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A cap-type Schiff base acting as a fluorescence sensor for zinc(II) and a colorimetric sensor for iron(II), copper(II), and zinc(II) in aqueous media†

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A simple and low cost chemosensor is described. This sensor could simultaneously detect three biologically important metal ions through fluorogenic (Zn^{2+}) and chromogenic (Fe^{2+}, Cu^{2+}, and Zn^{2+}) methods in aqueous solution. The sensor could function as a "turn-on" fluorescence receptor only to Zn^{2+} ions. In addition, the sensor could be successfully applied to the detection of intracellular Zn^{2+}. Meanwhile, the sensor displayed an obvious red color upon selective binding with Fe^{2+}. Therefore, the sensor could serve as a useful tool for the discrimination of Fe^{2+} from Fe^{3+} in aqueous media. Moreover, the sensor also showed color changes from yellow to colorless upon selective binding with Zn^{2+} and Cu^{2+}, respectively. The detection limit of the sensor for Cu^{2+} (1.5 μM) is far below the guidelines of the World Health Organization (30 μM) as the maximum allowable copper concentration in drinking water, and therefore it is capable of being a practical system for the monitoring of Cu^{2+} concentrations in aqueous samples. These results provide a new approach for selectively recognizing the most important three trace elements in the human body simultaneously, for Zn^{2+} by emission spectra and Fe^{2+}, Cu^{2+}, and Zn^{2+} by the naked eye.

Introduction

The design and synthesis of new chemosensors for the efficient detection of trace metal ions is among the most important research topics in environmental chemistry and biology.[1] Among the various types of sensors, fluorescent chemosensors for metal ions have attracted much attention due to their convenient use and high sensitivity, and they have been employed to clarify the real-time dynamics and various biological functions of targeted metal cations.[2–15] Furthermore, colorimetric methods are also extremely attractive because they allow naked-eye detection of color change without any use of a spectroscopic instrument.[16–26]

The zinc ion (Zn^{2+}) is the second most abundant essential trace element in the human body after iron; it is indispensable for mediating many enzyme-catalyzed reactions and, therefore, plays very important roles in a wide variety of physiological and pathological processes.[27–30] It is known that disorders of zinc metabolism are closely associated with many severe neurological diseases such as Alzheimer's disease (AD) and Parkinson's disease.[31–34] Therefore, the detection of Zn^{2+} in biological samples is of significant interest and importance. Over the past few decades, many different fluorescent chemosensors for Zn^{2+} have been developed, using quinoline,[35,36] anthracene,[37] and coumarin[38,39] as fluorophores. However, some of them require complicated syntheses and they are insoluble in aqueous media.

Iron is the most abundant essential trace element in the human body. Both Fe^{2+} and Fe^{3+} play vital roles in many biological processes.[40–42] Many biochemical processes at the cellular level, such as oxygen transportation and DNA synthesis, involve electron transfer between the two oxidation states of iron.[43] The levels of iron are related to some biochemical, pharmacological, and toxicological functions in biological systems, and either iron deficiency or overload can lead to human disorders and diseases.[43–48] Therefore, great efforts have been made to develop suitable detection methods for iron.

Copper is third in abundance (after iron and zinc) among the essential transition metal ions in the human body and plays an important role in various physiological processes.[49] Many proteins contain copper ions (Cu^{2+}) in the catalytic center. Several researchers have connected the cellular toxicity of Cu^{2+} to serious diseases including AD,[50] Indian childhood

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cirrhosis,[51] prion disease,[52] and Menkes and Wilson diseases.[53] Due to the extensive use of copper in modern society, Cu^{2+} is also a significant metal pollutant. The World Health Organization (WHO) has recommended the maximum limit of copper in drinking water to be 2 ppm (30 μM).[54]

Schiff bases are well known to be good ligands for metal ions and their synthesis is usually simple and easy.[55–59] Therefore, Schiff base derivatives incorporating a fluorescent moiety are appealing tools for optical sensing of metal ions.[60–64] Furthermore, we expected that if a Schiff base with a fluorescent moiety has a cap-type tripodal structure,[5,65,66] it would be a better metal ion sensor with more effective chelation to metal ions than a linear Schiff base. Indeed, the receptor *N*,*N*′,*N*″-tris(salicylidene)(2-aminoethyl)amine (**L**) with a cap-type structure was found to be an excellent fluorogenic and chromogenic sensor.

Herein, we report on a fluorogenic Zn^{2+} and chromogenic Fe^{2+}, Cu^{2+}, and Zn^{2+} sensor based on a cap-type Schiff base (**L**) with an effective chelation to metal ions. Importantly, **L** simultaneously sensed three biologically important metal ions through fluorogenic and chromogenic methods in aqueous solution. The sensor **L** could function as a "turn-on" fluorescent chemosensor for Zn^{2+}, which is capable of mapping zinc levels in cells for application to bioimaging. Moreover, **L** could display obvious color changes from yellow to red upon selective binding with Fe^{2+} and from yellow to colorless upon selective binding with Cu^{2+} and Zn^{2+}. In particular, it is worth noting that **L** could clearly discriminate Fe^{2+} from Fe^{3+} in aqueous media.

