

## A ratiometric fluorescent sensor for zinc(II) with high selectivity

Huili Chen<sup>a,b,\*</sup>, Yanbo Wu<sup>a</sup>, Yunfei Cheng<sup>b</sup>, Hong Yang<sup>b</sup>,  
Fuyou Li<sup>b</sup>, Pin Yang<sup>a</sup>, Chunhui Huang<sup>b,\*</sup>

<sup>a</sup> Institute of Molecular Science, Key Laboratory of Chemical Biology and Molecular Engineering of Education Ministry, Shanxi University, Taiyuan 030006, PR China

<sup>b</sup> Department of Chemistry, Laboratory of Advanced Materials, Fudan University, Shanghai 200433, PR China

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### Abstract

Spectroscopic studies revealed that the compound [*N,N'*-di(quinoline-2-methylene)-1,2-phenylenediiimine] (**1**) exhibited a rather high selectivity toward Zn<sup>2+</sup> over other metal ions, even Cd<sup>2+</sup>. In acetonitrile, the red shift of fluorescent emission from 396 nm to 426 nm upon zinc binding is due to the formation of a 1:1 metal/ligand complex.

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Zinc ion is the second most abundant heavy metal ion and well known to play an important role in cellular events including structural co-factors, regulator of enzymes, DNA binding, catalytic centers, and neuronal signal transmission [1]. However, zinc is a metal pollutant of the environment. Too much zinc may reduce the soil microbial activity causing phytotoxic effects [2] and it is a common contaminant in agricultural and food wastes [3]. Therefore, the detection of trace amounts of Zn<sup>2+</sup> is a significant issue in environmental and biological analysis. A large number of probes have been reported and used for intracellular measurements [4–9]. However, Zn<sup>2+</sup> and Cd<sup>2+</sup> have similar chemical properties, and the discrimination between them is very difficult [10]. Up to now, only a few examples of fluorescent chemosensor for Zn<sup>2+</sup>, which can distinguish Zn<sup>2+</sup> from Cd<sup>2+</sup> to some extent [11], have been reported.

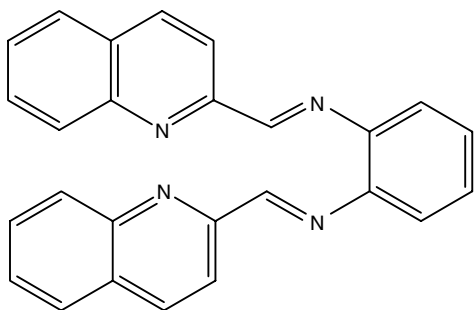
Owing to the simplicity and sensitivity, fluorescent sensors are often designed and synthesized for the detection of physiologically important ions, molecules [12], or harmful pollutants in the environment [13]. Recently, ratiometric fluorescence measurements have been conducted to detect metal ions, which can increase the selectivity and sensitivity and eliminate most or all of the possible variability due to differences in instrumental efficiency and content of effective dye [14]. Herein we report a novel Zn<sup>2+</sup> sensor **1** (Scheme 1) based on the ratiometric fluorescence measurement. Fluorescence studies show the high selectivity of **1** towards Zn<sup>2+</sup> over other metal ions, even Cd<sup>2+</sup>.

Sensor **1** was synthesized by a simple and straightforward method with moderate yields (55%) by condensation of 2-quinolinecarboxaldehyde with 1,2-phenylenediamine in methanol [15,16]. All metal ions are from acetate salts. In acetonitrile, the UV–vis absorbance spectrum of **1**, shows a strong absorbance at 347 nm ( $\epsilon = 4.85 \times 10^4 \text{ cm}^{-1} \text{ mol}^{-1}$ ) in the UV region. Titration of **1** against Zn<sup>2+</sup> led to a pronounced and red-shifted shoulder in the UV spectra ( $\Delta\lambda_{\text{max}} = 10 \text{ nm}$  shift) with a well-defined isosbestic point at 351 nm (Fig. 1).

To determine the magnitude of Zn<sup>2+</sup> induced fluorescence spectral changes of **1** as well as its binding affinity,

\* Corresponding authors. Address: Institute of Molecular Science, Key Laboratory of Chemical Biology and Molecular Engineering of Education Ministry, Shanxi University, Taiyuan 030006, PR China (H. Chen). Tel./fax: +86 21 55664621.

