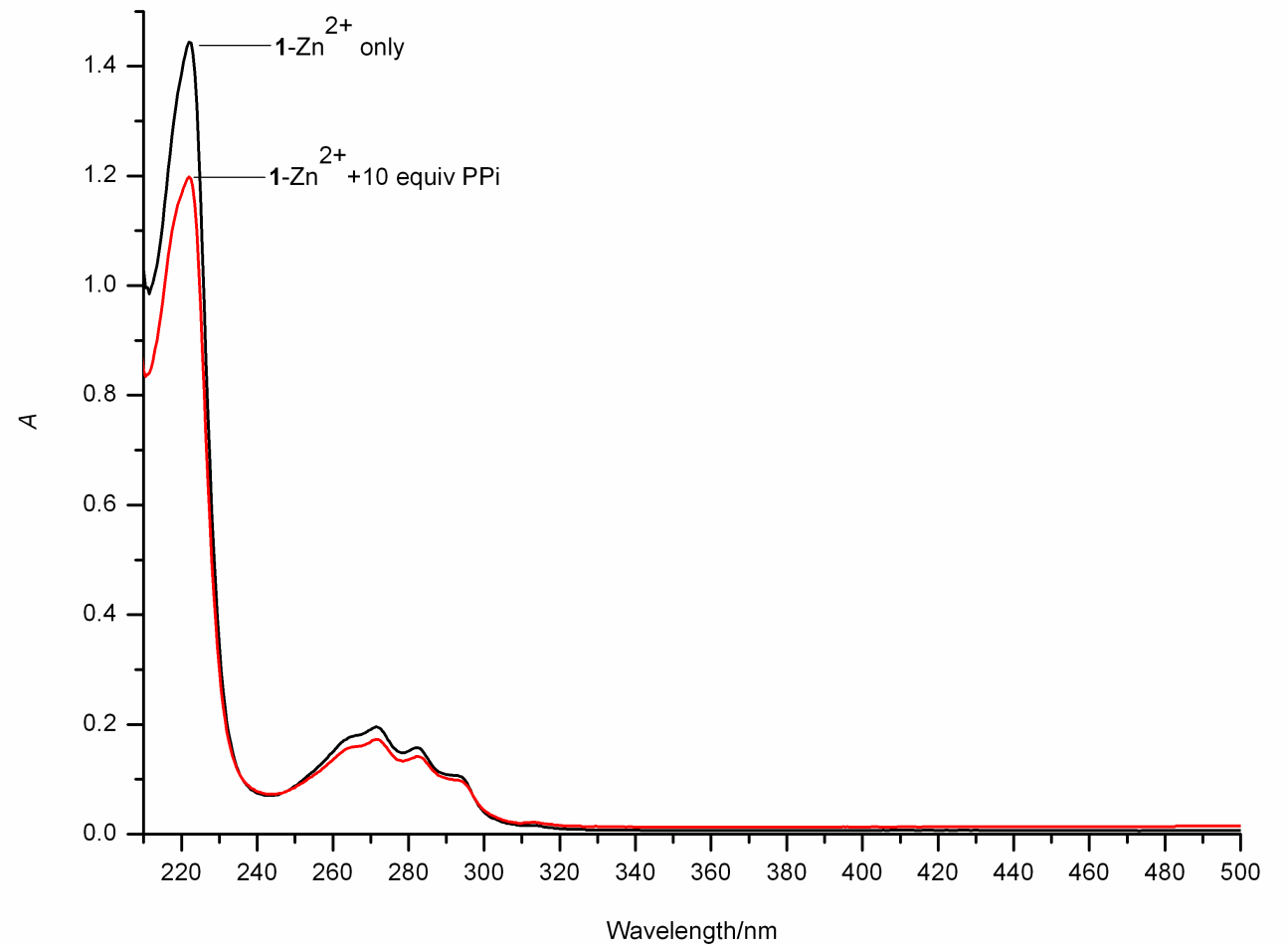

Fig. S9 UV/vis change of 20 μM **1**-Zn^{2+} upon addition of PPi in HEPES buffer (10 mM, pH 7.4).