Experimental section

General information

All the solvents and reagents (analytical grade and spectroscopic grade) were obtained from Sigma-Aldrich and used as received. ^{1}H NMR spectra were recorded using a Varian 400 MHz spectrometer and chemical shifts are recorded in ppm. Electrospray ionization mass spectra (ESI-MS) were collected using a Thermo Finnigan (San Jose, CA, USA) LCQ™ Advantage MAX quadrupole ion trap instrument by infusing samples directly into the source manually. Spray voltage was set at 4.2 kV, and the capillary temperature was at 80 °C. Absorption spectra were recorded at room temperature using Perkin Elmer model Lambda 2S UV/Vis spectrometer. Emission spectra were recorded using a Perkin Elmer LS45 fluorescence spectrometer.

Synthesis of sensor L. The tri-Schiff base compound, *N*,*N*′,*N*″-tris(salicylidene)(2-aminoethyl) amine (**L**), was prepared according to the literature method.[67,68] To a solution of salicylaldehyde (1 g, 8.20 mmol) in ethanol (20 mL) was added tris(2-aminoethyl)amine (0.40 g, 2.73 mmol) in absolute ethanol (20 mL). A yellow precipitate was formed immediately. The mixture was refluxed and stirred for 2 h. Then, the resulting solid was filtered off, washed with diethyl ether, and dried in air to obtain the desired compound.

Yield 1.13 g (90%); Anal. Calc. for $C_{27}H_{40}N_4O_3$: C, 69.20; H, 8.60; N, 11.96. Found: C, 69.10; H, 8.80; N, 11.90%; ESI-MS *m*/*z* ($M + H^+$): calcd, 459.23; found, 459.20; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.66 (s, 3H), 8.22 (s, 3H), 7.32 (t, 3H), 6.87 (m, 6H), 6.76 (t, 3H), 3.60 (t, 6H), 2.85 (t, 6H); ^{13}C NMR (500 MHz, $CDCl_3$, d, ppm): 166.09 (N=C), 116.68 (aromatic ring), 118.48 (aromatic ring), 131.75 (aromatic ring), 161.05 (aromatic ring), 57.89 (NCH_2CH_2), 55.80 (NCH_2CH_2); IR (cm^{-1}, KBr pellet): 3436 (b, ν_{O-H}, typical for intramolecular hydrogen bonded O–H), 3000–2800 (ν_{C-H}), 1632 ($\nu_{C=N}$), 1610, 1582, 1498, 1459, 1430, 1337, 756.

UV-vis titration. **L** (4.6 mg, 0.01 mmol) was dissolved in methanol (5 mL) and 30 μL (2 mM) of it was diluted to 2.97 mL with HEPES buffer (10 mM) to make a final concentration of 20 μM. $Zn(NO_3)_2$ and $Cu(NO_3)_2$ (0.1 mmol) were dissolved in methanol (5 mL) and 0.3–3 μL of the Zn^{2+} ion solution (20 mM) was transferred to the solution of **L** (20 μM) prepared above. $Fe(ClO_4)_2$ (0.1 mmol) was dissolved in methanol (5 mL). 0.6–6 μL of the Fe^{2+} ion solution (20 mM) was transferred to the solution of **L** (20 μM) prepared above. After mixing them, UV-vis spectra were taken at room temperature.

Fluorescence titration. **L** (4.6 mg, 0.01 mmol) was dissolved in methanol (10 mL) and 30 μL of this solution (2 mM) was diluted with 2.97 mL of HEPES buffer (10 mM) to make the final concentration of 10 μM. $Zn(NO_3)_2$ (30.0 mg, 0.10 mmol) was dissolved in methanol (5 mL) and 0.15–1.5 μL of this Zn^{2+} solution (20 mM) was transferred to each receptor solution (10 μM) to give 0–1 equivalent. After mixing for a few seconds, fluorescence spectra were taken at room temperature.

Competition with other metal ions. **L** (4.6 mg, 0.01 mmol) was dissolved in methanol (10 mL) and 30 μL of this solution (2 mM) was diluted with 2.97 mL of HEPES buffer (10 mM) to make the final concentration of 10 μM. MNO_3 (M = Na, K, 0.1 mmol) or $M(NO_3)_2$ (M = Mn, Co, Ni, Cu, Zn, Cd, Mg, Ca, Hg, 0.1 mmol) or $M(NO_3)_3$ (M = Al, Fe, Cr, 0.1 mmol) or $M(ClO_4)_2$ (M = Fe, 0.1 mmol) was dissolved in methanol (10 mL).

For Zn^{2+} ion, 3 μL of each metal solution (10 mM) was taken and added to 3 mL of the solution of receptor **L** (10 μM) to give 1 equivalent of metal ions. Then, 3 μL of Zn^{2+} solution (10 mM) was added into the mixed solution of each metal ions and **L** to make 1 equivalent. After mixing for a few seconds, fluorescence spectra were taken at room temperature.

For Fe^{2+} ion, 6 μL of each metal solution (20 mM) was taken and added to 3 mL of the solution of receptor **L** (20 μM) to give 2 equivalents of metal ions. Then, 6 μL of Fe^{2+} solution (20 mM) was added into the mixed solution of each metal ions and **L** to make 2 equivalents. After mixing them, UV-vis spectra were obtained at room temperature.

For Cu^{2+} ion, 3 μL of each metal solution (20 mM) was taken and added to 3 mL of the solution of receptor **L** (20 μM) to give 1 equivalent of metal ions. Then, 3 μL of Cu^{2+} solution (20 mM) was added to the mixed solution of each metal ions and **L** to make 1 equivalent. After mixing, UV-vis spectra were taken at room temperature.