E-mail addresses: huilichen@sxu.edu.cn (H. Chen), chuang@pku.edu.cn (C. Huang).



Scheme 1. Structure of [*N,N'*-di(quinoline-2-methylene)-1,2-phenylenediamine] (**1**).

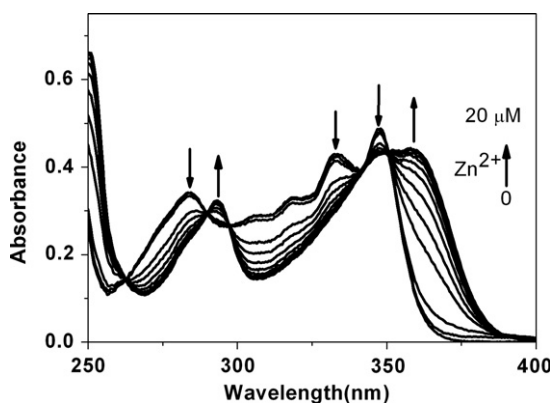


Fig. 1. Changes in UV-vis absorption spectra of **1** (10 μM) in acetonitrile with various amounts of Zn<sup>2+</sup> (0–20 μM).

we performed a detailed spectrofluorometric titration studies. With the increase of Zn<sup>2+</sup>, the intensity of the emission band at 396 nm decreased and a new band at 426 nm appeared gradually, corresponding to a red-shift of about 30 nm (Fig. 2).

For an excellent chemosensor, high selectivity is a necessary factor. The fluorescence titration of **1** with various metal ions shows excellent selectivity to Zn<sup>2+</sup>. For example, physiologically important metal ions which exist in living cells, such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, and K<sup>+</sup> do not give any response. Most heavy and transition metal ions, such as Pb<sup>2+</sup>, Ag<sup>+</sup>, Mn<sup>2+</sup>, and Cr<sup>3+</sup>, also have no interference.

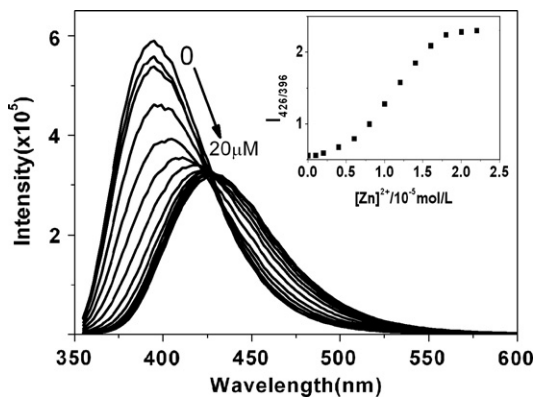
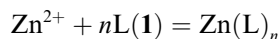


Fig. 2. Fluorescence emission ( $\lambda_{\text{ex}} = 351$  nm) spectra of **1** (10 μM) in acetonitrile. Inset: Titration curve of **1** with Zinc(II) (0–20 μM).

Fe<sup>3+</sup>, Ni<sup>2+</sup>, Co<sup>2+</sup>, and Cu<sup>2+</sup> obviously quench the fluorescence, which always meet in the other metal ion sensors [17]. Hg<sup>2+</sup> and Al<sup>3+</sup> induce slight fluorescence decrease without red-shift. Cd<sup>2+</sup>, a d<sup>10</sup> ion that often exhibits coordination properties similar to Zn<sup>2+</sup>, also shows a decrease on the fluorescence intensity of **1**, but the magnitude of the red-shift is less than that for Zn<sup>2+</sup>. The most dramatic effect of fluorescence profile of **1** is observed in the presence of Zn<sup>2+</sup>. As noteworthy from the data of Fig. 2, with increasing concentration of Zn<sup>2+</sup>, a red shift and a partial quenching of the fluorescence emission of **1** from 396 nm to 426 nm occurs. Both the quenching in fluorescence intensity and the red shift are not kinetically controlled as the time dependent incubation of Zn<sup>2+</sup> with **1** does not alter the emission intensity. This deduction is equally valid for the interaction of other metal ions with **1**, irrespective of their spectral modulating features. It is interesting that the emission point at 426 nm is also the isoemission point.