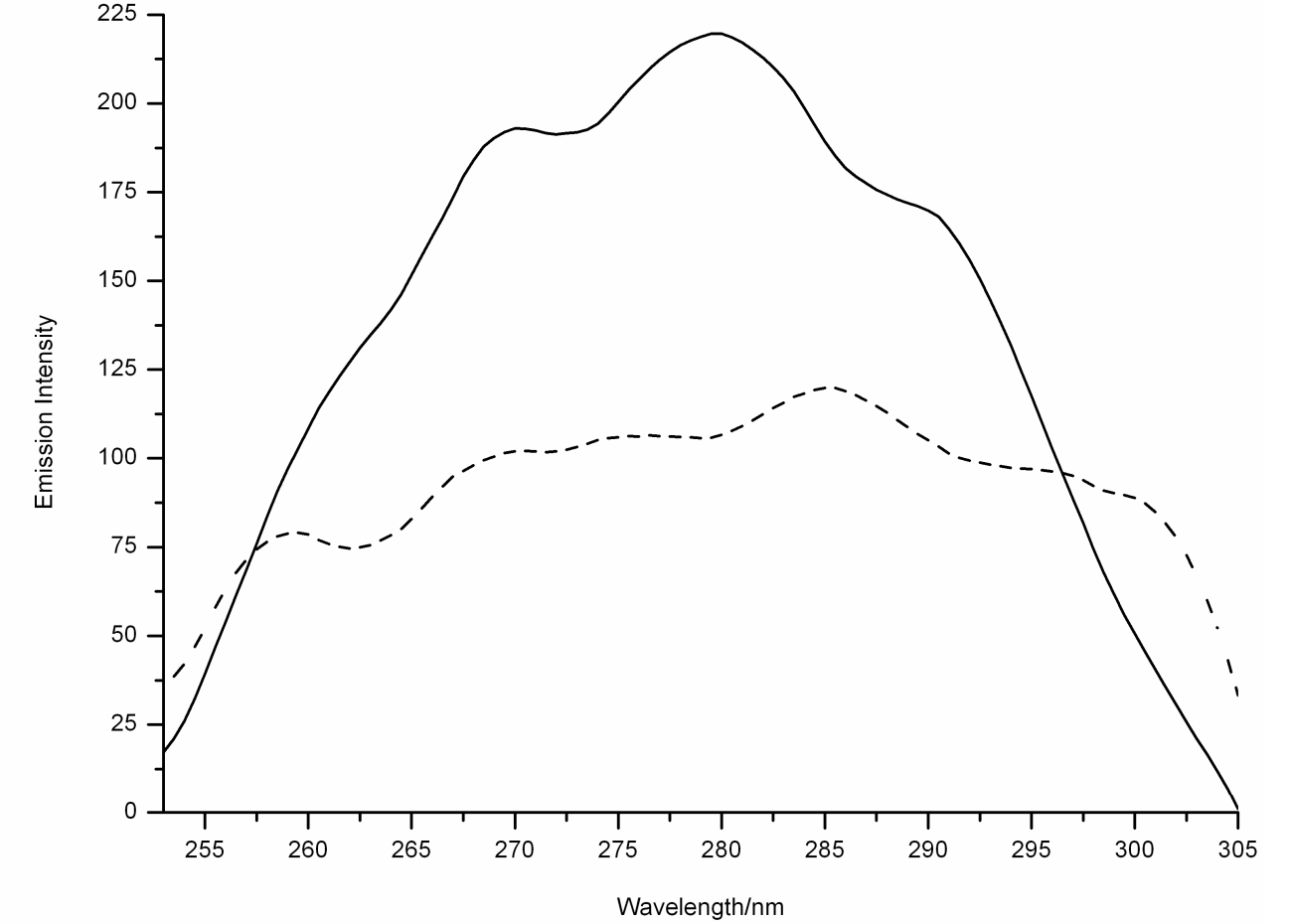

Fig. S10 Fluorescence excitation spectra of 20 μM **1**-Zn^{2+} with 0.5 equiv. of PPi in HEPES buffer (10 mM, pH 7.4). The solid line and the dashed line correspond to the excitation spectra monitored at the monomer emission (λ_{em}=337 nm) and the excimer emission (λ_{em}=415 nm), respectively.