Job plot measurements. **L** (45.9 mg, 0.10 mmol) was dissolved in methanol (10 mL). 12, 10.8, 9.6, 8.4, 7.2, 6.0, 4.8, 3.6,

2.4, 1.2, and 0 μL of the **L** solution were taken and transferred to vials. Each vial was diluted with HEPES buffer (10 mM) to make a total volume of 2.988 mL. Zn^{2+}, Fe^{2+}, and Cu^{2+} (0.10 mmol) were dissolved in methanol (10 mL). 0, 1.2, 2.4, 3.6, 4.8, 6.0, 7.2, 8.4, 9.6, 10.8, and 12 μL of the metal solution were added to each diluted **L** solution. Each vial had a total volume of 3 mL. After shaking, fluorescence or absorbance spectra were taken at room temperature.

Quantum yield measurements. **L** (45.9 mg, 0.10 mmol) and $Zn(NO_3)_2$ (30.0 mg, 0.10 mmol) were dissolved in methanol (10 mL). Quinine hemisulfate monohydrate (21.7 mg, 0.05 mmol) was dissolved in 0.1 M H_2SO_4 (10 mL). UV-vis spectra of **L** (20 μM) were taken with and without $Zn(NO_3)_2$ (20 μM) in HEPES buffer (10 mM). UV-vis spectra of quinine hemisulfate monohydrate (5, 10, 15, 20, 25 μM) were taken in 0.1 M H_2SO_4 solution. Fluorescence spectra of **L** (20 μM) were taken with and without $Zn(NO_3)_2$ (20 μM) in HEPES buffer (10 mM) after being excited at 362 nm. Fluorescence spectra of quinine hemisulfate monohydrate (2.3, 19.2 μM) were taken in 0.1 M H_2SO_4 solution after being excited at 362 nm (Φ = 0.065). Quantum yield was adjusted according to UV-vis absorption intensities.

Methods for cell imaging. The cell imaging test was carried out by the same method as our previous study.[69] Human dermal fibroblast cells in low passage were cultured in FGM-2 medium (Lonza, Switzerland) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin in the *in vitro* incubator with 5% CO_2 at 37 °C. Cells were seeded onto a 12 well plate (SPL Lifesciences, Korea) at a density of 2×10^5 cells per well and then incubated at 37 °C for 4 h after addition of various concentrations (0–200 μM) of $Zn(NO_3)_2$. After washing with phosphate buffered saline (PBS) two times to remove the remaining $Zn(NO_3)_2$, the cells were incubated with 10 μM **L** at room temperature for 30 min. The cells were observed using a microscope (Olympus, Japan). The fluorescent images of the cells were obtained using a fluorescence microscope (Leica DMLB, Germany) at the excitation wavelength of 515–560 nm.

Results and discussion

Fluorogenic Zn(II) sensing

To determine the practical applications, the fluorescence response behavior of **L** was examined upon treatment with various metal ions in 10 mM HEPES buffer–CH_3OH (99 : 1, v/v) (Fig. 1a). Only Zn^{2+} resulted in a pronounced fluorescence enhancement, whereas addition of other relevant metal ions, such as Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Al^{3+}, Cr^{3+}, Hg^{2+}, and Fe^{3+}, caused little fluorescence increase. The selectivity for Zn^{2+} with **L** was plotted as a bar graph in Fig. 1b.

The fluorescence titration for the binding of **L** with Zn^{2+} was carried out under simulated physiological conditions. Fig. 2 shows the change in fluorescence spectra of **L** upon addition of Zn^{2+}.

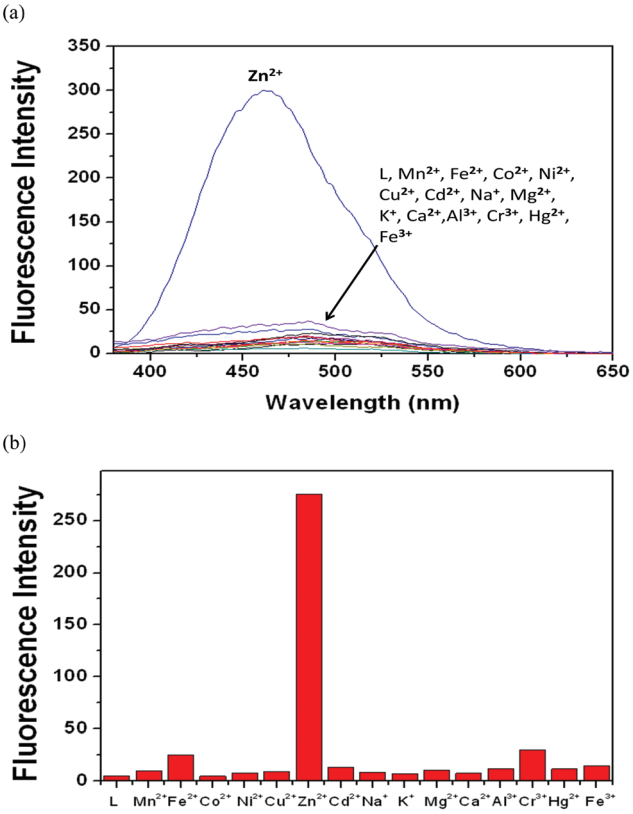


Fig. 1 Fluorescence spectra of **L** (10 μM) before and after addition of metal salts (10 μM) of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Na^{+}, K^{+}+, Mg^{2+}, Ca^{2+}, Al^{3+}, Cr^{3+}, Hg^{2+}, and Fe^{3+} in 10 mM HEPES buffer–CH_3OH (99 : 1, v/v). (b) Bar graph shows the relative emission intensity of **L** at 462 nm upon treatment with various metal ions.