Fig. 3 shows the dependence of the intensity ratios of **1** at 426 nm to that at 396 nm ( $I_{426}/I_{396}$ ) on the cations. It can be easily recognized that high selectivity for Zn<sup>2+</sup> is obtained by **1**. The competition experiments of Zn<sup>2+</sup> mixed with the metal ions show no significant variation in the ratio fluorescence intensity ( $I_{426}/I_{396}$ , Fig. 3), although Fe<sup>3+</sup>, Ni<sup>2+</sup>, Co<sup>2+</sup> and Cu<sup>2+</sup> have some fluorescence quenching in normal fluorescence intensity at 396 ± 15 nm. These results show that **1** can be utilized to detect Zn<sup>2+</sup>.

The stoichiometry of the predominant species in acetonitrile solution is determined by using the Job's plot method [18]. Considering a global equilibrium of Zn<sup>2+</sup> and *n* ligands Dq (**1**) on the form



where *n* is determined from the plot of the absorbance as a function of the mole fraction  $\chi$  of the added ligand. In the absorbance maximum

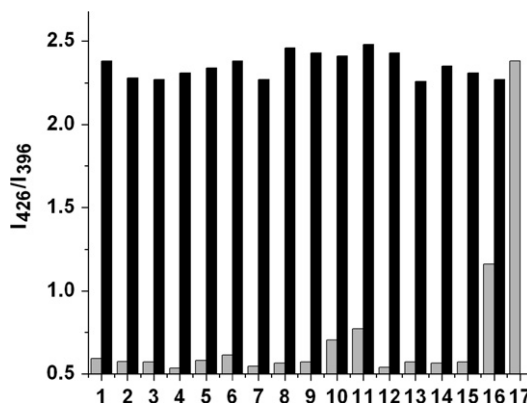


Fig. 3. M<sup>n+</sup>-selectivity profile of **1**: (grey bars) relative integrated emission intensity of **1** + 2 equiv. M<sup>n+</sup>; (black bars) relative integrated emission intensity of **1** + 2 equiv. M<sup>n+</sup>, followed by 2 equiv. Zn<sup>2+</sup>. 1, compound **1**; 2, Na<sup>+</sup>; 3, K<sup>+</sup>; 4, Ca<sup>2+</sup>; 5, Mg<sup>2+</sup>; 6, Al<sup>3+</sup>; 7, Ag<sup>3+</sup>; 8, Co<sup>2+</sup>; 9, Cr<sup>3+</sup>; 10, Cu<sup>2+</sup>; 11, Fe<sup>3+</sup>; 12, Hg<sup>2+</sup>; 13, Mn<sup>2+</sup>; 14, Ni<sup>2+</sup>; 15, Pb<sup>2+</sup>; 16, Cd<sup>2+</sup>; 17, Zn<sup>2+</sup>. Conditions: [**1**] = 10 μM in acetonitrile;  $\lambda_{\text{exc}} = 351$  nm.

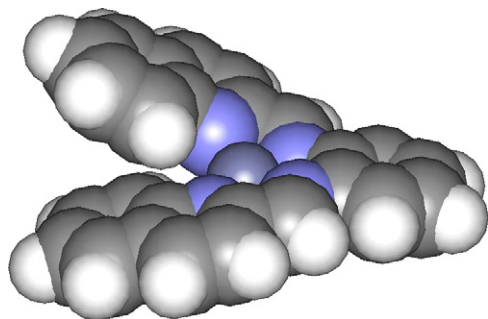


Fig. 4. A proposed structure for **1** + Zn<sup>2+</sup>.

$$n = \frac{\chi_{\max}}{1 - \chi_{\max}}$$

The formation of the adduct with a 1:1 Zn<sup>2+</sup> – **1** stoichiometry is evidenced, with log*K* = 5.30. The metal center binds to the four nitrogen atoms of **1** and forms a “Y-Type” conformation, which is illustrated in Fig. 4, in which the molecular modeling structure was energy-minimized using Chemoffice 7.0 MM2 utilities.

In summary, this paper shows the specific fluorescent behavior of **1** in acetonitrile solutions toward the Zn<sup>2+</sup> cation over other metal ions. Indeed, the free neutral **1** can detect Zn<sup>2+</sup> metal ion efficiently and selectively. The major species in solution exhibits a 1:1 Zn<sup>2+</sup> – **1** stoichiometry. Further work in this direction is in progress.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.inoche.2007.08.028.