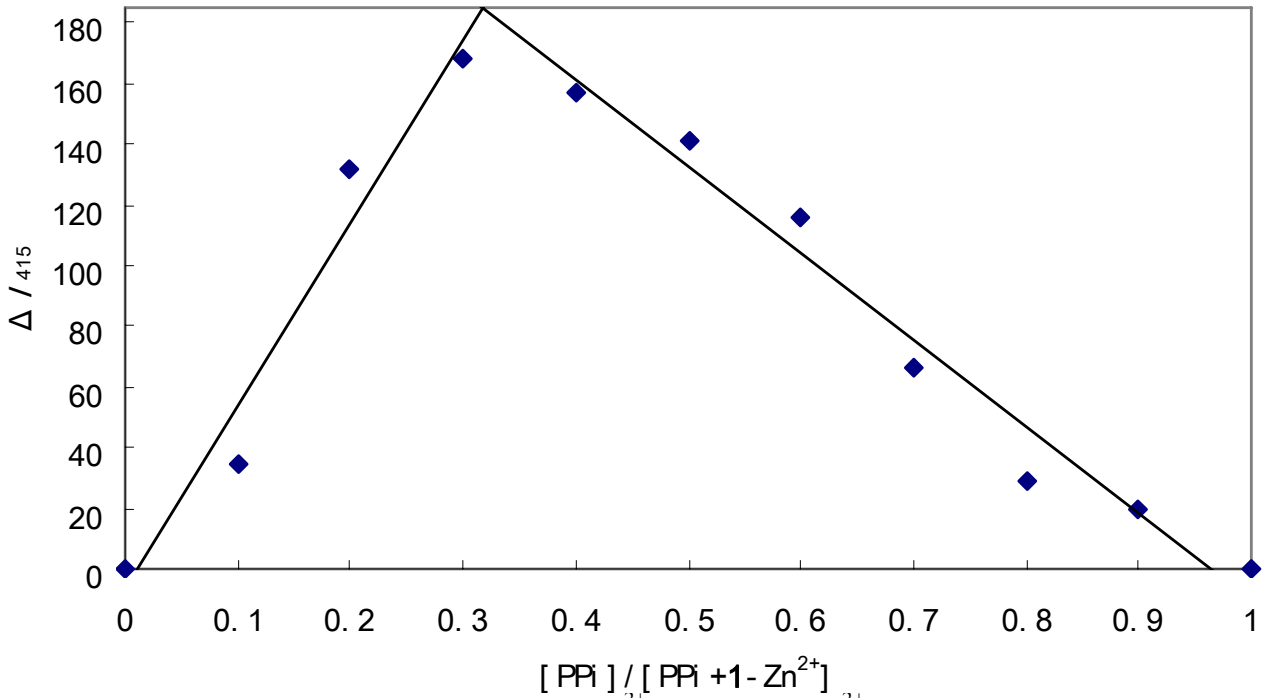

Fig. S11 Job's plot for the binding between **1**-Zn^{2+} and PPi. [**1**-Zn^{2+}]+[PPi]=20 μM.