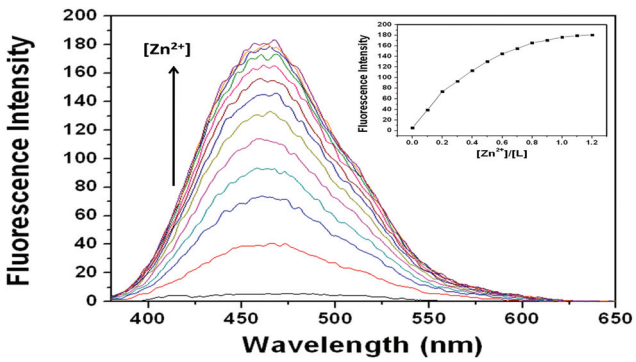
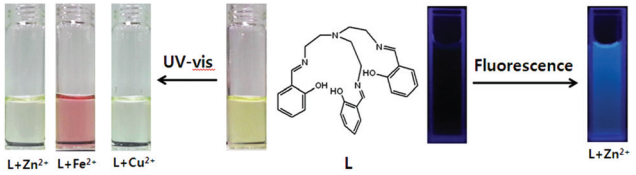


Fig. 2 Fluorescence spectra of **L** (10 μM, λ_{ex} = 362 nm) after addition of increasing amounts of Zn^{2+} ions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, and 1.2 equiv.) in 10 mM HEPES buffer–CH_3OH (99 : 1, v/v) at room temperature. Inset: intensity at 462 nm *versus* the number of equivalents of Zn^{2+} added.

While compound **L** alone displayed negligible fluorescence intensity (λ_{ex} = 362 nm), a Zn^{2+} titration experiment led to a prominent fluorescence enhancement, indicating a Zn^{2+}-selective OFF–ON type of fluorescent signaling behavior (Scheme 1). The fluorescence intensity of **L** increased 55-fold at a wavelength of 463 nm (Φ = 0.065). The increase in emission intensity is due to the formation of the **L**–Zn^{2+} complex,



Scheme 1 Fluorometric and colorimetric changes of **L** with Zn^{2+}, Cu^{2+}, and Fe^{2+}.

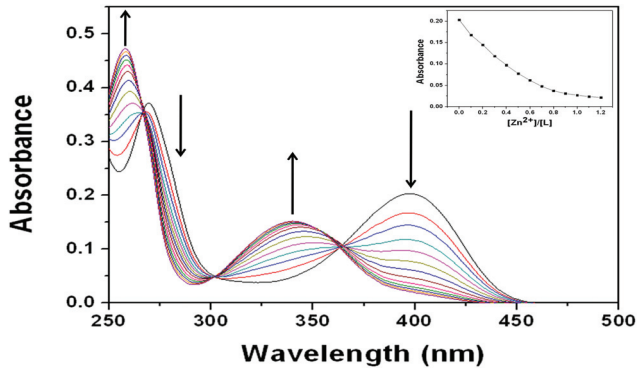


Fig. 3 Absorption spectra changes of **L** (20 μM) after addition of increasing amounts of Zn^{2+} ions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, and 1.2 equiv.) in 10 mM HEPES buffer–CH_3OH (99 : 1, v/v) at room temperature. Inset: absorption at 397 nm *versus* the number of equivalents of Zn^{2+} added.

causing the chelation-enhanced fluorescence effect[70,71] and inhibiting the C=N isomerization.[72–74]

A quantitative investigation of the binding affinity of **L** with Zn^{2+} was studied by UV-vis titration (Fig. 3). Upon the addition of Zn^{2+} (0–1 equiv.) to a solution of **L**, a gradual decrease of the absorption bands at 272 and 400 nm and a concomitant gradual increase of new absorption bands centered at 260 and 341 nm were observed with distinct isosbestic points at 267, 301, and 364 nm. Subsequently, upon further addition of Zn^{2+} (>1 equiv.), the absorption spectrum remained at a plateau. These observations imply the undoubted conversion of free compound **L** to the corresponding zinc complex.

The Job plot for the binding between **L** and Zn^{2+} exhibited a 1 : 1 stoichiometry for the **L**–Zn^{2+} complexation (Fig. S1†). Furthermore, the positive ion mass spectrum for the reaction of **L** with Zn^{2+} confirmed the 1 : 1 formation of the **L**–Zn^{2+} complex [*m*/*z*: 521.27; calcd, 521.15] (Fig. 4).