#### References

- [1] J.M. Berg, Y. Shi, *Science* 271 (1996) 1081.
- [2] A. Voegelín, S. Poster, A.C. Scheinost, M.A. Marcus, R. Kretzschmar, *Environ. Sci. Technol.* 39 (2005) 6616.
- [3] E. Callender, *Environ. Sci. Technol.* 34 (2000) 232.
- [4] G.K. Walkup, S.C. Burdette, S.J. Lippard, R.Y. Tsien, *J. Am. Chem. Soc.* 122 (2000) 5644.
- [5] T. Hirano, K. Kikuchi, Y. Urano, T. Higuchi, T. Nagano, *J. Am. Chem. Soc.* 122 (2000) 12399.
- [6] K.R. Gee, Z.L. Zhou, W.J. Qian, R.J. Kennedy, *J. Am. Chem. Soc.* 124 (2002) 776.
- [7] N.C. Lim, L. Yao, H.C. Freake, C. Bruckner, *Bioorgan. Med. Chem. Lett.* 13 (2003) 2251.
- [8] C.C. Woodrooffe, R. Masalha, K.R. Barnes, C.J. Frederickson, S.J. Lippard, *Chem. Biol.* 11 (2004) 1659.
- [9] E.M. Nolan, J. Jaworski, K.I. Okamoto, Y. Hayashi, M. Sheng, S.J. Lippard, *J. Am. Chem. Soc.* 127 (2005) 16812.
- [10] (a) F.A. Cotton, G. Wilkinson, *Advances in Inorganic Chemistry*, fifth ed., Wiley, New York, 1988, pp. 957–1358; (b) D. Dakternieks, *Coord. Chem. Rev.* 98 (1990) 279.
- [11] (a) N.C. Lim, L. Yao, H.C. Freake, C. Bruckner, *Bioorgan. Med. Chem. Lett.* 13 (2003) 2251; (b) N.C. Lim, J.V. Schuster, M.C. Porto, M.A. Tanudra, L. Yao, H.C. Freake, C. Bru2ckner, *Inorg. Chem.* 44 (2005) 2018; (c) M.D. Shults, D.A. Pearce, B. Imperiali, *J. Am. Chem. Soc.* 125 (2003) 10591.
- [12] Y.E. Spichiger-Keller, *US-Sensors and Biosensors for Medical and Biological Applications*, Wiley-VCH, New York, 1998.
- [13] C.F. Mason, *Biology of Freshwater Pollution*, second ed., Longman, New York, 2002.
- [14] J.R. Lakowicz, *Probe Design and Chemical Sensing Topics in Fluorescence Spectroscopy*, vol. 4, Plenum, New York, 1994.
- [15] K. Yliheikkilä, K. Lappalainen, P.M. Castro, K. Ibrahim, A.A. Surrah, M. Leskela, T. Repo, *Eur. Polym. J.* 42 (2006) 92.
- [16] To a solution of 2-quinolinecarboxaldehyde (4.7 g, 30 mmol) in MeOH was added a solution of 1,2-phenylenediamine (1.08 g, 10 mmol) in MeOH and stirred for 1.5 h. The raw product was purified by column chromatography over silica gel using petroleum ether/ethyl acetate mixture (3:1) as eluent. Yields: 55%. <sup>1</sup>H NMR: 8.573 (d, 2H, *J* = 8.8 Hz), 8.334 (d, 2H, *J* = 8.4 Hz), 8.128 (d, 2H, *J* = 8.4 Hz), 7.885 (d, 2H, *J* = 8.4 Hz), 7.786 ~ 7.747 (m, 4H) 7.596 (t, 2H, *J* = 8 Hz), 7.343 ~ 7.32 (m, 4H) Anal. Calcd. for C<sub>26</sub>H<sub>18</sub>N<sub>4</sub>, C, 80.81; H, 4.69; N, 14.50. Found, C, 80.46; H, 4.98; N, 14.32. MS (LDI-TOF; *m/e*), 386.
- [17] Y. Zheng, J. Orbulescu, X. Ji, F.M. Andreopoulos, S.M. Pham, R.M. Leblanc, *J. Am. Chem. Soc.* 125 (2003) 2680.
- [18] W.C. Vosburgh, G.R. Cooper, *J. Am. Chem. Soc.* 63 (1941) 437.