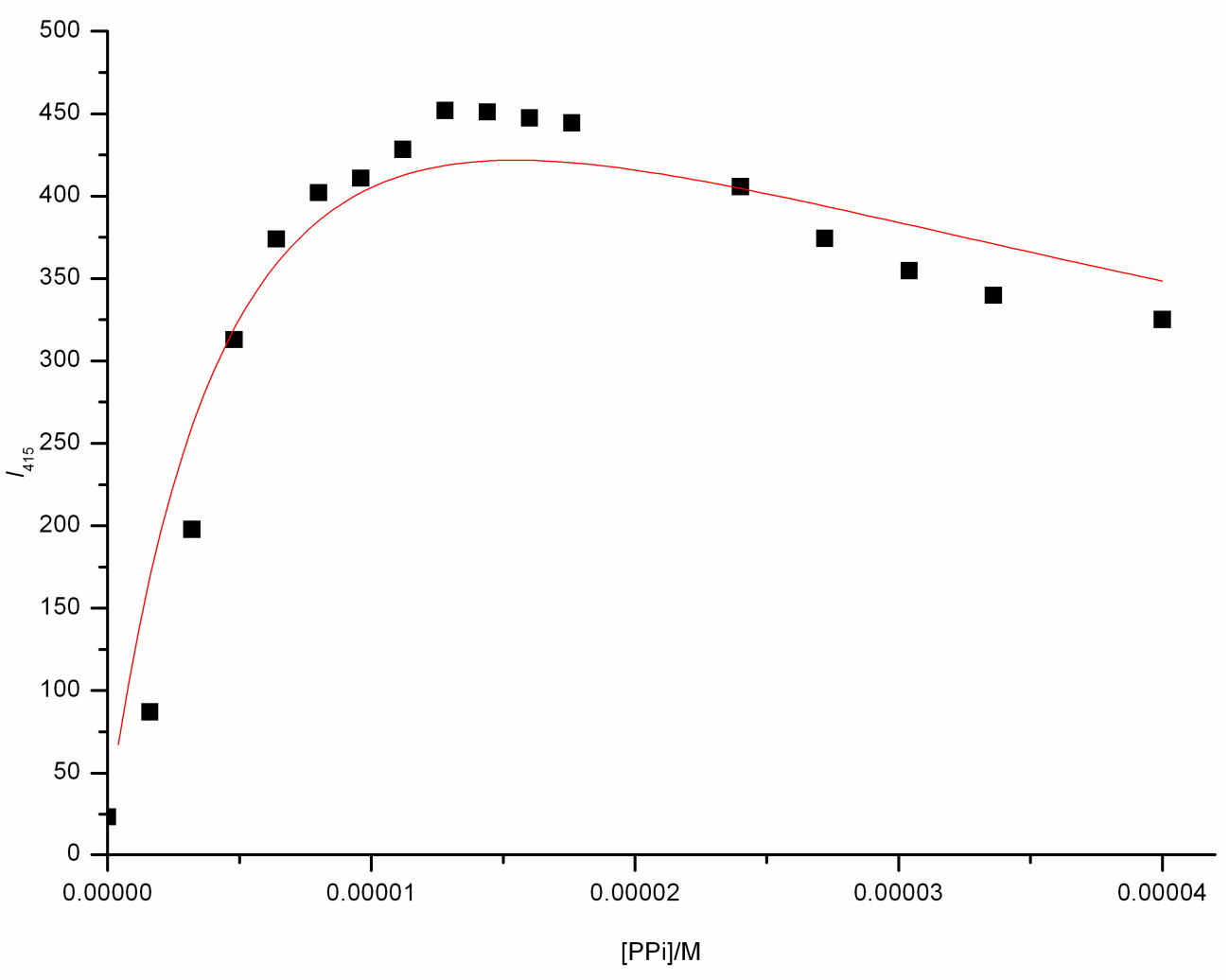

Fig. S12 The emission change of **1**-Zn^{2+} induced by PPi titration. The binding constant was obtained by nonlinear fitting to the data, and the red line is the resulted fitting line.