Based on the Job plot and ESI-mass spectrometry analysis, we propose the structure of a 1 : 1 complex of **L** and Zn^{2+}, as shown in Scheme 2. The binding constant of **L** with Zn^{2+} was calculated as log K_a = 4.52 on the basis of Benesi–Hildebrand analysis (Fig. S2†). This value is within the range of those (0.3–7.09) reported for Zn^{2+}-binding chemosensors.[59,60,69,75] The absorption titration profile of **L** with Zn^{2+} demonstrated that the detection limit of Zn^{2+} is 1.1 μM on the basis of $3\sigma/K$ (Fig. S3†).[76] The detection limit of **L** is far below the WHO guidelines for drinking water (76 μM).[77]

To examine the selectivity for Zn^{2+} in a complex background of potentially competing species, the fluorescence

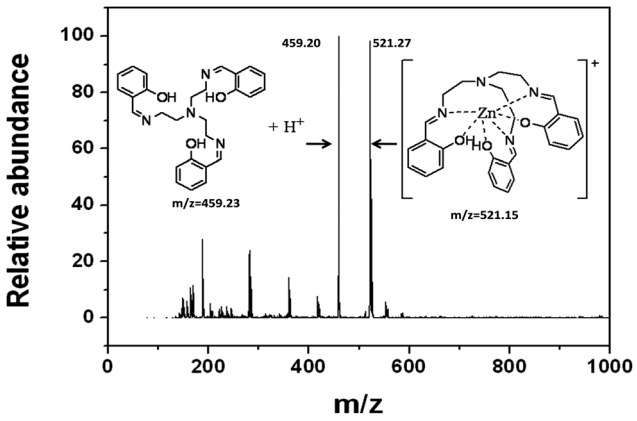
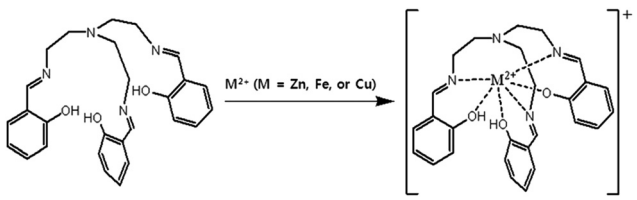


Fig. 4 Positive-ion electrospray ionization mass spectrum of **L** upon addition of 1 equiv. of Zn^{2+} in CH_3OH.



Scheme 2 Proposed structures of **L**–M^{2+} (M = Zn or Fe or Cu) complexes.

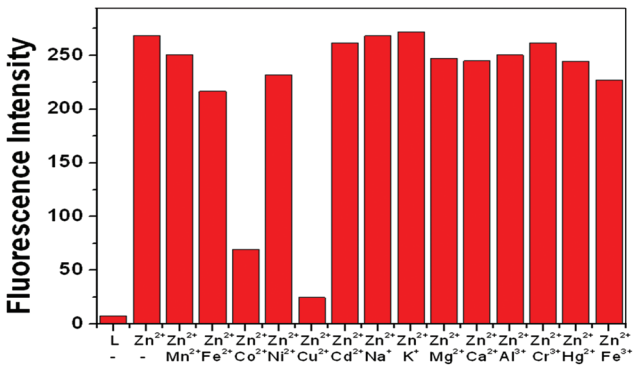


Fig. 5 Relative fluorescence of **L** and its complexation with Zn^{2+} in the presence of various metal ions. Response of **L** was included as controls. **L** alone, **L** + Zn^{2+}, **L** + Zn^{2+} + Mn^{2+}, **L** + Zn^{2+} + Fe^{2+}, *etc.* (Left to right). Conditions: **L**, 10 μM; Zn^{2+}, 10 μM; other metal ions, 10 μM (λ_{ex} = 362 nm).

enhancement of **L** with Zn^{2+} was investigated in the presence of other metal ions (Fig. 5). A background of most competing metal ions did not interfere with the detection of Zn^{2+} by **L** in HEPES buffer–CH_3OH (99 : 1, v/v), except for Co^{2+} and Cu^{2+}, which, respectively, quenched about 74 and 90% of the fluorescence obtained with Zn^{2+} alone. Nevertheless, **L** still had a sufficient "turn-on" ratio for the detection of Zn^{2+} in the presence of Co^{2+}, although Cu^{2+} interferes much with the binding of Zn^{2+} to **L**. These results indicate that **L** exhibits good selectivity for Zn^{2+} over competing relevant metal ions.

For biological applications, the pH dependence of the **L**–Zn^{2+} complex was examined. Over the pH range tested, the

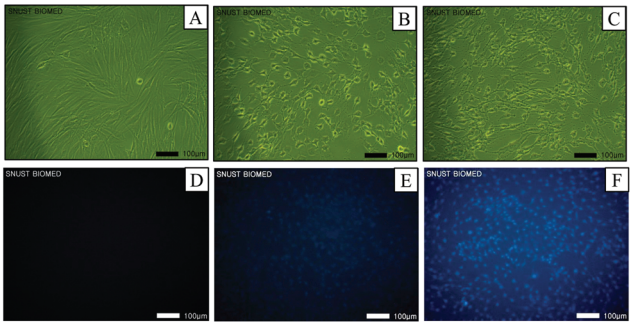


Fig. 6 Fluorescence images of fibroblasts cultured with Zn^{2+} and **L**. Cells were exposed to 0 (A and D), 100 (B and E), and 200 (C and F) μM $Zn(NO_3)_2$ for four hours and then later with 10 μM **L** for 30 min. The top images (A–C) were observed with the light microscope and the bottom images were taken with a fluorescence microscope. The scale bar is 100 μm.

fluorescence intensity of **L**–Zn^{2+} displayed strong pH dependence, as illustrated by its fluorescence intensity at 462 nm (Fig. S4†). An intense and stable fluorescence of **L**–Zn^{2+} in the pH range 6.0–12.0 warrants its application in monitoring intracellular Zn^{2+} without its being affected by changes in physiological pH values.

Based on pH dependence, subsequent experiments were conducted to test whether **L** could be used to visualize intracellular Zn^{2+} by fluorescence. Adult human dermal fibroblasts were first incubated with various concentrations of Zn^{2+} (0, 100, and 200 μM) for 4 h and then exposed to **L** (10 μM) for 30 min before imaging. The fibroblasts that were cultured with both Zn^{2+} and **L** exhibited fluorescence (Fig. 6), while those cells cultured without Zn^{2+} or without **L** did not exhibit fluorescence. The intensity and region of the fluorescence within the cell with **L** increased as the Zn^{2+} concentration increased from 100 to 200 μM. These results provide a new sensor **L** to image Zn^{2+} in cells, and, therefore, **L** is useful for determining the exposure level of cells to Zn^{2+}.

Chromogenic Fe(II), Cu(II), and Zn(II) sensing

Compared with other detection methods, colorimetric detection offers the advantages of simplicity, rapidity, and cost-effectiveness.[16] Therefore, colorimetric detection methods are extremely attractive in chemical and biological analyses. The cap-type tripodal receptor **L** showed color changes from yellow to red in the presence of Fe^{2+} and from yellow to colorless in the presence of Cu^{2+} and Zn^{2+} (Scheme 1), while other metals caused no change in color (Fig. 7a). Fortunately, **L**–Zn^{2+} and **L**–Cu^{2+} complex solutions could be distinguished using fluorescence spectroscopy, because the **L**–Zn^{2+} complex exhibits fluorescence but the **L**–Cu^{2+} complex exhibits no fluorescence. The UV-vis spectra changes of **L** toward various metal ions are shown in Fig. 7b. An absorption peak at 509 nm in the presence of Fe^{2+} is attributed to the metal-to-ligand charge-transfer,[78] which is responsible for the red color of the solution (Fig. 7b). Meanwhile, the absorption peak at 400 nm decreased in the presence of Cu^{2+} and Zn^{2+}. This is responsible for the color changes from yellow to colorless (Fig. 7b).

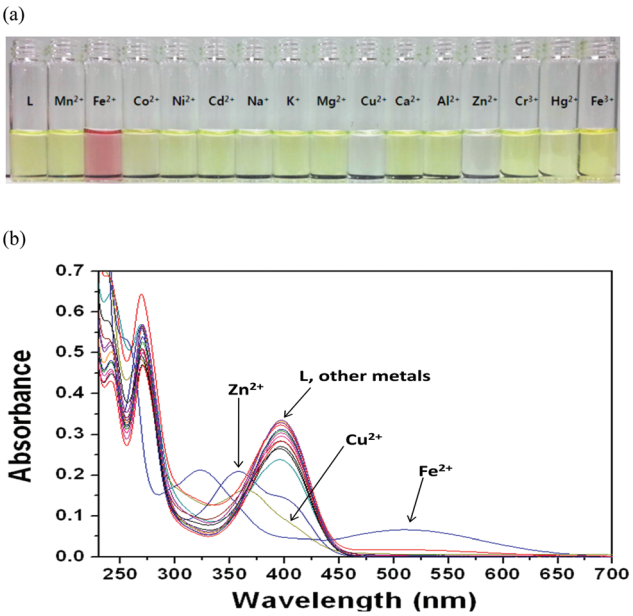


Fig. 7 (a) The color changes of **L** (50 μM) upon addition of various metal ions (2 equiv.). (b) Absorption spectra changes of **L** (20 μM) in the presence of 2 equivalents of different metal ions in 10 mM HEPES buffer–CH_3OH (99 : 1, v/v).

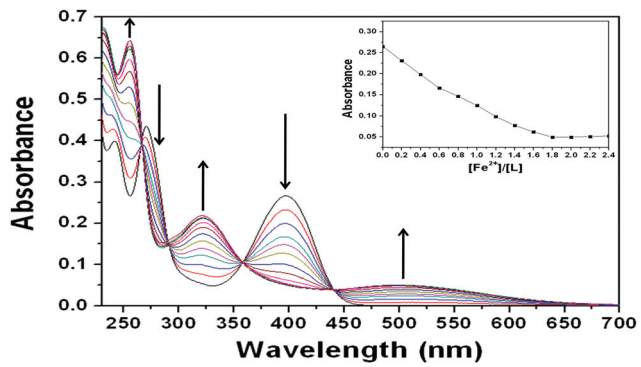


Fig. 8 Absorption spectra changes of **L** (20 μM) after addition of increasing amounts of Fe^{2+} ions (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, and 2.4 equiv.) in 10 mM HEPES buffer–CH_3OH (99 : 1, v/v) at room temperature. Inset: absorption at 400 nm *versus* the number of equivalents of Fe^{2+} added.

In order to understand the binding property of **L** and Fe^{2+}, UV-vis titrations of **L** with Fe^{2+} were carried out (Fig. 8). Upon the addition of Fe^{2+} (0–2 equiv.) to **L**, a gradual decrease of the absorbances at 272 and 400 nm and a concomitant gradual increase of new absorption bands centered at 257, 325, and 509 nm were observed, with distinct isosbestic points at 267, 290, 359, and 442 nm.

The latter indicate the formation of only one UV-active iron complex. Subsequently, the absorption spectra remained at a plateau upon further addition of Fe^{2+} (>2 equiv.).