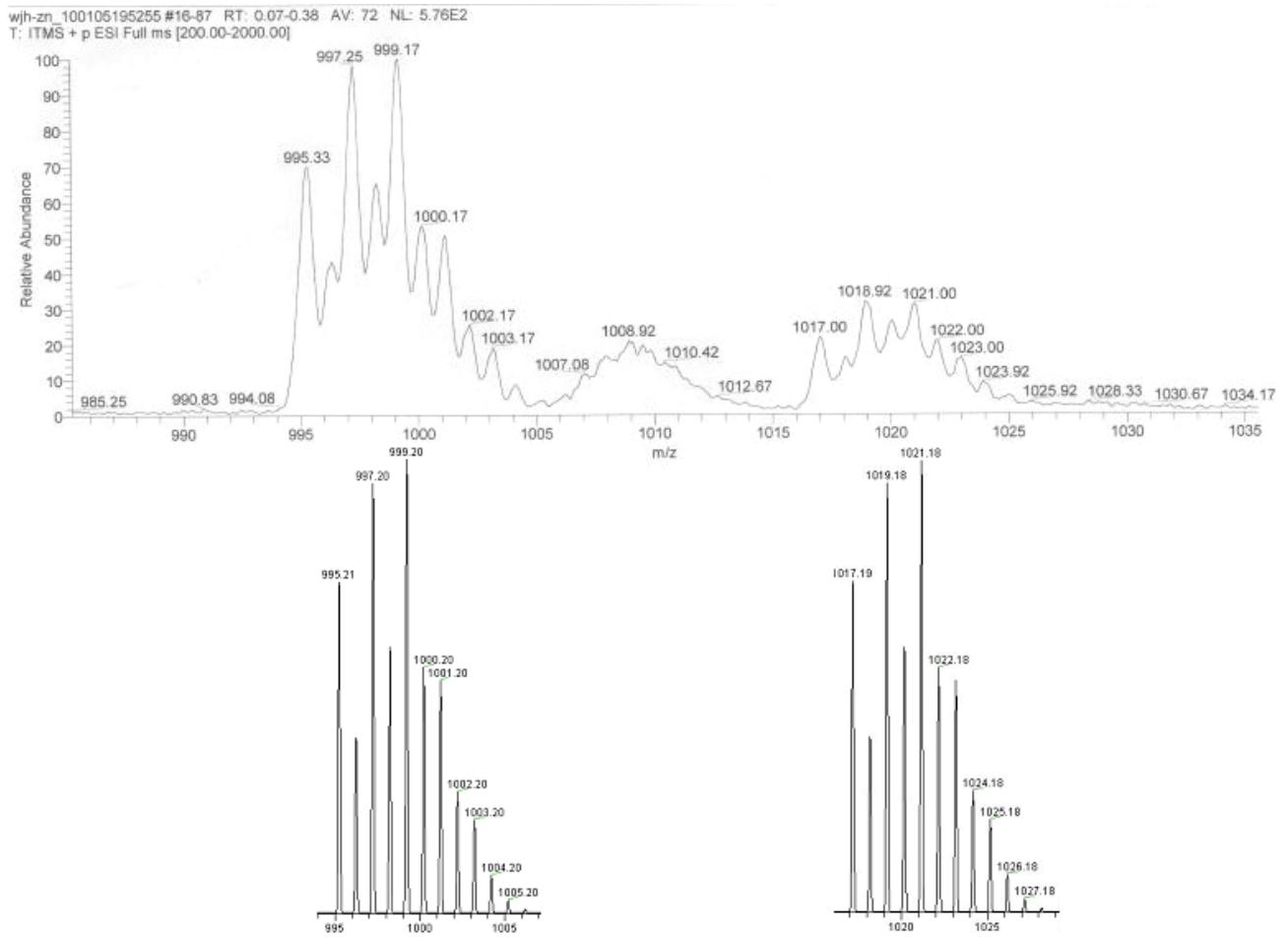

Fig. S13 Observed electrospray ionization(EI) mass spectrum and calculated isotope patterns for **1**-Zn^{2+}+PPi.

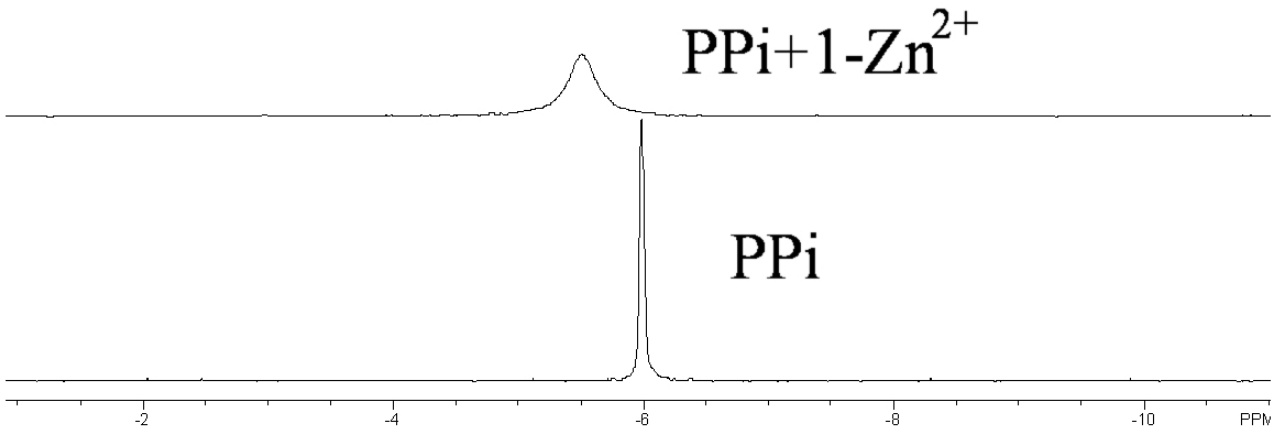

Fig. S14 ^{31}P NMR spectra of PPi (lower) and PPi in the presence of 2 equiv. of **1**-Zn^{2+} (upper) recorded in D_2O at room temperature.