The Job plot for the binding between **L** and Fe^{2+} exhibited a 1 : 1 stoichiometry for the **L**–Fe^{2+} complex formation (Fig. S5†). In order to further investigate the binding mode of the **L**–Fe^{2+} complex, the positive ion mass experiment was performed. However, it was not possible to get more information on the

binding mode of the **L**–Fe^{2+} complex. Based on the Job plot and the structures of similar types of iron complexes reported in the literature,[79–84] we therefore propose the structure of a 1 : 1 complex of **L** and Fe^{2+} as shown in Scheme 2. The binding constant of **L** with Fe^{2+} was calculated as log K_a = 4.30 (Fig. S6†), and the absorption titration profile of **L** with Fe^{2+} indicated that the detection limit of Fe^{2+} is 1.3 μM on the basis of $3\sigma/K$ (Fig. S7†).

To examine the selectivity for Fe^{2+} in the presence of potential competing species, the formation of **L** with Fe^{2+} was investigated in the presence of other metal ions (Fig. 9). The only metal ion that interfered with the detection of Fe^{2+} by **L** was Cu^{2+}; other metal ions had no effect. Nevertheless, Cu^{2+} still showed 20% absorbance. Importantly, Fe^{2+} could be clearly discriminated from Fe^{3+} by **L** in aqueous media. The discrimination between the 2+ and 3+ forms is important in order to understand the biological roles of iron. In addition, sensors capable of detecting Fe^{2+} by the naked eye in aqueous media are very rare.[85–87]

The pH sensitivity of Fe^{2+} detection by **L** was examined by absorption measurements (Fig. S8†). Whereas the absorption of **L**–Fe^{2+} was very weak at low and high pHs, the complex showed a significant response between pH 5 and 10, which includes the biologically relevant range of pH 6.0–7.6.

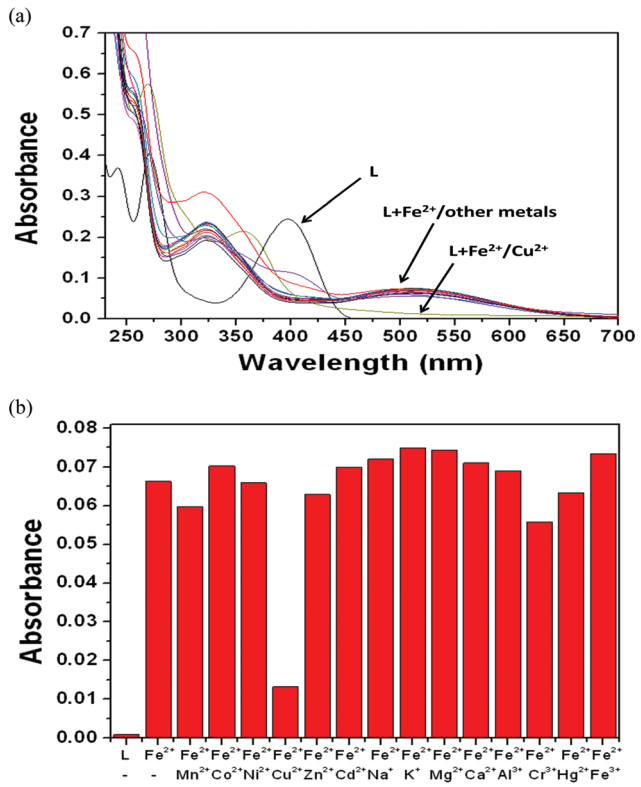


Fig. 9 (a) Absorption spectra changes of **L** and its complexation with Fe^{2+} in the presence of various metal ions in 10 mM HEPES buffer–CH_3OH (99 : 1, v/v). (b) Relative absorbance of **L** and its complexation with Fe^{2+} in the presence of various metal ions. Response of **L** was included as controls. **L** alone, **L** + Fe^{2+}, **L** + Fe^{2+} + Mn^{2+}, **L** + Fe^{2+} + Co^{2+}, *etc.* (left to right). Conditions: **L**, 20 μM; Fe^{2+}, 2 equiv.; other metal ions, 2 equiv.

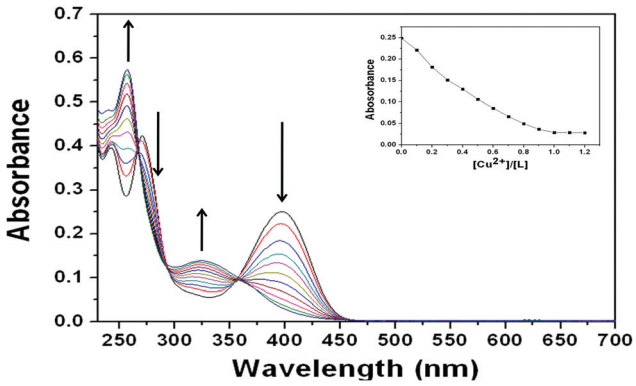


Fig. 10 Absorption spectra changes of **L** (20 μM) after addition of increasing amounts of Cu^{2+} ions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, and 1.2 equiv.) in 10 mM HEPES buffer–CH_3OH (99 : 1, v/v) at room temperature. Inset: absorption at 400 nm *versus* the number of equivalents of Cu^{2+} added.

The binding properties of **L** with Cu^{2+} were also studied by UV-vis titration (Fig. 10).

The addition of Cu^{2+} ions to a solution of **L** resulted in a decrease of the absorption bands at 275 nm and 400 nm and the appearance of new bands at 258 nm and 327 nm with a clear isosbestic point at 267 nm, 296 nm, and 360 nm, indicative of a conversion of **L** to a single **L**–Cu^{2+} complex. The Job plot for the binding between **L** and Cu^{2+} exhibited a 1 : 1 stoichiometry (Fig. S9†), and the positive ion mass spectrum of **L** upon addition of 1 equiv. of Cu^{2+} confirmed the formation of the Cu^{2+} + **L** complex [*m*/*z*: 520.27; calcd, 520.15] (Fig. S10†). Based on the Job plot and ESI-mass spectrometry analysis, we propose the structure of a 1 : 1 complex of **L** and Cu^{2+} as shown in Scheme 2. From the Benesi–Hildebrand equation, the association constant was found to be log K_a = 4.70 which is within the range of those (3.9–6.22) reported for Cu^{2+}-binding chemosensors (Fig. S11†).[88–91] The detection limit of receptor **L** for the analysis of Cu^{2+} was calculated to be 1.5 μM (Fig. S12†), which is far below the WHO guidelines (30 μM). This result suggests that **L** could be a powerful tool for the detection of copper in the drinking water.

When the selectivity for Cu^{2+} by **L** was examined in the presence of 1 equiv. of other metal ions such as Na^+, K^+, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+}, Hg^{2+}, Fe^{2+}, Al^{3+}, Fe^{3+}, and Cr^{3+}, the absorption spectra of **L** remained almost unchanged (Fig. 11), indicating that none of the other metals interfered with the detection of Cu^{2+} by **L**. This result strongly indicates that receptor **L** is an excellent chemosensor for the biologically important detection of Cu^{2+}.

Conclusions

We have described a unique fluorogenic Zn^{2+} and chromogenic Fe^{2+}, Cu^{2+} and Zn^{2+} chemosensor **L** based on a cap-typed tripodal Schiff base. It exhibited excellent selectivity for Zn^{2+}, Fe^{2+} and Cu^{2+}, by different methods, over competing relevant

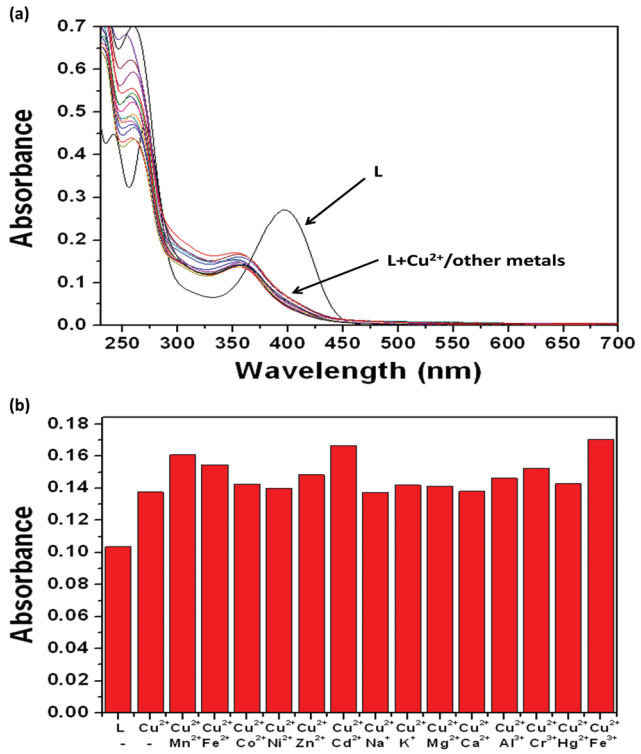


Fig. 11 (a) Absorption spectra changes of **L** and its complexation with Cu^{2+} in the presence of various metal ions in 10 mM HEPES buffer–CH_3OH (99 : 1, v/v). (b) Relative absorbance of **L** and its complexation with Cu^{2+} in the presence of various metal ions. Response of **L** was included as controls. **L** alone, **L** + Cu^{2+}, **L** + Cu^{2+} + Mn^{2+}, **L** + Cu^{2+} + Co^{2+}, *etc.* (left to right). Conditions: **L**, 20 μM; Cu^{2+}, 2 equiv.; other metal ions, 2 equiv.

metal ions in aqueous media. The sensor **L** could function as "turn-on" fluorescent chemosensor for Zn^{2+}. **L** is also capable of mapping zinc levels in cells. This class of fluorophores might be exploited as specific and effective sensors for intracellular Zn^{2+}. The detection limit of **L** for Zn^{2+} (1.1 μM) is far below the guidelines of the WHO (76 μM). Moreover, **L** displayed an obvious color change from yellow to red upon selective binding with Fe^{2+} and from yellow to colorless upon selective binding with Cu^{2+} and Zn^{2+}. Meanwhile, **L**–Zn^{2+} and **L**–Cu^{2+} complex solutions could be distinguished using fluorescence spectroscopy, because a **L**–Zn^{2+} complex has fluorescence but a **L**–Cu^{2+} complex has no fluorescence. The detection limit (1.5 μM) of **L** for Cu^{2+} is far below the guidelines of the WHO (30 μM), making it capable of being a practical system for the monitoring of Cu^{2+} concentrations in aqueous samples. In particular, it is worthwhile mentioning that Fe^{2+} could be clearly discriminated from Fe^{3+} by **L** in aqueous media. The discrimination between two 2+ and 3+ forms is important in order to understand the biological functions regulated by iron. Therefore, the results reported here provide a novel approach for the simultaneous selective recognition of the three most abundant transition metal ions (Zn^{2+}, Fe^{2+}, and Cu^{2+}) among the various metal ions in the human body.

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