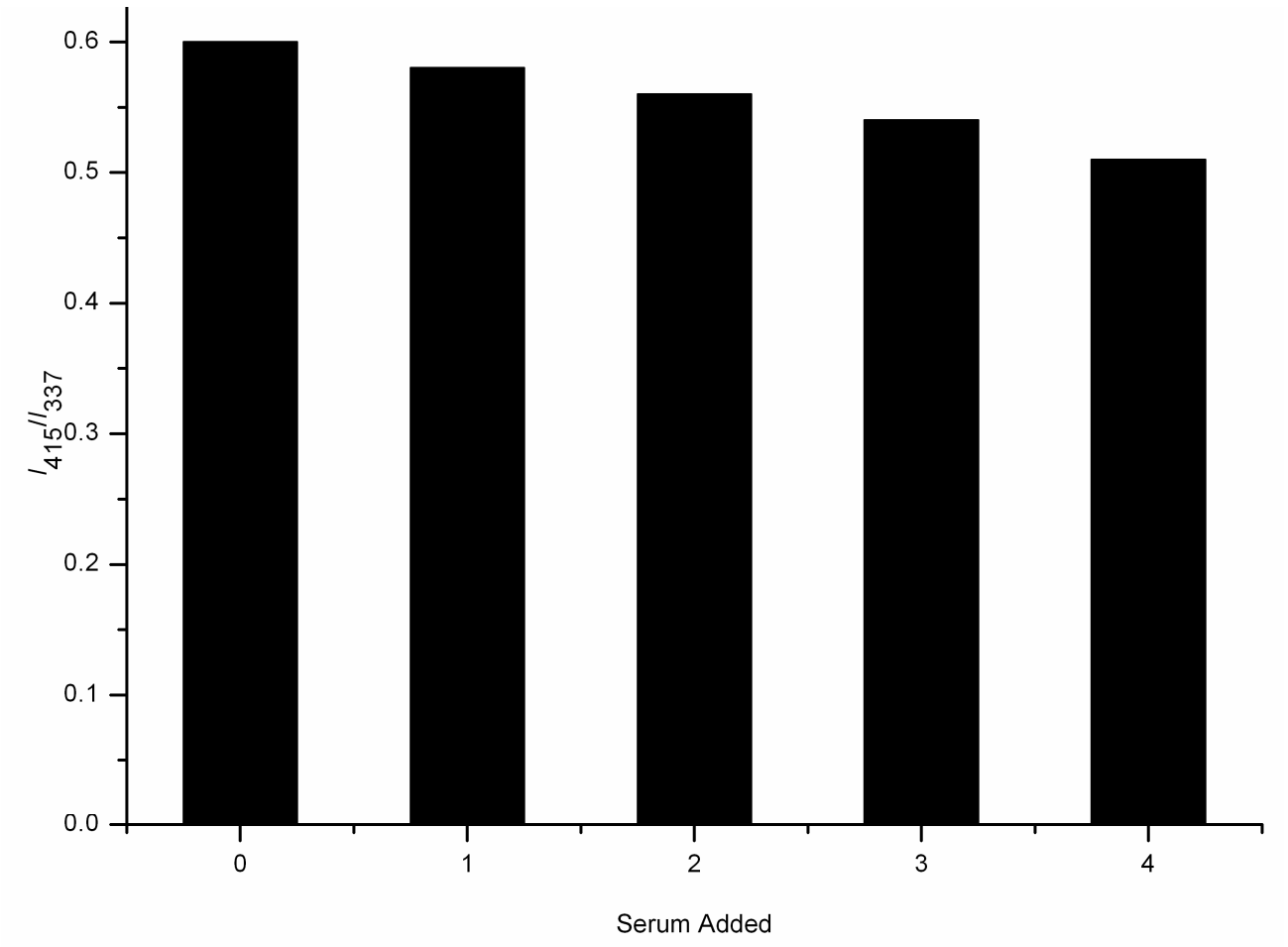

Fig. S15 Histogram showing the fluorescent response of 20 μM **1**-Zn^{2+} to PPi (0.5 equiv.) in the presence of various μL of serum in HEPES buffer (10 mM, pH 7.4). (0) 0 μL, (1) 20 μL, (2) 37.5 μL, (3) 75 μL, (4) 200 μL. λ_{ex}= 280 nm.